severity and Em and ACR severity and Er ($p < 0.0001$ for both comparisons). An ACR of G2 or G3 was more frequently resistant to steroid recycle therapy than an ACR of G1 ($p = 0.04$). There was no significant difference, however, in the Em ($p = 0.63$) or Er ($p = 0.38$) between ACR episodes that were responsive and resistant to steroid recycle therapy.

Correlation between blood and histologic eosinophilia

The ρ-value of Spearman's rank-correlation coefficient between blood and histologic eosinophil counts ranged between 0.35 and 0.45 (Table 3).

Uni- and multi-variate analysis

In univariate analysis, all of the P, B, V scores, Em, and Er were significantly higher in ACR group than in non-ACR group. Multivariate analysis, however, revealed only P, B, and V scores remained as significant factor related with ACR (Tables 4 and 5).

Discussion

The ideal method of diagnosing ACR is based on suspected ACR with high sensitivity before biopsy and confirmation of the diagnosis with high specificity. Our criterion to perform liver biopsy is an increase in all the liver function data. With this criterion, ACR was correctly diagnosed in 41% of all the biopsy cases (164/398). Unfortunately, absolute blood eosinophil count is not an ideal predictor of ACR because of its low sensitivity. The present result failed to reveal the equivalent impact on diagnosis of ACR between histologic eosinophilia and P, B, V scores. Histologic eosinophilia, however, might be useful for evaluating the severity of ACR after LDLT or differential diagnosis with hepatitis (11).

Table 3. Spearman's rank-correlation coefficient value (ρ) and p-value

Items	ρ-value	p-value
AECb vs. Em	0.37	<0.0001
AECo vs. Em	0.45	<0.0001
AECb vs. Er	0.35	<0.0001
AECo vs. Er	0.44	<0.0001

Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil; AECb, absolute eosinophil count three d before biopsy; AECo, absolute eosinophil count on the day.

Table 4. Univariate analysis for the predictor of ACR

Factor	ACR	No ACR	p-value
P score	1.31 ± 0.06	0.48 ± 0.05	<0.0001
B score	1.16 ± 0.05	0.32 ± 0.05	<0.0001
V score	1.29 ± 0.05	0.26 ± 0.05	<0.0001
Em	4.8 ± 0.60	1.2 ± 0.6	<0.0001
Er	26.0 ± 2.5	46.8 ± 2.6	<0.0001

ACR, acute cellular rejection; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil.

Table 5. Multivariate analysis for the predictor of ACR

Factor	Odds ratio	95% CI	p-value
P score	0.001	0.000007–0.06	0.002
B score	0.004	0.0001–0.06	0.0003
V score	0.0003	0.00000–0.006	<0.0001
Em	0.81	0.004–195.1	0.94
Er	0.55	0.01–16.5	0.74

ACR, acute cellular rejection; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil; CI, confidence interval.

This result is consistent with a previous study on deceased donor liver transplantation. The Royal Free Hospital group (6) used histologic eosinophilia in the diagnosis and grading of ACR. In their ACR grading system, the maximum eosinophil number in the portal tract was included in addition to the usual items, i.e. portal inflammation, endothelilitis, and bile duct damage. They were scored 0 to 3 according to the maximum eosinophil counts 0, 1–4, 5–9, 10, or more. Ben-Ari et al. (12) demonstrated that mean eosinophils per portal tract was different/depending on the degree of ACR; 0.41 in mild rejection specimens and 27.38 in moderate-to severe rejection specimens. Foster et al. (4) evaluated the average number of eosinophils per portal tract, which was 11.4 in the rejection group vs. 1.0 in the non-rejection group.

The present analysis revealed that eosinophilia did not correlate with the response to ACR. This might be due to the smaller numbers of subjects (52 responsive vs. 38 resistant) or the influence of steroids, which downregulate eosinophilia (13). The response of graft eosinophils to corticosteroids might be related to the role of various cytokines in the pathogenesis of rejection.

In conclusion, histologic eosinophilia is useful for confirming ACR after biopsy and for evaluating the severity of ACR after LDLT. Although the sensitivity of blood and histologic eosinophilia to predict ACR was low, the presence of eosinophilia can help the differential diagnosis of ACR. For this, clear quantification of eosinophilia with more appropriate cut-off value is needed by further analyses with a larger number of specimens.

Grant

Supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Grants-in-aid for Research on HIV/AIDS, a multicenter pilot clinical study to compare the safety and efficacy of a steroid free immunosuppression protocol with monoclonal anti-IL2R antibody in HCV positive living donor liver transplantation and Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan.

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ELSEVIER

Blood Eosinophilia After Living Donor Liver Transplantation for Hepatitis C Virus–Related Cirrhosis

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ABSTRACT

Background. Differentiating between acute cellular rejection (ACR) and recurrent hepatitis C virus after liver transplantation in hepatitis C virus–positive patients is difficult, but vital for preventing graft loss.

Methods. The blood eosinophil counts 3 days before or on the day of biopsy were retrospectively reviewed to evaluate their value for predicting ACR in 91 biopsy samples from 45 patients.

Results. Eosinophil counts on the day of biopsy were significantly higher in the ACR group (n = 20) than in the non-ACR (n = 71) group, although the difference was negligible 3 days before the biopsy. A relative eosinophil count of 2% or an absolute eosinophil count of 200 cells/mm^3 predicted ACR with a specificity of 94% or 96%, respectively.

Conclusions. Blood eosinophil count on the day of biopsy can be helpful in the diagnosis of ACR in patients who underwent living donor liver transplantation for hepatitis C virus–related cirrhosis.

A RECENT study[1] of liver transplantation reported that hepatitis C virus (HCV) infection is associated with a 23% increase in mortality and a 30% increase in the rate of graft failure. HCV-induced graft hepatitis and fibrosis/cirrhosis occur in 75% to 80% and 10% to 30% of recipients, respectively, after 5 years.[2] Once liver cirrhosis is established, the cumulative probability of developing clinical decompensation is approximately 50% after 1 year, and survival after decompensation is extremely short.[3] Cholestatic hepatitis occurs in approximately 10% of patients infected with HCV and leads to accelerated graft failure and death.[4] A current debate is whether the recipients of living donor liver transplantation (LDLT) are at risk for increased severity of HCV recurrence.[5–10] Acute cellular rejection (ACR), however, is a major complication that can lead to mortality. Thus, early diagnosis via liver biopsy is necessary to determine the appropriate treatment.

Differentiating ACR from recurrent HCV is critical, but difficult, especially in the early postoperative period.[11] Recently, Barnes et al reported that HCV-positive patients with ACR are less likely to have eosinophilia than HCV-negative patients with ACR. Thus, proposed that the eosinophil response might be suppressed in HCV-positive patients with ACR, and that ACR might be overdiagnosed if based on histopathology in these patients with normal eosinophil levels.[12] In the present study, we evaluated the efficacy of measuring eosinophil levels to diagnose ACR in HCV-positive LDLT patients.

MATERIALS AND METHODS

From June 1996 to June 2005, 80 HCV-positive patients underwent LDLT at the University of Tokyo Hospital. A total of 146 biopsies were performed in 64 (80%) patients during the 6-month period following LDLT. Preemptive interferon and ribavirin therapy was administered after LDLT. Protocol biopsies were not performed.[13] All biopsies were performed under the suspicion of ACR due to liver dysfunction. Blood chemistry was examined everyday or every other day during hospitalization, and either once every 2 weeks or once a month in the outpatient clinics after hospitalization. The following measures of liver function were analyzed: aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, and total bilirubin. When all measures were elevated compared with previous levels and bile

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0041-1345/07/$-see front matter
doi:10.1016/j.transproceed.2006.12.043

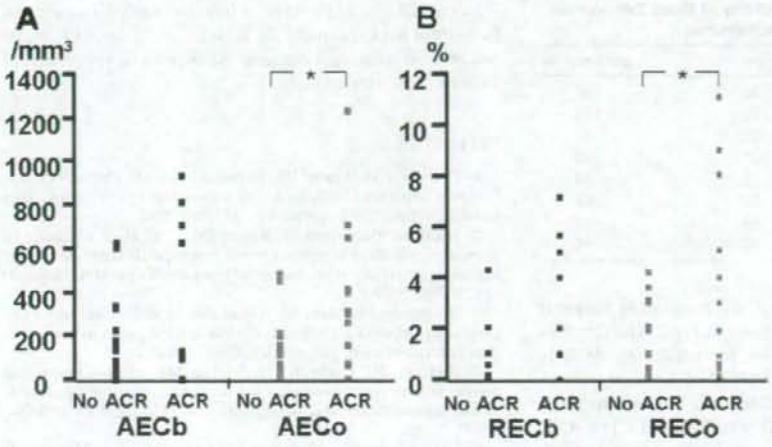

Fig 1. Distribution of absolute eosinophil counts (AEC, **A**) and relative (REC, **B**) in blood on 3 days before (black squares) and on the day of biopsy (grey squares). *$P < .0001$.

duct complication could be ruled out by ultrasonography, a biopsy was performed. ACR was diagnosed by experienced pathologists and graded into 4 classes according to the Banff scheme.[14]

Relative and absolute blood eosinophil counts obtained 3 days before the biopsy (RECb and AECb) and on the day of biopsy (RECo and AECo) were retrospectively reviewed. Transaminase levels on the day of biopsy were also reviewed. Eosinophilia was defined as REC ≥4% and/or AEC ≥400/mm³, as described previously.[6] Blood eosinophil data was successfully obtained from 91 (62%) biopsy samples from 45 patients.

Eosinophil counts of patients diagnosed with ACR were compared with those of patients without ACR. We then used a receiver operating characteristic curve to evaluate the sensitivity and specificity of eosinophilia in predicting ACR. All data were expressed as mean ± standard error. Statistical comparison of quantitative and qualitative data was performed using Wilcoxon's test and Fisher exact test, respectively. $P < .05$ was considered to be statistically significant.

RESULTS

Of 91 biopsies, ACR was indeterminate in 71 cases (non-ACR group) and mild and moderate ACR was confirmed in 18 and 2 cases, respectively (ACR group). Aspartate aminotransferase levels on the day of biopsy were not significantly different between the ACR and non-ACR groups (97 ± 16 IU/L vs 111 ± 9 IU/L, respectively; $P = .35$). Similarly, there was no significant difference in alanine aminotransferase levels between the ACR and non-ACR groups (169 ± 26 IU/L vs 164 ± 14 IU/L; $P = .90$).

Figure 1 shows the eosinophil counts of the ACR and non-ACR groups. Although RECb and AECb levels were higher in the ACR group, the difference was not statistically significant ($P = .14$ or $P = .18$, respectively). In contrast, RECo and AECo levels were both significantly higher in the ACR group ($P < .0001$ for both comparisons). The receiver

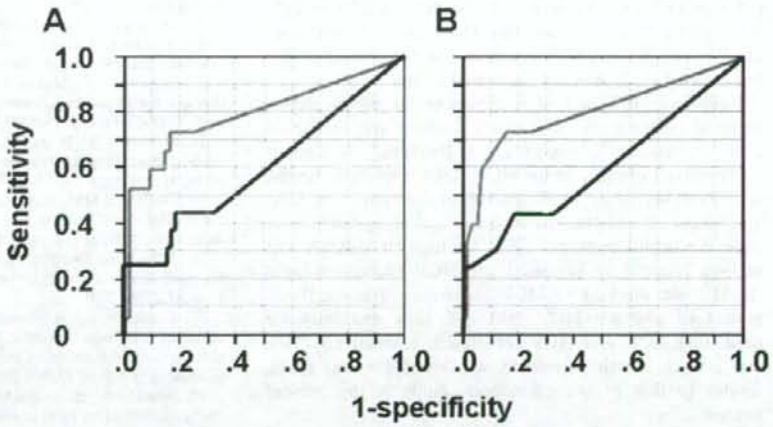

Fig 2. Receiver operating characteristic curve to evaluate the sensitivity and specificity of absolute **(A)** and relative **(B)** blood eosinophilia. Black line, eosinophil count 3 days before biopsy; grey line, eosinophil count on the day of biopsy.

Table 1. The Sensitivity and Specificity of Blood Eosinophilia to Predict Acute Rejection

	Sensitivity (%)	Specificity (%)
RECb ≥4%	20	100
AECb ≥400 cells/mm^3	20	100
RECo ≥4%	25	99
AECo ≥400 cells/mm^3	20	99
RECb ≥2%	25	89
AECb ≥200 cells/mm^3	20	96
RECo ≥2%	45	94
AECo ≥200 cells/mm^3	40	96

operating characteristic curve of the association between eosinophil counts and ACR is shown in Fig 2. The sensitivity and specificity of eosinophilia for predicting ACR is shown in Table 1. When the threshold of eosinophilia was set as REC ≥2% or AEC ≥200/mm^3, the sensitivity of eosinophilia for predicting ACR was 40% (REC) or 45% (AEC), whereas the specificity was 94% (REC) or 96% (AEC).

DISCUSSION

In the present study, we found that HCV-positive patients diagnosed with ACR had significantly higher REC and AEC levels on the day of biopsy than HCV-positive patients without ACR. These results indicate that measures of blood eosinophil levels might contribute to the differential diagnosis of ACR in HCV-positive recipients.

Few studies have evaluated whether postoperative eosinophilia predicts ACR.[15] Nagral et al[16] reviewed 129 biopsy cases after deceased donor liver transplantation. They reported that AEC levels 1 or 2 days before, or on the day of biopsy, predicted ACR with low sensitivity (30%–38%) and high specificity (83%–92%). Our current results are consistent with these findings; however, the present study was specifically limited to HCV-positive patients. The occurrence of eosinophilia was equal in comparison with 140 ACR episodes of HCV-negative patients in our subjects (33% vs 34% for relative blood eosinophilia, 27% vs 29% for absolute blood eosinophilia). The suggestion by Barnes et al[12] that the eosinophil response is suppressed in the presence of HCV was not supported by our study.

Recurrent HCV and ACR cannot be completely differentiated because the histological findings are similar or even overlapping between the 2 pathologies.[17] It remains controversial whether serum HCV-RNA level indicates the severity of hepatitis[18] of allografts. For differentiation, C4d, an end-product of the activated classical complement cascade, is a useful marker of ACR[19]; a high HCV-RNA titer in liver tissue[20] or increased anti-HCV immunoglobulin (Ig)M[21] are markers of HCV recurrence. Among these, both C4d and anti-HCV IgM had high specificity for predicting ACR and HCV recurrence, respectively (91% and 100%), but the sensitivity was low (68% and 82%), similar to that of the eosinophil counts in the present analysis.

In conclusion, evaluation of blood eosinophil count can be helpful for confirming ACR. REC ≥2% or AEC ≥200 cell/mm^3 convincingly suggest ACR with a specificity of 94% or 96%, respectively.

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World J Gastroenterol 2006 July 28; 12(28): 4461-4465
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Living donor liver transplantation to patients with hepatitis C virus cirrhosis

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Received: 2005-09-12 Accepted: 2005-11-18

Abstract

Living donor liver transplantation (LDLT) is an alternative therapeutic option for patients with end-stage hepatitis C virus (HCV) cirrhosis because of the cadaveric organ shortage. HCV infection is now a leading indication for LDLT among adults worldwide, and there is a worse prognosis with HCV recurrence. The antivirus strategy after transplantation, however, is currently under debate. Recent updates on the clinical and therapeutic aspects of living donor liver transplantation for HCV are discussed in the present review.

Key words: Hepatitis C virus; Living donor liver transplantation; Interferon; Rivabirin

Sugawara Y, Makuuchi M. Living donor liver transplantation to patients with hepatitis C virus cirrhosis. *World J Gastroenterol* 2006; 12(28): 4461-4465

http://www.wjgnet.com/1007-9327/12/4461.asp

INTRODUCTION

The use of live donors for liver transplantation was initiated more than a decade ago as a solution to the cadaveric donor shortage for pediatric recipients[1]. After the first successful case in an adult patient in 1994[2], this procedure is now widely applied to adult recipients, especially in countries where the availability of brain-dead donors is severely restricted[3] and also in the United States and European countries, due to a critical shortage of cadaveric organs. Improved surgical techniques and the introduction of new immunosuppressive agents have enhanced the long-term results of living donor liver transplantation (LDLT), leading to an increased demand for liver transplantation that exceeds the number of potential donor organs. In initial experiences with adult LDLT in Japan, the most common indication was cholestatic liver disease, including primary biliary cirrhosis and primary sclerosing cholangitis in Japan. The number of LDLT patients indicated for hepatitis C virus (HCV) has recently increased rapidly.

A recent study[4] of deceased donor liver transplantation (DDLT) reported that HCV infection was associated with a 23% increase in mortality and a 30% increase in the rate of graft failure. The poor results might be due to the recurrence of HCV disease in the graft[5]. HCV-induced graft hepatitis and fibrosis/cirrhosis occur in 75% to 80% and 10 % to 30% of recipients, respectively, at 5 years[6,7]. Once liver cirrhosis is established, the cumulative probability of developing clinical decompensation is close to 50% after 1 year and survival after decompensation is extremely short[8]. Cholestatic hepatitis occurs in approximately 10% of patients infected with HCV and leads to accelerated graft failure and death[9]. One of the hottest debates is the possibility of increased severity of recurrent HCV in LDLT patients. The benefit of LDLT might be offset if the outcome of LDLT for HCV patients is worse than that of DDLT. In this review, we describe current trends and controversies in LDLT for patients with HCV. Our results for LDLT and HCV are also reported.

CURRENT STATUS OF LDLT

According to the Japan Liver Transplantation Society[10], the number of adult patients (≥18 years old) is increasing annually, and has reached 300 in 2003. The most common indication for adults has been hepatocellular carcinoma (n = 311), followed by primary biliary cirrhosis (n = 255), and HCV-related cirrhosis without carcinoma (n = 113). The 1, 3, and 5 year survival rates of all the adult patients were 76%, 72%, and 69%, respectively. Those of HCV-positive patients were 76%, 73%, and 65%, respectively.

In the United States in 2000, there was a high level of enthusiasm for adult LDLT, with 49 centers performing at least one LDLT. Overall, in experienced centers, about a third of adults on the waiting list had a potential living donor and half of them had undergone LDLT; thus, LDLT might be applicable for up to 15% of individuals on the list[11]. The enthusiasm was, however, quickly tempered

Table 1 Comparison between LDLT and DDLT for hepatitis C virus cirrhosis

Study			N		Dif[1]	Protocol biopsy	Findings
Author	Year	Institution	LDLT	DDLT			
Gaglio[27]	2003	Colombia U.	23	45	Yes	No	Cholestatic hepatitis in 17% of LDLT and 0% of DDLT ($P = 0.001$). No significant difference in incidence of Rec.
Shiffman[28]	2003	Virginia Commonwealth U.	22	53	No	Yes	79% patient survival in LDLT and 91% in DDLT during 3 year (NS). No significant difference in inflammation score in liver specimen after 3 years
Russo[29]	2004	UNOS data	279	3955	No	No	87% 1-year patient survival in both.
Thuluvath[30]	2004	UNOS data	207	408	No	No	No significant difference in patient survival ($P = 0.6$).
Van Vlierberghe[31]	2004	Ghent U.	17	26	No	No	Rec in 35% of LDLT and 38% of DDLT during 1 year ($P = 0.1$)
Bozorgzadeh[32]	2004	Rochester U.	35	65	No	No	Rec in 77% of LDLT and 72% of DDLT during 1 year (NS), 89% patient survival in LDLT and 75% in DDLT during 39 mo (NS)

[1]Difference in short-term outcomes or severity of virus recurrence between living and deceased donor liver transplantation. Abbreviations: Rec, Virus recurrence; U, University; NS, not significant; UNOS, United Network for Organ Sharing.

by the death of a donor in 2002 in the United States[12]. Since 2001, the number of patients who have undergone LDLT has declined[13]. Currently less than 5% of all adult liver recipients use living donors. By July 2005, 2734 LDLT cases had been performed. There were 1761 adult patients and HCV was the most common indication. HCV is the most common indication for LDLT[14] and the number of HCV-positive patients is stable, approximately 100 per year between 2000 and 2002.

By the end of 2003, 1743 LDLT cases were recorded in the European Liver Transplantation Registry[15]. According to the Transplant Procurement Management[16], the number of LDLT peaked in 2003 and has gradually decreased over recent years. LDLT accounts for approximately 5% of the total liver transplants performed in Europe. Among the 806 LDLT cases from October 1991 to December 2001[17], the overall 5-year graft survival rate was 75%, better for children than for adults (80% *vs* 66% at 3 years). Cirrhosis secondary to HCV infection is a leading indication for LDLT among adults in Europe[18]. The number of LDLT patients is shown in the Table 1.

INDICATIONS

In areas with low deceased donor organ availability, the indications for LDLT are similar to those for DDLT. In contrast, in Western countries, LDLT is conducted in an attempt to alleviate the shortage of donor organs and to decrease the mortality among the patients awaiting transplants. That is, a balance needs to be achieved between the candidate's liver disease severity and the adequacy of a partial graft for transplantation. The candidate's liver disease should be advanced to the extent that transplantation is justified, but the liver disease cannot be so advanced that a partial graft will not provide adequate hepatic mass.

According to Russo's report[19] a substantial proportion of patients were United Network for Organ Sharing (UNOS) status 3 at the time of LDLT (43%). The policy at their centers prior to the implementation of a model for end stage liver disease (MELD)-based allocation was not to proceed with LDLT in patients meeting UNOS status 2A criteria. Their patient survival rate was 57% with an average stay of 23 d in the intensive care unit. In comparison, 1-year patient survival was 82% in DDLT recipients who were UNOS status 2A at the time of transplant[20].

The waiting list mortality increases in patients with advanced liver disease and patients with a MELD score of 25 have a 20% 3-mo mortality[21]. In general, it is uncommon to proceed with LDLT in patients with MELD scores above 25. Thus, depending on the region of the country and the average MELD score at the time of the transplant within the area served by the organ procurement organization, LDLT might offer patients transplantation before they die waiting for a deceased donor liver. The lower MELD score limit with LDLT is more controversial and varies from center to center. Russo[19] commented that they do not proceed with LDLT in candidates with MELD scores under 11.

LDLT AS A RISK FACTOR FOR RECURRENCE OF HCV

One study from Barcelona[22] reported that LDLT patients ($n = 22$) had younger donors, less graft steatosis, more frequent biliary complications, and earlier and more severe acute hepatitis compared with DDLT ($n = 95$) patients. A report from Colombia University[23] indicates that cholestatic hepatitis or severe HCV recurrence occurs more frequently in LDLT. These reports indicate that more intensive antiviral therapy might be necessary for recipients of living donor grafts.

The possible causes of HCV recurrence include HLA matching between donor and recipient. Because cellular immune reactions restricted by both HLA class I and II antigens are involved in the recognition of HCV peptides[24], HLA matching between donor and recipient could potentially increase damage to the graft from recurrent viral infections by facilitating host recognition of viral antigens[6]. Recently, a beneficial effect of a complete HLA-DQ mismatch was reported in 14 patients after transplantation for HCV cirrhosis[25]. Another possible cause might be related to liver regeneration[26], although recent data[27] did not support this hypothesis. *In vitro*, HCV internal ribosome entry site activity and replication are higher in actively dividing cells, and it is possible that

viral translation is enhanced by factors that stimulate the regeneration of hepatocytes. Moreover, there are experimental data suggesting that liver regeneration induces low density lipoprotein receptor expression, which might facilitate HCV entrance into the hepatocytes.

In contrast, comparable data between LDLT and DDLT for HCV was recently reported[28]. Russo and colleagues[29] compared patient and graft survival in recipients transplanted for chronic HCV who received a living donor organ (n = 279) and deceased donor organ (n = 3955) using the UNOS liver transplant database. One-year patient survival was 87% in both groups and 2-year patient survival was 83% and 81% in the living donor group and deceased donor group (P = 0.68), respectively. Similar results (DDLT, n = 480 *vs* LDLT, n = 207) were obtained from another analysis using the UNOS data base[30]. Analyses from the Mayo Clinic[31] and Gent University[32] also demonstrated no negative impacts of LDLT on the results of liver transplantation for HCV-related cirrhosis.

These data should be interpreted with caution, however, because of the important clinical distinction between LDLT and DDLT recipients. At the time of transplantation, the LDLT group recipients are far less sick than their DDLT group counterparts[33]. The LDLT (n = 35) and DDLT (n = 65) data from a single institution, Rochester University, were examined[34]. Patient survival, graft survival, rate of HCV recurrence, severity of HCV recurrence, graft loss from HCV, and interval for HCV recurrence in DDLT and LDLT were similar. It remains unclear, however, whether LDLT is truly disadvantageous compared to DDLT for HCV-positive patients because the number of cases or follow-up duration is not yet sufficient.

According to the data from Russo[29], from 1999 to 2000, the 1-year patient survival in the LDLT group increased from 69% to 90% (P = 0.04), and 1-year graft survival increased from 63% to 79% (P = 0.16). In contrast, in the DDLT group, 1-year patient and graft survival did not substantially change from 1999 to 2000. As a result, 1-year survival rates became similar between the LDLT and DDLT groups in 2000. The results indicated an experience effect and learning curve on outcomes after LDLT for HCV. Therefore, the initial reports indicating poorer results of LDLT might be due to technical problems from a lack of experience. Recent data indicating similar results between LDLT and DDLT might be due to the increased experience with LDLT. The multicenter adult to adult LDLT cohort study (A2ALL) might soon provide some answers to the questions about recurrent HCV after LDLT and DDLT[35].

MANAGEMENT OF HCV

Therapy for reccurrence in DDLT

If HCV recurs earlier and more severely after LDLT, a specific strategy for preventing the detrimental effects of HCV on living donor grafts must be developed. One strategy might be aggressive treatment for HCV. Treatment of recurrent HCV disease with interferon and ribavirin after DDLT is used in some centers[36-38]. One standard regimen includes interferon-alpha2b (3 MU × 3 per week) and ribavirin (1000 mg/d) for 6 mo. In a recent trial, polyethylene glycol-conjugated interferon therapy was used[35,39-44], with a sustained viral response rate ranging from 13% to 47%.

Preemptive therapy for HCV after DDLT

Preemptive therapy in the early post-transplantation period with interferon either alone or in combination with ribavirin has been attempted in DDLT, although its effectiveness is controversial. In one study, HCV-positive recipients were randomized within 2 wk of transplantation to receive either interferon alone (3 MU × 3 per wk, n = 30) or placebo (n = 41) for 1 year[39]. Only 17 patients could complete 1 year of interferon therapy. Eight patients (27%) in the interferon group and 22 (54%) of the untreated patients had recurrent hepatitis (P = 0.02). Patient and graft survival at 2 years did not differ between the groups, however, and the rate of viral persistence was not affected by treatment.

In another controlled trial[45], 24 recipients were randomized at 2 weeks post-transplantation to receive interferon (3 MU × 3 per wk) or placebo for 6 mo. There were no differences in graft or patient survival. There were no differences between groups in the incidence of histological recurrence or its severity differed between groups. Recurrent HCV was delayed 408 d in treated patients versus 193 d in the control cohort.

In a case series by Mazzaferro[46], 36 recipients were treated with interferon-alpha 2b (3 MU × 3 per wk) and ribavirin (10 mg/kg per d). They started treatment at a median of 18 d after the operation and treatment continued for 11 mo. After a median follow-up of 52 mo, the 5-year patient survival was 88%. Serum HCV RNA clearance was obtained in 12 patients (33%). They did not require further antiviral treatment because of negative HCV RNA in serum and normal liver histology for a median of an additional 36 mo. The former two randomized trials on preemptive interferon monotherapy demonstrated minimal benefits of the drug. In contrast, Mazzaferro reported more encouraging results, although their protocol brings into question how long therapy is needed once embarking on a preemptive strategy.

Re-transplantation

The approach to retransplantation for recurrent HCV varies widely among the transplant centers of DDLT[11]. The results after retransplantation for HCV (45% at 5 years) are poorer than that for other causes[47] (56%, P < 0.001). The patients with recurrent HCV in the early timing and graft failure within the first year have poor outcomes after retransplantation. These individuals should be considered contraindicated for retransplantation. The experience of retransplantation for HCV in LDLT has not been well accumulated.

OUR EXPERIENCE

We performed preemptive therapy for LDLT patients with HCV infection[48]. From 1996 to 2004, 67 patients underwent LDLT for HCV cirrhosis at the Tokyo University Hospital. The patients were 51 men and 16

women and their ages ranged from 23 to 63 years (median 55). The HCV genotype was 1b in 53 patients (79%). Forty-one patients (61%) had hepatocellular carcinoma. All the patients received the same immunosuppressive regimens with tacrolimus and methylprednisolone.

All the patients preemptively received antiviral therapy consisting of interferon α-2b and ribavirin, which was started approximately 1 mo after the operation. The therapy was continued for 12 mo after the first negative HCV RNA test. The standard regimen included interferon α-2b (3 MU × 3 per wk) and ribavirin (800 mg/d) for 6 mo. The patients were then observed without the therapy for 6 mo. The therapy was continued for at least 12 mo even if the HCV RNA test remained positive.

Therapy was discontinued when there was significant leukopenia (< 1500 /mL), thrombocytopenia (< 50000 / mL) despite application of granulocyte colony stimulating factor (Gran®, Sankyo, Co. Ltd., Tokyo, Japan), hemolytic anemia (hemoglobin < 8 g/L), renal dysfunction (serum creatinine > 20 mg/L), depressive psychological status, or general fatigue. The subjects were removed from the protocol if they did not continue the therapy for 12 mo due to adverse effects or could not start the therapy due to early death.

Blood counts and liver function tests were checked every 2 wk for the first month, and at 4 wk intervals thereafter. Serum samples were collected once a month for quantitative HCV RNA detection. Protocol liver biopsy was not performed. The log-rank test was used to compare the survival rate of the HCV-positive patients with the HCV-negative patients who underwent transplantation during the same period (n = 168).

A total of 28 patients were excluded from the analysis; 12 patients were removed from the protocol because of early death (n = 9) or because of drug cessation (n = 3). Another 16 patients are currently on the protocol and were therefore excluded from the analysis. Of the remaining 39 patients, 16 (16/39; 41%) obtained a sustained virologic response. The cumulative 5-year survival of the HCV-positive patients was 84%, comparable with that of patients negative for HCV (n = 168, 86%).

CONCLUSIONS

LDLT will remain an indispensable therapeutic tool for HCV related end stage liver disease and an alternative to DDLT. The association between LDLT and early HCV recurrence remains to be determined, although most of the recent papers suggest that live donor graft has no effect on short-term outcome or severity of virus recurrence. If living donor graft is associated with early HCV recurrence and consequently poorer graft survival, an aggressive antiviral protocol might improve the outcome of LDLT for HCV.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Grants-in-aid for Research on HIV/AIDS, a multicenter pilot clinical study to compare the safety and efficacy of a steroid free immunosuppression protocol with monoclonal anti-IL2R antibody in HCV positive living donor liver transplantation and Research on Measures for Intractable Diseases from the Ministry of Health, Labor and and Welfare of Japan.

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S- Editor Guo SY **L- Editor** Alpini G **E- Editor** Ma N

Cyclosporin A for Treatment of Hepatitis C Virus After Liver Transplantation

Cirrhosis secondary to hepatitis C virus (HCV) infection is a leading indication for liver transplantation. HCV recurrence, however, is nearly certain and might worsen patient/graft outcome. A recent paper (*1*) reported that HCV infection is associated with a 23% increase in mortality and a 30% increase in the graft failure rate. The goal of HCV management in the transplantation setting is to prevent graft loss due to recurrent HCV infection, raising questions about a possible role for immunosuppression regimens and antiviral therapy.

A previous report (*2*) indicated that cyclosporin A (CyA) and interferon-alpha2b might effectively inhibit HCV replication in vitro. The antiviral effects of CyA for patients with chronic HCV (*3*) and those for HCV recurrence after transplantation (*4*), however, are controversial. We conducted a pilot study of the use of CyA, interferon, and ribavirin for preemptive therapy of HCV after liver transplantation.

Until October 2003, 41 HCV-positive patients underwent liver transplantation from living donors at the University of Tokyo Hospital. The immunosuppression regimens consisted of steroids and tacrolimus (*5*). The targeted whole-blood tacrolimus level was 15 to 20 ng/ml during the seven days after living donor liver transplantation (LDLT), which was gradually tapered to 5 ng/ml six months after LDLT. Predonisolone was tapered to 0.05 mg/day/kg six months after LDLT but was not stopped. All of the HCV-positive patients preemptively received interferon-alpha2b and ribavirin therapy (*6*), which was started approximately one month after transplantation. HCV RNA level was measured by real-time-polymerase chain reaction (*7*) and Amplicor HCV (Roche Molecular Systems, Pleasanton, USA). Of the 41 patients, six died within two years; 14 obtained a viral response (<50 IU/ml by Amplicor HCV) at the end of the treatment period (one year) and the response was sustained for another six months without the antiviral therapy; 21 did not respond to the antiviral therapy. Of the 21 nonresponders, eight patients continued with the protocol, which called for a change from tacrolimus to CyA without changing the antiviral therapy. The targeted CyA trough level was 100 ng/ml.

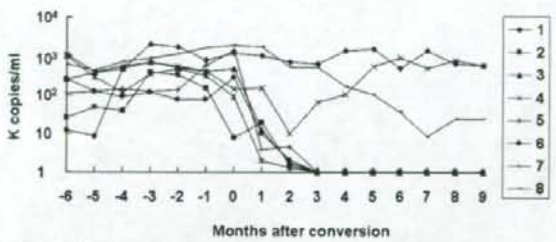

FIGURE 1. Change of the viral titer in the eight patients.

All of the patients were genotype 1b. The serum HCV titer was measured once a month by reverse transcriptase–polymerase chain reaction. The Institution Review Boards at University of Tokyo Hospital approved the protocol.

In five of the eight subjects (63%), the HCV titer was negative by Amplicor HCV within three months after the conversion and remained negative for another six months on CyA, interferon, and ribavirin (Figure 1). Liver and renal functions remained stable in all of the patients, and none of them had complications of acute cellular rejection after the conversion.

Our findings support the use of CyA in combination with interfron and rivabirin for the eradication of HCV in previous nonresponders. These findings suggest that a controlled study to confirm the benefit of CyA for preemptive treatment of HCV after liver transplantation is warranted.

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This work was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Grants-in-aid for Research on HIV/AIDS and Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan.

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Received 8 April 2006. Revision requested 12 April 2006.
Accepted 13 April 2006.

ISSN 0041-1337/06/8204-579
DOI: 10.1097/01.tp.0000229397.81425.51

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Hepatology Research 2008; **38**: 27–36 doi: 10.1111/j.1872-034X.2007.00217.x

Original Article

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

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Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts ≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

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Received 6 March 2007; revision 22 May 2007; accepted 14 June 2007.

that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment–insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average

Table 1 Baseline of hepatitis C virus patients with normal serum aminotransferase (ALT) received antiviral therapy

	ALT ≤ 30 U/L (group A)	ALT 31–40 U/L (group B)	*P*-value
No. patients	255	209	
Age	51.6 ± 13.0	53.5 ± 13.2	0.548*
Sex (male/female)	112/143	117/92	0.01**
BMI (kg/m^2)	21.6 ± 2.9	22.8 ± 3.0	<0.001*
HOMA-IR	2.5 ± 3.2	5.2 ± 6.5	0.093*
Genotype: 1/2/others	127/127/1	112/96/1	0.881**
Viral load: low/high	138/117	99/110	0.203**
G1 (low/high)	114/125		
G2 (low/high)	161/62		
Histology			
F stage (0/1/2/3/4)	29/166/48/11/1	22/122/57/6/2	0.169**
Grade (0/1/2/3)	25/187/41/2	7/159/43/0	0.046**
Fatty change† 0–1/2–4	232/23	161/48	0.033**
Iron load‡ 0/1–4	101/15	97/19	0.458**
Ferritin (ng/mL)	83.9 ± 103.7	118.8 ± 135.3	0.006*
PLT count (/μL)	19.2 ± 5.4	18.4 ± 6.1	0.059*
≥150 000/<150 000	204/51	141/68	0.002**
Hyaluronate (ng/mL)	60.8 ± 73.7	69.1 ± 73.0	0.249*
Duration of antiviral therapy (weeks)	25.6 ± 12.0	26.1 ± 12.1	0.297*
Effects of therapy			
SVR/non-SVR	142/113	99/110	0.075**

*P-values were calculated by Mann–Whitney-*U*-test. **Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in sex distribution ($P = 0.01$), BMI ($P = 0.01$), frequency of steatosis ($P = 0.033$), serum ferritin level ($P = 0.006$), grade of hepatic inflammation ($P = 0.046$), incidence of fatty change ($P = 0.033$), serum ferritin level ($P = 0.006$), and the incidence of low PLT counts ($P = 0.002$) between groups A and B. Values are expressed as mean ± SD.

ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet; SVR, sustained viral responders.

duration of therapy between 1995 and 2003 was 26 weeks for IFN monotherapy and 24 weeks for IFN/Riba combination therapy. In principle, 6–10 MU IFN was administered daily for 2 weeks and three times per week subsequently. The daily dosage of ribavirin was 600–1000 mg depending on body weight. Sustained viral responders (SVR) were defined as patients who were negative for serum HCV RNA 6 months after the completion of antiviral therapy.

All of the patients were divided into two groups (group A: ALT ≤ 30 U/L, group B: 31 U/L ≤ ALT ≤ 40 U/L) which were further divided into two subgroups based on PLT counts: group A-1 and B-1 (PLT counts ≥150 000/μL) and groups A-2 and B-2 (PLT counts <150 000/μL).

One hundred and twenty-nine HCV carriers with PNALT were enrolled to determine their long-term prognosis. These patients showed normal serum ALT levels (≤30 U/L) over a 12-month period on least three different occasions (PLT counts ≥150 000/μL, and body mass index [BMI] <25 kg/m^2). Thirty-nine patients received serial liver biopsies. The mean follow-up period of the 129 patients was 7.2 ± 3.2 years on 15 November 2006.

Statistical analyses

Data are expressed as mean ± SD. We compared continuous variables using the Mann–Whitney *U*-test. A frequency analysis and comparison between the groups were performed using the χ^2-test or Fisher's exact test and the Mann–Whitney *U*-test. ANOVA and Tukey's HSD procedure was used to determine the difference between multiple groups. All tests were two-tailed and *P*-values of less than 0.05 were considered significant. All statistical analyses were performed using Statistical Package of Services Solutions software, version 11.0 (SPSS, Chicago, IL, USA).

Table 2 Baseline of hepatitis C virus patients with less than 30 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/mL (group A-1)	PLT < 150 000/mL (group A-2)	*P*-value
No. patients	204	51	
Age	48.4 ± 12.7	58.7 ± 7.5	<0.001*
Sex (male/female)	90/114	22/29	1.000**
BMI (kg/m^2)	21.6 ± 3.0	21.3 ± 2.4	0.514*
HOMA-IR	2.8 ± 3.5	1.2 ± 0.8	0.598*
Genotype: 1/2/others	101/101/2	25/26/0	0.952**
Viral load: low/high	112/92	26/25	0.574**
Histology			
F stage (0/1/2/3/4)	29/142/27/6/0	1/25/21/3/1	<0.001**
Grade (0–1/2,3)	179/25	33/18	<0.001**
Fatty change† 0–1/2–4	188/16	44/7	0.582**
Iron load‡ 0/1–4	82/12	17/3	0.762**
Ferritin (ng/mL)	86.0 ± 112.1	73.9 ± 46.6	0.204*
PLT count (/μL)	21.0 ± 4.4	12.1 ± 2.5	<0.001*
Hyaluronate (ng/mL)	41.8 ± 56.1	112.5 ± 109.9	<0.001*
Duration of antiviral therapy (weeks)	25.7 ± 10.3	27.0 ± 9.9	0.503*
Effects of therapy			
SVR/non-SVR	115/89	27/24	0.66**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), grade of inflammatory activity ($P < 0.001$), PLT count ($P < 0.001$), and serum-hyaluronic acid ($P < 0.001$) between groups A-1 and A-2. Frequency of F2–4 patients was 16.2% in group A-1 and 51.6% in group A-2. Values are expressed as mean ± SD.

BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; PLT, platelet counts; SVR, sustained viral responders.

RESULTS

Demographic, clinical, and histological features of 464 HCV carriers with normal serum ALT

THE CHARACTERISTICS OF the 464 HCV carriers with normal serum ALT are shown in Table 1. There were significant differences in sex, frequency of steatosis, serum ferritin levels, BMI, and the incidence of low PLT counts (<150 000/μL) between groups A and B.

There were significant differences in age, fibrosis (F) stage, inflammatory activity, PLT counts, and serum hyaluronate between groups A-1 and A-2 (Table 2). The frequency of stage F2–4 patients was 16.2% in group A-1, and 49.0% in group A-2 (Table 2). In group B, there were significant differences in age, F stage, PLT counts, and serum hyaluronate between groups B-1 and B-2 (Table 3). There were no F4 patients in group A-1 and B-1, and the frequency of F3 patients was very low compared with those in groups A-2 and B-2 (2.6% *vs* 7.6%). The PLT counts decreased in proportion to the progression of liver fibrosis as follows; F0 ($n = 51$); $20.7 \pm 5.2 \times 10^4$/μL, F1 ($n = 288$); $19.8 \pm 5.6 \times 10^4$/μL, F2 ($n = 105$); $16.9 \pm 5.3 \times 10^4$/μL, F3 ($n = 17$); $15.9 \pm 4.6 \times 10^4$/μL, and F4 ($n = 3$); $11.3 \pm 3.8 \times 10^4$/μL.

Of the 464 patients, the frequency of the F0–1 stages was 80.1% and that of the F2–4 stages was 19.9% in patients with PLT counts ≥150 000/μL, and it was 50.4% and 49.6%, respectively, in patients with PLT counts <150 000/μL. In patients with PLT counts $\geq 17.0 \times 10^4$/μL, 80.8% were in stages F0–1 and 19.2% were in stages F2–4, and in patients with PLT counts $< 17.0 \times 10^4$/μL, 60.1% were in stages F0–1 and 39.9% were in stages F2–4.

The SVR rates of IFN therapy were 52.4% in F0–1 patients, 49.5% in F2–4 patients ($P = 0.896$ by Fisher's exact test), and 58.0% and 43.8% ($P = 0.592$) in IFN/Riba therapy, respectively.

In patients with genotype 1b and high viral load, the SVR rate was 12.5%. The SVR rate in genotype 2 patients was 60.4% in the IFN group and 67.7% in the IFN/Riba combination therapy group.

Table 3 Baseline of hepatitis C virus carriers with 31–40 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/mL (group B-1)	PLT < 150 000/mL (group B-2)	*P*-value
No. patients	141	68	
Age	48.2 ± 11.9	57.9 ± 7.5	<0.001*
Sex (male/female)	80/61	37/31	0.751**
BMI (kg/m^2)	22.9 ± 3.1	22.7 ± 2.6	0.08*
HOMA-IR	3.0 ± 2.0	8.2 ± 9.5	0.8.8*
Genotype: 1/2/others	82/58/1	30/38/0	0.095**
Viral load: low/high	64/77	35/33	0.542**
Histology			
F stage (0/1/2/3/4)	17/91/31/2/0	4/30/26/6/2	<0.001**
Grade (0–1/2,3)	116/25	50/18	0.114**
Fatty change† 0–1/2–4	111/30	50/18	0.10**
Iron load‡ 0/1–4	67/12	30/7	0.762**
Ferritin (ng/mL)	114.4 ± 116.1	127.2 ± 167.8	0.869*
PLT count (/μL)	21.5 ± 4.9	12.2 ± 2.1	<0.001*
Hyaluronate (ng/mL)	46.9 ± 35.4	100.7 ± 0.98.1	<0.001*
Administration of IFN (weeks)	26.1 ± 11.9	27.7 ± 11.4	0.983*
Effects of therapy			
SVR/non-SVR	64/77	35/33	0.409**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. In group B, there were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), PLT count ($P < 0.001$), and hyaluronic acid ($P < 0.001$) between B-1 and B-2. Frequency of F2–4 was 23.4% in B-1and 50.0% in B-2, respectively. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; IFN, interferon; PLT, platelet counts; SVR, sustained viral responders.

Demographic, clinical, and histological features of 129 HCV carriers with PNALT

The demographic and clinical features of the 129 HCV carriers with PNALT who were followed up for 7.2 years are shown in Table 4. Normal liver histology was noted in 17 patients, 102 showed minimal to mild CH, and 10 had moderate CH. Steatosis was seen in 7% and iron loading was noted in 12%.[18]

Of the 78 patients followed longer than 7 years (mean follow-up period; 10.4 ± 3.1 years), 11 (14%) had continuously normal ALT (G-1), 43 (55%) showed a transient elevation of ALT (G-2), and 24 (31%) changed to CH with continuously elevated ALT (G-3).

Thirty-nine patients received repeated liver biopsies (2–4 times). Of the 39 patients, six were in G-1, 17 were in G-2, and 16 were in G-3. The intervals between the first biopsy and the last biopsy in these three groups were 7.1, 7.8, and 7.2 years, respectively. The progression of the F stage was noted in two of six in G-1, six of 17 in G-2, and seven of 16 in G-3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.05, and 0.08 fibrosis unit. HCC was not detected in any patients during the follow-up periods.

Guidelines for the antiviral therapy of HCV carriers with normal serum ALT focused on the inhibition of the development of HCC

Considering the risk of progression to liver cirrhosis and the development of HCC, as well as the expected efficacy and various side-effects of antiviral therapy, an algorithm is needed for the management of HCV carriers with normal serum ALT. The progression rate of liver fibrosis stage was 0.05/year in HCV carriers with PNALT. The annual incidence of HCC in CH-C patients has been reported to be 0.5% at stages F0–F1, 1–2% at stage F2, 3–5% at stage F3, and 7% at stage F4.[4]

In principle, follow up without antiviral treatment is recommended for HCV carriers with PNALT (ALT ≤30 U/L) and PLT counts ≥150 000/μL, particularly in older patients (i.e. >65 years old), because over 90% show normal or minimal liver damage with good prognoses. However, antiviral therapy is not contraindicated for such patients since roughly 40% are infected with HCV genotype 2,[18] which suggests a high rate of SVR to the therapy with PEG-IFN/Riba.

As for the indication of antiviral therapy for HCV carriers with normal serum ALT (≤40 U/L), the PLT

Table 4 Characteristics of 129 HCV carriers with persistently normal ALT who received liver biopsy

	$n = 129$	Follow up over 5 years ($n = 78$)
Follow-up period (years)	7.2 ± 3.2	10.4 ± 3.1
Age (years)	48 (21–77)	45 (29–71)
Male ($n = 24$)	49.8 ± 16.4	42.3 ± 14.9
Female ($n = 105$)	47.2 ± 12.5	46.6 ± 11.6
Sex (male/female)	24/105	10/68
ALT (U/L)	8–30	9–30
Male ($n = 24$)	22.5 ± 5.7	21.1 ± 5.4
Female ($n = 105$)	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4$/mL)	15–31	15–31
Ferritin (ng/mL)	5–225	5–225
Male ($n = 24$)	76.2 ± 53.5	84.6 ± 59.2
Female ($n = 105$)	60.0 ± 43.3	66.6 ± 52.5
HCV genotype	G1 ($n = 58$), G2 ($n = 45$) Mixed and unclassified ($n = 16$)	
BMI (kg/m^2)	16–27	16–27
Male	22.2 ± 1.7	21.9 ± 1.9
Female	21.3 ± 2.2	21.0 ± 2.4

Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; PLT, platelet.

count is a good indicator for discriminating as to whether or not they have minimal to mild fibrosis or moderate to advanced fibrosis. Serum hyaluronate levels were significantly higher in HCV carriers with 31–40 U/L ALT having less than 150 000/μL PLT (Table 3). Advanced hepatic F stage, an elevated ALT level, old age (>65 years old), and sex (male) are important risk factors for the development of HCC.[6,18,30] We advocated an algorithm for such patients (Fig. 1) taking into consideration the risk of the progression to cirrhosis and the development of HCC. Therapy with PEG-IFN/Riba is the first-line treatment; therapy for 48 weeks is recommended for genotype 1 patients with high viral load and 12–24 weeks therapy for genotypes 2 and 1 with low viral load.

DISCUSSION

OUR PREVIOUS STUDY in 129 HCV carriers with PNALT demonstrated a predominance of females, higher frequency of genotype 2, minimal to mild liver histology, and very slow progression of hepatic fibrosis.[18] However, over 30% of these patients advanced to CH-C with elevated ALT levels during the 7-year follow up.

There are many reports concerning the natural course of liver fibrosis in CH-C patients, including those who are HCV carriers with normal serum ALT.[19,31–39] More than half of CH-C patients show progression of F stage from F1 to F2–4 within 10 years, and it was reported that the progression of liver fibrosis in HCV carriers with normal serum ALT was more rapid than was observed in the present study.[23] The main reason for the discrepancy between the report by Puoti *et al.*[23] and our results might be due to the definitions used for the normal range of serum ALT. In our previous study, the patients were HCV carriers with PNALT (ALT ≤ 30 U/L) and PLT counts ≥150 000/μL. On the other hand, the patients in the study by Puoti *et al.* had ALT levels ≤40 U/L, irrespective of PLT counts, in which cirrhotic patients might be included.[23] However, recent studies have demonstrated that normal ALT levels are less than 30 U/L[24] or 25 U/L in men[40] and less than 19 U/L[24] or 22 U/L in women.[40]

The present study demonstrated that the different distribution of hepatic F stage became remarkable when the A and B groups were divided into two subgroups according to their PLT counts. In HCV carriers with ALT levels ≤30 U/L, the frequency of stages F2–3 was 16.2% among those with PLT counts ≥150 000/μL; however, the frequency of stages F2–3 was 49.0% in those with PLT counts <150 000/μL. Conversely, in HCV carriers with ALT levels between 31 and 40 U/L, the frequency of stages F2–4 was 23.4% among those with PLT counts ≥150 000/μL and 50.0% in those with PLT counts <150 000/μL. The PLT count is a useful marker in dis-

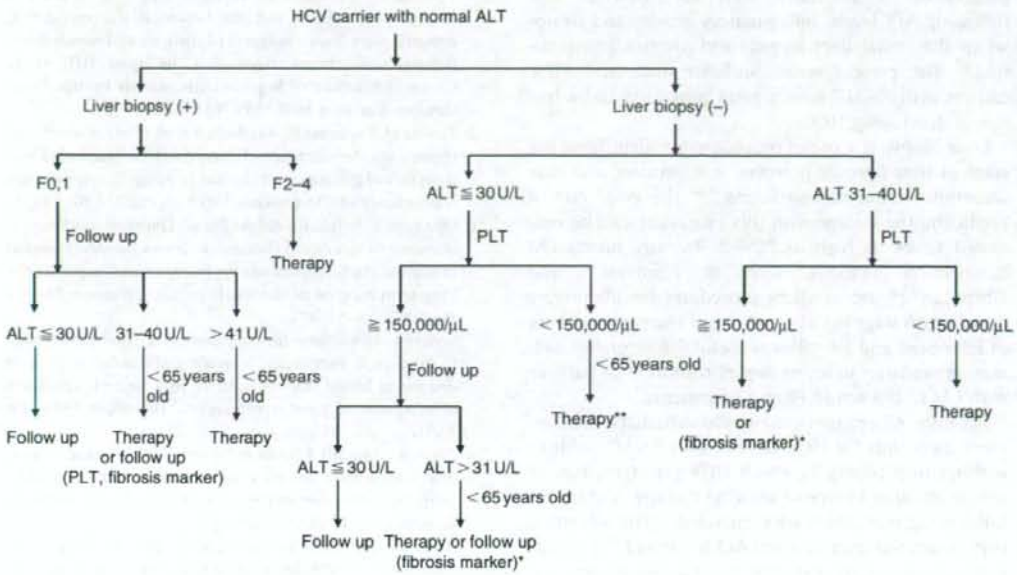

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral therapy, especially those with genotype 2 after the elevation of serum ALT levels. In patients who did not undergo liver biopsy, ALT and platelet (PLT) levels are good indicators for determining candidates for antiviral therapy. Older patients (>65 years) and/or patients having uncontrolled hypertension, diabetes mellitus, or anemia should not be treated with pegylated interferon and ribavirin. Combination therapy with pegylated interferon and ribavirin for 48 weeks is recommended for patients with genotype 1 and high viral load, and 12–24 weeks therapy is suggested for patients with genotype 2 and genotype 1 with low viral load. *,**Serum fibrosis markers, such as hyaluronate, might be useful to decide whether patients are candidates for antiviral therapy or not.

criminating between stages F0–1 and F2–4 F in HCV carriers with normal serum ALT (≤40 U/L). In the present study, the mean PLT count in F2 and F3 patients was 16.9 ± 5.3 ($\times 10^4/\mu L$) and 15.9 ± 4.6 ($\times 10^4/\mu L$), respectively. The distribution of the F stage was not significantly different between patients with PLT counts $\geq 15 \times 10^4/\mu L$ versus $< 15 \times 10^4/\mu L$ and $\geq 17 \times 10^4/\mu L$ versus $< 17 \times 10^4/\mu L$.

The SVR rate for genotype 1 patients with high viral load treated with either IFN monotherapy or IFN/Riba were 12.5% and 37.7%, respectively. In genotype 2 patients with high viral load, the SVR rate in the present study was better than the data of Japanese CH-C patients with elevated ALT levels in our previous paper.[6] It was not reasonable to compare the SVR rates between HCV carriers with normal serum ALT and CH-C with elevated ALT in the present study, because the total dosage of IFN and the duration of treatment were significantly different.

The annual incidence of HCC is correlated with the progression of liver fibrosis, that is, the stage of liver disease.[2–4,6] Sustained low serum ALT levels are also associated with a lower incidence of HCC.[2,6,41] PEG-IFN/Riba therapy is expensive and induces various side-effects. The present results indicate that most HCV carriers with normal serum ALT (≤40 U/L) and PLT counts ≥150 000/μL have minimal to mild liver damage, indicating a low risk for the progression to cirrhosis and the development of HCC. This was more remarkable in patients with ALT levels ≤30 U/L and PLT counts ≥150 000/μL. However, nearly half of the patients with PLT count <150 000/μL have F2 or F3 F stages, indicating a certain risk for the progression to cirrhosis and the development of HCC. Fibrosis