Liver Biopsy and the Evidence-Based Evaluation of Chronic Liver Disease

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Evidence-based Medicine

Although its origins may be traced to ancient times, the term "evidence-based medicine" first appeared in the medical literature in 1992 in a paper by Guyatt et al (1) who characterized it as a new approach or paradigm shift in the teaching and practice of medicine. According to Sackett et al (2) “evidence-based medicine is the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence-based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research.” Evidence-based medicine aims to apply the best available evidence gained from the scientific method to clinical decision making. It seeks to assess the strength of evidence of the risks and benefits of treatments (including lack of treatment) and diagnostic tests. Evidence quality can range from meta-analyses and systematic reviews of double-blind, placebo-controlled clinical trials at the top end, down to conventional wisdom at the bottom. Although not applicable to all aspects of medical practice, evidence-based medicine seeks to clarify those parts that are in principle subject to scientific methods and to apply these methods to ensure the best prediction of outcomes in medical diagnosis and treatment.

Methodology for the integration of evidence into the practice of medicine (3)

1. Formulation of specific questions regarding diagnosis, prognosis, causation or any other aspect of a clinical problem.
2. Search for relevant information in the medical literature.
4. Incorporation of best evidence into algorithms, protocols or guidelines.
5. Evaluation of the effectiveness of the algorithms, protocols or guidelines.

Evidence-based medicine categorizes different types of clinical evidence and rates them according to the strength of their freedom from the various biases that occur in medical research. For example, the strongest evidence for therapeutic interventions is provided by systematic review of randomized, double-blind, placebo-controlled trials with allocation concealment and complete follow-up involving a homogeneous patient population and medical condition. By contrast, patient testimonials, case reports, and expert opinions in the absence of data have the least quality as evidence because of the placebo effect and the biases inherent in observation and reporting of cases and personal opinions. Several systems for the stratification of evidence quality have been used. The American Association for the Study of Liver Diseases (AASLD) has adapted the stratification system of the American College of Cardiology and American Heart Association for use in practice guidelines related to liver disease, including the use of liver biopsy (4). In this system, levels of evidence include:

- **Level A** Data derived from multiple randomized clinical trials or meta-analyses
- **Level B** Data derived from a single randomized trial or nonrandomized studies
- **Level C** Only consensus opinion of experts, case studies or standard-of-care
Recommendations that are incorporated into practice guidelines are classified as:

**Class I**
Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation, procedure or treatment is beneficial, useful and effective

**Class II**
Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a diagnostic evaluation, procedure or treatment

**Class IIa**
Weight of evidence/opinion in favor of usefulness/efficacy

**Class IIb**
Usefulness/efficacy less well established

**Class III**
Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation/procedure/treatment is not useful/effective and in some cases may be harmful

Evidence-based AASLD Recommendations for liver biopsy (4)

1. Liver biopsy should be considered in patients in whom diagnosis is in question and when knowledge of a specific diagnosis is likely to alter the management plan (Class I, Level B).

2. Liver histology is an important adjunct in the management of patients with known liver disease, particularly in situations where (prognostic) information about fibrosis stage may guide subsequent treatment; the decision to perform liver biopsy in these situations should be closely tied to consideration of the risks and benefits of the procedure (Class I, Level B).

(Recommendations 3-24 deal with technical aspects, procedures, training, contraindications, and patient management.)

25. Because diagnosis, grading, and staging of non-neoplastic, diffuse parenchymal liver disease is dependent on an adequate sized biopsy, a biopsy of at least 2-3 cm in length and 16 gauge in caliber is recommended (Class I, level C).

26. It is recommended that if applicable, the presence of fewer than 11 complete portal tracts be noted in the pathology report, with recognition that diagnosis, grading and staging may be incorrect due to an insufficient sample size (Class I, level C).

27. If cirrhosis is suspected, a cutting rather than a suction needle is recommended (Class I, level C).

28. In clinical practice, use of a simple (e.g., Metavir or Batts-Ludwig) rather than complex (e.g., Ishak) scoring system is recommended (Class I, level C).

29. Liver biopsy is currently a fundamentally important tool in the management of patients with liver disease, important for diagnosis as well as staging of liver disease, and its use is recommended until clearly superior methodologies are developed and validated (Class IIB, Level C).
The Liver Biopsy as Evidence – The “Gold Standard”

Liver biopsy has frequently received the accolade of “gold standard”, a designation that dates to an era when knowledge of the causes and pathogenesis of liver disease was primitive by current standards and blood tests and radiologic procedures that could diagnose liver diseases were few. In the middle decades of the twentieth century, as hepatology was developing as a discipline, liver biopsy with a careful analysis of hepatic histology was the best test available, and the clinicians who were pioneers in the field had a keen interest in the subject.

As new diagnostic tests have become available, liver biopsy has typically been the standard against which they have been judged. However, although it may be a gold standard, liver biopsy is not 24 karat gold. In many liver diseases pathologic lesions, especially fibrosis, are not uniformly distributed in the tissue and may be missed if the biopsy is too small. This is best documented in chronic viral hepatitis (5) and in nonalcoholic steatohepatitis (6). As in other areas of pathology, interobserver and intraobserver variability are common (7), and the biopsy specimen itself may degrade diagnostic accuracy if the specimen is too small, fragmented or distorted to permit accurate assessment (8,9).

Even though liver biopsy is not a 24 karat gold standard, as the best standard available, it has frequently been used as a surrogate for clinical outcomes. Crawford (10) published his assessment of the evidence base for the use of liver biopsy and hepatic histopathology at a previous Hans Popper Society meeting. In his review of the literature there were 150 highly cited papers with a significant component of liver pathology published between 1948 and 2002. Many others of these took the form of expert opinions or descriptive case series. However, a significant number used histology of liver biopsies as a measure of outcome in clinical therapeutic trials or natural history studies. Indeed, histologic improvement was the predefined endpoint in studies that proved the efficacy of interferon based therapy for hepatitis C (11,12), antiviral drugs for hepatitis B (13-15), and more recently pioglitazone and vitamin E for nonalcoholic steatohepatitis (16,17).

Evidence for Usefulness of Liver Biopsy

For true evidence-based liver biopsy interpretation, the biopsy report needs to provide a diagnosis or interpretation that predicts the likelihood of a clinical outcome (death or hepatic decompensation) or response to therapy or provides other information relevant to the health, well being or prognosis of the patient. Improvement or worsening of serial biopsies is not sufficient in this regard.

The biopsy feature that correlates best with prognosis in virtually all liver diseases is the degree of fibrosis, which can be characterized as early, mid-stage or late (i.e. cirrhosis)
Life expectancy in relation to fibrosis staging in untreated patients with chronic liver diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Stage</th>
<th>Pathology</th>
<th>Life Expectancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Early</td>
<td>Portal-periportal fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>55-86% 5-year survival</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Early</td>
<td>Portal-periportal fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>84% 5-year survival</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>Early</td>
<td>Portal-periportal fibrosis</td>
<td>98% 5-year, 82% 10-year survival</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>85% 5-year, 62% 10-year survival</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>85% 5-year, 40% 10-year survival</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>Early</td>
<td>Portal-periportal fibrosis</td>
<td>90% 5-year survival</td>
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<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>70% 5-year survival</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>40% 5-year survival</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>Early</td>
<td>Perivenular-pericellular fibrosis</td>
<td>Uncertain</td>
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<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>54-69% 5-year survival</td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>Early</td>
<td>Perivenular-pericellular fibrosis</td>
<td>Uncertain</td>
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<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>Uncertain</td>
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<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>75% 5-year survival</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Early</td>
<td>Portal-periportal fibrosis</td>
<td>Uncertain</td>
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<tr>
<td></td>
<td>Mid</td>
<td>Bridging fibrosis</td>
<td>Uncertain</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>Cirrhosis</td>
<td>90% 5-year, 75% 10-year survival</td>
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### Other Liver Biopsy Features with Relevance to Clinical Outcomes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathology</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic hepatitis (18)</td>
<td>Moderate to severe cholestasis</td>
<td>22% 5-year survival</td>
</tr>
<tr>
<td></td>
<td>No to mild cholestasis</td>
<td>54% 5-year survival</td>
</tr>
<tr>
<td>Alcoholic cirrhosis (19)</td>
<td>Active alcoholic hepatitis</td>
<td>53% 5-year survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69% 5-year survival</td>
</tr>
<tr>
<td>Nonalcoholic fatty liver disease (20)</td>
<td>Simple steatosis</td>
<td>2.7% liver-related deaths in 18 yrs</td>
</tr>
<tr>
<td></td>
<td>Steatohepatitis</td>
<td>17.5% liver-related deaths in 18 yrs</td>
</tr>
<tr>
<td>Hepatitis B lamivudine therapy (21)</td>
<td>Pre-Rx Histology Activity Index = 0-4</td>
<td>9% HBeAg loss</td>
</tr>
<tr>
<td></td>
<td>Pre-Rx Histology Activity Index = 5-9</td>
<td>20% HBeAg loss</td>
</tr>
<tr>
<td></td>
<td>Pre-Rx Histology Activity Index ≥ 10</td>
<td>38% HBeAg loss</td>
</tr>
<tr>
<td>Hepatitis B lamivudine ±IFN vs placebo (22)</td>
<td>Histologic improvement (HAI ≥ 2)</td>
<td>1% liver-related events in 2 years</td>
</tr>
<tr>
<td></td>
<td>No histologic improvement</td>
<td>12% liver related events in 2 years</td>
</tr>
<tr>
<td>Hepatitis C Peginterferon Ribavirin Rx (23)</td>
<td>No or mild fibrosis</td>
<td>57% sustained virologic response</td>
</tr>
<tr>
<td></td>
<td>Bridging fibrosis/cirrhosis</td>
<td>44% sustained virologic response</td>
</tr>
<tr>
<td>Hepatitis C (Halt-C Trial)</td>
<td>Ishak Stage 2 fibrosis</td>
<td>4% Clinical outcomes in 6 years</td>
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<td></td>
<td>Ishak Stage 3 fibrosis</td>
<td>10% Clinical outcomes in 6 years</td>
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<td></td>
<td>Ishak Stage 4 fibrosis</td>
<td>16% Clinical outcomes in 6 years</td>
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<td></td>
<td>Ishak Stage 5 fibrosis</td>
<td>36% Clinical outcomes in 6 years</td>
</tr>
<tr>
<td></td>
<td>Ishak Stage 6 fibrosis</td>
<td>51% Clinical outcomes in 6 years</td>
</tr>
</tbody>
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### References:


Hans Popper Hepatopathology Society
Session on Evidence-Based Liver Biopsy Practice

“The Liver Biopsy in Modern Clinical Practice:
A Pediatric Point-of-View”

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I. Utility of Liver Biopsy
   A. Uncertain Diagnosis
   B. Overlapping diagnoses
   C. Conflicting diagnoses
   D. Procuring tissue for enzyme or chemical analysis
   E. Stage and Grade
   F. Prognosis
   G. Longitudinal Assessment
   H. No alternative method
   I. Unavailable alternative method
   J. Validation of other methods

II. Special Considerations in Pediatrics for Biopsy
   A. Higher risks
   B. Frequent need for general anesthesia
   C. Smaller needles/less tissue/diagnostic difficulty
   D. Hemorrhagic intolerance
   E. Cost of inpatient hospitalization for procedure
   F. Inability to verbalize potential complication symptom
   G. Consent/assent

III. Complications of Procedure
   A. Pain
   B. Hemorrhage
   C. Perforation of adjacent organ
   D. Bile leak
   E. Infection
   F. Seeding of biopsy tract with tumor cells

IV. Limitations to Interpretation
   A. Length and width of biopsy
   B. Number of passes
C. Method to obtain biopsy
D. Micro-and macro-heterogeneity
E. Location/lobe biopsied
F. Potential inability to reach focal lesion
G. Artifacts from preservation/sectioning
H. Intra- and inter-observer interpretation

V. Alternatives to Biopsy
A. Ultrasound imaging
B. MRI imaging
C. Metabolic screening
D. Noninvasive biomarkers
E. Noninvasive biophysical techniques
F. DNA sequencing
G. Biopsy of alternative tissue (bone marrow, salivary gland)

VI. Feature Evaluation Requiring Biopsy
A. Changes in cell type, location, number, relationships
B. Cell injury/death
C. Degree and type of inflammation
D. Identification and localization of storage material
E. Location and degree of fibrosis
F. Focal lesions of uncertain significance following imaging

VII. Differences in Pediatric and Adult Diseases
A. Genetic or developmental>acquired or neoplastic
B. Usually early presentation, milder or inconspicuous
C. Often without symptoms/suspected from parent disease
D. Usually lacking evidence-based guidelines
E. Lacking longitudinal data from childhood to adulthood
F. Fewer approved and tested therapies
G. Less drug-induced liver toxicity/drug interactions

VIII. Some Liver Diseases Unique to Children
A. Ductal plate and vascular malformations
B. Mitochondrial dysgenesis
C. Inborn errors of metabolism
D. Errors in protein formation (e.g. A1AT)
E. Type II autoimmune hepatitis
F. Acquired extrahepatic biliary atresia
G. Drug toxicity from placental exposure
H. Neonatal hemochromatosis
I. Hemophagocytic lymphohistiocytosis
J. Systemic viral infections causing neonatal FHF (e.g. enteroviruses)
K. Pediatric-predominant tumors

IX. Examples of Diseases in Continuum or Common with Adults
A. Problems after liver transplant
B. Nonalcoholic fatty liver disease
C. Viral hepatitis
D. Autoimmune hepatitis/PSC
E. Wilson’s disease
F. Hepatocellular carcinoma

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Bezerra JA, Balistrieri WF. Cholestatic syndromes of infancy and childhood. Semin Gastrointest Dis. 2001; 12: 54-65
Evidence-Based Evaluation of Liver Transplant Pathology –

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Goals:
1. Understand existing data and mechanisms of liver disease relating to the transplant patient.
2. Learn the salient features of pathology in the liver allograft through a foundation of knowledge of cell biology.
3. Develop an appreciation that features exist in the liver explant and liver allograft biopsy that have prognostic significance. Therefore, develop not only a “static” diagnostic modality but a “prognostic/biomarker” approach as well.
4. Appreciate that by understanding fundamental mechanisms, we can derive and figure out very complex processes.
5. Understand that we play a critical role in academic investigation and thus patient care.

Outline:
I. Establishment of Diagnosis of Liver Disease
   a. etiology of injury and stage
   b. malignancies
   c. mimics
II. Donor evaluation
   a. cadaveric
   b. living
III. Time zero liver biopsy
IV. Rejection
   a. acute
   b. chronic
   c. mimics
V. Recurrent disease
   a. hepatic
   b. malignancy

General Comments:
Liver transplantation has proven to be an effective modality to prolong life with good quality to many patients. Currently, organ procurement is a major limiting factor for treatment with many patients dying while on the wait list. Therefore, a fair and evidence based approach for placing and ranking patients on the wait list has been adopted at the
National level (United Network for Organ Sharing [UNOS] http://www.unos.org/ and Organ Procurement and Transplantation Network [OPTN] http://optn.transplant.hrsa.gov/). While the goal of maximizing liver allocation to maximally prolong a good quality of life to the most people is somewhat self-evident, development of the “formula” to achieve this goal is not so easy. As such, patient variables including overall health status, primary liver disease, presence (and multiplicity) of malignancies, age, and psychosocial factors are important determinants for consideration as a transplant candidate. The proper selection and transportation of the liver for engraftment are important for both short and long term outcomes. As well, the proper evaluation of post engraftment liver allograft biopsies is critical because our diagnosis not only dictates treatment but also offers prognostic information.

We play a fundamental role in all of the above steps. In addition, we serve continually for the care of these patients by leading and participating in large cohort studies to scientifically evaluate the validity of and develop new methods for the care of our patients. Pathologists are therefore an important member of the multidisciplinary transplant team. Our critically important role in the care of the patient with liver disease begins well in advance of the actual transplant event.

While we are fortunate to have many good studies to guide the field, we also have to recognize that not everything we do is in fact “scientific” or even “validated.” As well, we also must be aware that we exist in a very dynamic field and with changing technologies and practices, what was once deemed “true” may in fact be debunked. Things change. However, what does not change are the fundamental processes of science and cell biology. We have the opportunity to continually learn and evaluate in a critical manner our “beliefs” as applied to many things including how we deal with the pathology we “see” every day. While the principles applied to the evaluation and study of liver disease are applicable to patients of all ages, the probability of specific etiologies of disease certainly is age dependent.

In approaching the evaluation of patients with a liver allograft, we need to keep in mind that the relative probability of varying injury processes does in fact change over time. This temporal association should not, however, dictate our objective evaluation of the patient, but it does give us insights into underlying mechanisms of varying processes.

The diagram below displays in a rough graphical form the temporal patterns of three major processes at play in the liver allograft: 1) graft loss, 2) rejection, and 3) recurrent disease. Sorting out which and to what degree these injuries are occurring is a major challenge. The proper identification and ranking of these processes is critical.
**Patient Selection**

**Acute Liver Failure:** Patients with acute liver failure may be candidates for liver transplantation. In a few selected scenarios, time permitting, our evaluation of the native liver biopsy in the acute situation is needed. Our role in these situations is in large part to identify those diseases for which transplantation is contraindicated. Specifically, a patient with an unsuspected malignancy such as lymphoma may present with “acute liver failure,” the diagnosis of which is made from our examination of the native liver biopsy. Occasionally, patients with autoimmune hepatitis may present clinically with acute liver failure and prompt treatment with steroids may ward off the need for emergent transplantation. While a pan lobular hepatitis and at least some degree of fibrosis are helpful features for this diagnosis, they are by no means independently definitional of any one etiology. Many forms of acute toxic and other forms of liver injury may cause zone three necrosis. While one may think that the percentage of the native liver biopsy involved by necrosis would be correlative with patient outcomes, studies have shown otherwise.

**End-stage liver disease:**

**Malignancies:** How and by what criteria should we consider patients with cirrhosis and “tumors” for liver transplantation? On one hand, resection of hepatocellular and cholangiocarcinomas may afford a cure and/or prolonged patient survival. On the other hand, these same malignancies may, even with resection by liver transplantation, recur. The goal of liver transplantation in this setting is to choose those patients with malignancies who have the best chance for long term survival. In another words, to transplant those individuals with malignancies that have a low risk of recurrence. MRI and CT scans are used to image the livers of patients being considered for liver transplantation to detect any liver lesions. Because only a minority of these lesions is
biopsied, we rely upon imaging features to establish pre-operative diagnosis of these lesions. While these imaging modalities are very good, they are not perfect, as they may both over and under stage these malignancies based upon careful correlation with the explanted liver. The number and size of the putative hepatocellular carcinomas as assessed by imaging are key variables that correlate with patient outcomes post liver engraftment. Varying cut off points for each of these variables has been correlated with outcome data leading to several schemes, such as the Milan criteria which limit prioritization for OLT to those who have either a single tumor under 5 cm or three or less tumors each under 3 cm, without evidence of metastatic disease or vascular invasion.

Neoadjuvant therapy for hepaticellular carcinoma including transarterial chemoembolization, selective internal radiation therapy and radiofrequency ablation are instituted to bring into or maintain patients with in the current transplant guidelines. While patients have received liver transplantation because they have been so maintained, careful and complete pathologic examination of all lesions in the liver explant has shown that the vast majority of treated tumors have viable segments and that unsuspected non-treated carcinomas are present away from the main lesions. While neoadjuvant therapy has not proven as an effective tumor killing therapy, we do not know to what extent, if any, these treatment effects outcomes as controlled studies are difficult to do at best.

**Donor Selection**

**General Principles**: Short (weeks) and long term outcomes are dependent, in part, upon donor 1) age, 2) % of macro-vesicular steatosis, 3) presence of underlying liver disease, 4) size of liver. That “older” livers have worse outcomes than “younger” livers has interesting biologic implications. Specifically, this association implies that the liver, as with other systems, has a limited capacity/lifespan for regeneration. In addition to these variables, many other parameters influence graft survival such as cold and warm ischemic times. Immediately after liver engraftment with the influx of the patient’s blood into the organ, ischemic reperfusion injury always occurs; but the degree to which this injury is manifested and its effects on patient outcomes in the immediate post operative time period is dependent, in part, upon the amount of macro-vesicular, not micro-vesicular, steatosis in the allograft. The steatosis serves as a source of free radical generation which injures the sinusoidal endothelium impairing micro-vascular blood flow in the liver. It is not clear why the micro-vesicular feature is not associated with poor outcomes.

**Cadaveric**: Immediate assessment of the potential liver from a cadaveric donor is needed. One of the most important assessments made microscopically is the determination of amount of steatosis. If there is more than about 33% of the hepatocytes with macro-vesicular steatosis as assessed on the frozen section, then the organ may not be deemed suitable and therefore passed on for consideration to another center. It is important to understand the distinction of the macro versus micro steatotic vesicles.

The bile ducts are extremely sensitive to ischemic damage and therefore “non-beating” donors have a higher risk of developing bile duct strictures compared to “beating” and living donors.

**Living**: Because both the recipient and the donor are living people, the use of these donors is especially problematic. Ethical issues aside, the medical criteria for these donors is held at an extremely high level. While not all centers require that the potential
donors undergo a liver biopsy, our group has made it a standard practice. We have found surprising findings in these liver biopsies which have led to exclusion of these donors. While there is little “outcome” data per se to study the criteria applied to living donors, we have chosen to be very conservative.

**Rejection:**

As a response to the foreign liver, the patient may mount an immunologic response in which the patient’s lymphocytes are targeted to the allograft bile ducts, terminal venules and/or arteries. Because the hepatocytes are relatively devoid of HLA class II expression, they are not the primary target of this immune response. The severity of this cellular response (acute cellular rejection) is graded into categories of mild, moderate, and severe. Moderate and severe acute cellular rejection are treated with increased and/or alternate immunosuppressive agents because if left unabated graft loss may occur due to the succeeding evolution to late chronic rejection defined by the loss of bile ducts, central to portal bridging fibrosis, and/or accelerated atherosclerosis.

Acute rejection is most probable within the first year post transplantation with maximal probability in the first three months. As well, chronic rejection, because it may follow as a consequence of acute rejection, is most probable with in the first year post transplantation. However, both acute and chronic rejection may occur at any time even years later.

While the bile ducts and thus “portal type rejection” is the most common form, the terminal venule either in concert with the bile duct or in isolation is also a target of rejection.

**Recurrent Disease:**

**General Comments:** One of the important reasons to establish the disease in the native liver is to be able to recognize and assess recurrent and/or acquired disease in the allograft. There are diseases which never recur in the allograft such as Wilson’s disease, those that may recur such as autoimmune hepatitis, primary biliary cirrhosis and malignancies, and those that essentially always recur such as hepatitis C. The time course of recurrence and damage to the allograft may, especially in the case of hepatitis C, be accelerated relative to the native state. With the development of outstanding immunosuppressive regimes, recurrent disease and in particular recurrent hepatitis C is a huge clinical problem. Specifically, the fibrosis resulting from this injury is currently one of the major factors in liver allograft loss. Thus, research to understand the mechanisms and the development of predictive “biomarkers” of fibrosis in the liver allograft are extremely important.

**Hepatitis C and fibrosis:**

1) The rate of post-transplantation liver fibrosis is determined early and is constant
2) In early post transplant biopsies prior to the onset of detectable fibrosis (F0), we have established the following biomarkers of rapid fibrosis progression:
   - Hepatocellular apoptosis
   - CK19
   - Vimentin
3) These findings give insight into the basic cellular mechanisms of liver fibrogenesis and offer new tools for patient management.
Both hepatitis C and hepatic B may recur in the liver allograft in a rare but particular histologic and biochemical form termed “fibrosing cholestatic hepatitis.” The outcome data with these putative aggressive forms of hepatitis is somewhat variable. Initially, this form was thought to portend a poor outcome. However, other studies have shown differing outcome data perhaps in part due to variance in both the clinical and histologic definition of this entity.

**Malignancy:** Hepatocellular carcinomas definitely may recur and usually recur outside the liver allograft. Common sites of recurrence include the adrenal glands, intra-abdominal area, and the brain. The time frame of recurrence may vary from months to several years. As previously stated, the prognostic factors predictive of recurrence are based upon gross and microscopic features.

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Liver Biopsy for Cirrhosis

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Introduction

Cirrhosis and chronic liver disease (CLD) are ranked 12th in the top 15 causes of death in the United States, and account for 9.7 per 100,000 deaths. Furthermore, while overall life expectancy increased in 2007 compared with 2006, mortality from cirrhosis and CLD increased 3.4% in the same time period [1].

Cirrhosis: Definition

Cirrhosis is a term derived from Greek κιρρός, meaning tawny, or yellow, and linked to an amber-colored wine; the ancient Greeks described a disease of the liver with tan-yellow micronodules. The actual term, cirrhosis, is credited to Laennec, in the 1800s. For clinicians, the term implies end-stage liver disease with a variety of partially manageable complications (encephalopathy, ascites, bleeding) as well as life-threatening complications (bleeding and hepatocellular carcinoma). For pathologists, the term has evolved over the years to connote diffuse remodeling of the liver parenchyma that includes, in its simplest iterations, vascular remodeling with septal/scar formation surrounding nodules of hepatocytes with or without aberrant portal tract-like structures. Subtleties of this description can be found in discussions by leading experts [2, 3].

Underlying Causes of Cirrhosis in the United States: Overview

The leading etiologies for cirrhosis include, broadly:

(1) primary liver diseases, i.e. post-hepatitic, including viral hepatitides C, B, and D, autoimmune hepatitis;
(2) diseases that destroy the bile ducts, including PBC, PSC, IAD, Alagille's, biliary atresia;
(3) ingested toxins such as alcohol;
(4) inherited or acquired metabolic diseases and/or systemic diseases such as diabetes mellitus, nonalcoholic steatohepatitis, hereditary hemochromatosis, alpha-1-antitrypsin disease, Wilson disease, tyrosinemia, cystic fibrosis;
(5) diseases of the vascular outflow tract such as Budd-Chiari or cardiac sclerosis;
(6) drug-induced liver injury (DILI) including methotrexate, amiodarone and others;
(7) the diseases for which a cause has yet to be identified, i.e. cryptogenic.

The Role of Liver Biopsy for Cirrhosis

When a liver biopsy is done for which “cirrhosis” is the diagnostic request on the requisition, what does that tell the pathologist? Most often, it tells us two things: 1. That the patient has portal hypertension, i.e. encephalopathy, varices or variceal bleed, ascites,
splenomegaly, depressed platelets; 2. that the clinically “obvious” causes have likely been ruled out. Therefore, it is highly unusual in modern practice for a pathologist to receive a liver biopsy to "confirm" cirrhosis; rather, the primary reason currently is to determine the underlying cause(s). Often, therefore, the real question is determination of the underlying cause of clinically evident portal hypertension.

Thus, what causes portal hypertension (pHTN)? In the West, cirrhosis is by far the #1 cause. Other processes to be aware of, however, are previously unrecognized advanced biliary fibrosis, and advanced alcoholic hepatitis. Throughout the world, the #1 cause of pHTN is schistosomiasis with the resultant characteristic Symmer’s pipestem fibrosis. Over 200 million people worldwide are infected and it is estimated that up to 400,000 people currently living in the US have schistosomiasis, so this is not a problem to which we can be indifferent.

Classifications of Portal HTN[4]

pHTN is due to increased vascular resistance. One of the reasons this occurs is the peculiarity of the portal venous system: there are no valves. Thus increased pressures within this normally low-pressure system, are transmitted proximally. Increased pressures may occur from obstruction at 3 different sites: splenic vein thrombosis (pre-hepatic, extra-hepatic), cirrhosis (sinusoidal, intra-hepatic) and/or large outflow veins, right heart, IVC, constrictive pericarditis (post-hepatic). pHTN is commonly classified broadly into 2 (or 3) categories: pre-sinusoidal and hepatic, based on wedged hepatic vein measurements, or pre-sinusoidal, sinusoidal, and post-sinusoidal, based on the organ's structure. Utilizing the pressure gradient categories, pre-sinusoidal is further subdivided into extra-hepatic and intra-hepatic, and the latter is further subdivided into intra-hepatic and post-sinusoidal. These categories carry clinical significance: pre-sinusoidal causes of pHTN are not associated with abnormal liver function and liver failure following variceal bleeding is rare, whereas hepatic causes of pHTN will have abnormal liver function (and tests), and liver failure is common following a variceal bleed.

A pathologically-based classification of pHTN is cirrhotic and non-cirrhotic (NC-pHTN).

Pre-sinusoidal Portal Hypertension

This group of disease processes is characterized by wedged hepatic pressures that are either normal, or less than measured portal vein pressure. Examples include diseases characterized by portal infiltrates (sarcoidosis, schistosomiasis, PBC), developmental abnormalities (hereditary hemorrhagic telangiectasia, adult polycystic liver disease, congenital hepatic fibrosis), phlebosclerotic processes of the portal vein (hepatopetal sclerosing, biliary diseases, toxins, nodular regenerative hyperplasia).

The extra-hepatic causes of pre-sinusoidal portal hypertension include splenic vein thrombosis, pylephlebitis and other neoplastic and non-neoplastic disease processes that can lead to structural disruption of the main portal vein. This group of diseases will not be further discussed.

Hepatic Causes of Portal Hypertension
Some authors group these into sinusoidal and post-sinusoidal. This grouping of diseases are characterized by wedged hepatic pressures that either exceed, or are equal to measured portal pressure, but are not normal. Causes include many infiltrative processes (amyloid, alcoholic-induced sinusoidal fibrosis, Gaucher's disease, etc), malignancies of the sinusoids (epithelioid hemangioendothelioma, angiosarcoma) all forms of cirrhosis, and venous outflow disease, including cardiac causes.

Steps in Histologic Determination of Presence and Types of Portal Hypertension:

#1: Cirrhosis or Not

This is a process that can be simple and recognized by "gestalt" within the first minutes of evaluation, or it can be quite challenging, and take conscious efforts to work through. Fragmentation is no longer as reliable a feature as previously, unless the clinician is continuing to utilize a suction biopsy needle; the transjugular needle biopsy technique, and the percutaneous gun biopsy will not yield this type of specimen. Intact core biopsies must, therefore, be evaluated for other "clues": presence of vascular structures (portal tracts and outflow veins); vascular relationships/spacing; cord nodularity and cord integrity; nuclear features: bi-nucleates, homogeneity or anisonucleosis, and cytologic alterations of hepatocytes. One can be highly suspicious of cirrhosis when no well defined portal tracts and predictably-spaced outflow veins are present; increased numbers of vascular/lymphatic-like channels within enlarged fibrous tracts; increased numbers of ductular profiles within enlarged fibrous tracts; delicate fibrous outlines defining rounded edges of hyperplastic, nodular cords are also suggestive of cirrhosis. The presence of an ectatic open sinusoid-like vascular structure adjacent to a septum is a concern for approximation of an outflow vein and loss of intervening parenchyma.

Hepatocyte cords without an outflow vein present but that have small portal-tract like structures may represent a macro-regenerative nodule. Nodules or non-anatomic groups of hepatocytes with distinct but seemingly monoclonal cytologic alterations clearly different from neighboring hepatocytes, i.e. deeply oncocytic, fatty, clear, lack of stainable iron in an otherwise iron-loaded liver, small cell change, large cell change, may represent dysplastic hepatocytes or nodules. The reader is referred to expert reviews [5, 6] for more in-depth discussions of this latter and very important topic. Hepatocellular carcinoma may actually be encountered in unguided liver biopsies performed for "cirrhosis".

1a. If Cirrhosis is Present: Clues to Etiology

Having established cirrhosis, there are histopathologic features that may be clues to the underlying etiology for hepatitic (HBV/metabolic/toxic), cholestatic or vascular disease. Caveats, however, are that once the liver has undergone complete remodeling, acute and chronic cholestasis and vascular alterations may occur in the nodules due to local aberrations, and the vagaries of end-stage liver failure.
Cryptogenic cirrhosis is a term reserved for a biopsy diagnosis for which there is no known clinical or histopathologic etiology. Many disease processes, such as autoimmune hepatitis, alcoholic cirrhosis and nonalcoholic steatohepatitis can "burn-out" and lose features of activity; if the underlying cause has been previously identified, the biopsy is not cryptogenic.

**Regression of Cirrhosis:** Histopathologic lesions of “regressed cirrhosis” have been described [7]; these include thin, incomplete and perforated septa; hepatocyte growth into veins, and small thick collagen bundles in parenchymal sinusoids. These may be challenging lesions to discern in needle biopsies.

#2 If Not Cirrhosis: Clues to Diagnosis

## Noncirrhotic Portal Hypertension

NC-pHTN can be referred to by many rubrics: Idiopathic portal HTN (IPH), intrahepatic portal venopathy, hepatoportal sclerosis, nodular regenerative hyperplasia, and is a leading cause of pre-sinusoidal pHTN. These processes vary by the amounts of fibrosis and nodularity that define them. Clinically, there is increased intrahepatic portal pressure and features of pHTN; these changes are seen in the presence of patent extra-hepatic portal and hepatic veins, and in the absence of cirrhosis. Portal vein thrombosis is known to develop in the larger portal veins in late stages in up to 50%, and carries a worse prognosis. Overall, these entities have better outcomes than cirrhosis, if the clinical manifestations can be managed. A variety of systemic diseases, toxins and drugs are associated with NC-pHTN/IPH, including autoimmune conditions and vasculitides. Geographic differences between India and Japan have been clearly presented and may be related to poverty in the former and autoimmunity to sinusoidal endothelial cells in the latter. Recent work has shown different mechanisms of fibro-obliterative lesions of portal veins and fibrosis within the parenchyma.

Incomplete Septal Cirrhosis (ISC) is a term that, broadly, applies to with parenchymal nodularity and varying degrees of fibrous septal formation, but without the complete parenchymal remodeling of cirrhosis. There is more fibrosis in this disease process than in the other forms of IPH, poorly-defined nodules, increased periportal thin-walled vascular channels ("shunt vessels"). The latter have also been referred to as megasinusoids or herniated veins.

Nodular regenerative hyperplasia (NRH), another manifestation of NC-pHTN, is on the other end of the spectrum and is characterized by nodularity of the parenchyma without the fibrosis of cirrhotic septa. NRH can be challenging to diagnose by needle biopsy; but clues include alternating groups of “thick and thin” cords with nodules 1-3 mm, and compression of the outflow veins into a crescent moon shape. The nodularity may be noted initially by "dilated" sinusoids due to compression of atrophic cords between nodules[8]. Portal veins may be absent in the smallest portal tracts; periportal and perisinusoidal fibrosis may be noted irregularly. NRH may be associated with certain toxins and drugs, and systemic processes that injure the sinusoidal endothelium and result in impaired portal and sinusoidal circulation. This process has become increasingly recognized in patients following the use of various chemotherapeutics
including 6-MP, azathioprine, and oxaliplatin. Both NRH and HPS have been reported in AIDS.\[9]\n
Hepatoportal sclerosis is a third form of NC-pHTN that may be encountered. The biopsy may seem deceivingly "normal", or the portal tracts may be abnormally enlarged. Portal veins may appear enlarged, as the patent intraparenchymal vessels are bearing the pressure from the obliterated portal venous branches elsewhere. Alternatively, only scarred remnants of a portal vein branch may be present. The megasinusoids described above may be noted, as may cord atrophy, nodular parenchymal changes, and perisinusoidal fibrosis.\[4]\n
REFERENCES


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