Volumetric laser endomicroscopy for the detection of early Barrett's neoplasia
Swager, A.

Citation for published version (APA):
Chapter 5

Detection of buried Barrett’s glands after radiofrequency ablation with volumetric laser endomicroscopy

A Swager
DF Boerwinkel
DM de Bruin
DJ Faber
TG van Leeuwen

BL Weusten
SL Meijer
JJ Bergman
WL Curvers

Gastrointestinal Endoscopy
2016; 83(1):80-8
ABSTRACT

Background and aims
The prevalence and clinical relevance of buried Barrett's glands (BB) after radiofrequency ablation (RFA) in Barrett's esophagus (BE) are debated. Recent optical coherence tomography studies demonstrated a high prevalence of BBs. Direct histological correlation, however, has been lacking. Volumetric Laser Endomicroscopy (VLE) is a second-generation optical coherence tomography system capable of scanning a large surface of the esophageal wall layers with low-power microscopy resolution. The aim was to evaluate whether post-RFA subsquamous glandular structures (SGSs), detected with VLE, actually correspond to BBs by pursuing direct histological correlation with VLE images.

Methods
In vivo VLE was performed to detect SGS in patients with endoscopic regression of BE post-RFA. A second in vivo VLE scan was performed to confirm correct delineation of the SGSs. After endoscopic resection the specimens were imaged ex vivo with VLE. Extensive histological sectioning of SGS areas was performed, and all histology slides were evaluated by an expert BE pathologist.

Results
Seventeen patients underwent successful in vivo VLE (histological diagnosis before endoscopic treatment: early adenocarcinoma in 8 patients and high-grade dysplasia in 9). In 4 of 17 patients no SGSs were identified during VLE and a random resection was performed. In the remaining 13 patients (76%), VLE detected SGS areas, which were all confirmed on second in vivo VLE scan and subsequently resected. Most SGSs identified by VLE corresponded to normal histological structures (eg, dilated glands and blood vessels). However, one area containing BBs was found on histology. No specific VLE features to distinguish between BBs and normal SGSs were identified.

Conclusions
VLE is able to detect subsquamous esophageal structures. One area showed BBs beneath endoscopically normal-appearing neosquamous epithelium; however, most post-RFA SGSs identified by VLE correspond to normal histological structures. (Clinical trial registration number: NTR4056).
INTRODUCTION

In the past decade, endoscopic treatment of early neoplasia in Barrett’s esophagus (BE) has become the standard of care. Visible lesions suspicious for intramucosal neoplasia on endoscopic inspection are removed by endoscopic resection (ER). Residual flat Barrett’s epithelium, dysplastic or nondysplastic, can subsequently be treated with radiofrequency ablation (RFA). RFA uses radiofrequency energy to ablate the esophageal mucosa and superficial submucosa.1,2 This leads to eradication of intestinal metaplasia (IM) and any residual neoplasia and restoration of the normal esophageal squamous mucosa also known as neosquamous epithelium (NSE). RFA has been shown to be an effective treatment with reported complete eradication of neoplasia of 92 to 98% and complete eradication of IM of 83 to 93% with a favorable safety profile.3–6

A potential disadvantage of ablation of BE is the possible occurrence of so-called buried Barrett’s glands (BBs): residual subsquamous BBs that may remain hidden beneath the NSE. It is suggested that these glands may lead to recurrence of Barrett’s epithelium after treatment or may progress to dysplasia or cancer without being detected endoscopically.7,8 The detection of BBs with biopsy sampling of the NSE may be hampered by sampling error due to the scattered spatial distribution of BBs and insufficient depth of biopsy sampling.9–11

Optical frequency domain imaging (OFDI) is a new advanced imaging technique based on optical coherence tomography (OCT) that is able to image the superficial layers of the esophagus over a large surface area.12–14 Recent OCT studies in patients treated with RFA showed high rates of subsquamous glandular structures (SGSs) that might correspond to BBs compared to low rates in previous biopsy-based studies.15,16 These OCT studies are, however, hampered by some important methodological limitations. Most studies lack a direct correlation between histology and OCT images. In addition, structures suspicious for BBs were generally found within 5 mm proximal to the gastroesophageal junction (GEJ) where subsquamous structures are a normal finding.17 This raises the question whether the structures seen on OCT were “truly” BBs, or rather a physiological presence of columnar epithelial outgrowth at the GEJ, or non-Barrett’s-related glandular structures (eg, ducts of [sub]mucosal glands or blood vessels).

Volumetric laser endomicroscopy (VLE) (NVisionVLE Imaging System, NinePoint Medical Inc, Cambridge, Mass) is a new balloon-based OFDI imaging system that generates cross-sectional images over a large circumferential surface area and up to 3 mm deep into the esophageal wall. A major advantage of the VLE system is the possibility of imaging the entire distal esophagus at high-resolution and high-acquisition rates, making it an ideal device for investigating the presence of BBs in patients treated with RFA.

The aim of this study was to investigate the feasibility of VLE for the detection of SGSs beneath the NSE after RFA and to directly correlate the VLE findings with histology.
Chapter 5

METHODS

Setting
This study was conducted at the department of Gastroenterology and Hepatology of the Academic Medical Center in Amsterdam, a tertiary-care referral centre for patients with BE and early Barrett’s neoplasia, and was approved by the local medical ethics committee.

Patients
Patients were eligible if they underwent endoscopic follow-up after successful RFA with or without previous ER for BE with high-grade dysplasia (HGD) or early adenocarcinoma (EAC).

Inclusion criteria:
- Complete endoscopic regression of all Barrett’s epithelium. Complete endoscopic regression was defined as an endoscopically normal-appearing neo-Z-line on inspection with high-definition white-light endoscopy (WLE) and narrow band imaging (NBI) with no endoscopic signs of residual Barrett’s mucosa proximal to the neo-Z-line.
- Circumferential Barrett’s extent more than 2 cm before ablation therapy.
- Written informed consent obtained.

Exclusion criteria:
- Inability to undergo ER and/or obtain biopsies (eg, due to anticoagulation, coagulation disorders, varices).
- Significant stenosis of the esophagus.
- Erosive esophagitis.

Nvision VLE Imaging System
For more comprehensive technical details of the OFDI technique that is used in the Nvision VLE Imaging System, we refer to previous publications.\textsuperscript{18-20} For the first 10 patients enrolled in the study, a precommercial VLE imaging system was used that consisted of a disposable balloon-based optical probe, inflation system and imaging console. A commercial VLE imaging system was used for the remaining patients in the study (Fig. 1A). Differences between the 2 systems were mainly design changes for commercialization without significant effect on imaging properties, clinical use or patient safety. The major change is the smaller outer diameter of the balloon (20 mm instead of 25 mm) in the commercial system. The console has a display with a user interface, as well as wavelength-swept light source, optical receiver, interferometer, and data acquisition computer. The console generates near-infrared light (bandwidth range 1250-1350 nm) and transmits light through an optical fiber in the probe as a conduit for the laser light source. At its distal end is a polymer, noncompliant balloon with
Detection of buried Barrett’s with VLE

a soft tip. The balloon allows the optical probe to be placed in the centre of the esophageal lumen for in vivo imaging. Long-segment (6 cm) images can be acquired in 96 seconds over the entire circumference of the distal esophagus to a depth of 3 mm, with an axial resolution of 7 μm (comparable to low-power microscopy).

Figure 1. A. Commercial Nvision VLE Imaging System. B. In vivo placement of 3 orientation cautery marks (a-c) and 4 reference cautery marks (1-4) for delineation of an SGS area. 1 and 2 represent the 12 o’clock position endoscopically. C. Custom-designed tubular-shaped fixture for volumetric laser endomicroscopy scanning of an endoscopic resection specimen.
Endoscopic procedure and VLE scanning

All endoscopic procedures were performed by 2 expert endoscopists (J.B. and B.W.) with extensive experience in examining BE with advanced imaging techniques. Before this study, both endoscopists performed at least 4 VLE procedures in a VLE Barrett’s imaging pilot study. Moreover, all endoscopists, research nurses and researchers participating in this study received training on the VLE imaging system and interpretation of VLE images. 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), if necessary. The esophagus was first examined in overview with WLE and NBI by using a high-definition diagnostic gastroscope (GIF-HQ190, Olympus GmbH, Hamburg, Germany) for the detection of residual Barrett’s mucosa. For the first 10 patients enrolled in the study, after endoscopic examination the gastroscope was exchanged for a therapeutic gastroscope with a 3.7-mm working channel (ITQ190, Olympus GmbH) that enables introduction of the Nvision VLE optical probe. For the last 8 patients enrolled in the study, the optical probe was designed to fit down the working channel of high-definition diagnostic endoscope with a 2.8 mm working channel (GIF-HQ190, Olympus GmbH).

To orientate endoscopic view with VLE images, 3 cautery marks were placed profoundly at the 12 o’clock position at approximately 5 mm, 1 to 2 cm and 3 to 4 cm proximal to the upper end of the gastric folds (Fig. 1B) by using the tip of an endoscopic resection snare (SD-221L-25, Olympus GmbH) with pure monopolar coagulation current (forced coagulation effect 2, 40 W, Erbe Elektromedizin GmbH, Tübingen, Germany). Subsequently, the VLE probe was introduced through the working channel of the endoscope and positioned in the distal esophagus including the GEJ. Once the correct

![Flow diagram showing the methodology of the study. VLE, volumetric laser endomicroscopy; SGS, subsquamous glandular structures; ER, endoscopic resection.](image-url)
position was obtained, a VLE scan was performed, followed by review of the scan to check whether the 3 orientation marks were visible. If necessary, repositioning the optical probe and repeating VLE was performed until a good-quality scan was obtained. The scan was then searched for areas containing SGSs (for a definition of SGSs see the VLE assessment section). In case of multiple SGSs, the most suspicious area was determined by consensus of the endoscopist (J.B. or B.W.) and research coordinator trained in VLE (A.S.). Subsequently, its position in the esophagus was determined relative to the 3 orientation marks. The estimated location of the area of interest was then delineated with cautery marks according to the following protocol: 2 reference cautery marks were placed at the proximal border of the area and 2 marks, placed closer together, at the distal border (Fig. 1B). In case no SGSs were seen, a random area was delineated at the discretion of the endoscopists. After delineation, a second VLE scan of the distal esophagus was performed to confirm the presence of SGSs in the delineated area (Fig. 2). Based on the second in vivo scan and the position of the reference marks, ER was performed, including all 4 reference marks, per standard clinical practice, by using the ER-cap technique. For mucosal lifting, saline solution without epinephrine was used. After ER, the resection wound was inspected and photographed, and the ER specimen was retrieved.

**VLE assessment**

Validated criteria for SGSs indicating BBs have not been established; hence, broad criteria were defined as the existence of low-scattering round- to oval-shaped structures of unspecified size, localized in a subepithelial wall layer. SGSs identified on VLE were correlated by using the reference cautery marks placed during the endoscopic procedure. Postprocedure assessment of SGSs included the number, size, depth, longitudinal appearance (glandular structure continuing on a number of subsequent VLE slides), and distance from the GEJ by using Fiji (open source image processing program, http://fiji.sc/Fiji).

**Targeted histological evaluation of ER specimens**

All ER specimens were pinned on cork with a grid of 5-mm squares for reference. Based on the position of the cautery marks, the ER specimens were orientated in the in vivo position. After orientation and pinning, the resection specimens were placed in a custom-designed tubular-shaped fixture for ex vivo scanning (Fig. 1C). The optical probe was placed over the ER specimen, the balloon was inflated, and VLE scan was performed. The location of the SGSs on the ER specimen was determined and indicated on a macroscopic picture of the specimen. Finally, the ER specimen was placed in buffered formalin and fixed for a minimum of 24 hours. After fixation, the lateral sides of the bottom of the specimen were stained with 2 different colors to allow correct orientation on histology. If the ER specimen contained an area with SGSs, it was sectioned in such a way that the SGS area was included in 1 paraffin block. After embedding in paraffin blocks, 4-µm histology slides were sectioned. Blocks containing SGS areas were extensively sectioned in
4-µm slides until the entire tissue block was sectioned. Jumps of 3 to 5 slides (~12-20 µm) were omitted between every histology slide that was processed for analysis, resulting in, on average, 10 processed histology slides per tissue block. Apart from tissue blocks containing SGSs on VLE, the remaining tissue of the ER specimens (including randomly resected ER specimens without SGSs) were routinely sectioned every 2 to 3 mm. These tissue blocks, of which 1 histology slide was sectioned from each block, acted as negative controls. All slides were stained with hematoxylin and eosin for histological analysis and evaluated by an expert gastroenterology pathologist (S.M.) with extensive experience with early Barrett’s neoplasia. For correlation with VLE images, selected histology slides from areas containing SGSs on VLE were digitalized in high resolution tiff images (x10 magnification) by using the Olympus virtual slide scanner dotSlide (Olympus GmbH, Hamburg, Germany). The following histological parameters were noted: distribution of all layers (eg, mucosa, lamina propria, submucosa), the presence of columnar-lined glands beneath the NSE (number, size, and location and the presence of IM and dysplasia) and presence of other SGSs (blood vessels, dilated ducts of [sub]mucosal glands, lymphatic ducts, lymphocyte aggregates).

**Outcome measurements**
Detection of SGSs beneath NSE post-RFA treatment with VLE, by means of the presence of SGS areas identified on VLE and histological diagnoses of SGSs identified on VLE.

**Statistical analysis**
Statistical analysis was performed with SPSS Version 19 Software for Windows (IBM, Armonk, NY). For descriptive statistics the mean and standard deviation (SD) were used in case of a normal distribution of variables and the median and interquartile range (IQR) were used for variables with a skewed distribution.

**RESULTS**

**Patients**
Eighteen consecutive patients scheduled for endoscopic follow-up after RFA were enrolled. In 1 patient, the precommercial VLE system was unable to image during the procedure, and no ER was performed. Seventeen patients were included for analysis (mean age [SD], 62 years [8]; 15 men). The worst histological diagnosis before endoscopic treatment was EAC in 8 patients and HGD in 9. The median circumferential extent of the BE segment before RFA was 4 cm (IQR 2-9), and the median maximum of the BE segment was 7 cm (IQR 5-10). Fourteen patients were treated by ER and RFA (8 patients with EAC and 6 with HGD), whereas 3 patients (all with HGD) underwent RFA monotherapy. Patients had a median of 3 RFA treatments (IQR 2-4), and the median interval between the last RFA and study endoscopy was 21 months (IQR 12-58).

The anticipated inclusion of 20 patients was not reached due to the occurrence of 2 significant adverse events after ER (see the following).
Detection of buried Barrett’s with VLE

Endoscopy
None of the patients had visible Barrett’s epithelium on inspection with high-definition WLE and NBI nor significant stenosis or reflux esophagitis. All 17 patients underwent 2 VLE scans without adverse events.

Volumetric laser endomicroscopy
In 4 of 17 patients, no SGSs were identified during VLE imaging, and a random area within the distal esophagus was delineated and resected. In the remaining 13 patients (76%), VLE detected areas of SGSs. These 13 areas of SGSs were delineated by cautery marks, which were well visible on VLE in majority of the cases, followed by a repeated VLE scan, which confirmed correct delineation in all cases. All 13 areas of SGS were subsequently resected endoscopically.

The median distance of SGSs from the GEJ was 20 mm (IQR 11-35). Twelve SGS areas were localized 10 mm or more above the GEJ, one area containing SGSs was located less than 5 mm from the GEJ. The 13 SGS areas contained 249 different structures on manual count. The median size of in vivo SGS was 140 µm (IQR 80-260). The median depth location of the SGSs was 420 µm (IQR 230-850). Forty-four percent of the SGSs showed a longitudinal appearance.

Histological outcome ER specimens
All detected SGSs were enclosed in separate tissue blocks that were extensively sectioned for histological evaluation. Subsquamous columnar-lined glands with IM (also known as BBs) were detected in 1 tissue block and seen on 3 adjacent histology slides (Fig. 3) from an area of SGSs that was located at 11 mm from the GEJ. Histology of all other SGSs showed nonpathological structures such as blood vessels, dilated ducts of (sub)mucosal glands, lymphocyte aggregates and lymphatic ducts that likely explained the structures seen on VLE (Figs. 4 and 5). The median SGS depth on VLE corresponded to the lamina propria in the majority of the cases, as confirmed on corresponding histology slides. No apparent difference in VLE appearance or localization between SGSs correlated to BBs and other SGSs was noted.

In none of the histology slides of the routinely sectioned tissue blocks without SGS (55 in total) BBs were identified.

The overall prevalence of BBs on a per-patient analysis was 5.9% (1/17), and the frequency of BBs in an area of SGSs detected with VLE was 7.7% (1/13).

Adverse events
In 2 patients a late postprocedural bleeding from the ER site occurred 1 and 2 weeks after study endoscopy. In both patients, the source of the bleeding was treated during a repeat endoscopy; however, 1 patient required intensive care unit admission because of hemodynamic instability due to significant blood loss. At the time of these adverse events,
Figure 3. In vivo volumetric laser endomicroscopy (VLE) scan (A). Endoscopic picture of overview with narrow band imaging showing no residual Barrett’s within the neosquamous epithelium (B) and white-light endoscopic picture of the site after endoscopic resection (C). Buried Barrett’s glands on in vivo VLE (D), ex vivo VLE (E) and corresponding histology (F). Arrowheads indicate buried Barrett’s glands; asterisk indicates blood vessel.

18 patients had been enrolled in the study. The study initially aimed at enrolling 20 patients, but given the 2 significant bleeding events and the fact that inclusion of 2 additional patients was not expected to change the main outcomes, the study was terminated prematurely.

DISCUSSION

The phenomenon of subsquamous Barrett’s epithelium (BBs) after ablative therapy for BE has been described for multiple techniques (eg, photodynamic therapy, argon plasma coagulation, RFA).23–25 Compared with other ablative therapies, RFA is associated with a low
A recent systematic review reported a prevalence of 0.9% in random biopsies in more than 1000 patients that had been treated with RFA.\textsuperscript{25} These biopsy-based studies were recently contradicted by OCT studies claiming that the presence of SGS corresponded to BBs in as many as 63% of post-RFA patients.\textsuperscript{15}

This discrepancy may have 2 possible explanations: biopsy protocols are associated with sampling error or SGSs identified with OCT did not necessarily represent BBs. Assessment of the presence of BBs by random biopsies from the NSE is inevitably associated with sampling error because only a small area of the NSE is sampled. OCT overcomes the potential problems of sampling error because it allows for targeted localization of BBs. In addition, VLE compares favourably to OCT systems used in previous studies because of its capability to evaluate a larger volume of tissue: respectively, 63 mm (circumference) x 60 mm (length) x 3 mm

**Figure 4.** A. Histological image of detailed rendering of C; dilated duct of mucosal gland (arrowhead). B. Corresponding ex vivo volumetric laser endomicroscopy (VLE) scan of histology slide in C. In both transections 2 pins (asterisks) are visible as long straight structures on VLE and as pin holes on histology. Arrow in B indicates VLE balloon.
It has been suggested that biopsy sampling may not reach deeply enough in the subepithelial level to identify BBs. OCT visualizes deeper tissue layers and may therefore detect BBs that are outside the reach of biopsies. However, studies showed that a majority of the biopsies do reach the lamina propria (37-78%) and that there is no difference in biopsy depth between biopsies obtained from post-RFA NSE and untreated squamous epithelium proximal to the ablation zone.9,10 Furthermore, the current study suggests that most SGSs (lamina propria) are generally within the reach of biopsy depth.

In previous studies, our group performed random ERs of the NSE in 44 post-RFA patients without finding BBs in a single resection specimen.4,10 Strikingly, the only BBs that were detected in an ER were found after VLE guided ER in the current study. This shows the potential of VLE as a valuable imaging technique for targeting BBs.

Zhou et al described 114 subsquamous “Barrett’s glands” seen on OCT in 10 of 16 patients.15 However, histological correlation was only provided by 1 biopsy specimen and 1 ex vivo ER specimen obtained at the level of the GEJ. In the GEJ region, mucosal overlap is a normal phenomenon because cardiac mucosa may undermine the NSE. ER specimens obtained close to the junction may therefore result in a histological artefact of BBs.11

Figure 5. Corresponding histology (A) and ex vivo volumetric laser endomicroscopy scan (B) of lymphatic ducts (squares), lymphocyte aggregate (diamonds) in lamina propria and blood vessel (asterisks) in submucosa. Arrowheads indicate transition layer from the epithelium to the lamina propria.
In contrast to previous OCT studies on BBs, we used a stringent protocol including in vivo identification of SGSs with VLE followed by localization and reconfirmation of the VLE-targeted areas. Subsequently, ER of the targeted area was performed followed by ex vivo VLE imaging of the ER specimens to allow for optimal correlation between VLE images and histology. We found that the vast majority of SGSs detected by VLE in vivo did not correspond with BBs but represented normal anatomic structures such as (dilated) (ducts of) (sub)mucosal glands and blood vessels. This suggests that most SGSs identified by OCT in previous studies may not represent BB.

The high percentage of normal histological SGSs may partly be explained by the broad VLE assessment criteria. Furthermore, our patient population had a low BB prevalence, as supported by previously reported negligible BB rates.4,10 This most likely results from meticulous treatment and, in particular, stringent inclusion criteria for complete BE eradication in this study. Therefore, the prevalence of BBs might be different in a general practice based post-RFA population.

Despite the imaging potential of VLE for detecting BBs, the clinical relevance of BBs post-RFA is still in question. The prevalence of BBs in endoscopically normal NSE is rare and generally the identified BBs are not neoplastic. More importantly, most neoplastic recurrences of Barrett’s epithelium post-ablation are endoscopically visible. Of the few anecdotal reports of subsquamous neoplastic recurrences post-ablation treatment, it is not clear whether these were completely buried beneath the NSE or just had some squamous overgrowth. In all cases, lesions were identified endoscopically, thereby questioning the supposedly “buried” and “hidden” danger of BBs.11,26

The VLE appearance of the area containing BBs on histology was not different from the VLE appearance of other SGS areas. Cobb et al described in an OCT study on esophagectomy specimens that BBs can be characterized by a uniform double-band wall appearance.27 After reviewing all areas containing SGSs, we were not able to identify any SGSs with uniform double-band wall appearance. The axial resolution of VLE (7 µm) is, however, lower compared to the system used by Cobb et al (2 µm), and we can therefore not exclude the possibility that at a higher resolution this feature might have been apparent. Hence, specific VLE features to distinguish BBs from other histological structures could not be identified. Although this was not the aim of this study, criteria to differentiate BBs need to be established in order to improve the value of VLE for detecting BBs.

Limitations of this study coincide with the complexity of the protocol of performing multiple VLE scans that were correlated with histology to confirm correspondence between SGSs detected on VLE and histology. Exactly locating and delineating an endoscopic area by VLE findings with the systems used in this study was complex; however, in vivo markers placed around the SGS area were confirmed to be placed correctly by the second in vivo VLE scan in all cases. Future-generation VLE devices may include additional laser marking registration technology to reduce marking complexity.28
Two patients had late post-ER bleeding. No specific patient characteristics or procedure-related conditions were found as an evident cause of bleeding (eg, no anti-coagulation therapy). Beforehand no ethical objections were raised against this protocol since the ER-cap technique is a widely used safe technique; serious adverse events such as severe bleeding and perforation are rare (<3%).\textsuperscript{29,30} Furthermore, as mentioned earlier, we had performed ER of the NSE after RFA for study purposes in 44 patients without any adverse events before the current study.\textsuperscript{4,10}

With this pilot study, we provide reliable histological counterparts of carefully targeted areas containing SGSs detected with VLE. Only 1 VLE area detected in 17 post-RFA patients was shown to contain BBs on histology, which is consistent with previous negligible BBs rates from our patient population. Most SGSs identified by VLE corresponded to normal histological structures. Specific VLE features to distinguish between BBs and normal SGSs could not be identified in this study; therefore, further research is needed before VLE can be used for routine use in post-RFA patients. In addition, ER of endoscopically normal-appearing neosquamous epithelium should not be performed outside study protocols. Still, this study shows the potential of VLE-guided detection of subsquamous esophageal structures. Therefore, VLE may have a future role in the detection and management of early neoplasia in BE.
REFERENCES


