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Graft 2002; 5; 102

DOI: 10.1177/1522162802005002009

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Chronic Rejection after Human Liver Transplantation

Ulf P. Neumann, Jan M. Langrehr, and Peter Neuhaus

Survival figures after orthotopic liver transplantation (OLT) have markedly improved, with 1-year and 5-year patient survival rates approaching 90% and 80%, respectively. With increased short-term survival rates, chronic rejection (CR) has emerged as a major factor of morbidity and mortality after OLT. CR is characterized by the histological findings of ductopenia and decreased number of hepatic arteries in portal tracts in the presence of obliterative arteriopathy. However, in contrast to acute rejection, the incidence of CR is currently decreasing to 3% to 4%. The greatest risk factors for CR were acute or recurrent rejection episodes and retransplantation due to CRs. Other factors were underlying liver disease (autoimmune hepatitis, primary sclerosing cholangitis), cytomegalovirus (CMV) infection, and low levels of immunosuppression. Reasons for the decline of CR in clinical transplantations are unknown but may relate to improved immunosuppression. Outcome of CR after liver transplantation is poor and leads to graft failure more than 50% of the time. Treatments with tacrolimus and mycophenolate mofetil have shown to reverse CR, particularly when diagnosed in early histological stages.

ABBREVIATIONS

CR chronic rejection
OLT orthotopic liver transplantation

Ulf P. Neuhaus, M.D., Ph.D.
Chirurgische Klinik und Poliklinik Charité
Campus Virchow-Klinikum
Humboldt Universität zu Berlin
Augustenburger Platz 1
13353 Berlin
Germany
Tel.: +49.30.450.552001
Fax: +49.30.450.552900
email: peter.neuhaus@charite.de

Introduction

In contrast to other vascularized allografts, chronic liver allograft rejection is uncommon.¹⁻³ During the past decade, the incidence of chronic rejection (CR) has decreased to 3% in liver allograft recipients.⁴⁻⁶ Reasons for low incidence of CR are the unique immunologic properties of the liver and better recognition and control of acute rejection, but they may also be related to the remarkable regenerative capabilities.^{3,7-9} Nevertheless, CR still plays a role, and proper recognition and staging are essential for long-term management of liver transplant recipients. Furthermore, current concepts of calcineurin inhibitors and steroid-sparing immunosuppressive regimens have to be carefully evaluated so as not to increase the incidence of CR after orthotopic liver transplantation (OLT).

Definition of Chronic Rejection

Chronic liver rejection can be defined as an immunologic injury to the graft, which occurs after severe or persistent acute rejection and results in irreversible loss of bile ducts, arteries, and veins.^{4,10,11}

The onset of the disease is during the 1st year post-transplant and occurs only in rare cases in the long term after OLT.^{10,12} Late CR might also develop after a therapy refractory late acute rejection episode. From our experiences, this late appearance of CR mainly evolves in patients who are noncompliant to immunosuppressive therapy, or where immunosuppression is lowered for other reasons.

Clinical and Histopathological Features

CR is usually expected in patients with a history of acute rejection, increasing jaundice, and an increase of cholestatic markers.¹³ This pattern is the end-stage after unresolved acute rejection or multiple acute rejection.^{14,15} After treatment of acute rejection, these patients often experience an elevation of gamma-glutamyl transpeptidase, alkaline phosphatase, and bilirubin.¹² Imaging techniques such as CT scan and magnetic resonance imaging have not been able to distinguish CR and other causes of liver dysfunction, such as ischemic-type biliary lesions, reinfection of hepatitis C, and acute rejection. However, angiography could present rarifica-

tion and occlusion of intrahepatic vessels in some patients.^{16,17} Graft biopsy specimen during the course of the disease reveal bile duct atrophy/pyknosis, affecting the majority of bile ducts with or without bile duct loss. At present, in more than 50% of the portal tracts a foam cell obliterative arteriopathy or bile duct loss is found.^{2,18,19} Unfortunately, arteries with pathognomonic changes are rarely present in needle biopsies, and therefore obliterative arteriopathy is mainly observed in liver allografts removed at the time point of retransplantation or autopsy.² It has been proven in the past that there are distinctive early signs of CR in liver biopsy specimen, and lobular inflammation was present very early in the course of the disease.²⁰ Additional features are eosinophilic transformation of biliary epithelial cytoplasm, nuclear enlargement and hyperchromasia, and ducts without complete lining by biliary epithelial cells (Figs. 1-2).² The precise mechanism of CR is still unknown and is thought to be multifactorial. Examination of failed hepatic allografts showed lymphocytes in close contact to the degenerated bile duct cells, which suggests that a direct lymphocytotoxic reaction may play a role in bile duct damage. This has been confirmed by *in vitro* studies, showing direct lymphocytotoxic reactions against major histocompatibility complex (MHC) antigens.^{21,22} MHC I antigens are commonly expressed on bile duct epithelial cells, whereas MHC II is present only during acute rejection or other inflammatory conditions. Immunohistochemical stainings showed that the lymphocyte profile consists mainly of CD3⁺CD4⁺, CD3⁺CD4⁻-T cells, CD20⁺-B cells, and CD68⁺ macrophages.²³ Acute rejection, which initiates immune response damage, supports the hypothesis that several inflammatory cytokines such as tumor necrosis factor- α , interleukin-2, interleukin-6, and tumor growth factor- β are up-regulated during the course of the disease.^{6,23} With the destruction of small bile ducts, the intensity of inflammation diminishes.²⁴ Vice versa, the lack of increasing markers of the extracellular matrix, such as laminin and fibronectin,⁶ are responsible for the inability of the liver to recover from acute rejection. The up-regulation of proinflammatory cytokines may lead to activation of graft endothelium. As a consequence, endothelial and smooth muscle cell activation is

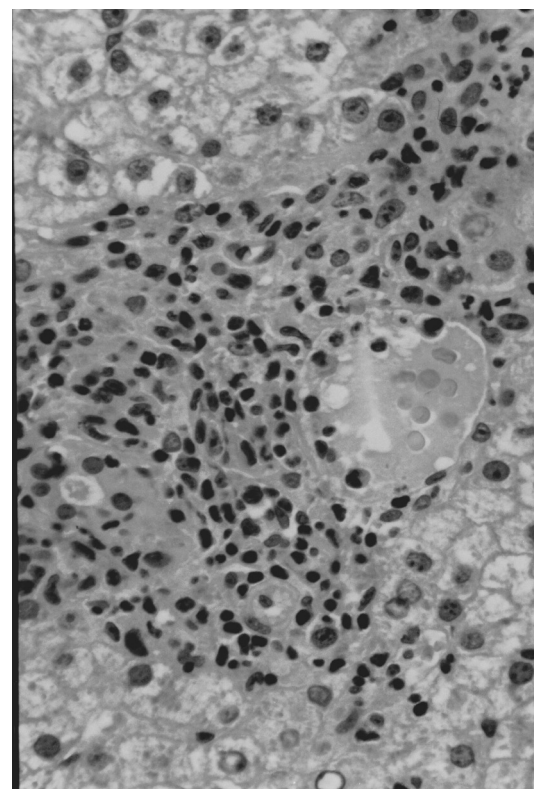


Figure 1. Early chronic rejection with intraepithelial lymphocytes and moderate bile duct damage. The biliary epithelium reveals eosinophilic transformation of the cytoplasm (Hematoxylin and eosin; original magnification $\times 250$).

generated, leading to graft arterial intimal thickening. With progressive arterial occlusion, even large bile ducts degenerate, presumably caused by ischemia of the biliary tree, which is mainly nourished by arterial vessels and not by the portal vein.

Incidence and Outcome of CR after OLT

Recent studies show that the incidence of CR decreased in the past years. It has been reported that, prior to 1990, the incidence of CR was up to 20%.²⁵ Currently, the large multicenter studies with tacrolimus immunosuppressive induction protocols reported the incidence of CR between 1.5% and 3.0%.²⁶ In our own center, the incidence of CR in 1000 liver transplant recipients was 2.4%. The reasons for the decreasing number of CR are not clear but may reflect improved immunosuppression in recent years. In contrast to these results in adult pa-

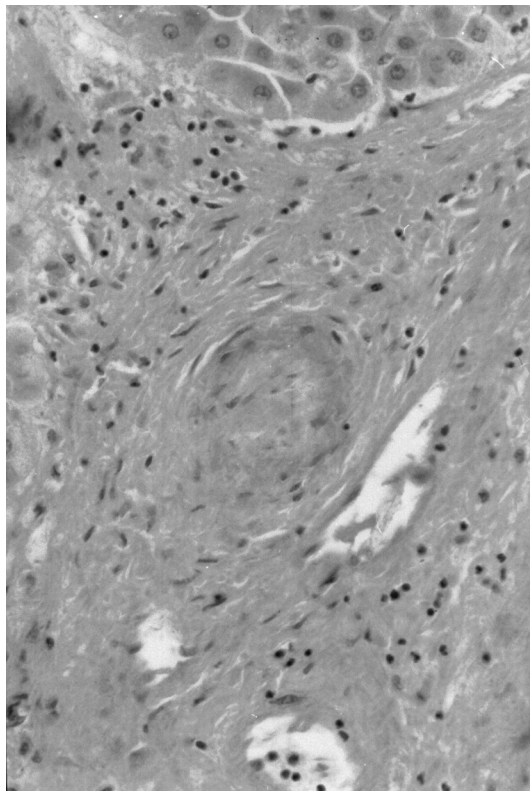


Figure 2. Late chronic rejection with bile duct loss of the portal tract and severe graft vasculopathy. The artery is completely obliterated.

tients, the number of CR in pediatric patients remains stable, between 8% and 12%.²⁷ Other factors that might play a role are an improved interpretation of histological findings in graft specimen, the improved knowledge and new markers on recurrence of viral hepatitis after OLT (which helps the clinician and the pathologist to distinguish between CR and recurrence of the origin disease), and better differential diagnosis to discern biliary obstruction, infections, and drug toxicity. In addition, recurrent primary biliary cirrhosis (PBC) may show the same clinical picture as described above for CR. However, in patients with recurrent PBC, the histological findings present florid duct lesions and staining of biliary epithelial cells with a monoclonal antibody directed to pyruvate dehydrogenase complex in combination with high antimitochondrial antibodies, which is unique.²⁸ In contrast, it is more difficult to distinguish a primary sclerosing

cholangitis from CR. Cholangiography, in patients with recurrent primary sclerosing cholangitis, commonly demonstrates intrahepatic strictures of the bile ducts. These findings can also be observed in CR. Recurrence of hepatitis C virus (HCV) and CMV infections can be diagnosed by DNA and RNA measurements of the virus in blood samples and liver specimen. Histological examination, which reveals ductopenia in the presence of typical obliterative arterial lesions, is nearly evidencing for CR.

In the past decade, a number of risk factors for the development of CR were reported; however, these could not be confirmed in more recent studies. The most important ones are retransplantation for graft failure due to CR and number and severity of acute rejection episodes.¹⁰ Other factors include the underlying disease (Fig. 3), where autoimmune cirrhosis and primary sclerosing cholangitis are at high risk to experience CR episodes.²⁹⁻³² Only in cyclosporine-treated patients have late onset of acute rejection,³³ male-female mismatch,³² younger recipient age,⁸ baseline immunosuppression, non-Caucasian race,³⁴ and absence of azathioprine been reported to increase the risk of CR. Whereas crossmatch and HLA mismatches have been identified as important risk factors for CR in kidney and heart transplantation, they remain controversial as risk factors for CR after liver transplantation. CMV infection has also been reported as a strong risk factor for the development of CR.³⁵ This is explained by MHC II antigens on bile duct epithels and consecutively enhanced immune and inflammatory response. At the same time, adhesion molecules have been shown to increase biliary endothelial cells. Another risk factor was the introduction of interferon- α therapy in HCV-positive patients for recurrent hepatitis C. However, of all these factors, we could only confirm a previous acute rejection episode and underlying disease as significant risk factors during a multivariate analysis of our own patient population.

Other factors that contribute to the development of CR include donor age > 40 years,¹⁵ which is described for heart and kidney transplant and could not be confirmed in our patients.

Treatment of CR

Early reports of the long-term follow-up of patients with CR showed that only 30% of the pa-

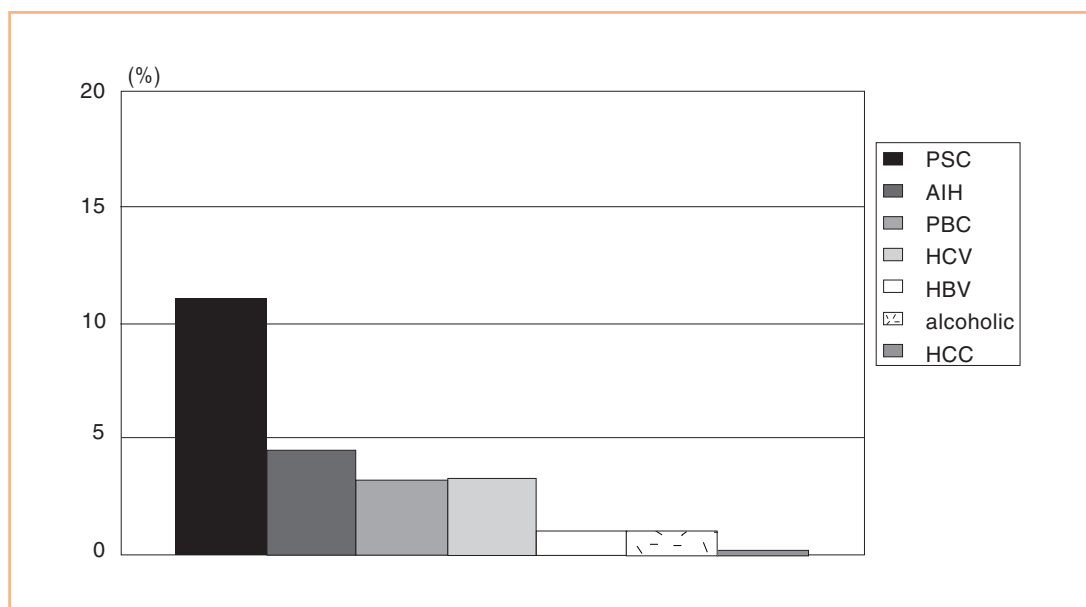


Figure 3. Incidence of chronic rejection depending on the primary diagnosis in 1000 transplants in Berlin. The risk for the development of chronic rejection is significantly increased in patients with primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). None of the patients with a hepatocellular carcinoma developed a chronic rejection during the course of the disease.

tients recover with conventional immunosuppressive drug regimens.^{9,36} The introduction of tacrolimus and mycophenolate mofetil (MMF) in clinical liver transplantation yielded new therapeutic approaches for CR.^{7,37-39} An important factor for outcome is the time point of diagnosis. Recent studies have shown that early treatment can reverse CR. Studies in large patient populations showed that tacrolimus rescue is effective to reverse CR. Two factors that significantly influence the outcome were identified in this study. Patients with a total bilirubin > 10 mg/dl and time between transplantation and tacrolimus rescue at > 90 days were at high risk for the development of graft failure. Mean total bilirubin levels in the responder group were 7.1 mg/dl and decreased to 3.4 mg/dl at the end of the study. Alternative treatment with MMF showed that 65% of the patients improved during the course of the disease with well-functioning grafts.³⁸ However, there is a lack of data on the combined treatment of tacrolimus and MMF. The overall 5-year survival figures of patients developing a CR in our own patient population was 40% and therefore significantly less when compared with patients without CR. Howev-

er, new therapeutic agents, such as Rapamycin and anti-B7 antibodies, have proven to be effective in preventing and treating transplant arteriopathy in several animal models. Their value in the clinical setting after OLT needs to be assessed in the future. The outcome of patients with bilirubin levels greater than 20 mg/dl and complete absence of bile ducts in histological specimens is still poor. In those patients, early retransplantation should be considered and increase of immunosuppressive therapy should be performed carefully to prevent severe complications from overimmunosuppression.

Summary

Frequency of CR has decreased in recent years, with a current incidence of less than 3%. Furthermore, today more than 50% of the patients respond to additional immunosuppressive therapy. One key factor for this is the early diagnosis of CR during the course of the disease. In contrast to this, the morbidity and mortality caused by side effects of immunosuppressive therapy is increasing. The frequency of de novo malignancies has been reported to be up to 15% after 5 years after OLT.⁴⁰ These

PRIMARY SCLEROSING CHOLANGITIS:

A chronic cholestatic liver disease of unknown cause.

data can be confirmed in our own patient population. Other important side effects are diabetes, hypertension, renal failure, and bone complications. Unfortunately, we have only been progressing slowly at identifying patients with high risk of severe immunologic complications. Up to now, there are only a few markers to measure alloreactivity. In the future, it will be necessary to perform individualized immunosuppressive treatment to reduce the risk of overimmunosuppression in the long term. Whether routine liver biopsies are helpful to adapt the individual immunosuppressive therapy is not yet clear and awaits to be proven in the future. However, when minimizing immunosuppressive regimens, early diagnosis and treatment is necessary to prevent severe complications from chronic rejection.

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