Practical Issues for Frozen Section Diagnosis in Gastrointestinal and Liver Diseases

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Abstract

Surgeons frequently utilize frozen sections as a tool to gain information intraoperatively. The use of frozen sections has certain benefits as well as limitations which vary according to the situation. After reviewing the available literature we provide the reader with an idea of when and how to use frozen sections in common situations involving the gastrointestinal tract and liver as well as address the benefits and limitations in each setting.

Key words


Introduction

Frozen sections are used to gain real time information intraoperatively about surgical specimens. Commonly, this includes gaining an immediate diagnosis, determining disease extent, analysis of unexpected findings and determining adequacy of tissue for histological analysis and special studies [1]. In addition to the immediate question at hand, the prudent surgeon and frozen section pathologist must both have insight into the purposes and shortfalls of this art. To this end we have taken several common situations in which one might expect a frozen section and expanded into why it is being done and what limitations it might have, specifically in regards to common gastrointestinal and liver disease settings. The hope is that both the frozen section pathologist and the clinician will gain a more thorough understanding of the use of frozen sections in these situations.

Intraoperative assessment of Hirschsprung disease

Frozen section analysis has long played an important role in the treatment of Hirschsprung disease (HD). Hirschsprung disease is a developmental disorder with microscopic absence of myenteric (Auerbach’s) and submucosal (Meissner’s) nerve plexuses along with hypertrophic nerve bundles. This absence of nerve plexuses results in clinical symptoms early in life including constipation, failure to defecate, abdominal distention and vomiting. Treatment is surgical, in the form of removal of the affected aganglionic bowel segment. From the perspective of the diagnostic physician as well as the surgeon, the greatest challenge in HD is determining where functional bowel ends and non-functional bowel begins. Many surgeons use frozen sections for this purpose.

However, evaluation of mucosal tissue and transitional zone colon for ganglia is widely recognized as difficult, and even more so on frozen section analysis. This is due to tissue sampling error, technical problems or interpretative error (difficulties in evaluating and recognizing ganglion cells themselves) [2] and requires a high degree of skill [3, 4]. Most institutions obtain multiple (as many as 10 or even more) “levels” or sections on any given tissue specimen being examined for HD by permanent sections following formalin fixation; it is often possible to get only one or two levels during frozen sections.

Surgeons should be aware of these concepts when they ask the pathologist to determine disease extent intraoperatively. As surgical standard of care moves from a two step to a one step procedure, pathologists are being asked to both diagnose HD and evaluate the extent via frozen section more frequently. It is true that such a one step procedure may have overall benefits [5], but in light of the above points surgeons should consider making a diagnosis and determining disease extent preoperatively by endoscopic tissue sampling, whenever possible.

Because of these issues, several groups have looked into the use of immunohistochemical or semi-quantitative methods for rapid frozen section evaluation using stains for items like tubulin and acetylcholinesterase [6-8].
Alternatively, some pathologists recommend Diff-Quick staining as a way to view ganglia more easily than with H&E alone. These new techniques, most of which are done in combination with traditional H&E staining, could give a better assessment of bowel innervation and ultimately lead to better results for the patient. In the vast majority of institutions, however, these techniques are not currently used for practical reasons.

Other groups have suggested that whenever intraoperative frozen sections are performed they should be evaluated by more than one pathologist [2]. It remains important for the surgeon to understand that there are limitations to the frozen section diagnosis and evaluation of HD, and that risks of intraoperative diagnosis must be taken into account along with benefits in terms of convenience and cost.

**Intraoperative diagnosis of pancreatic cancer**

Pancreatic cancer is a leading cause of cancer death in the United States with very low rates of long-term survival and surgical resection remaining the mainstay of therapy. The role of frozen sections in regards to pancreatic cancer falls under two main categories: for diagnostic purposes and for evaluation of disease extent.

Preoperative biopsy of a pancreatic mass has been a source of controversy for some time [9]. A recent review concluded that “preoperative biopsy of potentially resectable pancreatic tumors is not generally advisable, as malignancy cannot be ruled out with adequate reliability” [10]. It is in these situations in which frozen sections may yield the first histological data regarding pancreatic lesions. A tissue specimen obtained intraoperatively avoids the sampling problems associated with transcutaneous or endoscopic biopsies and is therefore superior in one way. However, frozen section analysis does not avoid the histological difficulties in diagnosis inherent in core biopsy or fine needle aspiration. A number of studies have shown that frozen section analysis of a pancreatic mass does not always yield a definitive cancer diagnosis [11-13]. While some data are more optimistic [14], a false negative biopsy is particularly devastating to a patient with adenocarcinoma whose only hope for survival is surgical resection. Accordingly, many surgeons choose to proceed with surgery based on gross findings alone, regardless of or in the absence of data from frozen sections. Further complicating the picture is the idea of panIN or pancreatic intraepithelial neoplasia, which is now recognized as a precursor lesion to invasive adenocarcinoma [15]. These lesions have a high prevalence in the healthy population and currently there are few recommendations to aid in management.

Frozen sections are an imperfect but useful tool in the evaluation of a pancreatic mass, but when frozen section and gross findings do not agree, especially in the context of a negative frozen section evaluation, surgical judgment is indispensable. Diagnostic information obtained from frozen section should be taken in combination with the surgeon’s gross assessment of the situation as well as treatment and patient goals, not as the sole indicator of further surgical action [16]. This will minimize misdiagnosis and ensure optimal patient care.

**Frozen sections to assess pancreatic cancer extent**

In addition to obtaining frozen sections for diagnostic purposes, they are also commonly obtained to assess tumor extent. Several studies exist which state the importance of a curative resection as the single most important factor for determining outcome in patients with pancreatic adenocarcinoma [17]. The practical utility of obtaining intraoperative frozen sections during surgery has been a source of disagreement among surgeons. One study found that intraoperative assessment of the pancreatic neck margin using frozen section analysis increased the likelihood of a resection with negative margins in patients with pancreatic cancer [18]. Interestingly, this same study found that there was no difference in survival between the groups with and without intraoperative frozen section. Another group also found that final margin status did not affect survival [19]. One hypothesis is that the lack of a prognostic influence of negative histological margins on permanent sections in the absence of frozen section analysis may be indicative of a less aggressive tumor. In contrast, frozen sections allow the surgeon to “tailor” a negative margin, but this is not indicative of survival because the grade of the tumor biology is more likely to be high [18].

Therefore, there is no absolute indication for frozen sections in this setting and the choice to utilize frozen assessment to define margins in pancreatic cancer will be left up to the discretion of the surgeon.

**Frozen section evaluation for diagnosis of unknown liver lesions**

One reason that frozen sections may be performed in the liver are for the diagnosis of an unknown liver lesion. The frozen section may be the first tissue sample of an unknown liver lesion, for example in liver lesions found incidentally during another procedure. Alternative scenarios are laparoscopic or open evaluation for primary or secondary liver masses for diagnostic purposes when a surgeon may fear the potential risk involved in fine needle aspiration or core biopsy [20]. The information gained from the frozen section is used to guide surgical or nonsurgical management. The accuracy of frozen section diagnosis of liver mass lesions is about 95%, similar to ovary and breast [21]. This study should give the surgeon some confidence that in the situation where a frozen section is the first approach to an unknown liver lesion, the results are dependable, although still less definitive than permanent sections following formalin fixation.

Once the nature of a liver lesion has been determined, frozen sections may also be used to help determine disease
Frozen sections as a tool to evaluate liver transplant organ quality

Another major use of frozen section evaluation of liver tissue is in the assessment of liver suitability for transplantation. Given the high prevalence of obesity, diabetes and fatty liver disease in the population, liver steatosis is common and is a major risk factor for primary dysfunction after transplantation [22]. Liver steatosis is typically described as the percentage of micro and macrovesicular fat. The two are distinct entities and a liver can exhibit all of one or a combination of the two. Typically macrovesicular fat is of greatest concern during transplantation. Greater amounts of macrovesicular fat in a liver specimen are associated with an increased risk of transplant failure. Livers with less than 30% macrovesicular fat tend to do favorably [23], whereas livers with >60% macrovesicular fat content are excluded when there are other options available [24, 25]. It is felt that livers with between 30% to 60% fatty infiltration should be used for transplantation, albeit cautiously with attention placed on other patient risk factors [24, 26]. Given the severe shortage of available livers, the more frequent use of severely steatotic livers has even been suggested, although this is controversial [27]. Of course, there is often a high opportunity cost of not doing a liver transplant, even if the only liver available is of poor quality. Maximizing utilization of steatotic livers is a difficult balancing act and one that is unlikely to become clear cut. Other factors, including degree of liver fibrosis and inflammation can also be evaluated on frozen section analysis and these parameters may also have prognostic value.

There are several other interesting studies in regards to frozen sections and liver transplantation. One found that intraoperative gross assessment of the liver by the surgeon alone is a poor indicator of fat content and fibrosis [28]. These data indicate that seemingly poor livers are being disposed of when in actuality they may have been transplantable. The authors conclude with a recommendation for surgeons to perform more frozen sections during liver transplantation.

Overall, when frozen sections are used in this context they tend to provide useful intraoperative information.

Frozen sections for esophageal tumors, cancer and dysplasia

Surgery remains the mainstay of treatment for esophageal adenocarcinoma and squamous cell carcinoma, although recurrence and mortality are high [29]. Primary diagnosis is not only rarely made intraoperatively, as the esophagus is accessible for preoperative biopsy. Patients with localized disease without metastases have the best prognosis for survival. Depending on the surgical indication, survival or effectiveness of palliation of patients with carcinoma of the esophagus is related to completeness of disease resection [30, 31]. Frozen section analysis is often utilized by surgeons to define and ensure clarity of resection margins for gastroesophageal resections, both distal (gastric) and proximal (esophageal), as well as to evaluate for disease spread. The extent of microscopic disease and gross disease may differ, making the accurate gross assessment of the resection margins challenging [32]. To this end, routine use of frozen sections as a way to verify complete resection of tumor has been recommended [33, 34] and is frequently used, although studies reporting the benefits of this are limited.

Frozen section analysis has been proposed as an alternative to gross examination for complete tumor resection. The proximal margin tends to be more often involved and a proximal resection margin of 12 cm has been recommended [34]. A macroscopic gastric or distal margin

<table>
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<tr>
<th>Site</th>
<th>Use</th>
<th>Pros</th>
<th>Cons</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Colon</td>
<td>Evaluate Hirschprung disease extent</td>
<td>Reduces workup, reduces cost, allows for simultaneous evaluation and treatment</td>
<td>Difficult pathological analysis, especially on frozen sections; may increase patient morbidity</td>
<td>It is best to avoid diagnosis and evaluation of HD via frozens whenever possible</td>
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<tr>
<td>Pancreas</td>
<td>Diagnose unknown lesions</td>
<td>Allows for simultaneous evaluation and treatment</td>
<td>Certain lesions in the pancreas are very difficult to differentiate between, especially chronic pancreatitis vs. carcinoma. May lead to misdiagnosis</td>
<td>Surgeon must maintain a high level of clinical suspicion</td>
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<tr>
<td>Pancreas</td>
<td>Evaluate margins and disease extent</td>
<td>May be done intraoperatively, improves chance of positive margin</td>
<td>Improved survival is not clear</td>
<td>Surgical discretion</td>
</tr>
<tr>
<td>Liver</td>
<td>Unknown lesion</td>
<td>May be done intraoperatively, good accuracy overall</td>
<td>Still not as good as permanent sections</td>
<td>Useful when indicated</td>
</tr>
<tr>
<td>Liver</td>
<td>Assess transplant quality</td>
<td>Gives better data than surgical gross assessment alone</td>
<td>Utilizes time and resources</td>
<td>Useful when indicated</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Assess disease extent. Rarely for intraoperative diagnosis</td>
<td>May help attain negative margins</td>
<td>Few studies indicating clear benefit</td>
<td>Surgical discretion</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>Assess unknown lesions</td>
<td>Helps to aid management</td>
<td>Limitations in intraoperative diagnosis on frozen sections</td>
<td>Surgical discretion</td>
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of 5 cm is recommended by one group who suggests frozen sections when a 5 cm macroscopic margin is unattainable [34]. The distal margin is less frequently involved as a portion of stomach can usually be taken if necessary. Recurrence at the site of anastomosis is related to the length of the proximal resection margin [31]. For squamous cell carcinoma, one study recommends resection margins of 1.0 cm for carcinoma involving the submucosa and muscularis propria or 3.0 cm for tumor involving the adventitia in potentially curative operations [35]. However, there are no studies that we know of attempting to elucidate the superiority of frozen section evaluation versus gross evaluation. Because of the technical difficulties involved in resecting a portion of esophagus out of a difficult milieu, it is reasonable that frozen section analysis may be of great assistance in patients in which desired gross resection margins prove difficult or impossible to obtain.

When receiving a frozen section, the pathologist should report not only the presence of carcinoma, but also dysplasia and metaplasia to the surgeon [33]. It has been recommended that prophylactic esophagectomy be undertaken in suitable candidates with high grade dysplasia of the esophagus [36]. Our extrapolation is that when high grade dysplasia appears on frozen sections at the margins, efforts should be made to remove this tissue whenever possible. Since Barrett’s esophagus without dysplasia, low grade dysplasia and high grade dysplasia are seen as a progressive continuum leading toward adenocarcinoma, removal of precursor lesions should be balanced with the likelihood and significance of subsequent morbidity and mortality and surgical discretion.

In addition, frozen sections can be used to differentiate a malignant tumor from a benign tumor when preoperative biopsy is unavailable. The most common benign tumor of the esophagus is the leiomyoma and it is important to rule out malignant processes, including carcinoma and leiomyosarcoma.

**Frozen section analysis of peritoneal lesions**

Finally, frozen sections are often used in the identification of unknown peritoneal lesions, particularly in the differentiation of benign versus malignant in order to guide surgical therapy. While mimickers of tumor implants make this an imperfect technique [33] it remains an indispensable tool to assist in the surgical management of patients.

**Conclusion**

Frozen sections are an important yet imperfect tool utilized by surgeons to influence judgment and commonly guide patient management [37]. As with any tool, understanding and respecting its limitations is important. As with all tests in medicine, it is important to remember that it should only be used when it may make a contribution to clinical management and patient care.

**Conflicts of interest**

None to declare.

**References**