Table 1 Inclusion criteria for liver transplantation

NIH sponsored study in the USA[94]	1. No AIDS-related opportunistic infections 2. CD4 counts should be > 100/mL for those without a history of opportunistic infection and > 200/mL for those with a history of opportunistic infection. 3. HIV-RNA should be undetectable. In the event that an undetectable HIV viral load is not achievable as a result of drug-induced hepatotoxic, an HIV clinician should predict the ability to control the HIV virus post-transplantation.
Spanish criteria[41]	1. No opportunistic infections 2. CD4 counts > 100/mL 3. HIV-RNA should be undetectable or suppressible with antiretroviral therapy.
O'Grady[95]	1. Absence of AIDS-defining illness after immune reconstitution 2. CD4 counts should be > 200/mL or > 100/mL in the presence of portal hypertension. 3. Absence of HIV viremia 4. Antiretroviral therapeutic options available if the HIV disease reactivates

AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus.

control HIV after the transplantation, based on a complete review of the antiretroviral history, HIV RNA history and resistance testing[43]. This issue is more controversial for patients with CD4 counts greater than 100/mL but who have detectable HIV that is multi-drug-resistant. Currently, most centers still consider this an exclusion criterion, although with more data demonstrating the safety of immunosuppression in the HIV-positive patient as well as an increasing number of antiretroviral agents, this exclusion criterion may be liberalized on a case by case basis[44].

SURGICAL RESULTS

Survival

Survival at 1 year post-transplantation ranges from 58% to 89% (Table 2). Ragni *et al*[32] reported 1, 2 and 3 year survival rates of 87%, 73% and 73% in 24 HIV-positive patients which were not statistically different from age and race-matched HIV-negative patients. Similar results[45] were reported based on an analysis of 15 HIV-positive recipients with a 3 year survival rate of 73% compared to 79% for HIV-negative recipients. Neff *et al*[33] reported that graft and patient survival rates in HIV-positive patients are similar to that of HIV-negative patients transplanted for the same indication. Studies that analyzed liver transplantation for a variety of reasons showed excellent outcome for ESLD irrespective of underlying HIV infection. Another report showed lower survival rates in 27 patients with 1, 3 and 5 year survival rates of 67%, 56% and 33% respectively[35]. A Spanish series found HIV-positive patient survival rates of 90% at 1 year and 67% at 3 years[46].

Two recently published studies comparing survival in HCV/HIV-co-infected and HCV-mono-infected transplant recipients reported a significantly lower survival rate in co-infected patients[35,42]. In a French study of 35 HCV/HIV-infected and 44 HCV-infected recipients, 2 and 5 year patient survival rates were statistically lower in co-infected patients, 73% *vs* 91% and 51% *vs* 81% respectively[42]. MELD was the only significant predictor for mortality and HIV infection did not predict survival. In a US study[35] of 27 HCV/HIV-infected and 41 HCV-infected recipients, the 3 and 5 year patient survival rates tended to be lower in co-infected patients, 56% *vs* 72% and 33% *vs* 72% respectively.

In a review of the United Network for Organ Sharing liver transplant database (between 1997 and 2006)[47], the 2 and 3 year survival rates in 138 HIV-positive recipients were 70% and 66% respectively. These outcomes were slightly worse than the 2 and 3 year survival rates of 81% and 77% respectively of the 30520 HIV-negative recipients ($P < 0.05$). The overall results of liver transplantation in HIV-positive patients are favorable but large prospective clinical trials providing insight into survival and clinical management are required.

Complications

Rejection episode rates in HIV-positive recipients are not different from those of HIV-negative recipients[42]. HIV-associated opportunistic infections and AIDS-related diseases are uncommon. Only a single case of Kaposi's sarcoma and multicentric Castleman's disease has been reported[48]. Death from infectious complications, however, is reported to be more frequent in HIV-positive recipients[45]. Importantly, no HIV disease progression has been reported and HIV replication is efficiently controlled by HAART[32,35,42,49,50]. An exception, though, is the report by Schreibman *et al*[45] that HIV-infected patients experienced significantly higher mortality from infectious complications (4 of 15 recipients). The results of HCC cases within Milan-criteria are encouraging and there are no reports of recurrences[33,35,45,48,49,51-54].

Prognostic factors

A recent report[42] identified high MELD scores at the time of transplant as predictive of a poor outcome. Early and severe HCV graft re-infection is a major determinant influencing post-transplant outcome[32,34,48,51-53,55-58]. Another report[35] showed that HAART intolerance is a significant predictor. Other risk factors include low pre-transplant body mass index and African American race[59].

POSTOPERATIVE MANAGEMENT

Immunosuppression and HAART

Cyclosporine (CyA) inhibits CD4 cell apoptosis and

Table 2 Survival, hepatitis C virus recurrence, and therapy in hepatitis C virus/human immunodeficiency virus co-infected liver transplant patients

References	Institution	Years	n	Genotypes	Time to recurrence (mo)	IFN and RBV doses	Time to therapy (mo)	SVR (%)	FCH (n)	Death (n)	Follow up (mo)
Prachialis 2001[58], Norris 2004[56]	King's College	95-03	7	NA	5	From 2 wk: IFN, 3 MU tiw and RBV, after 3 wk: Peg- IFN 180 μg/wk	0.5 (n = 2), 6 m (n = 1)	0	2	5	12
Rafecas 2004[51]	Hospital Universitari de Bellvitge	02-03	4	4, 1b, 1b, 1a	7	NA	5 (n = 3)	0	0	0	17
Moreno 2005[55]	Hospital Ramon (Madrid)	02-03	4	NA	1-6	NA	1-6	0	1	1	14-18
Radecke 2005[57]	University Hospital Essen	98-01	4	NA	3-8	NA	NA	NA	1	2	10-61
Vogel 2005[48]	Bonn University	97-04	4	1a (n = 2), 2a/2c, 3a	1-8	NA	5-15	50	0	0	NA
Neff 2003[33], Fung 2004[71], de Vera 2006[35]	Thomas E Starzl Transplantation Institute	97-05	27	1 (n = 16), 2 (n = 2), 3 (n = 1)	6	IFN and Peg -IFN, RBV 800 mg/d	2-50	27	6	14	27+-5
Castells 2007[53]	Hospital Universitari Vall d'Hebro'n (Barcelona)	02-05	9	1 (n = 7), 3 (n = 2)	3+-3	Peg-IFN 1.5 μg/kg, RBV 800-1000 mg/d	NA	14	0	1	15+-13
Schreibman 2007[45]	University of Miami	99-06	8	NA	NA	NA	NA	25	0	2	6-74
Vennarecci 2007[52]	Regina Elena Cancer Institute (Rome)	02-06	10	NA	NA	NA	NA	10	3	6	5-46
Wojcik 2007[88]	Medical University of Lodz (Poland)	97-06	4	1a (n = 2), 2a, 3a	1-3	Peg-IFN 180 μg/wk, RBV 200-1000 mg/d	1-3	100	0	0	21-54
Duclos-Vallee 2005[50], 2008[42]	Paul Brousse	99-05	35	1 (n = 20), 2 (n = 1), 3 (n = 9), 4 (n = 4)	0-3	Peg-IFN 50-180 μg/wk, and RBV 400-800 mg/d	0-3	16	3	13	44+-83
Stock 2003[43], Roland 2008[96]	University of California, San Francisco	00-03	6	NA	1-11	NA	1-11	NA	2	4	NA
Testillano 2009[97]	Hospital de Cruces (Vizcaya)	01-07	12	1 (n = 8), 3 (n = 4)	NA	NA	NA	50	2	4	NA
Hughes 2010[98]	Emory University School of Medicine	NA	5	1	2-12	Peg-IFN 135-180 μg/wk, and RBV 600 mg/d	2-12	40	2	2	6-48
Di Benedetto 2008[54], 2010[99]	University of Modena and Reggio Emilia	03-	13	1 (n = 3), 3a (n = 7), 4 (n = 3)	2-16	Peg-IFN 50-180 μg/wk, and RBV 400-800 mg/d	NA	0	2	4	1-14

HCV: hepatitis C; IFN: interferon; Peg-IFN: pegylated interferon; RBV: ribavirin; SVR: sustained virological response; NA: not available; FCH: fibrosing cholestatic hepatitis.

p55Gag processing by binding to cyclophilin A[60-62]. Some beneficial effects of the combination of HAART and CyA have been demonstrated [63] but low-dose CyA exhibits no benefits in patients with stable early HIV disease[64].

Mycophenolate mofetil (MMF) inhibits inosine monophosphate dehydrogenase and depletes the pool of deoxyguanosine triphosphate. MMF is expected to reduce HIV infection by both virological and immunological mechanisms[65-70]. Antagonism due to the inhibition of thymidine kinase has been reported with MMF plus the thymidine analogues zidovudine and stavudine. Mitochondrial toxicity of nucleoside reverse transcriptase inhibitors (NRTI) is potentially augmented by the effect of MMF. Mitochondrial toxicity and lactic acidosis are linked to the use of didanosine, stavudine and zalcitabine and are attributed to damage to mitochondrial polymerase[71].

Sirolimus (SRL) downregulates the expression of chemokine receptor 5 on T-cells which is required for the propagation of macrophage tropic strains of HIV-1[72]. SRL inhibits the progression of Kaposi's sarcoma and primary effusion lymphoma[40,73].

Interactions between HAART drugs [protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)] and calcineurin inhibitors or SRL are well described[74,75]. NNRTIs induce the expression of cytochrome P450, family A gene (CYP3A)[74]. PIs inhibit the production of cytochrome P450 enzymes or P-glycoprotein. CyA, tacrolimus and SRL are substrates of CYP3A4 and P-glycoprotein. PIs, therefore, increase

blood levels of CyA, tacrolimus and SRL, requiring dose reductions of 85 % to 99%[74-78] (Table 2). HAART without PIs may have fewer significant interactions with CyA, tacrolimus and SRL[79].

Efavirenz (NNRTI) induces the production cytochrome P450 enzymes[74] but when efavirenz or a nucleoside analogue combination are added to the treatment regimen, little change in the dosing of tacrolimus is required. In contrast, nelfinavir and lopinavir/ritonavir inhibit the first pass metabolism of tacrolimus, resulting in an increase in its elimination half-life and a reduction in its oral clearance[75].

Monitoring for HAART-associated hepatotoxicity is important. The use of NRTIs is associated with hepatic steatosis, mitochondrial dyfunction and fulminant hepatic failure[80,81]. PI-related hepatitis occurs in 5 % to 9% of patients[82] and has an aggressive course in HCV-positive patients[83]. Liver dysfunction is observed in up to 30% of patients taking NNRTIs[84]. A French study[50] reported evidence of mitochondrial dysfunction in 5 patients with severe recurrent HCV, with most patients developing mitochondrial dysfunction while on stavudine or stavudine plus didanosine and in patients concurrently using ribavirin.

To maintain virological control of HIV infection, quantitative HIV RNA and CD4 cell counts should be measured with the first assays at 1 mo after transplant and subsequent studies every 2 to 3 mo thereafter. If patients have persistent HIV viremia, resistance testing should be performed to determine treatment options[59].

HCV management

HCV recurrence is a significant problem following transplantation[35,42,50] although there are reports of spontaneous clearance of HCV post-transplantation[85]. HCV recurrence appears earlier in HIV-infected HCV patients than in HIV-uninfected HCV patients (median time 2 mo) and the rate of the progression of fibrosis is enhanced. In one controlled study, the proportion of patients with bridging fibrosis or cirrhosis at 2 and 5 years post-transplantation was 28% and 48% respectively for HCV-HIV co-infected patients versus 10% and 18% respectively in HCV-mono-infected patients[42]. HCV recurrence is attributed to graft loss (Table 2). The prognosis for patients with fibrosing cholestatic hepatitis is poor[42,53].

Pegylated interferon and ribavirin combination therapy is the mainstay for the management of recurrent HCV disease. The mitochondrial toxicity of HAART, however, increases when used in conjunction with ribavirin[86,87]. The rates of a sustained virological response (SVR) are low in co-infected patients, apart from one recent study that showed 100% SVR[88]. SVR occurs in only 11% to 27% of treated patients[35,42,88,89] (Table 2). Biochemical responses are obtained in more than half of patients but histological stabilization or improvement is rare in virological non-responders. Tolerability of the full dose therapy is limited, contributing to the poor SVR rates.

In the HCV-mono-infected population, viral factors (a high viral load before and after transplantation) and host factors (donor age > 50 years) are associated with a more severe recurrence of HCV[90]. Corticosteroid boluses are also associated with a severe HCV recurrence and should be avoided. Rapid corticosteroid withdrawal after transplantation should be avoided and may be associated with a more rapid progression of fibrosis[91]. The effects of immunosuppressant agents, including tacrolimus, CyA, MMF, anti-interleukin 2 receptor antibodies, SRL and azathioprine, on the severity of HCV recurrence are controversial[90].

Prophylaxis for opportunistic infections[59]

The risk of opportunistic infection in HIV-positive transplant patients seems to be similar to that of HIV-negative transplants. The ability to suppress HIV viral loads in patients on HAART is associated with the stabilization of, or improvement in, CD4 counts, which decreases opportunistic infection in HIV-positive patients[92].

Prophylactic regimens for preventing opportunistic infection include those against *Pneumocystis jirovecii* with trimethoprim-sulfamethoxazole, toxoplasmosis (for CD4 counts < 100), Mycobacterium avium complex (for CD4 counts < 50) and histoplasmosis and coccidioidomycosis. For patients at risk for primary toxoplasmosis due to donor infection, trimethoprim-sulfamethoxazole should be considered for primary prevention; dapsone or atovaquone in combination with pyrimethamine can be considered for patients intolerant to trimethoprim-sulfamethoxazole[71].

CONCLUSION

Clinical trials[35,41,48,56,71,88] suggest that liver transplantation in HIV/HCV co-infected patients is safe and that HIV infection does not influence the outcome. The United Network for Organ Sharing no longer considers HIV an absolute contraindication for transplantation. The French agency for Organ Distribution[93] has also concluded that there is no reason to consider HIV a contraindication. Spain has published a national policy[41] advocating liver transplantation for patients with HIV infection within defined criteria.

To improve the results of liver transplantation in HIV-infected individuals, better selection of candidates at an earlier stage of liver disease and optimization of donor and perioperative factors are needed. The natural history of HCV re-infection and treatment algorithms must also be determined as HCV recurrence is the most important concern. Better management of HAART after transplantation is also required.

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ORIGINAL ARTICLE

Pre-emptive antiviral therapy in living donor liver transplantation for hepatitis C: observation based on a single-center experience

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Summary

Reports of large series in living donor liver transplantation (LDLT) for hepatitis C virus infection (HCV) are scarce. Between 1996 and 2008, 105 LDLTs were performed at the University of Tokyo for HCV. Rapid induction of antiviral treatment with interferon (IFN) and ribavirin (RBV) was attempted per protocol regardless of the clinical presentation of recurrent HCV (pre-emptive treatment approach). Treatment was continued for 12 months after serum HCV-RNA became negative (ETR: end-of-treatment response) and judged as a sustained viral response (SVR) after another 6 months of negative results without treatment. A fixed treatment period was not defined unless an ETR was achieved (no-stopping approach). Flexible dose adjustments were allowed. Ninety-five patients were eligible for pre-emptive therapy. Forty-three (45%) patients experienced an ETR, and 32 (34%) achieved SVR. Nonadherence to full-dose INF and RBV had little impact on the viral response. Evaluation using the Kaplan–Meier method to incorporate the cumulative time-dependent nature of the no-stopping approach estimated SVR rate at 53% by the fifth year. Survival rate at 5 years was 79% for the HCV recipients and did not differ significantly from our non-HCV series. In LDLT for HCV, pre-emptive IFN–RBV-based treatment with the application of no-stopping approach is feasible and effective.

Introduction

Hepatitis C virus (HCV) is the major cause of chronic liver disease resulting in cirrhosis and liver failure in developed countries [1–3], including Japan [4]. It has become the leading indication for liver transplantation and will continue to be an important challenge [5–9]. Unfortunately, liver transplantation is not a cure for HCV infection. Re-infection is universal [10–12], and the histologic progression of HCV seems to be accelerated in comparison to that in nontransplant patients [13–15]. Large studies have demonstrated poorer survival outcomes in liver transplant recipients with HCV [16–19]. Although results of re-transplantation following graft failure in this patient group have been demonstrated with acceptable rate of success [20], this remains a challenging option in the era of organ shortage. Treatment of HCV recurrence generally follows the strategy for treating HCV in non-transplant patients. Experience with interferon (IFN)-based combination therapy has accumulated in liver transplant settings [21–23].

Earlier Western experiences have raised concerns that living donor liver transplantation (LDLT) might be disadvantageous for HCV-positive patients, leading to

more rapidly progressive recurrence of HCV after transplantation [24,25]. Recent studies suggest that the HCV kinetics is accelerated in LDLT as compared with deceased donor liver transplantation (DDLT). Schiano *et al.* [26] compared 11 LDLT patients with 15 DDLT patients; HCV-RNA levels rose more rapidly in the LDLT with greater biochemical changes. Another study by the Barcelona group focusing on the histologic aspects of HCV recurrence with a protocol biopsy reported more severe progression in LDLT as compared with DDLT [27]. Although this remains controversial [28–30], the concern has affected the decision-making process with regard to treating HCV in many transplant centers in the Far East where LDLT is predominantly performed, and where there is little hope for DDLT or re-transplantation.

The rationale for the early initiation of combined IFN-based treatment regardless of the clinical symptoms of recurrent HCV following transplantation (pre-emptive therapy) is to strike at a time when the total HCV viral load is relatively low and histologic damage is minimal [11,12]. Despite this theoretical advantage, the efficacy of pre-emptive therapy has not been determined in Western experience where DDLT is predominant [31–33]. The number of reports on the treatment of HCV in LDLT from high-volume Eastern centers is also limited [34,35]. Much remains to be elucidated regarding the form of application of INF-based treatment as well as its overall outcome in an LDLT setting. We herein report the results of our experience with the application of a pre-emptive therapy approach in LDLT.

Patients and methods

Patients

Between January 1996 and March 2008, 411 LDLTs were performed at the University of Tokyo. Of the 411 LDLTs, 336 were performed in adults, among whom 105 underwent LDLT for HCV. The clinical courses of these patients were studied prospectively. The median age of the patients was 55 years (range 23–66). The majority of patients were male subjects (76 men and 29 women), and the HCV genotype was 1b in 84 cases (80%). The median Model for End-Stage Liver Disease (MELD) score was 14 (range 6–48). Six patients were co-infected with HIV and 60 patients had hepatocellular carcinoma (HCC), 50 of whom were within the Milan criteria. As for 231 patients that underwent adult-to-adult LDLT for other indications, the median age of the patients was 55 years (range 18–67). The majority of patients were female subjects (110 men and 121 women). The median MELD score was 14 (range 6–41). Forty patients had HCC, 37 of whom were within the Milan criteria.

Our surgical technique for LDLT and the process of donor selection and evaluation are described elsewhere [36–39]. Splenectomy was performed at the time of LDLT to prevent the progression of thrombocytopenia under IFN-based antiviral therapy [40]. In line with the practice at majority of liver transplantation centers worldwide [41], tacrolimus-based immunosuppression regimen had been administered in our program for all indications including HCV. All patients initially received the same immunosuppressive regimens with tacrolimus (Prograf; Astellas Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone [42]. In brief, tacrolimus was administered by continuous intravenous infusion at a dose of 2.5 μg/kg/h just after the operation. After the whole blood level of tacrolimus reached 17–18 ng/ml, the dose was adjusted to maintain this level during the first week after the operation. Intravenous methylprednisolone was started during the operation (20 mg/kg/day), and was gradually tapered afterwards. When gastrointestinal function returned, tacrolimus and steroid were given orally. Steroid treatment was not discontinued in any of the patients. Conversion from tacrolimus to cyclosporine was performed in 34 patients (32%), mostly as a result of adverse events [43,44].

Before transplantation, the HCV genotype was determined [45]. After initiation of the combined therapy, blood counts and liver function tests were performed every 2 weeks for the first month, and at up to 4-week intervals thereafter. Serum samples were collected once per month for quantitative HCV-RNA detection. HCV-RNA was measured quantitatively immediately before and after liver transplantation by reverse-transcriptase polymerase chain reaction (Amplicor HCV; Roche Molecular Systems, Pleasanton, CA, USA). Serum HCV-RNA was considered negative when test results were negative (sensitivity <50 IU/ml).

IFN-based combination treatment for HCV

The current study includes patients from our previous pilot study describing the feasibility of our approach for the early initiation of antiviral treatment in HCV recipients [46]. In brief, treatment was initiated with low-dose IFN alpha2b and ribavirin (RBV) 400 mg/day promptly after improvement in general condition following liver transplantation, especially recovery of hematologic and renal function was recognized. More specifically, initiation was considered when the leukocyte number was ≥4000/ml, the platelet count was ≥50 000/ml, the hemoglobin was ≥8 g/l, and serum creatinine <2 mg/dl. Thereafter, the dosage was gradually increased as tolerated. Finally, pegylated (PEG)-IFN 1.5 μg/kg/week and RBV 800 mg/day are administered, depending on patient compliance.

The treatment is continued for 12 months after serum HCV-RNA becomes negative, which is defined as the end-of-treatment response (ETR). The response was considered to be a sustained viral response (SVR) after another 6 months of negative serologic results without antiviral treatment (Fig. 1). Serologic monitoring for HCV-RNA was consecutively performed on a monthly basis even after SVR was achieved to avoid unrecognized episodes or delayed diagnosis of relapse.

Flexible dose adjustments were made accordingly to avoid serious adverse events and to prevent any lapse in treatment. Actual levels of the given dosage at the time of data collection or final administration was recorded, represented by percentile, with 100% being the full target dose described above (i.e., PEG-IFN 0.5 μg/kg/week is represented as 33%, or RBV 200 mg/day is represented as 25%).

A fixed overall treatment period length was not defined and cessation resulting from adverse events was considered temporary unless an ETR was achieved. Poor virologic response alone was not considered an indication for discontinuation. Treatment was temporarily discontinued when there was significant leukopenia (<1500/ml), thrombocytopenia (<50 000/ml) despite administration of granulocyte colony-stimulating factor (Gran, Sankyo, Co. Ltd., Tokyo, Japan), hemolytic anemia (hemoglobin <8 g/l), renal dysfunction (serum creatinine <2 mg/dl), depressive psychologic status, or general fatigue affecting quality of life. Erythropoietin was given when recovery from anemia remained poor following cessation of antiviral treatment.

Treatment of acute cellular rejection

During the period of observation, biopsy-proven mild-to-moderate acute cellular rejections were confirmed in 27 (26%) patients and treated with a 20-mg/kg bolus of methylprednisolone intravenously with subsequent tapering of the dosage, which was decreased by 50% on each of the following days.

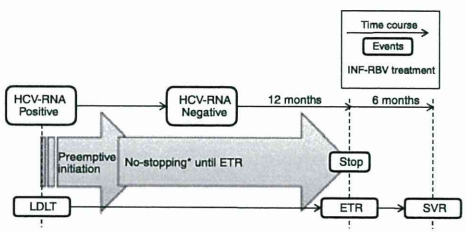

Figure 1. Diagram of combined interferon and ribavirin treatment following living donor liver transplantation at Tokyo University. HCV-RNA, status of hepatitis C virus RNA in the serum; IFN RBV, interferon and ribavirin therapy; LDLT, living donor liver transplantation; ETR, end of treatment response; SVR, sustained viral response. *Treatment with interferon and ribavirin was put on hold when serious adverse events occurred.

Statistical analysis

To clarify whether tolerability affected the outcome, tolerated rates of doses of INF and RBV were studied in accordance with the viral response and eradication. To clarify the time-dependant response, the cumulative rate of negative HCV-RNA, and of the ETR and SVR statuses were studied using the Kaplan–Meier method. The survival curves and cumulative viral response rates were compared using the log-rank test. Various clinical factors, including recipient and donor age and gender, MELD score, presence of HIV, HCV genotype, HCV-RNA viral titer prior to LDLT, occurrence of acute cellular rejection, and use of cyclosporine, were analysed for their effect on achieving an SVR and survival. A multivariate analysis was performed using the Cox proportional hazards model and a forward stepwise procedure. Continuous data were compared between groups using the Mann–Whitney *U*-test. Creation of figures including Kaplan–Meier curves, density-contour plots, box-and-whisker plots, and statistical calculations were performed using SAS software (SAS Institute, Cary, NC, USA). A P value of <0.05 was considered statistically significant.

Results

Applicability of IFN-based treatment using a pre-emptive approach

Among the 105 recipients with HCV who underwent LDLT during the observation period, 95 patients (90%) received IFN-based combination therapy with RBV, according to our early treatment regimen. Ten patients were not eligible for our pre-emptive approach; in the case of two patients, the reason was attributable to early death, in case of six attributable to lack of consent, and in case of one patient, attributable to negative HCV-RNA after transplantation. One other patient was excluded because of poor condition, including multi-organ failure, during the immediate post-transplant period, which resulted in subsequent renal failure necessitating maintenance hemodialysis. This patient eventually received IFN monotherapy for recurrent HCV, resulting in viral eradication 23 months after LDLT, but died from the progression of pulmonary hypertension before achieving the ETR. For the remaining 95 patients, the median period from LDLT to IFN/RBV initiation was 26 days (range 10 days–6 months). The median follow-up period was 45 months (range 1–122 months).

Episode of biopsy-proven acute cellular rejection was confirmed in 21 of the 95 patients. Episode of rejection

took place prior to, or after the initiation of IFN-based combination therapy in nine (9%), and 12 (13%) patients respectively. The median period from LDLT to the initiation of antiviral treatment in the nine patients was 28 days (range 21–59 days), whereas the median interval from LDLT to rejection episodes was 11 days (range 5–29 days). In the 12 patients in whom rejection took place after the initiation of IFN-based combination therapy, the median period from LDLT to the initiation of antiviral treatment was 17 days (range 12–52 days), and the median period to rejection episode from LDLT was 44 days (range 18–577 days).

Viral response

Among the 95 recipients that underwent IFN-based therapy, 51 (54%) patients had negative HCV-RNA results at least once, among whom 43 patients experienced a sustained response for 12 months (ETR). Six patients who reached the ETR eventually presented with a viral relapse, and did not achieve an SVR. At the time of data collection, 32 (34%) achieved an SVR. None of the recipients that achieved SVR have presented with a viral relapse during the observed period. The median time to achieve a negative HCV-RNA, ETR, and SVR under the treatment regimen was 12 months (range 2–63 months), 25 months (13–79 months), and 28 months (19–67 months) respectively.

Consistent with the nature of a treatment protocol without a defined time endpoint, the response rate tended to increase over time. At 3 years, negative HCV-RNA status was obtained in 63%, ETR in 48%, and SVR in 34%. By the fifth year, negative HCV-RNA status was obtained in 70%, ETR in 68%, and SVR in 53% (Fig. 2a).

Hepatitis C virus infection genotype 1b, use of cyclosporine, and a lower rate of tolerated RBV dose presented with significantly poorer outcomes (Table 1). Multivariate analysis revealed HCV genotype 1b as the only independent factor resulting in a significantly poorer viral

Table 1. Sustained viral response in patients with combined treatment and clinical factors.

	Factors	No.	%SVR at 5 years	*P*
R-age	≤55	50	58	0.85
	>	45	47	
R-gender	Male	68	57	0.39
	Female	27	40	
MELD	<15	49	54	0.75
	≥	46	51	
HIV	Positive	4	67	0.17
	Negative	91	52	
HCC	Positive	55	41	0.08
	Negative	40	66	
Genotype	1b	78	43	<0.0001
	Non-1b	17	85	
HCV-RNA titer	≤250 K IU/ml (5.4 log)*	40	49	0.29
	>	55	53	
ACR	Yes	21	37	0.27
	No	74	56	
D-age	≤35	46	47	0.35
	>35	49	61	
D-gender	Male	59	45	0.59
	Female	36	71	
CyA	Yes	64	35	0.02
	No	31	61	
INF dosage†	≥60%	48	55	0.36
	<60%	47	58	
RBV dosage†	≥50%	54	69	0.02
	<50%	41	33	

No., number of patients; %SVR, percentage of patients achieving sustained viral response; R-age, age of the recipient at the time of transplantation; R-gender, gender of the recipient; MELD, Model for end-stage liver disease score; HIV, human immunodeficiency virus; HCC, hepatocellular carcinoma; HCV-RNA, hepatitis C viral ribonucleic acid; ACR, acute cellular rejection; D-age, age of the donor at the time of transplantation; D-gender, gender of the donor; CyA, cyclosporine A.
*During the study period, quantification of real-time RT-PCR introduced for linear quantification and detection of HCV-RNA.
†Actual levels of the given dosage at the time of data collection or final administration was recorded represented by means of percentile, 100% being the per-protocol full target dose.

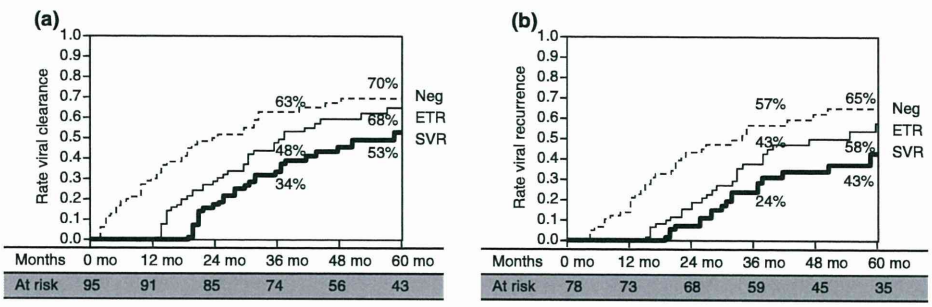

Figure 2. (a) Cumulative overall viral response depicted by Kaplan-Meier method. (b) Cumulative overall viral response of recipients with HCV genotype 1b depicted by Kaplan-Meier method. Neg, negative HCV-RNA; ETR, end of treatment response; SVR, sustained viral resposne; mo, months.

response (hazard ratio 0.263, 95% confidence interval 0.127–0.545, $P = 0.0003$). Of the recipients with an HCV genotype 1b, negative HCV-RNA status was obtained in 57%, ETR in 43%, and SVR in 24% at 3 years. By the fifth year, negative HCV-RNA status was obtained in 65%, ETR in 58%, and SVR in 43% (Fig. 2b).

Tolerability of IFN-based treatment

At the time of final data collection, a total of 24 (25%) patients had tolerated the full dose of INF, and eight (8%) patients had tolerated the planned full dose of RBV. The average dosage of INF tolerated among the 95 patients was 68% (SD 26%) of the full dose, and that of RBV was 41% (SD 24%, Fig. 3).

Tolerability in terms of rates of dosage of INF or RBV did not differ significantly between those with a viral response and those without (Fig. 4a–c). Lack of adherence to the planned target dose was common, but had no significant impact on the viral response within the observation period.

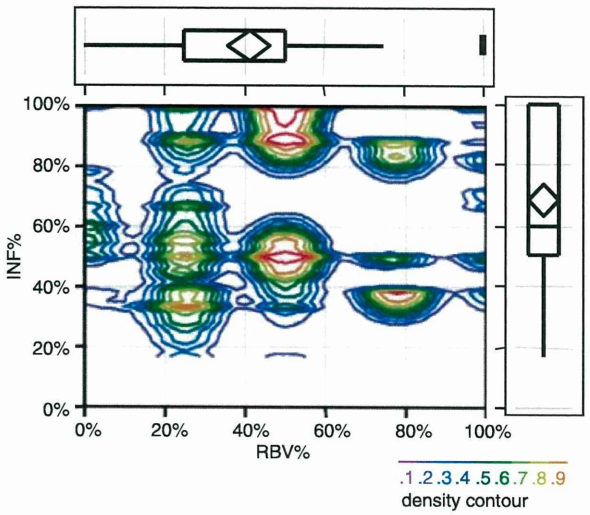

Figure 3. Actual tolerated rates of dosages of interferon (IFN) and ribavirin (RBV) by density contour plot. Box-and-whisker plots accompanying the vertical and horizontal axis represent the summery of IFN and RBV tolerated rates of dosages.

Survival

The overall mid-term rates of survival were not statistically different between HCV and non-HCV recipients (Fig. 5a). The short-term outcomes, however, were poorer in HCV recipients. At 2 years after transplantation, recipients with HCV presented with a significantly lower survival rate compared to non-HCV recipients (82% vs.

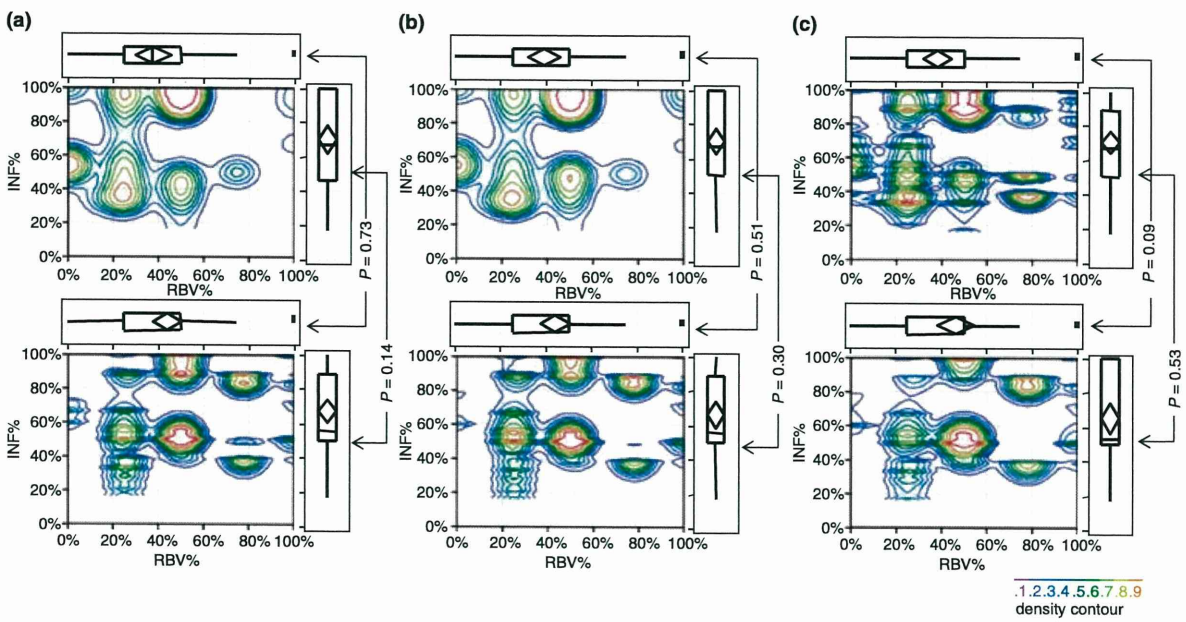

Figure 4. Actual tolerated rates of dosages of interferon (IFN) and ribavirin (RBV) by density contour plot according to viral response. Box-and-whisker plots accompanying vertical and horizontal axis represent the summery of IFN and RBV tolerated rates of dosages, respectively. Diamond in the box-plot represents the mean and 95% confidence interval. (a) Above: outcomes of patients that remained positive for HCV-RNA during the studied period. Below: outcomes of patients that demonstrated negative HCV-RNA results at least once during the studied period. (b) Above: outcomes of patients that did not achieve end-of-treatment (ETR). Below: outcomes of patients that achieved ETR. (c) Above: outcomes of patients that did not achieve sustained viral response (SVR). Below: outcomes of patients that achieved SVR (*P*-values by Mann-Whitney *U*-test).

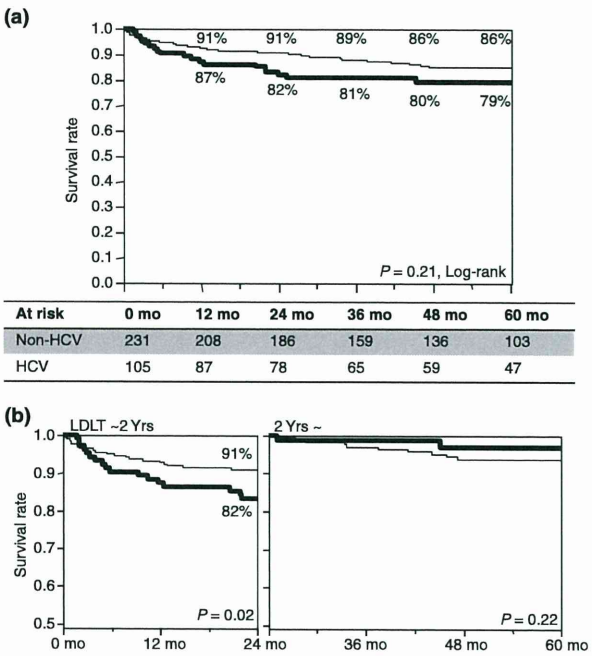

Figure 5. (a) Comparison of overall survival between hepatitis C virus infection (HCV) (n = 105) and non-HCV (n = 231) adult-to-adult LDLT recipients. Median follow up period of HCV, and non-HCV patients were 45 and 55 months, respectively. (b) Comparison of overall survival between HCV and non-HCV adult-to-adult LDLT recipients at 2 years post-LDLT and thereafter (bold lines indicate HCV patients). LDLT, living donor liver transplantation; mo, months.

91%, $P = 0.02$). Survival rate after the second year did not differ between HCV and non-HCV recipients (Fig. 5b).

Analysis of factors affecting the short-term survival rates indicated that viral titer prior to transplantation, viral response to treatment, acute cellular rejection, donor age, and donor gender were significant factors affecting the survival at 2 years (Table 2). Multivariate analysis revealed that a higher viral titer prior to transplantation, poor response to antiviral treatment, occurrence of acute cellular rejection, and older donor age were independently significant factors associated with poor survival (Table 3).

Discussion

In this study, as also considering the experience gained in the above-mentioned pilot series, data of a total of 105 adult patients with HCV that underwent LDLT at our institution over the past decade were collected and evaluated to validate our approach of pre-emptive treatment. Ninety-five patients were eligible and received pre-emptive antiviral therapy. The rate of complete viral eradication identified by an SVR within the observed follow-up period was comparable to a reported series of DDLT recipients with responsive treatment approaches (32 of 95 recipients, 34%). Unlike the outcome in the previously reported series with a fixed treatment period, however,

Table 2. Survival at 2-year after living donor liver transplantation and clinical factors.

	Factors	No.	%OS at 24 months	*P*
R-age	≤55	54	79	0.35
	>	51	86	
R-gender	Male	76	82	0.98
	Female	29	82	
MELD	<15	56	80	0.52
	≥	49	85	
HIV	Positive	6	67	0.23
	Negative	99	83	
HCC	Positive	60	79	0.39
	Negative	45	86	
Genotype	1b	84	82	0.85
	Non-1b	21	81	
HCV-RNA titer	≤250 K IU/ml (5.4 log)	49	92	0.02
	>	56	73	
Response to INF-RBV Tx	Yes	51	94	0.0005
	No	44	69	
ACR	Yes	27	63	0.0009
	No	78	89	
D-age	≤35	53	96	0.0002
	>35	52	67	
D-gender	Male	67	89	0.009
	Female	38	69	
CyA	Yes	34	76	0.29
	No	71	85	

No., number of patients; %OS, percentage of overall survival of patients; R-age, age of the recipient at the time of transplantation; R-gender, gender of the recipient; MELD, Model for end-stage liver disease score; HIV, human immune deficiency virus; HCC, hepatocellular carcinoma; HCV-RNA, hepatitis C viral ribonucleic acid; Response to INF-RBV Tx, Response to interferon ribavirin combination therapy indicated by negative serum HCV-RNA at one point or more; ACR, acute cellular rejection; D-age, age of the donor at the time of transplantation; D-gender, gender of the donor; CyA, cyclosporine A.

Table 3. Factors affecting survival at 2 years after living donor liver transplantation: a multivariate analysis.

Factors	Ratio	95% CI	*P*
Response to Tx	0.12	0.04–0.44	0.001
ACR	3.63	1.40–9.43	0.008
Age of the donor	8.20	1.84–36.6	0.006
HCV-RNA titer	3.30	1.04–10.5	0.04

Response to Tx, response to interferon combination therapy indicated by negative serum HCV-RNA at one point or more; ACR, acute cellular rejection; CI, confidence interval.

our current series indicates the possibility of improvement in the rate of viral eradication over a period of time with continued, non-stop application. Viral responses based on the Kaplan–Meier method demonstrate that a continued treatment is related to higher rates of viral response, as high as an expected rate of 70% for clearance of viremia, and 53% for SVR at 5 years post-LDLT (Fig. 2).

Another interesting implication of the results from our approach is the improvement in survival over the longer term. Extensive data on the outcomes of HCV patients after DDLT indicates that outcomes become poorer in later years when compared with non-HCV patients [16,17]. So far, this has not been the case for our LDLT series. The overall rate of survival after the second year following LDLT remains equivalent for HCV and non-HCV recipients (Fig. 5). In contrast to the acceptable mid-to-long term outcomes, however, our current series demonstrated poorer survival rates as compared with non-HCV recipients for the immediate short-term in HCV recipients. Analysis revealed higher viral titer prior to transplantation, poor response to antiviral treatment, occurrence of acute cellular rejection, and older donor age were significant risk factors for poorer short-term survival. This offers important insights for the management during this period.

Finally, our series demonstrated that adherence to the full target dose of INF or RBV is not mandatory. Patients who tolerated the full target dose were in the minority. The majority tolerated <70% of the intended dose of INF and less than half that of RBV (Fig. 3). The low tolerability for the target dose, however, did not have apparent disadvantage (Fig. 4). Reports in the recent literature suggest the benefits of sustained application of antiviral therapy at a lower dosage for normalizing liver function and preventing recurrent HCC in non transplant patients [47–49]. In the most recent report from the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) study, a randomized controlled trial of PEG-IFN alpha-2a at a dosage of 90 μg/week for 3.5 years in the treatment arm indicated that there was no significant difference between groups in the rate of progression of liver disease, defined as death, HCC, or hepatic decompensation [50]. The studied population, however, was predominantly patients with advanced fibrosis who had not had any response to previous therapy with PEG-IFN and RBV. On the other hand, most interestingly, the report described significantly improved serum aminotransferase levels, decreased serum HCV-RNA levels, and improved histologic necroinflammatory scores. Kuo *et al.* [51] reported a reduced risk of fibrosis progression, even among virologic nonresponders who underwent pre-emptive treatment that was limited to 48 weeks. These outcomes may support, in part, the application of prolonged treatment initiated pre-emptively in liver transplant recipients with un-injured liver grafts, and are encouraging to our approach.

In conclusion, pre-emptive antiviral treatment with combined IFN-based therapy is feasible and effective in LDLT for HCV. The application of a non-stopping, flexible dose adjustment approach for further improvement in the outcomes is warranted in the LDLT setting.

Authorship

ST, YS and MM: designed study. ST, YS, JK, NK, and MM: performed study. ST, YS, NY, JK: collected data. ST and YS: wrote the paper.

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Original contribution

High density of tryptase-positive mast cells in patients with renal cell carcinoma on hemodialysis: correlation with expression of stem cell factor and protease activated receptor-2☆

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Summary Patients on hemodialysis are at higher risk of renal cell carcinoma probably because of inflammatory and immune system disorders. The aim of this study was to clarify the pathologic roles of 2 phenotypes of mast cells, mast cell tryptase and mast cell chymase, and their correlation with stem cell factor and protease-activated receptor 2 in patients with renal cell carcinoma on hemodialysis. The densities of mast cell tryptase and mast cell chymase and expressions of stem cell factor and protease-activated receptor 2 were examined in 35 patients with hemodialysis-renal cell carcinoma and 39 with non–hemodialysis-renal cell carcinoma who were diagnosed and treated in our hospital. Protein expression was examined by immunohistochemistry. The proliferation index represented the number of Ki-67–positive cells. There were no significant differences in clinicopathologic features between the 2 groups. Mast cell tryptase densities in intratumoral (8.3 per high-power field) and peritumoral areas (8.7 per high-power field) were higher in hemodialysis-renal cell carcinoma than non–hemodialysis-renal cell carcinoma (2.7 and 5.3 per high-power field). No such significant correlations were detected in mast cell chymase. In hemodialysis-renal cell carcinoma, intratumoral mast cell tryptase density correlated with the proliferation index ($P = .039$ and $P = .008$, respectively) and also with stem cell factor and protease-activated receptor 2 expression. Our results emphasize the important roles of mast cell tryptase in cancer cell proliferation and recurrence in hemodialysis-renal cell carcinoma. Stem cell factor and protease-activated receptor 2 seem to up-regulate mast cell tryptase functions in these patients. The results suggest collaborative effects of stem cell factor, mast cell tryptase, and protease-activated receptor 2 on the malignant potential of hemodialysis-renal cell carcinoma.

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1. Introduction

Chronic inflammation is associated with carcinogenesis and tumor growth, although its role within the tumor microenvironment is not fully understood. Various inflammatory cells, such as macrophages and lymphocytes, are thought to regulate inflammation and the malignant process [1]. Mast cells (MCs) are key components of the immune system and crucial regulators of inflammation and immune response by releasing various bioactive substances [2,3]. Furthermore, MCs are thought to play some role in the carcinogenesis because their accumulation has been recognized in various cancers [1,4,5]. With regard to the pathologic significance of MCs, they are regarded as a "double-edged sword" in cancer-malignant aggressiveness because they can produce both cytokines with antitumoral activity as well as those with protumoral activity [5,6]. In fact, there are 2 conflicting opinions on the prognostic roles of MCs in patients with cancer [4,5,7,8]. One of the reasons for such difference is probably related to the effects of the local microenvironment (eg, cytokines and growth factors) on the differentiation, proliferation, and pathologic roles of MCs [9]. In general, MCs are classified into 2 phenotypes based on their neutral protease composition: MC tryptase (MC-t) (tryptase positive, chymase negative) and MC chymase (MC-tc) (tryptase and chymase positive) [10,11]. Several studies have investigated the clinical significance of MC-t and/or MC-tc in human cancer tissues [12,13]. The results of these studies indicate that the distribution and pathologic roles of MCs in human cancer tissue varies with cancer type [13]. In renal cell carcinoma (RCC), it seems that MCs plays important role in malignant aggressiveness [14,15]. However, there is little or no information on the clinical and pathologic significance of MC-t and MC-tc in RCC.

Patients on hemodialysis (HD) are reported to be at higher risk of malignancies, in particular, they are at approximately 100 times greater risk for RCC than age-matched general population [16]. The reason for the high risk is probably related to the exposure of the kidneys of patients with end-stage renal disease (ESRD) and HD to various protumoral factors, such as suppression of immune response and stimulation of inflammation [17]. However, there is little information on how these factors promote renal carcinogenesis and the malignant potential of RCC in HD patients (HD-RCC). Lacking also is the comparison of the pathologic features of cancer cells and the tumor microenvironment between HD-RCC and normal renal function (non–HD-RCC). With regard to MCs in RCC, MCs are present in renal biopsy tissues from patients with various forms of chronic glomerulonephritides and nephropathy, and their densities are higher than those in controls [17-19]. The recruited MCs probably play important roles in the pathogenesis and progression of chronic glomerulonephritides [18,20]. Based on these facts, MCs are presumed to play some roles in HD-RCC because the biologic activities of MCs are up-regulated in renal dysfunction.

MC development is regulated by various factors. Specifically, stem cell factor (SCF) is associated with cell proliferation, migration, and secretion of MCs under various pathologic conditions [21,22]. SCF expression is up-regulated in kidney tissues of chronic nephritis [18,19], and its serum levels in patients with ESRD are higher than those in healthy control [23]. Based on this background, we hypothesized that SCF expression is up-regulated in HD-RCC tissues and that it is involved in tumorigenesis.

Protease-activated receptors (PARs) constitute a unique branch of G protein-coupled receptor super family. PAR-2 was originally reported as a trypsin receptor; however, it is also known to be activated by other serine proteases including tryptase produced by MCs [24,25]. One of the main biologic roles of PAR-2 signaling of MC-t is the regulation of cell proliferation under physiologic and pathologic conditions including cancers [7,26]. Thus, PAR-2 is speculated to be involved in human cancers. In fact, PAR-2 immunoreactivities are higher in various cancer tissues than normal ones [7]. On the other hand, PAR-2 expression correlates with serum creatinine levels in patients with nephropathy and is mainly localized within proximal tubular cells in immunoglobin A nephropathy [19,27]. Thus, it is possible that PAR-2 expression in proximal tubular cells is modulated in the presence of renal diseases and that its overexpression could be associated with the malignant potential of RCC.

The main aim of the present study was to clarify the clinical and pathologic roles of MC-t and MC-tc in patients with HD-RCC. In addition, we estimated the densities of these phenotypes of MCs within and around the tumoral area in the same tissues. Based on the results, we also examined the relationship between SCF expression and the density of MC-t and between PAR-2 expression and cancer cell proliferation in HD-RCC tissues. Our results should be useful to our understanding of the pathologic characteristics of HD-RCC.

2. Materials and methods

2.1. Patients and tumor samples

The study subjects were patients with HD-RCC who underwent nephrectomy at our hospital between 1992 and 2009. Patients with HD-RCC with more than pT3 and/or metastasis were excluded because of the small number of such patients (n = 3). Thus, the study specimens (n = 35) were obtained from 26 patients with conventional RCC (74.3%), 6 with papillary RCC (17.2%), and 3 with chromophobe RCC (8.6%). Pathology-matched non–HD-RCC specimens were selected at random from patients with RCC with normal serum creatinine level (<1.1 mg/dL). We excluded from the control those patients with pT3

and pT4 tumors, metastasis, and elderly patients (>75 years) to match clinicopathologic features. Their clinicopathologic features are shown in Table 1. The study also included normal control specimens, representing 23 kidney tissues free of hydronephrosis that were obtained by surgery from patients with ureter tumors. All patients were evaluated by chest x-ray, ultrasonography, and computed tomography of the abdomen. Tumors were staged according to the 2004 TNM classification, and the grade was determined using the criteria of Fuhrman et al. [28]. The study protocol met the ethical standard of the human ethics review committee of Nagasaki University Hospital.

2.2. Immunohistochemistry and terminal deoxynucleotidyl transferase-mediated nick end labeling

We used antibody for MC-t (NeoMarkers, Fremont, CA), MC chymase (NeoMarkers), SCF (Immuno-Biological Laboratories Co, Gunma, Japan), PAR-2 (Santa Cruz Biotechnology, Santa Cruz, CA), Ki-67 (Dako, Carpinteria, CA), cleaved caspase-3 (R&D Systems, Inc, Abingdon, UK), and CD68 (Novocastra Laboratories, Newcastle, UK). Five-micrometer-thick sections were deparaffinized in xylene and rehydrated in ethanol. Antigen retrieval was performed, and then the sections were immersed in 3% hydrogen peroxide for 30 minutes. Sections were incubated overnight with the primary antibody at 4°C and then were washed in 0.05% Tween-20 in phosphate-buffered saline. The sections were then incubated with peroxidase using the labeled polymer method with Dako EnVision + Peroxidase (Dako) for 60 minutes. The peroxidase reaction was visualized with the liquid 3,3-diaminobenzidine tetrahydrochloride substrate kit (Zymed Laboratories, San Francisco, CA). Sections were counterstained with hematoxylin. Positive controls were tonsil for Ki-67, MC, and macrophage, liver cancer for SCF, and colon cancer for PAR-2. A consecutive section from each sample processed without the primary antibody was used as a negative control. In situ labeling for detection of apoptotic cells was performed as described previously [29]. We used the Apop Tag In Situ Apoptosis Detection Kit (Intergen Company, Purchase, NY) based on terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL).

Table 1 Patients' characteristics

	HD (n = 35)	Non-HD (n = 39)	*P*
Age at operation, y	54 (48-61)	67 (52-73)	.053
Male (%)/female	25/10 (71.4)	23/16 (59.0)	.263
pT stage			.213
T1a	25 (71.4)	22 (56.4)	
T1b	7 (20.0)	15 (38.5)	
T2	3 (8.6)	2 (5.1)	
Grade			.299
G1	15 (42.9)	20 (51.2)	
G2	13 (37.1)	16 (41.0)	
G3/4	7 (20.0)	3 (7.7)	

2.3. Evaluation

The number of MCs was counted in each case in 5 of intratumoral and peritumoral areas at high-power magnification of ×200, and the mean count of positive cells per high-power field (HPF) was calculated. Evaluation of expression of all molecules was assessed semiquantitatively, taking into account the percentage of positively stained cancer cells (at least 500 cells). In this study, SCF expression was considered positive if staining intensity was strong or moderate. The percentage of positively stained cancer cells was determined using a continuous scale. PAR-2 expression was quantified by the immunoreactive score (IRS) system, where IRS = staining intensity × percentage of positive cells. Staining intensity was determined as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was defined as follows: 0, negative; 1, 1% to 20%; 2, 21% to 50%; and 3, 51% to 100% positive cells. In addition, for statistical analysis, patients were divided into 2 groups based on the IRS, that is, negative and positive; those with IRS above the median level were considered the positive group. Similarly, patients were divided according to age at operation, taking the median age as the cutoff value. To evaluate the apoptotic cells, we used 2 parameters: the proportions of cleaved caspase-3–positive and TUNEL-positive cells [29]. All specimens were examined using a Nikon E-400 microscope, and digital images were captured (model DU100, Nikon, Tokyo, Japan). In addition, we used a computer-aided image analysis system (Win ROOF, version 5.0; MITANI, Fukui, Japan) to calculate the statistical variables. Slides were blindly evaluated twice at different times by 2 investigators (YM and SW) who were blinded to the clinical and pathologic data.

2.4. Statistical analysis

All data were expressed as median and interquartile range (IQR) based on the skewed distribution. The Mann-Whitney *U* test was used for comparisons of continuous variables. The χ^2 and Fisher exact tests were used for categorical comparison of the data. The Scheffé test was used for multiple comparisons of the data. Spearman correlation coefficient was used to determine the association between 2 continuous variables. The disease-free survival time was compared with Kaplan-Meier survival curve and log-rank test. All statistical analyses were 2 sided, and significance was defined as $P < .050$. All statistical analyses were performed on a personal computer with the statistical package StatView for Windows (version 5.0; Abacus Concept, Inc, Berkeley, CA).

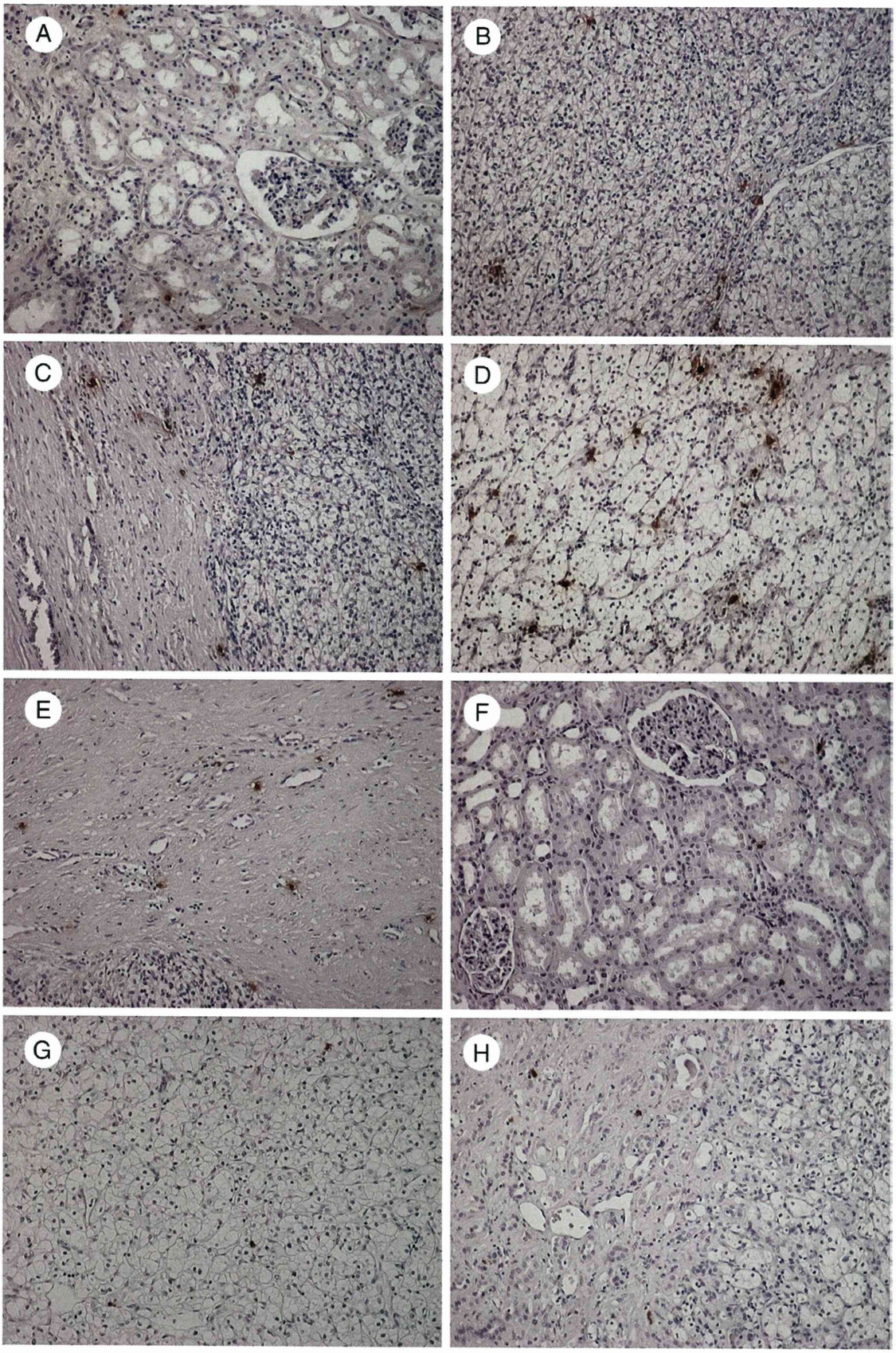

Fig. 1 (*continued*)

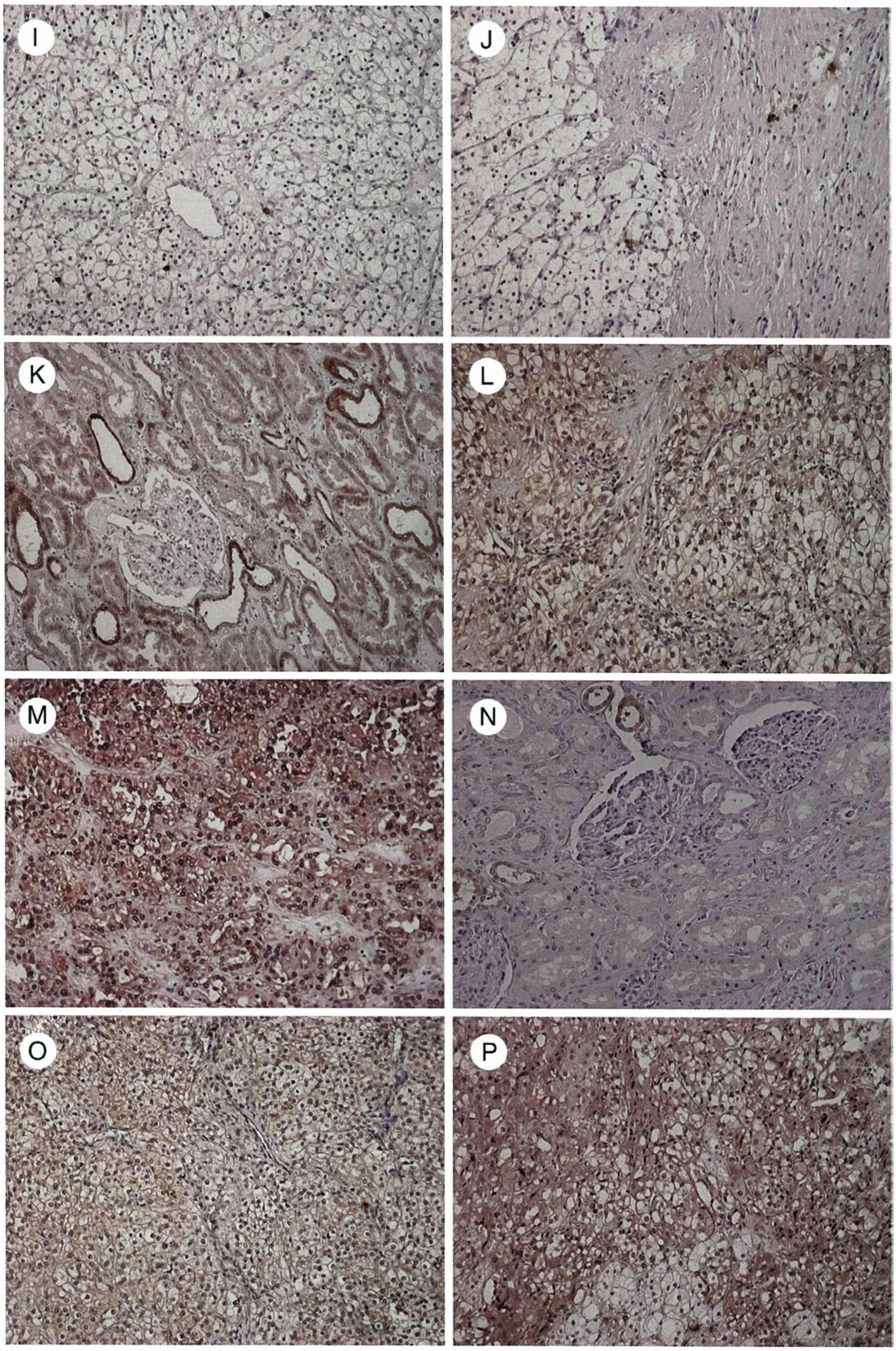

Fig. 1 (*continued*)