Staging of Superficial Esophageal Adenocarcinoma using Volumetric Laser Endomicroscopy

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A. Background, Innovation and Significance:

The prognosis and management of esophageal adenocarcinoma (EAC) is strongly associated with the stage of disease. Accurate staging is particularly important for superficial EAC given that T1a disease can be treated effectively with endoscopic therapy while T1b disease generally requires esophagectomy due to a higher risk of lymph node metastasis.[1] Our current practice relies on histologic assessment of endoscopic mucosal resection (EMR) specimens to determine depth of invasion. Endoscopic resection and in particular endoscopic submucosal dissection may place patients with T1b disease at an unnecessary risk. Complications such as bleeding have been reported in up to 33%, perforation in up to 5% and esophageal stenosis in up to 30% of patients undergoing resection.[2] Furthermore, up to 15% of patients who undergo EMR will eventually require surgery.[2] Volumetric laser endomicroscopy (VLE) is an imaging platform that uses infrared light to generate cross-sectional views of the human esophagus with microscopic resolution.[3] VLE has been used to detect dysplasia associated with Barrett's esophagus (BE) but its use in cancer staging has not been previously explored. [4, 5] We propose that VLE can accurately and comprehensively stage T1 EAC. The use of this technology may help select patients with invasive disease for esophagectomy while avoiding complications associated with endoscopic resection.

B. Specific Aims:

Accurate staging of superficial EAC (T1a vs. T1b) is critical in guiding treatment of early luminal cancer. We hypothesize that VLE can accurately and comprehensively stage T1 EAC. Thus the specific aims of this proposal are:

1. To correlate depth of cancer invasion measured using VLE to depth of invasion established by EMR (clinical gold-standard) in a retrospective cohort of T1 EAC patients.
2. To determine the diagnostic performance of VLE in staging T1 EAC in a prospective cohort of patients undergoing endoscopic evaluation.

C. Technology Review:

The VLE imaging system (Nine Point Medical, MA) consists of an imaging console, monitor and optical probe. The system's optical probe is centered by a balloon (diameter: 14 mm, 17 mm, 20 mm; length: 6 cm) that is deployed through a gastroscope's instrument channel. Imaging is performed by automatic helical pullback of the probe from the distal to the proximal end of the balloon over 90 seconds. A total of 1200 single cross-sectional frames are generated per scan. VLE images have an axial resolution of 7 μm and can reach an imaging depth of up to 3 mm, providing surface and subsurface microstructural detail. These characteristics make VLE an ideal imaging modality for staging T1 EAC.

The latest generation of VLE platform allows users to identify a region of interest and perform laser-markings that appear as superficial cautery marks under white-light endoscopy. The advantages of this approach are the ability to perform direct VLE to histology correlation and to demarcate an area for endoscopic resection. Imaging enhancing software provides an en-face VLE view, where anatomical landmarks can be easily identified. This software also superimposes signal

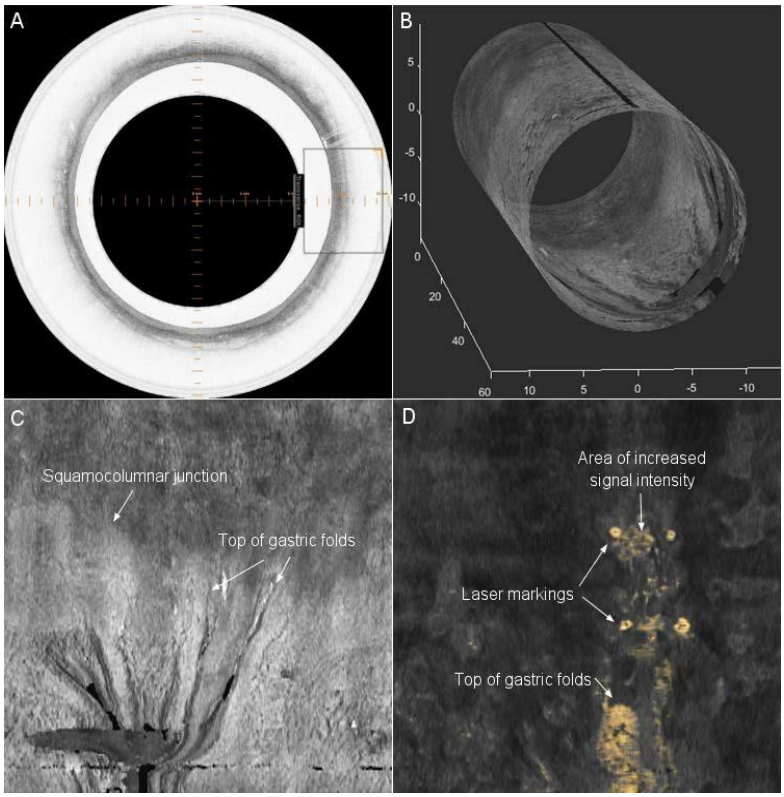


Figure 1: (A) Cross-sectional view of esophagus using VLE in patient with non-dysplastic BE **(B)** Three-dimensional rendering of VLE scan, **(C)** En-face view of VLE scan with labeled anatomical landmarks. **(D)** En-face view of VLE scan of patient with T1 EAC with image enhancing software. Area of increased signal intensity demarcated by laser markings corresponding to area of neoplasia. Gastric epithelium shows normal increased signal intensity.

intensity attenuation measurements allowing for recognition of areas of concern and quantification of degree of signal intensity.

A recent advancement in the field of volumetric laser endomicroscopy (VLE) is the release of an FDA approved image enhancement software termed Intelligent real-time image segmentation (IRIS) (Nine Point Medical, Bedford, MA). IRIS highlights established VLE features using a color-graded scale superimposed over cross-sectional and reconstructed en-face views. VLE IRIS features include (1) surface hyper-reflectivity, a pink graded color scale at the epithelial surface that represents the degree of signal decay over depth (signal attenuation), (2) hypo-reflective structures (epithelial glands), filled by a solid blue color and (3) lack of layered architecture represented by an orange graded color bar at the external rim of the VLE image. Our VLE console was recently upgraded with the IRIS software package. Following the study methodology of this IRB protocol we plan to use IRIS to locate and delineate neoplastic regions prior to biopsy or resection. This is a continuum of our work using a previous offline version of IRIS to measure VLE signal attenuation to determine stage (T1a vs T1b) of early esophageal adenocarcinoma. (Endosc Int Open. 2019 Apr;7(4):E462-E470) The use of IRIS is meant to facilitate VLE interpretation and does not impact patient care. VLE scans will be reviewed with and without IRIS enhancement.

D. Research Strategy:

a. Rationale:

We propose that the use of an advanced endoscopic imaging technology designed to image the esophagus in cross-section at microscopic resolution can accurately predict depth of invasion in patients with T1 EAC. An advantage of cancer staging using VLE is that it could prevent unnecessary endoscopic resection and associated complications. This technology also provides comprehensive assessment of the esophagus as compared to the current standard of practice that relies on a discrete area chosen for endoscopic resection to determine depth of invasion.

b. Preliminary Data and Published Data:

Our group has performed extensive work aimed at understanding the VLE features associated with BE dysplasia. These features include effacement of layered architecture, atypical gland conformations and increased epithelial (surface) VLE signal intensity.[4, 5] More recently we validated a computer algorithm that performs segmentation and reconstruction of a VLE scan into an en-face view with superimposed signal intensity profiles. (**Figure 1**) VLE signal decay is dependent upon several tissue optical characteristics.[6] This algorithm can be applied to estimate the depth of invasion based on degree of signal attenuation. **Figure 2** highlights an example of how depth of invasion can be correlated with VLE signal attenuation. We also reported on the use of this technology to detect subquamous esophageal adenocarcinoma.[7]

Our group has also performed work on histologic determinants of mortality for patients with T1 EAC and described that evidence of lymphovascular invasion and deep margin involvement are significantly associated with reduced overall survival.[8] Ultimately, the data obtained from the present study will help determine if histologic features such as lymphovascular invasion can also be quantified with VLE.

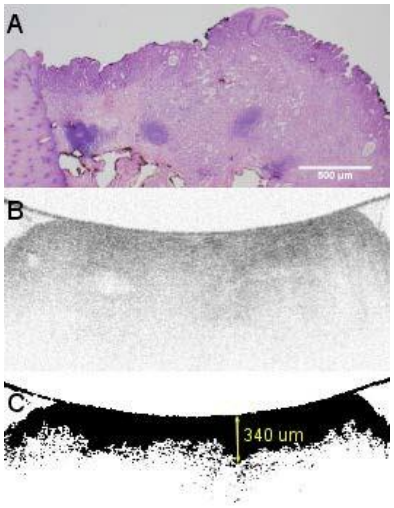
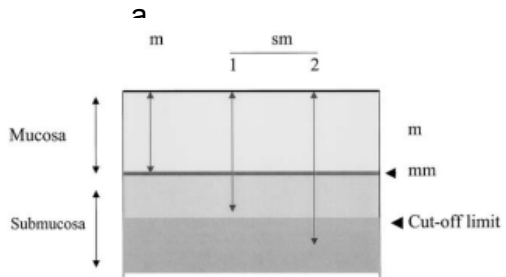


Figure 2: (A) endoscopic mucosal resection specimen of patient with T1a EAC and imaged with **(B)** VLE **(C)** Depth of invasion estimated by signal attenuation decay at 340 µm.

c. Study Design:

I. Aim1: VLE to histology correlation of T1 EAC depth of invasion



Endoscopic Mucosal Resection Database: Our group
platform for imaging EMR specimens using VLE.[4] Patients with BE
inically indicated EMR after participating in informed consent (IRB

ID:12-006665). The fresh EMR specimen is imaged with VLE and inked for orientation. Through an established pathology core lab protocol (Proc 053417.002) for sectioning of the EMR specimens we are able to obtain direct imaging-to- histology correlation Using this imaging platform we have imaged over 200 EMR specimens since 2012 of which 60 specimens were obtained from patients with T1 EAC (T1a EAC, N=45; T1b EAC, N=15).

b. **Histopathologic Review:** EMR histology will be reviewed systematically to assess grade of tumor differentiation, depth of invasion, and presence or absence of lymphovascular invasion. Depth of invasion will be manually quantified by

measuring distance (μm) from mucosal surface to deepest point of invasion. (**Figure 3**) The Paris classification suggests a cut-off of 500 μm to divide depth of submucosal invasion into superficial (sm1) and deep (sm2). This classification has been correlated to depth of invasion defined by layer involvement: (1) **T1a EAC:** tumor invasion into the lamina propria or muscularis mucosa and (2) **T1b EAC**: tumor invading into the submucosa.[9]

c. **Correlation of VLE Signal Attenuation to Depth of Cancer Invasion:** A validated computer algorithm will be used to quantify the degree of signal attenuation in the T1 EAC EMR specimen database identified above. VLE signal attenuation measurements will be correlated to depth of invasion as determined by histology (dB/μm). Based on these measurements, a cutoff signal attenuation value will be established to distinguish between superficial and deep invasion.

II. Aim 2: Diagnostic performance of VLE in staging T1 EAC

Patient Selection:

a. Patients referred to our institution for management of BE and early neoplasia will be prospectively enrolled in this study. Inclusion criteria include patients with T1 EAC who are undergoing endoscopic evaluation including EMR.

Inclusion:

- Patient over the age of 18
- Patient undergoing an upper endoscopy with prior-biopsy confirmed Barrett's Esophagus.
- Ability to provide written, informed consent
- No significant esophagitis (LA grade <B, C, and D)

Figure 4: Study flow diagram

Exclusion:

- Patients who have achieved complete remission of intestinal metaplasia (CR-IM)
- Patients without visible BE at the time of study EGD
- Patients for whom use of the NvisionVLE device would be in conflict with the instruction for use.
- Prior esophageal or gastric surgical resection
- Significant esophageal stricture requiring dilatation
- Patients who require anticoagulation for who biopsy would be contraindicated
- Patients who are known to be pregnant

b. Endoscopic Evaluation: Patients will undergo high-definition white light endoscopy/narrow band imaging with detailed inspection of the BE mucosa. The length of the BE segment will be measured using Prague criteria (circumferential length, maximal length). Area(s) of mucosal nodularity/irregularity will be classified (Paris classification) and their location recorded (o'clock position, distance from incisors). If clinically indicated, an area(s) will be chosen for EMR.

Figure4: Studyflow diagram

d. **Volumetric Laser Endomicroscopy**: A VLE scan will be performed. The VLE scan will be systematically reviewed based on a previously established protocol. If an area(s) is chosen for EMR during

Figure 4: Study flow diagram

endoscopic evaluation it will be identified and demarcated using VLE laser markings. If additional areas of concern are identified under VLE, they will be demarcated using VLE laser marking. All area(s) demarcated with VLE but not identified on endoscopic evaluation will undergo targeted biopsies. Area(s) identified under endoscopic evaluation and demarcated with VLE will undergo EMR as outlined below. Standard surveillance biopsies will be obtained across the remaining BE segment.

e. **Assessment of Cancer Depth of Invasion:** VLE scans will be analyzed using an automated computer algorithm to measure degree of signal attenuation over areas demarcated for EMR. The signal attenuation cut-off established from *aim1* will be used to classify cancers as superficial vs. deep. EMR specimens will be reviewed for grade of tumor differentiation, depth of invasion, and presence or absence of lymphovascular invasion. Depth of tumor invasion will be measured as outlined above and used to classify cancers as superficial vs. deep.

f. **Statistical Considerations and Data Analysis:** This is a prospective cohort pilot study to determine the diagnostic performance of VLE in staging T1 EAC. To achieve a specificity of 90% with a 95% confidence interval, we estimate that a total of 60 patients with T1 EAC will need to be enrolled in this study taking into account that approximately 75% of patients will be staged as having T1a disease on histology. Sensitivity, specificity, negative predictive value, positive predictive value and diagnostic accuracy will be calculated for VLE compared to histology as the gold-standard in staging T1 EAC. Kappa statistics will be performed to test for agreement of the diagnostic methods.

Anticipated Results, Interpretation and Potential Pitfalls:

In the first aim of this study we explore the correlation between VLE signal attenuation and depth of cancer invasion. We use VLE scans from an EMR database of T1 EAC patients obtained using a validated ex-vivo imaging platform. A novel computer algorithm is used to measure the degree of signal attenuation. We anticipate establishing a cut-off of signal attenuation value that correlates with histologic depth of cancer invasion (superficial vs. deep). A potential setback to this approach is the ability to accurately measure absolute histologic depth of invasion, especially in cases with duplicated muscularis mucosa. This setback is mitigated by our prior experience with histological assessment of EMR specimens in which we established a standardized approach to specimen review that will involve two independent gastrointestinal pathologists.

In the second aim of this study we validate the accuracy of VLE in staging T1 EAC compared to a histologic gold-standard. We anticipate that VLE will be able to distinguish between superficial and deep cancer with a >90% accuracy. A potential pitfall to this approach is that the signal attenuation cut-off will be established by imaging of EMR specimens. There is little variation in imaging characteristics between ex-vivo and in-vivo VLE imaging. However, if we find a broad distribution of signal attenuation measurement in EMR specimens we plan to perform preliminary validation of this approach using a cohort of T1 EAC patients (N=10) who have undergone in-vivo VLE and EMR as part of a previous research protocol.

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