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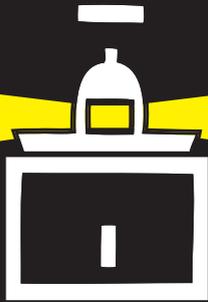
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THIRD GENERATION AUTOFLUORESCENCE ENDOSCOPY FOR THE DETECTION OF EARLY NEOPLASIA IN BARRETT'S OESOPHAGUS; A PILOT STUDY



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ABSTRACT

INTRODUCTION: in Barrett's oesophagus (BO), second-generation autofluorescence imaging (AFI-II) improves targeted detection of high-grade intraepithelial neoplasia (HGIN) and early cancer (EC), yet suffers from high false-positive (FP) rates. The newest generation AFI (AFI-III) specifically targets fluorescence in malignant cells and may therefore improve detection of early neoplasia and reduce FP-rate.

AIM: Compare AFI-III with AFI-II for endoscopic detection of early neoplasia in BO.

METHODS: BO patients with endoscopically inconspicuous neoplasia underwent two diagnostic endoscopies (AFI-II/AFI-III) in a single session. Endpoints: number of patients and lesions with HGIN/EC detected with AFI-II and AFI-III after white-light-endoscopy (WLE) and the value of reinspection of AFI-positive areas with WLE and narrow-band imaging (NBI).

RESULTS: 45 patients were included (38 males, age 65 years). 19 patients showed HGIN/EC. AFI-II inspection after WLE increased detection of HGIN/EC from 9 to 15 patients (47% to 79%); AFI-III increased detection from 9 to 17 patients (47% to 89%). WLE plus random biopsies diagnosed 13/19 (68%) HGIN/EC-patients. 104 abnormal AFI-areas were inspected; 23 (22%) showed HGIN/EC. AFI-II increased detection of HGIN/EC from 10 to 18 lesions (43 to 78%). AFI-III increased detection from 10 to 20 lesions (43 to 87%). FP-rate was 86% for AFI-II and AFI-III. Reinspection with WLE or NBI reduced FP-rate to 21% and 22% respectively, but misclassified HGIN/EC-lesions as unsuspecting in 54% and 31%, respectively.

CONCLUSIONS: This first feasibility study on third-generation AFI again showed improved targeted detection of HGIN/EC in BO. However, the results do not suggest AFI-III performs significantly better than conventional AFI-II.



INTRODUCTION

Endoscopic surveillance of patients with Barrett's oesophagus (BO) is recommended to detect early neoplasia (*i.e.* High Grade Intraepithelial Neoplasia (HGIN) and/or Early Cancer (EC)) at a curable stage [1]. When using standard endoscopy, however, it may be difficult to distinguish areas with early neoplasia within the normal Barrett's mucosa [2]. In the absence of visible abnormalities random four-quadrant biopsies are obtained every 1-2 cm of the BO [1], to allow for histological evaluation for the presence of neoplasia [2, 3]. Unfortunately, random biopsies are associated with significant sampling error and therefore occult malignant lesions in the BO may be missed [4, 5]. To increase the detection of early neoplasia in BO patients, different advanced imaging techniques have been studied. A promising approach is Endoscopic TriModal Imaging (ETMI), which incorporates white light endoscopy (WLE), autofluorescence imaging (AFI) and narrow-band imaging (NBI) in a single endoscopy system. AFI is based on the principle that certain endogenous substances such as flavins and collagen (*i.e.* *fluorophores*) emit fluorescent light when they are excited with short wavelengths of light. It has been demonstrated that early neoplasia has a different autofluorescence spectrum than non-neoplastic Barrett's tissue [6, 7]. When these spectra are computed into a pseudocolor image, early neoplastic areas show a violet colour, whereas the non-neoplastic Barrett's tissue appears green. Two uncontrolled studies have suggested that AFI increases the detection of early neoplasia, at the expense of a relatively high false positive rate [8, 9]⁸⁹. The third modality of the ETMI system, NBI, utilizes short wavelength light (essentially, blue light) to enhance the superficial imaging of the mucosa, resulting in an enhanced image of the relief, epithelial architecture and vascular structures. Initial studies yielded promising results in terms of distinguishing NBI images of non-neoplastic BO from those containing early neoplasia [10, 11]. We have recently conducted two randomized crossover trials comparing the ETMI system with standard video endoscopy. These studies showed that AFI significantly increases the targeted detection of early neoplasia and that subsequent inspection with NBI reduces the false-positive rate. The increased targeted detection of the ETMI system, however, can be fully compensated by obtaining random biopsies during standard endoscopy. In fact, targeted biopsies with ETMI was found to be inferior to targeted biopsies plus random biopsies with standard endoscopy [12-14].

The AFI-mode of the current ETMI system (AFI-II) uses an algorithm which computes the emitted autofluorescence spectra and reflected green light into a pseudocolour image. With the current algorithm, the detected autofluorescence is mainly produced by fluorophores in the submucosal layer [15]. Autofluorescence therefore is dependent on the thickness of the mucosa, since the excitation light has to pass through the mucosa to reach the submucosa and subsequently the emitted fluorescent light has to return through the mucosa to reach the esophageal lumen. This implies that the autofluorescence signal can also be altered by non-dysplastic changes of the mucosal architecture such as extensive tissue inflammation [9].

A different combination of fluorescence and/or reflectance signals may target the actual dysplastic parameters more adequately. Imaizumi *et al.* have suggested that by changing excitation wavelength and combining multiple fluorescence spectra, fluorophores in malignant

cells are specifically targeted, rather than tissue architectural changes [16]. Their research resulted in a new AFI algorithm which was subsequently incorporated in a third generation AFI endoscope (AFI-III) and combined with WLE and NBI in a third generation ETMI system.

We hypothesize that the new AFI-III system – compared to the conventional AFI-II – enhances the distinction between early neoplasia and inflammation in BO and thus reduces the amount of false positive lesions, allowing for targeted sampling and better detection of early neoplasia in BO.

This is the first uncontrolled feasibility study investigating the third generation AFI system for the detection of early neoplasia in a selected group of patients with BO.

METHODS

Setting

This pilot study was performed at the Academic Medical Centre (AMC) Amsterdam, a tertiary-care referral centre for the treatment of patients with early Barrett's neoplasia.

The study was approved by the Medical Ethical Committee of the AMC Amsterdam and all patients provided a written informed consent.

Patients

In line with previous pilot studies on earlier AFI systems, a total of 49 patients was considered sufficient in this pilot study to investigate the feasibility of the third generation AFI-system^{8,9}.

Patients were included if they met all the following inclusion criteria:

- Age over 18 years;
- Prior diagnosis of BO defined as columnar lined esophageal epithelium upon endoscopy and intestinal metaplasia upon histological assessment of esophageal biopsies;
- Confirmed diagnosis of EC, HGIN, LGIN or non-dysplastic BO;
- Written informed consent.

Patients were excluded if they met any of the following exclusion criteria:

- Active erosive esophagitis grade B or higher according to the Los Angeles classification of erosive oesophagitis [17];
- Advanced neoplastic lesion (*i.e.* any lesion considered not amendable to endoscopic treatment based its endoscopic appearance);
- Unable to undergo biopsy sampling (*e.g.* due to coagulation disorders, esophageal varices).

Technical background

Two separate ETMI systems were used in this study. The ETMI endoscopes (GIF-FQ260Z, Olympus Inc., Tokyo, Japan) provide real-time endoscopic images for high-resolution WLE, AFI and NBI, and allow for optical zooming (magnification 100x). The light sources (CLV-260SL, Olympus Inc., Tokyo, Japan) use sequential red, green and blue (RGB) illumination through a set of two filters: one filter switches between WLE, AFI or NBI. The second filter rotates and contains filters for WLE and NBI on the outer ring and AFI filters on the inner ring. The endoscopes contain two



separate high-quality monochromatic charge-coupled devices (CCD's): one for high-resolution WLE and NBI, and one for AFI. In the WLE and NBI mode the reflected RGB light is detected by a monochromatic CCD and transmitted to a video processor (Lucera, Olympus Inc., Tokyo, Japan), which is synchronised with the rotary filter. The processor then overlays the red, green and blue images to produce a high-quality WLE or NBI image. The WLE and NBI modalities of both ETMI systems used in this study are equal. The AFI mode of both systems is described separately below.

AFI-II-ETMI system

In the AFI-II mode, the AFI image is composed of total emitted autofluorescence after blue light excitation (390-470 nm) and green reflectance (540-560 nm). A filter is placed in front of the AFI-CCD to only allow passage of fluorescent light with a wavelength between 500 and 630 nm, eliminating the blue excitation light. The composed signal is then transmitted to the video processor and projected on the monitor in pseudocolours: normal, non-suspicious Barrett's mucosa appears green and suspicious areas have a dark purple appearance.

AFI-III-ETMI system

The AFI algorithm in the prototype AFI-III-ETMI system targets alterations in the metabolism of fluorescent NADH (hydrolyzed nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide), which is influenced by neoplasia related hypoxia. AFI-III induces fluorescence with dual narrow wavelength excitation combined with green reflectance: F_{380ex} ($\lambda = 380-390$ nm), F_{405ex} ($\lambda=400-420$ nm), G_{ref} ($\lambda = 540-560$ nm). The barrier filter in front of the AFI CCD allows passage of light between 450 and 570 nm. With the AFI-III-ETMI system, the endoscopist can choose from three distinct AFI modi, in which the images are constructed from different components of fluorescent and reflected light:

1. The first mode uses a two-colour image and is composed of "pure fluorescence": $[R, G, B] = [F_{380ex}, F_{405ex}, F_{380ex}]$
2. The second mode produces a full three colour image: $[R, G, B] = [F_{380ex}, F_{405ex}, G_{ref}]$
3. The third mode resembles the conventional AFI-II: $[R, G, B] = [G_{ref}, F_{405ex}, G_{ref}]$

Study design

All patients underwent two consecutive endoscopy procedures in one session. The first endoscopy was performed with the AFI-II-ETMI system. Subsequently, the AFI-II-ETMI system was replaced by the prototype AFI-III-ETMI system. All endoscopic procedures were performed in the AMC Amsterdam by one expert endoscopist (JB) with extensive experience in the use of advanced imaging and endoscopic treatment for neoplastic lesions in the oesophagus.

Endoscopic procedures

Patients were sedated by intravenous administration of propofol or midazolam (2.5-15 mg) supplemented with fentanyl (0.1-0.2 mg) if necessary.

The procedure was started with the AFI-II ETMI system. The oesophagus was first examined in overview with WLE and the length of the Barrett's segment was recorded according to the

Prague-classification system [18]. Then, detailed WLE inspection of the Barrett's segment was performed. For all detected lesions the location (distance from the incisors and endoscopic quadrant), diameter and lesion type according to the Paris classification [19]¹⁹ were recorded on a standardized CRF and still images obtained. Subsequently, the Barrett's segment was inspected with AFI-II for the presence of additional lesions. For all additional lesions primarily detected with AFI-II, the location, diameter and macroscopic appearance were recorded. All lesions were subsequently inspected using WLE and NBI in overview and magnification using a 4-mm soft clear cap, to document the following NBI characteristics: mucosal pattern (regular -including flat or absent- vs. irregular); vascular pattern (regular vs. irregular); abnormal blood vessels (present vs. absent); the mucosal relief (completely flat vs. granular/nodular); the overall NBI appearance (suspicious vs. not suspicious for neoplasia) [20,21]. Furthermore, an AFI-negative control area was identified and inspected with WLE and NBI. Then, the AFI-II-ETMI system was replaced by the AFI-III-ETMI system and prototype endoscope. All lesions, including the AFI-negative control area, initially found with WLE or AFI-II were inspected with AFI-III, followed by inspection of the remaining BO with AFI-III for the presence of additional lesions. During inspection with the AFI-III-ETMI system, all three AFI-III modes were used by the endoscopist to assess the oesophagus. Areas found positive with AFI-III that were not detected with WLE or AFI-II, were reinspected by reintroducing the AFI-II-ETMI system. All additional lesions detected with AFI-III were recorded and subsequently inspected with WLE and NBI. All lesions, including the AFI-negative control area, were sampled for histological correlation using a standard disposable biopsy forceps (FB-240K, Olympus, Tokyo). Finally, 4 quadrant random biopsies were obtained at 2 cm levels of the Barrett's segment. The prior biopsied targeted areas were avoided for random sampling.

Histological assessment

All hematoxylin and eosin (H&E) and - when available - all p53 stained slides of the biopsy specimens obtained at the referring centre were evaluated by an expert GI pathologist (MV) prior to inclusion.

All biopsies were fixed in formalin, embedded in paraffin and routinely cut and stained with haematoxylin and eosin (H&E). Histological assessment of all biopsies was performed by an expert GI-pathologist (MV), who recorded the presence of intestinal metaplasia and neoplasia according to the revised Vienna classification: no-dysplasia, indefinite for dysplasia, LGIN, HGIN or invasive cancer [22].

Study outcome parameters

- The number of additional patients with HGIN/EC detected with AFI-II and AFI-III after inspection with WLE;
- The number of additional lesions with HGIN/EC detected with AFI-II and AFI-III after inspection with WLE;
- False positive rates of AFI-II and AFI-III;
- Accuracy of reinspection of AFI-positive areas with WLE, expressed by reduction of false positive rate of AFI and misclassification of HGIN/EC lesions as unsuspecting;



- Accuracy of detailed inspection of WLE- and AFI-positive areas with NBI, expressed by reduction of false positive rate of AFI and WLE and misclassification of HGIN/EC lesions as unsuspecting.

Statistics

Statistical analysis was performed with SPSS 16.0.2/18 software for Windows. For descriptive statistics mean (\pm SD) was used in case of a normal distribution of variables, and median (IQR) was used for variables with a skewed distribution. Where appropriate, the student *t*-test, McNemar's test and the Mann-Whitney test were used.

RESULTS

Patients

From September 2010 until July 2011, 49 patients with BO (38 males, mean age 65 years [SD 12]) were included with the following indications (the histological diagnoses are based on review of the biopsies from the referring center by the study pathologist):

1. 10 BO patients were referred with HGIN/EC (8/2) and an endoscopically visible lesion;
2. 10 BO patients were referred with HGIN in the absence of an endoscopically visible lesion;
3. 13 BO patients were referred with low grade intraepithelial neoplasia (LGIN) in the absence of an endoscopically visible lesion;
4. 16 Patients with a non-dysplastic Barrett's oesophagus (NDBO) and no visible lesions.

Three patients were excluded during endoscopy due to unexpected more advanced stages of carcinoma upon first endoscopy after referral. One patient was excluded due to refractory oesophagitis, leaving a total of 45 patients for analysis.

Median circumferential Barrett's length was 4 cm (IQR: 1-9), median maximum Barrett's length was 6 cm (IQR: 4-10). Two patients were found to have grade A reflux esophagitis.

The worst histological diagnoses in these 45 patients were: HGIN in 19 patients, LGIN in 9 patients and NDBO in 17 patients.

Per patient analysis

Of the 19 patients with HGIN/EC, 9 patients had HGIN/EC lesions that were initially detected with WLE (9/19; 47%). AFI-II picked up an additional 6 patients with HGIN/EC, improving the targeted detection rate from 47% to 79% (15/19). Subsequent inspection with AFI-III resulted in the additional detection of 3 patients with HGIN in targeted biopsies, increasing overall targeted detection to 95% (18/19). The remaining patient was diagnosed with HGIN solely based on random biopsies out of multiple levels in a C3M5 Barrett's segment, while targeted biopsies out of two AFI positive lesions and a negative control area showed LGIN (*table 1*). If only AFI-III had been used next to WLE, an additional 8 patients with HGIN/EC would have been detected, resulting in an detection rate of 89% (17/19). The additional value of AFI-II and AFI-III over WLE was comparable (15/19 (79%) vs. 17/19 (89%) respectively, $p=0.25$).

Targeted biopsies after WLE yielded 9 patients with HGIN/EC. Without the use of both AFI systems, random biopsies would have detected 3 additional patients with HGIN. Therefore,

random biopsies increased the targeted detection rate of WLE from 47% to 63% (12/19). The contribution of all individual imaging modalities and random biopsies to the diagnosis of patients with HGIN/EC is summarized in table 1.

Per lesion analysis

In 45 patients, a total number of 104 abnormal areas (*figure 1*) and 41 negative control areas were inspected. Of the 104 abnormal areas, 23 (22%) showed HGIN/EC in targeted biopsies, 14 showed LGIN, 67 showed no dysplasia (Overall FP rate: 78% (81/104))

In total 23 areas were suspicious on WLE inspection, 10 lesions contained HGIN/EC (WLE FP-rate 57% (13/23)). Inspection with AFI-II picked-up 57 AFI-II positive areas of which 8 areas contained HGIN/EC (AFI-II FP-rate 86% (49/57)). Subsequent inspection with AFI-III resulted in the additional detection of 5 HGIN/EC lesions (*table 2*). If WLE had been followed only by AFI-III, an additional 71 AFI-III positives areas would have been detected of which 10 lesions contained HGIN/EC (AFI-III FP rate of 61/71; 86%). These figures suggest that there is no difference in false-positive rates between both AFI systems (86% for AFI-II vs. 86% for AFI-III, $p=0.289$) (*table 1*).

Forty-one areas, unsuspecting with both AFI-II and AFI-III, were included as negative controls. All areas were also considered unsuspecting with WLE and NBI. Thirty-five areas showed non-dysplastic mucosa, five areas contained LGIN, and in one patient with a C3M6 BO, 1 area showed HGIN. Four out of 8 random biopsies confirmed the multifocal distribution of HGIN in this patient. The negative predictive value for HGIN/EC of both systems combined therefore was 98% (40/41).

Concordant and discordant AFI-II/AFI-III cases

Table 3 shows the number of concordant and discordant AFI-II and AFI-III areas. Areas that showed suspicious AFI features with both AFI-systems demonstrated significantly more often HGIN/EC, compared to areas that were considered negative with both systems (19/90; 21% vs 1/41; 2%, $p=0.006$). Four areas were AFI-II positive and AFI-III negative, whereas 10 areas were considered AFI-III positive and AFI-II negative. There was no difference in the detection of neoplasia in the

Table 1. Detection of patients and lesions with HGIN/EC by WLE, AFI-II and AFI-III separately, and random biopsies, out of a total of 45 patients included in this study. AFI-II and AFI-III both reached a high false positive rate of 86%.

| | Detected with WLE | Additionally detected with AFI-II after WLE | Additionally detected with AFI-III after WLE | Additionally detected with RBx after WLE | Total number detected |
|---|-----------------------|---|--|--|-----------------------|
| Patients with HGIN/EC | 9 | 6 | 8 | 1 | 19* |
| Number of lesions detected | 23 | 57 | 71 | | 104* |
| Lesions with HGIN/EC | 10 | 8 | 10 | | 28 |
| False positive lesions | 13 | 49 | 61 | | 123 |
| False positive rate per modality (95% CI) | 13/23 (57%; 36-77) | 49/57 (86%; 77-95) | 61/71 (86%; 78-94) | | |

* the total number does not add up: some patients/lesions with HGIN/EC were diagnosed with both AFI-systems.
WLE: white light endoscopy; AFI: autofluorescence imaging; RBx: random biopsies; HGIN: high grade intraepithelial neoplasia; EC: early cancer; CI: confidence interval.

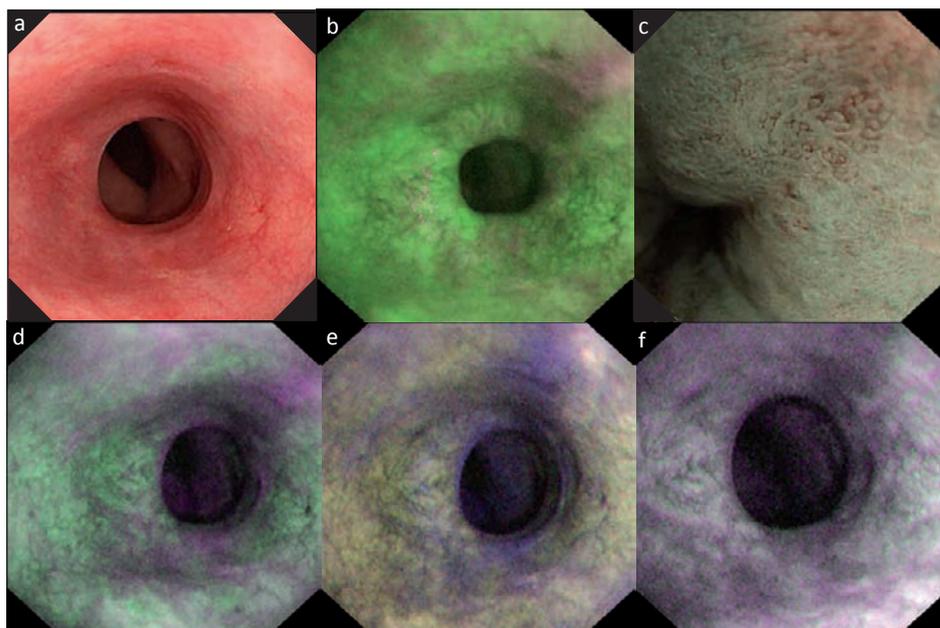


Figure 1a-f. Area containing HGIN in a patient referred with LGIN. The area was detected with AFI-II (b), considered unsuspecting with WLE (a) and suspicious with AFI-III (d-f) and NBI (c).

Table 2. The summary, distribution and (dis)agreement between AFI-II and AFI-III positive and negative areas and the yield of neoplasia (HGIN/EC).

| | AFI-II positive | AFI-II negative | Total |
|-------------------|-----------------|-----------------|-----------|
| AFI-III positive | 90 | 10 | 100 |
| Number of HGIN/EC | 19 (21%)* | 3 (30%)** | 36 (36%) |
| AFI-III negative | 4 | 41 | 45 |
| Number of HGIN/EC | 1 (25%)** | 1 (2%)* | 7 (16%) |
| Total | 94 | 51 | * p=0.006 |
| Number of HGIN/EC | 20 (21%) | 4 (8%) | ** p=0.85 |

Areas that were positive with both systems were significantly more often neoplastic than areas that were considered negative with both systems (19/90; (21%) vs 1/41; (2%), $p=0.006$). Discordant cases are shown in gray. In these discordant cases, there was no difference in the detection of neoplasia (neoplastic yield in AFI-II positive/AFI-III negative areas: 1/4 (25%) vs. AFI-II negative/AFI-III positive areas: 3/10 (30%), $p=0.85$).

discordant cases (neoplastic yield in AFI-II positive/AFI-III negative areas: 1/4 (25%) vs. AFI-II negative/AFI-III positive areas: 3/10 (30%), $p=0.85$).

The high number of concordant cases and the comparable neoplasia yield of the two systems for discordant cases suggests that both systems are comparable in terms of detecting neoplasia. This is also demonstrated by the yield of neoplasia in AFI positive areas for both systems individually (AFI-II 20/94 (21%) vs. AFI-III 22/100 (22%), table 2).

Reinspection of AFI-positive areas with WLE and NBI

Primary inspection with WLE resulted in 23 areas suspicious for early neoplasia of which 10 contained HGIN/EC.

Subsequent inspection with AFI (AFI-II and/or AFI-III) yielded 81 additional suspicious areas, 13 of which showed HGIN/EC. The false-positive (FP) rate of AFI after WLE inspection therefore was 84% (68/81).

These 81 AFI-positive areas were reinspected with WLE and 23 areas demonstrated subtle abnormalities. Six of these 23 areas contained HGIN/EC, the other 7 areas with HGIN/EC were considered normal upon reinspection with WLE. Reinspection with WLE thus reduced the FP-rate from 84% to 21% (17/81) at the expense of missing 54% (7/13) of lesions with HGIN/EC (*figure 2*).

All suspicious areas (WLE or AFI) were inspected in detail with NBI. Most of the 23 lesions identified primarily with WLE were also considered suspicious when inspected with NBI (21/23). NBI correctly identified all 10 HGIN/EC areas, yet also labelled 11/13 of the areas that did not contain HGIN/EC as suspicious. The reduction in FP-rate by NBI after primary inspection with WLE was therefore limited (WLE: 13/23 (57%); WLE+NBI: 11/21 (52%).

Of the 81 AFI-positive areas additionally detected after inspection with WLE, NBI graded 27 areas as suspicious for neoplasia. Nine of these 27 areas contained HGIN/EC (33%) vs. 4/54 AFI-positive areas which were considered normal upon inspection with NBI (7%).

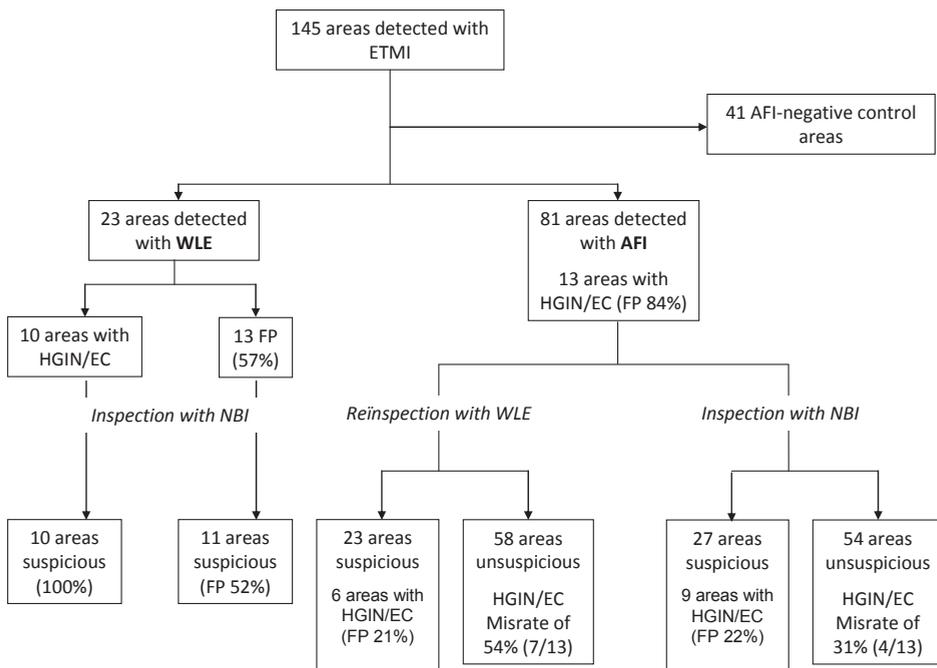


Figure 2. Flowchart of all areas detected by WLE and AFI, the amount of areas containing HGIN/EC and the additional value of (re)inspection with WLE and NBI. *FP: false positive.*



Detailed inspection with NBI thus reduced the FP-rate of AFI-positive areas from 84% (58/81) to 22% (18/81), yet at the expense of misclassifying 31% (4/13) areas containing HGIN/EC (*figure 2*).

Post-hoc evaluation of discordant AFI-II/AFI-III images

A post-hoc analysis of the still images of all discordant lesions as described in *table 2* was performed. In retrospect, 2 out of 4 areas considered AFI-II positive/AFI-III negative during real-time AFI, were now scored as both AFI-II and AFI-III positive. Contrary, 6 out of 10 AFI-II negative/AFI-III positive lesions were in retrospect regarded as positive with both AFI systems. In the post-hoc image analysis of discordant cases, 50% (2/4) and 60% (6/10) of the real-time gradings were thus changed.

DISCUSSION

This is the first feasibility study on the use of a third generation video-autofluorescence system for detection of early neoplasia in BO. By adjusting the excitation wavelengths and computing algorithms, this system aims to target fluorescent changes in malignant cells, in stead of architectural and spatial alterations due to secondary tissue reactions. We hypothesized that the AFI-III-ETMI system would increase the detection of dysplasia in Barrett's oesophagus and reduce the false positive rate associated with the AFI-II-ETMI system.

Our results again show that AFI markedly increases the detection of early neoplastic lesions in BO. Both AFI systems showed a comparable performance in detecting early neoplasia on a per patient basis. The added neoplastic yield of AFI-II over WLE was 32% and AFI-III improved the detection of HGIN/EC with 42%. In the per lesion analysis, AFI-II and AFI-III also achieved similar additional detection rates of 35% and 44%, respectively. In those few areas that were found to be positive with one AFI system and not with the other (discordant cases), no difference between both systems was observed in the yield of neoplasia.

As was shown in previous studies, AFI was associated with a high false-positive rate. In our study, both AFI-II and AFI-III had a FP-rate of 86%.

The tandem endoscopy design used in our current study may have introduced a systematic bias. Inspection with AFI-III can be influenced by previous inspection with AFI-II, which may lower the threshold for calling subtle AFI-III areas positive and thus overestimate both the detection rate and the false positive rate of AFI-III. A possible bias would be in favour of AFI-III for detecting early neoplasia, yet no differences in detection rates between both systems were observed. Post hoc evaluation of still images of subtle AFI-III false positive lesions did not reveal a lowered threshold for calling areas positive.

The added neoplastic yield of random biopsies over WLE in this study was less (16%) than observed in previously published reports [9, 12]. The tandem endoscopy design of the current study and previous feasibility studies may have led to an underestimation of the true value of random biopsies, since random sampling from suspicious areas was avoided per protocol. Certain AFI-positive areas would otherwise have been sampled by random biopsies. In our two previous randomized crossover trials with the AFI-II-ETMI system, targeted biopsies with ETMI

were in fact found to be inferior to targeted biopsies plus random biopsies with standard video endoscopy. This suggests that the additional yield of AFI over WLE can be largely compensated by obtaining random biopsies [13, 14].

WLE and AFI are used as red-flag techniques in order to highlight possible dysplastic areas in overview. Reinspection with WLE and NBI is used for the detailed inspection of AFI positive areas, to distinguish true positive areas (*i.e.* AFI positive areas containing HGIN/EC) from false positive areas (*i.e.* AFI positive areas not containing HGIN/EC). Previous studies, using a rather artificial setting of preselected still images with corresponding histology, suggested that NBI reduced the FP-rate significantly [20]. In subsequent studies, however, inspection with NBI was shown to be less effective: the reduction in FP-rate was found to be limited and/or a significant number of HGIN/EC lesions were considered to be NBI unsuspecting [13, 14]. In the current study, detailed inspection with NBI reduced the FP rate to 22%, but 4/13 lesions containing HGIN/EC were graded as NBI unsuspecting. This confirms the limited value for differentiation of AFI positive areas in BO with NBI.

The following limitations of our study must be addressed. As mentioned above, the most important limitation is the sequential tandem endoscopy design. Although both systems were individually evaluated by a single endoscopist during endoscopy, the assessment of the AFI-III-ETMI system may have been biased by the prior AFI-II inspection. A randomized crossover trial is the optimal design to study the performance of two imaging systems. However, given the anticipated small differences, a RCT would require a large number of patients. Therefore, a pilot study with a tandem design was considered the best option to study the feasibility of the AFI-III-system.

Furthermore, the lengthened inspection-time due to the addition of AFI-II and AFI-III was not corrected for. We cannot exclude the possibility that a prolonged inspection with WLE alone would have resulted in a comparable increase in the detection of HGIN/EC. Another limitation is that all procedures were performed by a single endoscopist with extensive experience in the detection of subtle abnormalities in BO, who was not blinded for the clinical history of the patient. This may have led to a high yield of dysplastic areas with WLE inspection. The additional value of AFI may therefore be different in community hospitals where there is a low pre-test likelihood of early neoplasia and endoscopists are generally less trained in detection of early neoplasia in BO. A recent randomized crossover study on the use of the AFI-II-ETMI system in community hospitals however showed no significant benefit of AFI over WLE with random biopsies [14].

Real-time AFI is influenced by various factors, such as distension of the oesophagus that influences the pseudocolour AFI-images. A post-hoc image analysis was therefore performed to assess the discordant cases (*table 2*), which showed that 50% and 60% of the original real-time AFI gradings were changed. Although the post-hoc analysis did not change the statistical outcome of the results reported above, it does illustrate the subjective nature of real-time AFI interpretation. Future studies should address this issue, since inter-observer variation may be a significant factor in the interpretation of AFI images. In this respect, the subjective assessment of AFI-III may be better than that of AFI-II. Some of the operating modi of AFI-III appear to offer improved brightness, contrast and image quality. A quantitative assessment of the image quality, colour and intensity, together with inter-observer agreement studies, may shed a new light on this issue.



In this first feasibility study of the AFI-III-ETMI system, we have again demonstrated that AFI increases the targeted detection of neoplasia in Barrett oesophagus. The additional value of AFI-III compared to the currently commercially available AFI-II system appears to be limited.

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