Impact and Prevention of Late Acute Rejection in Liver Transplant Recipients

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Abstract

Immunological issues related to liver transplantation continue to be a frequent event, mainly during the first six months and more frequently during the first three. To minimize immunosuppression therapy is a common practice due to patients frequently dying because of over-immunosuppression as a result of infection and disease recurrence. The incidence of late acute rejection, defined as rejection occurring between 90 and 180 days and beyond, has been established at between 7-23%. Noncompliant patients or those with low blood levels of immunosuppressive drugs have been the main cause of late acute rejection. Variables additionally identified with higher incidences of late acute rejection include: posttransplant lymphoproliferative disease, autoimmune cirrhosis, primary sclerosing cholangitis, primary biliary cirrhosis, female gender, and youth. The use of cyclosporine instead of tacrolimus, taking two drugs instead of three, and not including mycophenolate mofetil have been associated to late acute rejection as well. Pathological findings are quite similar to acute cellular rejection, with only slight differences: fewer blastic lymphocytes, greater interface activity, less venous subendothelial inflammation, and higher lobular activity. The practice of directly confronting patients with noncompliance or those who manipulate treatment may be helpful. The vast majority of them can be managed successfully with steroids and only a minority will need more aggressive immunosuppression. **(Trends in Transplant. 2010;4:29-35)**

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Introduction

The success of liver transplantation has traditionally been related to patient survival, which has been improving over time and can be considered as excellent. Survival rates in the USA, with only slight differences in Europe, reach numbers as high as 90 and 70% for one and five year follow-up, respectively. For some specific indications, such as cholestatic liver disease, survival is even better[1,2]. As long as the survival expectancy was not a matter of concern, most attention was paid to issues related to long-term follow-up. Recurrence of underlying disease, complications of immunosuppression and *de novo* diseases like metabolic syndrome are frequent and impair both the duration and quality of life among survivors. As a consequence, less emphasis is placed now on survival and more on issues dealing with medical management and rehabilitation after transplantation.

Immunological issues related to liver transplantation continue to be a frequent event[3]. The vast majority of acute rejection occurs during the first six months and more frequently during the first three. Patients frequently die due to over-immunosuppression as a result of infection and disease recurrence and this is the reason to minimize immunosuppression therapy. Consequently, some patients will be able to reach tolerance, but the greater percentage of them will be at continued risk of rejection, which means that immunosuppression therapy will be permanent. In spite of the fact that immunosuppressive therapy itself contributes to early mortality by increasing susceptibility to infection, rejection is one of the most common causes of graft loss after liver transplantation. This is a difficult balance that challenges both patients and clinicians.

Acute cellular rejection (ACR) and particularly its late presentation has been associated with both resistance to immunosuppressive therapy and higher rate of graft loss as a consequence of ductopenic rejection[4,5]. In this paper, we will review the significance of acute rejection in general, and then we will focus specifically on late acute rejection (LAR) by assessing long-term consequences and therapy strategy.

Terms and definitions

The routine use of liver biopsy has provided much information regarding the frequency of rejection and the definition of histological features[6]. Acute cellular rejection occurs most often during the first few weeks once liver transplantation has been performed. It affects as many as 70% of patients[7]. The timetable of ACR appears to be important, not only in regards to outcome but also the frequency. While the majority of ACR occurs during the first three months, a minority will appear after this period of time. The incidence of LAR has been established at 7-23%[5,8,9]. There is no consensus about when ACR may be considered as a late acute cellular rejection, but most authors define that as occurring between 90 and 180 days and beyond[9-11]. The differences in the definition of late onset and the different surveillance practices (center effect) might be the reasons that account for significant differences between centers.

Chronic rejection, as established by Banff Working Group, has been based more on morphologic features than on the time that it appears[12-14]. The overall incidence has been declining, with fewer than 2% of grafts failing as a result of chronic rejection[15].

Etiology and risk factors

Medical literature analyzing LAR is scarce and studies dealing with this issue are generally retrospective. Patient noncompliance has been pointed out as the most important cause of LAR. An early study assessing

noncompliance in liver, kidney, and heart patients indicated that this behavioral pattern is an important factor for late rejection and graft loss in all three groups[16]. Mor, et al. in a retrospective study of 375 consecutive liver transplants, mainly in the cyclosporine (CsA) era, either found noncompliance or low CsA levels as the main causes for LAR[10]. Interestingly, they found that in four out of 31 patients, biliary strictures are a risk factor for developing LAR. They explained this factor for both low blood levels because of lack of absorption, which relies on normal enterohepatic absorption, and biliary obstruction, which has been associated with the increased expression of major histocompatibility antigens on the biliary epithelium, which results in cytokine release from inflammatory cells in the portal tracts[17].

Low levels of CsA or tacrolimus as a cause of LAR has been confirmed in further studies dealing with rejection[11]. More recently, Akamatsu, et al. found that the use of cyclosporine instead of tacrolimus is related to developing LAR[8]. The importance of immunosuppression has been highlighted by an extensive study performed by Wiesner, et al. on 9,646 patients from the Scientific Registry of Transplant Recipients data base[18]. They defined LAR as a rejection appearing beyond six months after liver transplantation. In this study, patients with viral (HCV and HBV) and nonviral causes for liver transplantation were included. Four years after liver transplantation, a Kaplan Meier analysis showed that lower LAR rates were seen in patients who had been taking three versus two drugs. The addition of mycophenolate mofetil (MMF) to the immunosuppressive regimen was protective for all groups. Similarly, acute rejection during the first six months after transplantation was the greatest risk factor for development of LAR, reaching a hazard ratio of 3.8 and 3.3, depending on the study group (viral and nonviral patients). In addition, African Americans as compared to Caucasians had an increase risk. In this study, they were not able to detect any effect of tapering, continuation, or noncompliance with steroids on the part of the patient. In a case-control study performed during the cyclosporine era in 1996, it was reported that the majority of LAR episodes (83%) occurred in patients receiving less than 5 mg of prednisone, while patients receiving more than 5 mg represented only a minority (8%)[19]. Ten years later, Akamatsu, et al. showed a significant difference in LAR-free survival between patients on CsA-based immunosuppression protocols versus tacrolimus. Multivariate analysis revealed that the CsA-based regimen was the only independent predictor. Interestingly, while the human leukocyte antigen donor/recipient (HLA-DR) mismatching and positive T-lymphocytotoxic crossmatch had been proved to be an independent significant factor to predict early acute rejection, neither HLA nor lymphocytotoxic crossmatch were associated with LAR[8].

In a more recent study focused on clinical records of 1,604 patients, they identified that 305 (19%) developed LAR[20]. The occurrence of posttransplant lymphoproliferative disease (PTLD) was the only significant predictor for the development of LAR. The authors hypothesized that LAR may be caused by decreased immunosuppression, which is an important feature of the treatment of PTLD. In addition, variables related to higher incidences of LAR include: autoimmune cirrhosis, primary sclerosing cholangitis, primary biliary cirrhosis, female gender, and youth (Table 1). As previous studies have shown, sepsis, biliary duct problems, and chronic rejection can be potential indicators of future LAR development. As discussed above, infection of the liver and the biliary system is well known to enhance the expression of major histocompatibility complex (MHC) antigen on hepatocytes and biliary epithelium[17]. Interestingly, in this study almost all cytomegalovirus (CMV) infections were followed by LAR and most chronic rejections occurred after LAR. O'Grady, et al. reported a relationship between CMV infection and LAR and also with chronic rejection[21]. Cytomegalovirus infection

Table 1. Most important studies dealing with late acute rejection in liver transplantation

Author/center	Incidence transplants/ episodes	LAR definition	Risk factors	Progression to chronic rejection	Comments
Mor E, et al.[10] Baylor Univ. (1992)	375/31 (8.2%)	> 180 days	Low CsA levels Biliary strictures Malabsorption	1/31 chronic rejection	Retrospective 6% graft loss
Anand A, et al.[11] Birmingham (1995)	717/71 (9.9%)	> 30 days	Either low or subtherapeutic CsA levels in half of sample	16 (27%)	Retrospective 27% graft loss 2% re-transplanted
Yoshida E, et al.[19] Vancouver (1996)	21/5 (24%)	> 365 days	Either low CsA levels or prednisone	1 (8%)	8% graft loss
Ramji A, et al.[9] Vancouver (2002)	415/97 (23%)	> 180 days	Non viral etiology† CsA vs. Tacrolimus* Use of Steroids in EAR*	9 (9%)	(6%) re-transplanted
Florman S, et al.[5] New Orleans (2004)	531/43	> 365 days	Noncompliance Previous ACR PSC as etiology	7 (16%)	4 deaths
Wiesner RH, et al.[18] SRTR (2006)	9646 (2.5-5% per year)	> 180 days	Two drugs not including MMF Nonviral cause as etiology African American Previous acute rejection Younger age Year of transplant < 1995	NA	LAR patients had a HR of 1.98 for death
Akatmasu N, et al.[8] Tokio (2006)	204/15 (living donors) 7%	> 180 days	CsA instead of tacrolimus	0%	
Uemura T, et al.[20] Baylor Univ. (2008)	1604/305 (19%)	> 180 days	Autoimmune hepatitis* PBC* PSC* Non-metabolic and non re-transplanted patients* Younger age* PTLD†	9 (3%)	Poorer graft and survival in patients with LAR More sepsis (HR: 2.9) Chronic rejection (HR: 2.3) and PTLD (HR: 3.5)

LAR: late acute rejection; CsA: cyclosporin A; HR: hazard ratio; EAR: early acute rejection; ACR: acute cellular rejection; PB: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; PTLD: posttransplant lymphoproliferative disease; SRTR: Scientific Registry of Transplant Recipients.
*Significant in univariate analysis;
†Significant in multivariate analysis.

has been associated with three factors: the persistence of the virus in the bile duct epithelium in patients with chronic rejection, an indirect immune effect associated with enhanced expression of HLA antigens and, consequently, adhesion molecules on biliary endothelial cells[22].

Pathologic findings

Differences in MHC antigens trigger a specific inflammatory response soon after liver transplantation[14]. Tissue damage at the beginning of the liver transplant releases cryptic antigens that activate endogenous danger signals. During this process non-MHC antigens take over autoantibody production. These antigens are shared by the donor and recipient, while some are donor specific[12]. Obtaining protocol liver biopsies is controversial. Biopsy interpretation from the Banff working group suggested “it should include an assessment of six portal tracts and the findings should then be correlated with the original

disease, immunosuppression, liver test, viral serology, and immunology as well as radiology findings".

Late acute rejection may show different features of typical acute rejection. Four features have been identified: fewer blastic lymphocytes, greater interface activity, less venous subendothelial inflammation, and higher lobular activity[13]. These features resemble a chronic hepatitis pattern. Late acute rejection can also present as isolated perivenular inflammation and hepatocyte dropout. This phenomenon, the so-called "central perivenulitis", was recently assessed in 100 adult allograft liver recipients who had long-term follow-up and routine protocol liver biopsies[23]. This fact was identified in 28 and 27% of adult and pediatric liver transplant patients, respectively, at 658 days of mean follow-up[24]. It usually occurred in conjunction with portal ACR, where it represents a significant risk for the development of zone 3 fibrosis and a tendency toward the development of ductopenic chronic rejection. This is the main reason to consider central perivenulitis as an injury with a poor prognosis[25]. With the exception of these cases evolving to chronic rejection with ductopenia, subendothelial inflammation of portal or central veins is not a required condition for LAR diagnosis. According to the Banff protocol, late acute rejection is characterized by:

- inflammation containing lymphocytes, neutrophils, and eosinophils,
- venous subendothelial inflammation of portal or central veins or perivenular inflammation, and
- inflammatory bile duct damage.

The Banff working group recommended that the grading should be done according to the following features (with the exception of those cases where LAR presents isolated central venulitis):

- Minimal: perivenular inflammation involving a minority of central veins with patchy perivenular hepatocyte loss without confluent necrosis.
- Mild: the above process involves a majority of central veins.
- Moderate: a focal confluent perivenular hepatocyte dropout and mild/moderate inflammation without bridging necrosis.
- Severe: confluent perivenular hepatocyte dropout and inflammation involving a majority of hepatic venules with central to central bridging necrosis[13].

While mild and minimal cases may resolve spontaneously, more severe cases warrant aggressive treatment.

Prognosis and therapeutic approaches

Acute cellular rejection continues to be an important cause of morbidity, but no longer represents the main cause of graft loss[26,27]. In an early study by Dousset, et al.[4], they reported that 51% of their patients had experienced a single episode of ACR. The rate of steroid-resistant ACR was quite high (23%) and chronic rejection was quite low (4%). In this study, there was no significant difference between patients with a single episode of ACR and non-rejecting patients for liver biochemistry, dye clearances, and histological features. In contrast, patients with multiple episodes of ACR experienced significant impairment in liver biochemistry and increased histological damage. The authors highlighted that the deterioration of bromsulphthalein clearance was higher in these patients. This suggests that the biliary lesions represent the main feature

of the residual damage due to the rejection process. The rate of chronic rejection was similar between patients with a single episode and patients having recurrent ACR, which means that the liver injury may be present in the absence of chronic rejection. As the single episode of acute rejection was not followed by liver injury in long-term follow-up, it suggests the possibility of lighter immunosuppression. Nevertheless, regarding liver deterioration after recurrent ACR, this factor could indicate the need for heavier immunosuppression treatment.

Late acute rejection is associated with very well known risk factors. Mor, et al. identified that 35% of their patients were noncompliant and this behavior was more often seen in younger patients or in those of a lower socioeconomic group. The practice of directly confronting patients with noncompliance issues may help to identify those patients who are the least compliant, in other words, those that manipulate dosage to minimize side effects[10]. In contrast to kidney patients, the vast majority of them respond to steroid therapy without graft loss[29]. They pointed out that patients resistant to steroids may benefit from more aggressive therapy. Anand, et al.[11] reported that 51% of their LAR episodes had complete response to steroid therapy, 30% had partial response, and 20% had no response (Table 2). The most important finding was that 27% of their patients (16/59) developed chronic rejection and graft loss. Delayed response to therapy during an earlier episode of ACR and centrilobular necrosis or bile duct loss at the time of LAR were associated with a high risk of progression to graft failure[28]. Akatmasu, et al. found that to reduce prednisone below a critical although low dose one year after liver transplant increases the risk of LAR. All of their patients, with the exception of one, presented a complete response to methylprednisolone[8]. Ramji, et al. in his Canadian group[9] reported a weak tendency to find less LAR in transplanted patients due to viral cirrhosis and taking tacrolimus, although the multivariate analysis did not find a robust association. Wiesner, et al. found that lower LAR rates were present in patients treated with MMF, tacrolimus, and steroids in all pretransplantation diagnostic groups studied (viral versus nonviral patients) as compared with those patients treated with tacrolimus plus steroids. They highlighted the risk factors for developing LAR. The importance of their findings may help clinicians identify and avoid unnecessary hospitalizations and also prevent specific groups, such as HCV patients, from receiving steroid boluses, which have been associated with more aggressive recurrence[18].

Table 2. Late acute rejection outcome after treatment

Author/center	Response
Mor E, et al.[10] Baylor Univ. (1992)	94% complete response either steroids/OKT3 6% (1 Chronic rejection and graft loss 1 sepsis and death
Anand A, et al.[11] Birmingham (1995)	51% complete response 29% partial response 20% nonresponse
Yoshida E, et al.[19] Vancouver (1996)	92% complete resolution with methylprednisolone/OKT3
Florman S, et al.[5] New Orleans (2004)	65% complete resolution with methylprednisolone/OKT3
Akatmasu N, et al.[8] Tokyo (2006)	100% complete response to steroid therapy

In other conditions, such as liver transplantation from living donors[8], 15 patients who developed LAR were successfully treated with steroid recycle therapy. Only two of them had to receive MMF and T-cell monoclonal antibody administration. None of them developed chronic rejection. More recently, Uemura, et al. reported more disappointing results related to prognosis and treatment[20]. As has been pointed out earlier in this article, this group found an association between PTLD and developing LAR. They found patient and graft survival to be lower in the LAR group. They highlighted the importance of being aware of

immunosuppression in these specific groups of patients.

Conclusions

The incidence of LAR is quite variable and depends on the definition and early detection. It appears to be associated with a nonviral etiology and with either noncompliant patients or patients with subtherapeutic immunosuppressive drug levels. To take three drugs, including MMF, instead of two drugs prevents patients from developing LAR. The vast majority of them can be managed successfully with steroids and only a minority will need more aggressive immunosuppression. Likewise, only a few patients will later develop chronic rejection and graft loss.

References

1. Roberts MS, Angus DC, Bryce CL, Valenta Z, Weissfeld L. Survival after liver transplantation in the United States: a disease-specific analysis of the UNOS database. Liver Transpl. 2004;10:886-97.
2. Burroughs AK, Sabin CA, Rolles K, et al. European Liver Transplant Association. 3-month and 12-month mortality after first liver transplant in adults in Europe: predictive models for outcome. Lancet. 2006;367:225-32.
3. Klintmalm GBG, Nery JR, Husberg BS, Gonwa TA, Yillery GW. Rejection in liver transplantation. Hepatology. 1989;10:978.
4. Dousset B, Conti F, Cherruau B, et al. Is acute rejection deleterious to long-term liver allograft function? J Hepatol. 1998;29:660-8.
5. Florman S, Schiano T, Kim L, et al. The incidence and significance of late acute cellular rejection (>1000 days) after liver transplantation. Clin Transplant. 2004;18:152-5. **One of the few articles dealing specifically with LAR appearing after long-term follow-up.*
6. Snover DC, Sibley RK, Freese DK, et al. Orthotopic liver transplantation: a pathological study of 63 serial liver biopsies from 17 patients with special reference to the diagnostic features and natural history of rejection. Hepatology. 1984;4:1212-22.
7. Wiesner RH, Demetris AJ, Belle SH, et al. Acute hepatic allograft rejection: incidence, risk factors, and impact on outcome. Hepatology. 1998;28:638-45.
8. Akamatsu N, Sugawara Y, Tamura S, et al. Late-onset acute rejection after living donor liver transplantation. World J Gastroenterol. 2006;12:6674-7. **This article is the only one focused on LAR in patients from liver living donors.*
9. Ramji A, Yoshida EM, Bain VG, et al. Late acute rejection after liver transplantation: the Western Canada experience. Liver Transpl. 2002;:945-51.
10. Mor E, Gonwa TA, Husberg BS, Goldstein RM, Klintmalm GB. Late-onset acute rejection in orthotopic liver transplantation-associated risk factors and outcome. Transplantation. 1992; 54:821-4. **One of the first articles identifying non compliance and low dosage as a cause of LAR.*
11. Anand AC, Hubscher SG, Gunson BK, McMaster P, Neuberger JM. Timing, significance, and prognosis of late acute liver allograft rejection. Transplantation. 1995;60:1098-103.
12. Banff Working Group; Demetris AJ, Adeyi O, Bellamy CO, et al. Liver biopsy interpretation for causes of late liver allograft dysfunction. Hepatology. 2006:489-501. ***The reference for liver biopsy interpretation from the Banf working group.*
13. Sebagh M, Blakolmer K, Falissard B, et al. Accuracy of bile duct changes for the diagnosis of chronic liver allograft rejection: reliability of the 1999 Banff schema. Hepatology. 2002;35:117-25.
14. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology. 1997;25: 658-63.
15. Jain A, Demetris AJ, Kashyap R, et al. Does tacrolimus offer virtual freedom from chronic rejection after primary liver transplantation? Risk and prognostic factors in 1,048 liver transplantations with a mean follow-up of 6 years. Liver Transpl. 2001; 7:623-30.
16. Schweizer RT, Rovelli M, Palmeri D, Vossler E, Hull D, Bartus S. Noncompliance in organ transplant recipients. Transplantation. 1990;49:374-7.
17. Steinhoff G. Major histocompatibility complex antigens in human liver transplants. J Hepatol. 1990;11:9-15.
18. Wiesner RH, Steffen BJ, David KM, Chu AH, Gordon RD, Lake JR. Mycophenolate mofetil use is associated with decreased risk of late acute rejection in adult liver transplant recipients. Am J Transplant. 2006;6:1609-16. ***An interesting study based on a large cohort from the SRTR identifying specific immunosuppression schedules associated to LAR.*
19. Yoshida EM, Shackleton CR, Erb SR, et al. Late acute rejection occurring in liver allograft recipients. Can J Gastroenterol. 1996;10:376-80.
20. Uemura T, Ikegami T, Sanchez EQ, et al. Late acute rejection after liver transplantation impacts patient survival. Clin Transplant. 2008;22:316-23.
21. O'Grady JG, Alexander GJ, Sutherland S, et al. Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bile-duct syndrome after liver transplantation. Lancet. 1988;2:302-5. **A significant study identifying a link between CMV infection and acute cellular rejection.*
22. Ustinov J, Loginov R, Bruggeman C, Suni J, Häyry P, Lautenschlager I. CMV-induced class II antigen expression in various rat organs. Transpl Int. 1994;7:302-8.
23. Krasinskas AM, Demetris AJ, Poterucha JJ, Abraham SC. The prevalence and natural history of untreated isolated central perivenulitis in adult allograft livers. Liver Transpl. 2008;14:625-32.
24. Abraham SC, Freese DK, Ishitani MB, Krasinskas AM, Wu TT. Significance of central perivenulitis in pediatric liver transplantation. Am J Surg Pathol. 2008;32:1479-88.
25. Neil DA, Hubscher SG. Histologic and biochemical changes during the evolution of chronic rejection of liver allografts. Hepatology. 2002;35:639-51.
26. Adams DH, Neuberger JM. Patterns of graft rejection following liver transplantation. J Hepatol. 1990;10:113-19.
27. Quiroga J, Colina I, Demetris AJ, Starzl TE, Van Thiel DH. Cause and timing of first allograft failure in orthotopic liver transplantation: a study of 177 consecutive patients. Hepatology. 1991;14:1054-62.
28. Neil DA, Hubscher SG. Delay in diagnosis: a factor in the poor outcome of late acute rejection of liver allografts. Transplant Proc. 2001;33:1525-6.
29. Basadonna GP, Matas AJ, Gillingham KJ, et al. Early versus late acute renal allograft rejection: impact on chronic rejection. Transplantation. 1993;55:993-5.