Advanced endoscopic imaging of esophageal neoplasia; old looks and new visions

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EFFECTS OF AUTOFLUORESCENCE IMAGING ON DETECTION AND TREATMENT OF EARLY NEOPLASIA IN PATIENTS WITH BARRETT’S ESOPHAGUS

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ABSTRACT

BACKGROUND & AIMS: Five prospective studies have shown that autofluorescence imaging (AFI) increases targeted detection of high-grade intraepithelial neoplasia (HGIN) and intramucosal cancer (IMC) in Barrett’s esophagus (BE). We aimed to assess the clinical relevance of AFI-detected lesions and the impact on management of patients with early neoplasia in BE.

METHODS: Data on patients, endoscopy and histology were extracted from databases of 5 prospective AFI studies and related to treatment outcome and follow-up.

ENDPOINTS: diagnostic value of AFI (proportion of surveillance patients with HGIN/IMC detected by AFI-targeted biopsies only), and therapeutic value of AFI: proportion of patients with any HGIN/IMC lesion detected with AFI that changed initial therapeutic plans based on white-light endoscopy (WLE) or random biopsies (RBx).

RESULTS: 371 BE patients (mean age 65 yrs, 305 males), referred for surveillance (211) or work-up for early neoplasia (160), were enrolled. HGIN/IMC was diagnosed in 147 patients. In 211 patients undergoing surveillance, 39 showed HGIN/IMC: WLE detected 23, RBx detected 11; AFI detected 5 patients. The diagnostic value of AFI was 5/211 (2%).

In 24 patients, HGIN/IMC was diagnosed with AFI only. In 33 patients, AFI detected additional HGIN/IMC apart from primary WLE/RBx detected lesions. In 57 patients, AFI lesions were treated: 26 underwent radiofrequency ablation and showed full remission of neoplasia; 31 underwent endoscopic resection, showing IMC in 6 cases. The therapeutic value was 6/371 (2%).

CONCLUSIONS: Given the low clinical impact of AFI-detected lesions on the diagnosis of early neoplasia and therapeutic decision-making, the role of AFI in routine BE surveillance and work-up is limited.
INTRODUCTION

Barrett’s esophagus (BE) is a condition in which the normal squamous mucosa of the esophagus has been replaced by columnar epithelium with intestinal metaplasia. BE develops as a result of long standing gastro-oesophageal reflux disease and carries an increased risk of developing oesophageal adenocarcinoma. Malignant transformation from intestinal metaplasia to invasive adenocarcinoma is a gradual process, through a series of premalignant stages: low grade intraepithelial neoplasia (LGIN), high grade intraepithelial neoplasia (HGIN) and early intramucosal cancer (IMC). These early lesions have a negligible risk of lymph node metastasis and are therefore amenable for curative, minimal invasive endoscopic therapy, such as endoscopic resection or radiofrequency ablation. Therefore, Barrett’s patients are recommended to undergo regular endoscopic surveillance to detect and possibly treat these early lesions, which are often difficult to detect with white light endoscopy (WLE).

Autofluorescence imaging (AFI) is an advanced imaging modality that may improve the detection of early neoplastic lesions in BE. AFI is based on the principle that certain endogenous substances such as flavins and collagen (i.e. endogenous fluorophores) emit fluorescent light when excited with short wavelengths of light. Influenced by physiological and pathophysiological alterations in biochemistry and structure, normal oesophageal tissue, Barrett’s mucosa, and early neoplasia emit distinct fluorescent spectral signatures. In recent years, various endoscopic systems, incorporating a form of autofluorescence imaging have been studied. Early reports showed promising results for light-induced fluorescence endoscopy (LIFE) or autofluorescence endoscopy (AFE). An important limitation of many of these techniques is combination with, or comparison to, suboptimal white light technology, such as fiberoptic endoscopes. The performance of autofluorescence was, therefore, overestimated in these reports.

The latest generation autofluorescence technology (autofluorescence imaging, AFI) was combined with high resolution WLE and NBI into a multimodal endoscopy system (endoscopic trimodal imaging; ETMI). The resolution of the AFI pseudocolour images was markedly improved, while the HR-WLE image provided optimal WLE inspection.

Our group has conducted three uncontrolled prospective studies on AFI: one feasibility study on the first prototype AFI endoscope; one international, multicentre feasibility study on the ETMI-system (incorporating second generation AFI); and one feasibility study comparing second and third generation AFI. These studies suggested that AFI markedly increased the targeted detection of early neoplasia. In a recent randomized crossover trial comparing ETMI with standard WLE, targeted biopsies with AFI again proved to be superior to targeted biopsies with WLE for the detection of early neoplasia. However, the improved targeted detection with AFI was compensated by obtaining random biopsies according to the Seattle protocol in the WLE arm. A second randomized crossover trial with the same design was performed in a community hospital setting in order to avoid possible bias introduced by highly experienced endoscopists and a high-risk population, and included only confirmed LGIN cases. The results of this study, however, were comparable to the first randomized crossover study.

Although ETMI did not significantly improve the overall detection of early neoplasia compared to standard endoscopy with random biopsies, all aforementioned studies demonstrated...
a significant improvement in targeted detection of early neoplasia by AFI. The additional lesions detected by AFI may influence subsequent endoscopic treatment and thus be of clinical relevance. However, none of the five studies presented data on the management of AFI-detected early neoplastic lesions. The issue thus remains whether lesions found by AFI influence the initial management plan based on WLE and random biopsies. We therefore aimed to investigate the clinical relevance and impact of AFI for the diagnosis and management of early Barrett’s neoplasia by pooling all available data from the original databases and including prospectively collected data on treatment and follow-up of all patients.

METHODS
Pooling of data
The original databases of five prospectively conducted trials on AFI were retrieved and assessed for patient demographics, endoscopic data and pathology records (Table 1).

Two feasibility studies were performed at the Academic Medical Centre (AMC, Amsterdam, the Netherlands), a tertiary referral centre for diagnosis and treatment of early neoplasia in BE.8,9 The randomized, multicentre studies and one multicentre feasibility study were performed at the AMC, Mayo Clinic (Jacksonville, Florida, USA), Mayo Clinic (Rochester, Minnesota, USA), Queens Medical Centre (Nottingham, UK) and general hospitals in the Amsterdam region.3,6,7 For the purpose of these studies, the histological evaluation of all study biopsies and endoscopic resection specimens was reviewed by gastrointestinal pathologists with expertise in early Barrett’s neoplasia. Discrepancies in initial histological interpretation were resolved by consensus evaluation.

Data from the five databases were pooled into a single database (Statistical Package for Social Sciences 18.02, SPSS Inc, Chicago, IL, USA) and evaluated for:

- Age;
- Gender;
- Extent of the Barrett’s segment, according to the Prague C&M criteria;
- Histological diagnosis prior to referral;
- Histological diagnosis of targeted biopsies obtained from lesions first identified with WLE;
- Histological diagnosis of targeted biopsies obtained from lesions first identified with AFI;
- Histological diagnosis of random biopsies (RBx);
- Histological diagnosis of treatment-related specimens (in case of endoscopic mucosal resection (EMR)) or follow up biopsies (in case of radiofrequency ablation (RFA)).

Endoscopic treatment protocol
The treatment protocol for patients with confirmed early neoplasia in BE in the participating centres was as follows: in patients in whom a WLE detected lesion with HGIN/IMC was found (predominantly Paris type 0-IIa, 0-IIc or a combination), EMR was performed: either stepwise radical resection of the lesion including the remaining Barrett’s segment, or EMR followed by RFA of the remaining Barrett’s segment. In patients with flat lesions containing HGIN (Paris
type 0-IIb), the lesion was either treated with EMR for the purpose of obtaining histology, or with RFA of the lesion and the remaining Barrett’s segment, at the discretion of the performing endoscopist. In case of HGIN found in random biopsies without a visible lesion, the lesion and remaining Barrett’s segment were eradicated by RFA.

In case an EMR specimen demonstrated submucosal invasion, positive deep resection margins, poorly/non-differentiated cancer or lymphovascular invasion, patients were referred for additional treatment (surgery, chemoradiation therapy).

Treatment outcomes and follow up

All patients underwent treatment in the participating tertiary referral centres with extensive expertise in endoscopic treatment of early Barrett’s neoplasia (AMC, Mayo Clinic Florida, Mayo Clinic Rochester, Queens Medical Centre). In these centres all patients who undergo endoscopic treatment are prospectively followed. Information on treatment modality and treatment outcome was collected and incorporated in the study database. In case of surgery or endoscopic mucosal resection, the histological diagnosis of the resection specimens was obtained. In case of RFA or surveillance without treatment, the histological diagnosis of the biopsies during follow-up endoscopies was collected.

Endpoints of the pooled analysis

- The “additional diagnostic value” of AFI, defined as the proportion of patients with HGIN/IMC detected by AFI-targeted biopsies only (i.e. no HGIN/IMC detected in WLE-targeted biopsies or RBx) during endoscopic surveillance.
- The “additional therapeutic value” of AFI, defined as the proportion of patients in whom a relevant change of therapy was based on a HGIN/IMC lesion that was primarily detected with AFI.

Ethical Considerations

All individual studies used in this pooled analysis were approved by the medical ethical committees of the participating centres, and all patients involved provided written informed consent. All authors had access to the clinical data and approved the final version of the manuscript.

RESULTS

A total of 371 patients were included (mean age 65 years [SD 11], 305 males) with the following indications: endoscopic surveillance (n=184), follow-up after endoscopic treatment (n=27), or work-up of early neoplasia (n=160). The histology of the AFI endoscopy showed HGIN/IMC in 147/371 patients (40%) (table 1).

The additional diagnostic value of AFI

In order to assess the additional diagnostic value of AFI, we excluded the 160 patients referred for work-up of early neoplasia, because a recent diagnosis of HGIN/IMC had already been made in these patients. Therefore, 211 patients were available for analysis of the diagnostic value of AFI (the “surveillance group”).
In 39/211 patients (18%), HGIN/IMC was detected. In 23 of these patients (59%), HGIN/IMC was initially diagnosed in lesions detected with WLE. In 11 patients (28%), HGIN/IMC was diagnosed based on RBx only. In 5 patients (13%), HGIN/IMC was diagnosed only in AFI-targeted biopsies and not detected with either WLE or RBx.

The “additional diagnostic value” of AFI for early neoplasia in BE in this cohort of 211 patients was therefore limited to 5 patients (5/211; 2%), in whom AFI led to the detection of HGIN/IMC that was missed by WLE or RBx (figure 2).

Table 1. Overview of the patient characteristics from the 5 studies included in this study.

<table>
<thead>
<tr>
<th></th>
<th>AFI-I feasibility Kara et al</th>
<th>AFI-II feasibility Curvers et al</th>
<th>AFI-II RCT Curvers et al</th>
<th>AFI-II RCT general practice Curvers et al</th>
<th>AFI-III feasibility Boerwinkel et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients included</td>
<td>371</td>
<td>60</td>
<td>84</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>Male</td>
<td>305 (82%)</td>
<td>54/60 (90%)</td>
<td>70/84 (83%)</td>
<td>71/87 (81%)</td>
<td>79/99 (80%)</td>
</tr>
<tr>
<td>Barrett length C&amp;M (cm) *</td>
<td>C4M7</td>
<td>C2M7</td>
<td>C4M7</td>
<td>C4M7</td>
<td>C2M5</td>
</tr>
<tr>
<td>HGIN/IMC</td>
<td>147/371 (40%)</td>
<td>22/60 (37%)</td>
<td>55/84 (65%)</td>
<td>30/87 (34%)</td>
<td>24/99 (24%)</td>
</tr>
<tr>
<td>WLE detected</td>
<td>88/147 (60%)</td>
<td>14/22 (64%)</td>
<td>33/55 (60%)</td>
<td>16/30 (53%)</td>
<td>16/24 (67%)</td>
</tr>
<tr>
<td>RBx detected</td>
<td>35/147 (24%)</td>
<td>2/22 (9%)</td>
<td>18/55 (33%)</td>
<td>5/30 (17%)</td>
<td>7/24 (29%)</td>
</tr>
<tr>
<td>AFI detected</td>
<td>24/147 (16%)</td>
<td>6/22 (27%)</td>
<td>4/55 (7%)</td>
<td>9/30 (30%)</td>
<td>1/24 (4%)</td>
</tr>
</tbody>
</table>

* mean * median

Figure 1. White light (left) and autofluorescence imaging (right) image of a neoplastic lesion in a Barrett’s esophagus. The neoplastic area absorbs a substantial part of the excitation light and the fluorescent light has to travel through neoplastic degenerated mucosal structures. Therefore neoplasia demonstrates a violet decolouration, which is essentially a loss of fluorescence signal. Non-neoplastic Barrett’s mucosa appears green.

In 39/211 patients (18%), HGIN/IMC was detected. In 23 of these patients (59%), HGIN/IMC was initially diagnosed in lesions detected with WLE. In 11 patients (28%), HGIN/IMC was diagnosed based on RBx only. In 5 patients (13%), HGIN/IMC was diagnosed only in AFI-targeted biopsies and not detected with either WLE or RBx.

The “additional diagnostic value” of AFI for early neoplasia in BE in this cohort of 211 patients was therefore limited to 5 patients (5/211; 2%), in whom AFI led to the detection of HGIN/IMC that was missed by WLE or RBx (figure 2).
The additional therapeutic value of AFI

The additional therapeutic value of AFI was defined as the proportion of patients in whom a relevant change of therapy was based on a HGIN/IMC lesion that was primarily detected with AFI. In the surveillance group (n=211), 39 patients (18%) were diagnosed with HGIN/IMC. In 5 patients, these lesions were detected with AFI only. In 6 patients in whom HGIN/IMC was initially diagnosed in a lesion detected with WLE or in RBx, an additional lesion containing HGIN/IMC was detected with AFI.

In the work-up group (n=160), 108 (68%) patients were diagnosed with HGIN/IMC. In 19 patients, the diagnosis was based on AFI-guided biopsies alone. In 27 patients in whom HGIN/IMC was initially diagnosed in a lesion detected with WLE or in RBx, an additional lesion containing HGIN/IMC was detected with AFI.

In both groups (n=371), one or more HGIN/IMC lesions were detected with AFI that were not primarily seen with WLE in a total of 57 patients (5+6+19+27). The additional therapeutic value of AFI was based on the therapeutic approach and outcome of these 57 patients (Table 2).

In 26 patients, the AFI lesions (pre-treatment histology: 26 HGIN) were flat and were ablated with RFA. All patients achieved complete remission of neoplasia and intestinal metaplasia. None of the 26 patients treated with RFA showed signs of disease progression or recurrence during follow-up (median follow-up: 45 months, IQR: 34–58).

In 31 patients, AFI prompted EMR of the AFI detected lesion. All lesions were re-inspected with WLE after detection with AFI, and were classified as either non-visible or 0-IIb flat-type lesions and thus...
would have been treated by RFA if only WLE was used. Histology of the EMR-specimens showed HGIN or lesser degrees of neoplasia in 25 patients and IMC in 6 patients (figure 3). Histology showed well differentiated intramucosal cancer, not reaching into the muscularis mucosa (m2) in all 6 cases. None of the 6 EMR-specimens with IMC showed submucosal invasion, positive deep resection margins, poorly/non-differentiated cancer or lymphovascular invasion.

Since the current treatment strategy for IMC is EMR, without AFI these 6 patients would have been unjustly treated with RFA. Therefore, the “additional therapeutic value” of AFI for early neoplasia in this pooled cohort of BE patients was limited to 6/371 (2%).

DISCUSSION

This is the first study to evaluate the impact of autofluorescence imaging (AFI) on the diagnostic and therapeutic management of early neoplasia in patients with Barrett’s esophagus. Data from 3 uncontrolled prospective AFI studies and 2 randomized crossover trials comparing standard video endoscopy with AFI were pooled, including follow-up data on endoscopic treatment, and assessed for the additional diagnostic and therapeutic value of AFI.

In 211 patients where AFI was performed as part of endoscopic surveillance or follow-up after endoscopic treatment, the additional diagnostic value of AFI for early neoplasia was 2%. This finding suggests that most lesions with clinically relevant early neoplasia can be detected with standard white light endoscopy (WLE). Flat, endoscopically inconspicuous neoplasia can be detected with random quadrantic biopsies or with AFI, but the additional value of AFI after WLE and random biopsies (RBx) is limited.

In these 211 patients HGIN/IMC was found in 18% of cases. This reflects the tertiary referral bias of the participating centres and the inclusion of patients at higher risk for developing early neoplasia (i.e. confirmed LGIN, follow-up after endoscopic treatment). Patients referred

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Table 2. Breakdown of all patients with HGIN/IMC in a lesion that was detected solely by AFI, treatment modality and outcome of treatment.

<table>
<thead>
<tr>
<th>Surveillance</th>
<th>Neoplasia detected by AFI alone</th>
<th>Additional neoplasia detected by AFI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>11</td>
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<thead>
<tr>
<th>Work-up</th>
<th>Neoplasia detected by AFI alone</th>
<th>Additional neoplasia detected by AFI</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>19</td>
<td>27</td>
<td>46</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome of AFI histology:</th>
<th>IMC</th>
<th>HGIN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>8</td>
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<tr>
<th>Treatment of neoplasia:</th>
<th>RFA</th>
<th>EMR</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>15</td>
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<table>
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<tr>
<th>Treatment outcome EMR:</th>
<th>IMC</th>
<th>HGIN</th>
<th>≤LGIN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>6</td>
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<table>
<thead>
<tr>
<th>Number of patients:</th>
<th>5</th>
<th>6</th>
<th>11</th>
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</table>

| Table 2. Breakdown of all patients with HGIN/IMC in a lesion that was detected solely by AFI, treatment modality and outcome of treatment. |
for surveillance of NDBE showed HGIN/IMC in 11/75 (15%), while patients with LGIN showed HGIN/IMC in 21/109 (19%). In patients referred for follow-up after treatment, HGIN/IMC was diagnosed in 7/27 (26%), demonstrating the increasing likelihood of detecting early neoplasia in these subgroups. In general, however, AFI did not impact substantially the overall diagnosis of neoplasia in this surveillance population. In a community Barrett’s surveillance population, with a lower a-priori chance of early neoplasia, we postulate that the additional diagnostic value of AFI will be even lower than the 2% found in the current series. Furthermore, the false positive ratio and the number of unnecessary biopsies are also likely to increase. The pooled false positive rate for all studies included in the current analysis was 78%. On a per lesion basis, AFI showed the highest false positive rate in community hospitals with an intermediate risk population. The patients that were detected with HGIN/IMC by AFI only were referred for surveillance of NDBE after previous endoscopic therapy (2) and LGIN (3). Based on prior endoscopies and the WLE/RBx diagnosis during the AFI endoscopy, all 5 patients would have been sent away for a 1 year surveillance interval. No data are available on the consequences of missing flat HGIN/IMC, yet one may argue that these patients are still amendable for curative endoscopic therapy after one year. However, in light of the current discussion on (cost)effectiveness of endoscopic surveillance for BE and the possible extension of surveillance intervals, every individual surveillance endoscopy will have increasing impact.

A possible bias may have been introduced by the expertise of the endoscopists involved in the included trials. They have a trained eye for detecting early neoplasia with WLE, which may have underestimated the diagnostic performance of AFI. Moreover, they were not blinded to the patient’s clinical history, which – in this high risk population – may have resulted in over-scrutinizing the esophagus for very subtle abnormalities, potentially reducing the additional value of AFI. On the other hand, the sequential order of inspection with WLE prior to AFI may have biased the AFI assessment, overlooking subtle WLE abnormalities and overestimating the detection rate of AFI. In none of the studies, a control arm with double WLE inspection was included. In addition, AFI positive areas were avoided during random sampling in all studies, underestimating the diagnostic value of random biopsies. Therefore, the resultant overall bias has likely favoured an artificial increase in the diagnostic effect of AFI, rather than an under appreciation of its performance.

All five AFI studies have shown an increase in the targeted detection of AFI for early neoplasia. We hypothesized that these AFI lesions may be relevant for the overall management of patients with neoplasia. AFI may detect a lesion, initially not seen with WLE, leading to the decision of performing an EMR. When the EMR shows submucosal infiltration, AFI has had a substantial impact on the management of the patient. Therefore, we evaluated the histological outcome of AFI-guided biopsies and the outcome of the subsequent therapy, and correlated this to the postulated outcome of a therapy based on WLE and/or random biopsy diagnosis, without AFI.

In 57 patients, AFI detected an area with HGIN/IMC that was not detected by WLE/RBx. In 31 patients, the AFI lesion was removed by EMR. The endoscopic resection specimen showed HGIN or lesser degrees of neoplasia in 25 cases. In 6 patients, the EMR specimen showed IMC. Was the resection of these 6 cancerous AFI lesions of clinical importance for adequate treatment of these
patients? Histological assessment of the 6 IMC cases did not reveal submucosal invasion, poorly/non-differentiated cancer or lymphovascular invasion. This makes the distinction from HGIN less relevant in terms of risk for lymphnode metastasis and therefore on the impact on therapeutic management. Since all lesions that are detected primarily with AFI are endoscopically inconspicuous and flat, the difference between HGIN and superficial IMC may be less clinically important. Moreover, the interobserver variability among pathologists for differentiating HGIN from IMC in biopsies is generally poor15,16 and even in surgical resection specimens the agreement is only fair17. In the absence of a WLE detected lesion, or after resection of the lesion, additional flat neoplasia – including AFI-positive lesions – may therefore be adequately treated with RFA. Indeed, previous studies from our group have demonstrated that after removal of the endoscopically detected primary lesion, the remainder of the Barrett’s segment does not harbour more severe grades of dysplasia10,11.

In the 26 patients in whom RFA of the AFI lesions (26 HGIN) was performed, no adequate histological assessment of the AFI lesions was made, which is a limitation of the current study.

Figure 3. WLE (a) and AFI image (b) of an AFI-positive area that showed HGIN upon biopsy and subsequent EMR. WLE (c) and AFI (d) image of an AFI-positive area that showed IMC following EMR. These areas were not detected by WLE.
However, after a median follow up of 45 months none of these patients showed either local recurrence or metastatic spread, supporting the intramucosal character of the ablated lesions. In summary, in our cohort of 371 BE patients, AFI guided the decision to perform an EMR that showed IMC in 6 patients only. Although the clinical relevance of IMC in these cases may be disputable, we consider these 6 lesions relevant, given the current guidelines for treatment of flat neoplasia. We therefore conclude that, after adequate inspection with WLE, lesions identified with AFI rarely contain more advanced stages of neoplasia that have an impact on clinical therapeutic decision making.

Despite recent developments in advanced imaging for the detection of early neoplasia in Barrett’s esophagus, white light endoscopy with systematic random biopsies remains the gold standard. Relevant abnormalities are generally visible with high resolution WLE upon careful examination, yet often remain undetected by endoscopists. A training program, which is currently being developed by the international workgroup for the classification of oesophagitis (IWGCO238), may increase the WLE detection of relevant lesions. However, in the absence of visible abnormalities – which represents the majority of Barrett’s patients – an improved risk-stratification model, rather than random biopsies and standard histological assessment, is a dire necessity.

Can AFI play a role in such a model? Recent studies have shown that, irrespective of the presence of dysplasia in the actual biopsy sample, AFI positivity was associated with an increased content of putative biomarkers and dysplasia. The use of a biomarker panel on a limited number of AFI targeted biopsies may thus effectively classify Barrett’s patients according to their dysplasia status, avoiding laborious random sampling19,20. However, the results of our study suggest that autofluorescence imaging currently is of limited value in routine endoscopic surveillance or work-up of early Barrett’s neoplasia.

REFERENCES


