Frozen Section of the Gastrointestinal Tract, Appendix, and Peritoneum

Mamoun Younes, MD

- **Context.**—Intraoperative consultation is frequently requested by surgeons operating on the gastrointestinal tract, appendix, and peritoneum. In this setting, the pathologist's diagnosis plays a central role in determining whether a resection is needed, and if so, how much to resect and whether it was adequate. There is no room for errors in the frozen section laboratory, because a small mistake can have serious consequences. To my knowledge, no recent books or publications in the literature have dealt with this important topic.

- **Objective.**—To review the intraoperative consultation of the gastrointestinal tract, appendix, and peritoneum.

- **Data Sources.**—The MEDLINE database was queried for keywords, including gastrointestinal, esophagus, stomach, esophageal, gastric, small intestine, and all other names of the gastrointestinal tract, peritoneum, and appendix in combination with frozen section. All suitable articles were retrieved and reviewed. This literature search and my personal experience formed the basis of this review.

- **Conclusions.**—The role, value, and limitations of frozen section and gross consultation are different for different areas of the gastrointestinal tract, even for the same types of lesions. Close interaction with the surgeon and knowing what is intended from the frozen section, what will be done following a certain diagnosis, and what is the minimal information needed from the pathologist at the time of frozen section are essential for proper patient management.

With few exceptions, the main indications for intraoperative consultation in the evaluation of gastrointestinal tract specimens are to evaluate the adequacy of resection (margin check), diagnosis (what is it?), and to evaluate the extent of disease (such as tumor spread). In many instances, all or some of the surgeon's questions can be answered by gross examination, which when done appropriately can save time and expense without compromising patient care.

**ESOPHAGUS**

Almost all esophagectomy or esophagogastrectomy specimens are performed for squamous cell carcinoma, adenocarcinoma of the esophagus, or adenocarcinoma of the esophagogastric junction. In many cases, the gastric portion is generous and the gastric margin is far enough from the tumor that gross evaluation of the gastric margin is adequate (Figure 1). It has been suggested that for a microscopically negative gastric margin, at least 5 cm of macroscopically normal gastric mucosa from the distal tumor margin is needed. The esophageal portion of the specimen is rarely as generous, and the proximal margin is often within 2 cm from the proximal edge of the tumor. The proximal margin should be submitted for frozen section evaluation, even if normal-appearing squamous mucosa is identified grossly (Figure 1). It is not uncommon to see carcinoma extending underneath an otherwise unremarkable mucosa. In resections for squamous cell carcinoma, at least 3 cm of grossly negative esophageal margin from the proximal end of the tumor is required to ensure a microscopically negative margin. However, squamous cell carcinoma can be multifocal (Figure 2), and therefore gross evaluation of the proximal margin may not be adequate. In resections for adenocarcinoma, the presence of Barrett metaplasia with typical goblet cells on frozen section of the proximal margin (Figure 3) should be reported to the surgeon, even if no dysplasia is seen. Whenever possible, the proximal margin should consist of benign squamous mucosa. Although deep (radial or circumferential) margin involvement has been reported to be associated with worse prognosis by some investigators, evaluation of this margin by frozen section is not necessary at the present time, since the surgeon is unlikely to do anything different.

Frozen section is rarely performed for the diagnosis of esophageal epithelial tumors, since diagnosis is made on preoperative biopsy. Frozen section is sometimes requested on nonepithelial tumors to determine their type and whether the tumor is benign or malignant. Unlike in the stomach and intestine, typical leiomyomas are the most common mesenchymal tumors of the esophagus, and therefore such diagnosis can be made on frozen section. However, it should be noted that gastrointestinal stromal tumors (GISTs) do occur in the esophagus and should be taken into consideration before making a diagnosis of leiomyoma. Leiomyosarcomas are usually large, high-grade tumors and should be recognizable as malignant on frozen section.
Preoperative radiation therapy is being used increasingly in the treatment of esophageal carcinoma. In the absence of clinical information, radiation atypia at the resection margin could be mistaken for high-grade dysplasia or carcinoma in situ, leading to unnecessary additional resections. The pathologist should ask whether the patient was subjected to such treatment, prior to declaring that dysplasia is present at the margin.

**STOMACH**

Unlike the esophagus, frozen section is requested on most gastrectomy specimens for diagnosis, as well as for evaluation of margins. Frozen section is usually performed to evaluate the resection margin in partial or total gastrectomy specimens for gastric cancer. Margin evaluation by frozen section is also performed on resections for GISTs, which are increasingly removed by laparoscopy with a limited margin of normal tissue.

In addition to adequacy of tumor resection margins, evaluation of surgical margins is performed to ensure completeness of antrectomy. Antrectomy is performed as treatment for hypergastrinemia-associated gastric carcinoids, and both chronic atrophic gastritis and endocrine cell (G-cell) hyperplasia are usually present. These carcinoids tend to be small and multiple (Figure 4), and they usually regress following antrectomy. Therefore, the presence of carcinoid at the resection (antrectomy) margin should not necessarily lead to re-excision of the proximal margin. In these cases, the distal margin should consist of small intestinal (duodenal) mucosa. The proximal margin should consist ideally of oxyntic gastric mucosa; however, since this is often atrophic, the adequate proximal margin should contain no identifiable G cells on hematoxylin-eosin stain. This finding should be confirmed later on permanent sections with immunostaining for gastrin.

Frozen section is performed for the diagnosis of gastric cancer if such diagnosis was suspected on clinical grounds, but biopsy results were negative. Signet ring cell carcinoma and margins of resection for this cancer can be difficult to evaluate on hematoxylin-eosin stain. Rapid immunostaining for cytokeratin and rapid mucin stains have been proposed as an aid to frozen section in difficult cases. Additional prospective studies are needed to assess the practicality of these tests and whether their routine use actually improves diagnosis in a significant number of cases.

Frozen section is also done for gastric ulcers suggestive of malignancy, perforation, or high-grade dysplasia/intra-mucosal carcinoma complicating atrophic gastritis with intestinal metaplasia. Metastatic carcinoma can involve the stomach and may not be identified as such if appropriate clinical history is not available at the time of frozen section. Large cell lymphoma can be accurately diagnosed on frozen section with the aid of cytologic imprints, but can be extremely difficult at times. Large cell lymphomas may be associated with gastric ulcer, just like carcinoma, and treatment of this lymphoma with radiation (especially when transmural) may lead to perforation. Mesenchymal tumors are better diagnosed as GISTs on frozen section, regardless of how benign-appearing they may be and regardless of their size. When dealing with resected specimens for such lesions, the resection margin should be evaluated and the surrounding mucosa should be inspected for other lesions. In our experience, several partial gastrectomies for GIST also grossly had atrophic gastritis, and in 1 case this was positive for high-grade epithelial dysplasia (Figure 5). Frozen section is also performed for evaluation of gastric ulcers (benign vs malignant), some of which may be multiple or perforated. For clinically benign gastric ulcers, which did not respond to conservative treatment, frozen section is performed to identify nerve and ganglion cells in vagotomy tissue and to ensure completeness of antrectomy.

In cases of gastrectomy for extensive atrophy and intestinal metaplasia, in which dysplasia but no carcinoma was identified on preoperative biopsy, it may not be possible to make a diagnosis of carcinoma on frozen section owing to the known and unavoidable sampling error. When no mass lesion can be identified by palpation, frozen section should focus on the margins in these cases.

When faced with a poorly differentiated neoplasm that cannot be further characterized on frozen section, the pathologist should communicate this opinion to the surgeon, adding that the final diagnosis is deferred to permanent sections and special studies if needed. Inflammatory fibroid polyps are rarely seen and can be distinguished from GIST on frozen section by the abundance of eosinophils in the former. Ectopic gastric mucosa may show large, cystically dilated glands filled with mucinous or amorphous material and may be surrounded by muciphages that could be confused with signet ring cells (Figure 6, A). Additional sampling will show small, benign-appearing gastric glands in the wall of some of these cysts (Figure 6, B).

**SMALL INTESTINE**

Margins are evaluated for resections carried out for tumors, such as carcinomas or GISTs, and sometimes benign lesions, such as inflammatory bowel disease (IBD) and ischemia. Because primary carcinomas of the small bowel are uncommon, there are no conclusive studies regarding the length of resection margin considered adequate by gross examination in these cases. In cases of IBD, resections are most often performed because of complications, whether benign or malignant. The presence of microscopic disease at the margin of resection for Crohn disease is not considered to be of clinical significance, and therefore these margins should not be frozen. In some cases, a diagnosis of ulcerative colitis or Crohn disease is established for the first time by frozen section during surgery. The possibility of adenocarcinoma complicating IBD should be always kept in mind, and margins grossly involved by IBD may be considered for frozen section to rule out carcinoma or high-grade dysplasia when segmental resection is done.

Most frozen sections for resected small intestine are requested for the diagnosis of tumors. Primary tumors of the small intestines include adenocarcinoma, lymphoma, neuroendocrine tumors (mainly carcinoids), and GISTs. Many of the carcinomas evaluated by frozen section are metastatic. Sites of origin include the pancreas, ovary, endometrium, bladder, colon, and gallbladder, among others. Not all polyloid tumors are epithelial or GISTs. Polyloid melanomas can be seen in the gastrointestinal tract, including the small intestines (Figure 7), and may grossly resemble carcinoma. Although rare, diverticulitis with perforation can occur in the small intestine. The resulting fibrosis may be confused with a mesenchymal tumor (Figure 8).

Several cases of benign ulcers and inflammation, mostly
due to fistula or scarring from Crohn disease, are evaluated by frozen section for clinical suspicion of malignancy. Marked uniform infiltration by small lymphocytes can be grossly (Figure 9, A) and microscopically (Figure 9, B) suspected for lymphoma. Heterotopic pancreas, which is relatively uncommon, may be symptomatic, causing ulceration and obstruction in the stomach and duodenum, but is generally asymptomatic when present in the jejunum.14,15 Because of its yellow lobulated appearance, it can be grossly mistaken for submucosal lipoma; however, this misdiagnosis does not impact patient management.

Adenomyoma may be confused with invasive or metastatic adenocarcinoma. However, the glands in adenomyoma are of variable size and shape, lack a desmoplastic stromal reaction, and are lined by uniform epithelial cells with benign nuclei (Figure 10, A and B). Endometriosis, which is not infrequent, can be differentiated from adenomyoma and carcinoma by the endometrial stroma associated with the glands in the former.

Biopsies of the small intestinal serosa or serosal nodules are often submitted for frozen section analysis to rule out metastatic carcinoma, for diagnosis of an unsuspected lesion encountered during surgery for other reasons, or for staging laparotomy. Although some of these biopsies turn out to be malignant, many are benign and show only fibrosis, fat necrosis, or adhesions.

**APPENDIX**

Primary adenocarcinomas of the appendix are rare. The presenting symptoms are usually those of acute appendicitis. A diagnosis of appendiceal adenocarcinoma is rarely made preoperatively, and it has been recommended that any appendix that does not show a typical inflamed appearance be submitted for frozen section diagnosis.34-38 Therefore, it is not surprising that many of the appendices submitted for frozen section turn out to be benign with a variable degree of inflammation (Figure 11, A and B). A diagnosis of primary adenocarcinoma of the appendix on frozen section leads to a right hemicolectomy, even when the appendiceal margin is negative. The margin is important to evaluate when appendiceal carcinoids and adenomas are present. Other benign lesions encountered at frozen section include endometriosis, diverticulitis, mucinous cystadenoma, and mucosal hyperplasia.

**COLON**

In many instances, the margins of colectomy specimens can be evaluated grossly. In resections for primary adenocarcinoma of the colon, the distal resection margin should be at least 2.5 cm from the distal edge of the tumor to be considered negative by gross examination.19,20 When a preoperative diagnosis is made on the colonic tumor, there is no need to perform a frozen section on the tumor itself, except for the evaluation of the deep margin if it is suspected to be involved grossly. In this case, the deep margin should be inked prior to sectioning and examination by frozen section. In most cases, gross identification of the tumor is sufficient. For example, “a fungating tumor measuring 3 cm in greatest dimension is identified” is an adequate diagnosis in the majority of cases.

Frozen section is performed for the diagnosis of colon tumors occasionally, and when it is done for margin evaluation, it is usually done on the closest margin or when the distal margin is less than 2.5 cm from the tumor. It is also done when metastatic carcinoma is suspected, which can be present as serosal implants or may be intramural involving all layers of the colonic wall. In some cases, it is not possible to determine the site of primary cancer when multiple organs are involved on frozen section. Such diagnosis should be deferred to permanent sections and special studies, such as immunohistochemistry.

Frozen section is often requested also when a tumor is clinically suspected to be an adenocarcinoma, but the preoperative biopsy reveals only an adenoma. In these cases, there is a great potential for sampling error. In our experience, many cases diagnosed as adenoma on frozen section turn out to have at least adenocarcinoma invasive into submucosa. We have seen 2 cases in which a tumor diagnosed as adenoma on frozen section showed adenocarcinoma invasive to the serosa on permanent sections after the entire lesion was submitted. Perhaps, in these cases, submitting a single section of the tumor for frozen section may not be adequate. When frozen section shows only adenoma, the potential of sampling error should be indicated in the frozen section report.

Endoscopic polypectomy is now an acceptable procedure for treatment of larger polyps. In these cases, when adenocarcinoma is found on histologic examination of sections of routinely processed polypectomy specimens, colectomy is usually performed whenever this routine examination shows incomplete excision, poorly differentiated carcinoma, lymphatic invasion, positive polypectomy margin, or when the level of carcinoma invasion within the polyp cannot be determined.21-24 The resulting colectomy specimen is submitted for intraoperative consultation, and the pathologist is asked to identify the polypectomy site. When removing larger polyps endoscopically, some gastroenterologists inject the polypectomy site with India ink. The uptake of this ink by macrophages leads to “tattooing” of the serosal aspect at the site of the polyp...

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**Figure 1.** Ulcerating adenocarcinoma at the distal esophagus. The distal (gastric) margin is far from the tumor, is unremarkable by visual inspection and palpation, and could be safely determined to be negative for malignancy. The short esophageal margin, showing a pearly white normal-appearing mucosa, should be submitted in its entirety for frozen section evaluation.

**Figure 2.** Multifocal esophageal squamous cell carcinoma.

**Figure 3.** Barrett metaplasia without dysplasia at the proximal resection margin for esophageal adenocarcinoma. This is not considered a satisfactory proximal margin (hematoxylin-eosin, original magnification ×40).

**Figure 4.** Multiple small carcinoids in a stomach with atrophy.

**Figure 5.** High-grade epithelial dysplasia is present in a partial gastrectomy specimen for gastrointestinal stromal tumor (hematoxylin-eosin, original magnification ×40).

**Figure 6.** Ectopic oxyntic mucosa in the stomach. Cystic areas can be bordered by macrophages (A), but additional sampling reveals residual glands (B) (hematoxylin-eosin, original magnification ×40).
Figure 7. Polypoid melanoma in small intestine. These lesions are grossly indistinguishable from adenocarcinoma.

Figure 8. Fibrosis resulting from a perforated small intestinal diverticulitis that became walled off (hematoxylin-eosin, original magnification ×10).

Figure 9. Crohn disease, with focal transmural inflammation. A, A firm tan area is seen extending from the mucosa into the adipose tissue. B, Microscopic examination showed sheets of small lymphocytes (hematoxylin-eosin, original magnification ×20). This was not a lymphoma.

Figure 10. A, Adenomyoma of small intestine. The glands are intimately associated with hypertrophic muscle fibers, have different shapes and sizes, and lack a desmoplastic stromal response (hematoxylin-eosin, original magnification ×2). B, At higher magnification, the glands are lined by epithelial cells with benign nuclear features (hematoxylin-eosin, original magnification ×40).

ectomy, which makes it easier for the surgeon to identify the segment of colon to be resected. Some gastroenterologists do not tattoo the polypectomy site, either because they did not get into the habit of doing it or because they did not suspect the polyp to be malignant. In these cases, the surgeon may not get the correct segment resected the first time. The pathologist should examine the serosal surface for tattoos and report the finding, as well as examine
Diverticulitis (especially with rupture or perforation) and other inflammatory conditions (including IBD, especially Crohn disease) may produce stenosis and mass lesions that may clinically and radiographically resemble carcinoma. Conversely, sometimes an ulcerating invasive carcinoma may be associated with extensive inflammation and fibrosis in the pericolonic adipose tissue, resembling an inflammatory condition. A frozen section may be necessary to determine whether such a lesion is benign or malignant. As in the small intestine and stomach, large cell lymphoma may be seen and correctly diagnosed on frozen section with the assistance of cytologic imprints. Low-grade lymphomas should not be diagnosed on frozen section. Rather, these should be deferred to permanent sections. Regional lymph nodes and liver biopsies are sometimes submitted for frozen section to determine the spread of carcinoma.

Rarely, intestinal tuberculosis may cause mass lesion and intestinal obstruction. If a history of tuberculosis is available, touch imprints may be used for the diagnosis to avoid contaminating the equipment and unnecessarily increasing the potential of exposure to the organisms. 25

Colonic endometriosis may be seen as serosal implants or may involve variable portions of the bowel wall, extending all the way to the mucosa in some cases. When involving the muscularis propria, it can be associated with fibrosis or muscle hypertrophy, giving a mass effect. It is distinguishable from adenocarcinoma by the presence of endometrial stroma. Ischemic bowel disease may produce strictures, masses, edema, or multiple ulcers. Although many of these conditions can be accurately diagnosed on frozen section as ischemic, a descriptive diagnosis with “negative for dysplasia or malignancy” is sufficient.

Submucosal lipoma may present as a polypoid mass (Figure 12); however, cut section should allow for gross diagnosis. Resections for IBD should be carefully inspected for carcinoma or dysplasia. Serosal biopsies are submitted for frozen section usually to exclude metastatic carcinoma or implants from intra-abdominal malignancy. These are often benign, revealing scarring or fat necrosis.

**RECTUM**

As in the colon, frozen sectioning for the diagnosis of rectal cancer is rarely needed. Instead, most frozen sections are performed to evaluate the resection margin. The distal margin in resections for rectal cancer is usually shorter than that in colectomies, and therefore the distal margin is almost always submitted for frozen section. The deep (radial) margin is also submitted when involvement is suspected by gross inspection. Occasionally, a biopsy of rectal lesion is submitted to frozen section if preoperative biopsy is not diagnostic, especially when doing colectomy for right-sided cancer and a decision of whether to perform a total colectomy needs to be made in the presence of a synchronous rectal mass.

Frozen section is also done on rectal polyps. A shaved polypectomy margin sometimes reveals adenoma or even carcinoma on frozen section, leading to surgical resection. Carcinoids, endometriosis, diverticulitis, and IBD are also encountered on frozen section. We encountered 1 case that was submitted for frozen section as a “submucosal mass,” but frozen section showed hemorrhoids with a thrombosed vessel. Rectal prolapse can be identified by distortion of the glands, fibrosis, and granulation tissue. As in

Figure 11. A, In many cases, appendicitis may look grossly like a mass. B, Even after sectioning, fibrosis in the appendiceal wall may look like an infiltrative malignancy. (A is a photograph of fresh specimen, and B is a section of the same specimen after formalin fixation.)

Figure 12. Polypoid submucosal lipoma in colon.

the mucosal surface for ulcers or residual tumor. If needed, a suspected polypectomy site may be submitted for frozen section to confirm the presence of residual adenoma or carcinoma, or simply granulation tissue. However, caution should be taken since ulcers unrelated to biopsy sites may coexist in the same colon.
the colon, the pathologist may be asked to identify previous biopsy or polypectomy site.

Because many rectal cancers are successfully treated with preoperative radiotherapy, it is not surprising that some resected specimens sent for intraoperative consultation with a diagnosis of rectal cancer show no mass lesions when opened in the frozen section room. If the surgeon did not provide this information, the pathologist should ask about the patient’s history of radiation. It is important to let the specimen fix well before taking sections for routine histopathologic examination. In many cases, no tumor is found even after thorough microscopic examination.

**PERITONEUM**

Frozen sections are usually performed for the diagnosis of metastatic carcinoma or implants during surgery for something else. Metastatic carcinomas to the peritoneum include adenocarcinomas from ovary, pancreas, and colon; papillary serous carcinomas from ovary; urothelial carcinoma; and squamous cell carcinoma. Many mimickers of tumor implants, including fat necrosis, benign cysts, dystrophic calcifications, fibrosis, fibrous adhesions, hyalinized nodule, acute and chronic inflammation, and foreign body granulomas, are seen on frozen section. Leiomyoma, primary serous tumor of low malignant potential (whether primary peritoneal or implants from ovarian primary), high-grade sarcoma, and primary mesothelioma are rarely seen.

Special thanks go to Juan Lechago, MD, PhD (Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, Calif) for his review and valuable comments.

**References**