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

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FLUORESCENCE SPECTROSCOPY INCORPORATED IN AN OPTICAL BIOPSY SYSTEM FOR THE DETECTION OF EARLY NEOPLASIA IN BARRETT'S ESOPHAGUS

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ABSTRACT

INTRODUCTION: Endoscopic surveillance is recommended for patients with Barrett's esophagus (BE) to detect high-grade intraepithelial neoplasia (HGIN) or early cancer (EC). Early neoplasia is difficult to detect with white light endoscopy and random biopsies are associated with sampling error. Fluorescence spectroscopy has been studied to distinguish non dysplastic Barrett's epithelium (NDBE) from early neoplasia. The optical biopsy system (OBS) uses an optical fiber integrated in a regular biopsy forceps. This allows real-time spectroscopy and ensures spot-on correlation between the spectral signature and corresponding physical biopsy. The OBS may provide an easy-to-use endoscopic tool during BE surveillance.

METHODS: In BE patients undergoing endoscopy, areas suspicious for neoplasia and endoscopically non-suspicious areas were investigated with the OBS, followed by a correlating physical biopsy with the optical biopsy forceps. Spectra were correlated to histology and an algorithm was constructed to discriminate between HGIN/EC and NDBE using smoothed linear dicriminant analysis. The constructed classifier was internally cross-validated and correlated to the endoscopist's assessment of the BE segment.

RESULTS: A total of 47 patients were included (39 males, age 66 yrs): 35 BE patients were referred with early neoplasia and 12 patients with NDBE. A total of 245 areas were investigated with following histology: 43 HGIN/EC, 66 low-grade intraepithelial neoplasia, 108 NDBE, 28 gastric or squamous mucosa. Areas with LGIN and gastric/squamous mucosa were excluded. The area under the ROC curve of the constructed classifier was 0.78. Sensitivity and specificity of OBS alone were 81% and 58% respectively. When OBS was combined with the endoscopist's assesssment, sensitivity was 91% and specificity 50%. If this protocol would have guided the decision to obtain biopsies, half of the biopsies would have been avoided, yet 4/43 areas containing HGIN/EC (9%) would have been inadvertently classified as unsuspicious.

CONCLUSION: In this study, the OBS was used to construct an algorithm to discriminate neoplastic from non-neoplastic BE. Moreover, the feasibility of OBS with the constructed algorithm as an adjunctive tool to the endoscopist's assessment during endoscopic BE surveillance was demonstrated. These results should be validated in future studies. In addition, other probe based spectroscopy techniques may be integrated in this optical biopsy forceps system.

INTRODUCTION

In patients with Barrett's esophagus (BE), endoscopic surveillance is recommended to detect neoplasia at an early stage[1]. Malignant degeneration of Barrett's epithelium is thought to occur through a series of phenotypic cellular changes detected and graded on microscopy; beginning with non-dysplastic intestinal metaplasia (IM), then low- (LGIN) and high-grade intraepithelial neoplasia (HGIN), and eventually early cancer (EC) may arise[2,3]. When using standard white light endoscopy, however, it may be difficult to distinguish areas with early neoplasia (*i.e.* HGIN and/or EC) within the normal Barrett's mucosa[4]. Thus, in the absence of visible abnormalities random four-quadrant biopsies are obtained every 1-2 cm of the BE, to allow for histological evaluation for the presence of neoplasia (Seattle protocol)[4,5]. However, random biopsies are associated with a high rate of sampling error and early neoplasia can therefore be missed[6]. Moreover, the extensive biopsy protocol poses significant burden on patients, endoscopists and health care costs, due to prolonged endoscopy time and high costs of histopathological processing of the high number of obtained biopsies. Lastly, in light of recently published literature, in which the progression rate of non-dysplastic BE to HGIN/EC was shown to be significantly less than previously assumed, the cost effectiveness of endoscopic surveillance in its current form is under much debate[7,8].

To increase the detection rate of early neoplasia during endoscopic surveillance of BE patients, different imaging techniques have been developed. In this respect, roughly two goals can be distinguished: first and foremost, suspicious lesions will have to be identified in the BE, which requires a "red flag" imaging modality to draw attention to a certain area of interest. Second, a differentiating tool will have to be able to distinguish between truly suspicious areas (i.e. harboring HGIN/EC) or false positive areas that are in fact non-neoplastic. Fluorescence spectroscopy has been studied in pursuit of both goals. Fluorescence is based on the principle that when light of an appropriate wavelength hits a molecule (fluorophore), it may reach an excited electronic state. When the molecule relaxes back to its ground state, the molecule emits light of lower energy and thus of longer wavelength[9]. Influenced by fluctuations in biochemistry and structure, each tissue has a distinct fluorescent spectrum. Normal esophageal tissue, Barrett's intestinal metaplasia and dysplasia have a different spectral signal[10]. Therefore, fluorescence spectroscopy can be integrated in a diagnostic system for differentiating dysplastic from non-dysplastic tissue[11].

The Optical Biopsy System (OBS, SpectraScience Inc., San Diego, CA, USA) is a probe-based spectroscopy system, utilizing excitation light of a single wavelength. The light is delivered to the tissue through an optical fiber, after which the emitted light is collected by the same fiber and passed to a processing system with a spectrometer, computer and user-interface console. The optical fiber is integrated in a modified standard biopsy forceps (Optical Biopsy Forceps, SpectraScience Inc., San Diego, CA, USA). The OBS allows for real-time, in-vivo spectroscopic measurements (optical biopsy) and provides spot-on correlation with the histology of the corresponding physical biopsy.

We hypothesized that we could construct a spectroscopy-based differentiating algorithm using the optical biopsy system, and that the OBS may improve the endoscopist's ability to detect and distinguish suspicious lesions in BE, while reducing the need for extensive biopsy protocols during surveillance endoscopies.

The aim of this first feasibility study with the OBS for the detection of early neoplasia in Barrett's esophagus was to develop a tissue differentiating algorithm and correlate the discriminating properties of the OBS with the constructed algorithm to the endoscopist's assessment of the Barrett's esophagus.

METHODS

Patients

Consecutive patients with known Barrett's esophagus were scheduled for an imaging endoscopy if they met all of the following selection criteria:

- Age 18 – 80 years;
- BE with a minimal circumferential length of 1 cm;
- Referred with non-dysplastic BE (NDBE), with neoplasia detected in random biopsies, or with subtle lesions suspicious for neoplasia (HGIN/EC);
- Signed informed consent.

Patients were not eligible for this study if they met any of the following exclusion criteria:

- Prior history of surgical or endoscopic treatment for esophageal neoplasia;
- Presence of erosive esophagitis (Los Angeles classification ≥B);
- Inability to obtain biopsies (*e.g.* due to anticoagulation, coagulation disorders, varices);

The study was conducted at the department of Gastroenterology and Hepatology of the Academic Medical Centre (AMC) Amsterdam, in close collaboration with the AMC department of Biomedical Engineering and Physics. The department of Gastroenterology and Hepatology of the AMC is a tertiary-care referral centre for patients with Barrett's esophagus and the detection and treatment of early Barrett's neoplasia.

The study protocol was approved by the medical ethics committee of the AMC. All patients signed informed consent.

Optical Biopsy System

The Optical Biopsy System (OBS) consists of a low power 405 nm solid state laser, a spectrometer and light-conducting optics, an analyzing computer with a touch screen user-interface console and a foot pedal to activate the spectroscopic measurement sequence.

The Optical Biopsy Probe is a disposable, modified standard biopsy forceps, with a single integrated optical fiber suitable for transfering both the excitation light to the tissue and the emitted light from the tissue. When obtaining a fluorescence spectrum, a so called optical biopsy, the optical biopsy forceps is opened, which exposes the optical probe that protrudes between the jaws of the forceps (*figure 1*).

The 405 nm laser induces tissue autofluorescence of specific fluorophores that are associated with cell metabolism, such as flavins and porphyrins, and also of structural attributes such as collagen and elastin[12]. During the 6 excitation pulses, the system is capable of both exciting the tissue and collecting the emitted fluorescent signal. The spectrometer collects all 6

Figure 1. Optical biopsy forceps: when the jaws of the biopsy forceps are opened, the optical probe protrudes and enables spot-on correlation between the scanned area and the corresponding histology. *Image courtesy of SpectraScience Inc., San Diego, CA, USA.*

fluorescence spectra. The data acquisition process for one optical biopsy – six excitation cycles – takes approximately 1-2 seconds.

Endoscopic Procedures

All endoscopic procedures were performed by two expert endoscopists (JB, BW) with extensive experience in the use of advanced imaging techniques and endoscopic treatment of early Barrett neoplasia.

Patients were sedated by intravenous administration of Propofol or Midazolam (2.5-15 mg) supplemented with Fentanyl (0.1-0.2 mg) or Pethidine (25-50 mg) when necessary. The esophagus was first examined in overview with white light endoscopy (WLE) using a standard therapeutic endoscope (1TQ 160, Olympus Inc., Tokyo, Japan) and the length of the Barrett segment was recorded according to the Prague-classification system[15]. Then, detailed WLE inspection was performed to detect macroscopic lesions within the Barrett's segment and the endoscopist's suspicion for neoplasia was recorded, based on the mucosal and vascular appearance with WLE combined with magnification endoscopy. For all lesions suspicious for neoplasia and for 2 additional areas not suspicious for neoplasia, the location (distance from the incisors and endoscopic quadrant), diameter and lesion type according to the Paris classification[13] was recorded on a standardized case record form and still images were obtained. Subsequently, all identified areas were investigated with the optical biopsy forceps, passed through the working channel of the endoscope. A transparent cap (Ø12.4mm, protruding length 3-4mm, Olympus Inc., Tokyo, Japan) was attached to the tip of the endoscope to ensure a fixed minimal distance to the mucosa and to ease the biopsy procedure. The optical biopsy forceps was opened with the optical probe positioned on the mucosal surface. By pressing the foot pedal, the fluorescence spectra were measured. This was followed by the collection of a physical biopsy specimen, obtained by closing the optical biopsy forceps.

Biopsy protocol

Per patient, 5 areas in the BE segment were selected for an optical plus physical biopsy using the OBS, aiming to include 3 areas suspicious for neoplasia (if present) and 2 non-suspicious areas. Subsequently, random physical biopsies were collected of the BE according to the Seattle protocol (4 quadrants, every 2 cm), using a standard biopsy forceps (Olympus Inc., Tokyo, Japan).

Histological evaluation

All formalin fixed biopsies were embedded in paraffin, cut, and stained with hematoxylin and eosin (H&E). All slides were routinely evaluated by a junior pathologist, supervised by a senior pathologist. For the purpose of this study all biopsies were reviewed by an expert GI-pathologist, blinded for the endoscopic findings, who recorded the presence, grade and distribution of inflammation and intestinal metaplasia and neoplasia according to the WHO classification for gastrointestinal tumours: no-dysplasia (NDBE), indefinite for dysplasia, LGIN, HGIN or invasive cancer (EC)[14].

Construction of the algorithm

All data in this study was propectively obtained. For this study, samples were grouped by histological diagnosis: HGIN and EC samples were combined into a single category for "neoplastic". NDBE and indefinite comprised the "non-neoplastic" group. All LGIN, squamous and gastric samples were excluded from the analysis, in order to construct a differentiation algorithm for the extremes of the histological spectrum: HGIN/EC *vs*. NDBE.

The OBS spectrometer reports the obtained light intensity returned at 1 nm increments from wavelengths 475 nm to 675 nm, yielding 201 individual energy measurements for each tissue specimen. These 201 measurements were used to build a classification rule to distinguish neoplastic from non-neoplastic tissue. This classification rule is a "linear classifier" that turns the 201 intensity values of each spectrum into a single numerical score. Higher values indicate higher levels of suspicion that the spectrum is that of neoplastic tissue, rather than non-neoplastic. For the purpose of this study, a smoothed linear discriminant analysis (LDA) function was used to construct the classifier as follows:

Write x_j for the spectral energy at wavelength j. A linear classifier uses a score $S = \Sigma_j \, \beta_j x_j$, where the β_j are coefficients to be fitted to the data. The classifier categorizes the tissue as suspicious if S exceeds a cutoff C and as not suspicious otherwise[15].

The full data-set served as a learning set to calculate the numerical score for each of the spectra. Leave-one-out cross-validation was used to ensure that the performance figures were unbiased even though the entire data set was used in the learning. The cut off value for the current analysis was set to achieve a sensitivity of at least 80%, using repeater operating characteristic (ROC) curves.

Based on previous pilot studies by our group and the leave-one-out cross-validation method of the LDA, we speculated that a total of 250 independent data points would provide sufficient data to construct the classifier[16]. Given the low absolute prevalence of early neoplasia in BE, we included a majority of patients with known neoplasia in order to obtain a balanced neoplastic sample size.

Outcome parameters

1. Sensitivity, specificity and negative predictive value (NPV) of the OBS algorithm for the discrimination between non-neoplastic and neoplastic tissue (HGIN/EC);
2. Sensitivity, specificity and NPV of WLE inspection by the endoscopist in combination with the OBS algorithm for the discrimination between non-neoplastic and neoplastic tissue (HGIN/EC).

RESULTS

Patients

A total of 47 patients were included (39 males, age 66 yrs [SD 10], median BE length C2 (IQR: 1-4), M4 (IQR: 2-8). Thirty-five BE patients were referred for work-up of early neoplasia (HGIN/EC), and 12 patients were referred for surveillance of NDBE. Three patients showed a grade A esophagitis during the study endoscopy.

Twenty-one patients were diagnosed with HGIN/EC, 13 with LGIN, and 13 with NDBE. A total of 245 areas were investigated with the OBS, with the following endoscopic appearance as assessed by the endoscopist: 164 non-suspicious and 81 suspicious for neoplasia. The histology of the corresponding physical biopsies was: 43 HGIN/EC, 66 LGIN, 108 NDBE, 26 gastric mucosa, 2 squamous mucosa.

Performance of the Optical Biopsy System

A total of 151 investigated areas and corresponding histological diagnoses were included in the analysis (*figure 2*), each providing spectra with intensity values at 201 distinct wavelengths per area.
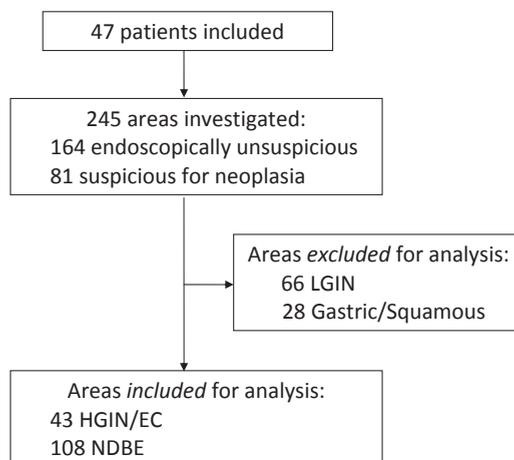

Figure 2. Flowchart of included patients (47), investigated areas (245) and corresponding histological diagnoses. A total of 151 optical biopsy system (OBS) spectra and corresponding histology were included in the final analysis. Of the 43 areas with HGIN/EC, 29 were endoscopically suspicious for neoplasia. Of the 108 NDBE areas, 85 were endoscopically unsuspicious. *LGIN: low grade intraepithelial neoplasia; HGIN; high grade intraepithelial neoplasia; EC: early cancer; NDBE: non-dysplastic Barrett's epithelium.*

The cross-validated ROC curve of the smoothed LDA is shown in *figure 3*, and has an area under the curve (AUC) of 0.78. The threshold giving 80% sensitivity for the OBS was used to evaluate the performance, presented in *table 1*.

Sensitivity for the OBS alone for the discrimination of neoplastic from non-neoplastic tissue was 81% (95% Confidence interval (CI): 66-97), with a specificity of 58% (95%CI: 56-61) and a negative predictive value (NPV) of 89% (95%CI: 75-99). If the OBS would have guided the decision to obtain biopsies, 63/108 of biopsies would have been avoided. However, 8/43 areas containing HGIN/EC (19%) would have been inadvertently classified as unsuspicious and not sampled.

Since the OBS was used as an adjunctive tool to the endoscopist's assessment, the performance of the endoscopist alone was evaluated (*table 2*) and the combination of the endoscopist's assessment and the OBS (*table 3*).

White light endoscopic inspection by the endoscopist had a sensitivity of 67% (95%CI: 44-83), a specificity of 79% (95%CI: 63-87) for discriminating NDBE from early neoplasia, with a NPV of 86% (95%CI: 69-92). With the OBS as an adjunctive tool, the endoscopist and OBS combined showed a sensitivity of 91% (95%CI: 84-100), a specificity of 50% (95%CI: 27-55) and a NPV of 93% (95%CI: 80-100). This resulted in a 50% reduction of the number of biopsies (54/108), while classifying 4/43 (9%) of neoplastic areas as unsuspicious.

Table 1. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), including 95% confidence intervals (CI) of the optical biopsy system (OBS) for the discrimination of neoplasia from non-neoplastic tissue.

		Histology			
		Neoplastic	Non neoplastic	Total	
OBS	Suspicious	35	45	80	PPV 44% (95% CI 51-73)
	Not suspicious	8	63	71	NPV 89% (95% CI 75-99)
	Total	43	108	151	
		Sensitivity 81% (95% CI 66-97)	Specificity 58% (95% CI 56-61)		Concordance 65% (95% CI 51-73)

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), including 95% confidence intervals (CI) of the assessment by the endoscopist for the discrimination of neoplasia from non-neoplastic tissue.

		Histology			
		Neoplastic	Non neoplastic	Total	
Endoscopist	Suspicious	29	23	52	PPV 65% (95% CI 37-73)
	Not suspicious	14	85	99	NPV 86% (95% CI 67-93)
	Total	43	108	151	
		Sensitivity 67% (95% CI 44-83)	Specificity 79% (95% CI 63-87)		Concordance 76% (95% CI 65-85)

Table 3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), including 95% confidence intervals (CI) of the adjunctive use of the optical biopsy system (OBS), combined with the endoscopist's assessment.

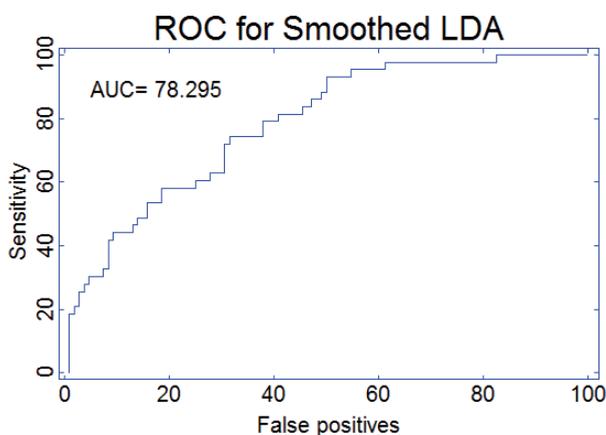|		Histology			
		Neoplastic	Non neoplastic	Total	
Endoscopist + OBS	Suspicious	39	54	93	PPV 42% (95% CI 29-56)
	Not suspicious	4	54	58	NPV 93% (95% CI 80-100)
	Total	43	108	151	
		Sensitivity 91% (95% CI 84-100)	Specificity 50% (95% CI 28-55)		Concordance 62% (95% CI 50-72)

Figure 3. Receiver operating characteristic (ROC) curve of the smoothed linear discriminant analysis (LDA) performed on the values obtained by including 201 measures (intensity per wavelength) per investigated area (n=151). *AUC: area under the ROC curve.*

DISCUSSION

In recent years, much attention has been given to endoscopic wide-field fluorescence imaging systems, such as autofluorescence imaging (AFI). Despite promising first reports, two randomized crossover trials could not confirm the superiority of AFI over standard WLE for the detection of early neoplasia in BE[17,18]. Moreover, the clinical impact of AFI in terms of detecting lesions that are relevant for therapeutic decision making, seems limited[19]. Therefore, wide-field detection of early lesions still relies on white light endoscopy and random biopsies. Recently, attention again has shifted towards probe-based fluorescence spectroscopy techniques that may aid the endoscopist in determining where and when to sample and increase the yield of random biopsies. This is the first report on a newly developed Optical Biopsy System (OBS) that uses a custom made optical biopsy forceps in patients with BE. One of the drawbacks of spectroscopy studies has been the sampling error associated with the technically challenging correlation of

the scanned area and the corresponding physical biopsy. The optical biopsy forceps used in the current study ensures a spot-on correlation and thus solves this problem. Theoretically, multiple spectroscopy techniques using several light sources may be integrated in one device that uses the optical biopsy forceps. Such a device would offer low-complexity investigation of the esophageal mucosa – so called optical biopsies – to evaluate the suspicion on prevalent neoplasia. Compared to the current practice of obtaining four-quadrant biopsies every 2 cm of the Barrett segment, this may increase the yield of random biopsies, since only areas considered suspicious by the device would require a physical biopsy.

The results of this feasibility study in patients with early neoplasia in BE suggests that the fluorescence based OBS can effectively discriminate early neoplasia from NDBE, and potentially reduce the large number of physical biopsies required. However, the OBS misclassifies a substantial number of neoplastic lesions as non-suspicious. In its current form, the OBS alone thus cannot guide the decision whether or not to obtain physical biopsies.

In daily practice, any probe-based tool that will be used during BE surveillance, will serve as an adjunct to the endoscopist's assessment. In our study, the combination of the endoscopist's assessment of abnormalities and the OBS was able to reach acceptable sensitivity (91%), while reducing the number of physical biopsies by half. With 10% of truly neoplastic areas missed by this protocol, the miss rate is comparable to previously studied endoscopic modalities, such as AFI[17].

The optimal performance of the algorithm, obtained by maximimizing the sum of the sensitivity and specificity, was reached at the point on the ROC curve that gave 81% sensitivity and 58% specificity. Depending on the clinical application of the OBS, a different threshold may be chosen to change the implicit tradeoff between sensitivity and specificity.

This study has some limitations that need to be addressed. First of all, due to the character of the study, the total number of data points for analysis was too small to provide an independent learning and validation data set. A validated method to construct a robust classifier with a limited number of datapoints is a leave-one-out cross-validation of all samples. In this, each sample in turn is removed from the data set; all modelling steps are applied to the remaining samples, and the fit so obtained is used to predict the held-out sample. These hold-out predictions are then used to quantify the method's performance. In this way, all available data are used for learning, and all available data are used for checking model fit, without incurring the bias that results from "resubstituting" the learning data set into the classifier developed in that set[20,21].

Second, since this was a feasibility study we considered the extremes of the "neoplastic spectrum" – i.e. NDBE vs. HGIN/EC – the most relevant histological subtypes. Pathological evaluation of early neoplasia and especially of LGIN is known to be subject to high inter-observer variability[22]. All LGIN samples were therefore excluded, which impacts on the external validity of this study.

Third, the majority of patients included in this study were referred for work-up of early neoplasia, resulting in a high a-priori chance of detecting early neoplasia. Moreover, all procedures were performed by expert endoscopists that have a trained eye for detecting subtle abnormalities in BE. This may have overestimated the sensitivity of the endoscopist, and hence also of the adjunctive use of the OBS (91%). In a surveillance population and in community hospitals with no specific interest in BE, the combined sensitivity (OBS + endoscopist's assessment) may be

lower. During surveillance, especially in a population with a low a-priori chance of detecting early neoplasia, the main requirement of an adjunctive tool is a high sensitivity (>90%): rare cases of early neoplasia should be detected. Moreover, as stated in the recently published ASGE PIVI (*preservation and incorporation of valuable endoscopic innovations)* on imaging in BE, in a low-prevalence population the negative predictive value (NPV) is also an important metric[23]. The protocol of the endoscopist in combination with the OBS demonstrated a NPV of 93%, which likely increases in a low risk population.

Lastly, due to the experimental set-up of the study, a standard therapeutic endoscope was used to allow for larger manoeuvrability in the 3.7 mm working channel. However, with a reduced WLE quality compared to HR-WLE in the current diagnostic endoscopes, this may have led to an underestimation of the performance of the endoscopist.

Autofluorescence spectroscopy may not be adequate to distinguish the minute and focal intracellular changes that are associated with very early neoplasia. Inflammation may potentially disturb the fluorescent signal, as was shown by Kara et al[24]. However, other probe based spectroscopy techniques may detect other specific neoplastic characteristics[25]. An OBS can potentially combine multiple spectroscopy techniques, and has many potential benefits to increase the (cost-)effectiveness of BE surveillance. In a hypothetical biopsy protocol, the endoscopist would obtain physical biopsies when either the endoscopist, the OBS or both would consider an area suspicious. The endoscopist may decide not to take a physical biopsy if both the endoscopist and the OBS rule an area not suspicious. With the optical biopsy forceps, large numbers of random optical biopsies may be obtained in a short time-frame with only a few physical biopsies to be sent in for histopathological evaluation. In a surveillance population with a low a-priori chance of early neoplasia, this protocol may increase the yield of random biopsies substantially.

In conclusion; in this study we have constructed a fluorescence spectroscopy-based algorithm using the optical biopsy system, to discriminate between non-neoplastic Barrett's mucosa and early neoplasia. Furthermore, we demonstrated the feasibility of the optical biopsy system to aid the endoscopist during surveillance endoscopy. Moreover, the yield of (random) biopsies may be increased, while reducing the number of biopsies required. In its current form, the OBS alone was not able to reach clinical applicable performance due to the high number of neoplastic lesions misclassified. Despite these results, the optical biopsy forceps has the potential to incorporate multiple small-field spectroscopy modalities and find future applications in surveillance endoscopy for Barrett's esophagus. Subsequent studies on the OBS may therefore involve multi-spectroscopy set-ups in a low-risk population with a random optical biopsy protocol, during which high numbers of optical biopsies and corresponding histology will be obtained to assess the value of the OBS.

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