



## THE RESULTS OF THE HISTOLOGICAL EXAMINATION OF THE LIVER IN THE POST-TRANSPLANTATION PERIOD

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### ABSTRACT

*Background: Liver transplantation has been accepted as an effective therapeutic option for patients with acute or chronic end-stage liver diseases. Histological abnormalities are commonly present in late post-transplant biopsies from adult and pediatric liver allograft recipients. Material and methods: For 5.5 years, biopsies were performed in 28 patients after liver transplantation, among them 19 women, 9 men, and 1 6-year-old child. The cause of terminal liver disease in 35.7% of these patients was viral hepatitis D, in 21.4% was primary biliary cirrhosis, in 17.85% was hepatitis B virus, in 14.2% was hepatitis C virus, and in 10.7% was autoimmune hepatitis. Results: The result of a morphological study of the liver showed that in 50% of patients with changes in biochemical parameters, acute and chronic rejection is diagnosed. Biliary complications were found in 10.7% of cases. Recurrent liver diseases were confirmed in 17.9% of patients after liver transplantation. Among the patients, 3.6% had confirmed cholangitis and 7.1% were diagnosed with liver steatosis. Conclusion: Histopathologic assessment of allograft liver biopsies plays an important role in the differential diagnosis of post-transplantation complications.*

**KEYWORDS:** liver transplantation, rejection, recurrent liver disease

### INTRODUCTION

Liver transplantation has been accepted as an effective therapeutic option for patients with acute or chronic end-stage liver diseases. However, during the post-operative course, liver transplant recipients will face rejection due to alloantigens and a number of complications. Among these complications, hepatic artery thrombosis, intrahepatic biliary injury, preservation-reperfusion injury, opportunistic infection, and immunosuppressive drug-induced hepatic injury are critical for allograft poor liver function. Clinically, the complications do not have specific symptoms and signs, although supervision of blood biochemistry, Dop-

pler ultrasound, and radiologic imaging has some value for the evaluation of graft liver dysfunction; the final diagnosis still relies on liver biopsy evaluation [Hübscher SG, 2011].

These biopsies covered a number of complications following liver transplantation (LT). Histological abnormalities are commonly present in late post-transplant biopsies from adult and pediatric liver allograft recipients. Recurrent disease is the most commonly recognized cause of abnormal graft histology in late biopsies from adults, but is very uncommon in the pediatric population. The features of recurrent disease may be modified by the effects of immunosuppression and interaction with other graft complications, resulting in changes that are complex and difficult to interpret [Hübscher SG, 2011].

Liver biopsy can provide a diagnosis in ~90% of patients with unexplained abnormal liver function tests [Hübscher SG, 2011]. In transplant recipients, liver biopsy is the gold standard for the

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diagnosis of acute rejection [Saad WE, et al., 2013; Chau TN, et al., 2002].

The aim of the present study was to assess allograft liver biopsies for the differential diagnosis of post-transplantation complications.

**SUBJECTS AND METHODS**

The program of liver transplantation in Kazakhstan started in 2011. In Almaty through August 2017, we performed 97 transplants from living donors and 15 from cadaveric donors.

Liver transplant recipients undergoing liver biopsy between 2012 and 2016 were retrospectively analyzed. A total of 39 liver biopsies in 28 patients after orthotopic liver transplantation (OLT) fulfilled the inclusion criteria. The biopsies were classified according to the main histological diagnosis. All biopsies were clinically indicated.

The 28 post-transplantation patients included 19 women, 9 men, and 1 6-year-old child. The causes of terminal liver disease in these patients was viral hepatitis D in 35.7%, primary biliary cirrhosis in 21.4%, hepatitis B virus in 17.85%, hepatitis C virus in 14.2%, and autoimmune hepatitis in 10.7%. In the table 1 presents the patient characteristics and transplantation data, including demographic data, transplantation data, and indications for OLT from the study cohort (n=28).

**RESULTS AND DISCUSSION**

Histological assessments continue to play an important role in the diagnosis and management of liver allograft rejection. Since 1997, with the introduction of the Banff Classification of liver

allograft rejection, most centers have assumed a unified approach to the diagnosis and grading of acute cellular rejection [Emetris, JD, et al., 1997]. Although the prevalence of acute rejection (AR) is declining, 20-40% of patients still have one or more episodes requiring treatment with additional immunosuppression [Demetris A, et al., 2000]. These episodes usually occur during the first 3 months of transplantation and the diagnosis at this time is generally easy. The updated Banff schema published in 2000 is also widely used for the diagnosis and staging of chronic rejection (CR) [Shaked A, et al., 2009].

In the table 2 presents histological diagnoses of 28 liver biopsies obtained >3 months post-transplant during a 5.5-year period (2011-2017) at the Department of Hepatopancreatobiliary Surgery and Liver Transplantation, National Scientific Center of Surgery, Almaty, Kazakhstan.

TABLE 2.

Histological diagnoses	
Demographics	Number of patients
Gender	19 female, 9 male
Age (years)	27 (6-52)
Main diagnosis	
Rejection	
Acute indefinite	3
Mild	4
Moderate	3
Severe	3
Chronic	1
Total	14 (50%)
Biliary obstruction/cholestasis	3 (10.7%)
Recurrent disease	
Primary Biliary Cirrhosis	3
Hepatitis C virus	1
Hepatitis B virus	1
Total	5 (17.9%)
Cholangitis	1 (3.6%)
Steatosis	2 (7.1%)
Reperfusion damage	1 (3.6%)
Graft dysfunction	2 (7.1%)

Note: Scale for rejections: acute indefinite -RAI, mild - RAI 4-5, moderate - RAI 6-7, severe -RAI 8-9,

TABLE 1.

Indications for orthotopic liver transplantation		
	Number of patients	Percentage
Hepatitis C virus	4/28	14.2
Hepatitis B virus	5/28	17.85
Hepatitis D virus	10/28	35.7
Primary biliary cirrhosis	6/28	21.4
Autoimmune hepatitis	3/28	10.71
Type of liver transplantation performed		
Living donor	19/28	67.86
Cadaveric donor	9/15 %	60

The majority of patients were treated with a triple therapy, and only 3.6% (1/28) received monotherapy. Among those with severe rejection was a patient with PBC-AIH overlap syndrome. The patient was refractory to methylprednisolone. Simultaneously with the rejection, the patient developed end-stage renal failure. In the second case, severe ACR developed in a patient with recurrent HBV infection. The patients with RAI 3-4 did not receive pulse therapy (methylprednisolone) as they had biliary and septic complications.

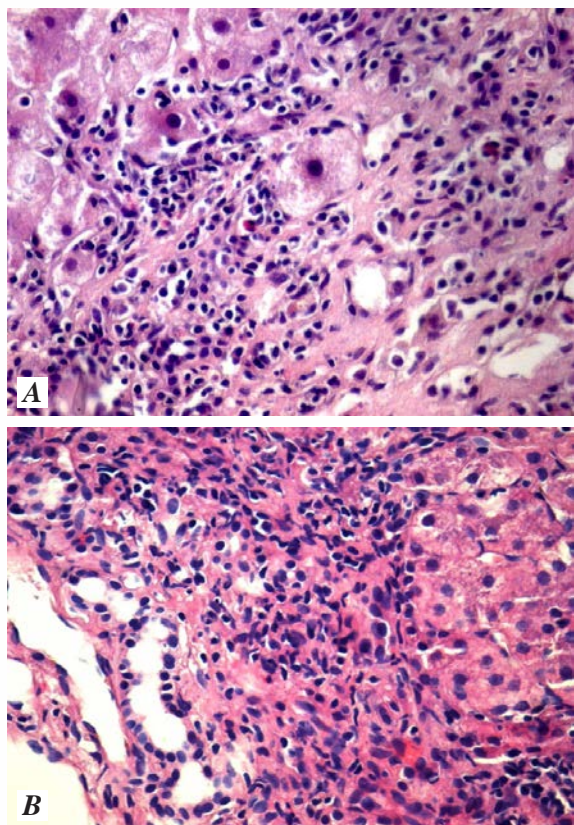
One patient with severe rejection (RAI 8) interrupted immunosuppressive therapy for 1.5 months 1.5 years after LT. He had cirrhosis-caused hepatitis B and D and biliary stricture 6 months after LT (Figures 1A and 1B).

**Recurrence of hepatitis C:** In the diagnosis of recurrent HCV (excluding other causes of graft dysfunction), liver biopsies are used to assess disease severity and progression. Histological abnormalities are often present in protocol biopsies from HCV-positive patients who are clinically healthy, with apparently normal graft function [Sebagh M,

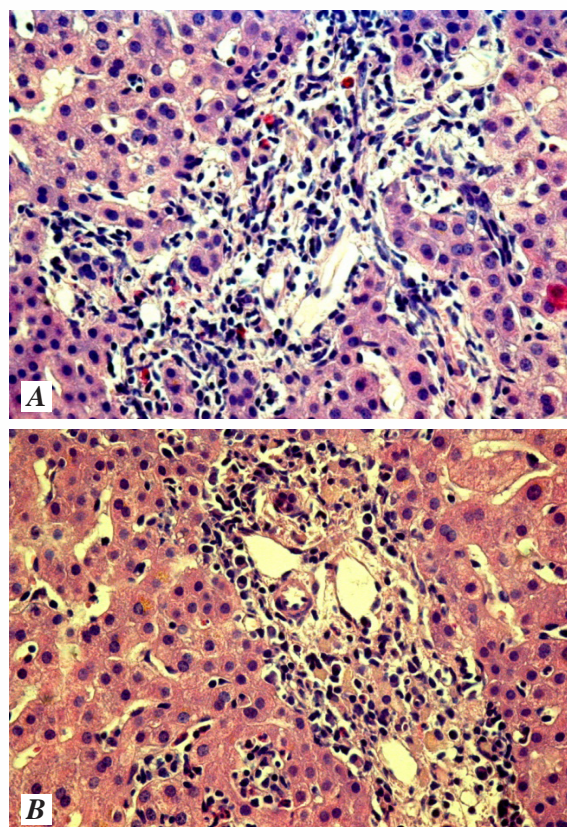
et al., 2003] and the changes seen in these specimens may have implications for prognosis and treatment [Roche B, et al., 2010]. For example, the presence and severity of fibrosis at one year have been shown to be predictive for subsequent progression to cirrhosis and graft failure [Firpi RJ, et al., 2010; Neumann UP, et al., 2004; Samuel D, et al., 2007]. This information may help identify patients who are most likely to benefit most from antiviral therapy [Pagadala M, et al., 2009].

Recurrent hepatitis C was found in a patient with genotype 1b. This patient received pegylated interferon and other direct-action antiviral drugs. The reason for recurrent hepatitis B was the interruption of antiviral therapy 9 months after LT (Figures 2A and 2B).

**Recurrent primary biliary cirrhosis:** Four patients transplanted for PBC were followed for up to 3.5 years. Some 1.5 years after surgery, 3 patients experienced elevations of ALT, alkaline



**FIGURE 1.** Acute rejection crisis with (A) moderately affected bile duct lesions, (B) venous endothelial sites with small foci of central perivenulitis. HE X 200.



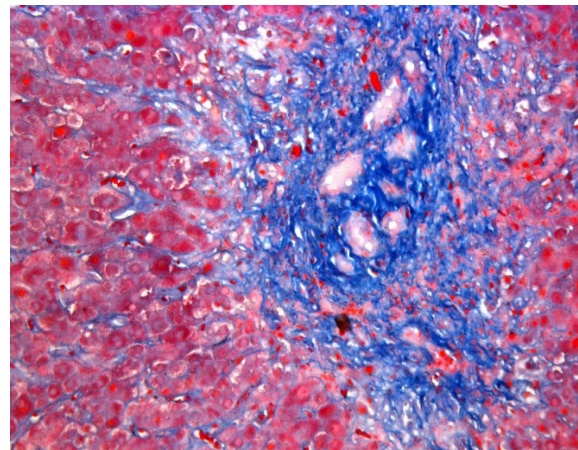
**FIGURE 2A.** Liver biopsy 1 year 2 months post-transplant. Recurrent hepatitis C (A) with moderately pronounced inflammatory activity. In the portal tracts, a moderate infiltrate consisting of mononuclear cells was determined. (B) There is marked perivenular inflammation with foci of necrosis of individual hepatocytes. HE X 100.

phosphatase up to 2 norms, but a liver biopsy diagnosed graft dysfunction. Two patients had diabetes, and 1 patient had biliary stricture 2 years after transplantation. Titers of AMA fell to undetectable levels shortly after transplantation in all recipients; however, values similar to or higher than those before transplantation were observed over time. Biopsies revealed portal inflammation, lymphoid aggregates, granulomas, bile duct injury, and ductopenia. The histology was not suggestive of chronic rejection (Figures 3 and 4).

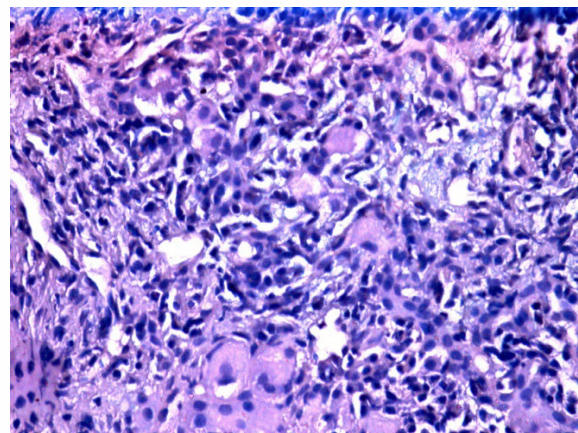
**Non-alcoholic fatty liver disease:** The distinction between recurrent and de novo NAFLD is often difficult. Liver transplant patients are at risk for developing a number of features of the metabolic syndrome, such as diabetes mellitus, weight gain, hypertension, and hyperlipidemia and are thus predisposed to the development of NAFLD [Watt KD, 2010; Watt KD, et al., 2010]. Steatosis in the donor liver has also been identified as a risk factor for the development of steatosis in late post-transplant biopsies, although the mechanism for this is uncertain [Ong J, et al., 2001]. Several studies have identified cases of NAFLD that appear to have arisen de novo following liver transplantation [Watt KD, 2010; Ong J, et al., 2001]. However, some of these have occurred in patients who were transplanted for cryptogenic cirrhosis and/or had risk factors for the metabolic syndrome prior to transplantation and could thus be regarded as having recurrent rather than de novo disease [Dumortier J, et al., 2010; Seo S, et al., 2007]. Interactions between hepatitis C infection, insulin resistance, and NAFLD also appear to be important in the pathogenesis of recurrent HCV and de novo NAFLD [Abdelmalek MF, et al., 2007; Desley AH Neil, et al., 2010].

In the 2 cases of NAFLD, in 1 patient the cause was metabolic changes that occurred de novo. The second patient was a 6-year-old child. The most likely cause in this case was steatosis of the donor liver. The indications of the liver biopsy in the child were high rates of transaminases throughout the post-transplant period (1 year 3 months) (Figures 5 and 6).

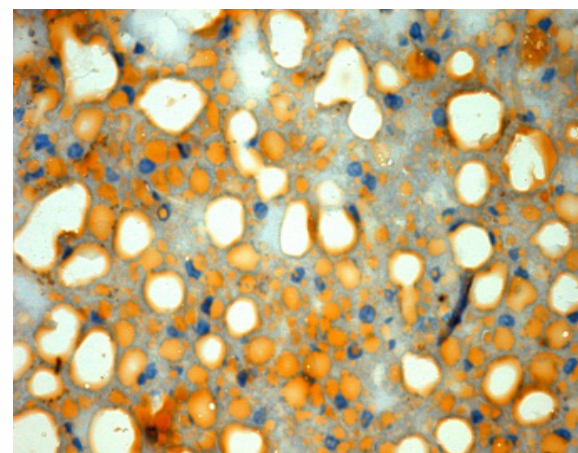
The result of a morphological study of the liver showed that in 50% of patients with changes in biochemical parameters, acute and chronic rejection is diagnosed. Biliary complications were



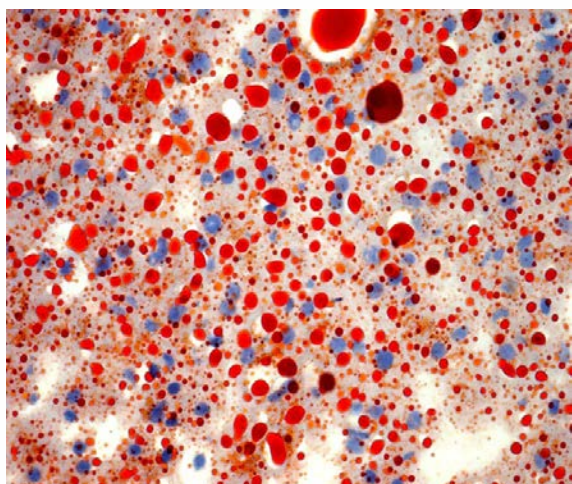
**FIGURE 3.** Liver biopsy 2 years post-transplant. Recurrent PBC. In the portal tract, there is a proliferation of coarse fibrous connective tissue with the formation of short septa and the compression of newly formed bile ductules.



**FIGURE 4.** Recurrent PBC. There is proliferation of the small bile ducts and foci of the ductules with marked lymphocytic infiltration. Stepwise necrosis and moderately pronounced dystrophic changes of individual hepatic cells formed.



**FIGURE 5.** Large-droplet steatosis of the transplanted liver with 74% displacement of the nuclei of individual hepatocytes to the periphery in a 6-year-old patient after 1 year post-transplant. Oil red X 100.



**FIGURE 6.** Large-droplet steatosis in 52% of the transplanted liver in a 54-year-old patient after 3 years post-transplant. Oil red X 100.

found in 10.7% of cases. Recurrent liver diseases were confirmed in 17.9% of patients after liver transplantation. Among the patients, 3.6% had confirmed cholangitis and 7.1% were diagnosed with liver steatosis.

An evaluation of allograft liver biopsy at the National Scientific Center of Surgery named after A.N.Syzganov revealed that although acute and chronic rejection was a major factor in liver dysfunction, all cases of acute rejection were promptly diag-

nosed and cured via close cooperation between clinicians and pathologists [Saad WE, et al., 2013]. The overall occurrence of chronic rejection was 3.6%.

The evaluation found a 17.9% rate of recurrent liver disease. The results of liver biopsies at various transplantation centers showed that the frequency of recurrent liver diseases varies from 13.2% to 20% [Hübscher SG, 2011; Sebah M, et al., 2003]. The incidence of biliary complications found during biopsy was 10.7%.

#### CONCLUSION

Liver histology remains the gold standard test for the diagnosis of allograft dysfunction [19], rejection of the transplant, NAFLD, and recurrence of viral hepatitis. Therefore, histopathologic assessments of allograft liver biopsies play an important role in managing liver transplantation patients.

Many common post-transplantation complications cannot be differentiated by clinical, paraclinical, and imaging studies; in many situations, more than one cause contributes to graft dysfunction. Hence histopathologic assessment of allograft liver biopsies plays an important role in the differential diagnosis of post-transplantation complications, identifying the cause of graft damage, and subsequently initiating appropriate therapeutic intervention.

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