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.

Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus co-infection: update in 2013

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Abstract Because of the progress of anti-retroviral therapy (ART) for human immunodeficiency virus (HIV), mortality due to opportunistic infection resulting in AIDS has been remarkably reduced. However, meanwhile, half of those patients have died of end-stage liver cirrhosis due to hepatitis C virus (HCV) with liver cirrhosis and early occurrence of hepatocellular carcinoma. Recently, in 2013, non-cirrhotic portal hypertension due to ART drugs or still unknown mechanisms have become problematic with early progression of the disease in this patient population. Liver transplantation (LT) could be one treatment of choice in such cases, but the indications for LT perioperative management, including both HIV and HCV treatments and immunosuppression, are still challenging. In this review, we update the literature on HIV/HCV co-infection and LT as well as recent effort for modifying allocation system for those patients.

Keywords Co-infection · Hepatitis C virus · HIV · Human immunodeficiency virus · Liver transplantation

Introduction

The causes of death of human immunodeficiency virus (HIV) infected patients have dramatically changed since 1995. A major background factor behind these trends is the improved HIV control achieved with anti-retroviral therapy (ART) [1]. Despite dramatic reduction of death due to acquired immunodeficiency syndrome (AIDS), co-infected hepatitis C virus (HCV)-related death due to liver failure or hepatocellular carcinoma (HCC) became a serious problem, not only in Japan but all over the world, including England

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[2]. In Japan, in the late 1980s, contaminated blood products for hemophilia caused co-infection by HIV and HCV. In such cases, liver transplantation (LT) is the only possible treatment option to achieve long-term survival, but several modifications of perioperative management are required recently for better outcome.

In this review, the outcome and the points of management of LT for HIV/HCV co-infected patients were reviewed to save relatively young patients with HIV/HCV co-infection bearing HCC [3, 4], non-cirrhotic portal hypertension (NCPH) [5–7], and decompensated liver cirrhosis [8, 9]. An updated critical review of the literature in 2013 was performed, and new information on problems and results for LT for HIV/HCV co-infection were included.

Upcoming topics regarding LT indications for HIV/HCV co-infection in 2013

Non-cirrhotic portal hypertension

In HIV/HCV coinfected patients, liver failure due to HCV hepatitis was enhanced by ART-related hepatotoxicity, especially manifesting as non-cirrhotic portal hypertension [5–7]. One of the ART drugs, Didanosin (DDI), has been suspected for serious morbidity. Thus, not only in cases with deteriorated liver function, such as in Child–Pugh B or C cases, but also even in Class A cases, patients' liver function can easily deteriorate abruptly [10, 11]. The actual natural course of pure NCPH is unknown, because it can be modulated with HCV or other causes and reported as only case series. However, an important study regarding "Non-cirrhotic portal hypertension in HIV mono-infected patients without HCV" was published in 2012 [12]. All five patients had portal hypertensive symptoms such as ascites or variceal bleeding after ART medication. We need to await their prognostic information, since it can be extrapolated into HIV/HCV co-infected patients after successful HCV eradication.

Therefore, all HIV/HCV co-infected patients should be carefully followed up so as not to miss the opportunity for LT. Recently, in Japan, a scoring system was created for listing a deceased donor LT for those patients with HIV/HCV co-infection due to previous contaminated blood products.

Hepatocellular carcinoma

Recently it became evident that HCC in HIV/HCV co-infected patients develop HCC at a very early stage of life, such as in the 30s and 40s [3, 4]. The molecular mechanism of its development still remains unclear, but surveillance in those patients should be considered for HCC strictly. In Japan, HIV/HCV co-infected hemophilic patients have been undergoing periodic examination for liver-related disease on a research basis. Early detection could contribute to treatment choices such as liver resection or liver transplantation. Regardless of the infectious status of HIV, treatment strategy for HCC in HIV/HCV infected patients should be the same in HCV mono-infected patients. Namely, whether liver resection could be performed or not should be based on the liver functional reserve. Also radio frequency ablation and transarterial chemoembolization can be selected according to the location, size and number of HCC.

Current results of LT for HIV/HCV co-infected patients in 2013

Indications for LT

As HCV mono-infected patients, LT should be considered when patients develop deteriorated liver function as indicated by a Child–Pugh score of class B or C in co-infected patients. Recently, Murillas et al. reported that the Model for End-stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients [13]. HIV/HCV co-infected patients might be considered for LT before their MELD score increases to achieve comparable results with HCV mono-infected patients. Several studies showed that aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients [14, 15], but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse (ARFI) imaging to check for liver stiffness has been introduced as an effective and noninvasive modality to determine patients' candidacy for LT [16, 17].

Regardless of the presence of hemophilia, the indications and methods for performing liver transplantation remains unchanged for patients with HIV/HCV co-infection. In fact, after a successful liver transplantation, hemophilia can normally be cured. Usually, the conditions for liver transplan-

tation are as follows: (1) AIDS symptoms have not surfaced; (2) CD4+ T lymphocyte count is 150–200/μl or above; and (3) as a result of ART, the amount of HIV RNA in the blood by PCR method is below the level of sensitivity of the assay.

In HIV/HCV co-infected patients, current studies show that a count of more than 100/μl CD4+ T lymphocytes is acceptable [18, 19], because patients generally have portal hypertension, which can cause leukocytopenia. In such patients, the ratio of CD4/CD8 is reported to be a realistic marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher [20].

In 2013, based on the evidence of rapid progression of the liver cirrhosis and portal hypertension in patients with HIV/HCV co-infection, a ranking system for waiting list of deceased donor LT has been set up in Japan. Even HIV/HCV co-infected liver cirrhotic patients with Child–Pugh class A can be listed for LT as "point 3" because of NCPH nature. Also co-infected patients with Child–Pugh class B and C can be listed as "point 6" and "point 8" based on the data from our HIV/AIDS project team of the Ministry of Health, Labor, and Welfare of Japan, and world literatures [21–23]. It is basically considered for previous victims of contaminated blood products for hemophilia.

Results of LT for patients with HIV/HCV co-infection

In the United States and Europe, liver transplantation from deceased donors has been performed in HIV patients since the 1980s. At that time, the outcomes of LT were very poor [11]. Recent series of reports are listed in Table 1 [24–31]. The reality is that, in addition to those listed therein, there have been many sporadic reports, such as reviews, expectations for liver transplantation, and assessment of indications.

In general, most reports concluded that the results were 10% worse than in the cases with HCV mono-infection, with a 3-year survival of around 60–70%. Recently, a 5-year patient survival of around 50% was reported, and there is debate whether these results can be accepted for patients of a younger age and were co-infected through previous use of a contaminated blood product. In Japan, the Tokyo group reported six cases of living donor liver transplantation (LDLT) between 2001 and 2004 [32]. Terrault et al. reported that older donor age, combined kidney-liver transplantation, an anti-HCV positive donor, and a body mass index <21 kg/m² were independent predictors of graft loss [33]. After LT, several studies showed that acute cellular rejection was more frequent and more severe in HIV/HCV co-infected patients than in HCV mono-infected patients, possibly due to difficulties in achieving optimal immuno-suppression because of interactions between antiretroviral agents and immunosuppression.

Table 1 Updated outcome of liver transplantation for HIV positive recipients

Authors	Year	Country	n	Patient survival (%)			
				1 year	3 years	5 years	
Duclos-Vallee et al. [25]	2008	France	35	–	73	51	
Tsukada et al. [32]	2011	Japan	6	66	66	50	Only LDLT, only hemophilia
Terrault et al. [33]	2012	US	89	76	60	–	
Miro et al. [26]	2012	Spain	84	88	62	54	
Anadol et al. [27]	2012	Germany	32	90	65	60	
Harbell et al. [28]	2012	USA	125	91	67	–	
Baccarani et al. [31]	2012	Italy	32	–	79	69	
Di Benedetto et al. [46]	2012	Italy	30	75	65	50	with HCC
Ragni et al. [29]	2013	USA	15	71	38	–	only hemophilia

HCC hepatocellular carcinoma, *LDLT* living donor liver transplantation

Lowered outcome can be presumed from previous reports. Final mortality (graft loss) after LT was usually due to infection and multiorgan failure. As in Miro's report the causes due to the higher proportion of organs from donation after cardiac death (DCD) donors, higher rate of combined liver-kidney transplantation, increased rate of acute cellular rejection, HBV co-infection and infection. However, it was of note that there was no death due to infections related to HIV.

Preoperative management of HIV/HCV in liver transplantation

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 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. Also ART can be toxic for the virgin graft, which underwent ischemia/reperfusion injury and liver resection in a donor. Once it is settled down after liver transplant, especially in LDLT cases, ART can be resumed with meticulous adjustment with calcineurin inhibitors.

Actually, after LT, ART should be restarted as soon as possible, because HIV-RNA appears at 3 to 30 days after ART is stopped [34], but the timing of restart of ART depends on the patient's condition, including liver function [35]. As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not yet sufficiently regenerated, 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) [36]. ART administered after LT should be the same as the preLT regimen, but the majority of ART drugs, including protease inhibitors and non-nucleoside reverse transcriptase inhibitors, have interactions with calcineurin inhibitors (CNI) or mammalian

target-of-rapamycin (mTOR) [37], so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. It can easily overshoot beyond the therapeutic level. Currently, a novel HIV-1 integrase inhibitor, raltegravir, is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs [38, 39]. Therefore, the current recommended strategy in the light of LT could be to try raltegravir as ART before LT and see if HIV can be controlled with raltegravir. If it is the case, CNI could be used as usual after LT. However, if raltegravir cannot control HIV or cannot be applied due to other reasons, meticulous management of CNI (e.g. once a week administration with frequent trough monitoring) or Mycophenolate mofetil protocol should be considered. In fact, the novel protease inhibitor anti-HCV drug, telapreir, has the same character as ART drugs for HIV, and transplants team learn to overcome such drug interactions when post-LT HCV mono-infected patients are treated with telaprevir.

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 in 2013. 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 [40, 41]. As mentioned above, the novel protease inhibitor telaprevir is currently being introduced as an effective drug to achieve sustained viral response (SVR) of 70%, even in genotype 1b, with Peg-IFN/ribavirin in a non-transplant setting [42], but this drug is metabolized via cytochrome P450, 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 or even without Peg-IFN 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 for HIV/HCV co-infected patients. Additionally, mutational status of the IL28 B genotype should be investigated before interferon therapy for both donor and recipient.

Immunosuppression

Several reports have demonstrated both the in vitro and in vivo effectiveness of rapamycin in reducing HIV replication [43–45]. 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. Mycophenolic acid, a selective inhibitor of the de novo synthesis of guanosine nucleotides in T and B lymphocytes, has been proposed to inhibit HIV replication in vitro by depleting the substrate (guanosine nucleotides) for reverse transcriptase. Using these drugs, a more effective regimen of immunosuppression with ART may be established. However, more information needs to be obtained to establish concrete immunosuppressive protocol.

As to steroids, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. In HIV/HCV co-infected patients, a steroid-free protocol may play a beneficial role in preventing both HIV and HCV recurrence after LT [47, 48].

Hepatocellular carcinoma

Liver transplantation has been performed also for indication of HCC. The most updated study indicated that the existence of HCC did not change the outcome of LT provided that HCC was downstaged preoperatively for UCSF criteria [49]. Also for these cases sirolimus tended to be used as primary immunosuppressive agents. This encouraging result awaits further reports [50].

Conclusions

The above is an overview of liver transplantation performed to date in HIV/HCV- co-infected patients. Although, the results are 10% lower in patient survival after LT than those for HCV mono-infected patients, LT could be feasible in selected cases with HIV/HCV co-infection after careful evaluation within suitable stages of the disease. In light of the fact that most HIV/HCV co-infected patients in Japan are the victims of contaminated blood products, it is believed that the importance of liver transplantation will increase in the future in the context of medical relief as well.

Our investigating team under the Ministry of Health, Labor, and Welfare of Japan has made all possible efforts to clarify the appropriate timing to put HIV/HCV co-infected patients on a waiting list for LT.

References

1. Eguchi S, Soyama A, Hidaka M, Takatsuki M, Muraoka I, Tomonaga T, 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. Darby SC, Ewart DW, Giangrande PL, Spooner RG, Rizza CR, Dusheiko GM, 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;350:1425–31.
3. Merchante N, Merino E, Lopez-Aldeguer J, Jover F, Delgado-Fernandez M, Galindo MJ, et al. Increasing incidence of hepatocellular carcinoma in HIV-infected patients in Spain. HIV/AIDS. 2013;56:143–50.
4. Cusinato CT, Koetz AP, Barcellos NT, Wolff FH. The prevalence of cirrhosis and hepatocellular carcinoma in patients with human immunodeficiency virus infection. Hepatology. 2013;57:249–57.
5. Vispo E, Moreno A, Maida I, Barreiro P, Cuevas A, Albertos S, et al. Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. AIDS. 2010;24:1171–6.
6. 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.
7. Kovari H, Ledergerber B, Peter U, Flepp M, Jost J, Schmid P, 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.
8. Merchante N, Girón-González JA, González-Serrano M, Torre-Cisneros J, García-García JA, Arizcorreta A, et al. Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. AIDS. 2006;20:49–57.
9. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, 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.
10. 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.
11. de Vera ME, Dvorchik I, Tom K, Eghtesad B, Thai N, Shakil O, 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.
12. Jackson BD, Doyle JS, Hoy JF, Roberts SK, Colman J, Hellard ME, et al. Non-cirrhotic portal hypertension in HIV mono-infected patients. J Gastroenterol Hepatol. 2012;17:1512–19.

13. Murillas J, Rimola A, Laguno M, de Lazzari E, Rascón J, Agüero F, et al. ESLD-HIV Working Group Investigators. The model for end-stage liver disease score is the best prognostic factor in human immunodeficiency virus 1-infected patients with end-stage liver disease: a prospective cohort study. Liver Transpl. 2009;15:1133–41.

14. Rullier A, Trimoulet P, Neau D, Bernard PH, Foucher J, Lacoste 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.

15. Ragni MV, Moore CG, Soadwa K, Nalesnik MA, Zajko AB, Cortese-Hassett A, et al. HHH Study Group. Impact of HIV on liver fibrosis in men with hepatitis C infection and haemophilia. Haemophilia. 2011;17:103–11.

16. 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.

17. Merchante N, Rivero-Juárez A, Téllez F, Merino D, José Ríos-Villegas M, Márquez-Solero M, 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.

18. Miro JM, Torre-Cisnero J, Moreno A, Tuset M, Quereda C, Laguno M, 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.

19. 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.

20. Xia XJ, Liu BC, Su JS, Pei H, Chen H, Li L, 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.

21. Takatsuki M, Eguchi S, Soyama A, Kanematsu T, Nakao K, Shirasaka T, et al. Evaluation of portal hypertension and prognosis of patients with HIV-HCV co-infection through comtaminated blood product. Acta Hepatol Japonica (KANZO). 2012;53:586–90 (in Japanese).

22. Soyama A, Eguchi S, Takatsuki T, Hidaka M, Muraoka I, Kanematsu T. Analysis of hepatic functional reserve in HIV_HCV co-infected patients. Acta Hepatol Japonica (KANZO). 2012;53:403–8 (in Japanese).

23. López-Diéguez M, Montes ML, Pascual-Pareja JF, Quereda C, Von Wichmann MA, Berenguer J, et al. GESIDA 37/03-FIPSE 36465/03-NEAT IG5 Study Group. The natural history of liver cirrhosis in HIV-hepatitis C virus-coinfected patients. AIDS. 2011;25:899–904.

24. Schreibman I, Gaynor JJ, Jayaweera D, Pyrsopoulos N, Weppler D, Tzakis A, et al. Outcomes after orthotopic liver transplantation in 15 HIV-infected patients. Transplantation. 2007;84:697–705.

25. Duclos-Vallee JC, Feray C, Sebagh M, Sebagh M, Teicher E, Roque-Afonso AM, 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.

26. Miro JM, Montejo M, Castells L, Rafecas A, Moreno S, Agüero F, 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.

27. Anadol E, Beckebaum S, Radecke K, Paul A, Zoufaly A, Bickel M, et al. Orthotopic liver transplantation in human-immunodeficiency-virus-positive patients in Germany. AIDS Res Treat. 2012;2012:4–9.

28. Harbell J, Fung J, Nissen N, Olthoff K, Florman SS, Hanto DW, et al. Surgical complication in 275 HIV-infected liver and/or kidney transplantation recipients. Surgery. 2012;152:376–81.

29. Ragni MV, Devera ME, Roland ME, Wong M, Stosor V, Sherman KE, et al. Liver transplant outcomes in HIV(+) haemophilic men. Haemophilia. 2013;19:134–40.

30. O'Grady JG. Liver transplantation in human immunodeficiency virus/hepatitis C virus-coinfected patients: response needed! Liver Transpl. 2012;18:617–18.

31. Baccarani U, Adani GL, Bragantini F, Londero A, Comuzzi C, Rossetto A, 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.

32. Tsukada K, Sugawara Y, Kaneko J, Tamura S, Tachikawa N, Morisawa Y, et al. Living donor liver transplantations in HIV- and hepatitis C virus-coinfected hemophiliacs: experience in a single center. Transplantation. 2011;91:1261–4.

33. Terrault NA, Roland ME, Schiano T, Dove L, Wong MT, Poordad F, 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.

34. García F, Plana M, Vidal C, Cruceta A, O'Brien WA, Pantaleo G, et al. Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. AIDS. 1999;13:F79–86.

35. Neff GW, Bonham A, Tzakis AG, Ragni M, Jayaweera D, Schiff ER, et al. Orthotopic liver transplantation in patients with human immunodeficiency virus and end-stage liver disease. Liver Transpl. 2003;9:239–47.

36. Castells L, Escartín A, Bilbao I, Len O, Allende H, Vargas V, et al. Liver transplantation in HIV-HCV coinfected patients: a case-control study. Transplantation. 2007;83:354–8.

37. Frassetto LA, Browne M, Cheng A, Wolfe AR, Roland ME, Stock PG, et al. Immunosuppressant pharmacokinetics and dosing modifications in HIV-1 infected liver and kidney transplant recipients. Am J Transplant. 2007;7:2816–20.

38. 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.

39. Tricot L, Teicher E, Peytavin G, Conti F, Calmus Y, Barrou B, et al. Safety and efficacy of raltegravir in HIV-infected transplant patients cotreated with immunosuppressive drugs. Am J Transplant. 2009;9:1946–52.

40. 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;183:1112–15.

41. Sulkowski MS, Sherman KE, Dieterich DT, Bsharat M, Mahnke L, Rockstroh JK, et al. Combination therapy with telaprevir for chronic hepatitis C virus genotype 1 infection in patients with HIV: a randomized trial. Ann Intern Med. 2013;159:86–96.

42. Polard E, Camus C, Abault AY, Turlin B, Arvieux C, Messner M, 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.

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, Le N, Reardon E, Dominique JK, 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: 10411–16.

46. Di Benedetto F, Di Sandro S, De Ruvo N, Montalti R, Ballarin R, Guerrini GP, et al. First report on a series of HIV patients undergoing rapamycin monotherapy after liver transplantation. Transplantation. 2010;89:733–8.

47. Klintmalm GB, Davis GL, Teperman L, Netto GJ, Washburn K, Rudich SM, 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.

48. Marubashi S, Umeshita K, Asahara T, Fujiwara K, Haga H, Hashimoto T, et al. Steroid-free living donor liver transplantation for HCV – a multicenter prospective cohort study in Japan. Clin Transplant. 2012;26:857–67.

49. Di Benedetto F, Tarantino G, Ercolani G, Baccarani U, Montalti R, De Ruvo N, et al. Multicenter Italian experience in liver transplantation for hepatocellular carcinoma in HIV-infected patients. Oncologist. 2013;18:592–9.

50. Baccarani U, Adani GL, Tavio M, Viale P. Liver transplantation for hepatocellular carcinoma: the impact of human immunodeficiency virus infection. Hepatology. 2011;53:2138–9.

ORIGINAL ARTICLE

The Outcomes of Patients with Severe Hyperbilirubinemia Following Living Donor Liver Transplantation

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Abstract

Background Prolonged hyperbilirubinemia (HB) follow-
ing living donor liver transplantation (LDLT) can be a risk
factor for early graft loss and mortality. However, some
recipients who present with postoperative hyperbilirubi-
nemia do recover and maintain a good liver function.
Aim The purpose of this study was to investigate the risk
factors for hyperbilirubinemia following LDLT and to
identify predictors of the outcomes in patients with post-
transplant hyperbilirubinemia.
Methods A total of 107 consecutive adults who under-
went LDLT in Nagasaki University Hospital were inves-
tigated retrospectively. The patients were divided into two
groups according to postoperative peak serum bilirubin
level (HB group: ≥30 mg/dl; non-HB group: <30 mg/dl).
These two groups of patients and the prognosis of patients
in the HB group were analyzed using several parameters.
Results Seventeen patients (15.9 %) presented with
hyperbilirubinemia, and their overall survival was signifi-
cantly worse than patients in the non-HB group ($n = 90$).
Donor age was significantly higher in the HB group
($P < 0.05$). Of the 17 patients in the HB group, nine sur-
vived. The postoperative serum prothrombin level at the
time when the serum bilirubin level was >30 mg/dl was
significantly higher in surviving patients ($P < 0.01$).
Conclusions The use of a partial liver graft from an aged
donor is a significant risk factor for severe hyperbilirubi-
nemia and a poorer outcome. However, those patients who

maintain their liver synthetic function while suffering from
hyperbilirubinemia may recover from hyperbilirubinemia
and eventually achieve good liver function, thus resulting
in a favorable survival.

Introduction

Hyperbilirubinemia following living donor liver trans-
plantation (LDLT) can be caused by several mechanisms,
such as initial poor function, acute cellular rejection, sur-
gical complications, small-for-size syndrome, drug toxic-
ity, among others. Hyperbilirubinemia has also been
reported to be a risk factor for early graft loss and mortality
[1]. However, some recipients can overcome hyperbiliru-
binemia, and these patients subsequently achieve and
maintain a good liver function after their eventual recovery
from hyperbilirubinemia. The aim of this study was to
retrospectively clarify the risk factors for the development
of postoperative severe hyperbilirubinemia and to identify
any predictors for the outcomes in patients who present
with hyperbilirubinemia following LDLT.

Patients and Methods

We retrospectively analyzed the data of 107 consecutive
adult patients (67 males, 40 females, median age 55 years,
age range 16–68 years) who underwent LDLT in the
Department of Surgery of Nagasaki University Hospital
between November 1997 and January 2010. The etiologies

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of the liver disease were hepatitis C virus infection (35 patients), hepatitis B virus infection (25 patients), non-viral causes (40 patients), and fulminant liver failure (7 patients) (Table 1). During this period, we occasionally treated patients with a postoperative bilirubin level of >20 mg/dl. Marubashi et al. [1] reported that a postoperative peak serum bilirubin level of >27 mg/dl could be a predictor of short-term graft outcome. Therefore, we defined those patients who had presented with a postoperative peak serum bilirubin level of >30 mg/dl as having hyperbilirubinemia (HB group); the remaining patients formed the non-HB group.

The two groups of patients were compared for preoperative serum bilirubin level; donor age; the postoperative peak alanine aminotransferase (ALT); model for end-stage liver disease (MELD) score; graft weight (GW)/standard liver volume ratio [SLV; SLV (ml) = 706.2 × body surface area (m^2) + 2.4] [2]; type of graft; development of acute cellular rejection [as proven by biopsy within postoperative day (POD) 60]; ABO compatibility; the development of biliary complications. We defined a biliary complication as anastomotic stenosis that needed interventions by means of balloon dilatation, stent placement, or re-operation. We divided the types of grafts into those for the right lobe and left lobe, respectively. The right lobe included the right lateral sector, and the left lobe included the left lateral segment.

In the HB group, we compared surviving and non-surviving patients for all of the above-mentioned parameters as well as for serum prothrombin [PT (%)] and creatinine levels at the time when the serum bilirubin level was >30 mg/dl. In the HB

Table 1 Indication for liver transplantation

Cause of liver disease	Total (n = 107)	HB group (n = 17)	Non-HB group (n = 90)
Liver cirrhosis (hepatitis virus C)	35	6	29
Liver cirrhosis (hepatitis virus B)	25	4	21
Alcoholism	11	2	9
Primary biliary cirrhosis	8	3	5
Fulminant hepatitis	7	0	7
Liver cirrhosis (non-B non-C)	6	0	6
Primary sclerosing cholangitis	3	0	3
Budd–Chiari syndrome	1	0	1
Caroli's disease	1	0	1
Graft failure	4	2	2
Others	6	0	6

HB Hyperbilirubinemia

group, no patients received administration of fresh frozen plasma at the time of diagnosis. We used log-rank test for survival comparison. Group data were compared with the Mann–Whitney U test, and differences between proportions of categorical data were compared with the χ^2 test. Furthermore, several factors detected in the univariate analysis with P values of <0.15 were entered into a multivariate analysis. We used multivariate logistic regression analysis for the multivariate analysis. A P value <0.05 was considered to be statistically significant.
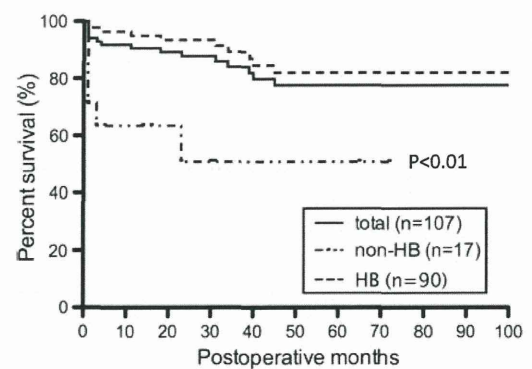
Results

Of the 107 consecutive adult patients who underwent LDLT at our hospital during the study period, 17 (15.9 %) met our criteria for HB and were included in the HB group; the remaining 90 patients (84.1 %) formed to the non-HB group. The overall survival rate was significantly different between the groups (P < 0.01) (Fig. 1). Time-zero biopsies showed no apparent differences between patients in the HB and non-HB group. Protocol biopsy was not performed postoperatively except in cases of cellular rejection or recurrence of hepatitis was suspected. The median donor age was significantly higher in the HB versus the non-HB group [50 (range 22–63) vs. 36 (19–67) years, respectively; P < 0.05], and ABO incompatibility was identified as a risk factor for posttransplant hyperbilirubinemia. The median preoperative serum bilirubin level tended to be higher in the HB group than in the non-HB group [5.4 (range 1.1–39.5) vs. 3.3 (0.6–42.7) mg/dl, respectively; P = 0.06]. The median postoperative peak ALT level was significantly higher in the HB group than in the non-HB group [569 (range 120–1,907) vs. 339 (79–3,359) IU/l,

Patients at risk

HB group	17	8	6	4	3	3	3	2	1	1	1
Non-HB group	90	70	61	49	36	31	20	18	15	11	4

Fig. 1 Kaplan–Meier curves of the postoperative survival of patients with hyperbilirubinemia (*HB* group) and without hyperbilirubinemia (*non-HB* group)

Table 2 Analysis of predictive factors for hyperbilirubinemia (univariate analysis)

Predictive factors	HB group[a] (n = 17)		Non-HB group[a] (n = 90)		P value
GW/SLV (%)	39.9 (24.9–56.3)		44.1 (23.6–85.3)		0.139
Donor age (years)	50 (22–63)		36 (19–67)		0.035
MELD score	22 (9–32)		18 (7–40)		0.217
Preoperative serum total bilirubin (mg/dl)	5.4 (1.1–39.5)		3.3 (0.6–42.7)		0.061
postoperative peak ALT(IU/l)	569 (120–1,907)		339 (79–3,359)		0.02
	+ (n)	%	+ (n)	%	
Acute cellular rejection (<POD 60)	5/17	29	26/90	29	0.804
Biliary complication	0/17	0	18/90	20	0.07
Type of graft					
Right lobe	10/17	59	36/90	40	
Left lobe	7/17	41	54/90	60	0.241
ABO incompatibility	6/17	35	9/90	10	0.01

GW/SLV Graft weight/standard liver volume ratio, *MELD* model for end-stage liver disease, *POD* postoperative days, *ALT* alanine aminotransferase

[a] Values are presented as the median with the range in parenthesis

Table 3 Multivariate analysis of postoperative hyperbilirubinemia

Preoperative risk factors	Yes/no	P value
GW/SLV (%)	–	0.107
Donor age (years)	–	0.0125
Preoperative serum total bilirubin (mg/dl)	–	0.032
ABO incompatibility	Yes	0.163

respectively; $P = 0.02$]. There were no significant differences in the GW/SLV, MELD score, type of graft, and incidence of biliary complication and acute cellular rejection between the groups (Table 2). The multivariate logistic analysis identified donor age ($P = 0.0125$) and preoperative serum bilirubin level ($P = 0.032$) as preoperative risk factors for postoperative hyperbilirubinemia (Table 3).

Of the 17 patients in the HB group, nine were alive at the writing of this manuscript. The results of the comparison between surviving and non-surviving patients are shown in Table 4. The median postoperative PT (%) at the time when the serum bilirubin level was >30 mg/dl was significantly higher in surviving patients than in those that did not survive [52 (range 26–59) vs. 33.5 (20–60) %, respectively; $P < 0.01$]. The median postoperative serum creatinine level at the time when the serum bilirubin level exceeded 30 mg/dl tended to be lower in surviving patients than in those that had not survived [1.2 (range 0.5–2.9) vs. 1.86 (0.4–3.1) mg/dl, respectively; $P = 0.06$]. There were no significant differences between surviving patients and non-surviving patients for donor age, GW/SLV, preoperative serum bilirubin level, MELD score, postoperative duration when the serum bilirubin level was >30 mg/dl, ABO incompatibilty, and acute cellular rejection (Table 4). The multivariate logistic analysis was not performed because of the small number of patients. Table 5 summarizes the characteristics and the postoperative course of patients in the HB group. Eight patients did not survive—one patient due to severe acute cellular rejection and seven patients due to infection. The indications for liver transplantation for non-survivors were liver cirrhosis (hepatitis C virus; 3 patients), primary biliary cirrhosis (3 patients), (hepatitis B virus; 1 patient), and graft failure (1 patients) (Table 5). None of these patients had suffered from short-term recurrence of viral hepatitis and hepatocellular carcinoma after transplantation. One patient (Table 5, case no. 10) was considered to be small-for-size syndrome with massive ascites and prolonged hyperbilirubinemia without arterial or portal occlusion and rejection. However, she had maintained PT (%) and survived. Although postoperative biopsies were performed for 11 patients in the HB group, no specific causes of hyperbilirubinemia were detected besides the findings of acute cellular rejection or recurrent hepatitis.

Discussion

In this study, we analyzed the risk factors for postoperative HB and the prognosis of patients who belonged to the HB group. Our results indicate that the donor age was most strongly correlated with the development of HB. A multivariate analysis also identified donor age and patient preoperative total bilirubin level as significant risk factors for post-transplant HB. The outcome of liver transplantation from aged donors is controversial. Some studies have shown that the outcomes of using grafts from donors older than 50 years without additional risk factors are similar to those of using grafts from donors younger than 50 years [3, 4]. However, the data from a registry of the Japanese Liver Transplantation Society show that patients who received a graft from an older donor had a significantly

Table 4 Comparison of risk factors for mortality in HB group (univariate analysis)

Risk factors	Surviving group[a] (n = 9)		Non-surviving group[a] (n = 8)		P value
GW/SLV(%)	40 (24.9–56.3)		39.2 (26.9–48.4)		0.847
Donor age	50 (22–61)		50.5 (22–63)		0.847
MELD score	22 (13–32)		22 (9–40)		1
Preoperative total bilirubin (mg/dl)	3.2 (1.9–39.5)		14.2 (1.1–28.7)		0.289
Timing of diagnosing HB	19 (5–28)		17 (6–32)		0.885
Prothrombin time (%) at HB diagnosis	52 (26–59)		33.5 (20–60)		0.004
Serum creatinine (mg/dl) at HB diagnosis	1.2 (0.5–2.9)		1.86 (0.4–3.1)		0.067
	+ (n)	%	+ (n)	%	
ABO incompatibility	3/9	33	3/8	38	1
Acute cellular rejection (<POD 60)	2/9	22	3/8	38	0.619

[a] Values are presented as the median with the range in parenthesis

Table 5 Characteristics and postoperative courses of patients in HB group

Case no.	Gender	Age	Indication for transplantation	ABO incompatibility	GW/SLV	Timing of diagnosing HB	Prothrombin (%) at HB diagnosis[a]	Outcomes	Cause of death
1	Male	63	B-LC, HCC	+	36.9	36	45	Dead	Infection
2	Female	61	PBC	+	26.9	26	25	Dead	Infection
3	Female	61	C-LC, HCC	−	43.6	12	29	Dead	Infection
4	Female	62	PBC	−	38.4	45	31	Dead	Infection
5	Male	57	C-LC, HCC	−	40	18	37	Dead	Infection
6	Male	57	C-LC, HCC	−	48.4	15	36	Dead	Infection
7	Male	41	PBC	−	44.6	16	31	Dead	ACR
8	Female	56	Graft failure	+	36.3	14	43	Dead	Infection
9	Female	54	C-LC, HCC	+	41.2	28	61	Alive	
10	Female	59	C-LC, HCC	−	24.9	26	45	Alive	
11	Male	58	B-LC, HCC	−	29.7	17	46	Alive	
12	Male	56	B-LC, HCC	−	44.2	37	76	Alive	
13	Female	53	C-LC	+	40	11	55	Alive	
14	Male	22	Graft failure	−	56.3	5	41	Alive	
15	Male	52	B-LC, HCC	+	36.1	34	52	Alive	
16	Male	62	Alcoholism	−	43.5	19	60	Alive	
17	Female	46	Alcoholism	−	37.8	17	34	Alive	

C-LC Liver cirrhosis type C, B-LC liver cirrhosis type B, PBC primry biliary cirrhosis, ACR acute cellular rejection

[a] At the time when the serum bilirubin level was >30 mg/dl

worse survival [5]. Notable findings of two studies which investigated non-transplanted aged livers were: 40 and 50 % decreases in vascular inflow and biliary flow, respectively, impairment of energy- and microtubule-dependent transport processes, with reduced endoplasmic reticulum mass, cumulative pigmented waste deposition, and a reduced ability to scavenge reactive oxygen intermediates [6, 7].

It has been reported that patients who receive a graft from an aged donor tend to have a greater incidence of delayed graft function [8, 9]. A multivariate analysis also revealed that the use of these grafts is associated with an increased incidence of recurrent hepatitis C [10]. A relative

poorer regeneration of the liver graft from an aged donor has also been reported [11]. Taken together, these findings indicate that clinicians should be aware that the use of grafts from aged donors could lead to the development of severe hyperbilirubinemia by a multifactorial mechanism.

The HB group included significantly more patients who had undergone ABO blood type-incompatible LDLT. The outcomes of ABO blood type-incompatible LDLT have improved over the years, and many institutes have adopted ABO-incompatible LDLT owing to the various treatments that can be used to overcome antibody-mediated rejection (AMR). AMR is the result of a circulatory disturbance that is caused by injury to the endothelium due to an antibody–

antigen–complement reaction. The typical clinical manifestations of AMR are hepatic necrosis and intrahepatic biliary complications [12]. Although no patients in our study had developed hepatic necrosis or apparent intrahepatic biliary complications with the prophylaxis, including rituximab and plasma exchange, our results suggest that patients undergoing ABO-incompatible LDLT may have a greater chance of developing postoperative severe hyperbilirubinemia.

The prognosis of the HB group was significantly worse than that of the non-HB group. Marubashi et al. [1] reported devastating outcomes in patients with a postoperative peak serum bilirubin level of >27 mg/dl, with eight of their grafts resulting in early graft loss within 1 year. In contrast, we experienced a number of patients with severe hyperbilirubinemia post-LDLT who eventually recovered their liver function; in fact, nine of the 17 patients in the HB group survived. Therefore, we investigated the perioperative parameters to clarify the risk factors for decreased survival. Our analysis revealed that the postoperative PT (%) at the time when the serum bilirubin level exceeded 30 mg/dl for the first time was significantly correlated with the prognosis based on the univariate analyses. Based on these results, the patients who were able to maintain their liver synthesis function were able to recover their liver function despite a temporal deterioration in bilirubin excretion.

Cholestasis has been recognized as a clinical manifestation of small-for-size graft syndrome, and the improvement of temporal cholestasis in proportion to the liver regeneration can be expected in cases of partial liver graft transplantation. We tried to exclude small-for-size syndrome with massive ascites. Although there is no consensus on the definition of small-for-size syndrome, there was one patient in the HB group who was suspected to have small-for-size syndrome, and she recovered spontaneously [normal range PT (%)] [13, 14]. In fact, GW/SLV was not a significant risk factor for the development of hyperbilirubinemia in our present study.

In addition, the postoperative serum creatinine level at the time when the serum bilirubin level exceeded 30 mg/dl for the first time tended to be lower in surviving patients. Acute kidney injury following liver transplantation has been reported to be associated with a worse outcome [15]. It is not hard to comprehend that HB patients with multiple organ dysfunction would have be a worse prognosis.

In conclusion, the use of a partial liver graft from an aged donor is considered to be a significant risk factor for postoperative severe hyperbilirubinemia. Although the outcomes of the HB patients were worse than those for the non-HB group, we should recognize that recovery is possible even from severe hyperbilirubinemia in those patients who are able to maintain their liver synthetic function during the postoperative course.

Conflict of interest None.

References

1. Marubashi S, Dono K, Nagano H, et al. Postoperative hyperbilirubinemia and graft outcome in living donor liver transplantation. *Liver Transpl*. 2007;13:1538–1544.
2. Urata K, Kawasaki S, Matsunami H, et al. Calculation of child and adult standard liver volume for liver transplantation. *Hepatology*. 1995;21:1317–1321.
3. Ikegami T, Nishizaki T, Yanaga K, et al. The impact of donor age on living donor transplantation. *Transplantation*. 2000;70:1703–1707.
4. Grande L, Rull A, Rimola A, et al. Outcome of patients undergoing orthotopic liver transplantation with elderly donors (over 60 years). *Transpl Proc*. 1997;29:3289–3290.
5. Japanese Liver Transplant Registry. Liver transplantation in Japan—registry by the Japanese Liver transplantation Society (in Japanese). *Isyoku*. 2010;45:621–632.
6. Wynne HA, Cope LH, Mutch E, et al. The effect of age upon liver volume and apparent liver blood flow in healthy man. *Hepatology*. 1989;9:297–301.
7. Wynne HA, Goudevenous J, Rawlins MD, et al. Hepatic drug clearance: the effect of age using indocyanine green as a model compound. *Br J Clin Pharmacol*. 1990;30:634–637.
8. Yoshizumi T, Taketomi A, Uchiyama H, et al. Graft size, donor age, and patient status are the indicators of early graft function after living donor liver transplantation. *Liver Transpl*. 2008;14:1007–1013.
9. Rull R, Vidal O, Momblan D, et al. Evaluation of potential liver donors: limits imposed by donor variables in liver transplantation. *Liver Transpl*. 2003;9:389–393.
10. Cameron AM, Ghobrial RM, Hiatt JR, et al. Effect of nonviral factors on hepatitis C recurrence after liver transplantation. *Ann Surg*. 2006;244:563–571.
11. Ono Y, Kawachi S, Hayashida T, et al. The influence of donor age on liver regeneration and hepatic progenitor cell populations. *Surgery*. 2011;150:154–161.
12. Egawa H, Teramukai S, Haga H, Tanabe M, Fukushima M, Shimazu M. Present status of ABO-incompatible living donor liver transplantation in Japan. *Hepatology*. 2008;47:143–152.
13. Kiuchi T, Kasahara M, Uryuhara K, et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation*. 1999;67:321–327.
14. Felix D, Panco G, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant*. 2005;5:2605–2610.
15. Barri YM, Sanchez EQ, Jennings LW, et al. Acute kidney injury following liver transplantation: definition and outcome. *Liver Transpl*. 2009;15:475–483.

HEPATOLOGY

Significance of hepatitis B virus core-related antigen and covalently closed circular DNA levels as markers of hepatitis B virus re-infection after liver transplantation

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Abstract

Background and Aim: Currently, hepatitis B virus (HBV) re-infection after liver transplantation (LT) can be almost completely suppressed by the administration of HBV reverse transcriptase inhibitors and hepatitis B immunoglobulins. However, after transplantation, there is no indicator of HBV replication because tests for the serum hepatitis B surface antigen and HBV-DNA are both negative. Therefore, the criteria for reducing and discontinuing these precautions are unclear. In this study, we examined the serum HBV core-related antigen (HBcrAg) and intrahepatic covalently closed circular DNA (cccDNA) in order to determine if these could be useful markers for HBV re-infection.

Methods: Thirty-one patients underwent LT for HBV-related liver disease at Nagasaki University Hospital from 2001 to 2010. Of these, 20 cases were followed up for more than 1 year (median follow-up period, 903 days). We measured serum HBcrAg and intrahepatic cccDNA levels in liver tissue. In addition, in nine cases, we assessed the serial changes of HBcrAg and intrahepatic cccDNA levels from preoperative periods to stable periods.

Results: We examined serum HBcrAg and intrahepatic cccDNA levels in 20 patients (35 samples). HBcrAg and cccDNA levels were significantly correlated with each other ($r = 0.616$, $P < 0.001$). From a clinical aspect, the fibrosis stage was significantly lower in both HBcrAg- and cccDNA-negative patients than in HBcrAg- or cccDNA-positive patients.

Conclusions: HBcrAg and cccDNA were useful as HBV re-infection markers after LT. Keeping patients' HBcrAg and cccDNA negative after LT might contribute to long-term graft survival.

Authors' Contributions:
Toshihisa Matsuzaki: acquisition of data, study concept and design, statistical analysis, writing of manuscript.
Tatsuki Ichikawa: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content.
Masashi Otani: critical revision of the manuscript for important intellectual content.
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Kazuhiko Nakao: study supervision, critical revision of the manuscript for important intellectual content.

Introduction

Liver transplantation (LT) is an established procedure for the treatment of end-stage liver disease. However, the recurrence of hepatitis B virus (HBV) is implicated in life-threatening graft failure.[1] Therefore, the prevention of HBV recurrence following LT is a serious concern. The advent of hepatitis B immunoglobulins (HBIg) and the HBV reverse transcriptase inhibitor (RTI) was a major breakthrough in the management of HBV recurrence. Currently, an ideal recurrence rate for HBV has been observed in patients who received HBIg and RTI combination therapy.[2] However, several studies have reported that HBV can be detected in the transplanted liver and peripheral blood mononuclear cells of recipients even when they have a hepatitis B surface antigen (HBsAg)-negative status.[3] Therefore, prophylaxis currently must be continued for the patient's lifetime. However, there are concerns with the long-term administration of HBIg and RTI with respect to safety, medical costs, and resistant mutations of HBV.[4] In order to discontinue the prophylaxis, several groups have attempted to vaccinate LT recipients against HBV, but most of these studies involve relatively low seroconversion rates because of the immunosuppressive environment.[5]

Recently, new agents against HBV, such as adefovir and entecavir, which hardly develop resistant mutations, have become available. Some have reported that HBIg can be discontinued after LT by using the new anti-HBV agents even if the vaccination does not succeed.[6] Angus *et al.* reported that when adefovir dipivoxil was substituted for low-dose HBIg, all patients were alive at the study completion without recurrence.[7] In addition, low-risk cases, such as those with fulminant hepatitis, and hepatitis B core antibody (HBcAb)-positive donors are not necessary for the administration of high-dose HBIg.[8] However, after transplantation, RTI and HBIg may mask the appearance of HBV-DNA, regardless of the presence of intrahepatic HBV covalently closed circular DNA (cccDNA). These factors make it difficult to detect HBV dynamics following LT, and we are therefore unable to determine the feasibility of the discontinuation of prophylaxis.

Recently, a new enzyme immunoassay that detects hepatitis B core-related antigen (HBcrAg) has been reported.[9] HBcrAg changes in parallel with HBV-DNA in the serum and has a wide detection range.[10] Moreover, its levels are correlated with the intrahepatic cccDNA levels of patients with chronic hepatitis B.[11] In addition, we previously reported on the usefulness of HBcrAg in patients receiving anti-HBV prophylaxis following LT.[12]

Therefore, in this study, we simultaneously measured serum HBcrAg and intrahepatic cccDNA levels in liver tissue and studied the HBV dynamics in patients following HBV-related LTs.

Methods

Patients and samples. From 2001 to 2010, a total of 31 patients with HBV-related end-stage liver disease underwent LTs at Nagasaki University Hospital, Nagasaki, Japan. Of these, we enrolled 20 patients who could be followed up for more than approximately 1 year (median 902 days; range 323–2456 days). There were 17 men and 3 women, with a median age of 56.5 years (range 33–68 years). All 20 patients were diagnosed with liver cirrhosis, and 12 were diagnosed with hepatocellular carcinoma. In addition, two patients were coinfected with the hepatitis C virus (Table 1).

Table 1 Baseline clinical features of the enrolled patients

Case	Age	Gender	Indication disease	HBV-DNA	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb	Donor HBcAb	HBcrAg
1	55	F	LC-B	< 2.6	> 2000	0.2	36.0	0.0	> 100.0	5.0	6.0
2	56	M	LC-B	< 2.6	> 2000	2.3	0.6	82.4	99.9	5.0	4.2
3	48	M	LC-B, HCC	< 2.6	562.5	0.1	1.1	57.7	> 100.0	31.3	5.0
4	60	M	LC-B	< 2.6	1789	0.1	0.2	97.6	> 100.0	70.1	5.8
5	59	M	LC-B, HCC	< 2.6	> 2000	0.1	0.1	> 100.0	> 100.0	5.0	3.2
6	57	M	LC-B, HCC	3.9	188.5	0.5	0.8	54.0	> 100.0	10.3	5.1
7	56	M	LC-B, HCC	< 2.6	> 2000	0.1	1.4	75.4	> 100.0	91.9	5.6
8	68	M	LC-B, HCC	< 2.6	> 2000	0.2	0.1	> 100.0	> 100.0	5.0	3.0
9	33	F	LC-B	3.0	> 2000	0.2	0.2	81.5	99.9	99.6	5.5
10	58	M	LC-B, HCC	3.0	> 2000	0.1	0.1	93.6	> 100.0	93.4	5.1
11	59	M	LC-B	< 2.6	378.3	0.3	0.1	61.6	> 100.0	93.0	3.8
12	57	M	LC-B + C, HCC	< 2.6	519.9	0.1	0.1	> 100.0	99.9	5.0	2.0
13	49	M	LC-B	< 2.6	> 2000	0.1	0.9	52.9	> 100.0	34.1	5.2
14	65	F	LC-B	6.9	> 2000	0.2	0.1	> 100.0	> 100.0	5.0	6.8
15	55	M	LC-B, HCC	< 2.1	> 2000	0.2	0.1	99.3	> 100.0	31.6	4.5
16	46	M	LC-B + C	4.3	1100.4	0.2	0.1	> 100.0	> 100.0	81.9	3.7
17	59	M	LC-B, HCC	< 2.1	> 2000	0.1	0.1	99.2	> 100.0	38.6	3.7
18	51	M	LC-B, HCC	2.1	> 2000	0.2	0.4	62.8	99.4	50.0	4.7
19	67	M	LC-B, HCC	3.9	> 2000	0.1	34.3	60.2	> 100.0	91.1	6.3
20	54	M	LC-B, HCC	2.1	> 2000	0.1	104.8	37.4	> 100.0	9.7	4.3

HBV, hepatitis B virus; HBcAb, hepatitis B core antibody; HBcrAg, hepatitis B core-related antigen; HBeAb, hepatitis B envelope antibody; HBeAg, hepatitis B envelope antigen; HBsAb, antibody against hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LC, liver cirrhosis; LC-B, LC due to HBV; LC-B + C, LC due to HBV-HCV coinfection.

All patients had been receiving RTI since preoperative periods. The HBsAg was negative in all donors, but eight donors were HBcAb-positive (cut-off, 50%), which was suggested to be due to prior exposures to HBV.

The prophylactic infusion of HBIg was administered to all patients according to a fixed-dose schedule; 10 000 units were given intravenously at the anhepatic period during the operation and the next day after the living donor LT (LDLT). Afterwards, 2000 units of HBIg were given routinely in order to keep the serum hepatitis B surface antibody (HBsAb) titers above 100 units/L. After the LDLT, serum HBsAg, hepatitis B envelope antigen (HBeAg), and HBV-DNA were not detected in any of the patients in this study.

Serum samples and biopsy specimens were obtained from 20 patients who received protocol biopsies 1 year after the LDLT at Nagasaki University Hospital after providing informed consent. Nine patients were followed up from the preoperative period to the stable period. Serum samples were obtained at the following three specified intervals: (i) in the preoperative period, samples were obtained just before the operation; (ii) in the postoperative period, samples were obtained during the operation of LT; and (iii) in the stable period, samples were obtained during admission for protocol biopsy. Liver tissue samples were obtained during the following three specified procedures: (i) biopsy from explanted liver during the operation; (ii) time-zero biopsy from the implanted liver during the operation; and (iii) protocol biopsy 1 year after the LDLT.

Serological markers for HBV.
HBsAg, HBsAb, HBeAg, hepatitis B envelope antibodies (HBeAb), and HBcAb levels were assessed by the chemiluminescence enzyme immunoassay (CLEIA) method using a commercially available enzyme immunoassay kit (Lumipulse, Fuji Rebio, Inc., Tokyo, Japan). Serum concentrations of HBV-DNA were determined using a polymerase chain reaction (PCR) HBV monitoring kit (Roche Diagnostics K.K., Tokyo, Japan), which had a quantitative range from 2.6 to 7.6 log copies/mL.

HBcrAg test.
Serum HBcrAg levels were measured by a CLEIA HBcrAg assay kit (Fujirebio, Inc.) with a fully automated analyzer system (Lumipulse System, FujiRebio, Inc.). HBcrAg concentrations were expressed as units/mL (U/mL). In this study, HBcrAg values were expressed as log U/mL, and the cut-off value was set at 3.0 log U/mL.[9,13]

Measurement of cccDNA.
Liver tissues were stored at $-80°C$ before DNA extraction. HBV-DNA was extracted using a high pure PCR template preparation kit (Roche Diagnostics K.K.). The concentration of purified DNA was measured at an absorbance of 260 nm.

cccDNA levels were measured with the real-time PCR method. With reference to a previous study,[11] we designed two oligonucleotide primers, cccF2 (5'-CGTCTGTGCCTTCTCATCTGA-3', nucleotides: 1424-1444) and cccR4 (5'-GCACAGCTTGGAGGC TTGAA-3', nucleotides: 1755-1737), and a cccP2 probe (5'-FAM-ACCAATTTATGCCTACAG-MGB-3', nucleotides: 1672-1655). Reaction volume (20.0 μL) containing 500 ng of extracted DNA,

0.5 μmol/L of each primer, 0.2 μmol/L of the probes, and Light-Cycler TaqMan Master (Roche Diagnostics K.K.) was administered. The initial activation step was heated at 95°C for 10 min. The subsequent PCR conditions consisted of 60 cycles of denaturation at 95°C for 10 s, and annealing and extension at 60°C for 30 s per cycle. Real-time PCR was performed in a LightCycler (Roche Diagnostics K.K.). Serial dilutions of a plasmid containing an HBV monomer were used as quantitation standards.

Liver histology.
Liver histology was evaluated by the same two pathologists. The degrees of necroinflammation and fibrosis were assessed based on the New Inuyama classification.[14] The degrees of rejection were assessed with the Rejection Activity Index according to the Banff working classification of hepatic allograft pathology.[15]

Liver function test.
Blood biochemical tests were performed in all patients, and liver function was evaluated. Liver function was assessed using Pugh's modification of Child's scoring system.[16]

Statistical analyses.
Student's t-tests and Fisher's exact tests were used for comparisons between groups of parametric quantitative data, and Mann–Whitney U-tests were used for comparisons between independent groups of non-parametric data. Categorical variables were compared with chi-square tests. The correlations between continuous variables were analyzed by the Pearson's correlation test. Two-tailed P values less than 0.05 were considered statistically significant.

Results

Correlation between HBcrAg and cccDNA.
The correlation between HBcrAg and cccDNA levels in all 35 samples is summarized in Figure 1. A statistically significant positive
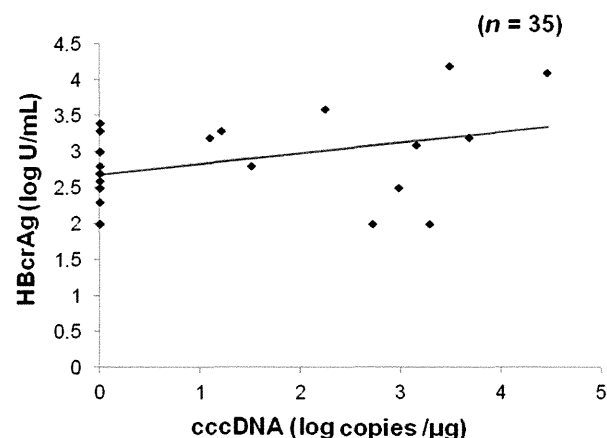

Figure 1 Correlation between serum hepatitis B core-related antigen (HBcrAg) and intrahepatic hepatitis B virus covalently closed circular DNA (cccDNA). $r = 0.616$, $P < 0.001$ ($y = 0.40x + 2.62$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.
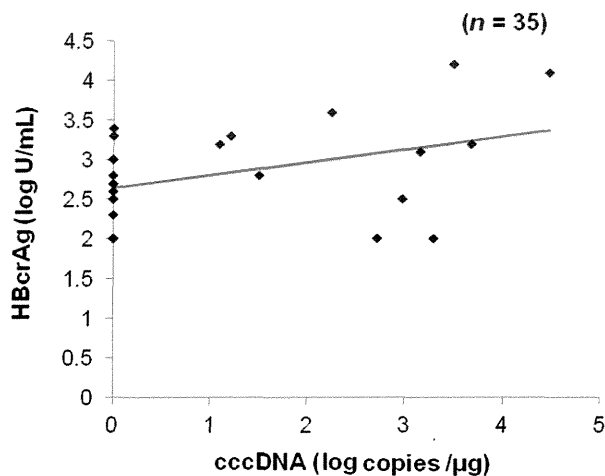
–69–

Figure 2 Correlation between hepatitis B core-related antigen (HBcrAg) and covalently closed circular DNA (cccDNA) levels after transplantation. $r = 0.402$, $P = 0.046$ ($y = 0.16 \times + 2.64$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.
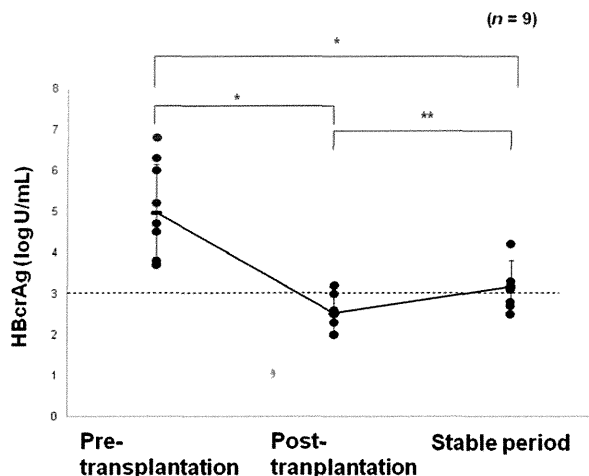

Figure 3 Serial changes of the hepatitis B core-related antigen (HBcrAg) levels. HBcrAg levels are represented as mean values; the closed circles show the values of the HBcrAg levels in all phases. The error bars indicate standard deviations. The detection range is above 3.0 log U/mL. In order to obtain the mean value, the values of 3.0 log U/mL or less, and 2.0 log U/mL or more were added to the calculation. The mean values of HBcrAg levels dropped during the postoperative period but then gradually increased again during the stable period ($*P < 0.001$ and $**P = 0.035$ indicate the significant differences between each period).

correlation was observed ($r = 0.616$, $P < 0.001$). Similarly, in the 23 samples that were obtained after LT only (that is, preoperative state samples were excluded), HBcrAg levels were significantly correlated with cccDNA levels (Fig. 2, $r = 0.402$, $P = 0.046$). These results supported the hypothesis that HBcrAg can be useful as an HBV marker instead of cccDNA after LT.

Serial changes in HBcrAg and cccDNA levels. HBcrAg and cccDNA levels showed similar dynamics during each period (Figs 3,4). All nine cases had positive levels of HBcrAg. However, seven of them were negative for HBV-DNA. During the post-transplantation period, HBcrAg levels of seven cases and cccDNA levels of eight cases became negative. Subsequently, HBcrAg and cccDNA levels of five cases became positive again during the stable period. These dynamics implicated the re-infection of HBV in the graft liver.

Comparisons of the clinical features of HBcrAg and cccDNA levels. We divided patients into two groups according to their status of HBcrAg and cccDNA, and investigated their clinical features (Table 2). Positive group includes the patients with positive cccDNA or HBcrAg, negative group includes the patients with both negative.

In comparisons between the positive group and negative group, the number of patients being treated with entecavir was significantly lower in negative group ($P = 0.022$). Additionally, the stage of the graft liver was significantly lower ($P = 0.012$) in negative group. The grafts of the HBcrAg- and cccDNA-negative patients were in good condition in the lower fibrosis stages (median 0; range 0–1).

Discussion

In the present study, we demonstrated the usefulness of HBcrAg and cccDNA as markers of HBV after transplantation. As in our
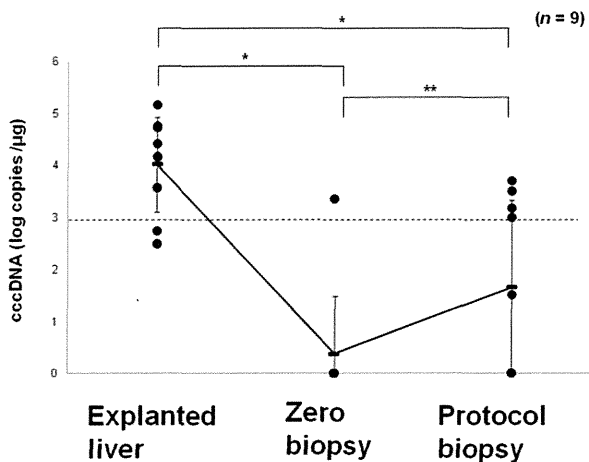

Figure 4 Serial changes of the covalently closed circular DNA (cccDNA) levels. cccDNA levels are represented as mean values; the closed circles show the values of the cccDNA levels in all phases. The error bars indicate standard deviations. The mean values of the cccDNA levels dropped during the time-zero biopsy but then gradually increased during the protocol biopsy ($*P < 0.001$ and $**P = 0.078$ indicate the significant differences between each period).

previous report,[12] we suggest that HBcrAg, which is a newly developed enzyme immunoassay,[9] is a possible method for detecting the dynamics of HBV after LT. However, HBcrAg consists of HBcAg, HBeAg, and p22cr, which is generated from cccDNA,

Table 2 Comparisons of the clinical features of HBcrAg and cccDNA levels

HBcrAg/cccDNA status	Positive group	Negative group	Positive versus negative
Patient M/F	10/2	7/1	NS
Day after transplantation[†]	854 (323–2163)	1674.5 (353–2456)	NS
Age[†]	55.5 (33–68)	56.5 (48–65)	NS
Serum HBV-DNA positive at LT (p/n)	7/5 (58.3%)	2/6 (33.3%)	NS
Serum HBeAg positive at LT (p/n)	1/11 (8.3%)	1/7 (14.3%)	NS
HBcAb-positive donor (p/n)	7/5 (58.3%)	1/7 (14.3%)	NS
Blood incompatibly (p/n)	1/11 (8.3%)	1/7 (14.3%)	NS
Presence of HCC at LT (p/n)	9/3 (75%)	7/1 (87.5%)	NS
RTI for prophylactic therapy after LT			
Use of LAM	3/12 (25%)	4/8 (50%)	NS
Use of ETV	9/12 (75%)	1/8 (12.5%)	$P = 0.022$
Use of ADV	0 (0%)	2/8 (25%)	NS
Use of LAM + ADV	0 (0%)	1/8 (12.5%)	NS
Immunosuppression after LT			
Use of TAC	10/12 (83.3%)	5/8 (62.5%)	NS
Use of CYA	0 (0%)	2/8 (25%)	NS
Use of MMF	2/12 (16.6%)	0 (0%)	NS
Use of TAC + MMF	0 (0%)	1/8 (12.5%)	NS
Liver function test			
Serum albumin (g/L)[‡]	39.2 (4.7)	40.0 (4.8)	NS
Child–Pugh score[†]	5.0 (5.0–9.0)	5.0 (5.0–6.0)	NS
Histology of LB			
Grade[†]	1.0 (0.0–3.0)	0.5 (0.0–1.0)	NS
Stage[†]	1.0 (0.0–3.0)	0.0 (0.0–1.0)	$P = 0.0027$
RAI score[†]	2.5 (0.0–5.0)	1.5 (0–4)	NS

Fisher's exact test for categorical variables.

[†]Mann–Whitney *U*-test for non-normally distributed variables, expressed as median (range).

[‡]Student's *t*-test for normally distributed variables, expressed as mean (SD).

ADV, adefovir; cccDNA, covalently closed circular DNA; CYA, cyclosporin A; ETV, entecavir; HBV, hepatitis B virus; HBcAb, hepatitis B core antibody; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HCC, hepatocellular carcinoma; LAM, lamivudine; LB, liver biopsy; LT, liver transplantation; MMF, mycophenolate mofetil; n, negative; NS, not significant; p, positive; RAI, Rejection Activity Index; RTI, reverse transcriptase inhibitor; SD, standard deviation; TAC, taclolimus.

and thus, it is questionable if HBcrAg truly reflects the viral pattern of HBV. Therefore, we designed this study to examine the usefulness of further analysis of cccDNA, which truly functions as a reservoir of HBV replication.

In the results of this study, a positive correlation between HBcrAg and cccDNA was shown, and this was consistent with a previous report on chronic hepatitis B.[11] These findings suggest the usefulness of monitoring HBV dynamics of patients after LTs because examinations of serum HBcrAg are less invasive methods compared with examinations of cccDNA levels in liver tissue. HBcrAg enables us to frequently check the HBV dynamics of patients, and it contributes to a reduction in the risk of HBV reactivation.[13]

However, as shown in Table 2, the results of the HBcrAg and cccDNA levels were not matched in 35% (7 of 20) of the patients. This may be due to a problem with the sensitivity of these two markers. We should use these markers cautiously because HBV might exist even if these were negative. Suzuki *et al.* reported that among the 13 patients with negative results for HBsAg, HBeAg, and HBV-DNA, all had positive results with cccDNA, while HBcrAg was positive in only seven patients.[11] In addition, cccDNA was also examined in a limited way because it was

extracted from tissue from only a small part of the liver. Moreover, some reports have suggested that cccDNA can be detected in extrahepatic sites,[17] and thus, it is impossible to determine whether HBV exists with only one method. Therefore, we preferred to assess HBV dynamics with these two methods in order to overcome problems with sensitivity.

Interestingly, in the group with negative results for both of the two markers, the fibrosis stage was significantly lower compared with the other. This might reflect HBV activity after the LT. In addition, it was considered that keeping the two markers negative after LT may suggest the possibility of an extension of graft survival. But we observed only a limited period, further study of long-term outcome will be required.

The goal of this study was to determine the criteria for the appropriate prophylaxis of HBV related to LT with these two markers. Lenci *et al.* reported that 80.1% of the patients with undetectable intrahepatic cccDNA levels did not exhibit signs of HBV recurrence, even after withdrawal of the prophylaxis.[18] We thought that it might be possible to select patients more efficiently and correctly by using a method that combines examinations of HBcrAg and cccDNA. We observed one patient with both HBcrAg- and cccDNA-negative discontinued antiviral therapy.

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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.

EDITORIAL

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

Susumu Eguchi

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].

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].

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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.