Although the patient stopped antiviral therapy, he has not relapsed for 29 months (data not shown).

In conclusion, HBcrAg and cccDNA were helpful for the monitoring of HBV dynamics after LT and keeping a negative status of these markers might contribute to graft survival. In addition, using these methods, the criteria for the discontinuation of HBV prophylaxis could be clarified in the future.

## References


1 Samuel D, Muller R, Alexander G *et al.* Liver transplantation in European patients with the hepatitis B surface antigen. *N. Engl. J. Med.* 1993; **329**: 1842–7.
2 Markowitz JS, Martin P, Conrad AJ *et al.* Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. *Hepatology* 1998; **28**: 585–9.
3 Roche B, Feray C, Gigou M *et al.* HBV DNA persistence 10 years after liver transplantation despite successful anti-HBS passive immunoprophylaxis. *Hepatology* 2003; **38**: 86–95.
4 Saab S, Waterman B, Chi AC, Tong MJ. Comparison of different immunoprophylaxis regimens after liver transplantation with hepatitis B core antibody-positive donors: a systematic review. *Liver Transpl.* 2010; **16**: 300–7.
5 Angelico M, Di Paolo D, Trinito MO *et al.* Failure of a reinforced triple course of hepatitis B vaccination in patients transplanted for HBV-related cirrhosis. *Hepatology* 2002; **35**: 176–81.
6 Degertekin B, Han SH, Keeffe EB *et al.* Impact of virologic breakthrough and HBIG regimen on Hepatitis B recurrence after liver transplantation. *Am. J. Transplant.* 2010; **10**: 1823–33.
7 Angus PW, Patterson SJ, Strasser SI, McCaughan GW, Gane E. A randomized study of adefovir dipivoxil in place of HBIG in combination with lamivudine as post-liver transplantation hepatitis B prophylaxis. *Hepatology* 2008; **48**: 1460–6.
8 Takaki A, Yagi T, Iwasaki Y *et al.* Short-term high-dose followed by long-term low-dose hepatitis B immunoglobulin and lamivudine therapy prevented recurrent hepatitis B after liver transplantation. *Transplantation* 2007; **83**: 231–3.
9 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J. Clin. Microbiol.* 2002; **40**: 439–45.
10 Rokuhara A, Sun X, Tanaka E *et al.* Hepatitis B virus core and core-related antigen quantitation in Chinese patients with chronic genotype B and C hepatitis B virus infection. *J. Gastroenterol. Hepatol.* 2005; **20**: 1726–30.
11 Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. *J. Med. Virol.* 2009; **81**: 27–33.
12 Fujimoto M, Ichikawa T, Nakao K *et al.* The significance of enzyme immunoassay for the assessment of hepatitis B virus core-related antigen following liver transplantation. *Intern. Med.* 2009; **48**: 1577–83.
13 Tanaka E, Matsumoto A, Suzuki F *et al.* Measurement of hepatitis B virus core-related antigen is valuable for identifying patients who are at low risk of lamivudine resistance. *Liver Int.* 2006; **26**: 90–6.
14 Ichida F, Tsuji T, Omata M *et al.* New Inuyama classification; new criteria for histological assessment of chronic hepatitis. *Int. Hepatol. Commun.* 1996; **6**: 112–9.
15 Demetris AJ, Batts KP, Dhillon AP *et al.* Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25**: 658–63.
16 Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br. J. Surg.* 1973; **60**: 646–9.
17 Mazet-Wagner AA, Baclet MC, Loustaud-Ratti V, Denis F, Alain S. Real-time PCR quantitation of hepatitis B virus total DNA and covalently closed circular DNA in peripheral blood mononuclear cells from hepatitis B virus-infected patients. *J. Virol. Methods* 2006; **138**: 70–9.
18 Lenci I, Tisone G, Di Paolo D *et al.* Safety of complete and sustained prophylaxis withdrawal in patients liver transplanted for HBV-related cirrhosis at low risk of HBV recurrence. *J. Hepatol.* 2011; **55**: 587–93.

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LETTER TO THE EDITORS

# False Positivity for the Human Immunodeficiency Virus Antibody After Influenza Vaccination in a Living Donor for Liver Transplantation

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**TO THE EDITORS:**

Because of increased productivity and availability, more people have had the chance to undergo prophylactic influenza vaccination. It has been reported that influenza vaccination has cross-reactivity with human immunodeficiency virus (HIV) antibody assays, but this information is not well known in the field of transplantation.[1] Recently, we experienced a case of living donor liver transplantation in which a healthy donor candidate was frightened and was further screened for the HIV antibody.

The patient was a 43-year-old female who was a candidate for partial liver donation for her husband, who was suffering from hepatocellular carcinoma associated with hepatitis B liver cirrhosis. She had never undergone a blood transfusion or abused drugs before her screening for living partial liver donation. According to her laboratory results, she was positive for the HIV antibody (1.7 cut off index). Otherwise, all data, including hepatitis B antibody results, were within normal limits. It was found that she had undergone vaccination for influenza 1 week before the screening. She was referred to a specialist in HIV infection, and western blotting for all antibodies (GP160, GP110/120, P68/66, P55, P52/51, GP41, P40, P34/31, P24/25, and P18/17) was negative. HIV RNA was undetectable in her blood (<40 copies/mL). Thus, she was considered to be HIV-negative with a high level of confidence and subsequently donated the left lobe of her liver. The recipient remained negative for the HIV antibody even after living donor liver transplantation.

With the prevalence of influenza vaccination and organ donation, physicians should keep in mind that recent inoculation with any brand of influenza vaccine is associated with a false-positive screening assay for HIV antibodies.[2]

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## REFERENCES

1. Erickson CP, McNiff T, Klausner JD. Influenza vaccination and false positive HIV results. N Engl J Med 2006;354:1422-1423.
2. Mac Kenzie WR, Davis JP, Peterson DE, Hibbard AJ, Becker G, Zarvan BS. Multiple false-positive serologic tests for HIV, HTLV-1, and hepatitis C following influenza vaccination, 1991. JAMA 1992;268:1015-1017.

The protocol for our living donor liver transplantation received a priori approval by the institutional review committee.

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***Review Article***

# Liver transplantation for HIV/hepatitis C virus co-infected patients

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Since the introduction of antiretroviral therapy (ART) in the mid-1990s, AIDS-related death has been dramatically reduced, and hepatitis-C-virus (HCV)-related liver failure or hepatocellular carcinoma has currently become the leading cause of death in HIV/HCV co-infected patients. Liver transplantation may be one of the treatments of choices in such cases, but the indications for transplantation, perioperative management including both HIV and HCV treatments, immunosuppression and the prevention/treatment of infectious complications are all still topics of debate. With the improved understanding of the viral behaviors of both HIV and HCV and the development of novel strategies, especially to avoid drug interactions between ART and immunosuppression, liver transplantation has become a realistic treatment for HIV/HCV co-infected patients.

**Key words:** hepatitis C virus, HIV, liver transplantation

## INTRODUCTION

IN JAPAN, IN the late 1980s, contaminated blood production of coagulation factor for hemophilia caused co-infection of HIV and hepatitis C virus (HCV). Actually, greater than 90% of HIV-infected patients have HCV as well.[1]

After antiretroviral therapy (ART) was introduced in the late 1990s, successful control of HIV was achieved in most cases and death due to AIDS was dramatically reduced, but HCV-related death due to liver failure or hepatocellular carcinoma became a serious problem, not only in Japan, but all over the world.[2–6] In such cases, liver transplantation (LT) is the only treatment option to achieve long-term survival, but several modifications of perioperative management are required. In this review, the outcome and the points of management of LT for HIV/HCV co-infected patients were reviewed.

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## REPORTED OUTCOME OF LT FOR HIV/HCV PATIENTS

THE REPORTED OUTCOMES of LT for HIV and HIV/HCV co-infected patients from Western countries after the introduction of ART are summarized in Table 1.[7–11] In general, most reports concluded that the results were worse than in the cases with HCV mono-infection, with a 3-year survival of approximately 60–70%. In Japan, the Tokyo group reported six cases of living donor liver transplantation (LDLT) between 2001 and 2004, of whom four died.[12] These unfavorable outcomes are likely related to the difficulties of determining the indications for LT and of perioperative management, including HIV/HCV treatment and the prevention and treatment of infectious complications. Terrault *et al.* reported that older donor age, combined kidney–liver transplantation, an anti-HCV positive donor and a body mass index of less than 21 kg/m$^2$ were independent predictors of graft loss.[10] After transplantation, several studies showed that acute cellular rejection was more frequent and severer in HIV/HCV co-infected patients than that in HCV mono-infected patients, possibly due to the difficulties in achieving optimal immunosuppression because of interactions between antiretroviral agents and immunosuppression.[10,11]

**Table 1** Outcome of liver transplantation for HIV/hepatitis C virus co-infection

| Authors | Publication year | Country | *n* | Patient survival (%) | | |
|---|---|---|---|---|---|---|
| | | | | 1 year | 3 years | 5 years |
| de Vera *et al.*[7] | 2006 | USA | 27 | 67 | 56 | 33 |
| Schreibman *et al.*[8] | 2007 | USA | 15 | 73 | 73 | – |
| Duclos-Vallee *et al.*[9] | 2008 | France | 35 | – | 73 | 51 |
| Terrault *et al.*[10] | 2012 | USA | 89 | 76 | 60 | – |
| Miro *et al.*[11] | 2012 | Spain | 84 | 88 | 62 | 54 |

## SPECIAL ISSUES REGARDING LT INDICATIONS FOR HIV/HCV CO-INFECTION

### ART-related non-cirrhotic portal hypertension

IN HCV MONO-INFECTED patients, LT should be considered when the patients develop deteriorated liver function as indicated by a Child–Pugh classification of B or C. In HIV/HCV co-infected patients, liver failure due to HCV hepatitis was generally enhanced by ART-related hepatotoxicity, especially non-cirrhotic portal hypertension.[13–15] Accordingly, not only in cases with deteriorated liver function but also in class A cases, the patients can easily develop severe liver dysfunction suddenly,[16,17] so that all HIV/HCV co-infected patients should be carefully followed up so as not to miss the chance for LT. Also, Murillas *et al.* reported that Model for End-Stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients,[18] so that HIV/HCV co-infected patients may be considered for LT before MELD score increase to achieve comparable results with HCV mono-infected patients. Several studies showed the aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients,[19,20] but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse imaging to check for liver stiffness has been introduced as an effective and non-invasive modality to determine patients' candidacy for LT.[21–23]

### Count of CD4$^+$ T lymphocytes

Generally, the count of CD4$^+$ T lymphocytes has been required to be more than 200/μL to perform general elective surgeries in HIV-infected patients,[24] but in HIV/HCV co-infected patients, current studies show that a count of more than 100/μL is acceptable,[25,26] because patients generally have portal hypertension which can cause pancytopenia. In such patients, the ratio of CD4/CD8 is reported to be a feasible marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher.[27]

### Preoperative infections

In regard to latent opportunistic infections that occur before LT, they are not absolute contraindications when they can be expected to be controlled.[28] Infections regarded as contraindications for LT included uncontrollable multidrug resistance HIV infection, chronic *Cryptosporidium enteritis*, progressive multifocal leukoencephalopathy and lymphoma.[29]

## MANAGEMENT OF HIV/HCV IN LT

### Management of HIV

THE NUMBER OF HIV RNA copies before LT is suggested as an independent risk factor of postoperative mortality, so that HIV should be controlled sufficiently before LT.[30] Accordingly, in the patients who are under consideration to receive LT, ART can be safely stopped before LT because HIV is generally well-controlled for a long period by ART. After LT, ART should be restarted as soon as possible because HIV RNA appears at 3–30 days after ART is stopped,[31] but the timing of restart of ART depends on the patient's condition, including liver function.[32] As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not sufficiently regenerated yet, ART cannot be started. Castells *et al.* reported in their case–control study that ART was started at a median of 8 days after LT (range, 4–28 days).[33] In principle, the ART administrated after LT should be the same as the pretransplant regimen, but the majority of ART drugs including protease inhibitor (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) have interactions with calcineurin inhibitors

(CNI) or mammalian target of rapamycin (mTOR),[34] so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. Currently, a novel HIV-1 integrase inhibitor, raltegravir (RAL), is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs.[35,36]

### Management of HCV

The treatment strategy for HCV in HIV/HCV co-infected patients is the same as in HCV mono-infected patients. Combination therapy of pegylated interferon (PEG IFN) and ribavirin is the standard treatment both before and after LT. The timing of the induction therapy after LT is controversial. A Tokyo group proposed early induction as a preemptive therapy before patients develop hepatitis,[37] while several other reports showed favorable results when the treatment was administrated only after the development of hepatitis was confirmed by liver biopsy.[38,39] Theoretically, the treatment should be started as soon as possible, because in HIV/HCV co-infected patients, HCV recurrence may be accelerated in an immunocompromised state.[30,40] The novel protease inhibitor, telaprevir, is currently introduced as an effective drug to achieve sustained viral response of 70%, even in genotype 1b, with PEG IFN/ribavirin in a non-transplant setting,[41] but this drug is metabolized via cytochrome P450 as a substrate, as are CNI and various protease inhibitors of ART for HIV. Close monitoring of the CNI trough level should be performed, and although triple therapy with telaprevir/PEG IFN/ribavirin is currently reported to be effective to prevent HCV recurrence after LT in HCV mono-infected cases, special attention should be paid when this regimen is adapted in HIV/HCV co-infected patients.

## IMMUNOSUPPRESSION

AS PREVIOUSLY MENTIONED, many factors including ART, anti-HCV treatment and an HIV-related immunocompromised state make post-LT immunosuppressive treatment difficult. Many ART drugs, both PI and NNRTI, cause instability in the blood concentration of CNI through the cytochrome P3A4 (CYP3A4)-related metabolism. Most PI cause the overconcentration of CNI by inhibiting CYP3A4, while most NNRTI cause decreased levels of CNI by stimulating CYP3A4.[29,42] As mentioned earlier, RAL is introduced as a key drug in LT in HIV positive patients, because the metabolism of this drug is not related to CYP450, so it does not affect the blood concentration of CNI. Several reports have demonstrated both the *in vitro* and *in vivo* effectiveness of rapamycin in reducing HIV replication,[43–45] and Di Benedetto *et al.* found that rapamycin monotherapy was significantly beneficial in long-term immunosuppression maintenance and HIV control after LT.[46] Mycophenolate mofetil is expected to be an effective immunosuppressive drug because of its efficacy in reducing HIV infection by both virological and immunological mechanisms.[47–49] Using these drugs, a more effective regimen of immunosuppression with ART may be established.

In regard to the steroid, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. Also, in HIV/HCV co-infected patients, steroid-free protocol may be beneficial to prevent both HIV and HCV recurrence after LT.[50,51]

## CONCLUSIONS

LIVER TRANSPLANTATION FOR HIV/HCV co-infected patients remains challenging, but with recent developments in perioperative management and novel drugs for both HIV and HCV, the results are likely to be improved.

## REFERENCES

1 Eguchi S, Soyama A, Hidaka M *et al.* Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan. *Surg Today* 2011; **41**: 1325–31.
2 Weber R, Sabin CA, Friis-Moller N *et al.* Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006; **166**: 1632–41.
3 Rosenthal E, Pialoux G, Bernard N *et al.* Liver-related mortality in human-immunodeficiency-virus-infected patients between 1995 and 2003 in the French GERMIVIC Joint Study Group Network (MORTAVIC 2003 Study). *J Viral Hepat* 2007; **14**: 183–8.
4 Bica I, McGovern B, Dhar R *et al.* Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; **32**: 492–7.
5 Darby SC, Ewart DW, Giangrande PL *et al.* Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. *Lancet* 1997; **350** (9089): 1425–31.
6 Thio CL, Seaberg EC, Skolasky R *et al.* HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet* 2002; **360** (9349): 1921–6.

7 de Vera ME, Dvorchik I, Tom K *et al.* Survival of liver transplant patients coinfected with HIV and HCV is adversely impacted by recurrent hepatitis C. *Am J Transplant* 2006; **6**: 2983–93.
8 Schreibman I, Gaynor JJ, Jayaweera D *et al.* Outcomes after orthotopic liver transplantation in 15 HIV-infected patients. *Transplantation* 2007; **84**: 697–705.
9 Duclos-Vallee JC, Feray C, Sebagh M *et al.* Survival and recurrence of hepatitis C after liver transplantation in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology* 2008; **47**: 407–17.
10 Terrault NA, Roland ME, Schiano T *et al.*, Solid Organ Transplantation in HIV: Multi-Site Study Investigators. Outcomes of liver transplant recipients with hepatitis C and human immunodeficiency virus coinfection. *Liver Transpl* 2012; **18**: 716–26.
11 Miro JM, Montejo M, Castells L *et al.*, Spanish OLT in HIV-Infected Patients Working Group Investigators. Outcome of HCV/HIV-coinfected liver transplant recipients: a prospective and multicenter cohort study. *Am J Transplant* 2012; **12**: 1866–76.
12 Tsukada K, Sugawara Y, Kaneko J *et al.* Living donor liver transplantations in HIV- and hepatitis C virus-coinfected hemophiliacs: experience in a single center. *Transplantation* 2011; **91**: 1261–4.
13 Vispo E, Moreno A, Maida I *et al.* Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. *AIDS* 2010; **24**: 1171–6.
14 Mendizabal M, Craviotto S, Chen T, Silva MO, Reddy KR. Noncirrhotic portal hypertension: another cause of liver disease in HIV patients. *Ann Hepatol* 2009; **8**: 390–5.
15 Kovari H, Ledergerber B, Peter U *et al.*, Swiss HIV Cohort Study. Association of noncirrhotic portal hypertension in HIV-infected persons and antiretroviral therapy with didanosine: a nested case-control study. *Clin Infect Dis* 2009; **49**: 626–35.
16 Merchante N, Girón-González JA, González-Serrano M *et al.* Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. *AIDS* 2006; **20**: 49–57.
17 Ragni MV, Eghtesad B, Schlesinger KW, Dvorchik I, Fung JJ. Pretransplant survival is shorter in HIV-positive than HIV-negative subjects with end-stage liver disease. *Liver Transpl* 2005; **11**: 1425–30.
18 Baccarani U, Adani GL, Bragantini F *et al.* Long-term outcomes of orthotopic liver transplantation in human immunodeficiency virus-infected patients and comparison with human immunodeficiency virus-negative cases. *Transplant Proc* 2011; **43**: 1119–22.
19 Rullier A, Trimoulet P, Neau D *et al.* Fibrosis is worse in HIV-HCV patients with low-level immunodepression referred for HCV treatment than in HCV-matched patients. *Hum Pathol* 2004; **35**: 1088–94.
20 Ragni MV, Moore CG, Soadwa K *et al.*, HHH Study Group. Impact of HIV on liver fibrosis in men with hepatitis C infection and haemophilia. *Haemophilia* 2011; **17**: 103–11.
21 Resino S, Sánchez-Conde M, Berenguer J. Coinfection by human immunodeficiency virus and hepatitis C virus: noninvasive assessment and staging of fibrosis. *Curr Opin Infect Dis* 2012; **25**: 564–9.
22 Merchante N, Rivero-Juárez A, Téllez F *et al.* Liver stiffness predicts clinical outcome in human immunodeficiency virus/hepatitis C virus-coinfected patients with compensated liver cirrhosis. *Hepatology* 2012; **56**: 228–38.
23 Sánchez-Conde M, Miralles P, Bellón JM *et al.* Use of transient elastography (FibroScan®) for the noninvasive assessment of portal hypertension in HIV/HCV-coinfected patients. *J Viral Hepat* 2011; **18**: 685–91.
24 Davison SP, Reisman NR, Pellegrino ED, Larson EE, Dermody M, Hutchison PJ. Perioperative guidelines for elective surgery in the human immunodeficiency virus-positive patient. *Plast Reconstr Surg* 2008; **121**: 1831–40.
25 Miro JM, Torre-Cisnero J, Moreno A *et al.* [GESIDA/GESITRA-SEIMC, PNS and ONT consensus document on solid organ transplant (SOT) in HIV-infected patients in Spain (March, 2005)]. *Enferm Infecc Microbiol Clin* 2005; **23**: 353–62.
26 O'Grady J, Taylor C, Brook G. Guidelines for liver transplantation in patients with HIV infection (2005). *HIV Med* 2005; **6** (Suppl 2): 149–53.
27 Xia XJ, Liu BC, Su JS *et al.* Preoperative CD4 count or CD4/CD8 ratio as a useful indicator for postoperative sepsis in HIV-infected patients undergoing abdominal operations. *J Surg Res* 2012; **174**: e25–30.
28 Grossi PA. Update in HIV infection in organ transplantation. *Curr Opin Organ Transplant* 2012; **17**: 586–93.
29 Joshi D, O'Grady J, Taylor C, Heaton N, Agarwal K. Liver transplantation in human immunodeficiency virus-positive patients. *Liver Transpl* 2011; **17**: 881–90.
30 Ragni MV, Belle SH. Impact of human immunodeficiency virus infection on progression to end-stage liver disease in individuals with hemophilia and hepatitis C virus infection. *J Infect Dis* 2001; **183**: 1112–5.
31 García F, Plana M, Vidal C *et al.* Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. *AIDS* 1999; **13**: F79–86.
32 Neff GW, Bonham A, Tzakis AG *et al.* Orthotopic liver transplantation in patients with human immunodeficiency virus and end-stage liver disease. *Liver Transpl* 2003; **9**: 239–47.
33 Castells L, Escartín A, Bilbao I *et al.* Liver transplantation in HIV-HCV coinfected patients: a case-control study. *Transplantation* 2007; **83**: 354–8.
34 Frassetto LA, Browne M, Cheng A *et al.* Immunosuppressant pharmacokinetics and dosing modifications in HIV-1 infected liver and kidney transplant recipients. *Am J Transplant* 2007; **7**: 2816–20.
35 Armstrong MJ, Corbett C, Rowe IA, Taylor GP, Neuberger JM. HTLV-1 in solid-organ transplantation: current

challenges and future management strategies. *Transplantation* 2012; **94**: 1075–84.
36 Tricot L, Teicher E, Peytavin G *et al.* Safety and efficacy of raltegravir in HIV-infected transplant patients cotreated with immunosuppressive drugs. *Am J Transplant* 2009; **9**: 1946–52.
37 Sugawara Y, Tamura S, Yamashiki N *et al.* Preemptive antiviral treatment for hepatitis C virus after living donor liver transplantation. *Transplant Proc* 2012; **44**: 791–3.
38 Tanaka T, Selzner N, Therapondos G, Renner EL, Lilly LB. Virological response for recurrent hepatitis C improves long-term survival in liver transplant recipients. *Transpl Int* 2013; **26**: 42–9.
39 Ueda Y, Takada Y, Marusawa H, Egawa H, Uemoto S, Chiba T. Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. *Transplantation* 2010; **90**: 661–5.
40 Polard E, Camus C, Abault AY *et al.* Retransplantation for acute liver failure due to combined antiviral agents in an HIV-HCV coinfected liver transplant recipient. *Transplantation* 2005; **80**: 1136–8.
41 Barritt AS 4th, Fried MW. Maximizing opportunities and avoiding mistakes in triple therapy for hepatitis C virus. *Gastroenterology* 2012; **142**: 1314–23.
42 Sugawara Y, Tamura S, Kokudo N. Liver transplantation in HCV/HIV positive patients. *World J Gastrointest Surg* 2011; **3**: 21–8.
43 Lin YL, Mettling C, Portales P, Reynes J, Clot J, Corbeau P. Cell surface CCR5 density determines the post-entry efficiency of R5 HIV-1 infection. *Proc Natl Acad Sci U S A* 2002; **99**: 15590–5.
44 Weissman D, Dybul M, Daucher MB, Davey RT Jr, Walker RE, Kovacs JA. Interleukin-2 up-regulates expression of the human immunodeficiency virus fusion coreceptor CCR5 by CD4+ lymphocytes in vivo. *J Infect Dis* 2000; **181**: 933–8.
45 Heredia A, Amoroso A, Davis C *et al.* Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV beta-chemokines: an approach to suppress R5 strains of HIV-1. *Proc Natl Acad Sci U S A* 2003; **100**: 10411–6.
46 Di Benedetto F, Di Sandro S, De Ruvo N *et al.* First report on a series of HIV patients undergoing rapamycin monotherapy after liver transplantation. *Transplantation* 2010; **89**: 733–8.
47 Chapuis AG, Paolo Rizzardi G, D'Agostino C *et al.* Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. *Nat Med* 2000; **6**: 762–8.
48 García F, Plana M, Arnedo M *et al.* Effect of mycophenolate mofetil on immune response and plasma and lymphatic tissue viral load during and after interruption of highly active antiretroviral therapy for patients with chronic HIV infection: a randomized pilot study. *J Acquir Immune Defic Syndr* 2004; **36**: 823–30.
49 Coull JJ, Turner D, Melby T, Betts MR, Lanier R, Margolis DM. A pilot study of the use of mycophenolate mofetil as a component of therapy for multidrug-resistant HIV-1 infection. *J Acquir Immune Defic Syndr* 2001; **26**: 423–34.
50 Marubashi S, Umeshita K, Asahara T *et al.* Steroid-free living donor liver transplantation for HCV – a multicenter prospective cohort study in Japan. *Clin Transplant* 2012; **26**: 857–67.
51 Klintmalm GB, Davis GL, Teperman L *et al.* A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. *Liver Transpl* 2011; **17**: 1394–403.

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EDITORIAL

# Is low central venous pressure effective for postoperative care after liver transplantation?

**Susumu Eguchi**

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The central venous pressure (CVP) has been regarded as an important factor for reducing blood loss and the blood transfusion rate during major hepatectomy, and can be controlled by positive end-expiratory pressure (PEEP) or certain drugs and the optimal positioning of the patient [1–4].

In this issue of *Surgery Today*, Wang et al. [5] describe the beneficial effects of lowering the CVP for achieving a better postoperative outcome compared with conventional fluid management in deceased donor liver transplantation based on a prospective randomized controlled study. They report that the low CVP group showed (1) less intraoperative blood loss, (2) a decreased need for intraoperative blood transfusion, (3) fewer lung-related complications at 1 month postoperatively, (4) a shorter intubation period and (5) equal patient survival at 1 year after liver transplantation. A previous retrospective study showed intraoperative blood transfusion to be a risk factor for postoperative lung complications [6]. The present study was done in a prospective, randomized manner, which yielded the same results as those seen in the previous retrospective study. The methods used to reduce the CVP in the present study were the use of the Fowler position, fluid restriction and drugs (e.g., nitroglycerin, furosemide and somatostatin). These methods have also been used in previous studies to reduce the intraoperative CVP, and therefore they appear to be valid for this kind of study [2].

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Although the results provided in the article were of high importance, lowering the CVP during liver transplantation might still be controversial and may have ambivalent aspects with regard to the lack of a relationship between the early complication rates, including renal, hepatic and pulmonary complications, and the CVP following liver transplantation [7–10]. For example, apart from the reduced pulmonary complication rate, and the lower blood loss and blood transfusion rate, what would be the influence of lowering the CVP on the postoperative care following liver transplantation? If blood product administration during the intensive care period is increased, then the policy to limit CVP during surgery would be in vain. Therefore, the readers will also want to know: How would the perfusion in the organ be affected? How would the lactate level in the blood after LT be affected, not only at the end of surgery but also during the postoperative period? How would the post-transplant blood product requirements be affected?

In fact, the period in which the CVP is lowered may be of importance. For example, Feng et al. [7] reported that a low CVP during the pre-anhepatic phase reduced the intraoperative blood loss, protected the liver function and it also had no detrimental effects on the renal function after LT. On the other hand, Cywinski et al. reported that a low CVP during the post-anhepatic phase was not associated with any benefit in terms of immediate postoperative allograft function, graft survival or patient survival [10]. In addition, the cut-off value for CVP monitoring in previous studies varied between 5 and 10 mmHg.

We therefore await further reports from other investigators before drawing any definitive conclusions about the above-mentioned issues, since liver transplant surgery, especially partial liver transplantation, is often affected by multiple factors [11].

## References


1. Hasegawa K, Takayama T, Orii R, Sano K, Sugawara Y, Imamura H, et al. Effect of hypoventilation on bleeding during hepatic resection: a randomized controlled trial. Arch Surg. 2002;137:311–5.
2. Zhu P, Lau WY, Chen YF, Zhang BX, Huang ZY, Zhang ZW, et al. Randomized clinical trial comparing infrahepatic inferior vena cava clamping with low central venous pressure in complex liver resections involving the Pringle manoeuvre. Br J Surg. 2012;99:781–8.
3. Rahbari NN, Koch M, Zimmermann JB, Elbers H, Bruckner T, Contin P, et al. Infrahepatic inferior vena cava clamping for reduction of central venous pressure and blood loss during hepatic resection: a randomized controlled trial. Ann Surg. 2011;253:1102–10.
4. Westerkamp AC, Lisman T, Porte RJ. How to minimize blood loss during liver surgery in patients with cirrhosis. HPB (Oxford). 2009;11:453–8.
5. Wang B, He HK, Cheng B, Sei K, Min S. Effect of low central venous pressure on postoperative pulmonary complications in patients undergoing liver transplantation. Surg Today. 2012. doi: 10.1007/s00595-012-0419-y (Epub ahead of print).
6. Pirat A, Ozgur S, Torgay A, Candan S, Zeyneloğlu P, Arslan G. Risk factors for postoperative respiratory complications in adult liver transplant recipients. Transplant Proc. 2004;36:218–20.
7. Feng ZY, Xu X, Zhu SM, Bein B, Zheng SS. Effects of low central venous pressure during preanhepatic phase on blood loss and liver and renal function in liver transplantation. World J Surg. 2010;34:1864–73.
8. Saner FH, Olde Damink SW, Pavlaković G, Sotiropoulos GC, Radtke A, Treckmann J, et al. How far can we go with positive end-expiratory pressure (PEEP) in liver transplant patients? J Clin Anesth. 2010;22:104–9.
9. Cywinski JB, Mascha E, You J, Argalious M, Kapural L, Christiansen E, Parker BM. Central venous pressure during the post-anhepatic phase is not associated with early postoperative outcomes following orthotopic liver transplantation. Minerva Anesthesiol. 2010;76:795–804.
10. Schroeder RA, Collins BH, Tuttle-Newhall E, Robertson K, Plotkin J, Johnson LB, Kuo PC. Intraoperative fluid management during orthotopic liver transplantation. J Cardiothorac Vasc Anesth. 2004;18:438–41.
11. Eguchi S, Soyama A, Hidaka M, Takatsuki M, Muraoka I, Tomonaga T, Kanematsu T. Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan. Surg Today. 2011;41:1325–31.

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***Original Article***

# Disease recurrence plays a minor role as a cause for retransplantation after living-donor liver transplantation for primary biliary cirrhosis: A multicenter study in Japan

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*Aim:* To clarify the role of disease recurrence as a cause of graft loss after living-donor liver transplantation (LDLT) for primary biliary cirrhosis (PBC), we investigated explant grafts, as well as the native liver and liver biopsy specimens, of patients who underwent retransplantation.

*Methods:* Of 516 patients who underwent LDLT for PBC and were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation.

*Results:* Seven patients undergoing retransplantation later than 6 months after primary liver transplantation (LT) were enrolled. All seven patients were female, with ages ranging from 34–57 years, and Model for End-Stage Liver Disease scores ranging 10–28. The right lobe was used as graft in one and the left lobe in six. The initial immunosuppression regimen was tacrolimus in six and cyclosporin in one. The period between the primary LT and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation. The primary causes of primary graft loss revealed by histological examination of the explant livers were chronic rejection in three, portal thrombus and/or steatohepatitis in three and outflow block in one. PBC recurrence was observed in 3 and the stage was mild in all.

*Conclusion:* PBC recurrence has a small impact as a cause of graft loss after LDLT.

**Key words:** histology, living-donor liver transplantation, primary biliary cirrhosis, recurrence, retransplantation

## INTRODUCTION

PRIMARY BILIARY CIRRHOSIS (PBC) is a major indication for liver transplantation (LT). Because autoimmune mechanisms possibly contribute to the etiology of PBC, the possibility of recurrence after transplantation and the impact on the clinical course have been reason for considerable concern. Rates of recurrence have been reported to range 9–35% in deceased-donor LT in Western countries.[1] In living-donor liver transplantation (LDLT) in Japan, the rates have been reported to range 1–40% on the basis of histological evidence.[2–6] However, this range is not reliable because routine liver biopsy is not standard. Furthermore, the impact of recurrence on the clinical course is unclear. The proportion of grafts lost due to disease recurrence was reported to be 2% 10 years after transplantation by Rowe *et al.*[7] On the other hand, Charatcharoenwitthaya *et al.* reported that recurrent PBC was not associated

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with death or retransplantation.[8] There have been no reports of graft failure secondary to recurrent PBC in Japan, either.[2–6]

The difficulty of performing histological diagnosis of recurrent PBC using needle biopsy specimens is a barrier for studying the impact of recurrent PBC, although histological examination is the gold standard.[6,9,10] Heterogeneity of histological changes is a major hurdle for diagnosis on the basis of needle biopsy specimens. To overcome this problem, we conducted a multicenter study using whole hepatic grafts explanted during retransplantation for PBC.

## METHODS

OF 516 PATIENTS who underwent LDLT for PBC and who were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation. The demographic data of the recipients and primary donors and information on the clinical courses were obtained.

A current author (Y. N.) performed histological investigation of the native liver, the liver biopsy specimens if present, and the explant grafts. The diagnosis of acute cellular rejection (ACR) and chronic rejection was made according to the Banff criteria.[11,12] Staging of PBC was based on the Nakanuma staging system.[13]

This study was approved by the Ethical Committee of Tokyo Women's Medical University as the central office of the multicenter study, or at each institution if necessary, and it conforms to the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008).

## RESULTS

OF THE NINE patients who underwent retransplantation, two died within 6 months after retransplantation. One died due to graft failure secondary to severe acute rejection and another due to small-for-size syndrome. In both cases, we examined the clinical courses and explanted livers, and confirmed the diagnoses. We enrolled the remaining seven patients in this study.

The demographic and operative data of the recipients and primary donors and the clinical courses are shown in Table 1. All patients were female and had histories of pregnancies. Human leukocyte antigen DR8 was detected in all recipients except no. 5 and in the donors of recipients no. 3, 6 and 7. The donor was the patient's husband in two cases, son in three, sister in one and mother in one.

Primary immunosuppression was performed with a triple regimen consisting of calcineurin inhibitor, steroids and antimetabolites (azathioprine, mizoribine) in three patients, and calcineurin inhibitor and steroids in four patients. The calcineurin inhibitor was tacrolimus in all patients except no. 6 in which cyclosporin was converted to tacrolimus 1 year after transplantation.

All patients were treated with ursodeoxycholic acid (UDCA) and no. 1 and 7 with bezafibrate prior to primary transplantation. All patients were given UDCA after transplantation and only no. 3 was given bezafibrate transiently.

Patients 1, 4, 6 and 7 continued to complain of fatigue even after transplantation. Postoperative complications are shown in Table 1. The period between the primary transplantation and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation.

Histological findings of the native liver, the liver biopsy specimens and the explant grafts are summarized in Table 2. The stage of PBC of the native liver was 4 in all patients except no. 7. The primary causes of primary graft loss were chronic rejection in three (no. 2, 3 and 6), portal thrombus in one (no. 7), nonalcoholic steatohepatitis (NASH) in one (no. 4), portal thrombus and NASH in one (no. 5), and outflow block in one (no. 1). Briefly, submassive necrosis from ischemic etiology and liver cirrhosis of chronic congestive etiology were observed in no. 1. Foamy cell arteriopathy, duct loss with degenerative epithelial damage with severe cholestasis, and centrilobular and C-C and P-C bridging fibrosis were observed in no. 2. In both patients 4 and 5 with NASH, the stage had progressed from stage 2 in the biopsy specimens to stage 3 in the explanted livers.[14] Portal vein thromboembolism and altered intrahepatic circulation was also observed in no. 5. Marked centrilobular necrosis and hemorrhage with mild inflammation and fibrosis and portal venopathy with repeated thromboemboli were observed in no. 7.

Recurrence of PBC was observed in no. 2, 6 and 7 in the specimens of on-demand needle or wedge biopsies and confirmed in the explanted livers (Figs 1–3). Histological progression of PBC was very mild or mild and the recurrence was not the main cause of graft failure. We evaluated: (i) mononuclear inflammatory infiltrates; (ii) formation of lymphoid aggregates; (iii)

**Table 1** Demographic data, operative data and clinical courses

| Patient no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Age (years) | 52 | 40 | 34 | 37 | 47 | 47 | 57 |
| Time from diagnosis to LT (months) | 22 | 3 | 60 | 55 | 65 | 132 | 99 |
| AMA | >320 | 80 | 40 | 80 | NA | Negative | 160 |
| Anti-M2 (mg/dL) | 1859 | 1550 | NA | NA | NA | NA | 152 |
| IgM (mg/dL) | 1037.8 | 172.8 | 426 | 115 | 340 | NA | 524 |
| IgG (mg/dl) | 1945.7 | 884.2 | 1774 | 1373 | 2921 | NA | 180 |
| ANA | 640 | ± | Negative | ± | Negative | 320 | NA |
| Child–Pugh score | 7 | 8 | 11 | 12 | 12 | 14 | 10 |
| MELD score | 10 | 11 | 17 | 24 | 22 | 28 | 11 |
| Primary donor | | | | | | | |
| Relation | Husband | Mother | Husband | Sister | Son | Son | Son |
| Age (years) | 50 | 60 | 34 | 47 | 19 | 20 | 23 |
| Sex | Male | Female | Male | Female | Male | Male | Male |
| Operative variables | | | | | | | |
| Blood type combination | Compatible | Identical | Identical | Compatible | Compatible | Compatible | Identical |
| GRWR | 1.00 | 0.95 | 0.88 | 0.77 | 1.07 | 0.58 | 0.90 |
| Graft type | Left | Right | Left | Left | Left | Left | Left |
| Operation time (min) | 751 | 550 | 665 | 615 | 730 | 680 | 870 |
| Cold ischemic time (min) | 82 | 38 | 56 | 53 | 111 | 95 | 131 |
| Warm ischemic time (min) | 53 | 44 | 33 | 40 | 38 | 45 | 41 |
| Blood loss (g) | 2400 | 2470 | 850 | 10 320 | 6190 | 8005 | 4500 |
| Postoperative complications | Hemoperitoneum, biliary stenosis, ACR, hepatic vein stenosis | Biliary stenosis, ACR, EBV infection | Chronic rejection | ACR | ACR Artery-portal shunt | Biliary leakage and stenosis | Portal vein thrombosis |
| Time of retransplantation (months) | 39 | 24 | 36 | 88 | 120 | 20 | 11 |
| Outcome of retransplantation | Dead (49 days) | Alive | Dead (59 days) | Alive | Alive | Dead (15 days) | Dead (284 days) |
| Causes of death | Lung bleeding | | Graft failure | | | Graft failure | Graft failure |

ACR, acute cellular rejection; AMA, antimitochondrial antibody; ANA, antinuclear antibody; EBV, Epstein–Barr virus; GRWR, graft recipient weight ratio; Ig, immunoglobulin; LT, liver transplantation; MELD, Model of End-stage Liver Disease; NA, not applicable.

**Table 2** Histological findings of the native liver, biopsy specimens and explanted liver

| Patient no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| PBC staging of native livers | | | | | | | |
| Stage | 4 | 4 | 4 | 4 | 4 | 4 | 2 |
| Bile duct loss | 3 | 3 | 3 | 3 | 3 | 2 | 1 |
| Fibrosis | 3 | 2 | 3 | 3 | 3 | 3 | 1 |
| Orcein deposition | 3 | 2 | 3 | 3 | 3 | 2 | 1 |
| Hepatitis activities | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| Cholangitis activities | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Needle biopsies | | | | | | | |
| | Congestion at 6 months | Suspected rPBC (duct loss and hepatitis) at 20 months | No biopsy | rPBC (cholangitis) and NASH at 71 months | rPBC (cholangitis and granuloma) and NASH at 90 months | No biopsy | ACR at 9 months |
| Main diagnosis | Outflow block | Chronic rejection | Chronic rejection | NASH | PVT and NASH | Chronic rejection | PVT |
| PBC recurrence | No | Mild (mild chronic cholangitis) | No | Mild (focal duct damage and portal fibrosis) | Mild (focal duct loss and portal inflammation) | No | No |

ACR, acute cellular rejection; NASH, non-alcoholic steatohepatitis; PVT, portal vein thrombosis; rPBC, recurrence of PBC.

epithelioid granuloma; and (iv) bile duct damage according to Neuberger's criteria for the diagnosis of recurrent PBC based on liver histology.[15] In patient no. 2, biopsy showed (i) and (iv) (probable recurrence) and the explanted liver showed (i), (ii) and (iv) (definite recurrence); in no. 6, biopsy showed (i), (ii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence); and in no. 7, biopsy showed (i), (iii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence).

## Case report of three patients with histological diagnoses of recurrent PBC

Patient no. 2 had refractory ACR requiring steroid pulse therapy on postoperative day (POD) 12, 36, 43, 97, 103, 420 and OKT3 monoclonal antibody on POD 434. Liver dysfunction associated with biliary dilatation developed 20 months after LDLT and we performed hepaticojejunostomy and wedge liver biopsy, which revealed suspected recurrence of PBC. Immunosuppression consisted of tacrolimus (3.0 mg/day), steroid (5 mg) and mizoribine (50 mg). Immunoglobulin M was 136, antimitochondrial antibody (AMA) 80 and anti-M2 152 mg/dL. Aggressive liver failure developed despite increased immunosuppression thereafter. She underwent retransplantation 24 months after LDLT.

In patient no. 4, alkaline phosphatase (ALP) began to increase 65 months after LDLT and liver dysfunction developed thereafter. Liver biopsy was performed 71 months after LDLT. Immunosuppression consisted of tacrolimus (2.0 mg/day) and steroid (5 mg). Aspartate aminotransferase (AST) was 44, ALP 432, γ-glutamyltransferase (γ-GT) 17, total bilirubin 1.7 mg/dL, AMA 80 and AMA-M2 155 mg/dL. Tacrolimus was changed to Neoral (Cyclosporine; Novartis, Basel, Switzerland), and mycophenolate mofetil (MMF) (2000 mg/day) was added. Ascites developed 1 year after and liver failure developed. She underwent retransplantation 88 months after LDLT.

In patient no. 5, liver dysfunction developed (AST, 82 IU/L; ALP, 685 IU/L) 50 months after LDLT and was successfully treated with steroid pulse therapy. Liver dysfunction developed and liver biopsy was performed 90 months after LDLT. Total bilirubin was 1.2 mg/dL, AST 57 IU/L, ALP 585 IU/L and γ-GT 48 IU/L. AMA and M2 were not measured. Immunosuppression consisted of tacrolimus only (4.0 mg/day), and MMF (2000 mg) was added thereafter. Portal hypertension started to develop. Radiological examinations yielded a diagnosis of artery-portal shunt of segment 3 of the graft. Shunt

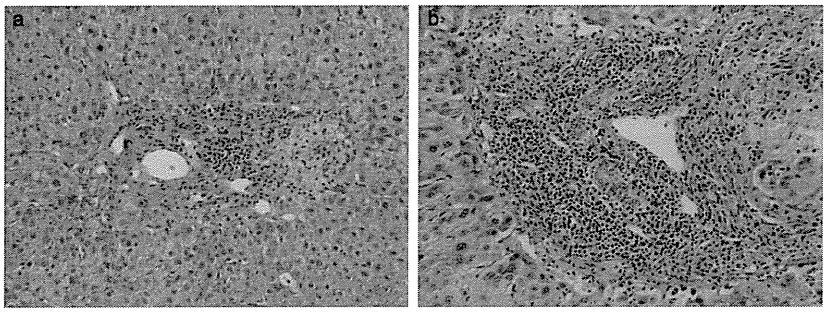


**Figure 1** Histological findings of patient no. 2. (a) Wedge liver biopsy at postoperative month 20. Suspected recurrence of primary biliary cirrhosis (PBC) with bile duct loss and mild lobular and portal hepatitis. (b) Second explant liver (allograft). Suspected recurrence of PBC with moderate portal hepatitis and minimal bile duct damage (hematoxylin–eosin, original magnification ×200).

occlusion using metallic coils failed and led to liver failure. She underwent retransplantation 120 months after LDLT.

## DISCUSSION

HISTOLOGICAL EXAMINATION IS the gold standard for recurrent PBC. Hubscher *et al.* reported the histological features to be mononuclear portal inflammation, portal lymphoid aggregate, portal granulomas and bile duct damage.[9] These findings are observed also in complications other than recurrent PBC. Lymphoid aggregate can be observed in chronic hepatitis, and bile duct damage and/or vanishing bile duct can be observed in chronic rejection or in the end stage of chronic cholangitis. Foamy cell arteriopathy, which is another specific feature of chronic rejection, is seldom observed on needle biopsy. Duct loss without portal granuloma suggests chronic rejection. The current study focusing on explanted allografts was conducted to avoid these uncertain factors.

Recently, late cellular rejection, chronic hepatitis, and de novo autoimmune hepatitis were discussed as causes of late liver allograft dysfunction.[16] Haga *et al.* reported perivenular lymphoplasmacytic infiltration in a case of their series, which simulated autoimmune hepatitis rather than typical PBC. In our series, ANA was strongly positive prior to primary transplantation in two patients but there were no such findings.

The incidence of recurrent PBC increased along with long-term follow up. Montano-Loza *et al.* studied the cumulative probability of PBC recurrence after LT.[17] Their histological study was not based on protocol biopsy. The overall 5- and 10-year probability of recurrence was 13% and 29%, respectively, in their series. They analyzed risk factors for recurrence and the clinical impacts. Although PBC transplant recipients receiving cyclosporin have a lower risk of disease recurrence, the development of recurrent PBC had no impact on long-term patient survival during 10 years of follow up. The incidence in LDLT based on protocol biopsy was 40% during 10 years of follow up.[3] Besides the increasing incidence, progression of recurrent PBC is still a concern, although progression of recurrent PBC was slow within 10 years of follow up in our series. In Japanese registries of LT, some cases of mortality after 10 years have been reported but information about their causes is not available.[18] A precise study of these cases is required to reveal the risks including recurrence in long-term follow-up.

Protocol biopsies for early diagnosis of recurrent PBC may not be essential to improve clinical courses of

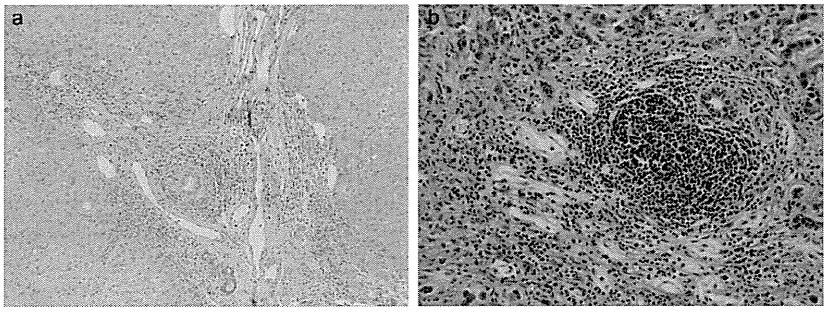


**Figure 2** Histological findings of patient no. 4. (a) Needle liver biopsy at postoperative month 71. Recurrence of primary biliary cirrhosis (PBC) with non-suppurative cholangitis and moderate portal hepatitis and fibrosis. (b) Second explant liver (allograft). Suspected recurrence of PBC with focal duct damage and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×150; [b] ×200).

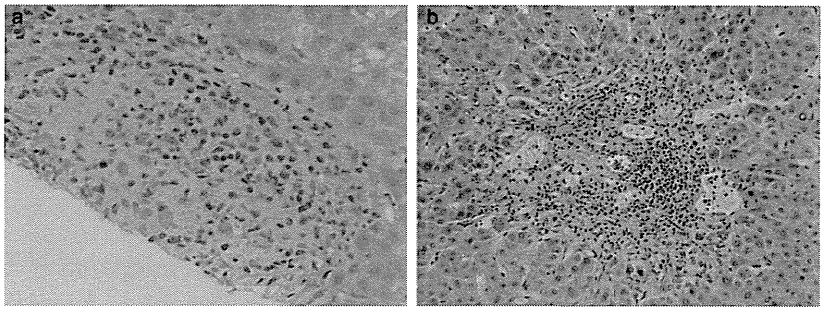


**Figure 3** Histological findings of patient no. 5. (a) Needle liver biopsy at postoperative month 90. Recurrence of primary biliary cirrhosis (PBC) with focal cholangitis and epithelioid granuloma. (b) Second explant liver (allograft). Suspected recurrence of PBC with bile duct loss and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×250; [b] ×200).

patients after LT for PBC. Timely biopsies and suitable radiological examinations, when hepatic chemistries deteriorate, are important to improve the clinical course within 10 years after transplantation.

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## REFERENCES

1 Silvera MG, Talwalker JA, Lindor KD, Wiesner RH. Recurrent primary biliary cirrhosis after liver transplantation. *Am J Transplant* 2010; **10**: 720–6.
2 Hashimoto E, Shimada M, Noguchi S *et al.* Disease recurrence after living donor liver transplantation for primary biliary cirrhosis: a clinical and histological follow-up study. *Liver Transpl* 2001; **7**: 588–95.
3 Hashimoto E, Taniai M, Yatsuji S *et al.* Long-term clinical outcome of living-donor liver transplantation for primary biliary cirrhosis. *Hepatol Res* 2007; **37**: S455–61.
4 Takeishi T, Sato Y, Ichida T, Yamamoto S, Kobayashi T, Hatakeyama K. Short-term outcomes of living-related liver transplantation for primary biliary cirrhosis and its recurrence: report of five cases. *Transplant Proc* 2003; **35**: 372–372.
5 Morioka D, Egawa H, Kasahara M *et al.* Impact of leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. *Liver Transpl* 2007; **13**: 80–90.
6 Kaneko J, Sugawara Y, Tamura S *et al.* Long-term outcome of living donor liver transplantation for primary biliary cirrhosis. *Transpl Int* 2012; **25**: 7–12.
7 Rowe IA, Webb K, Gunson BK, Mehta N, Haque S, Neuberger J. The impact of disease recurrence on graft survival following liver transplantation: a single center experience. *Transpl Int* 2008; **21**: 459–65.
8 Charatcharoenwitthaya P, Pimentel S, Talwalkar JA *et al.* Longterm survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl* 2007; **13**: 1236–45.
9 Hubscher SG, Elias E, Buckels JA, Mayar AD, McMaster P, Neuberger JM. Primary biliary cirrhosis. Histological eveidence od disease recurrence after liver transplantation. *J Hepatol* 1993; **18**: 173–84.
10 Haga H, Miyagawa-Hayashino Aya TK *et al.* Histological recurrence of autoimmune liver diseases after living-donor liver transplantation. *Hepatol Res* 2007; **37**: S463–S469.
11 International panel. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25**: 658–63.
12 Demetris AJ, Adams D, Bellamy C *et al.* Update of the international banff schema for liver allograft rejection: working recommendation for the histopathologic staging and reporting of chronic rejection. An international panel. *Hepatology* 2000; **31**: 792–9.
13 Nakanuma Y, Zen Y, Harada K *et al.* Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. *Pathol Int* 2010; **60**: 167–74.
14 Kleiner DE, Brunt EM, Van Natta M *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313–21.
15 Neuberger J. Recurrent primary biliary cirrhosis. *Liver Transpl* 2003; **9** (6): 539–46.
16 Banff Working Groups, Demetris AJ, Adeyi O, Bellamy CO *et al.* Liver biopsy interpretation as cause of late liver allograft dysfunction. *Hepatology* 2006; **44**: 489–501.
17 Montano-Loza J, Wasilenko S, Bintner J, Mason AL, Cyclosporine A. Protects against primary biliary cirrhosis recurrence after liver transplantation. *Am J Transplant* 2010; **10**: 852–8.
18 The Japanese Liver Transplant Society. Liver Transplantation in Japan – Registry by the Japanese Liver Transplantation Society. *Ishoku* 2012; **46**: 524–36.

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Original Paper

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# Evaluation of immune function under conversion from Prograf to Advagraf in living donor liver transplantation

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**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

## Summary

**Background:** Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf in liver transplantation, there have been no reports showing the change of immune function after conversion. The aim of this study is not only to analyze the safety and efficacy of conversion from Prograf to Advagraf, but also to evaluate the immune function using the ImmuKnow assay.

**Material/Methods:** Of the 168 living donor liver transplantation (LDLT) patients, 21 recipients whose liver function was stable after discharge in outpatient clinic and who agreed to conversion from Prograf to Advagraf were enrolled in this study. Liver, renal, and immune functions were retrospectively reviewed.

**Results:** There were no significant differences in liver and renal function after conversion from Prograf to Advagraf. The intracellular adenosine triphosphate levels before and after conversion were 263±157 and 256±133 ng/ml, respectively, and there was also no significant difference in immune function. None of the recipients showed adverse effects, rejection, or severe infection during the study. It should be further noted that none of the recipients had to increase the dose of Advagraf, while five of 21 recipients (24%) were able to reduce the dose of Advagraf during this study.

**Conclusions:** Conversion from Prograf to Advagraf in LDLT can be performed safely and effectively without affecting liver, renal, and immune function.

**Key words:** Advagraf • tacrolimus • ImmuKnow • LDLT

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**Tables:** 1
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**References:** 21

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## BACKGROUND

Immunosuppressive therapy is essential to preserve graft function in solid organ transplant recipients [1]. Prograf (Astellas Pharma, Inc.), which is a calcineurin inhibitor developed as an oral twice-daily medicine containing tacrolimus, has been the standard therapeutic regimen all over the world [2]. However, the oral twice-daily regimen has led to non-compliance, and non-compliance causes life-threatening rejection and late graft dysfunction [3,4]. To prevent this, Advagraf (Astellas Pharma, Inc.), a modified tacrolimus formation, was developed as an oral once-daily medicine. At present, conversion to Advagraf therapy has been accepted in various stable organ transplant recipients [5–11].

However, there have been no reports that show the actual changes of immune function after conversion. The ImmuKnow assay (Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay, Cylex, Inc., USA), which was approved by the Food and Drug Administration in 2002, has been shown to be capable of directly measuring the global immune response, especially T-cell-mediated immunity in transplant recipients. This assay has been shown to reliably distinguish between immune profiles of overimmunosuppression and underimmunosuppression and has been reported to be a convenient, non-invasive, *in vitro* assay, and to be effective as an immune monitoring tool for organ transplant recipients [12,13]. The aim of this study is to analyze the safety and efficacy of conversion from Prograf to Advagraf using not only liver and renal function but also immune function using the ImmuKnow assay.

## MATERIAL AND METHODS

### Patients

A total of 168 recipients underwent living donor liver transplantation (LDLT) from August 1997 to September 2011 at Nagasaki University Hospital. Of these recipients, 21 who underwent conversion from Prograf to Advagraf were enrolled in this study. They included 13 men and 8 women, with a median age at transplantation of 59 (range, 2–73). Original diagnoses included 3 hepatitis C virus (HCV) cirrhosis, 7 hepatitis B virus (HBV) cirrhosis, 5 alcoholic liver cirrhosis, and 6 others. Of these patients, 8 had hepatocellular carcinoma. The characteristics of the patients are shown in Table 1.

**Table 1.** The characteristic of the recipients.

| Variable | Recipients (n=21) |
|---|---|
| Gender (male: female) | 13: 8 |
| Age | 59 (2–73) |
| Original diagnosis* | HBV-LC: 2<br>HBV-LC/ HCC: 5<br>HCV-LC/ HCC: 3<br>Alcoholic LC: 5<br>BA: 4<br>FHF: 2 |
| Duration between LDLT and conversion (months) | 33 (7–171) |
| Duration after conversion (months) | 8 (3–29) |
| Dose of Advagraf at conversion (mg/day) | 2 (1–4) |

* HBV – hepatitis B virus; HCV – hepatitis C virus; LC – liver cirrhosis; HCC – hepatocellular carcinoma; BA – biliary atresia; FHF – fulminant hepatic failure.

### Protocol of immunosuppressant

The baseline protocol of immunosuppressants consisted of Prograf and steroids. The steroids were discontinued three to six months after staged reduction, as long as the liver function was stable without rejection. Prograf was initiated at the dose of 1 mg twice a day after transplantation, and regulated to adjust the desired tacrolimus trough level, 10–15 ng/ml within one month after transplantation and 5–10 ng/ml thereafter. In the outpatient clinic, Prograf was gradually reduced as long as the liver function was stable, and maintained at a minimal dose to prevent both adverse effects and rejection. The indications of the conversion were that liver functions had been stable for at least the three previous months in the outpatient clinic before conversion and that the recipient's fully informed consent to conversion was given. The initial dose after conversion to Advagraf started with the dose equivalent to the dose of Prograf at conversion.

### Laboratory evaluation

Tacrolimus trough (Tac), total bilirubin (T-Bil), alanine aminotransferase (ALT), estimated Glomerular Filtration Rate (eGFR), serum creatinine (Cr), and fasting blood sugar (FBS) levels were recorded just before conversion and at the last follow-up and evaluated retrospectively.

### The ImmuKnow assay

The immune function was evaluated using Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay (Cylex, Inc. USA). This assay

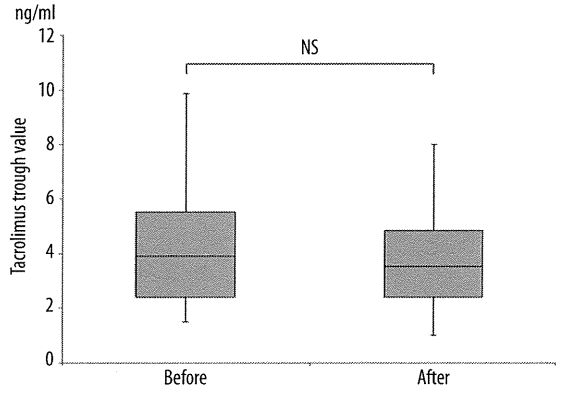


**Figure 1.** The change of the tacrolimus trough level before and after conversion. Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively and there was no significant difference in Tac.

was performed according to the manufacturer's protocol [14]. A whole blood sample was collected from each recipient just before conversion and at the last follow-up. The blood sample was collected into an 8-ml sodium heparin vascutainer tube and tested within 10 hours. The whole blood was diluted with a sample diluent, added to a microtiter plate well, and incubated with phytohemagglutinin for 15 to 18 hours in a 37°C, 5% $CO_2$ incubator. The following day, CD4+ cells were positively selected within the microwells with magnetic particles coated with anti-human CD4 monoclonal antibody (Dynabeads, Dynal, Oslo, Norway) and a strong magnet (model 1050 magnet tray, Cylex, Inc., Columbia, MD) and washed to remove residual cells. A lysing reagent was added to release intracellular adenosine triphosphate (ATP). A luciferin/luciferase mixture was then added to the cell lysate. Within 10 minutes after the addition of the enzyme, released ATP was measured with a GloRunner™ Microplate Luminometer (Turner Biosystems CA).

### Statistical analysis

Results for continuous variables were expressed as the median (range). Data for continuous variables were compared using the Mann-Whitney U test. We set statistical significance at $p<.05$.

## Results

Change in Tac level and liver functions after conversion.

As shown in Figure 1, the Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively, and there was no significant difference in Tac. Figure 2 shows liver function. The serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively, and the serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

### Change in renal functions and FBS levels after conversion

Figure 3 shows renal function and FBS level. The serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 $m^2$, the serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, and the serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal function or FBS level.

### Change in ATP levels after conversion

Figure 4 shows the immune function. The ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function. In addition to these results, none of the recipients showed adverse effects, rejection, or severe

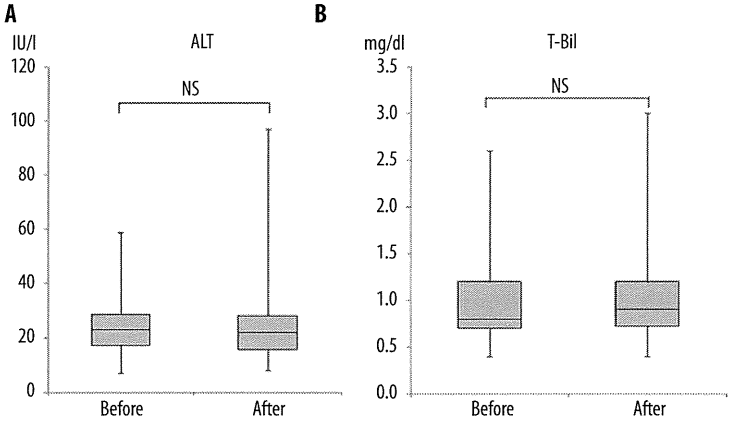


**Figure 2.** The change of liver functions before and after conversion. (**A**) Serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively. (**B**) Serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

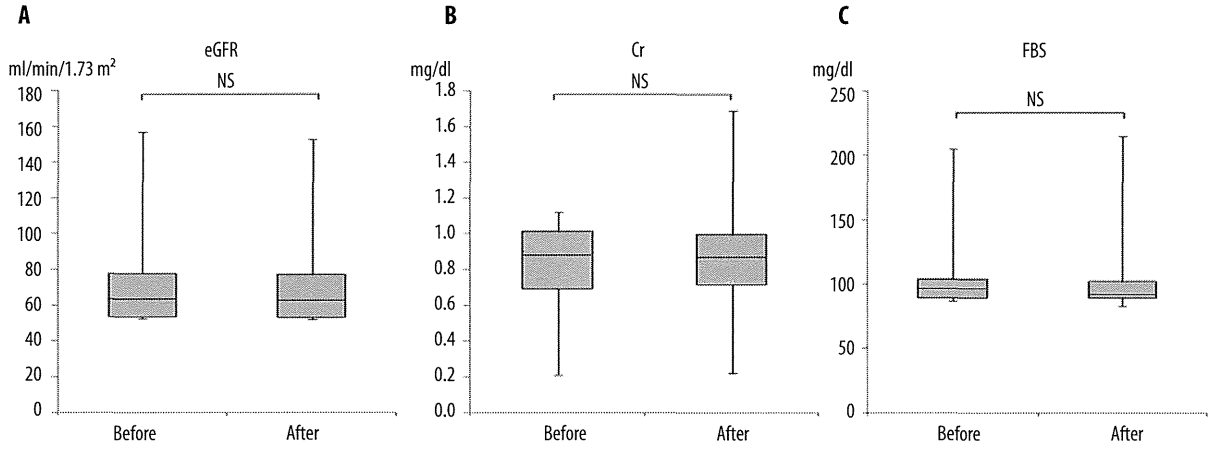


**Figure 3.** The change of renal functions and FBS before and after conversion. (**A**) Serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 m², respectively. (**B**) Serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, respectively. (**C**) Serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal functions or FBS level.

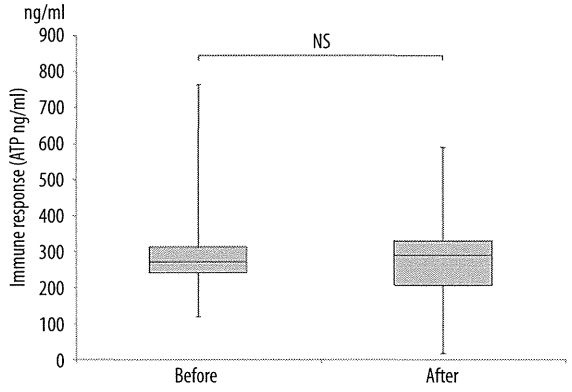


**Figure 4.** The change of immune function before and after conversion. ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function.

infection during the study. It should also be noted that none of the recipients had to increase the dose of Advagraf, and five of the recipients (24%) could reduce the dose of Advagraf without rejection during this study.

## Discussion

Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf with regard to liver and renal function [8–11], the actual immune function has not yet been clarified. Liver transplantation has been the standard therapeutic option for end-stage liver diseases and reduces the mortality and morbidity of end-stage liver diseases as reflected in the 1- and 5-year survival rates [15–17]. This is mainly the result of improved immunosuppression due to the introduction of a calcineurin inhibitor. Prograf was the immediate-release form of tacrolimus and the oral twice-daily medicine used to prevent various complications in solid organ transplantations and has been accepted as the standard therapeutic regimen all over the world [2,18,19]. However, the estimated rates of nonadherence to immunosuppressive regimens in solid organ transplant recipients range from 15 to 55% [15–17]. Nonadherence has been identified as a leading cause of preventable graft loss [3,4]. It has been proposed that simpler dosing regimens, such as an oral once-daily regimen, may help to improve adherence in transplant recipients [20]. In fact, the prolonged-release form of tacrolimus (Advagraf) was developed as an oral once-daily medicine, and some data have shown that an oral once-daily regimen was associated with an increased likelihood of patient adherence compared with an oral twice-daily regimen [21]. Some reports have evaluated liver and renal function before and after conversion and have shown that the conversion can be applied to liver transplant recipients [8–11]. This study was also able to suggest that conversion does not affect liver and renal function, which is consistent with previous reports.

Additionally, we adapted the ImmuKnow assay to evaluate of the actual immune function. This assay was approved by the US Food and Drug Administration in 2002 for measuring CD4+ T cell immunity [5]. A meta-analysis by Kowaski et al. reported that this assay was useful in monitoring the immune response and assessing the relative risk of infection and rejection [6]. However, no reports have evaluated the safety and efficacy of conversion from Prograf to Advagraf with regard to immune function using this assay. As a result, there

was no significant difference in immune function before and after conversion; this result suggested that conversion also did not affect immune function. In addition, it was important that none of the recipients showed adverse effects, rejection, or severe infection and none had to increase the dose of Advagraf, while five of 21 recipients (24%) were even able to reduce the dose of Advagraf during this study. In our policy of immunosuppression, especially in long-term cases, we reduce and maintain the dose of immunosuppressant as long as possible, keeping the lowest level of tacrolimus needed to prevent rejection. According to the results of this study, Advagraf might be a feasible treatment for avoiding an overdose of tacrolimus.

## Conclusions

This study suggested that the conversion of Advagraf can be safely and effectively applied to stable LDLT recipients without affecting liver, renal, and immune function.

### Disclosure

The authors have no conflicts of interest or funding to disclose.

## References:


1. Chapman JR: The KDIGO clinical practice guideline for the care of kidney transplant recipients. Transplantation, 2010; 89: 644–45
2. Weiler N, Thrun I, Hoppe-Lotichius M et al: Early steroid-free immunosuppression with FK506 after liver transplantation: long-term results of a prospectively randomized double-blinded trial. Transplantation, 2010; 90: 1562–66
3. Gaston RS, Hudson SL, Ward M et al: Late renal allograft loss: noncompliance masquerading as chronic rejection. Transplant Proc, 1999; 31: 21S–23S
4. Denhaerynck K, Dobbles F, Cleemput I et al: Prevalence, consequences, and determinants of nonadherence in adult renal transplant patients: a literature review. Transpl Int, 2005; 18: 1121–33
5. van Hooff JP, Alloway RR, Trunečka P, Mourad M: Four-year experience with tacrolimus once-daily prolonged release in patients from phase II conversion and *de novo* kidney, liver, and heart studies. Clin Transplant, 2010; 25: E1–12
6. Iaria G, Sforza D, Angelico R et al: Switch from twice-daily tacrolimus (Prograf) to once-daily prolonged-release tacrolimus (Advagraf) in kidney transplantation. Transplant Proc, 2011; 43: 1028–29
7. Calia R, Lai C, Aceto P et al: Effects of switching from twice-daily to once-daily tacrolimus formulation on quality of life, anxiety, and transplant benefit perception after kidney transplantation. Transplant Proc, 2011; 43: 1020–23
8. Marin-Gomez LM, Gomez-Bravo MA, Alamo-Martinez JA et al: Evaluation of clinical safety of conversion to Advagraf therapy in liver transplant recipients: observational study. Transplant Proc, 2009; 41: 2184–86
9. Trunečka P, Boillot O, Seehofer D et al: Once-daily prolonged-release tacrolimus (ADVAGRAF) versus twice-dailiy tacrolimus (PROGRAF) in liver transplantation. Am J Transplant, 2010; 10: 2313–23
10. Comuzzi C, Lorenzin D, Rossetto A et al: Safety of conversion from twice-daily tacrolimus (Prograf) to once-daily prolonged-release tacrolimus (Advagraf) in stable liver transplant recipients. Transplant Proc, 2010; 42: 1320–21
11. Perrakis A, Schwarz K, Yedibela S et al: Impact of the conversion of the immunosuppressive regimen from Prograf to Advagraf or to Sirolimus in long-term stable liver transplant recipients: indication, safety, and outcome. Transplant Proc, 2011; 43: 3702–7
12. Kowalski RJ, Post DR, Schneider MC et al: Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant, 2003; 17: 77–88
13. Kowalski RJ, Post DR, Mannon RB et al: Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. Transplantation. 2006; 82: 663–68
14. Sottong PR, Rosebrock JA, Britz JA, Kramer TR: Measurement of T-lymphocyte responses in whole-blood cultures using newly synthesized DNA and ATP. Clin Diagh Lab Immunol, 2000; 7: 307–11
15. O'Carroll RE, McGregor LM, Swanson V et al: Adherence to medication after liver transplantation in Scotland: a pilot study. Liver Transplant, 2006; 12: 1862–68
16. Butler JA, Roderick P, Mullee M et al: Frequency and impact of nonadherence to immunosuppressants after renal transplantation: a systematic review. Transplantation, 2004; 77: 769–76
17. Vasquez EM, Tanzi M, Benedetti E, Pollak R: Medication noncompliance after kidney transplantation. Am J Health Syst Pharm, 2003; 60: 266–69
18. Kelly PA, Burckart GJ, Venkataramanan R: Tacrolimus: a new immunosuppressive agent. Am J Health Syst Pharm, 1995; 52: 1521–35
19. Spencer CM, Goa KL, Gillis JC: Tacrolimus. An update of its pharmacology and clinical efficacy in the management of organ transplantation. Drugs, 1997; 54: 925–75