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Innovative gastric endoscopic muscle biopsy to identify all cell types, including myenteric neurons and interstitial cells of Cajal in patients with idiopathic gastroparesis: a feasibility study (with video)

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Abstract

Background and Aims—The pathophysiology of some gastrointestinal neuromuscular diseases (GINMD) remains largely unknown. This is in part due to the inability to obtain ample deep gastric wall biopsies that include the intermuscular layer of the muscularis propria (MP) to evaluate the enteric nervous system, interstitial cells of Cajal (ICC) and related cells. We report on a novel technique for gastric endoscopic muscle biopsy (gEMB).

Methods—Patients with idiopathic gastroparesis were prospectively enrolled in a feasibility study using a novel “no hole” gEMB. Main outcome measures were technical success, adverse events and histological confirmation of the intermuscular layer, including myenteric neurons and ICC. The gEMB was a double resection clip–assist technique. A site was identified on the anterior wall of the gastric body as recommended by the International Working Group on histological techniques. Endoscopic mucosal resection was performed to unroof and expose the underlying

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MP. The exposed MP was then retracted into the cap of an over-the-scope clip. The clip was deployed and the pseudopolyp of MP created was resected. This resulted in a “nohole” gEMB.

Results—Three patients with idiopathic gastroparesis underwent gEMB. Patients had severe delayed gastric emptying with a mean of 49 ± 16.8% of retained gastric contents at 4 hours. They had no history of gastric or small-bowel surgery, and did not use steroids or other immunosuppressive drugs. The gEMB procedure was successfully performed with no procedural adverse events. Postprocedure abdominal pain was controlled with nonsteroidal anti-inflammatory agents and opioid analgesics. Mean length of resected MP was 10.3 ± 1.5 mm. Mean procedure time was 25.7 ± 6 minutes. Tissue samples on hematoxylin and eosin (H&E) stain confirmed presence of both inner circular and outer longitudinal muscle as well as the intermuscular layer. H&E stain showed reduced myenteric ganglia in 1 patient. In 2 patients, specialized immunohistochemistry was performed, which showed marked decrease in myenteric neurons as delineated by an antibody to Protein Gene Product (PGP) 9.5 and severe decrease in ICC across the muscle layers. At 1 month follow-up, upper endoscopy showed a well-healed scar in 2 patients and minimal ulceration with retained clip in 1 patient. CT abdomen confirmed the integrity of the gastric wall in all patients. Due to lack of an immune infiltrate in the resected samples, patients were not considered suitable for immunosuppressive or steroid therapy.

Conclusions—gEMB is feasible and easy to perform, with acquisition of tissue close to surgical samples to identify myenteric ganglia, ICC and multiple cell types. The ability to perform gEMB represents a paradigm shift in endoscopic tissue diagnosis of gastric neuromuscular pathologies.

The pathophysiology of many gastrointestinal neuromuscular diseases (GINMD) including idiopathic gastroparesis and functional dyspepsia, is not well understood[1,2]. There is growing evidence to support an underlying heterogeneous neuromuscular pathology in patients with gastroparesis and emerging evidence for cellular changes in functional diseases such as functional dypspesia[3,4, 5]. Endoscopic mucosal-based biopsies do not allow for evaluation of the myenteric plexus or interstitial cells of Cajal (ICC) networks that lie within the muscularis propria (MP) or the intermuscular layer of the MP. Currently, we rely on surgical approaches such as laparoscopic wedge biopsy to obtain sufficient tissue samples of the gastric wall.

A readily available, effective, and safe endoscopic technique that enables ample deep gastric wall biopsies to include the intermuscular layer of the MP for evaluation of the enteric nervous system, immune cells and ICC may provide invaluable insights into the pathogenesis of these disorders. The aims of this study were to (1) determine the efficacy of an innovative gastric endoscopic muscle biopsy (gEMB) technique; (2) identify the muscle layers included in the resected specimen and the presence of myenteric ganglia and ICC; (3) determine the procedural and long-term safety of the technique and (4) identify neuromuscular pathologic changes in patients with idiopathic gastroparesis.

METHODS

Patients

Patients were prospectively enrolled in this feasibility study approved by the Institutional Review Board. Patients diagnosed with symptomatic refractory idiopathic gastroparesis

were recruited. Only patients with documentation within the last 2 years of delayed gastric emptying with >30% retained gastric contents at 4 hours based on 296 kcal solid-liquid, fat-containing standard meal gastric emptying test were included. Patients were excluded if there was a history of oropharyngeal, esophageal, gastric, or small-bowel surgery, esophageal stricture, abdominal radiation therapy, percutaneous endoscopic gastrostomy or jejunostomy, coagulopathy, and use of steroids or immunosuppressive drugs.

Patients were admitted after the procedure for 24-hour observation. Patients were maintained on clear liquids on day 1 of the procedure, and the diet was advanced as tolerated thereafter. Omeprazole 40 mg twice daily orally for 4 weeks and perioperative antibiotics were administered. Patients were contacted by phone 48 hours and 1 week after the procedure. At 1 month follow-up, a repeat upper endoscopy to review the mucosal aspect of the resection site and a CT abdomen to assess the integrity of the resection site were performed.

Description of Technique

gEMB involves a double resection clip-assist technique. A site was identified along the anterior wall of the gastric body, midway between the greater and lesser curves, as recommended by the International Working Group on histological techniques which is a consensus document by expert neurogastroenterologists[6]. From an endoscopic perspective the anterior wall is ideal both from safety (avoiding the gastroepiploic and gastric vessels) and ease of procedure performance. Endoscopic mucosal resection (EMR) using the band ligation approach (Duette; Cook Endoscopy, Bloomington, Ind) was initially performed to unroof and expose the underlying MP. An over-the-scope clip (Padlock Clip – 11 mm; Aponos, Kingston, NH) was fitted on a diagnostic upper endoscope (GIF-180; Olympus America, Center Valley, Pa) and advanced to the exposed MP. A tri-pronged tissue retraction device (OTSC Anchor, Ovesco Endoscopy, Tübingen, Germany) was passed through the working channel of the endoscope and deployed through the MP, which was then retracted and suctioned into the cap of the clip. Once an adequate amount of tissue was entrapped within the cap, the clip was deployed. The pseudopolyp of MP created was resected using hot snare (Acusnare; Cook Endoscopy, Bloomington, Ind). Resection was done approximately 5 mm above the deployed clip to ensure ample tissue remained for a secure closure. This approach resulted in a “no-hole” gEMB. Effective closure of the biopsy site was confirmed during endoscopy by visible closure without disruption, sustained distension of the gastric lumen with insufflation of CO_2, and absence of radiopaque contrast leakage into the peritoneal cavity after intragastric injection determined fluoroscopically. The resected specimens of MP, as well as mucosa and submucosa from the EMR, were retrieved, pinned, measured, and submitted for histology.

RESULTS

We report on 3 female patients with a mean age of 33 ± 8.5 years with symptomatic refractory idiopathic gastroparesis who underwent gEMB. Predominant symptoms were nausea, vomiting, abdominal pain, and weight loss that were ongoing for several years. Severe delayed gastric emptying with a mean of 49 ± 16.8% of retained gastric contents at 4 hours based on 296 kcal solid-liquid, fat-containing standard meal gastric emptying test was

documented in study patients. A double resection clip-assist gEMB was performed successfully in all patients (Figures 1A and 1B). There were no intraprocedural adverse events. The mean size of resected MP was 10.3 ± 1.5 mm (Figure 2). The mean size of resected mucosa and submucosa was 22 ± 6.1 mm. Mean procedure time from endoscope intubation to retrieval of tissue specimens was 25.7 ± 6 minutes.

Tissue samples on hematoxylin and eosin (H&E) stain confirmed the presence of MP with inner circular and outer longitudinal muscle, as well as intermuscular layer in all patients (Figure 3). Abnormalities were noted in 2 out of 3 (66%) patients. H&E stain showed reduced myenteric ganglia in 1 patient. In 2 of the 3 patients, additional specialized immunohistochemistry was carried out, which showed a marked decrease in myenteric neurons in both patients as delineated by an antibody to Protein Gene Product (PGP) 9.5 (Figure 4A) and a severe decrease in Kit (CD 117) positive ICC across the muscle layers (Figure 4B). Immunohistochemical stain for CD3 showed no increase in T-cells, either in the myenteric plexus or smooth muscle. Smooth muscle actin was normal. Trichrome stain showed no muscle fibrosis. Mucosa and submucosa tissue samples showed submucosal fibrosis of unclear clinical significance in 1 patient.

In all 3 patients, upper abdominal pain with pleuritic component was experienced post-procedure which was controlled with nonsteroidal anti-inflammatory agents and opioid analgesics. No fever or leukocytosis was noted. Given this represented our initial experience, CT abdomen and chest CT angiography 48 hours after the procedure was performed in 1 patient with left upper quadrant abdominal pain. The studies were negative for intra-abdominal pathology and pulmonary embolism. Repeat upper endoscopy in this same patient showed ulceration and retained clip at the biopsy site. Patients were contacted at 48 hours and 1 week after the procedure. A visual analog scale was used for pain assessment, and patients reported moderate abdominal pain at 48 hours and mild pain at 1 week.

Repeat upper endoscopy and CT abdomen were performed at 1 month per study protocol. All patients reported baseline symptoms at 1 month. Endoscopy showed a well-healed scar in 2 patients and minimal residual ulceration with a retained clip in 1 patient (Figures 5A and 5B). A CT abdomen examination showed negative results in all patients.

DISCUSSION

The etiology of several GINMD, including functional GI disorders, remains largely unknown[7,8,9,10]. Surgically obtained gastric muscle biopsy specimens from patients with gastroparesis have shown a decrease in ICC in the majority of patients, changes in macrophages, and a variable decrease in nerve fibers[11]. We have previously reported on different experimental endoscopic techniques for deep gastric wall biopsies to include the entire MP[12,13]. Our group introduced the “no hole” concept (close, then cut) to eliminate the risk of peritonitis from leaked gastric contents[14]. However, the techniques were limited by lack of adequate sample size and/or safety issues. Percutaneous endoscopically assisted transenteric full-thickness biopsy using a 14-gauge needle for assessing histopathological abnormalities in GINMD showed abnormalities in 44% of patients[15]. Recently an EUS-guided fine-needle aspiration of the gastric antrum MP using a 19 gauge core-needle was

shown to be safe with adequate tissue for histologic assessment of myenteric plexus in 54.5% and ICC in 81% of patients[16,17]. The limitations of these 2 techniques are the small sample volume of MP and the fragmented samples that can impair proper tissue orientation and impact diagnostic yield. The use of confocal laser endomicroscopy for optical histologic imaging is promising, but depth of imaging may not be sufficient in the stomach and the applicability of dye-based methods in humans is unclear[18,19,20,21].

The selection of a star-shaped, over-the-scope clip was intentional because when deployed, the flat clip configuration facilitates easy snare placement without entrapment of the clip prongs (Figure 6). Other preferences for the selection of this closure device included a simple pushbutton deployment, a clip diameter of 11 mm allowing for atraumatic peroral intubation, and a cap depth of 10 mm allowing for substantial tissue capture. The trigger wire is located alongside the endoscope shaft, thus freeing the working channel for use of additional accessories and suction of luminal contents. The double resection technique is fundamental for deep gastric wall biopsies due to the relative thickness of the gastric wall. Exposing the underlying MP after EMR ensures the entire muscle layer is captured in the subsequent resection. All devices used are commercially available in the United States.

Large tissue samples of the entire MP, closer to the criterion standard of surgically acquired specimens (≥10 mm), with myenteric ganglia and ICC were obtained in all patients. Harberson et al reported on laparoscopic full-thickness gastric antral biopsy specimens obtained from the anterior wall of the stomach in patients with gastroparesis[2]. The range in size of tissue samples were from 0.3 × 0.3 × 0.3 cm to 1.5 × 1.0 × 1.0 cm. The postprocedure abdominal pain may be explained by thermal effect on the serosa causing inflammation, edema and pain akin to postpolypectomy syndrome. Abnormalities with reduction of myenteric plexus and loss of ICC were noted, which are considered hallmark features of gastroparesis[22]. Due to the lack of an immune infiltrate in the resected specimens, patients were not considered suitable candidates for immunosuppressive or steroid therapy.

Based on our initial experience, the minimally invasive gEMB approach appears to be safe, effective, and easy to perform. This innovative gEMB method using a double resection clip-assist technique represents a paradigm shift in endoscopic tissue diagnosis. Our ability to acquire large tissue samples allows for both quantitative and qualitative analysis of multiple cell types. Future research may contribute to our knowledge of the pathophysiology of GINMD and other inflammatory and neoplastic conditions, leading to potential targeted and curative therapies and likely challenging current clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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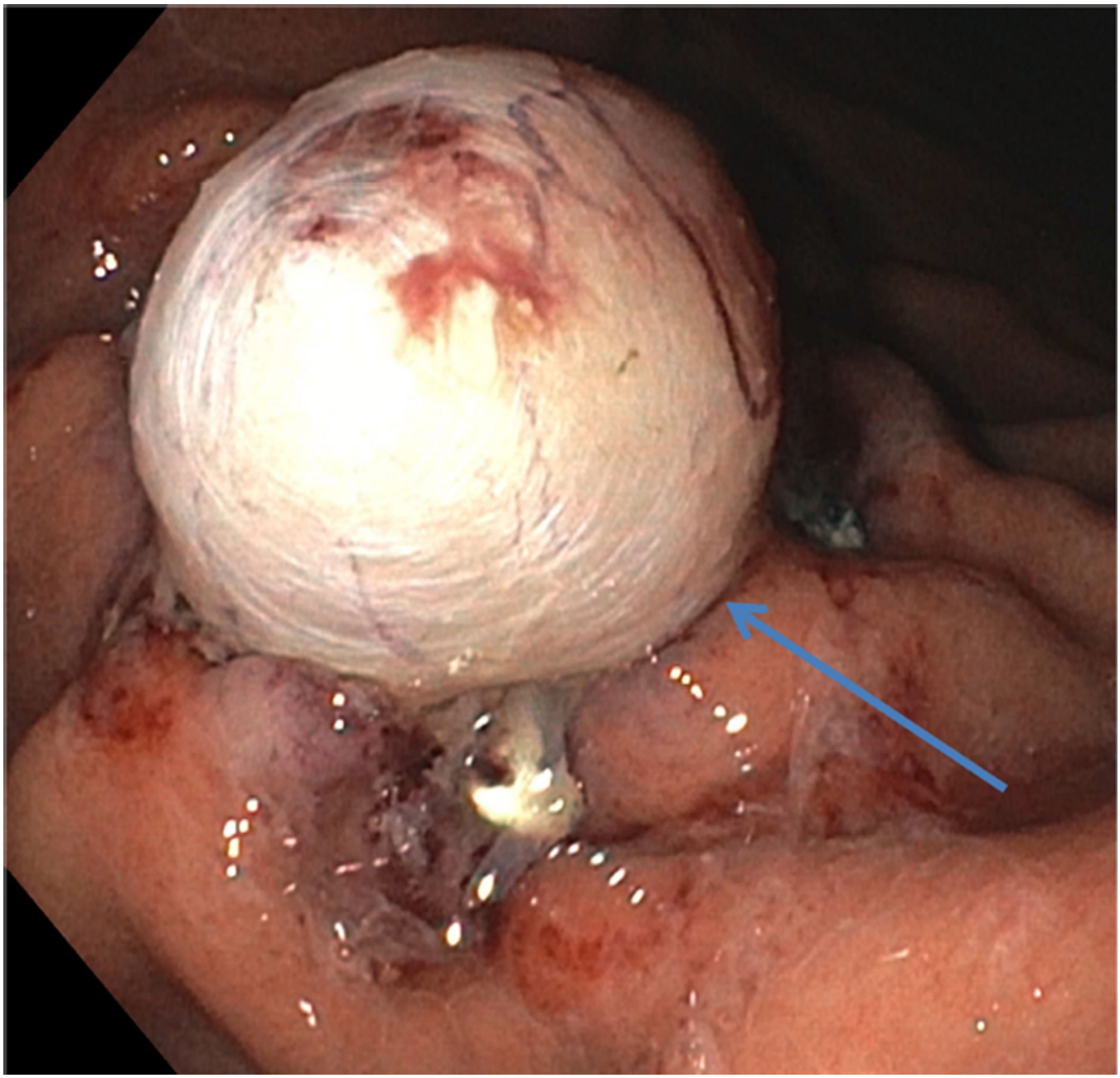
ACRONYMS

GINMD	Gastrointestinal neuromuscular disease
MP	Muscularis propria
ICC	Interstitial cells of Cajal
gEMB	Gastric endoscopic muscle biopsy
H&E	Hematoxylin and Eosin
PGP	Protein Gene Product
CM	Circular muscle
LM	Longitudinal muscle
SM	Submucosa
MYP	Myenteric plexus



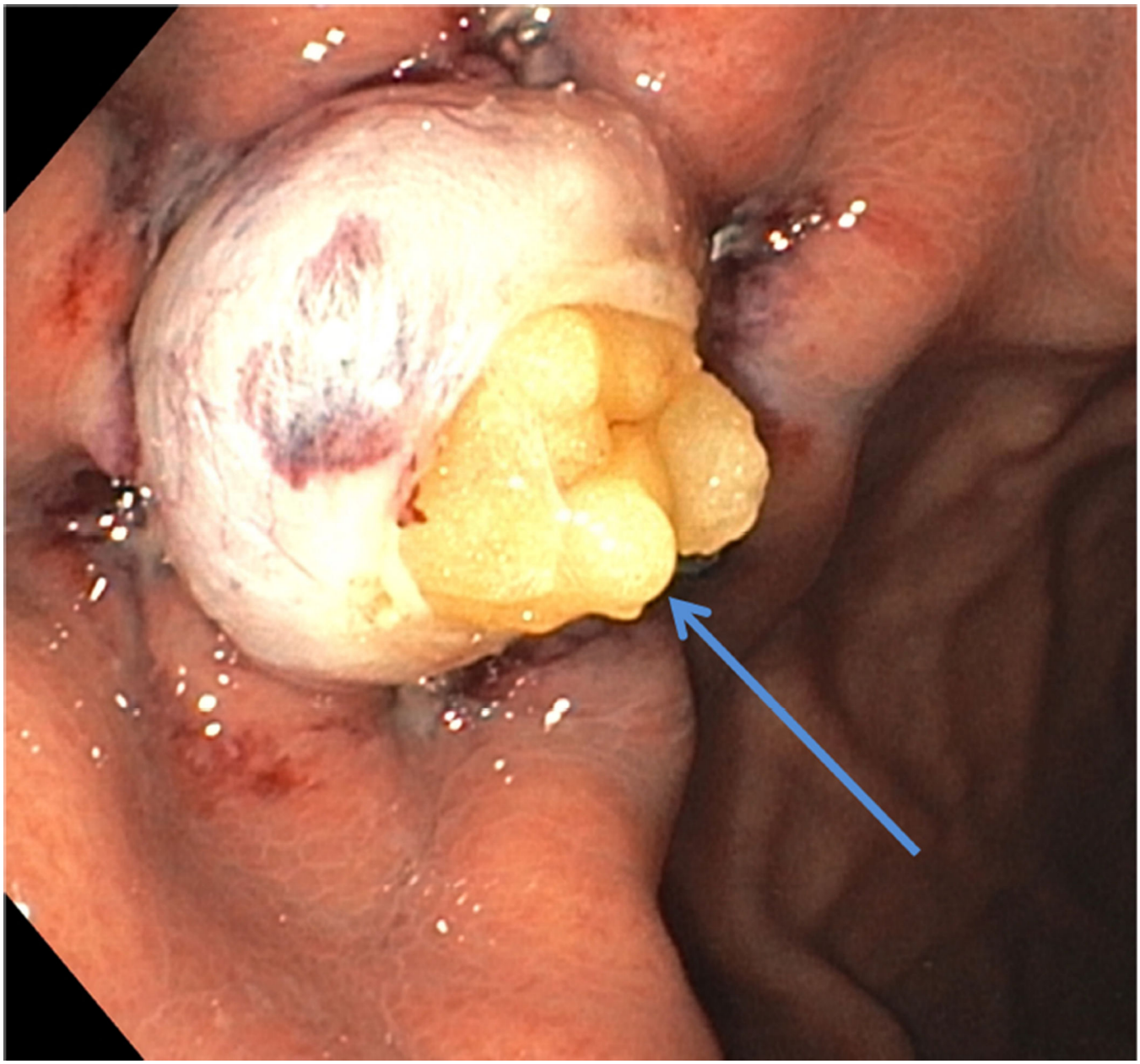


FIGURE 1.

A Pseudopolyp of muscularis propria (arrow) created after clip deployment

B After resection of muscularis propria with exposed omentum *(arrow)*

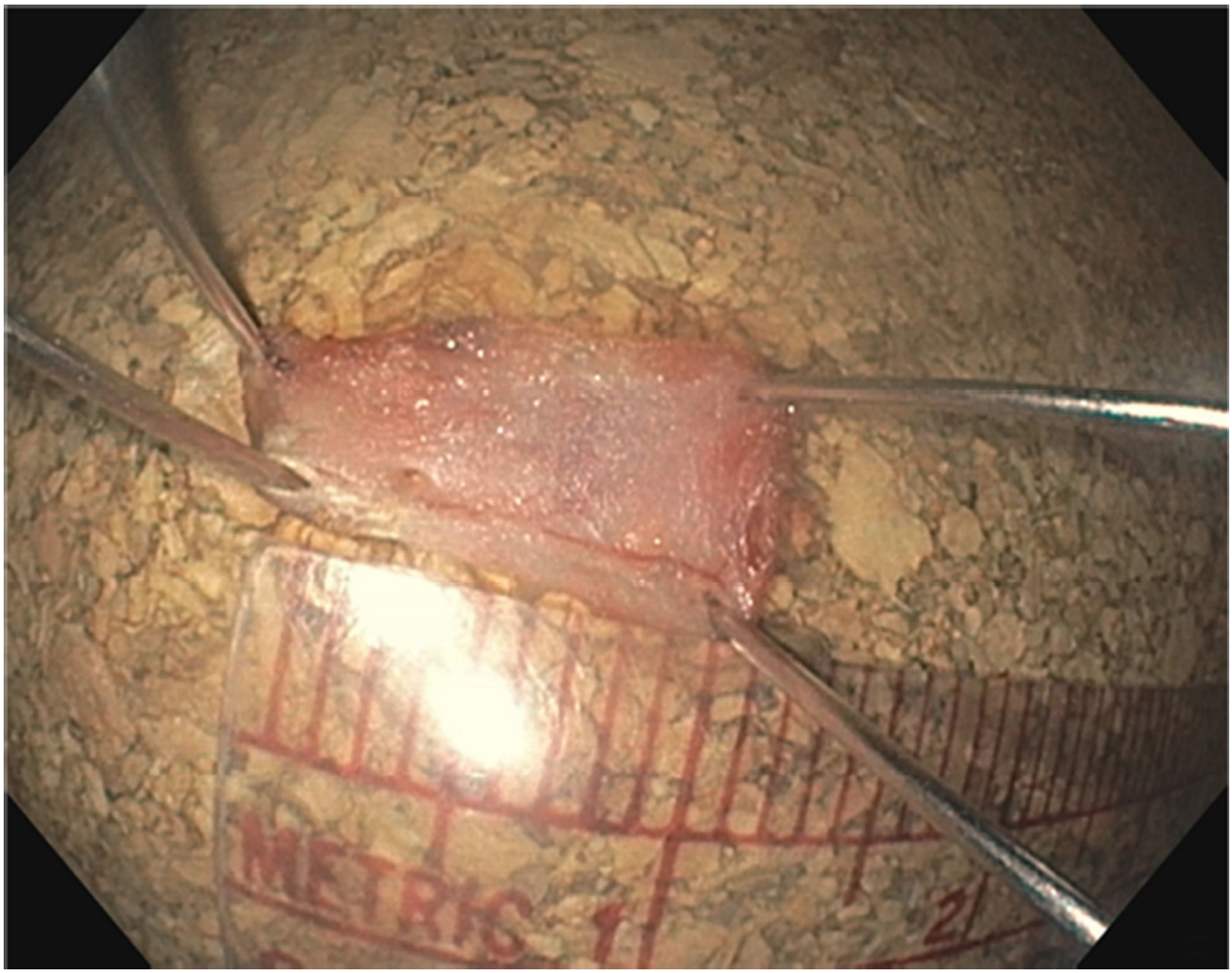


FIGURE 2.
Resected muscularis propria specimen

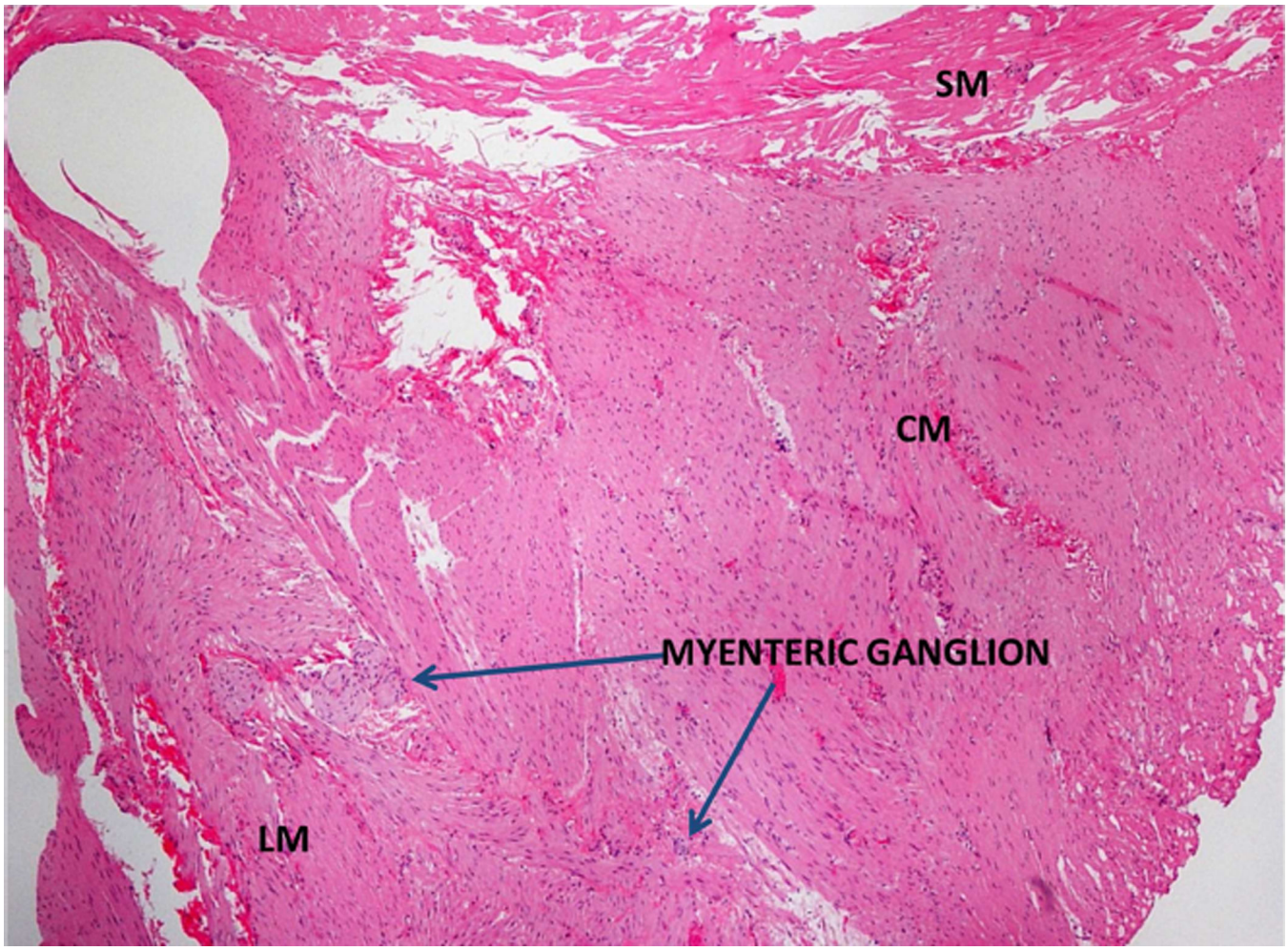
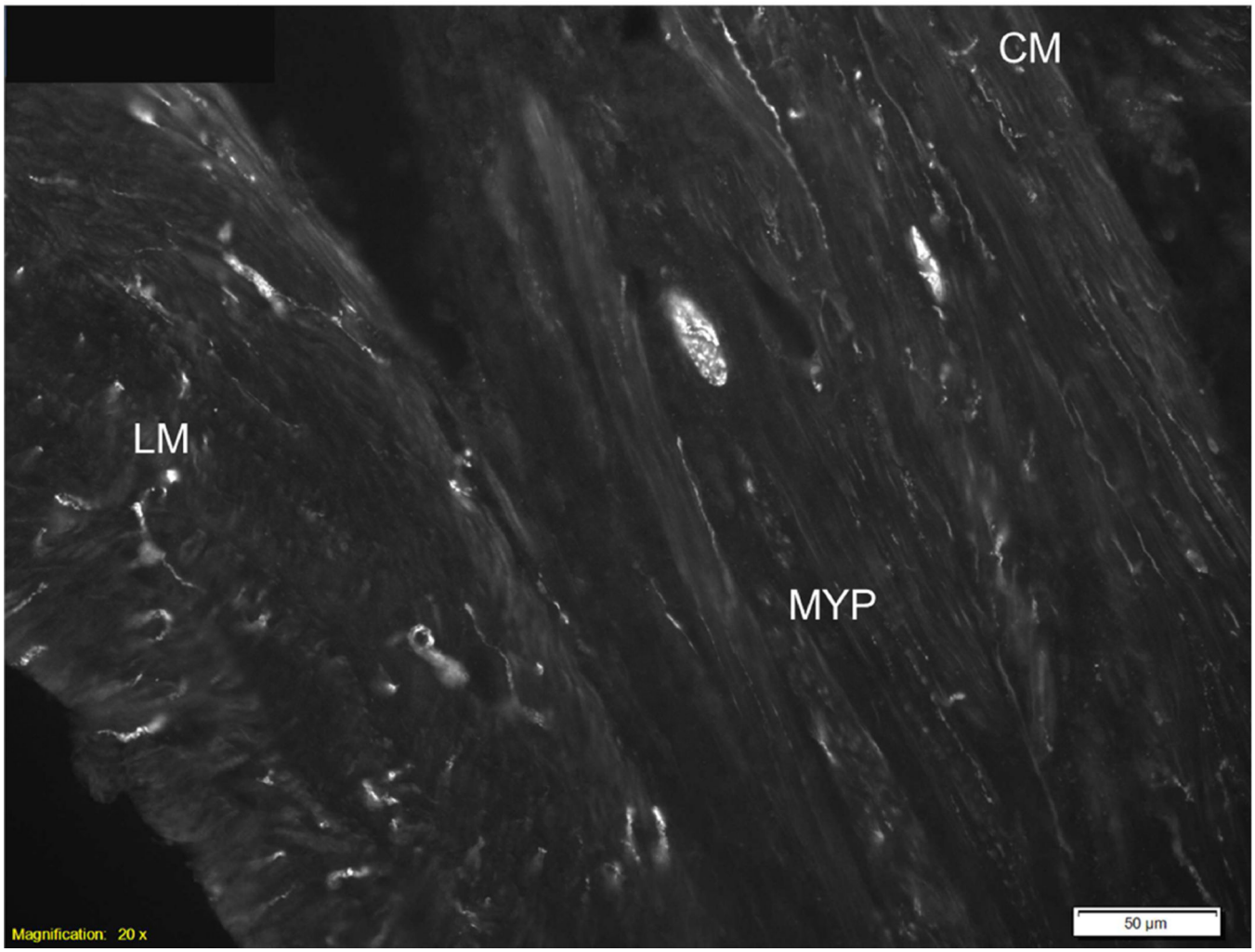


FIGURE 3.

Hematoxylin and eosin (H&E) staining of the muscularis propria and myenteric ganglia. Tissue shows the presence of the circular muscle (CM), myenteric ganglia, longitudinal muscle (LM) and submucosa (SM). Scale bar = 40 μm



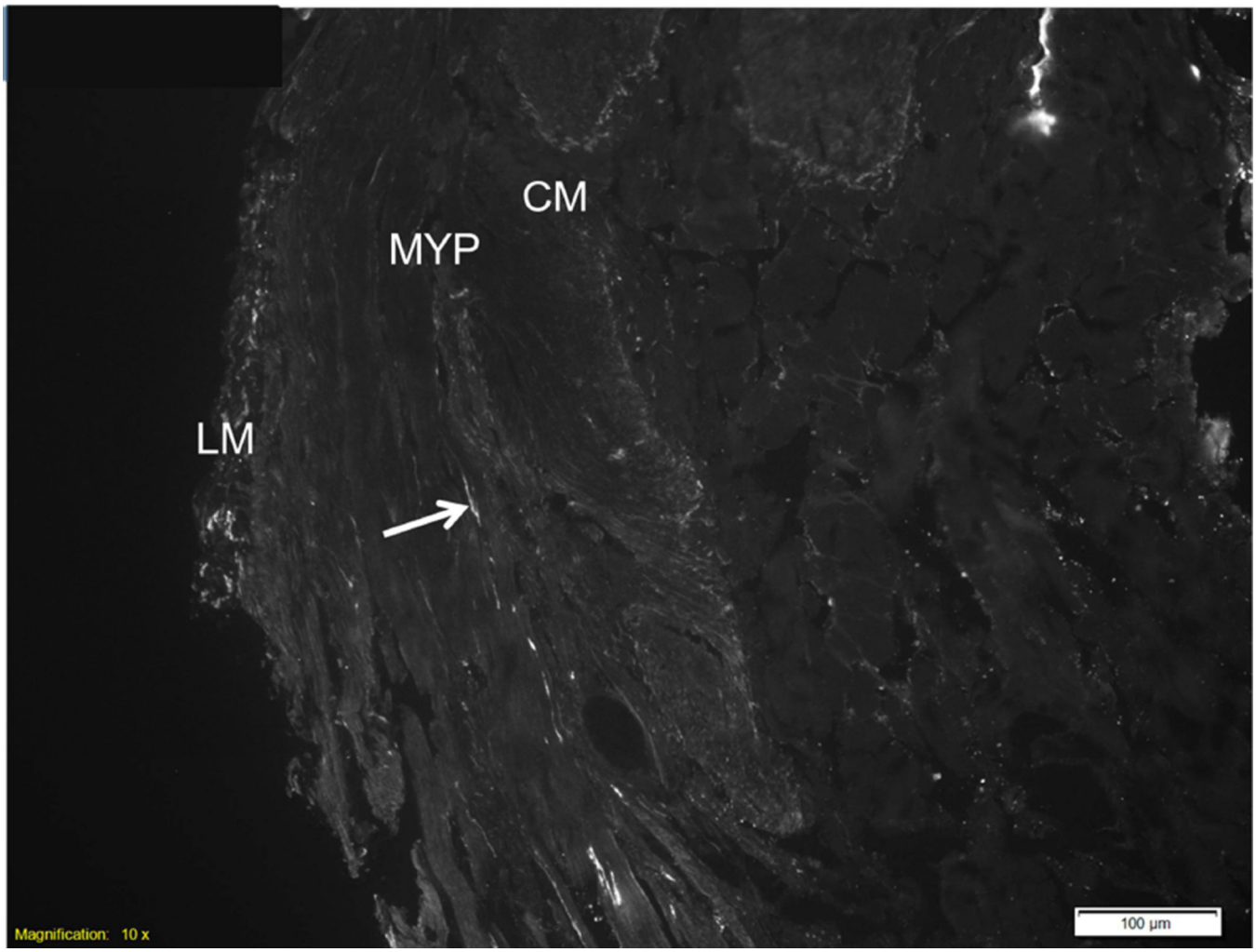
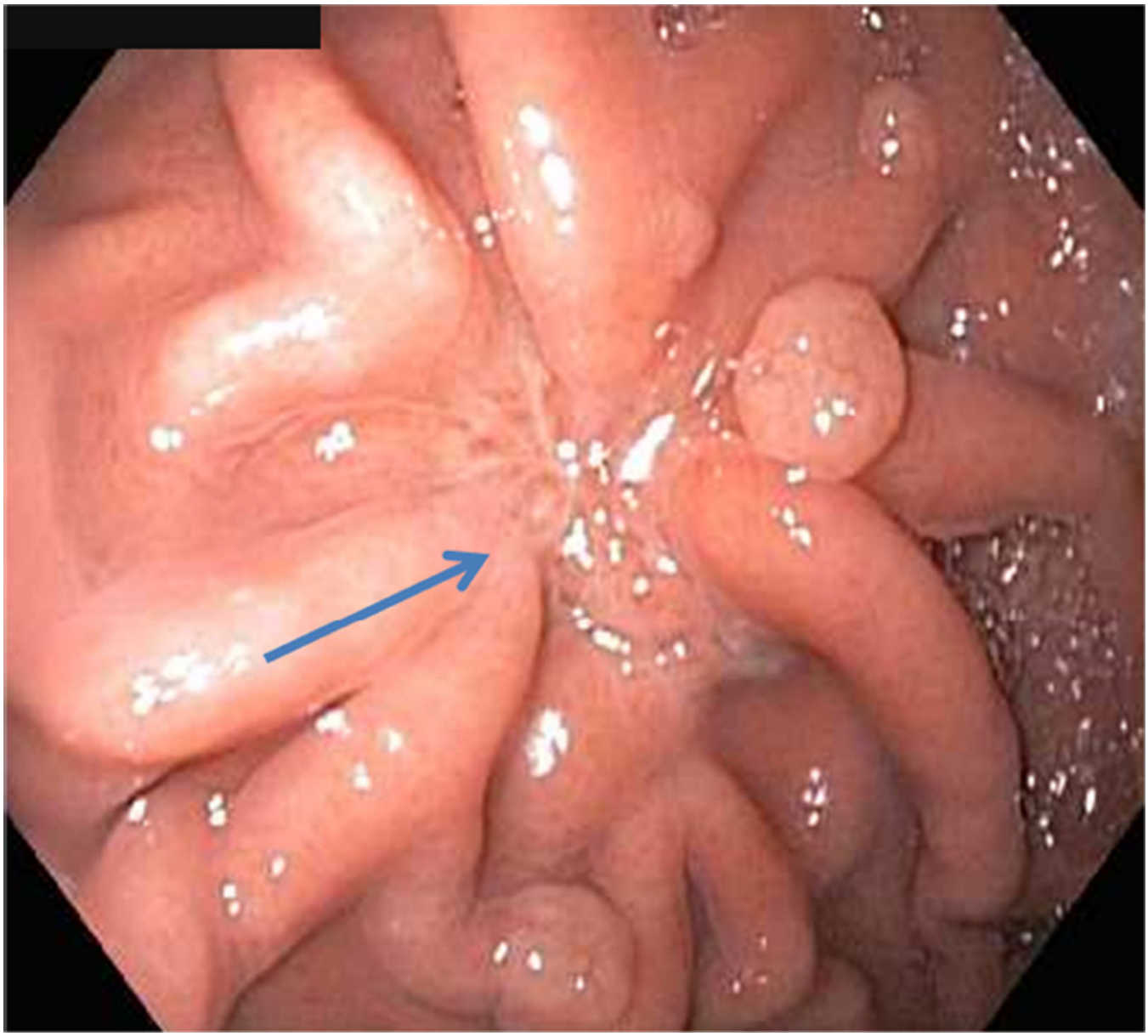


FIGURE 4.

A Myenteric region showing marked decreased in Protein Gene Product 9.5 (PGP 9.5) positive neurons. CM = circular muscle; LM = longitudinal muscle; MYP = myenteric plexus. Scale bar = 50 µm.

B Intermuscular layer of the muscularis propria showing marked decrease in Kit (CD 117) positive interstitial cells of Cajal (ICC). CM = circular muscle; LM = longitudinal muscle; MYP = myenteric plexus. Scale bar = 100 µm.



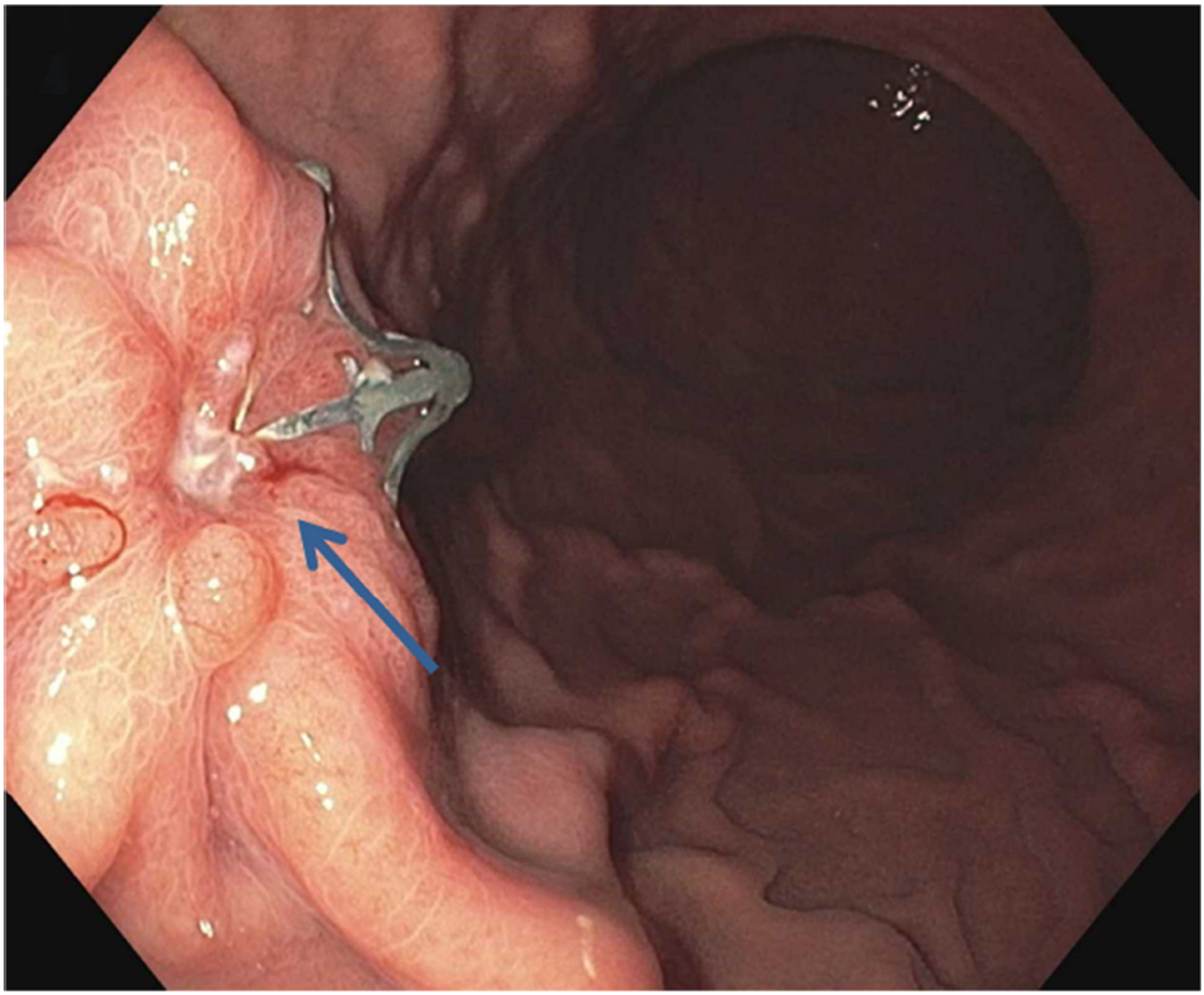


FIGURE 5.

A Endoscopy at 1 month showing well-healed scar *(arrow)*

B Endoscopy at 1 month showing ulceration and retained clip *(arrow)*

FIGURE 6.
Star-shaped over-the-scope clip