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Optical detection of field cancerization in the buccal mucosa of patients with esophageal cancer

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Abstract

Introduction: Esophageal cancer is an increasingly common type of neoplasm with a very poor prognosis. This prognosis could improve with more early tumor detection. We have previously shown that we can use an optical spectroscopy to detect field cancerization in the buccal mucosa of patients with laryngeal cancer. The aim of this prospective study was to investigate whether we could detect field cancerization of buccal mucosa of patients with esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC).

Methods: Optical measurements were performed in vivo using a novel optical technique: multidiameter single-fiber reflectance (MDSFR) spectroscopy. MDSFR spectra were acquired by a handheld probe incorporating three fiber diameters. Multiple absorption and scattering parameters that are related to the physiological and ultrastructural properties of the buccal mucosa were derived from these spectra. A linear discriminant analysis of the parameters was performed to create a combined biomarker σ to discriminate oncologic from non-oncologic patients.

Results: Twelve ESCC, 12 EAC, and 24 control patients were included in the study. The median value of our biomarker σ was significantly higher in patients with ESCC (2.07 [1.93–2.10]) than control patients (1.86 [1.73–1.95], $p = 0.022$). After cross-validation σ was able to identify ESCC patients with a sensitivity of 66.7% and a specificity of 70.8%. There were no significant differences between the EAC group and the control group.

Conclusion: Field cancerization in the buccal mucosa can be detected using optical spectroscopy in ESCC patients. This may be the first step towards non-invasive ESCC cancer screening.

Introduction

Esophageal cancer (EC) is an increasingly common type of neoplasm with a very poor prognosis. Worldwide, an estimated 450,000 new EC cases and 400,000 deaths occurred in 2012, making it the 8th most common type of cancer[1]. The vast majority of EC are squamous cell carcinoma (ESCC) or adenocarcinoma (EAC). Early diagnosis and treatment of (pre)cancerous lesions could greatly improve the overall patient outcome[2]. Unfortunately, about 60% of patients are diagnosed with an incurable locally advanced or metastatic EC[3].

A promising new approach for cancer detection is focused on field cancerization (FC). FC is the notion that the initial tissue changes that lead to a neoplasm, do not only occur in the tumor site itself, but instead affect an entire organ or tract[4]. These tissue changes include alterations in the microvasculature and the tissue nanoscale architecture, such as the organization of the

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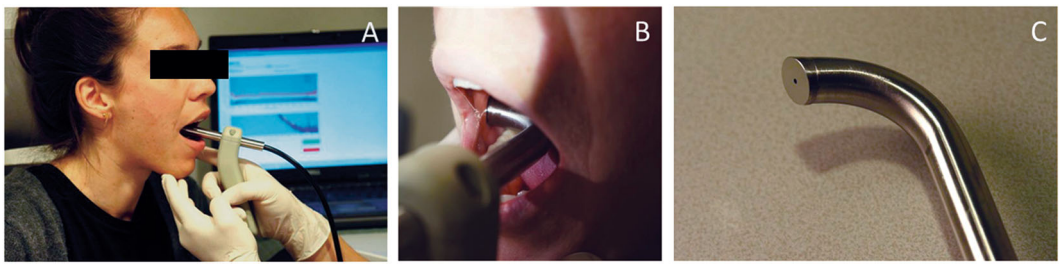

Fig. 1 Application of the multidiameter single-fiber reflectance spectroscopy probe on the buccal mucosa. a Overview picture with in the background spectra on laptop. **b** Detail of probe contact with buccal mucosa. **c** Detail of probe tip angled at 15 degrees. 180 × 46 mm (300 × 300 DPI)

cytoskeleton and the size and structure of cell nuclei and organelles[5,6]. In the case of EC, it is presumed that FC encompasses the entire upper aerodigestive tract. This is supported by the high incidence of second primary tumors in patients with esophageal, but also head and neck and lung, carcinoma[7]. Optical techniques, such as reflectance spectroscopy, have the potential to detect tissue changes caused by FC. Accurate optical detection of FC in an easily accessible, non-invasive anatomic location, such as the buccal mucosa, could potentially be used to detect distant EC[4,8,9].

A number of studies investigated similar approaches for early tumor detection. The first study analysed cells of the cytologically normal proximal esophagus of patients with distal EAC ex vivo with partial wave spectroscopy[10]. Esophageal adenocarcinoma (EAC) patients were shown to have a 1.8-times higher ($p = 0.01$) disorder strength, a parameter that is closely related to FC, than non-oncologic controls. A different ex vivo optical technique was used to detect FC in rectal mucosa biopsies to diagnose colorectal neoplasia[11]. This could predict the presence of an advanced adenoma with a promising sensitivity of 100% and a specificity of 80%. An final interesting study used a new in vivo optical technique to detect FC in the buccal mucosa of lung cancer patients[12]. Their optical biomarker was able to predict the presence of lung cancer with a sensitivity of 79% and a specificity of 83%. These studies illustrate the promise of optical detection of FC at a distant anatomic site than the actual malignancy.

Our group has recently developed a novel optical technique, multidiameter single-fiber reflectance (MDSFR) spectroscopy, which enables non-invasive quantification of the optical properties of tissue using a simple fiber-optic probe. MDSFR spectroscopy combines data from multiple single fiber reflectance (SFR) spectra. One SFR spectrum contains the combined information on how much light has been absorbed and scattered in tissue. From such a reflectance spectrum, the tissue absorption coefficient (μ_a) can be quantified. Spectral deconvolution of μ_a yields measurements of several physiological parameters. Successive SFR measurements with two or more fiber diameters enables the quantification of two scattering parameters, γ and μ_s', that are influenced by the angular scattering probability (phase function)[13–15]. γ and μ_s' are closely related to the nanoscale architecture of tissue[16]. In a previous study, we used MDSFR spectroscopy to detect FC in the buccal mucosa of patients with laryngeal cancer[17]. The blood oxygen saturation and BVF were lower in the buccal mucosa of patients with cancer than the control group. The combined parameter α was able to predict the presence of a tumor with a sensitivity of 78% and a specificity of 74%.

This study describes the first attempt to use reflectance spectroscopy of the buccal mucosa to assess if FC is present in EC. Again, we do this by measuring the optical properties of the buccal mucosa of patients with and without cancer. Differences in the values of our absorption and scattering parameters could indicate the presence of FC. The presence of FC may then be used to identify patients with EC. If proven feasible, this study would be the first step toward implementing this method as a detection tool for EC.

Methods

Subjects and examination procedure

This prospective study was approved by the Medical Ethics Committee of the Erasmus MC Cancer Institute. Patients were recruited from the outpatient clinic of the Gastroenterology and Hepatology department between December 2015 and January 2017. Clinical parameters such as: gender, age, medical history, smoking (pack-years), and TNM-stage of tumor were collected using the electronic medical record (CSC-iSOFT, Virginia, USA). The oncologic group of patients consisted of patients with primary and untreated esophageal squamous cell carcinoma (ESCC) and EAC. Tumors of all TNM-stages were included. The ESCC and EAC were confirmed by an endoscopic examination and histopathology. The non-oncologic control group consisted of patients that underwent endoscopic examination for a variety of complaints, e.g., gastro-esophageal reflux, dysphagia, and abdominal pain. The absence of an occult, unexpected malignancy or Barrett's esophagus was confirmed during

the endoscopic examination. Patients with a medical history of head and neck or lung cancer were excluded from all study groups. Informed consent forms were signed before inclusion in this study by all patients.

The multidiameter SFR measurements of the buccal mucosa were performed before the endoscopic examination (Fig. 1). All measurements were done by a single investigator (OB). The probe tip was gently placed in contact with the buccal mucosa, after disinfecting the fiber bundle with Tristel Trio (Tristel Solutions Ltd, Snailwell, UK). Five consecutive MDSFR measurements were performed without moving the probe tip. The total duration of these measurements was approximately forty seconds.

MDSFR device

The absorption and scattering properties of the buccal mucosa were quantified with a custom made MDSFR spectroscopy device. In a previous paper, we have described it in detail[18]. In summary, MDSFR spectroscopy uses one fiber bundle for both light delivery and collection. The fiber has 19 cores of 200 μm fibers. Each fiber in the fiber bundle is trifurcated at the proximal end into a fiber delivering light from a halogen lamp, a fiber delivering light from a 365 nm and 405 nm LED, and a fiber collecting light to the spectrometer. At the fiber tip, the fibers are bundled into three concentric groups compromising one, six, and twelve fibers. To avoid collection of specular reflection, they are polished at an angle of 15 degrees. The last 10 cm of the fiber bundle is at the distal end encased in a 12 mm diameter curved metal housing. This metal housing ensures optimal application on buccal mucosa (Fig. 1). A series of fiber optic interconnects and three computer-controlled shutters enable illumination and spectroscopic detection of independent fiber groups. This allows sequential SFR measurement of 200, 600, and 1000 μm to be made without moving the probe. Additional fluorescence measurements are made by illuminating all fibers in the bundle by the 365 nm LED and consecutively the 405 nm LED. The entire device is portable and approved for use in the outpatients clinic. A detailed description of the calibration procedure has been described previously[18].

The nature of FC requires that the tissue optical properties are measured superficially. The maximal sampling depth of MDSFR spectroscopy (500 μm) is well matched with the thickness of the epithelial layer of the buccal mucosa (250–350 μm) and the underlying vascularized layer of the lamina propria (300–350 μm)[19,20].

Spectral analysis

A previous paper by our group describes the complete analysis of spectra in detail[21]. First, the individual SFR spectra of the 200, 600, and 1000 μm fibers are used to calculate the tissue absorption properties. The absorption-corrected spectra of multiple fiber diameters were then combined to determine the tissue scattering properties: μ_s' (mm^{-1}), and γ (−). Next, we can extract four physiological parameters from the 1000 μm SFR fit: microvascular blood oxygen saturation (StO_2 (%)), blood volume fraction (BVF (%)), mean vessel diameter (VD (mm)) and tissue bilirubin concentration ($[BIL]_{tis}$ (μmol/L). Finally, the intrinsic fluorescence is calculated from the raw fluorescence spectrum using the optical properties that are previously measured with MDSFR spectroscopy. This quantity is given by the product of the absorption coefficient of the tissue fluorophores at the excitation wavelength $\mu^f_{a,x}$ and their quantum efficiency across the emission spectrum Q (−).

Statistical analysis

The optical parameters were calculated by averaging the five buccal mucosa measurements taken per patient weighted by the individual confidence intervals of the fitted parameters. Twelve parameters were analysed: StO_2, BVF, VD, $[BIL]_{tis}$, μ_s' at 450 and 800 nm, μ_s' power law scattering parameter, γ at 450 and 800 nm, average γ and intrinsic fluorescence under 365 and 405 nm excitation. Our quantitative variables were not normally distributed. We thus report our results as median value and interquartile range (IQR). Differences between two groups were analysed using the *t*-test (normally distributed data) or the Mann-Whitney *U* test (non-normally distributed data). Qualitative data was reported as counts and percentages, and differences between groups were analysed using the chi-squared test or the Fisher's exact test. Binary logistic regression was used to investigate if the outcome parameters could predict the presence of a malignancy. The age at measurement was included in the analysis as a covariate. We standardized our data to a standard normal distribution ($x_{new} = (x - \mu)/sd$, where μ is the mean and sd is the standard deviation of parameter x) to compute a biomarker to identify EC patients. A linear discriminant analysis of the parameters was performed to create a combined biomarker σ A ROC-curve of σ was created to perform a sensitivity and specificity analysis. A leave-one-out cross-validation was performed to test the robustness of σ. There was no missing data. Statistical analysis was performed using SPSS version 21 (IBM Co., Armonk, NY, USA) and the cut off point for significance was $p < 0.05$.

Results

Forty-eight patients were included in this study: 12 patients with ESCC, 12 patients with EAC and 24 control patients (Table 1). The percentage of males was higher in both the ESCC 7/12 (58.3%) and EAC group 11/12 (91.7%), than in the controls 10/24 (41.7%), $p = 0.004$. The median age of the patients was 69.9 (64.1–74.8) years in

Table 1 Patient characteristics

	Controls ($n = 24$)	ESCC ($n = 12$)	EAC ($n = 12$)
Male gender *n* (%)	10 (42)	7 (59)	11 (92)[a]
Age median (IQR)	61 (55–69)	70 (64–74)[b]	68 (65–72)[b]
Smoking PY median (IQR)	4 (0–30)	28 (6–30)[c]	16 (6–34)
Smoking status *n* (%)			
Never	11 (46)	2 (17)[b]	3 (25)
Past	9 (38)	6 (50)	7 (58)
Current	4 (17)	4 (33)	2 (17)

ESCC esophageal squamous cell carcinoma, *EAC* esophageal adenocarcinoma, *IQR* inter quartile range, *PY* pack-year
[a]*p*-value < 0.05 compared to controls
[b]*p*-value < 0.1 compared to controls
[c]*p*-value is NOT significantly different ($p = 0.182$). *p*-values calculated with χ^2 test (gender and smoking status) and Mann-Whitney *U* test (age and smoking PY)

Table 2 TNM-classification and location of ESCC and EAC tumors

	ESCC (*n* (%))	EAC (*n* (%))
T-stage		
I	2 (17)	2 (17)
II	–	1 (8)
III	9 (75)	9 (75)
IV	1 (8)	–
N-stage		
0	8 (67)	4 (33)
I	2 (17)	4 (33)
II	2 (17)	4 (33)
III	–	–
M-stage		
0	11 (92)	11 (92)
1	1 (8)	1 (8)
Location		
Upper	2 (17)	–
Middle	6 (50)	–
Lower	4 (33)	12 (100)

ESCC esophageal squamous cell carcinoma ($n = 12$), *EAC* esophageal adenocarcinoma ($n = 12$), – 0 cases

the ESCC group and 68.3 (64.8–71.8) years in the EAC group. This was higher than the 61.2 (54.9–69.0) years in the control group. The median amount of pack-years was 27.5 (6.3–30.0), 16.5 (6.3–33.8), and 4.0 (0.0–30.0) in the ESCC, EAC and control group respectively.

Table 2 shows the TNM-classification and location of the ESCC and EAC. T-stage was equally divided over the two types of EC. ESCC were staged as T1 in two (16.7%), T3 in nine (75.0%), and T4 in one (8.0%) cases and EAC were staged as T1 in two (16.7%), T2 in one (8.3%), and T3 in nine (75.0%) cases. Most tumors were not metastasized to regional lymph nodes, N0 in eight (66.6%) patients in ESCC group and four (33.3%) patients in EAC group. Tumors were staged N1 and N2 in two (16.7%) cases in the ESCC group and in four (33.3%) cases in the EAC group. Distant metastasis (M1) was found in one ESCC and one EAC patient. ESCC was located in the upper, middle, and lower esophagus in two (16.7%) six (50.0%) and four (33.3%) cases. All EAC tumors were located in the lower esophagus.

The intra-patient variation of the five consecutive measurements varied between 3.7 and 24.8% deviation from the mean for the twelve MDSFR parameters. This variation was similar to measurements in two previous studies[17,22]. μ_s' at 450 nm varied 9.6%, at 800 nm 8.6%, and the average μ_s' parameter varied 17.9%. The values of the three γ-parameters varies from 3.7 to 5.2%. The absorption parameters StO_2, BVF, VD, and $[BIL]_{tis}$ varied slightly more with intra-patient variations 8.0, 22.5, 18.2 and 24.8% respectively.

Based on a linear discriminant analysis of all the MDSFR parameters, μ_s' at 450 nm and μ_s' at 800 nm were combined into biomarker σ. μ_s' at 450 nm and μ_s' at 800 nm were the only two paramaters that were significantly different between the ESCC group and the controls. All other parameters showed no significant difference between these two groups. Sigma had a 4% bigger area under the curve than μ_s' at 450 nm alone. It also significantly increased the sensitivity/specificity ratio.

Figure 2 shows that biomarker σ was significantly higher in patients with ESCC than non-oncologic controls: 2.07 (1.93–2.10) vs. 1.86 (1.73–1.95), $p = 0.022$. Logically, individual values of μ_s' at 450 nm ($p = 0.033$) and 800 nm ($p = 0.029$) were also higher in the ESCC group than the control group (Fig. 2). Figure 3 shows a ROC-curve of σ for the ESCC group with an area under the curve of 75.7% (95% CI: 57.4–94.0). Biomarker σ was able to differentiate patients with ESCC from controls with a sensitivity of 66.7% and a specificity of 83.3%. A leave-one-out cross-validation was performed to test the robustness of sigma to predict patients with ESCC. This slightly decreased the diagnostic performance to a sensitivity of 66.7% and a specificity of 70.8%. Interestingly, there was no correlation between smoking (packyears) and biomarker sigma ($r^2 = = 0.0275$ and standard error of estimate = 0.1826).

There were no significant differences in all paramaters between the EAC group and the control group.

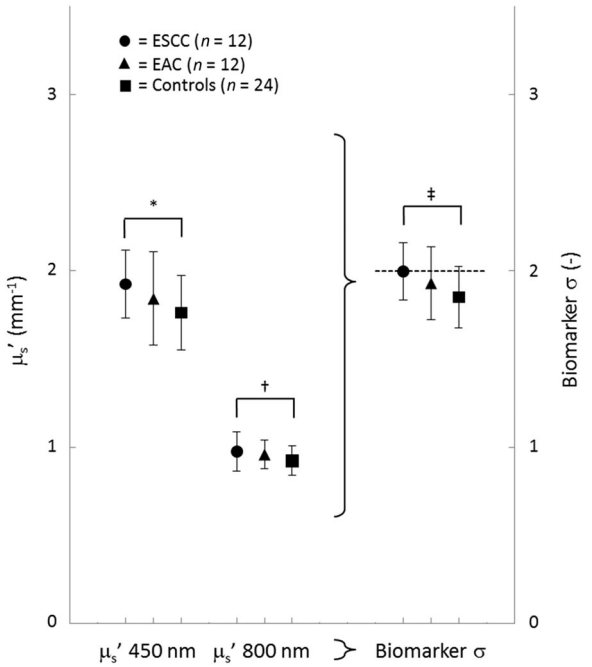

Fig. 2 Values of μ_s' at 450 nm and 800 nm and biomarker σ (combination of μ_s' at 450 nm and 800 nm). Circles, triangles, and squares represent means and error bars represent standard deviation. ESCC esophageal squamous cell carcinoma, EAC esophageal adenocarcinoma. *$p = 0.030$, †$p = 0.045$ and ‡$p = 0.012$. *p*-values were calculated with a binary logistic regression with 'age' as a covariate. 87 × 97 mm (300 × 300 DPI)

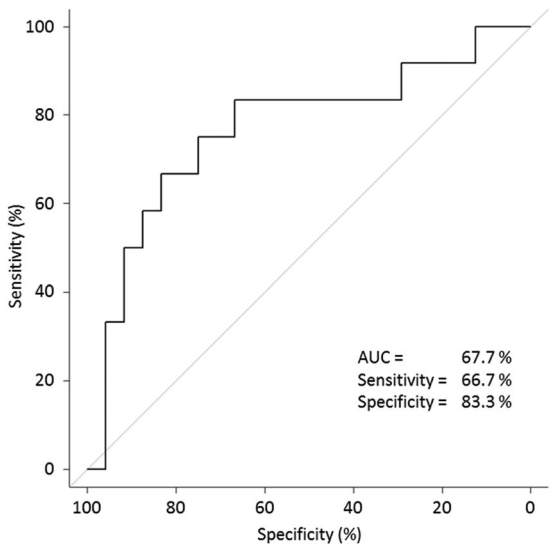

Fig. 3 ROC curve of biomarker σ (composed of μ_s' at 450 and 800 nm). AUC area under the curve. 87 × 86 mm (300 × 300 DPI)

Discussion

This study demonstrates that FC is present in the buccal mucosa of patients with ESCC and that it can be detected using optical spectroscopy. Multiple absorption and scattering parameters were measured with MDSFR spectroscopy. We found that our biomarker σ which is a combination of μ_s' at 450 and 800 nm, was significantly higher in patients with ESCC than in non-oncologic controls. Sigma was able to identify patients with ESCC with a sensitivity of 67% and a specificity of 70.8%. Unfortunately, σ could not distinguish patients with EAC from controls.

Our main result showed the first proof that the buccal mucosa of patients with ESCC is altered. The increase of scattering parameter μ_s' indicates that alterations in the nano-architecture of the buccal mucosa have occurred. Studies have shown that an increase in scattering events correlates with increase of the local density of macromolecules and changes in their organisation[23]. These alterations are key elements of FC[24]. Our findings confirm the results of a similar study that used in vivo low-coherence enhanced backscattering spectroscopy of the buccal mucosa to identify patients with lung cancer[12]. Their results also suggest that it is possible to detect nano-architectural changes in the buccal mucosa in patients with a tumor of the upper aerodigestive tract. Their biomarker was able to identify patients with lung cancer with a promising sensitivity of 79% and a specificity of 83% in their testing set. In a recent study, utilizing MDSFR in patients with head and neck squamous cell carcinoma cancer, we found that the physiological parameters (blood oxygen saturation and BVF) were altered instead of scattering parameters such as μs'[17]. It is not yet fully understood how this can be explained, since laryngeal and EC patients share the same risk factors. It might be that the FC has a different signature for different types of malignancies. However, this hypothesis has to be tested.

The results of the present study are promising with regard to the use of MDSFR spectroscopy as an innovative tool for early cancer detection. We chose a threshold that resulted in a higher specificity than sensitivity. This will result in a lower number of false positives and thus a lower number of falsely diagnosed patients. On the other hand, this will result in a relative high number of false negatives, which means that the test will miss some patients with malignancies[25]. A cost-effectiveness analysis will have to be performed in a later stage to decide the appropriate threshold and applicability of σ for detecting ESCC.

Our approach, using MDSFR spectroscopy of the buccal mucosa to identify patients with EC was more effective for ESCC than EAC. This difference is expected because FC in tissue partly develops due to exposure to carcinogens. The carcinogens of ESCC and EAC differ. As such, the main risk factors for ESCC are smoking and alcohol use, while the main risk factor for EAC is gastric reflux. This

also explains why all EAC were located in the lower esophagus, whereas most ESCC were located in the upper and middle esophagus (Table 2). However, evidence for FC of EAC was recently shown in a study in which cytologically normal proximal esophageal squamous cells were obtained by brushings during endoscopy. The disorder strength of these samples was significantly higher in patients with distal EAC ($p < 0.01$) and patients with distal Barrett's esophagus ($p < 0.01$) than healthy controls. This indicates that proximal squamous cells might undergo changes that are caused by distal EAC or Barrett's esophagus[10]. An important issue to address while discussing an EC detection tool is that although ESCC is the predominant histological type of EC worldwide, this is not the case in many developed countries. In developed countries the incidence of EAC has been exceeding that of SCC for some time with percentages reported of up to 80%. This highlights the need for a screening method for EAC in the 'western world'. Unfortunately MDSFR buccal mucosa spectroscopy did not show to be effective for EAC based on our results. It might however show value in high ESCC incidence regions in Asia[2].

In the present study we did not fully investigate the effect of smoking. Smoking is known to cause mucosal changes, some of which can lead to FC[9]. The exact relationship between smoking induced mucosal changes and FC is unknown, e.g., patient A with 20 PY could have extensive FC while patient B with the same amount of PY has normal mucosa. This is underlined by the fact that the lifetime risk of smokers to develop for instance lung cancer is only 10%[26]. Ideally, in the present study, the amount of pack years and distribution of current-smokers, past-smokers, and non-smokers should have been matched between the EC group and the controls. Although these differences in our study were not significantly different, they were not matched. This could have positively influenced our discriminative power to identify ESCC patients (Fig. 3). Also due to the small number of patients per group we were not able to take the amount of pack years into our multivariate analysis of σ. However we were able to do this in a previous study in which we tested the discriminative power of α to identify laryngeal cancer patients[17]. In that study smoking pack years did influence the results, but not to a significant degree. Therefore, we believe that optical detection of FC still shows promise for detecting ESCC patients.

There are a number of other potential limitations that should be considered. One is the relatively small number of patients per group, which might have had an influence in multiple ways: (a) it prevents us from making definitive statements about the discriminative power of the optical detection of FC, (b) it could lead to an underestimation of the significance of differences between groups (p-value), (c) we were not able to test the discriminative power of σ on an independent training-set, and (d) we were only able to correct our statistical analysis for one covariate: age. However, age was also the only borderline significantly different baseline patient characteristic. Another possible limitation is that the investigator who performed the measurements (OB) was not blind to the oncologic status of the patients. A final point of attention is the fact that the majority of patients in this cohort had advanced tumors (T3). For a screening tool that ultimately has an effect on patient survival the test performance characteristics should be tested and found adequate in patients with early EC, preferably T1 or T2. Survival in these patients is significantly better.

In conclusion, we have demonstrated that the reduced scattering coefficient, μ_s', is increased in the buccal mucosa of patients with ESCC. This increase could be used to discriminate between patients with and without ESCC based on an optical measurement of the buccal mucosa. To our knowledge, this is the first proof of the concept that it is possible to detect ESCC by detecting FC in the buccal mucosa. A larger study is now needed before definitive conclusions on the potential role of MDSFR spectroscopy detecting for ESCCC can be drawn.

Study Highlights

1. WHAT IS CURRENT KNOWLEDGE

- And increase in early tumor diagnosis will improve esophageal cancer survival.
- Field cancerization detection could be used to identify patients with unknown tumors.

2. WHAT IS NEW HERE

- Patients with esophageal squamous cell carcinoma have field cancerization in their buccal mucosa.
- This can be detected with detected in vivo with reflectance spectroscopy.
- Reflectance spectroscopy may be uses as a non-invasive esophageal cancer screening method.

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Competing interests

Guarantor of the article: Oisín Bugter.
Specific author contributions: A.A. and D.R. contributed to study conception. All authors contributed to the study design. O.B. performed the data collection. O.B., A.A., and D.R. did or supervised data analysis. All authors interpreted the data. O.B., M.S., and D.R. wrote sections of the initial manuscript. O.B. designed

figures and tables. All authors critically reviewed iterations of the manuscript and approved the final draft for submission.

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