Approach to Intraoperative Consultation for Donor Liver Biopsies

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Context.—As demand for organs to treat end-stage liver disease increases, donor livers once deemed only marginally suitable for donation are being considered for transplantation. Pathologists are increasingly being asked to evaluate these livers for acceptability. This article provides guidelines for frozen section evaluation of livers for transplantation.

Objective.—This article concentrates on the histopathologic features of transplant suitability with appropriate clinicopathologic correlation for the practicing pathologist. Recommendations for proper handling and sampling of tissue are discussed. Relative and absolute contraindications as well as artifacts and benign conditions are emphasized.

Data Sources.—Sources include a compilation of the authors’ experiences in academic and community liver transplantation centers. In addition, relevant medical literature was reviewed, as well as Web sites specializing in organ transplantation, such as Transplant Pathology Internet Services and the Organ Procurement and Transplantation Network.

Conclusions.—Malignancy and extensive necrosis in the liver are absolute contraindications to transplantation. Evaluation of macrosteatosis, fibrosis, hepatitis, and necrosis depends on the severity of disease and correlation with the clinical situation. Donor age of greater than 60 years does not preclude transplantation. Artifacts and benign conditions need to be understood to prevent wastage of precious organs and to ensure that an appropriate organ is provided for the recipient.


In 2011, 6341 orthotopic liver transplants were performed in the United States; however, more than 16 000 patients are currently registered on the liver transplant waiting list. The increasing use of orthotopic liver transplantation to treat end-stage liver disease has resulted in a shortage of available livers. Therefore, attempts have been made to expand the donor pool, including increasing the number of donation after cardiac death donors, and using livers considered to be “extended donor criteria.” Extended-criteria livers have one or more factors that increase the risk of initial poor function or primary nonfunction, such as elderly donors (older than 60 years), steatotic grafts, and donors with viral hepatitis, among others. The choice of the donor organ influences both the immediate and long-term function of the allograft liver. Different transplant centers use different clinical/laboratory criteria for acceptance of liver allografts. Frozen section examination can include or exclude extended donor criteria livers. As the number of extended-criteria livers has increased, so has the frequency of intraoperative consultation with frozen section to determine the suitability of these organs for transplantation.

Pathologists are increasingly called upon to evaluate these livers for transplant suitability, often after normal working hours or on weekends. Therefore, it is critical for the pathologist to become familiar with the frozen section criteria for donor organ suitability. However, other than the Web site of Transplant Pathology Internet Services, sponsored by the University of Pittsburgh, there are few resources available to the practicing pathologist. This article offers a succinct review of the histologic criteria used to assess livers for transplantation. Absolute and relative histologic contraindications as well as benign entities that could be confused with more serious conditions are emphasized (see Table).

CLINICAL RISK FACTORS AND DONOR ELIGIBILITY

In order to provide clinicopathologic correlation of frozen section findings, the pathologist needs to be familiar with the clinical factors that affect graft suitability. Several clinical risk factors have been identified that increase the risk of transplantation failure. These factors have been incorporated into a quantitative donor risk index that assigns a numerical value to the transplantation risk of the graft. In light of the rising demand for donor livers, several risk factors that may be associated with primary allograft nonfunction or early allograft failure have been given new consideration.

One way that the donor pool has widened considerably has been to accept grafts from older individuals. Contrary to...
Prior research, evidence suggests that allografts from advanced-age donors (older than 60 years) are as functional as allografts from younger donors. A key condition to preserving function in livers from older donors is to minimize cold ischemic time—the time between chilling of an organ after its blood supply has been cut and the time it is warmed by restoring the blood supply.

Other clinical conditions that in the past may have led to organ deferral include obesity, alcohol abuse, acute infections, hypotension, hypoxemia, cardiovascular disease, and chronic renal failure. Some studies have shown that these conditions do not significantly change the survival rates of the grafts. However, other studies suggest that having several risk factors can negatively impact postoperative graft success and should be evaluated on an individual basis. The pathologist’s role at the time of frozen section is to identify histologic features in the context of the other donor risk factors that will ultimately determine the suitability of the graft for transplantation.

INTRAOPERATIVE EVALUATION

General Considerations

Communication between the surgeon and pathologist regarding the reason for frozen section evaluation is essential because most organs from otherwise healthy donors do not require intraoperative consultation. Whenever possible, the pathologist should have knowledge of the gross appearance of the liver; however, it is not uncommon to receive a biopsy without knowledge of the gross findings.

A 1.5-cm² subcapsular wedge or 2.0-cm-long needle core biopsy from the anterior inferior edge of the liver is advocated in the literature for diffuse processes. Our preference is a needle biopsy, which eliminates artifacts due to sampling of the capsule and samples the parenchyma more deeply. When mass lesions are sampled, a separate biopsy of the background liver is recommended to evaluate for chronic liver disease or other contraindications to transplant.

Submitting the biopsy immediately fresh in a sterile container is preferred; gauze or saline should be discouraged because they can introduce artifacts that can lead to incorrect biopsy interpretation.

Finally, frozen section slides should be retained by the recipient institution to assist in evaluating the recipient’s posttransplant course. If procurement is performed at another facility, the material may be accessed through the organ procurement agency.

Acceptable and Unacceptable Criteria for Liver Transplantation Based on Frozen Section Histology

<table>
<thead>
<tr>
<th>Finding</th>
<th>Acceptable for Transplantation</th>
<th>Unacceptable for Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis—macrovesicular</td>
<td>&lt;30% hepatocytes involved</td>
<td>≥30% hepatocytes involved</td>
</tr>
<tr>
<td>Steatosis—microvesicular</td>
<td>Any type</td>
<td>N/A</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td>In positive recipients:</td>
<td>In all recipients:</td>
</tr>
<tr>
<td></td>
<td>Grade &lt;2 Batts and Ludwig</td>
<td>Grade ≥2 Batts &amp; Ludwig</td>
</tr>
<tr>
<td></td>
<td>Grade &lt;5 mHAI (Ishak/Knodell)</td>
<td>Grade ≥5 mHAI (Ishak/Knodell)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Stage &lt;2 Batts and Ludwig or mHAI (Ishak/Knodell)</td>
<td>Stage ≥2 Batts and Ludwig or mHAI (Ishak/Knodell)</td>
</tr>
<tr>
<td>Granulomas</td>
<td>“Burned-out” or fibrotic/calcified granulomas</td>
<td>Active granulomas, caseating or noncaseating</td>
</tr>
<tr>
<td>Nonspecific portal infection</td>
<td>Mild</td>
<td>&gt;Mild (particularly if the viral hepatitis status of the donor is unknown)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>&lt;10% of liver area</td>
<td>≥10% of liver area</td>
</tr>
<tr>
<td>Malignancy</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: mHAI, modified hepatic activity index; N/A, not applicable.

Macrovesicular Steatosis

Steatosis is a common finding in livers evaluated for transplantation and has been reported in 9% to 26% of biopsied donor livers. Steatosis can be microvesicular or macrovesicular. Microvesicular steatosis is defined by multiple small lipid droplets in the hepatocyte without nuclear dislocation, as compared with macrovesicular steatosis, which is usually composed of a large cytoplasmic lipid vacuole that displaces the nucleus peripherally. Macrovesicular steatosis is most often centrilobular, though it may become panlobular when more than two-thirds of the parenchyma is affected. The degree of steatosis is subdivided into mild (<30% of hepatocytes), moderate (30%–60% of hepatocytes), and severe (>60% of hepatocytes). Severe macrovesicular steatosis involving 60% or more of the hepatocytes increases posttransplant complications and qualifies as an absolute contraindication for liver transplantation. Risk factors for macrovesicular steatosis include alcoholism, diabetes mellitus, hypertension, obesity, various drugs (such as amiodarone and methotrexate), cellular toxins, malnutrition, and anoxia.

The posttransplant effects of steatosis are not completely understood but are thought to be associated with greater susceptibility to ischemia. Buildup of fat deposits in the hepatocytes results in an increased cell volume, which may cause sinusoidal obstruction and is more frequently found in the elderly. Ischemia/reperfusion injury of donor livers with severe macrovesicular steatosis may cause lysis of steatotic cells. The liberated lipid droplets may then form cystic spaces in liver sinusoids, which may result in damage to the graft and loss of function by disrupting hepatic microcirculation, with resultant foci of parenchymal necrosis.

There is some debate whether livers with less severe forms of macrovesicular steatosis are acceptable for transplant. Some studies have encouraged greater caution before approving even mildly macrosteatotic livers. Although these livers may maintain functionality, they become more susceptible to bleeding and fibrinolysis following transplantation. One large study of 390 frozen section biopsy specimens found that 13% of grafts with greater than 30% macrovesicular steatosis had primary nonfunction, whereas only 2.5% of livers without steatosis showed primary nonfunction. Typically, donor livers that have greater than 30% macrovesicular steatosis should be deferred, although the decision is ultimately left to the transplant surgeon (Figure 1).

It is important to note that the severity of steatosis can be easily misinterpreted at frozen section. Fresh biopsies are...
necessary for making an accurate assessment. Air drying quickly diminishes the amount of fat detected and may compromise the accuracy of the interpretation. Also, placing the biopsy on a towel or gauze can cause fat to leach out of the hepatocytes, resulting in an underestimation of steatosis. Conversely, placing the biopsy in saline can cause an overestimation of steatosis because of the freezing of water droplets in the tissue (Figure 2). If a significant accumulation of artifacts are present and are misinterpreted, they could result in the exclusion of an otherwise acceptable organ.

Supplemental methods of lipid evaluation at the time of frozen section have been examined in an attempt to mitigate the potential of confounding artifacts. Although oil red O increases the amount of fat visualized, it has its drawbacks, including artifacts (staining of sinusoids as well as vacuoles) and its lack of availability at the time of frozen section. Computer image analysis may provide a more accurate assessment of hepatic steatosis in the future.

Figure 1. Liver wedge biopsy showing approximately 30% of hepatocytes with macrovesicular steatosis in a predominantly pericentral distribution between 2 portal tracts (left and right edges). At our institution this organ would be deferred from transplant (hematoxylin-eosin, original magnification X40).

Figure 2. Water artifact mimicking steatosis following submission in saline. The frozen section slide has water vacuoles that simulate fat droplets and marked distention of sinusoids mimicking lipopeliosis (hematoxylin-eosin, original magnification X400).

Hepatitis
Serologic test results for viral hepatitis are usually known to the procurement agency and the surgeon prior to transplantation, and thus many would-be donors are deferred prior to coming to the pathologist’s attention. Viral hepatitis, particularly hepatitis C, is a contraindication to transplantation in an uninfected recipient. However, recipients who are already infected with hepatitis C can receive similarly infected livers to help expand the donor pool. Outcomes of liver transplantation from hepatitis C–positive donors with mild inflammation/fibrosis to hepatitis C–positive grafts suggest similar results to hepatitis C–negative grafts. Chronic hepatitis B–infected recipients can receive livers from hepatitis B core antibody–positive donors because they will receive antiviral prophylaxis. Studies have shown success using prophylactic antiviral therapy to prevent transmission of hepatitis B from donor to recipient. Therefore, the history of viral hepatitis is a relative contraindication and must be discussed with the transplant surgeon to determine if the graft is otherwise suitable for transplant into a similarly infected recipient.

On frozen section, cases with more than mild activity, that is, grade 2 or greater in the Batts and Ludwig classification or modified hepatic activity index score of 5 or more in the Ishak/Knodell classification, are deferred from transplantation.

Fibrosis
Hepatic fibrosis plays a role in long-term allograft survival, particularly when transplanting livers from hepatitis B– or C–positive donors into hepatitis B– or C–positive recipients. Fibrosis greater than portal fibrosis (stage 2 or greater in the Batts and Ludwig and Ishak/Knodell classifications) are generally considered unsuitable for transplantation.

Liver Necrosis
Donor livers are at risk for necrosis at various points throughout transplantation. If the liver has undergone ischemia prior to the pretransplantation biopsy (ie, “shock liver” secondary to exsanguination), cellular death may be evident in the frozen section. However, storage of the biopsy in saline may cause distortion of the morphology including cytoplasmic clumping and edema in the extracellular space, making it difficult to detect preexisting necrosis. These artifacts can be avoided by obtaining a fresh biopsy. Ischemic injury can also occur at the time of transplantation and is correlated with increasing cold ischemic time, particularly in donation after cardiac death donor livers. This may result in ischemic changes in follow-up biopsies that were not identified at the time of initial frozen section. In one study, biopsies obtained at time zero (immediate postreperfusion period) demonstrated that apoptotic hepatocytes and centrilobular (zone 3) necrosis were predictors of allograft failure. Although there is no consensus for the amount of necrosis that is acceptable, a cutoff of 10% diffuse necrosis (ignoring focal subcapsular necrosis) has been suggested.

Malignancy
Any malignancy found in the liver at frozen section is a contraindication to transplantation. Hematopoietic malignancies may be particularly difficult to diagnose on frozen section. In case of doubt, it is preferable not to use the organ for transplantation.
Central nervous system malignancies in the donor have not been found to metastasize to the transplanted liver, provided the blood-brain barrier has not been breached by a prior biopsy or surgery. Therefore, a history of central nervous system malignancy in the donor is not a contraindication for transplantation.

**MORPHOLOGIC FEATURES THAT ARE NOT CONTRAINdicATIONS TO TRANSPLANTATION**

**Microvesicular Steatosis**

Microvesicular steatosis is a common finding and can occur following warm ischemia. Currently, there is little evidence to suggest that microvesicular steatosis can harm the graft; common practice is to disregard microvesicular steatosis and focus on the degree of macrovesicular steatosis in pretransplant biopsies.

**Nonspecific Mononuclear Portal Inflammation**

Nonspecific mononuclear portal inflammation is often present after sepsis or following several days in the intensive care unit. The differential diagnosis of mononuclear portal inflammation is broad and should not automatically be associated with viral hepatitis. If mononuclear portal inflammation is present and serologic tests for viral hepatitis are negative, the liver can be accepted for transplantation.

**Iron**

Two types of hepatic iron deposition can be seen: Kupffer cell and hepatocellular iron. Kupffer cell siderosis (secondary iron overload) is evident on frozen section slides and corresponds to increased erythrocyte breakdown such as blood transfusion. Kupffer cell siderosis poses no risk of adverse outcome following transplantation.

Hepatocellular siderosis is identified on frozen section as a fine to coarse, dark brown pigment in periportal hepatocytes, and in severe cases becomes panlobular in distribution. It is graded using a grading scheme ranging from 0 to 4+ depending on the relative amount of intrahepatic iron as well as its distribution (Scheuer classification). Mild siderosis (grade 1+/4) may only be detected by Perl/Prussian blue stain performed on permanent sections. Mild siderosis can be seen in severe Kupffer cell siderosis, alcoholic liver disease, nonalcoholic fatty liver disease, and hepatitis B and C. Severe hepatocellular siderosis (>2+/4) is more worrisome and is seen in conditions of primary iron overload, that is, hereditary hemochromatosis. There is not much literature available regarding the implications of transplanting a severely siderotic liver into a nonhemochromatosis patient. In rare cases when this happens, subsequent posttransplant biopsies show that the iron is readily metabolized into the Kupffer cells and portal macrophages. Additionally, nonhemochromatosis transplant recipients have increased iron due to transfusion and to nonfunction of their native liver, and that may predispose those patients to certain opportunistic infections. In our center, donor livers with hepatocyte siderosis up to 2+/4 are accepted for transplantation.

**Other Pigments**

Lipofuscin is a granular brown pigment that is a product of fatty acid oxidation, associated with aging and certain disease states. In the liver, it is a product of the centrilobular hepatocytes and is finely granular (Figure 3, A). These features contrast with iron, which, in the early stages of hemochromatosis, is predominately perportal in distribution (Figure 3, B).

Bile can also be mistaken for lipofuscin or iron. Bilirubinostasis is a result of cholestasis, and is manifested as green to golden brown granules in perivenular hepatocytes. How-
ever, it is often associated with canicular bile and hepatocyte rosette formation in longstanding cases, helping to differentiate bilirubin from lipofuscin (Figure 3, C).

Focal Nodular Hyperplasia

Focal nodular hyperplasia is a discrete subcapsular mass, typically between 1.0 and 3.0 cm (although larger ones have been reported), and is usually an incidental finding. Histologically, these lesions can be mistaken for cirrhosis, especially if the pathologist does not know that the lesion is focal. When focal nodular hyperplasia is suspected, biopsy of a uninvolved area should be requested to rule out cirrhosis. Focal nodular hyperplasia is not a contraindication for transplantation.

Granulomas

Occasionally, small, firm, white, well-circumscribed nodules in the liver are sampled to exclude a primary or metastatic malignancy. These nodules are often old fibrotic granulomas from a remote infection with histoplasmosis. Some of these "histoplasmosmas" have no clinical significance and are not a contraindication to transplantation. On the other hand, active granulomas are a contraindication.

CONCLUSIONS

Pathologists play an increasing role in frozen section diagnosis of livers for transplantation as the demand for organs increases. Intraoperative consultation has immediate and significant consequences for the transplant recipient. This article is intended as a guide to the general pathologist to prevent an inappropriate organ from being transplanted with deleterious results. Benign conditions that should not be confused with significant lesions are also presented in order to prevent unnecessary wastage of precious organs.

References