# STRUCTURAL INTEGRITY AND IDENTIFICATION OF CAUSES OF LIVER ALLOGRAFT DYSFUNCTION OCCURRING MORE THAN 5 YEARS AFTER TRANSPLANTATION

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Running Title: Long surviving human liver allografts.

## ABSTRACT

The clinicopathologic features of liver allograft dysfunction occurring in 51 symptomatic recipients after more than 5 years survival (mean 7.1 years) with the same hepatic allograft was compared to a similar group of 14 asymptomatic patients (mean survival 9.9 years), who underwent a non-clinically indicated protocol liver biopsy evaluation. Predictably, patients who had clinically indicated biopsies more frequently showed histopathologic alterations (76% vs. 36%, $p < 0.002$). After detailed clinicopathologic correlation the changes in the symptomatic patients were primarily attributed to definite or presumed viral hepatitis in 17/51 (33%) patients, 11 of whom had recurrent viral disease; non-viral recurrent original disease in 7/51 (14%), obstructive cholangiopathy 3/51(6%), and acute cellular and/or chronic rejection 11/51 (22%) patients. In 13/51 (25%) of the symptomatic patients the clinical and pathologic abnormalities were minimal. Long term liver allograft survival in 9/14 (64%) of the asymptomatic patients was associated with minimally abnormal histologic alterations. Two of the asymptomatic patients had obstructive cholangiopathy, two others recurrence of the original disease and one possible viral hepatitis. Viral hepatitis types B and C, alcoholic liver disease, autoimmune hepatitis, granulomatous hepatitis (NOS), and probably primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) were shown to recur after hepatic transplantation.

The histopathologic changes associated with acute cellular and chronic rejection frequently overlapped with other syndromes causing late dysfunction, such as chronic viral or autoimmune hepatitis, PBC or PSC and more than one insult could be identified in 15 of the cases, which made the differential diagnosis of causes of late liver allograft dysfunction much more difficult

than early after hepatic transplantation. It is important to correlate the biopsy findings with the liver injury tests, the results of viral and autoimmune antibody serologic studies, review of previous biopsies, and to be aware of the original disease, recent changes in immunosuppression and results of therapeutic intervention(s) in order to correctly identify the causes of liver allograft dysfunction in this patient population.

Key words: Liver transplantation, histopathology, late dysfunction, diagnosis, tolerance, recurrent disease, alcoholism, hepatitis

## INTRODUCTION

Orthotopic liver transplantation is now a well accepted form of therapy for patients with endstage liver disease[40]. Many large centers report patient survival rates which range from 70-90% at one year and there is a more gradual attrition or even flattening of allograft and patient survival curves after 2-3 years, compared to kidney or heart allografts where progressive deterioration because of rejection is the rule. However, long term morbidity and allograft dysfunction are not uncommon, so that an increasing number of physicians will be faced with the problem of correctly identifying and then treating liver allograft dysfunction in their patient population, guided by a core needle biopsy and other laboratory or diagnostic tests.

While the clinical and histopathologic features of early (< 2 years) causes of liver allograft dysfunction are well described[6,10,12,14,21,23,25,33,34,38,39,44,46,47] those associated with late dysfunction are more limited in scope[3,5,7,13,17,18,19,22,27,29,30,31,35,37] and few studies specifically address the problems associated with a clinicopathologic differential diagnosis in long-term survivors[3,5,7,15,17,19,22,27,35]. None have reviewed the material from patients surviving for more than 5 years. Moreover, recognizing baseline changes in long-surviving allografts is important for differential diagnosis and in immunosuppressive drug withdrawal trials[31,36]. The following study is designed with two goals in mind: a) to identify the histopathologic features and causes of late liver allograft dysfunction; and b) to determine if long term, stable allograft livers in patients without clinical signs or symptoms of dysfunction develop any histopathologic changes attributable to prolonged engraftment that would otherwise not be present in age-matched controls.

## MATERIALS AND METHODS

### *Patient Selection*

One thousand eight hundred and thirty-three liver transplant operations were completed in 1431 patients (686 males and 745 females) at the University of Colorado or University of Pittsburgh before Dec. 31, 1988. The cumulative five year patient and allograft survival rates were 65% and 50%, respectively, for females, and 58% and 43%, respectively, for males($p < 0.012$ for patient and $p < 0.0062$ for allograft survival; female vs male; log-rank test). Of the long term survivors, 51/174 who have undergone liver biopsy evaluation for hepatic injury or dysfunction occurring more than 5 years after transplantation were randomly chosen from an in-house computerized database. Hereafter, these 51 patients who underwent indicated biopsies will be called the "symptomatic" group; it consisted of 32 females and 19 males, with an average age of 42.8 years (range 23-65 years) and a mean survival of 7.1 years at the time of biopsy (Table 1). The age and distribution of original disease was representative of the total group of long term survivors. An additional 14 "asymptomatic" patients were randomly selected from a group of 59 patients, who were well, survived for an average of 9.9 years (range 5-16 years) with the same liver and underwent liver protocol biopsy evaluation to test for hematolymphoid chimerism.[41]. There were 10 females and 4 males in this group, with an average age of 45.7 years (range 25-69 years). One patient was initially classified as asymptomatic but later was found to have had signs and symptoms of allograft dysfunction and switched to the "symptomatic" group.

Complete information about the donor age (average age 21; range 11-39 years), sex (27=M; 24=F), ABO blood group, HLA type and crossmatch was available in 56 of the 65 total

patients and showed no significant differences between the two groups.

Backtable biopsies obtained from twenty donors who died because of a cerebrovascular accident (n=9), motor vehicle accident (n=4) or other trauma (n=7) served as normal controls. None had known history of liver disease and serologic studies for HBV, HCV and HIV were negative.

***Immunosuppressive Regimens***

All of the patients originally received a combination of cyclosporin A (Sandoz, Basel, Switzerland) and corticosteroids as baseline immunosuppression ; twelve (12) of the patients also received azathioprine (Imuran$^{R}$, Burroughs Wellcome, Research Triangle Park, NC). Rejection episodes generally were treated with a 1 gram "bolus" of methylprednisolone or a tapering "recycle" of prednisone. "Steroid-resistant" rejection episodes were treated with a 3-10 day course of OKT3 (Ortho Pharmaceuticals, Raritan, NJ). Twenty five of the patients in the symptomatic group but no asymptomatic patients were switched from a baseline immunosuppression of CyA to FK506 (Fujisawa Pharmaceuticals, Japan) from 2-10 years after transplantation for acute cellular or chronic rejection[4] (n=18), CyA toxicity (n=5) or viral hepatitis (n=2). Two of the asymptomatic patients have subsequently been completely weaned from all immunosuppressive drugs for 6-8 months without experiencing rejection.

*Histopathologic Studies*

All patients were followed until 06/01/93 and any biopsy or failed allograft obtained from these patients after >5 years survival with the same liver and before 06/01/93 was reviewed. There were 106 needle biopsies and 6 allograft hepatectomy specimens obtained between 5 and 18 years after transplantation. The slides were reviewed together by two of the authors (OP, AJD) in a systematic fashion, without knowledge of the indication for biopsy, liver injury tests, viral serologic data, clinical events or original disease. The histopathologic features listed on Table 2 were assessed, the results recorded and a histopathologic diagnosis(es) rendered. When more than one diagnosis was given, they were listed in order of importance with the one perceived to be the most significant listed first. A chi-squared exact test was used to compare the frequency of histopathologic findings between two different groups: the first comparison was between symptomatic and asymptomatic patients; the second between seronegative patients and those who were either HBsAg+ or anti-HCV positive, using second generation RIBA assays.

*Clinicopathologic Determination of Cause of Allograft Dysfunction*

The original diagnosis was based only on the observed histopathologic parameters. The final *retrospective* diagnosis(es) was based on the histopathologic findings, and the clinical profile, serologic data, and if given, response to therapy including follow-up biopsies. All of the patients had at least 6 months of follow-up after the index biopsy and post-transplant serologic

studies for HBV and HCV were available in 59/65 patients.

## RESULTS

### *Clinicopathologic Events Before 5 Years after Transplantation*

At the time of study, the primary liver allograft was in place for more than 5 years in 42/51 (82%) of the symptomatic patients and in all of the asymptomatic patients. The second 6/51 (12%) or third 3/51 (6%) allograft had functioned for > 5 years in the remaining symptomatic patients. The causes of previous allograft failure(s) in the 9 symptomatic patients who had non-primary allografts in place for more than 5 years included cellular rejection (n=4), chronic rejection (n=2), primary dysfunction (n=2) and a massive biopsy-induced subcapsular hematoma (n=1). Three of these patients required a third liver allograft because of chronic rejection (n=2) and a combination of ischemic injury and severe CMV hepatitis (n=1). All of the patients in both groups experienced at least one episode (range 1-5; mean 2 $\pm$ 1) of histopathologically documented acute cellular rejection in the studied allograft within the first five years after transplantation.

Other significant events noted during the first 5 years of followup in these patients included: eighteen of the symptomatic and one asymptomatic patient required reconstruction and/or balloon dilatation of the biliary tree for strictures or obstruction; three symptomatic patients developed endstage kidney disease because of cyclosporine toxicity and underwent renal

transplantation; two other symptomatic patients developed an Epstein-Barr Virus (EBV)related lymphoproliferative disorder, one of which resolved while the other evolved into Hodgkin's Disease[28], that was successfully treated with a MOPP regimen of chemotherapy.

***Patient and Allograft Survival after Five Years***

All but two of the symptomatic patients currently are alive, 5-18 years after transplantation. One died from sepsis and multiorgan failure while awaiting hepatic retransplantation, 2275 days after her first liver allograft had been destroyed by hepatic artery thrombosis and subsequent sepsis. The second patient died of bleeding esophageal varices 6631 days after his first allograft became cirrhotic because of recurrent chronic HCV infection. All of the asymptomatic patients are alive with their original allograft an average of 9.9 years (range 5-16 years) after transplantation.

There were 6 failed allografts removed at the time of hepatic retransplantation from the symptomatic group of patients between 6 and 10 years survival with the same liver (mean=7 years). Chronic rejection was the insult solely responsible for allograft failure in 3 of the patients, two of whom also had superimposed hepatic artery thrombosis. In one other, both chronic rejection and hepatitis B contributed to allograft failure. Changes suggestive of recurrent primary sclerosing cholangitis were seen in one (see below), and a combination of chronic HCV hepatitis and portal vein thrombosis destroyed the allograft in the final patient.

### *Signs and Symptoms of Late Liver Allograft Dysfunction*

In most recipients monitoring of liver allograft function included: bimonthly tests for biochemical evidence of liver injury, yearly viral hepatitis serology screens and yearly physical exams. The most common sign of late liver allograft dysfunction was an elevation of the liver injury test above baseline values for that patient, which included: 1) jaundice or total bilirubin > 2mg/dl; 2) increased canalicular enzymes (ALP and γ-GTP) or transminases greater than 50% over the lowest value in the preceding month. In total, 19/51 (37%) of the patients who had indicated biopsies also had at least one of the following symptoms: fever (5/51; 10%); abdominal pain, nausea, vomiting or loss of appetite (9/51; 18%) or jaundice (7/51; 14%). In the more ill patients, the presenting symptoms at the time of biopsy included gastrointestinal bleeding (2/51;4%), and confusion and lethargy (4/51; 8%).

### *Histopathologic Findings*

Piecemeal necrosis, bile duct loss, thickened plates, lobular disarray, hepatocyte necrosis, lobular inflammation, Kupffer's cell hypertrophy and cholestasis (Table 2) were the histo*pathologic* changes present in a higher incidence in biopsies from the symptomatic patients ($p < 0.002$), when compared to biopsies from the asymptomatic patients. The changes present in a higher incidence in biopsies from HBV and/or HCV seropositive patients than in seronegative patients included piecemeal necrosis, bridging fibrosis, bile duct inflammation/damage and lobular disarray, hepatocyte necrosis, lobular inflammation and

Kupffer's cell hypertrophy (Table 2) .

In general, the portal inflammation tended to be slightly more intense in the symptomatic patients, but in both groups consisted of lymphocytes, macrophages and fewer plasma cells. Eosinophils were much less common than early after transplantation, being present in 2/14 (14%) of asymptomatic and 6/51 (12%) of the symptomatic patients.

*Clinicopathologic Diagnosis of Late Dysfunction*

The final retrospective diagnosis showed that viral hepatitis and recurrence of the original disease accounted for 47% of the episodes of allograft dysfunction occurring more than 5 years after transplantation (Table 3). In another 13/51 (25%) symptomatic patients the clinical and pathologic findings were minimal and no specific diagnosis was given. Acute cellular and/or chronic rejection accounted for 22% of late dysfunction syndromes, although another 16% of patients were thought to have acute cellular rejection as a secondary diagnosis. The remaining 3 (6%) patients had obstructive cholangiopathy. The results of the liver injury tests obtained at the time of biopsy, segregated according to the primary final clinicopathologic diagnosis is shown in Table 4. The clinical and pathologic features of each of the causes of dysfunction are discussed in greater detail below.

**Rejection:** Acute cellular rejection was the primary pathologic diagnosis in 2/51 (4%) patients and the secondary diagnosis in 8/51 (16%) others, all of whom were from the symptomatic group and showed non-selective, concomitant elevations of liver injury tests (Table 4). The

histopathologic characteristics were similar to those seen early after transplantation and included a predominantly mononuclear but mixed portal inflammatory infiltrate, with bile duct damage and/or central vein phlebitis (Figure 1). Subendothelial inflammation of portal or central veins was not observed, and periportal hepatocellular necrosis, spotty hepatocyte necrosis and lobular regenerative activity was more prominent than in cellular rejection seen early after transplantation (personal observation, Figure 1). Lobular disarray as a manifestation of acute cellular rejection alone was distinctly unusual. The primary diagnosis in the 8 patients with acute cellular rejection as a secondary diagnosis was chronic rejection (n=3; see below), chronic hepatitis (n=4; 2 HCV, 2 NANBNC) and minimal change (n=1).

Chronic rejection was the primary histopathologic diagnosis in 9/51 (18%) patients all of whom were symptomatic, usually because of a preferential elevation of γ-glutamyltranspeptidase and alkaline phosphatase (see Table 4). One patient also had cholangiographic evidence of intrahepatic biliary tract strictures. Histopathologic changes included mild chronic portal inflammation, hyalinization of the portal tract connective tissue, bile duct loss in > 25% of the triads, and other biliary epithelial cell alterations which included eosinophilic transformation of the cytoplasm, an increased nuclear:cytoplasmic ratio because of nuclear enlargement, uneven spacing of the nuclei and change from a dense nuclear chromatin pattern to an open, reticular chromatin profile with the appearance of nucleoli. In the lobules, mild spotty lobular necrosis without significant lobular disarray, mild mixed sinusoidal inflammation, sinusoidal foam cell clusters (n=3), Kupffer's cell hypertrophy (n=9), occasional apoptotic bodies (n=4), cholestasis (n=4), and steatosis (n=2) were present.

Followup of both patients with a primary diagnosis of acute cellular rejection showed that

treatment with increased immunosuppression resulted in improved liver injury tests and decreased liver allograft inflammation on rebiopsy. Of those with a secondary diagnosis of acute cellular rejection (n=8), 2 required retransplantation because of chronic rejection, 1 improved clinically and biochemically after a switch to FK506 but a background of chronic rejection persisted, and one improved after treatment with corticosteroids. Two patients with a primary diagnosis of hepatitis but a secondary diagnosis of cellular rejection biochemically responded to increased immunosuppression. One spontaneously improved while another improved after interferon treatment.

Three of the 9 patients with chronic rejection showed stable or improved liver injury tests after a switch to FK506. One patient developed intrahepatic bile duct strictures, one has stable elevation of liver injury tests, one required retransplantation because of chronic rejection, one non-compliant patient has shown worsening of liver injury tests and three remaining patients were accounted for above.

**Biliary Tract Complications:** Obstructive cholangiopathy was the primary diagnosis in 5 patients, three of whom were symptomatic. One of the "asymptomatic" patients had a long history of biliary tract anastomotic strictures. As expected, the liver injury tests in this group of patients showed a profile similar to chronic rejection, with a preferential concomitant elevation of the "canalicular" enzymes ALP and γ-GTP, which were ≥ 3X the minimally elevated levels of ALT and AST (Table 4).

The histopathologic changes in this group of patients was similar or identical to those seen in non-allografted livers with bile duct obstruction or stricturing, and confirmed by

cholangiography in all of the patients, three of whom were successfully treated with operative reconstruction and/or balloon dilatation of the biliary tree.

**Viral Hepatitis:** Definite or presumed chronic viral hepatitis was the primary diagnosis in 17 symptomatic and one asymptomatic patient, four of whom also had co-existent acute cellular rejection as a secondary diagnosis. In 11 of these patients, the viral disease in the allograft represented a recurrence of the original disease: two had HBV, 7 HCV, and 2 were presumed to be non-A, non-B, non-C, because the same histopathologic findings were present in the original liver, and HBV, HCV and auto-antibody serologic studies were negative. Seven cases represented de novo hepatitis; one patient had HBV, 2 HCV and 4 were presumed to have non-A, non-B, non-C because of the histopathologic findings (see below) and lack of a sustained response to increased immunosuppression therapy.

Liver injury tests in this group of patients showed minimal to marked but non-specific elevations of both hepatocellular (ALT and AST) and canalicular (ALP and γ-GTP) enzymes. When the patients with co-existent cellular rejection were excluded, there was less of an increase in the canalicular enzymes.

Histopathologic changes included chronic portal inflammation often with formation of lymphoid nodules, damage of only an occasional bile duct, no bile duct loss, minimal to marked piecemeal necrosis, minimal to moderate lobular disarray, inflammation, periportal or midzonal predominant macrovesicular steatosis, hepatocyte necrosis and lobular regenerative changes (Figure 2). Significant portal fibrosis was present in 11 of the patients, 8 of whom showed focal portal-portal or portal-central bridging.

Seven of the patients with hepatitis were placed on α-interferon (α-IFN), 5 of whom experienced clinical and biochemical improvement within 6 months of beginning therapy. However, one other patient treated with α-IFN died from variceal hemorrhaging and the last patient required hepatic retransplantation because of recurrent HBV induced cirrhosis, complicated with chronic rejection. Follow up in the 11 remaining patients not treated with α-IFN showed that after lowering immunosuppression 3 developed rejection (see above). One other patient developed bile duct strictures requiring balloon dilatation and one patient lost his allograft because of recurrent HCV and portal vein thrombosis. Two patients spontaneously improved without therapeutic intervention, one of whom serologically cleared HBsAg, which had been present for at least 3 years after transplantation. Three other patients showed chronic viral hepatitis with persistent low grade activity, whereas one progressively deteriorated because of hepatitis. The remaining patient was lost to follow-up.

**Minimal Chronic Changes, Not Otherwise Specified:** There were 22 (13 symptomatic, 9 asymptomatic) patients in whom the biopsy findings were minimal and could not be classified into a specific histopathologic diagnosis. All but 2 were seronegative for HBV and HCV infection. Even though this group of patients had normal, or only mildly abnormal liver injury tests, relatively subtle changes in their biopsies could be used to distinguish them from liver biopsies obtained from age matched normal controls, even when the pathologists were blinded as to the origin of the slides.

Altogether a slight increase in mononuclear portal or perivenular inflammation was present in 15/22 (68%), and portal arterial and arteriolar mural myocyte hypertrophy/hyperplasia and

hyalinization in 12/22 (55%) (Figure 3). Mild and *more subtle* intralobular regenerative activity was identified in 18/22 (82%) patients and mostly characterized by thickening of the plates (Figure 4). Biliary epithelial cell alterations, similar, but not as advanced as those described for chronic rejection, were seen in 11/22 (50%) patients. In 1/22 (5%) more abnormal biliary epithelial alterations and portal inflammation were present, eliciting a secondary diagnosis of acute cellular rejection.

Two biopsies from the clinically well patients with negative viral serologic studies and normal liver injury tests contained focally dense lymphocytic infiltrates, without any evidence of bile duct injury or piecemeal necrosis. No specific therapeutic intervention was undertaken in this group of patients and allograft function remained stable and good for at least 6 months after the biopsy.

***Recurrence of Original Disease***

Recrudesence of a chronic viral hepatitis was the most common recurrent disease, being responsible for 11/18 (61%) cases, all of whom were just described above in detail. Four patients had recurrent alcoholic liver disease, 1 probable autoimmune chronic active hepatitis and one granulomatous hepatitis, NOS. Two patients were suspected of developing reappearance of PSC and one PBC. Unfortunately, the design of this study did not afford the opportunity to assess the true incidence of recurrent disease. The histopathology and clinicopathologic correlation for each of these disorders is given in more detail below.

**Alcoholic Liver Disease:** Alcoholic relapse was strongly suspected as the primary cause of the liver histopathology in 3 symptomatic and one asymptomatic patient from the 7 total who underwent transplantation for this indication. They all were sero-negative for HBV or HCV virus infection and there was no other apparent explanation for the biopsy findings detailed below. Liver injury tests in this group of patients showed a selective rise in γ-GTP without a concomitant increase in ALP (Table 4). However, the ALT:AST ratio was approximately 1. Two of the four patients denied alcohol abuse, although one of these two had blood alcohol levels of 98 mg/dl at the time of hospitalization. The other patient experienced fluctuating levels of γ-GTP between 150-420 IU/L without apparent cause, which spontaneously declined during hospitalization without specific intervention. The remaining two patients admitted to recidivism.

The biopsy histopathology typical of 3/4 patients is shown in Figure 5. One of these patients also showed focal portal-central bridging fibrosis with early regenerative micronodularity, Mallory's hyaline and megamitochondria.

**Primary Biliary Cirrhosis:** Probable recurrent primary biliary cirrhosis was diagnosed in a 60 year old female, who was 1/16 (6%) patients who underwent transplantation for this indication and shared one HLA B and one HLA DR locus with the female donor. The diagnosis was largely based on hepatic histopathology (Figure 6), since the liver injury tests were only transiently abnormal. Normal cholangiographic studies, PBC in the original liver, and the exclusion of acid-fast bacteria, fungal infection or drug reactions were then used to further substantiate the diagnosis. Serologic studies for anti-mitochondrial antibodies were presumed to be positive and not repeated, because of the previously reported near universal re-elevation of this

disease marker in PBC patients[7]. The mildly abnormal liver injury tests spontaneously returned to normal shortly after biopsy and have since remained normal for more than 2 years.

**Primary Sclerosing Cholangitis:** Recurrence of "primary" sclerosing cholangitis(PSC) was first suspected between 5-6 years after transplantation in 2/6 (33%) patients, both of whom also had a history of ulcerative colitis. One patient had a total colectomy before liver transplantation and the colitis remained active up to the present time in the other patient. Both patients had ABO identical donors, negative lymphocytotoxic crossmatch results, and an "obstructive" liver enzyme profile at presentation. There was no obvious technical or mechanical explanation for the strictures observed on cholangiographic studies, but neither case was felt to be radiographically "classic" for PSC.

The liver biopsies showed changes suggestive of obstructive cholangiopathy, including mild portal expansion because of mild portal fibrosis, and acute and chronic pericholangitis. The biliary epithelium showed atrophic changes with eosinophilic transformation of the cytoplasm.

One of these patients who was suspected of being non-compliant, eventually required hepatic retransplantation after 7 years (Figure 7).

**Granulomatous Hepatitis:** A 44 year old male with negative viral hepatitis and autoantibody serologic studies was asymptomatic when a liver biopsy obtained 9 years after transplantation showed portal fibrosis and 2 portal-based and several non-caseating intralobular granulomas, without bile duct involvement or loss. The original liver showed mixed micro- and macronodular cirrhosis, with numerous non-caseating granulomas. No micro-organisms could be identified with

special stains in either the native liver or the hepatic allograft biopsy specimen. Ultimately, no specific cause of the granulomatous hepatitis could be found and the case was classified as recurrent granulomatous hepatitis, not otherwise specified.

**Autoimmune Chronic Active Hepatitis:** Late dysfunction was attributed to recurrent autoimmune chronic active hepatitis, in a 44 year old HLA B8 and DR3 positive male, who was negative for serologic evidence of HBV, but positive for anti-nuclear antibodies (ANA) before and after transplantation. The donor organ was matched for these disease-associated HLA antigens and a liver biopsy obtained 11 years after transplantation showed chronic portal inflammation with a prominent plasmacytic component, and active piecemeal necrosis without significant bile duct damage and no bile duct loss. However, antibodies to the HCV were detected 2 years after the biopsy was obtained. The final diagnosis was recurrent autoimmune and chronic HCV infection.

***Prospective versus Retrospective Diagnosis***

There were 11/65 (17%) cases where the final retrospective diagnosis was different from the original diagnosis. In 3 cases, an original diagnosis of mild acute cellular rejection was switched to viral hepatitis because of positive serologic studies and lack of a sustained response to additional steroid therapy. In one case, the converse was true, an original diagnosis of hepatitis was switched to acute cellular rejection. However, even in retrospect, in some cases the separation of hepatitis from acute cellular or even early chronic rejection was less than certain.

In 3 cases the original descriptive diagnosis(es) were replaced by firm diagnosis(es) of disease recurrence: [alcoholic (n=2); granulomatous hepatitis, (NOS n=1)], in 2 cases the possibility of both chronic rejection and biliary strictures was originally raised and later, evidence of chronic rejection could not be substantiated and the patient had PSC as an original disease. In one case, it was difficult to separate or determine if recurrent HCV or recurrent alcohol abuse was primarily responsible for dysfunction. And finally, an original diagnosis of hepatitis was replaced by one of obstructive cholangiopathy.

## DISCUSSION

The most common indication for liver allograft biopsy in patients who survive > 5 years with the same organ is elevated liver injury tests; 37% of patients present with physical symptoms. The histopathologic changes in this patient population can be attributed to viral hepatitis or recurrent original disease 47% of the time, while cellular and chronic rejection together account for only 22% of allograft dysfunction episodes, which is much different than early after transplantation. Evolution of the causes of dysfunction over time can be explained by the early manifestation of operative or preservation-related injuries, and the dynamic nature of the immunologic interface between the liver allograft and the recipient[8,41].

Our findings are quite similar to those recorded by Nakhleh et al[27] who studied liver allograft biopsies taken from recipients in the cyclosporine era who had survived an average of 3-4 years after transplantation. Porter[33], who, working with Starzl[42,43] did much of the pioneering work in liver transplant pathology, reported more chronic rejection and less hepatitis in a group of pediatric and adult patients from the pre-cyclosporine era. Hubscher et al[22] and Eid[13] on the other hand, reported less rejection and more normal or minimally abnormal biopsies in a relatively large series of patients who had survived a median of 18 months and 12 months after transplantation, respectively. Differences in the profile of original diseases in the recipient populations, immunosuppressive regimens and study designs make it impossible to directly compare results, but more protocol biopsies in both of the later series and identification of patients with "isolated ductopenia" by Hubscher[22] probably account for this seemingly small disparities.

The syndromes resulting in late liver allograft dysfunction frequently have overlapping histopathologic features; mononuclear or mixed portal inflammation with varying degrees of bile duct damage and piecemeal necrosis can be found in acute cellular and chronic rejection, PBC, chronic viral and autoimmune hepatitis. Such changes when mild, may even be non-specific, but in general, when the portal inflammation is mixed and is associated with damage of more than an occasional bile duct, and there is mononuclear infiltration in and around the connective tissue sheath of the terminal hepatic venules, a diagnosis of rejection is favored. Conversely, nodular portal lymphoid aggregates or portal inflammation associated with damage of only an occasional bile duct, lymphoplasmacytic portal inflammation directed at periportal hepatocytes (piecemeal necrosis), and lobular disarray and lobular inflammation are features in favor of a diagnosis of hepatitis. The usefulness of this paradigm is supported by previous studies[5] and the correlation between the "blind" histopathologic readings and the serologic tests for HBV and HCV infection, but the final diagnosis must take into account all of the clinical and laboratory data.

Even so, arriving at that final definitive diagnosis in a long term liver allograft recipient is even more difficult than early after liver transplantation and in the end, the diagnosis may still be left open to subjective judgment. For example, subendothelial inflammation of the portal or central veins and portal eosinophilia are fairly reliable rejection-related findings in the first several months. In long term recipients, portal eosinophils are present in many chronic inflammatory liver diseases and when present with mild ductular proliferation and adequate immunosuppressive levels, obstructive cholangiopathy should be first excluded. Endotheliitis is less often encountered in long term survivors with acute cellular rejection, and not at all in

chronic rejection[6,10,14,18,21,23,25,27,30,34,38,39,44,46]. The portal infiltrate associated with late acute cellular rejection also is often not well confined within the limits of the portal tracts as is seen early after transplantation making separation from chronic viral hepatitis more difficult. Moreover, the presence of a virus or autoantibodies does not provide immunity from rejection[9], and more than one process can simultaneously affect the allograft. Finally, reliance on serologic markers alone for detection of HCV infection may underestimate the total number of infected patients and add to the difficulties.

Liver injury test profiles can assist in the differential diagnosis. Preferential elevation of the "canalicular" enzymes (ALP and γ-GTP) was more frequently associated with chronic rejection and biliary tract obstruction. The 4 patients with alcoholic relapse showed isolated increases of γ-GTP, without a concomitant rise of ALP, similar to alcoholics in the general population. Hepatitis on the other hand showed elevation of both ALT/AST and ALP/γ-GTP, but overlapping rejection and "cholestatic" forms of hepatitis likely contribute this non-specific pattern of enzyme elevation. It is the authors' opinion that clinicopathologic correlation, awareness of the original disease, review of previous biopsies, and monitoring the effect of therapeutic intervention(s), in addition to a careful review of the index biopsy, is important in arriving at the correct diagnosis (Table 5). Similar conclusions have been drawn by most investigators studying long term survivors[3,5,7,13,17,18,19,22,27,29,30,31,35,37]. In some instances, empiric increases of immunosuppression and follow-up data may be of value in establishing the diagnosis, and in fact, supported the validity of our final retrospective diagnosis.

Viral hepatitis types B and C, alcohol-related injury, autoimmune and granulomatous hepatitis (NOS), and primary biliary cirrhosis appear histopathologically similar or identical to

the same diseases in native livers and recur after transplantation. Reappearance of primary sclerosing cholangitis was strongly suspected since we could find no other cause of the biliary cirrhosis and large extrahepatic bile duct inflammation in an allograft which failed after 7 years, even when the gross and histopathologic examination was complete. In contrast to the report by Hubscher et al[22] who showed a recurrence rate of 16% and in some cases severe allograft liver injury from PBC, the pathologic changes in the single patient in this series was minimal. We remain intrigued by this difference in the reported incidence of PBC recurrence and disease severity.[7,22,32] It is likely related to the addition of azathioprine or to lower baseline cyclosporin levels.[7,22,32]

In 24% of symptomatic patients there was no identifiable clinical, serologic or histopathologic cause of the usually mild dysfunction. Biopsies from these patients were for the most part, indistinguishable from those obtained from the long term asymptomatic recipients. Frequent monitoring of liver injury tests, combined with a lower biopsy threshold, may account for the relatively high frequency of biopsies with minimal pathologic changes.

In contrast, there were two biopsies from asymptomatic, HCV and HBV negative patients with normal and stable liver injury tests, that contained significant chronic portal inflammation but no detectable tissue damage. Whether this represents very indolent: chronic NANBNC viral hepatitis, undetected hepatitis C virus infection, rejection or an immunologic adaptation of the recipient to the allograft similar to that seen in cardiac[1] (Quilty lesions) and renal[20] allografts, is uncertain. The important message illustrated by such cases is that mononuclear inflammation alone in the absence of bile duct, vascular or hepatocyte damage can be associated with normal and stable liver allograft function and need not be treated with additional immunosuppression.

Therefore, a protocol biopsy before attempting to wean immunosuppressive therapy is strongly encouraged[31,36,41].

Many long term stable liver allografts develop histopathologic changes that are not otherwise present in age-matched controls, albeit they are minimal deviations from normal. Possible explanations for the mild lymphocytic inflammation were discussed above; the intrahepatic arteriolar changes can be seen as a result of diabetes, hypertension and perhaps direct drug related injury from chronic cyclosporine[11] and corticosteroid therapy. The biliary epithelial cell and mild intralobular regenerative changes were far more subtle, the latter being insufficient for the diagnosis of nodular regenerative hyperplasia, a disorder originally described in patients with autoimmune diseases[2], and later found with increased frequency after anabolic steroid and Azathioprine administration[16,24,26]. In this series, only 12/65 of the patients were maintained on Azathioprine and all were on either low dose, or no corticosteroids. Therefore, immunologically-mediated perivenular or sinusoidal endothelial cell injury[45] or other immunologic perturbations in the recipient similar to that seen in autoimmune disorders[8] is a possible cause of the regeneration that cannot be dismissed out of hand.

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TABLE 1. Patients and original disease at the time of transplantation.

| | Asymptomatic | Symptomatic |
|---|---|---|
| Female/Male | 10/4 | 32/19 |
| Age (at time of transplant) | 36.6 | 36.1 |
| Original Disease | | |
| Primary biliary Cirrhosis (PBC) | 6 | 10 |
| Cryptogenic cirrhosis | 1 | 8 |
| Alcoholic cirrhosis | 1 | 6 |
| Metabolic Diseases* | 2 | 5 |
| Chronic HCV | 2 | 5 |
| Primary Sclerosing Cholangitis | 0 | 6 |
| Autoimmune Chronic Hepatitis | 0 | 4 |
| Chronic HBV | 0 | 4 |
| Other | 1 | 3 |
| Fibrolamellar Carcinoma | 1 | |
| Total Number of Patients | 14 | 51 |

*Tyrosinemia and Porphyria (n=1), α-1-anti-Trypsin deficiency (n=1), Wilson's disease(n=4), Hemochromatosis(n=1).

**TABLE 2. Histopathologic changes categorized according to the reason for biopsy.**

| | | Symptomatic[a] / Asymptomatic | p value | Sero[b]+ / Sero - | p value |
|---|---|---|---|---|---|
| **PORTAL TRACT** | | | | | |
| Inflammation: | Mild | 27/9[c] | 0.80 | 8/23 | 0.39 |
| | Moderate | 21/4 | | 7/17 | |
| | Severe | 3/1 | | 0/4 | |
| Eosinophils | | 6/2 | 0.80 | 2/5 | 1.0 |
| Piecemeal Necrosis | | 19/2 | 0.12 | 8/11 | 0.01 |
| Bridging Fibrosis | | 10/1 | 0.43 | 6/4 | 0.02 |
| Bile Duct Loss (> 25% of triads) | | 13/1 | 0.17 | 4/9 | 1.0 |
| Subendothelial inflammation | | 0/0 | 1.0 | 0/0 | 1.0 |
| Bile duct inflammation/damage | | 40/9 | 0.31 | 16/27 | 0.006 |
| Ductular/cholangiolar proliferation | | 14/2 | 0.49 | 5/9 | 0.50 |
| **LOBULE** | | | | | |
| Thickened plates | | 50/11 | 0.03 | 15/40 | 1.0 |
| Disarray | | 15/1 | 0.16 | 11/4 | 0.0001 |
| Necrosis | | 21/2 | 0.11 | 9/12 | 0.07 |
| Inflammation | | 39/2 | 0.001 | 12/25 | 0.13 |
| Kupffer cell hypertrophy | | 40/4 | 0.0009 | 14/26 | 0.06 |
| Cholestasis | | 12/1 | 0.27 | 3/7 | 1.0 |
| Fatty change | | 14/4 | 1.0 | 3/14 | 0.35 |
| Hepatocytes anisonucleosis | | 19/4 | 0.75 | 3/16 | 0.22 |
| Sinusoidal dilatation | | 10/3 | 1.0 | 1/11 | 0.15 |
| Iron deposition | | 2/1 | 1.0 | 0/3 | 0.56 |
| **VASCULAR** | | | | | |
| Arteriolar thickening/hyalinization | | 20/8 | 0.36 | 8/18 | 0.77 |
| Obliterative arteriopathy | | 4/0 | 0.57 | 0/4 | 0.33 |
| Central vein phlebitis | | 13/3 | 1.00 | 3/13 | 0.20 |
| Central vein sclerosis | | 11/1 | 0.28 | 4/8 | 0.72 |
| **Total number of patients** | | 51/14 | | 16/43 | |

[a]A comparison of the histopatholgic findings in symptomatic versus asymptomatic patients.
[b]A comparison of patients who were either seropositive for HbsAg or anti-HCV to those seronegative for both viruses.
[c]Most of the portal inflammation in this group was minimal.

**TABLE 3. Breakdown of final retrospective primary clinicopathologic diagnoses in the symtomatic and asymptomatic patients. Patients are listed only by the primary diagnosis.**

| CLINICOPATHOLOGIC DIAGNOSIS | Symptomatic[1] (no.=51) | Asymptomatic[2] (no.=14) |
|---|---|---|
| Cellular Rejection | 2 ( 4%) | 0 ( 0%) |
| Chronic Rejection | 9 (18%) | 0 ( 0%) |
| Obstructive Cholangiopathy | 3 ( 6%) | 2 (14%) |
| Minimal Changes, NOS | 13 (25%) | 9 (64%) |
| Viral Hepatitis | 17 (35%) | 1 ( 7%) |
| HCV | 9 | |
| HBV | 3 | |
| NANBNC | 5 | 1 |
| Recurrent Original Disease | 18 (35%) | 2 (14%) |
| Alcoholic | 3 | 1 |
| PSC | 2 | |
| PBC | 1 | |
| Autoimmune Hepatitis | 1 | |
| Granulomatous Hepatitis, NOS | | 1 |
| Viral Hepatitis (see above) | 11 | |

[1]Patients had clinical symptoms or elevated liver injury tests as an indication for biopsy.
[2]Patients were asymptomatic and had normal liver injury tests; biopsies were done by protocol.

**TABLE 4. Liver injury test listed according to the primary clinicopathologic diagnosis.**

| Diagnosis | TBILI (mg/dl) (nl=0.3-1.5) | AST (IU/L) (nl ≤ 40) | ALT (IU/L) (nl ≤ 40) | ALP (IU/L) (nl=40-125) | γ-GTP (IU/L) (nl ≤ 65) |
|---|---|---|---|---|---|
| Minimal Changes | 0.6 ± 0.3 (0.2-1.0) | 1 =± 62 (13-264) | 100 ± 84 (22-323) | 173 ± 114 (74-573) | 246 ± 167 (39-947) |
| Obstructive Cholangiopathy | 2.4 ± 2.1 (0.8-7.3) | 72 ± 25 (43-119) | 73 ± 24 (46-292) | 331 ± 187 (145-647) | 320 ± 167 (119-635) |
| Chronic Hepatitis | 1.3 ± 1.4 (0.2-6.8) | 204 ± 214 (35-895) | 193 ± 242 (30-961) | 133 ± 76 (21-239) | 237 ± 235 (45-944) |
| Alcohol injury | 0.9 ± 0.2 (0.7-1.2) | 99 ± 60 (42-198) | 108 ± 62 (56-226) | 89 ± 49 (44-156) | 375 ± 245 (178-705) |
| Cellular Rejection[a] | 1.1 ± 0.9 (0.4 - 3.5) | 116 ± 97 (11-314) | 90 ± 61 (14 - 204) | 194 ± 263 (39- 780) | 231 ± 205 (77 - 628) |
| Chronic Rejection | 2.4 ± 2.2 (0.5 - 9.5) | 113 ± 64 (45 - 229) | 184 ± 174 (67 - 362) | 496 ± 360 (178 - 1192) | 736 ± 286 (30 - 2270) |

[a]Includes patients with a primary *and* secondary diagnosis of acute cellular rejection.

**Table 5. Paradigm used for clinicopathologic evaluation of liver biopsies from long term survivors**

| | Portal Tract Findings | Lobular Findings | Vascular Findings | Liver Injury Tests | Clinical History |
|---|---|---|---|---|---|
| **Cellular Rejection** | mononuclear/mixed inflammation<br>more than an occasional damaged bile duct<br>"moth-eaten" limiting plate | Kupffer cell hypertrophy<br>mild sinusoidal lymphocytosis<br>minimal to no lobular disarray<br>+/- cholestasis | perivenular inflammation,<br>central dropout/congestion<br>± subendothelial inflammation | ↑ ALT/AST<br>↑ γGTP/ALP<br>↑→ TB | inadequate immunosuppression<br>"viral" syndrome |
| **Chronic rejection** | minimal/mild inflammation<br>chronic bile duct changes<br>(see text)<br>bile duct loss<br>hyalinization of connective tissue | Spotty necrosis, cholestasis<br>foam cell clusters<br>central hepatocyte ballooning/dropout<br>central cholestasis | foam cell obliterative<br>arteriopathy[a], perivenular<br>sclerosis,<br>sinusoidal foam cell clusters | ↑↑ γGTP/ALP<br>↑ AST/ALT<br>↑→ TB (early)<br>↑↑ TB (late) | inadequate immunosuppression<br>chronic rejection in previous graft<br>cellular rejection unresponsive to treatment<br>positive crossmatch |
| **Obstructive Cholangiopathy** | intra-epithelial neutrophilic/eosinophilic inflammation, periductal edema<br>duct/cholangiolar proliferation<br>+/- fibrosis | sinusoidal neutrophil clusters<br>+/- cholestasis | none | ↑↑ γGTP/ALP<br>→↑ AST/ALT<br>↑→ TB | "difficult" biliary anastomosis<br>hepatic artery stenosis/thrombosis<br>original disease = PSC |
| **Viral Hepatitis** | mononuclear/mixed inflammation<br>piecemeal necrosis,<br>cholangiolar proliferation<br>nodular lymphoid aggregates<br>only occasional damaged bile duct | spotty necrosis, disarray, swelling<br>steatosis(periportal/midzonal)<br>mononuclear inflammation/Kupffer's cell hypertrophy | none | ↑↑ ALT/AST<br>→→ γGTP/ALP<br>↑↑ γGTP/ALP in cholestatic form | original disease = HCV, HBV, cryptogenic, autoimmune cirrhosis<br>positive HCV, HBV serologic/PCR tests<br>biochemical response to α-interferon therapy |
| **Alcohol Abuse[b]** | +/- mononuclear/mixed inflammation | mixed steatosis (central predominant)<br>"acute foamy degeneration"<br>perivenular sclerosis, sinusoidal fibrosis<br>+ iron deposition | perivenular sclerosis<br>sinusoidal fibrosis | ↑ γGTP, → ALP<br>↑→ AST/ALT<br>↑→ TB | original disease = alcohol abuse<br>improved liver injury test on hospitalization w/o intervention<br>positive blood alcohol test<br>R/O other causes of steatohepatitis |
| **Primary biliary cirrhosis**[15,22,29] | mononuclear inflammation<br>non-caseating granulomas<br>bile duct damage/loss<br>cholangiolar proliferation | Kupffer's cell hypertrophy/granuloma<br>mild inflammation<br>periportal copper deposition (late) | +/- focal arterial thickening | ↑ γGTP/ALP<br>↑→ ALT/AST<br>→ TB | original disease - PBC |

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; γ-GTP = gamma glutamyl transpeptidase; TB = total bilirubin.

[a] uncommonly detected in needle biopsies.

[b] other causes of non-alcoholic seatohepatitis, if present, should be excluded.

FIGURE LEGENDS

Figure 1. Acute cellular rejection in a patient who initially was "asymptomatic", then developed liver dysfunction as a result of immunosuppressive weaning. The portal tract (PT, inset) and peri-central vein (CV) inflammation and liver injury tests worsened after CyA/steroid withdrawal and improved after restarting treatment.

Figure 2. Chronic viral hepatitis type C often appeared histopathologically similar to that seen in non-transplanted livers as shown here, with lymphoid nodules in the portal triads, piecemeal necrosis, sinusoidal lymphocytosis and spotty lobular necrosis. In other cases of HCV, the inflammation was mild and neutrophilic predominant, but marked cholangiolar proliferation, more typical of a "cholestatic hepatitis" was seen.

Figure 3. Hepatic arteriolar thickening and hyalinization, with intact bile ducts was seen in patients with minimal other changes and was attributed to hypertension, diabetes or chronic cyclosporin injury, similar to the lesions seen in the kidney.

Figure 4. **A)** Mild intralobular regenerative activity, characterized by thickening of the plates and even focal nodule formation was a frequent but subtle finding in many of the long term survivors, even in those without symptoms or other pathologic changes. **B)** A reticulin stain shows the subtle areas of intralobular regenerative change with thickening of the plates.

Figure 5. Recurrent alcohol abuse was most frequently (3/4 cases) associated with centrilobular mixed steatosis with "foamy" degeneration of hepatocytes (inset), lobular neutrophil clusters,

perivenular and subsinusoidal fibrosis and Kupffer's cell iron deposition. One other patient who admitted to alcoholic relapse showed moderate reticuloendothelial iron deposition, portal and sinusoidal fibrosis, but no steatosis. As always, other causes of steatohepatitis should be excluded.

Figure 6. This periductal granuloma with minimal duct damage was seen in a biopsy obtained 6 years after transplantation in 1/16 patients whose original disease was PBC and in no other patients, regardless of the original disease.

Figure 7. Possible recurrent sclerosing cholangitis (with a component of rejection?) was suspected in this failed allograft (1100 gm) removed 7 years after transplantation. **A)** There was a well-developed biliary-type cirrhosis, with decreased bile ducts and deposition of copper-associated protein at the edge of the regenerative nodules (inset), but no classic "fibro-obliterative" duct lesions. **B)** Sections through the liver hilum showed chronic inflammation of large bile ducts and mural fibrosis, but no obliterative arteriopathy.