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 determinant factors 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 filed 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 hepacellular 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.

References


Demetris, A J; Eghtesad, ; Marcos, ; Ruppert, K ; Nalesnik, M A*; Randhawa, P ; Wu, T ; Krasinskas, A ; Fontes, P; Cacciarelli, T ; Shakil, A O ; Murase, N ; Fung, J J ,PHD†; Starzl, T E. Recurrent Hepatitis C in Liver Allografts: Prospective Assessment of Diagnostic Accuracy, Identification of Pitfalls, and Observations About Pathogenesis, The American Journal of Surgical Pathology Issue: Volume 28(5),May 2004,pp 658-669

Dixon LR, Crawford JM. Early histologic changes in fibrosing cholestatic hepatitis C. Liver Transpl. 2007 Feb;13(2):219-26

Jakate, Shriram , Yabes, Annoel; Giusto, Debora; Naini, Bita MD; Lassman, Charles ; Yeh, Matthew ; Ferrell, Linda D. Diffuse Cirrhosis-like Hepatocellular Carcinoma: A Clinically and Radiographically Undetected Variant Mimicking CirrhosisThe American Journal of Surgical Pathology Issue: Volume 34(7), July 2010, pp 935-941


