# Histology Utility in Liver Graft Surveillance: What About Normal Liver Tests?

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ABSTRACT

Introduction. In liver transplantation, late graft dysfunction can have several causes, particularly rejection, infection, vascular, biliary complications, and others, usually suspected by abnormal liver tests. However, normal liver tests do not confirm a normal graft and liver biopsy could identify unexpected features with repercussions in immunosuppressive therapy. The aim of this study was to determinate the histological abnormalities in patients 10 years after liver allograft transplantation with sustainably normal liver tests and evaluate the changes in immunosuppressive therapy triggered by histological data.

Material and Methods. A retrospective analysis of liver allograft recipients was performed in an adult liver transplantation center with graft histological characterization 10 years after transplantation. Patients with abnormal liver tests and retransplantation were excluded.

Results. We evaluated 39 patients with repeatedly normal liver tests. Familial amyloid polyneuropathy (n = 27) was the mainly indication for liver transplantation. Allograft histological dysfunction was observed in 13 (21.7%) patients. In 3 patients we observed chronic hepatitis, signs of cellular rejection in another 3 patients, and histological features suggesting autoimmune hepatitis in 7 patients. The diagnosis of de novo autoimmune hepatitis was proposed according to contemporaneous positive autoantibodies. Changes in immunosuppressive treatment were proposed in 7 patients.

Conclusion. Allograft histological dysfunctions 10 years after liver transplantation were observed in 21.7% of patients despite normal liver tests. Although the histological features led to alterations of immunosuppressive therapy in half of the cases, the absence of enzymatic tests changes makes monitoring a challenging process.

HEPATIC allograft rejection is an important cause of morbidity and even mortality in transplantation and can be classified in early acute, acute, and late rejection.

Although the prevalence of acute rejection is declining, 20% to 40% of patients still require treatment with additional immunosuppressive drugs during the acute phase [1]. Early acute cellular rejection mostly occurs within 3 months of the liver transplantation and it is suspected by abnormal liver tests and confirmed by liver histology. The typical histological findings are portal-based inflammation associated with bile ducts and portal venules inflammation. In severe cases, it can also occur isolated central perivenulitis in the absence of significant portal inflammation [2]. After the first months post–liver transplantation, the graft tends to normalize and the immunosuppressive therapy can be titrated to its steady dose. Cirrhosis is the final consequence

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of several insults that led to liver transplantation, namely alcohol, hepatitis B and C, and autoimmune liver disease, among others. Graft monitoring must be assertive including liver enzymes and histological features. However, when protocol liver biopsies were performed, several unspecific features, such as nonspecific portal and/or lobular inflammation, idiopathic chronic hepatitis, and a range of architectural and vascular changes were observed with no correlation with liver tests [3]. The importance of these histological abnormalities has been the target of several studies [2,4,5].

Outcomes from acute phase post–liver transplantation have improved and late histological changes are emerging as a common problem in long-term survivors. Factors that influence long-term liver graft abnormalities are complications related to chronic use of immunosuppressive therapy (cardiovascular disease, renal impairment, or malignancy) and liver dysfunction caused by recurrence of the liver disease, onset of de novo disease, or idiopathic chronic inflammatory graft abnormalities [5]. Thus, late graft dysfunction and late mortality may be reduced by minimization of immunosuppressive therapy to avoid unnecessary side effects while graft function is preserved [6,7]. Because liver tests have a low sensitivity and specificity to detect graft dysfunction, it is difficult to titrate immunosuppressive therapy to its minimal dose based only on these analytic abnormalities [4,6,8].

The main aim of this study was to determine late graft histological abnormalities 10 years after liver transplantation in patients with normal liver tests. We also evaluated changes in immunosuppressive therapy due to histological abnormalities.

## METHODS

This retrospective study evaluated adult patients who survived 10 years after liver transplantation performed from 1995 to 2004 who had been followed in a liver transplantation center in the North of Portugal. We excluded retransplantation, autoimmune, and viral hepatitis as cause of transplantation.

Before liver transplantation, all patients were screened for hepatitis B and C virus and for autoimmune auto-antibodies (antinuclear, anti-smooth muscle, anti-mitochondrial, anti-liver/kidney microsomal, anti-liver cytosolic antigen type 1, and anti-neutrophil cytoplasmic antibodies). These tests were repeated at the same time of the biopsy. Biopsy was performed in patients 10 years after liver transplantation according to the liver biopsy protocol used in this center. Liver enzymes of the patients included were sustainably normal for a period of more than 1 year. These tests consisted in measuring of total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase. Abnormal liver tests were defined as a repeatedly elevated level of liver enzymes 2 times the superior normal limit.

The histology reports were reviewed by an attending pathologist and were categorized according to the histopathological findings as normal, nearly normal (minimum portal and/or parenchymal mononuclear infiltrates, mild bile ductular proliferation, mild portal fibrosis or less than 10% steatosis), cellular rejection, chronic nonspecific hepatitis, and with features suggestive of autoimmune hepatitis [3].

## RESULTS

We studied 39 patients from a total of 215 who had been transplanted between 1995 and 2004 who also had a 10-year protocol liver biopsy (Fig 1). Fifty one percent were males with a mean age at transplantation of 37 $\pm$ 10.2 years and a mean age at the time of biopsy of 50.5 $\pm$ 10 years. The transplantation indication was familial amyloid polyneuropathy in 27 patients, acute fulminant hepatitis in 2, and cirrhosis in 10 patients.

From these 39 patients, only 26 had normal or nearly normal histological features. We observed graft histological abnormalities in 13 patients, 3 with cellular rejection, 3 with unspecific chronic hepatitis, and 7 with features suggestive of autoimmune hepatitis. In all the patients of this last group (n = 7) contemporaneous autoimmune autoantibodies were positive (Fig 2).

In five of seven patients with the proposed diagnosis of de novo autoimmune hepatitis, the immunosuppressive therapy was changed. In two patients we added prednisolone and in the other three patients we switched the immunosuppressive regimen to tacrolimus and prednisolone based on the gravity of histological abnormalities.

## DISCUSSION

Histological abnormalities are commonly present in late post-transplantation biopsy specimens from adult and pediatric liver allograft recipients from patients who appear to have a good clinical graft function and normal liver tests [4,5,9]. Berenguer et al reported chronic hepatitis in almost 15% of patients grafted for non–hepatitis C related cirrhosis after 1 year and 33% after 5 years post-transplantation [9]. Heneghan et al reported nonspecific inflammation in 68% of patients grafted for hepatitis C, 25% for alcoholic liver disease, and 22% for cryptogenic cirrhosis, suggesting that the prevalence of severe recurrent disease in patients who underwent transplantation for cryptogenic cirrhosis was low. In contrast, patients who underwent transplantation for hepatitis C virus–related cirrhosis had a significant risk of developing progressive fibrosis and cirrhosis [10]. Burra et al identified mostly fatty liver disease in patients undergoing transplantation for alcoholic cirrhosis [11]. Abraham et al found clinically significant histological abnormalities in 11.5% of specimens from all biopsies performed in patients with normal liver tests during 5-year follow-up examinations, mainly with non-alcoholic fatty liver disease [3].

It is well known that recurrent or newly onset liver disease after transplantation can be present despite normal liver tests [3]. Sebagh et al estimated that the sensitivity and specificity of normal liver tests are 75% and 54%, respectively, drawing attention to the need for implementation of liver recipient biopsy protocols [4].

In this study, at 10-year post–liver transplantation, we confirmed that 13 patients had graft histological abnormalities despite normal liver tests. The most common histological features were compatible with autoimmune hepatitis, which was reported in seven patients. In five of