＜短　　報＞

岡山県における肝炎ウイルス検診陽性者の医療機関受診等に関する追跡調査

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緒言：平成14年から18年までの5年間に老人保健法に基づく保健事業として40歳から70歳までの受診対象者に対して5歳刻みで節目検診が行われ，また節目検診の対象者とならないが肝炎ウイルス感染の可能性が高い者（過去に肝機能異常を指摘されたことのある者，広範な外科的処置を受けたことのある者又は妊娠・分娩時に多量に出血したことのある者であって定期的に肝機能検査を受けていない者，基本健康診査の結果，ALT（GPT）値により要指導とされた者）に対して節目外検診が行われた．これらの検診により新たな肝炎ウイルス感染者が見いだされたが，その後の医療機関受診状況や肝炎に対する治療状況については，岡山県が平成17年度に市町村の協力の下で調査を行って以来，実態が把握されていないのが現状であった．田中らは「広島県における検診結果」として広島県12市町において聞き取り調査を行い，平成21年度報告書に報告している[5]．それによると，HBVキャリアにおいては，回答率を考慮した医療機関受診率48％，把握されているHBVキャリア709名中440名から回答があった（回答率62.1％）．また，HCVキャリアにおいては，回答率を考慮した医療機関受診率65％，把握されているHCVキャリア630名中439名から回答があった（回答率69.7％）．また，医療機関受診率に関しては，HBVキャリアにおいては「現在受診中」が62％，「以前受診した」が15％，「受診していない」が23％であり，HCVキャリアにおいては「現在受診中」が80％，「以前受診した」が13％，「受診していない」が7％にすぎなかった．

そこで，厚生労働科学研究費補助金肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入に関する研究」の一環として，岡山県における平成14年度から18年度までの節目・節目外検診で新たに見いだされた肝炎ウイルス感染者について，その後の医療機関受診状況や肝炎に対する治療状況を把握する目的でアンケートによる追跡調査を行ったので報告する．

対象と方法：対象は岡山県において平成14年度から18年度までの検診（節目・節目外）で肝炎ウイルス感染が判明した2,566人（B型974人，C型1,592人）のうち，調査可能であった24市町村において既に追跡調査等が行われていた肝炎ウイルス感染者を除いた1,352人（52.7％）（B型549人，C型803人）であった．調査用紙は各市町村より直接肝炎ウイルス陽性者へ郵送され，また一部の市町村（笠岡市58人，勝央町11人）においては保健師が直接聞き取り調査を行った．回答後の調査用紙は肝炎ウイルス陽性者から匿名の形で本研究の事務局である川崎医科大学肝胆膵内科学研究室へ直接郵送された．

アンケートは以下の項目について調査を行った．1. 在住市町村. 2. 陽性と通知されたのはB型肝炎ウイルス（HBV）かC型肝炎ウイルス（HCV）か. 3. 性別と年齢. 4.「肝炎ウイルス感染の可能性が高い」と通知を受けて医療機関を受診したか否か. 5. 受診していない場合その理由. 6-1. 受診した場合は受診先がかかりつけ医か専門医療機関か. 6-2. 受診先での診断名. 7. 現在も通院を継続しているか否か. 8. 通院を中止した場合はその理由. 9. 治療を受けている場合は主な内容.

結果：調査を行った1,352人のうち716人（53％）より回答が得られた．このうち11人は既にウイルス性慢性肝炎として医療機関に通院していながら検診を受けており，8人は調査用紙の返送はあったものの無回答であり，1人は肝炎ウイルス陰性という回答であったため，

る HCV キャリア 630 名中 439 名から回答があった（回答率 69.7％）．それによると医療機関受診率に関しては，HBV キャリアにおいては「現在受診中」が 62％，「以前受診した」が 15％，「受診していない」が 23％ であった．一方 HCV キャリアにおいては「現在受診中」が 80％と高く，「以前受診した」が 13％，「受診していない」は 7％ にすぎなかった．B 型陽性者の通院中断の理由として，担当医から「通院しなくてもよい」と言われた割合が 71.8％ 認めた点も今後の課題と考えられる．したがって，抗ウイルス療法が適切に行われていない可能性もあり，ウイルス肝炎治療のガイドラインに準じた治療の啓発をさらに推進する必要があると考えられた．現在, B 型肝炎ウイルス感染者の肝発癌危険因子はウイルス量（HBV-DNA）であることが明らかにされているので[2]，トランスアミナーゼの値でフォローを中断するようなことは慎むべきであり，こうした点は肝臓専門医以外の医師にもっと広く啓発していく必要があると考えられる．

わが国の肝癌患者は高齢化が進んでおり，岡山県も例外ではない．今回の調査においてもアンケート回答者の平均年齢は高齢であった．肝炎ウイルス陽性の高齢者は肝癌の高危険群であり[3]~[5]，医療機関での経過観察あるいは治療が極めて重要である．これを実現するためには全県的な肝炎ウイルス陽性者の把握とともに地域に密接した保健活動を行政も含めてさらに推進していく必要がある．

結論：岡山県において平成 14 年度から 18 年度に実施された肝炎ウイルス検診（節目・節目外検診）で見出された陽性者について，その後の医療機関受診状況や受療状況を把握する目的で追跡調査を行った．肝炎ウイルス検診陽性者に対する医療機関への受診勧告や通院継続率の引き上げなどが今後の更なる課題と考えられた．

索引用語：ウイルス性肝炎，健康診断，追跡調査

文献：1）田中純子, 他.「平成 21 年度　厚生労働科学研究費補助金　肝炎等克服緊急対策研究事業　肝炎状況・長期予後の疫学に関する研究　研究報告書」2010, p11—14　2）Chen CJ, Yang HI, Su J, et al. JAMA 2006; 295: 65—67　3）Ikeda K, Saitoh S, Arase Y, et al. Hepatology 1999; 29: 1124— 1130　4）Kasahara A, Hayashi N, Mochizuki K, et al. Hepatology 1998; 27: 1394—1402　5）Tseng TC, Liu CJ, Yang HC, et al. Gastroenterology 2012; 142: 1140—1149

英文要旨

A follow-up survey of hepatitis virus carriers after notification of their infection in Okayama prefecture

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In Okayama prefecture we investigated by questionnaires whether hepatitis B virus (HBV) (n = 549) or hepatitis C virus (HCV) carriers (n = 803) consulted a doctor after they had been informed of their infection at their initial checkup for viral hepatitis between 2002 and 2006. The ratio of patients who consulted a doctor after notification of infection was 38.4% (211/549) of HBV carriers and 49.4% (397/803) of HCV carriers, respectively. Among those patients, 53.1% of HBV carriers and 73.4% of HCV carriers were on follow care at the start of this investigation. These results indicated a need to establish a more effective follow up system for hepatitis virus carriers following notification at the initial medical checkup.

Key words: virus hepatitis, medical checkup, follow-up survey

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ORIGINAL ARTICLE

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

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Abstract

Background Prolonged hyperbilirubinemia (HB) following living donor liver transplantation (LDLT) can be a risk factor for early graft loss and mortality. However, some recipients who present with postoperative hyperbilirubinemia 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 underwent LDLT in Nagasaki University Hospital were investigated 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 significantly 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 survived. 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 hyperbilirubinemia 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.

Keywords Living donor liver transplantation ·
Hyperbilirubinemia · Partial graft · Small-for-size graft
syndrome · Acute cellular rejection

Introduction

Hyperbilirubinemia following living donor liver transplantation (LDLT) can be caused by several mechanisms, such as initial poor function, acute cellular rejection, surgical complications, small-for-size syndrome, drug toxicity, among others. Hyperbilirubinemia has also been reported to be a risk factor for early graft loss and mortality [1]. However, some recipients can overcome hyperbilirubinemia, 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

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.
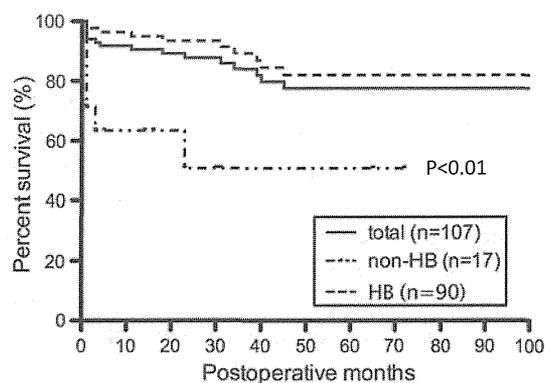
Results

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,

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

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

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

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.

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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.
Motohisa Akiyama: critical revision of the manuscript for important intellectual content.
Eisuke Ozawa: critical revision of the manuscript for important intellectual content.
Satoshi Miuma: critical revision of the manuscript for important intellectual content.
Sadayuki Okudaira: acquisition of data, critical revision of the manuscript for important intellectual content.
Tomayoshi Hayashi: acquisition of data, critical revision of the manuscript for important intellectual content.
Naota Taura: critical revision of the manuscript for important intellectual content.
Hisamitsu Miyaaki: critical revision of the manuscript for important intellectual content.
Susumu Eguchi: critical revision of the manuscript for important intellectual content.
Takashi Kanematsu: critical revision of the manuscript for important intellectual content.
Hajime Isomoto: critical revision of the manuscript for important intellectual content.
Fuminao Takeshima: critical revision of the manuscript for important intellectual content.
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 adminis-

tration 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
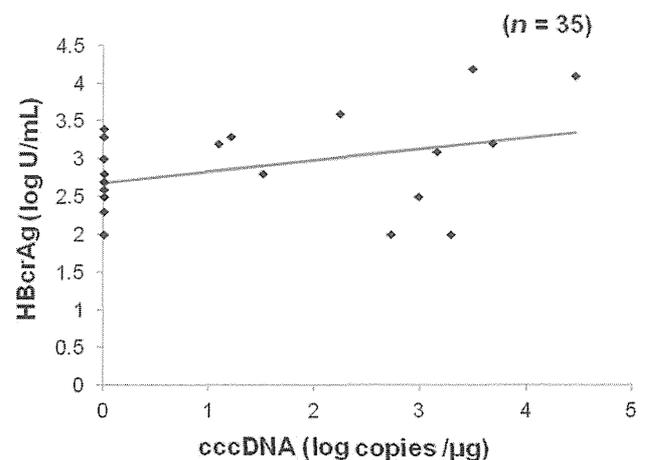

Figure 1 Correlation between serum hepatitis B core-related antigen (HBcrAg) and intrahepatic hepatitis B virus covalently closed circular DNA (cccDNA). $r = 0.616$, $P < 0.001$ ($y = 0.40x + 2.62$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.
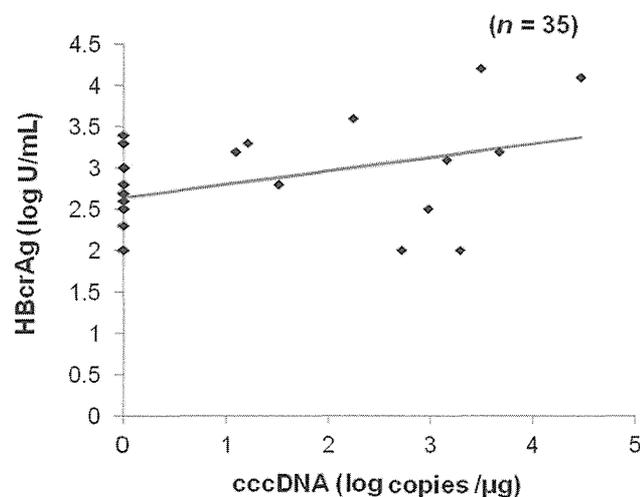
Figure 2 Correlation between hepatitis B core-related antigen (HBcrAg) and covalently closed circular DNA (cccDNA) levels after transplantation. $r = 0.402$, $P = 0.046$ ($y = 0.16 \times + 2.64$). Straight lines indicate the correlation between HBcrAg and cccDNA levels.

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
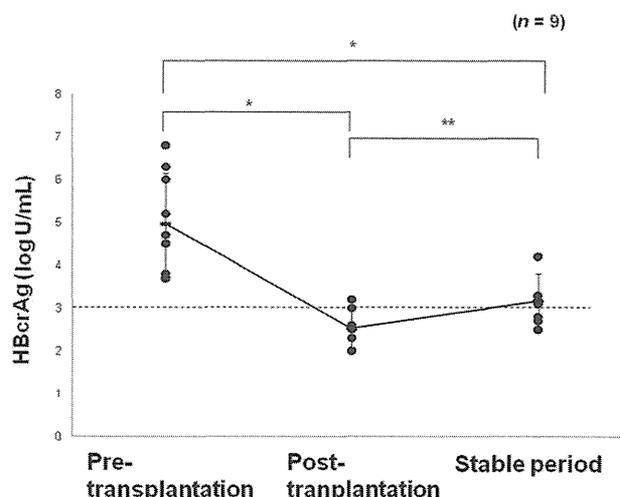

Figure 3 Serial changes of the hepatitis B core-related antigen (HBcrAg) levels. HBcrAg levels are represented as mean values; the closed circles show the values of the HBcrAg levels in all phases. The error bars indicate standard deviations. The detection range is above 3.0 log U/mL. In order to obtain 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).
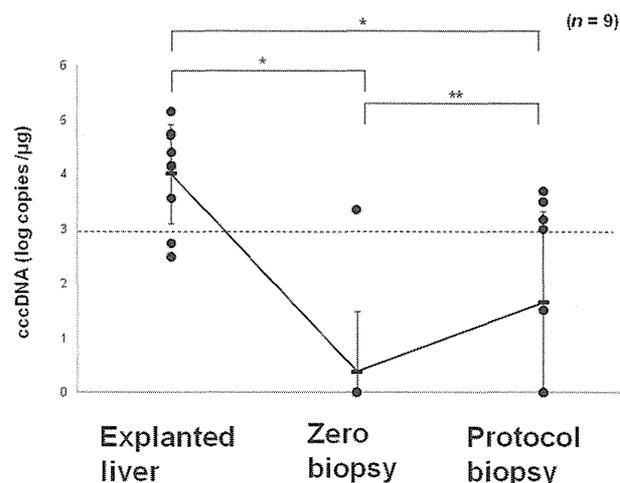

Figure 4 Serial changes of the covalently closed circular DNA (cccDNA) levels. cccDNA levels are represented as mean values; the closed circles show the values of the cccDNA levels in all phases. The error bars indicate standard deviations. The mean values of the cccDNA levels dropped during the time-zero 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.

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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

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

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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].

This comment refers to the article available at
doi:10.1007/s00595-012-0419-y.

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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].

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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.

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/m² 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.

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