

Video Article

Diagnosis of Neoplasia in Barrett's Esophagus using Vital-dye Enhanced Fluorescence Imaging

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Abstract

The ability to differentiate benign metaplasia in Barrett's Esophagus (BE) from neoplasia *in vivo* remains difficult as both tissue types can be flat and indistinguishable with white light imaging alone. As a result, a modality that highlights glandular architecture would be useful to discriminate neoplasia from benign epithelium in the distal esophagus. VFI is a novel technique that uses an exogenous topical fluorescent contrast agent to delineate high grade dysplasia and cancer from benign epithelium. Specifically, the fluorescent images provide spatial resolution of 50 to 100 μm and a field of view up to 2.5 cm, allowing endoscopists to visualize glandular morphology. Upon excitation, classic Barrett's metaplasia appears as continuous, evenly-spaced glands and an overall homogenous morphology; in contrast, neoplastic tissue appears crowded with complete obliteration of the glandular framework. Here we provide an overview of the instrumentation and enumerate the protocol of this new technique. While VFI affords a gastroenterologist with the glandular architecture of suspicious tissue, cellular dysplasia cannot be resolved with this modality. As such, one cannot morphologically distinguish Barrett's metaplasia from BE with Low-Grade Dysplasia via this imaging modality. By trading off a decrease in resolution with a greater field of view, this imaging system can be used at the very least as a red-flag imaging device to target and biopsy suspicious lesions; yet, if the accuracy measures are promising, VFI may become the standard imaging technique for the diagnosis of neoplasia (defined as either high grade dysplasia or cancer) in the distal esophagus.

Video Link

The video component of this article can be found at <http://www.jove.com/video/50992/>

Introduction

Over the last forty years, the incidence of esophageal adenocarcinoma (EAC) has increased significantly^{1,2}; yet due to late diagnosis, the five-year survival rate is less than 20%³. The current standard of endoscopic surveillance in BE, the precursor to EAC, is white light endoscopy with random four-quadrant forceps' biopsies of the segment. Unfortunately, this technique often misses neoplasia, which can be flat, subtle and difficult to differentiate on standard white light imaging⁴. While there has been success in using confocal laser microscopy to highlight cellular features *in vivo*, lesions can still be missed due to the decreased field of view⁵. Having a 'bridge' technology that can highlight areas for further confocal microendoscopic imaging would be markedly valuable.

Consequently, an enhanced red-flag imaging modality that improves the ability to target and biopsy early neoplasia in BE would be instrumental in detecting EAC at an early, curable stage and could lead to more effective treatment and subsequently improved survival rates. VFI is a novel technique that combines high-resolution epithelial imaging with exogenous topical fluorescent contrast, proflavine, to highlight glandular morphology and delineate neoplasia (high grade dysplasia and cancer) in the distal esophagus in hopes of improving the *in vivo* diagnosis⁶. Upon excitation of the proflavine, which concentrates within cell nuclei shortly after application, the fluorescent images provide spatial resolution of 50 to 100 μm and a field of view up to 2.5 cm, allowing endoscopists to visualize glandular morphology. As a result, this approach enables gastroenterologists to distinguish classic Barrett's metaplasia, which has continuous, evenly-spaced glands and an overall homogenous morphology, from BE with neoplasia, which has obliteration of the glandular architecture. Here we describe the protocol of this new technique with a multispectral endoscope, and provide representative results to demonstrate the utility of this device in depicting the morphological transformation from benign metaplasia to high-grade dysplasia and cancer.

Protocol

NOTE: Informed consent was obtained from the patients. Also, this research has been performed in compliance with all institutional, national, and international guidelines for human welfare.

1. Prepare Computer

1. Turn laptop on and connect USB from the DVI2USB Capture Card.

2. Prepare Monitor

1. Connect DVI cable to monitor and PinP to allow the endoscopist to view videos from these screens rather than the computer.
2. Make sure the standing monitor is on and is set to DVI.
3. Connect PinP to the back of the Olympus system then press the input button on the Olympus system to view the image on the large monitor mounted on the wall.

3. Laser Diode Driver Setting

1. Check to make sure the laser diode driver is OFF. It should only be turned on a couple minutes before imaging is performed.

4. Power Strip

1. Plug in laptop, processor, laser diode driver, DVI splitter, and epiphan capture card into the power strip.

5. Run MDE Widefield on the Laptop Desktop

1. Hit 'Current Folder'.
2. Type patient number and initials and press 'Create Patient Folder'.

6. Prepare Cap and Filter

1. When handling filter, always use gloves and Kimwipes to minimize the transfer of oil and debris to the filter. Gauze or alcohol swabs may leave fibers that can interfere with imaging.
2. Place a few Kimwipes onto a table to create a platform to lay down the filter and cap. This disinfected area should be easily accessible to the gastroenterologist during the procedure.
3. Lay down the filter and cap and keep Kimwipes close by for use during the procedure.

7. Patient Preparation

1. Consent patient on use of VFI and proflavine dye before arriving to endo suite.
2. Position patient for upper endoscopy procedure.
3. Proceed with standard white-light imaging using a multispectral digital microscope (MDM)⁷.

8. Insert and Spray Proflavine

1. After finishing with white-light imaging, insert and spray proflavine dye over tissue of interest. 1-5 mm should be sufficient depending on the area of the Barrett's tissue. By spraying the proflavine dye at this step, ample time (at least 1 min) is given for sufficient tissue absorption before VFI. Proflavine, which is covered under an investigational new drug application from the FDA (IND 102,217), is a fluorescent contrast agent that concentrates within cell nuclei shortly after application. Although VFI cannot resolve individual cellular morphology, when the laser diode pans over tissue, the reflected light enables the endoscopist to appreciate overall glandular morphology.

9. Turn on Laser Diode

1. Turn on the laser diode. Do this at least 2 min before imaging begins.

10. Prepare Endoscope for VFI

1. Withdraw the endoscope completely.
2. Prior to handling the endoscope, ensure that the assistant has two pairs of gloves on.
3. Have the endoscopist hold the endoscope in front of the assistant who is next to the Kimwipe platform detailed above.
4. With Kimwipes, have the assistant clean the tip of the endoscope. Make sure to clean the front surface gently and also clean a few centimeters along the side of the endoscope for easier handling.

5. After cleaning, have the assistant dispose of the outer pair of gloves.
6. Have the assistant put on the filter. Insert the short cylindrical protuberance on the filter into its complementary hole in the endoscope. Keep the endoscope vertical to aid the process.
7. Holding the filter in place, slide the cap over the filter and push it down over the tip of the endoscope. Make sure the cap is secure and pushed completely on with the edges flush with the endoscope. Make sure the lip of the cap slightly extends over the tip of the endoscope. Lastly, make sure the filter is still in place and flush with the tip of the endoscope.

11. Insert the Endoscope Back into the Esophagus and Image

12. Remove Endoscope from Esophagus

1. Using Kimwipes carefully pull the cap and filter off the endoscope. Throw away the cap and clean the filter for the next case.

13. Clean Filter

1. Gently clean the filter with Kimwipes.
2. Fill a small cup with Cydex and submerge the filter for 12 min. Every few min turn the filter over.
3. Gently clean the filter with Kimwipes.
4. Submerge filter, this time in sterile water. After 5 min, use a squirt bottle and lightly spray sterile water over the filter.
5. Place filter on a few Kimwipes and let it dry.
6. Once dry, place in storage container in between Kimwipes.

Representative Results

Figure 1B depicts classic Barrett's Esophagus with no dysplasia surrounded on the borders by normal squamous epithelium. Beginning with the flatter squamous tissue, which is peripherally located and indicated by the blue arrows, a homogenous area of dull fluorescence is present with no glandular architecture. The green arrows indicate a circular green line surrounding the squamous tissue. This outline is artifact resulting from the cap of the endoscope. Moving to the centrally located Barrett's tissue, glandular structures can be defined as green fluorescence surrounding a darker lumen. Although some glands are elongated, there is little distortion between adjacent glands, as the width of the glands is similar and the edges are clearly defined. Lastly, the glands and the lumens are evenly spaced with no clumping or crowding present.

Figure 2B depicts Barrett's Esophagus with Low-Grade Dysplasia. It is important to note that although there is Low-Grade Dysplasia present, this cannot be visualized by morphological criteria via this imaging modality. Thus, based on morphological patterns, this tissue is still classified as benign. While the homogenous glands and lumens in the yellow oval are suggestive of mere metaplasia, the blue oval indicates an area of largely coalesced glands. That is, the thickness of the glands have increased, while the darker luminal cavity has become thin and nearly absent. These crowded and slightly distorted glands, however, have discrete borders and tend to be homogenous. Moreover, there is no effacement present as the edges of the glands are smooth, thus the tissue is still benign. The red arrow indicates tissue that is out of focus and in video can be readily distinguished. Lastly, the black arrow shows bubbles that are artifact.

In **Figure 3B**, the red oval indicates the most prominent area with high grade dysplasia. These glands are crowded as they have thin irregular borders along with areas of tissue with near effacement of the glandular architecture. That is, the glands are no longer distinct, but rather fusing together, with their lumens being small and irregular. Although small, the continued presence of some lumens likely indicates high-grade dysplasia as it is more often in invasive cancer where the lumens are completely lost. In contrast to the high-grade dysplasia, the yellow oval highlights malignant tissue. Here, the glandular architecture is obliterated and lumens are largely absent.

Figure 4B depicts a centrally located adenocarcinoma. Notice first the cancer within the red oval and the complete obliteration of glandular architecture with luminal absence. This obliteration can be further appreciated when comparing the cancer to the tissue indicated by the blue arrow, which possesses some glandular framework. On the left, the gray rectangle highlights squamous epithelium, which can be better appreciated in video when the endoscope pans over it in its entirety. This squamous tissue is a flat homogenous area of dull fluorescence with no glandular architecture.

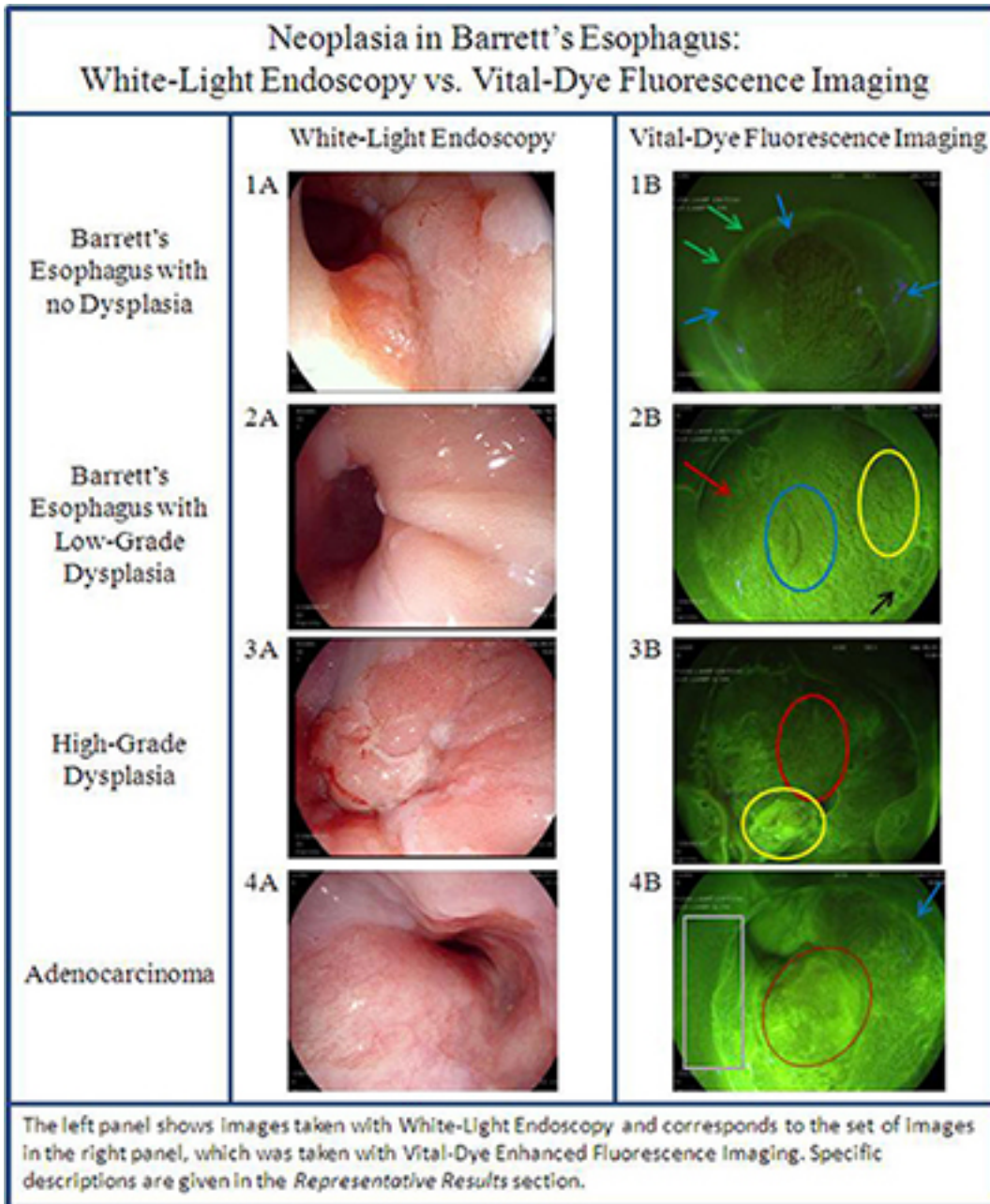


Figure 1. Neoplasia in Barrett's Esophagus: White-light endoscopy vs. vital dye fluorescence imaging. (Left) Images taken with white-light endoscopy. (Right) Corresponding images taken with vital-dye enhanced fluorescence imaging. [Please click here to view a larger version of this figure.](#)

Discussion

With standard endoscopic surveillance, neoplasia in BE is often missed⁸ because benign metaplasia can be indistinguishable from high-grade dysplasia and adenocarcinoma. As a tool that would better enable gastroenterologists to remedy this currently unavoidable error, vital-dye enhanced fluorescence imaging highlights a tissue's glandular morphology thereby providing a distinct feature to differentiate the tissue types. Moreover, by providing a field of view up to 2.5 cm, VFI enables endoscopists to pan over the entire distal esophagus efficiently and systematically, making suspicious lesions more prominent.

To utilize this novel technique, first spray a topical contrast agent, proflavine, over the tissue of interest. Then, after removing the endoscope a 495-nm long-pass filter needs to be securely capped to the MDM tip. Finally, after reinserting the endoscope near the tissue of interest and then switching from the white light to the laser diode, a green fluorescence provides *in vivo* imagery of the tissue's glandular framework.

In cases of continuous suboptimal fluorescence, using fresh proflavine can improve results as well as turning on the laser diode a few minutes before actual usage and adjusting its voltage. Separately, for patient safety, make certain the cap has been pushed completely over the filter,

such that the filter is flush with the tip of the endoscope. This, in turn, will enhance image quality by preventing mucus buildup behind the filter itself.

While VFI is able to characterize glandular morphology, a tangible improvement to white-light endoscopy, it cannot resolve cellular dysplasia. That is, the spatial resolution of 50-100 μm makes VFI incapable of differentiating benign metaplasia from low-grade dysplasia. To distinguish these diagnoses, higher resolution devices that visualize cellular traits, such as nuclear polarity and cellular crowding, are needed^{9,10}. As an example, high-resolution microendoscopy is a more novel technique that can differentiate neoplastic tissue on a cellular level as nuclei are illuminated bright white and nuclear to cytoplasmic ratio can be appreciated. Narrow band imaging is another option, but it requires use of two different scopes and processors and follow-up administration of either proflavine or fluorescein for microendoscopy; in contrast VFI allows immediate confocal imaging with one scope and one processor, lengthening anesthesia time by approximately 6-8 min. However, because these latter devices focus on minute tissue areas and consequently miss neoplasias, VFI, in addition to diagnosing neoplasia, may also act as a widefield, red-flagging device for the placement of higher-resolution probes. Thus, the future of endoscopic screening for Barrett's dysplasia will likely involve a combination approach of widefield surveillance technology, like VFI, along with a cellular classification technology like microendoscopy.

Disclosures

There is nothing to disclose.

Acknowledgements

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