Basic studies of vascular images as seen with indocyanine green-guided infrared ray electronic endoscopy as compared with microangiography

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Objective: The depth of blood vessels as seen with infrared ray electronic endoscopy (IREE) and whether these vessels are arteries or veins remains unclear. In the present study, we evaluated how blood vessels as seen with IREE images were related to structure in the gastric wall.

Methods: We chose 7 consecutive patients who underwent gastrectomy after they underwent IREE in our hospital. Blood vessels in the gastric wall as seen with indocyanine green (ICG)-guided IREE were compared with those using microangiography in matched pairs.

Results: Nearly all blood vessels were depicted as a single vascular plexus as seen with IREE, corresponding to the venous plexus in the submucosa. The minimal diameter of submucosal venous plexuses able to be observed was 0.2 mm, currently considered the detection limit of IREE. The IREE images showed no evidence of the other layer. Vascular plexuses could be observed as seen with the IREE images in all of the patients and were intimately related to the severity of mucosal atrophy in the stomach.

Conclusions: Vascular plexuses as seen with ICG-guided IREE images were mainly medium-to-large venous plexuses located in the submucosa. The visibility of vessels using the IREE images depends on various factors.

Key words: infrared ray electronic endoscopy, indocyanine green, gastric vascular structure

Introduction

Recently, techniques using special light sources, such as narrow-band imaging and infrared systems, have been widely used to estimate the extent and invasion depth of lesions in gastric cancer and various other diseases. In particular, near infrared light deeply penetrates tissues, allowing blood vessels in the gastric wall to be observed endoscopically. In addition, the use of a bandpass filter (BPF) after the injection of indocyanine green (ICG) selectively enhances blood vessels in the gastric wall, allowing them to be visualized. However, the depth of blood vessels as observed using infrared ray electronic endoscopy (IREE) and whether these vessels are the arteries or veins remain unclear. We used microangiography to determine whether blood vessels observed with IREE are arteries or veins and to assess their thickness and depth in the gastric wall.

Materials and Methods

This study was approved by the Institutional Review Board at Kitasato University and was performed according to the Declaration of Helsinki as amended in Somerset West. And all patients gave written informed consent.

Seven consecutive patients who underwent gastrectomy after undergoing IREE in Kitasato University East Hospital from 1991 to 1992 were enrolled in this study.

IREE

We used an infrared ray electronic endoscope (Olympus Optical Co., Ltd., Tokyo), that was a modification of the electronic endoscope EVIS 200 system. A BPF was attached to a xenon lamp, serving as the light source (Figure 1). The wavelength of light penetrating the BPF was extremely narrow (803 ± 17 nm). This wavelength
is nearly identical to the peak absorption point of intravenously injected ICG (805 nm). Therefore, infrared rays with a wavelength that selectively passes through the BPF can penetrate a red-green-blue filter and illuminate. Infrared rays can very deeply penetrate tissue in the gastric wall. Because ICG is systemically circulated into blood vessels in the gastric wall and infrared rays with the same wavelength as ICG are selectively absorbed, blood vessels in the gastric wall appear to be black and translucent. The electronic endoscope has a charge-coupled device (CCD) built into the scope tip, and the light source sequentially radiates red, green, and blue light on the mucosa. The CCD captures the reflected light, converts the image into electrical signals, and reconstructs the image on the monitor. Since the CCD is sensitive to invisible infrared rays, blood vessels in the gastric wall can be visualized.

**Intravenous injection of ICG**

After conventional observation, ICG (3 mg/kg) was rapidly injected intravenously with manual pressure. Immediately thereafter, observations were begun. Twenty to 30 seconds later, the same field was compared while switching between conventional and infrared images.

**Comparisons with microangiograms**

The resected stomachs of 7 patients underwent microangiography with the use of a barium-gelatin solution to determine the location, i.e., layer in the gastric wall, and the diameter of blood vessels in the gastric wall that were visualized with IREE. Contrast medium was injected into the left gastric vein until it was basically distributed to the capillary plexus in the mucosa, and soft radiography was performed. The gastric wall was carefully sliced with a scalpel into 3 layers: the mucosa, the submucosa, and the muscularis propria and deeper layers. Vascular plexuses were examined in each of these layers, and their characteristics were compared with images obtained by using IREE. Blood-vessel caliber was also examined, and blood-vessel thickness was compared with the IREE images to determine the detection limits of blood vessels in the gastric wall.

**Results**

**Relation of IREE images to atrophy in the gastric mucosa**

Figure 2b shows severe atrophic mucosa on conventional endoscopy. It was barely visible, but also in Figure 2a the IREE image was clearly visualized blood vessels. IREE images clearly showed vessels in the gastric wall that were unable to be depicted using conventional endoscopy, as well as blood vessels in the atrophic gastric mucosa that were barely visible with conventional endoscopy. The ability to visualize blood vessels tended

![Figure 1. System for infrared-ray electronic endoscopy (IREE)](image)
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**Figure 2.** The vascular network is more clearly depicted using IREE than with conventional endoscopy.

**Figure 3.** Specimens of the stomach fixed in formalin (a, c, and e) and their respective microangiographic images (b, d, and f), obtained after intravenous injection of a gelatin-barium solution into the resected stomach.

a, b. Layer of the mucosa and muscularis mucosa  
c, d. Submucosal layer  
e, f. Layer of the propria muscularis and serosa
to correlate with the severity of mucosal atrophy. Net-like vascular plexuses seen in the atrophic gastric mucosa with conventional endoscopy were more clearly depicted using IREE images. This was in contrast to blood vessels that were slightly indistinct in non-atrophic gastric mucosa. The more severe the atrophy, the more clearly blood vessels were visualized using IREE. Near fundic glands in regions of the gastric mucosa with mild atrophy, i.e., regions with thick mucosa, blood vessels were usually not depicted using IREE images. These findings clearly showed that the thickness of the mucosa is an important determinant of the ability to visualize deep vessels.

**Comparison of the course, depth, and thickness of blood vessels using IREE images with microangiograms findings according to the layer of the gastric wall**

To estimate the size of the veins in the gastric wall that were visualized using IREE, we compared vascular plexuses using IREE with those using microangiography according to the layer of the gastric wall.

**Mucosa and muscularis mucosae**

Figure 3a shows the macroscopic view of the mucosa and muscularis mucosae and Figure 3b shows vascular images of the mucosae and muscularis mucosae. Radial vascular plexuses were present in subdivisions of the stomach with a blood vessel of moderate thickness in the center. These vessels were the so-called collecting venules. Vascular plexuses in subdivisions of the stomach were independent. There were no vascular plexuses linking different subdivisions of the stomach. These vessels had extremely small calibers. The maximal caliber of most collecting venules was 100 μm or less. Such characteristic vascular plexuses were not found using IREE or conventional endoscopy.

**Submucosa**

The gastric wall was divided at the border between the submucosa and the muscularis propria to observe vessels in the submucosa. Figure 3c shows the macroscopic view of the submucosa and Figure 3d shows the vascular images of the submucosa. Vascular plexuses are most abundant in this submucosal layer. Veins with a caliber ranging from 20 to 30 μm up to 2 to 3 mm form networks and gradually become thicker as they converge toward the greater and lesser curvatures. These veins finally form main trunks at regular intervals along the greater and lesser curvatures and pass through the muscularis propria to form outflow pathways. Figure 4a shows IREE images and Figure 4b shows the microangiographic images. When the venous plexuses in this layer (Figure 4b) were compared in matched pairs with the IREE images (Figure 4a), there was almost exact correspondence. Vascular plexuses as seen using IREE (Figure 4a) and the corresponding microangiographic images of submucosal blood vessels are shown in Figure 4b. IREE images "A-C" correspond to submucosal microangiograms "A-C," respectively. Vessels between IREE images "A" and "B" extend superiorly towards the lesser curvature, and disappear in "D." These findings show that these vessels penetrate the muscularis propria at this region and proceed to the subserosa and lesser omentum, flowing into the left and right gastric veins. These findings were evidenced as the vascular plexuses

![Figure 4. Comparison of IREE images (a) with the corresponding microangiograms (b). Submucosal blood vessels using IREE images (A-G) corresponded to the vessels using microangiograms (A-G).](image)
were clearly seen in all of the 7 samples.

Blood vessels extending from regions "A-C" to the greater curvature also disappear at regions "E-G" consistent with the microangiographic findings for "E-G." Blood vessels at these sites pass through the muscularis propria and flow into the gastroepiploic veins in the subserosa. Vessels that pass from the submucosa to the muscularis propria are no longer found using IREE images. The vascular plexuses using IREE images were thus shown to be submucosal vessels, especially veins. The smallest-caliber vessels in this region that could be identified using IREE images appeared to be about 200 to 300 μm as seen microangiograms. The range of the minimum caliber of detectable vessels depended on the angle of branching.

Muscularis propria and serosa
The macroscopic view (Figure 3e) and microangiography (Figure 3f) of the muscularis propria, subserosa, and serosa showed vessels running parallel to bundles of oblique muscles, circular muscles, and longitudinal muscles.

Branched vessels were seen in the subserosa. These vessels head toward the lesser curvature, gradually becoming thicker, converge, and flow into thick, longitudinal gastric veins. The caliber of these vessels ranged from 20 to 30 μm to up to 2 mm. These vessels were poorly detected using IREE images. Blood vessels in the muscularis propria or deeper regions were discovered to be poorly visualized with IREE.

These results demonstrated that our system for IREE permits the visualization of deep blood vessels in the gastric wall. Most of the vessels able to be visualized were thick submucosal veins measuring 0.2 mm or more in diameter. Thin vessels measuring 0.1 mm or less in diameter and vessels in the muscularis propria or deeper regions were poorly detected using IREE.

Discussion
Clinical attempts to use infrared rays were made by Massopust et al. in 1953,6 who studied breast diseases, and by Gibson in 1965,7 who performed studies in the field of ophthalmology. In Japan, IREE was used clinically by Niwa8 in 1970. Various infrared techniques have been used to observe vascular plexuses in the gastric wall.9-12 Although observed blood vessels were considered to be veins, their location was difficult to identify. In 1989, a direct-illumination-type intragastric IREE system was developed, with a xenon lamp as the light source. The use of a light guide permitted examination of the intragastric region without limitation.13 In 1992, Nagao et al.5 developed an ICG-guided IREE system, which produced very clear vascular images. Gradual improvements in IREE have thus permitted the visualization of deep vessels in the gastric wall, and new systems are now ready for clinical application. However, the depth and minimal detectable thickness of these blood vessels and whether they are arteries or veins have not been studied previously. Clarifying these issues will be essential for clinical diagnosis by using IREE in the future.

We used an EVIS 200 system that was improved in cooperation with the Olympus Optical Company. A CCD that was very sensitive to near-infrared rays (760 to 1400 nm) as well as visible light (400 to 760 nm) was built into the tip of an electronic endoscope. Infrared rays more deeply penetrate tissues than visible light does, allowing information on deeper tissues to be obtained. ICG has a wavelength of 780 nm in water, but promptly binds to proteins in blood, resulting in a wavelength of 805 nm. A very narrow BPF, with a wavelength of 803 ± 17 nm, matched to that of ICG, is equal to peak absorption wavelength. This is an ideal system that allows minute quantities of ICG in blood located in deep regions of the gastric wall to be observed macroscopically.

We compared the findings on IREE with those on microangiography of the resected stomach. Arteries and veins in the gastric wall usually run parallel to each other. Veins are capacitive vessels, which are usually thicker than arteries. Vascular plexuses observed using IREE were reticular or branched. These vessels were usually unaccompanied by other vessels, and rarely was a thin parallel vessel noted.

After the intravenous injection of contrast medium, microangiographic specimens of the resected stomach were divided along the borderline between the muscularis mucosae and the submucosa and the borderline between the muscularis propria and the submucosa. Vascular plexuses seen using IREE images completely matched the submucosal vascular plexuses using microangiograms. In contrast, the fine vascular plexuses in the mucosa and muscularis mucosae were poorly visualized using with IREE. Microangiography showed that blood vessels in the muscularis propria ran along muscle bundles. These vessels were thin veins measuring only about 0.1 mm in diameter, running obliquely, circularly, and longitudinally. These blood vessels were not depicted using IREE.

Subserosal blood vessels form branch-like vascular plexuses flowing towards the lesser and greater curvatures. These vessels were not detected using IREE.
We, therefore, concluded that blood vessels depicted using IREE were submucosal venous plexuses. This conclusion was also supported by the fact that images of blood vessels that passed through the muscularis mucosa and flowed towards the lesser and greater curvatures were no longer depicted using IREE. When the resolution of IREE was evaluated on the basis of vascular caliber, the smallest veins seen with IREE were found to be about 0.1 mm in diameter on microangiography. This is considered the detection limit of the current technique. Our findings indicate that intramucosal blood vessels usually cannot be observed using IREE. However, these vessels can be visualized if blood is pooled to some extent in association with conditions such as hemangioma, telangiectasis, and some early gastric cancers.

In addition to the resolution of the IREE system, atrophy and the thickness of the gastric mucosa are important factors related to the visualization of vessels in the gastric wall. As reported, increasing severity of atrophy is associated with clearer visualization of vascular plexuses. In patients without atrophy, vascular plexuses are poorly visualized. Vascular plexuses in the fundic glands of the gastric body are not depicted because the mucosa is very thick. However, vascular plexuses are depicted even in the gastric body if atrophy is severe. Atrophy and the thickness of the gastric mucosa are, therefore, intimately related to the resolution of IREE images used for to examin venous plexuses.

In consideration of these findings, we previously reported that the extent of submucosal invasion by gastric cancer can be estimated on the basis of interruption or tapering of vascular plexuses. We also analyzed pooling of ICG, associated with the extent of invasion of early gastric cancer. In patients with depressed-type early gastric cancer, we reported that tumor staining on ICG-guided IREE images is caused by increased inflow of ICG due to increased circulation in the mucosal vascular bed and stasis of blood flow or pooling in the same area.

Our study demonstrated that vascular plexuses depicted using ICG-guided IREE are mainly medium-to-large venous plexuses located in the submucosa. The visibility of blood vessels using ICG-guided IREE is apparently influenced by factors such as vessel depth and diameter, differences between arteries and veins, gastric mucosal thickness, and tissue-specific differences in the penetration of infrared rays.

References