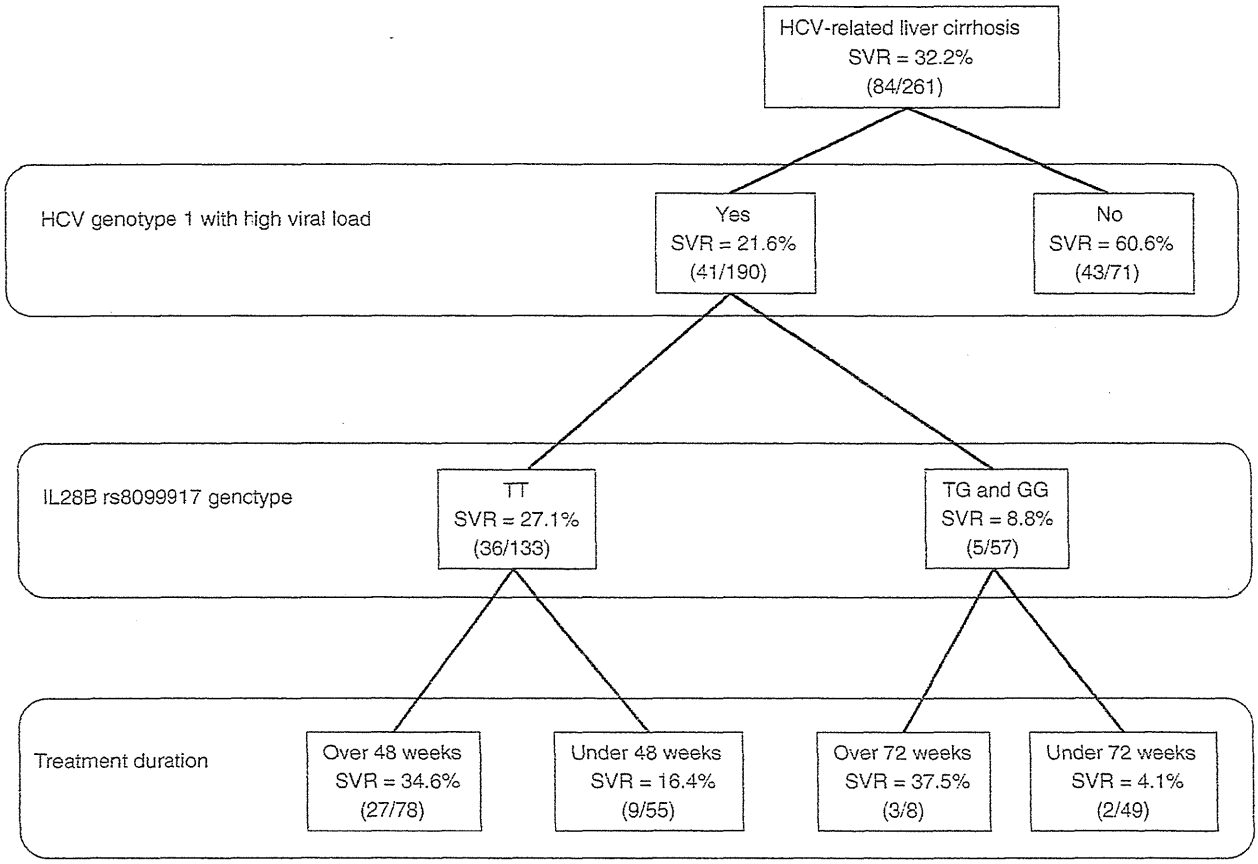

Figure 2 SVR in HCV-related liver cirrhosis patients treated with interferon plus ribavirin. In patients with G1H and the IL28B TT allele, the SVR rate of those who were treated for over 48 weeks was significantly higher than those treated for less than 48 weeks ($P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR rate of patients treated for over 72 weeks was significantly higher than those treated for less than 72 weeks ($P = 0.010$). G1H, genotype 1 with high viral load; HCV, hepatitis C virus ; IL28B, interleukin 28B rs8099917; SVR, sustained virological response.

enced by a polymorphism at IL28B rs8099917. In contrast, SVR rates in non-G1H were higher than those in G1H, irrespective of IL28B genotype. This is the first report to demonstrate that an IL28B polymorphism can influence SVR rate in patients treated with IFN plus ribavirin combination therapy for G1H HCV-related LC. These results suggest that HCV genotypes, viral load and IL28B polymorphism should be taken into when determining antiviral therapy for HCV-related LC. In patients with HCV-related LC, IL28B genotyping may be a useful tool to determine the best antiviral therapy.

Recently, host genetic variation near the IL28B on chromosome 19, which encodes IFN-λ-3, have been shown to be associated with SVR to PEG IFN plus ribavirin in patients infected with HCV genotype 1.[11–13] Although some investigators have shown that IL28B polymorphisms are associated with a favorable response to treatment in patients with non-1 genotype infection, the association between the variants in IL28B and SVR in non-1 genotype-infected patients remains controversial.[19–25] IL28B polymorphisms are also a strong predictive factor for spontaneous HCV clearance.[26,27] However, the precise mechanism associated with the action of IL28B polymorphisms has not been fully elucidated.

Pegylated IFN plus ribavirin combination therapy has become the standard of care treatment for chronic HCV infection. The SVR rates range 42–46% in patients with HCV genotype 1 or 4 infection and 76–82% in patients with HCV genotype 2 or 3 infection, respectively.[9,28,29] However, in patients with HCV-related LC the SVR rate is even lower than in non-LC patients, reflecting reduced

tolerance to the therapy.[8–10] Although patients with HCV-related LC are difficult to treat, patients who achieved SVR showed a lower rate of liver-related adverse outcomes and improved survival.[8–10] Moreover, a randomized controlled trial showed that patients with HCV-related LC who received long-term PEG IFN treatment had a lower risk of HCC than controls.[30] Thus, IFN treatment for HCV-related LC is an effective means of preventing HCC, irrespective of whether SVR is achieved. In this study, the SVR was very low in patients with G1H and the TG or GG allele. Therefore, for these patients, long-term administration of maintenance IFN should be considered to reduce the risk of developing of HCC even if SVR is unlikely to be achieved.

Patients with advanced liver disease have a higher rate of adverse events when taking IFN and ribavirin combination therapy than patients with mild disease. Adverse events, such as neutropenia, thrombocytopenia and anemia, often require dose reduction of IFN or ribavirin. Previous studies have demonstrated that in patients with HCV-related LC, the rate of dose reductions in IFN and ribavirin range 6.9–20.6% and 16.7–27.1%, respectively.[31–33] In our study, IFN and ribavirin dose reductions were needed in 51.3% and 53.6% of patients, respectively. These are higher than those reported in other studies, but the discontinuation rate was slightly lower (12.6%).[33] Many patients required reductions in the doses of IFN and/or ribavirin early in the treatment period because of adverse events, but ultimately were able to tolerate long-term administration. It might be safer to start low-dose antiviral therapy with IFN plus ribavirin in HCV-related LC and titrating the dose upward as tolerated with the aim of long-term treatment, rather than beginning with the full dose and risking adverse events that would curtail antiviral therapy.

In patients infected with HCV genotype 1, previous studies have demonstrated that SVR rates of late virological responders (HCV RNA detectable at 12 weeks and undetectable at 24 weeks after the start of treatment) could be improved when treatment was extended to 72 weeks, compared with the standard treatment duration of 48 weeks, largely as a result of reducing post-treatment relapse rates.[34–37] In this study, the SVR rate in patients who had an LVR was significantly lower than those who achieved RVR or cEVR. However, the duration of treatment in the patients with a LVR was significantly longer than those who achieved cEVR or RVR. Individual physicians determined the duration of treatment based on the time at which serum HCV RNA became undetectable, accounting for the improved SVR rates in those receiving extended courses. Nevertheless, the safety and effectiveness of more than 48 weeks of antiviral therapy in patients with HCV-related LC has not been examined. We found that patients with the IL28B rs8099917 genotype TT, treatment of more than 48 weeks achieved a higher SVR rate than treatment of less than 48 weeks, and in those with the TG or GG alleles SVR rates were greater in those who received more than 72 weeks of treatment. The response to treatment is a very important guide of treatment duration in HCV-related LC. Further prospective studies using larger numbers of patients matched for race, HCV genotype, viral load and treatment durations would be required to explore the relationships between IL28B polymorphism and the treatment response to combination therapy in patients with HCV-related LC.

Recently, new trials of IFN-free combination therapy with direct-acting antivirals (DAA) such as protease-inhibitor, non-structural (NS)5A inhibitor or NS5B polymerase inhibitor nucleotide analog have shown a strong antiviral activity against HCV.[38–40] A previous study reported that the IL28B genotype can affect the response to an IFN-free regimen, but this result has been unclear in other regimens.[38–40] In a study of Japanese patients with HCV genotype 1b infection, dual oral DAA therapy (NS5A inhibitor and NS3 protease inhibitor) without IFN achieved an SVR rate of 90.5% of 21 patients with no response to previous therapy and in 63.6% of 22 patients who had been ineligible for treatment with PEG IFN.[41] However, lack of a virological response to DAA was also seen in patients with no response or partial response to previous therapy. In these patients with viral resistance to DAA, the combination therapy with IFN and DAA may be a means of eliminating HCV, and IL28B genotyping may be a useful tool in determining the best antiviral therapy and duration of treatment.

This study had certain limitations. Selection bias cannot be excluded, considering the retrospective nature of the work. However, all patients had well-established cirrhosis and had received IFN plus ribavirin in hepatitis centers throughout Japan. Our patients received a variety of IFN treatments (IFN-α, IFN-β and PEG IFN), several different doses of IFN and ribavirin, and several treatment durations. In the intention-to-treat analysis, the overall SVR rate was 32.2%; in patients with G1H it was 21.6% but was 60.6% in those with non-G1H. Interestingly, the overall SVR rate in this study was similar to that found in previous studies of patients with advanced fibrosis or cirrhosis treated with IFN or PEG IFN plus ribavirin.[8–10] Thus, although there were some

limitations, our findings contribute to providing valuable information to guide clinical decisions.

In conclusion, the combination therapy with IFN plus ribavirin in Japanese patients with non-G1H HCV-related LC was more effective than those with G1H and not influenced by IL28B polymorphism. However, in patients with G1H, IL28B polymorphism may be a strong predictive factor for SVR. Extending treatment may provide a better outcome in those with the IL28B TT allele treated for more than 48 weeks and in those with the TG/GG alleles treated for more than 72 weeks.

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Trial Registration: Evaluation of lenalidomide (Revlimid) to Treat Subjects with Cutaneous Lupus Erythematosus (CLE). Identified: #NCT00633945. http://www.clinicaltrial.gov/ct2/show/NCT00633945

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Biphasic skin reactions during telaprevir-based therapy of Japanese patients infected with hepatitis C virus

To the Editor: Telaprevir-based triple therapy, comprising 12 weeks' telaprevir administration with peginterferon and ribavirin, is highly effective for chronic hepatitis C.[1,2] However, telaprevir-related skin reactions are more severe than those related to peginterferon and ribavirin.[3] Telaprevir generally causes mild or moderate rash that occasionally progresses to serious skin reactions, including drug rash with eosinophilia and systemic symptoms and Stevens-Johnson syndrome.[2-4] To characterize the dermatologic adverse reactions associated with telaprevir, we enrolled 61 patients with chronic hepatitis C who were receiving telaprevir-based triple therapy at Toranomon Hospital as part of the phase 3 study in Japan.

Drug eruptions were noted in 83.6% (51/61) of patients and were classified according to severity as grade 1 in 37.7%, grade 2 in 44.3%, and grade 3 in 1.6% of patients.[2] The onset of the eruptions revealed a biphasic pattern (Fig 1). Early-phase reactions (EPRs) were noted in 62.3% (38/61) of subjects within 10 days of treatment initiation, while late-phase reactions (LPRs) were sporadically observed in 37.3% (23/61) of subjects between days 19 and 60. Both EPRs and newly occurring LPRs developed in 15 of the 23 patients with LPRs.

The clinical features of EPRs and LPRs were different (Fig 2). In a typical case of EPR, the skin reaction was millet-sized disseminated erythema, frequently accompanied by itching; the lesions generally occurred in a symmetrical manner and tended to develop in intertriginous regions (Fig 2, *A*). EPRs were relatively mild and tended to recover spontaneously. The EPRs disappeared without medication in 3 cases and were treated with topical and oral antihistamines in 7 cases. Others were generally managed with topical corticosteroids and oral antihistamines. EPRs resolved completely in all patients except 2, in whom a few papules persisted even after early erythema had resolved; both of these patients experienced new development and aggravation of erythema around day 40, which is indicative of LPRs.

LPRs involved injection-site erythema accentuated by reddish papules, microvesicles, and concrescence of erythema with severe itching, lower limb purpura, and increase by approximately 10% in the eosinophil count (Fig 2, *B*). Among the 23 patients with LPRs, 8 required only topical corticosteroids, and 3 required both topical corticosteroids and oral antihistamines. Eleven patients with widespread erythema that was likely to progress to grade 3 without signs of severe drug eruption, such as systemic symptoms (e.g., pyrexia), mucous membrane lesions, and organ derangement, were treated with an oral corticosteroid along with topical corticosteroids and oral antihistamine, without discontinuing telaprevir-based triple therapy. In these cases,

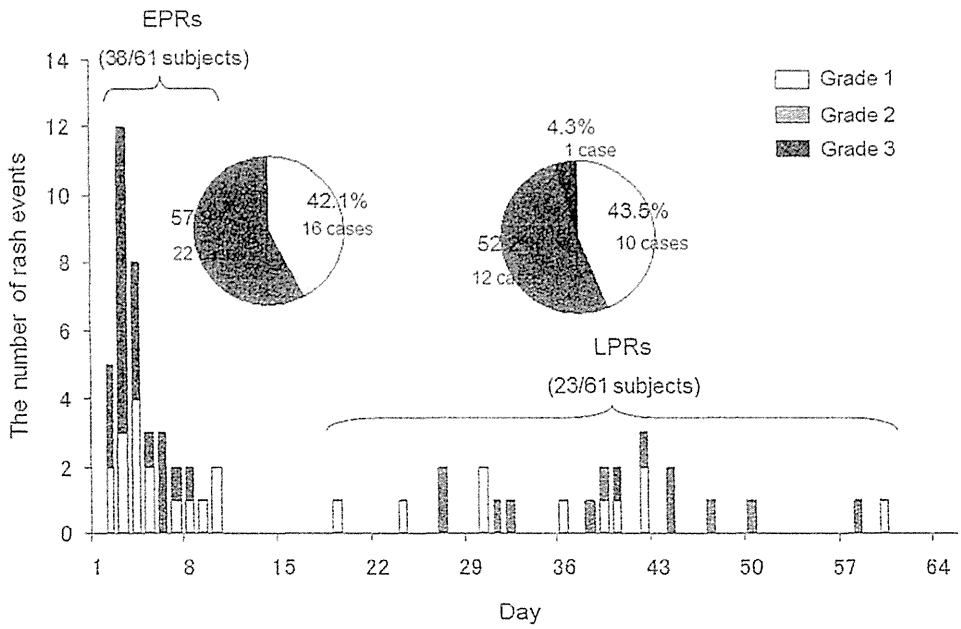

Fig 1. Time of onset of drug eruptions during administration of telaprevir-based triple therapy for hepatitis C. *EPRs*, Early-phase reactions; *LPRs*, late-phase reactions.

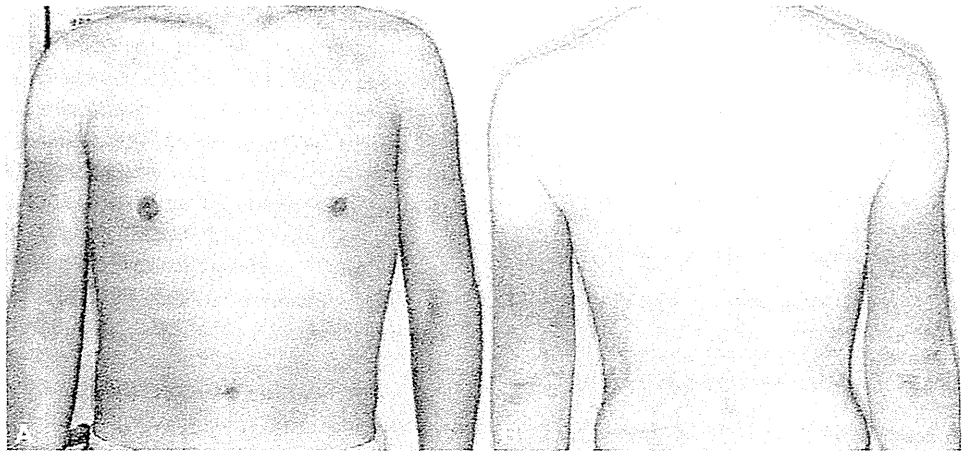

Fig 2. Comparison of early- and late-phase skin reactions in telaprevir-based triple therapy for hepatitis C. **A**, Skin reaction during the early phase (onset day: day 3, date of photo: day 4). **B**, Skin reaction during the late phase (onset day: day 31, date of photo: day 53).

prednisolone was orally administered at doses of 10 to 30 mg, which was gradually decreased without discontinuing telaprevir-based triple therapy; prednisolone was finally discontinued between 8 and 30 days, under careful observation. None of our patients had serious drug eruption. Telaprevir was continued in all patients except 1 who complained of strong itching sensation on day 64 without severe skin lesions.

Chronic hepatitis C is a fatal disease that can cause liver cancer. To continue the telaprevir-based triple therapy, good collaboration between hepatologists and dermatologists is important to appropriately evaluate skin symptoms and minimize the risk of serious drug eruptions.

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The diagnostic challenge of vulvar squamous cell carcinoma: Clinical manifestations and unusual human papillomavirus types

To the Editor: Treatment for early stage vulvar cancer lesions is readily available with limited morbidity; advanced stages of the disease, however, require interventions with serious morbidity. We sought to characterize clinical presentation of vulvar carcinoma to better understand reasons for delayed diagnosis and to determine which human papillomavirus (HPV) subtypes are causative.

Twenty-three cases of invasive vulvar squamous cell carcinoma (SCC) were identified using the pathology databases at the University of Pennsylvania. DNA was extracted from formalin-fixed paraffin embedded samples. HPV-PCR products were detected with PGMY-GP+-primer system. HPV-PCR products were then cloned and sequenced. NCBI-BLAST analysis revealed the presence of different HPV types.[1] Clinical data were extracted from the medical record (Table I).

Table I. Demographic and lesion characteristics

Age at diagnosis (median, IQR)	61	(43-76)
Smoking status (n, %)		
Never smoker	8	35%
Past smoker	6	26%
Current smoker	7	31%
Smoking status unknown	2	9%
Medical history (n, %)		
History of HIV infection	3	21%
History of genital warts	6	27%
History of diabetes	5	22%
History of inflammatory bowel disease	0	0%
History of organ transplant	2	9%
History of other cancer	4	17%
History of autoimmune disease	0	0%
History of eczema	1	4%
History of prior systemic treatments (n, %)		
Yes	3	14%
None/unknown	20	86%
History of topical treatments to vulvar area (n, %)		
Yes	8	35%
None/unknown	15	65%
History of abnormal Paps (n, %)		
Yes	8	35%
None/unknown	15	65%
History of concurrent or previous vulvar dermatoses diagnosed clinically (n, %)		
Yes	4	83%
None/unknown	19	17%
Previous vulvar symptoms (n, %)		
Yes	17	74%
None/unknown	6	26%
Lesion size in cm (median, IQR)*		
Type of provider who first evaluated patient (N)		
Ob/gyn	18	78%
Other	5	22%
Time from initial presentation in medical system to biopsy in months (Median, IQR)†	1	(0-2)

IQR, Interquartile range.
*Data available for 15 of 23 specimens.
†One day to 1 month rounded up to 1 month.

Studies have shown that vulvar cancer patients often have lengthy medical contact and treatment for vulvar symptoms prior to diagnosis.[2,3] Most patients in our study (74%) were aware of vulvar symptoms

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Short Communication

Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: An experimental animal study

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Aim: Treatment of hepatocellular carcinoma located on the liver surface is frequently difficult because direct puncture of the tumor must be avoided during needle insertion. The aim of this study was to investigate the utility of a no-touch pincer ablation procedure that uses a multipolar radiofrequency ablation (RFA) system for a tumor located on the liver surface.

Methods: The experimental animals were three pigs, and RFA was performed with two internally cooled bipolar electrodes. Three ablative procedures were compared: linear insertion at regular 13-mm intervals (pattern 1; virtual target tumor size, <10 mm); fan-shape insertion, maximum interval 20 mm (pattern 2; virtual target tumor size, <15 mm); and 25 mm (pattern 3; virtual target tumor size, <20 mm). All electrodes were inserted at a 30-mm depth. For patterns 1 and 2, ablation was performed on three other parts of the liver, and for pattern 3, ablation was performed on two other parts.

Results: For the median transverse and longitudinal diameter to the shaft, with the pattern 1 procedure, the ablative areas were 32 mm × 30 mm, and with the pattern 2 procedure, the ablative areas were 27 mm × 30 mm with carbonization of the liver surface. In contrast, with the pattern 3 procedure, the ablative areas were 45 mm × 26 mm; however, the ablative margin did not reach the surface, and carbonization was not apparent.

Conclusion: The no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small nodules that protrude from the liver surface.

Key words: bipolar, hepatocellular carcinoma, multipolar, no-touch ablation, radiofrequency ablation

INTRODUCTION

AMONG THE AVAILABLE treatment options for hepatocellular carcinoma (HCC), surgical resection is generally considered to be a local eradication method that can provide a satisfactory long-term outcome.[1–8] Recent advances in imaging procedures have led to increased detection of early-stage HCC and to improved survival due to the increased identification of patients in whom hepatic resection is possible.[9,10]

For patients who are not eligible for surgery for various reasons (e.g. lack of sufficient liver function for surgical resection), percutaneous local therapy is a viable therapeutic option. Several local ablation therapies are available, including percutaneous ethanol injection, percutaneous acetic acid injection, cryotherapy, percutaneous microwave coagulation therapy and radiofrequency ablation (RFA). In addition to surgical resection, local ablation therapies, particularly RFA, are considered to be local eradication methods for HCC that can provide good long-term outcomes.[11] Therefore, in recent years, RFA has become a widely used option for the primary treatment of small-size HCC. However, we often

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encounter cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver surface. In addition, a relationship between percutaneous local approaches to HCC (including tumor biopsy) and tumor seeding has been reported previously,[12,13] and with regard to the risk of treatment-related tumor seeding, the following risk factors have been reported: tumor size, tumor location (subcapsular portion), α-fetoprotein level, tumor stage and histopathological grade.[14,15] Therefore, a no-touch approach to local therapy may be considered an ideal treatment method for HCC.

Recently, a multipclar ablation system became available. Until now, in Japan, monopolar electrodes have typically been used, and the present cases are usually treated with some technical arrangement. For example, in the case of using a multi-tined expandable electrode, after obliquely inserting the electrode to avoid direct puncture of the target tumor, the multi needles are expanded toward the target tumor via non-tumor tissue, or in the case of using an internally cooled electrode, multiple insertions are made to avoid direct puncture of the target tumor, and RFA is performed after each insertion. However, these methods do not always provide enough of a treatment effect due to the influence of uncertain treatment procedures and natural, direct puncture to a tumor is indispensable. In contrast, a multipolar ablation system that uses an internally cooled bipolar electrode can combine the use of one to three electrodes at the same treatment session. When three electrodes are used, this system can treat large tumors; however, in the case of small tumors, it is not really necessary to use three electrodes to treat the target tumor. In addition, when we used this multipolar ablation system, usually electrodes were inserted into HCC, but in theory, this system can use no-touch ablation. However, to our knowledge, there are no technical reports that describe a non-direct punctual RFA method that uses a bipolar ablation system for HCC located on the liver surface. In this experimental animal study, we assumed that a small (<20 mm) HCC nodule protruded from the liver surface, and examined proper pincer ablation methods using two internally cooled bipolar electrodes.

METHODS

Summary of experimental procedures

WE USED A bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH [Telto, Germany]) and two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). RFA was applied in the livers of three normal female domestic pigs (each pig's weight was 60 kg) under general anesthesia maintained until killing. The abdomen was opened so that the needle could be inserted under an ultrasonography (US) guide directly into the upper region of the liver where the thickness was larger than 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed two to three times in each liver for evaluation of the "no-touch pincer ablation procedure". After the experiments were completed, the animal was killed, and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Protocol of the no-touch pincer ablation procedure

We used a bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH), and all ablation procedures were performed with two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). Internal liquid circulation of the applicator enables the efficiency of coagulation to be increased. The delivery rate was set to 30 mL/min of saline solution at room temperature. The liquid flow was provided by a triple peristaltic pump, which is part of the system. The electrodes were operated by a power control unit working at 470 kHz and providing a maximum output power of 250 W (OLYMPUS Winter & Ibe GmbH). In this study, output power and total energy in each session were fixed at 60 W and 25 kJ, respectively, according to the dosimetry table for the bipolar RFA system (CelonPOWER System; OLYMPUS Winter & Ibe GmbH).

With regard to the ablation protocol, we performed the following three types of ablation procedure: linear insertion, at regular 13-mm intervals (pattern 1); fan-shape insertion, maximum interval of 20 mm (pattern 2); and 25 mm (pattern 3). All electrodes were inserted at a 30-mm depth from the liver surface under a US guide (Fig. 1). Each ablation procedure was performed for the following number of times: pattern 1, three sessions; pattern 2, three sessions; and pattern 3, two sessions. In this study, we assumed that the size of the virtual target tumor was less than 10 mm in pattern 1,

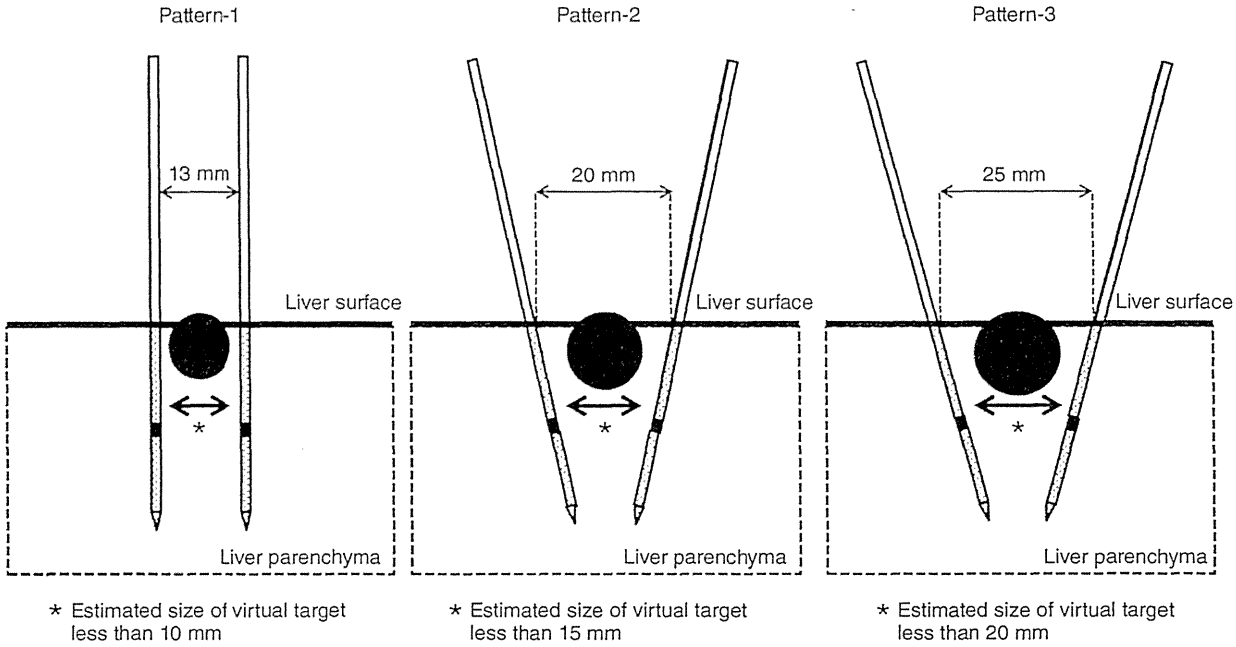


Figure 1 Protocol for a pincer ablation procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface.

less than 15 mm in pattern 2 and less than 20 mm in pattern 3.

Measurement procedure of the ablative margin

After completion of the experiments, the animal was killed and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone).

Statistical analysis

The size of the ablated zone and the duration of ablation were compared among the three groups with the Kruskal–Wallis test. All values are expressed as medians. A *P*-value of less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Features of the no-touch pincer ablation procedure

THE THREE TYPES of pincer ablation procedure applied to the pig liver were performed in the area shown in Figure 2(a).

Table 1 summarizes the features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface.

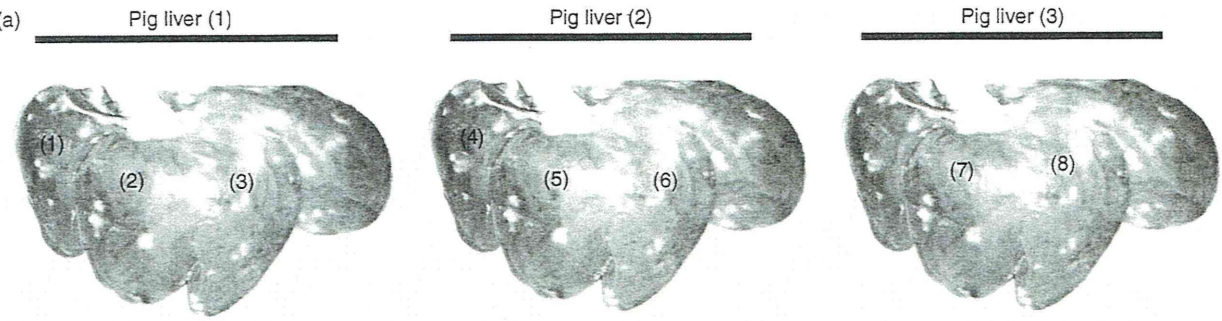
In the median (range) transverse and longitudinal diameter to the shaft, ablative areas were: pattern 1, 32 (27–35) mm × 30 (30–35) mm; pattern 2, 27 (25–35) mm × 30 (30–32) mm; and pattern 3, 45 (40–50) × 26 (25–27) mm. There were no significant differences in the size of each ablative area among the three ablation procedures. However, with the pattern 3 procedure, the transverse diameter to the shaft was larger than with the other procedures, and as a result, the ablative form was flatter. On the other hand, patterns 1 and 2 acquired sufficient ablative areas that covered the liver surface with carbonization of the surface; however, with pattern 3, the ablative areas did not reach the liver surface, and carbonization of the liver surface was not apparent (Fig. 2b–d).

In addition, there were no significant differences among ablation procedures in the duration of ablative time.

DISCUSSION

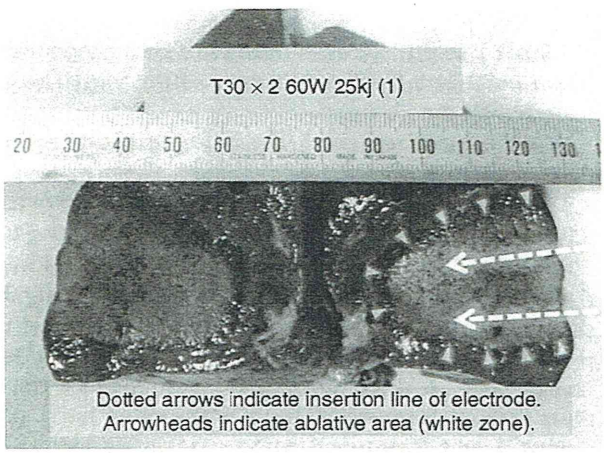
WE OFTEN ENCOUNTER cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver

(a)



Ablative pattern	*Location*
Pattern-1: linear insertion, at regular 13-mm intervals	(1)(2)(5)
Pattern-2: fan-shape insertion, maximum interval, 20 mm	(3)(4)(7)
Pattern-3: fan-shape insertion, maximum interval, 25 mm	(6)(8)

(b) Representative ablative images: pattern-1



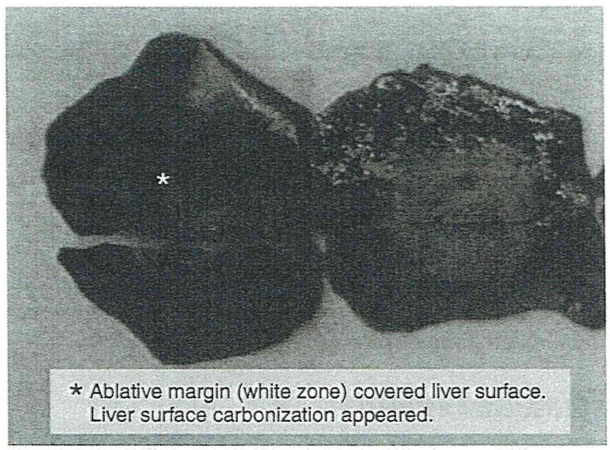
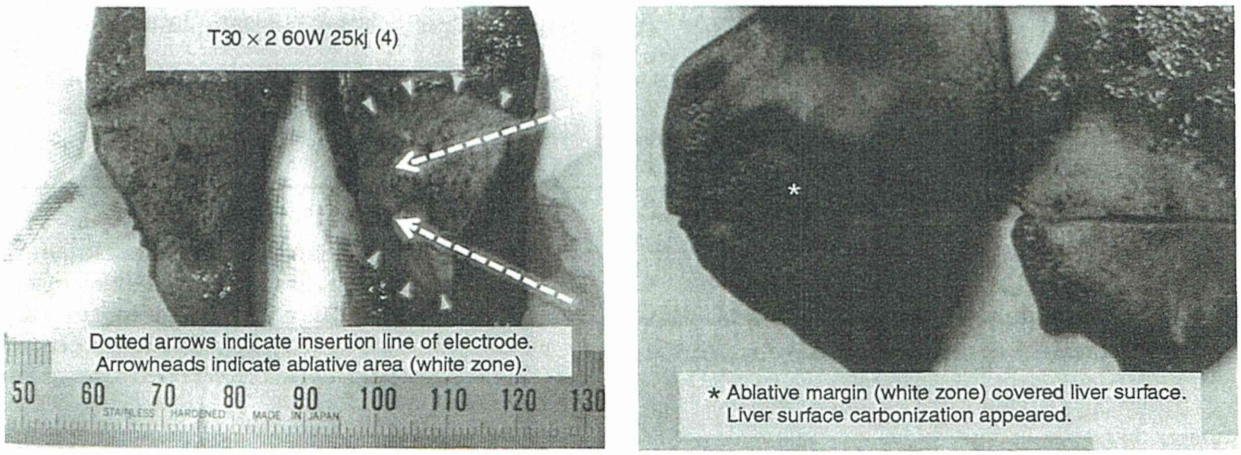


Figure 2 (a) Schema of the ablative areas of each pincer ablation procedure in the three pig livers. (b) One of the ablative shapes and the margin achieved with the pattern 1 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we inserted the electrodes linearly (maximum interval for each electrode was 13 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (c) One of the ablative shapes and the margin achieved with the pattern 2 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 20 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (d) Ablative shape and margin achieved with the Pattern 3 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 25 mm). The ablative area close to the liver surface was larger than with the other procedures. However, the ablative margin did not cover the liver surface, and carbonization of the liver surface was not apparent.

(c) Representative ablative images: pattern-2



(d) Representative ablative images: pattern-3

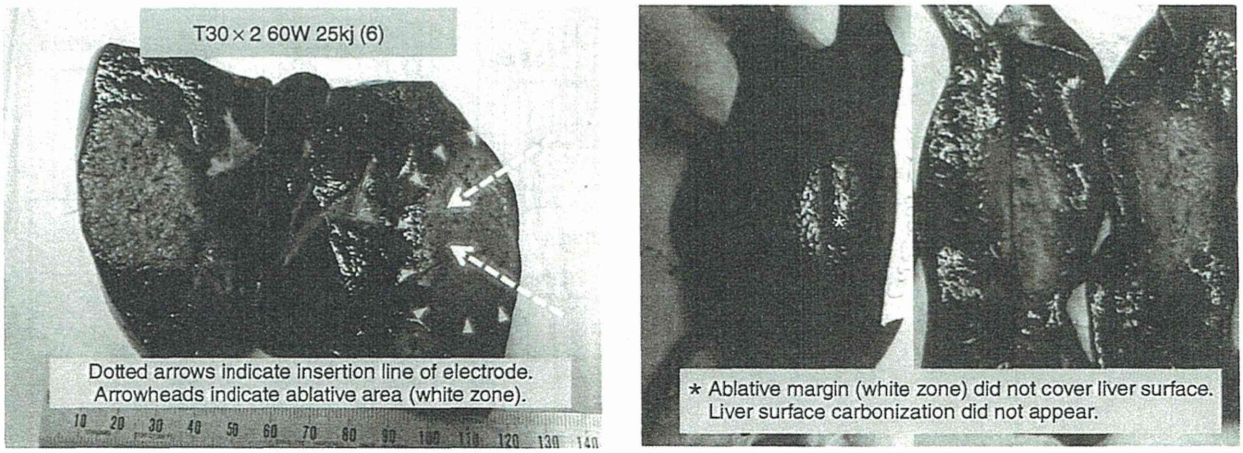


Figure 2 *Continued*

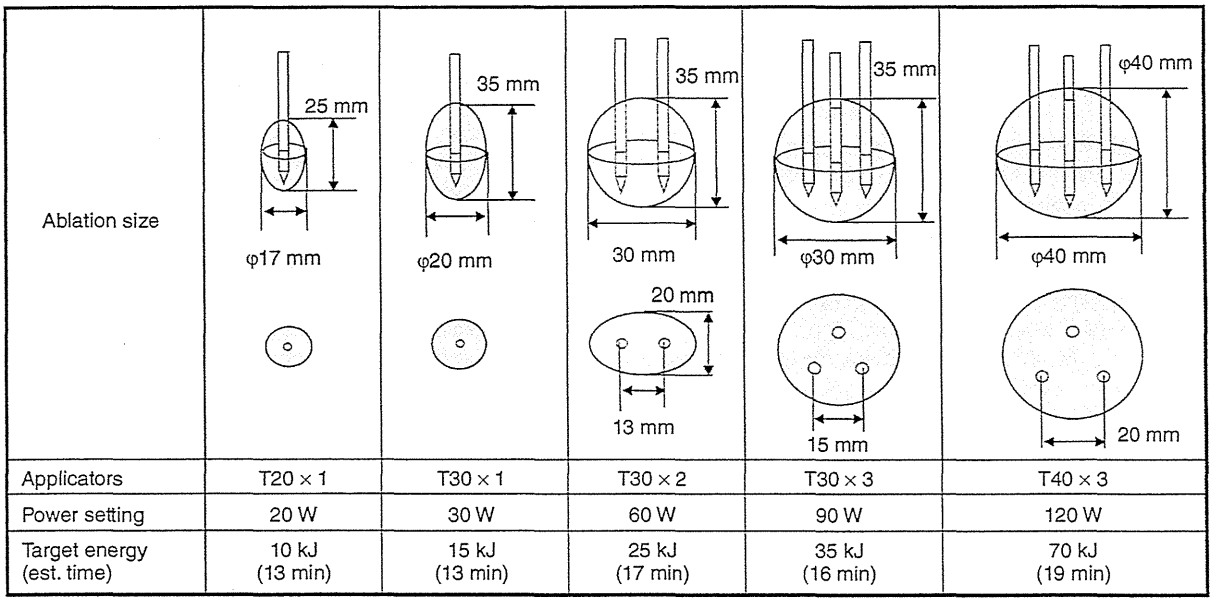
surface. In these situations, a multipolar ablation system that uses internally cooled bipolar electrodes may be suitable for treatment. With a multipolar ablation system, we can combine the use of one to three electrodes at the same treatment session, and when three electrodes are used, this system can treat a large tumor. However, in the case of small tumors (<20 mm), it is not really necessary to use three electrodes for treatment of the target tumor. However, in the dosimetry table of this bipolar system in Figure 3, which was made from previously reported early clinical data[16] and basic analysis, when two internally cooled bipolar electrodes are used (30 mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH), the recommended interval of each electrode in this system was 13 mm. With this regulation, we can treat only small tumors (<13 mm) when we perfume no-touch pincer ablation using two electrodes. Therefore, in this study we assumed a virtual target tumor with a tumor diameter less than 20 mm, and investigated the efficacy of a no-touch pincer ablation procedure and the maximum size of the tumor using two internally cooled bipolar electrodes for nodules that

Table 1 Features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface

	Pattern 1			Pattern 2			Pattern 3		*P*
	1	2	3	1	2	3	1	2	
Duration	13′46″	13′16″	12′58″	14′38″	13′50″	13′30″	13′05″	12′40″	$P = 0.151$
Ablated area									
Transverse diameter, mm	27	35	32	25	27	35	45	40	$P = 0.113$
Longitudinal length, mm	35	30	30	32	30	30	27	25	$P = 0.102$
Ablated area covered liver surface	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
Liver surface carbonization appeared	Yes	Yes	Yes	Yes	Yes	Yes	No	No	

protrude from the liver surface. In addition, we investigated only the fan-shape insertion method at a maximum interval of 20–25 mm. The reason for this is that in an actual RFA procedure, it is occasionally difficult to insert two electrodes in the same intercostal space for slightly large nodules that protrude from the liver surface; therefore, in this study, we examined a fan-shape ablation method that assumed two different intercostal approaches. Our results showed that with the pattern 3 treatment procedure, we could not acquire a sufficient ablative margin to the side of the liver surface. From these results, tumors of 20 mm or more may not be suitable for a no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

In contrast, with the pattern 1 and 2 treatment procedures, we acquired a sufficient ablative margin to the side of the liver surface with carbonization of the liver



- The data are based on Frericks et al., Radiology (2005) 237: 1056–1062. The reported average efficacy was ~0.5 millitre ablation volume per kilojoule.
  From these data, the required energy for an ablation sphere or ellipsoid of given diameter was calculated.
- The application of blood flow interruption (e.g. Pringle's manoeuvre, embolization) allows for a significant reduction of the target energy.

Disclaimer: this dosimetry table does not replace the monitoring of actual ablation sizes. The ablation diameters are approximations based on statistical data; they are not guaranteed for individual clinical cases. Ablation size and shape as well as the procedure time may significantly vary due to tumor physiology and vascular structure. A deviation from the recommended applicator distances may also have an impact on the ablation dimensions.

Figure 3 Dosimetry table for the CelonPOWER system (in Japan).

surface. These results may indicate that tumors of less than 15 mm are candidates for the no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

Finally, this experimental animal study had some limitations. First, the number of animals was very small, and the target tumor was a virtual tumor. Second, an additional examination regarding a no-touch linear insertion procedure for maximum intervals of 20 mm and 25 mm for each electrode was not enforced. Third, we could not investigate the same fan-shape ablation procedure using monopolar RFA in this study, because we assumed it would be too difficult to carry out a two-step insertion method using a monopolar electrode under the influence of a first ablation for nodules that protrude from the liver surface. Fourth, we could not investigate the pathological changes in the ablative area in this study. Therefore, with only these study results, it may not be possible to draw conclusions regarding the utility of the fan-shape insertion method using a bipolar RFA device. To solve these problems, we must carry out an additional large-scale study that includes pathological examination in the near future.

Finally, to summarize the points to be noted at the time of performing the pincer ablation procedure, first, we should insert the needle carefully under US guidance, because in this procedure, measuring the distance of the needle tip from the liver surface and the two needle intervals on the liver surface correctly is the most important point.

Second, with this procedure, we should pay attention to the risk of thermal damage to the visceral peritoneum. Therefore, if possible, thermal protection using measures such as artificial ascites should be considered.

Third, in this study, we did not observe a portal or hepatic vein thrombus in the ablative area. However, this study was performed mainly in the vicinity of the liver surface, and usually this area does not include large vessels. Therefore, we need to use caution as with monopolar ablation when we ablate near large vessels.

In conclusion, the no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small HCC tumors that protrude from the liver surface.

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Hepatology Research

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Original Article

Effectiveness and safety of reduced-dose telaprevir-based triple therapy in chronic hepatitis C patients

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Aim: To compare the early virological effectiveness, sustained virological response and safety of telaprevir 1500 mg/day with telaprevir 2250 mg/day, when combined in triple therapy with pegylated interferon and ribavirin in Japanese patients with high viral loads of genotype 1 hepatitis C virus.

Methods: The telaprevir 2250 mg/day and 1500 mg/day groups each contained 60 patients matched by age, sex and history of previous interferon-based treatment. Serum levels of genotype 1 hepatitis C virus RNA, hemoglobin levels, drug adherence and drug discontinuation rates were monitored during and after triple therapy.

Results: Patients receiving telaprevir 1500 mg/day had significantly lower telaprevir adherence and lower initial ribavirin dose but similar or superior pegylated interferon and ribavirin adherence and a lower rate of telaprevir discontinuation than did those receiving telaprevir 2250 mg/day. The early virological responses and sustained virological response rates were similar in both groups. Hemoglobin levels decreased to a greater extent in patients treated with telaprevir 2250 mg/day.

Conclusion: Compared to triple therapy including telaprevir 2250 mg/day, that including telaprevir at a reduced dose of 1500 mg/day was associated with lower rates of anemia and similar antiviral efficacy. Such a regimen may meaningfully improve sustained virological response rates, especially among female and elderly Japanese patients.

Key words: chronic hepatitis, hepatitis C virus, pegylated interferon, ribavirin, telaprevir

INTRODUCTION

APPROXIMATELY 170 MILLION people are chronically infected with hepatitis C virus (HCV) worldwide,[1] and approximately 30% develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC).[2,3] Currently, interferon (IFN) is the only antiviral drug capable of eliminating HCV infection. The present standard of care (SOC) for patients infected with HCV genotype 1, the most prevalent global genotype, is pegylated interferon (PEG IFN) combined with ribavirin (RBV) for 48 weeks.[4] However, sustained virological response (SVR), defined as the reduction of serum HCV RNA to undetectable levels 24 weeks after the completion of therapy, is achieved in only 42–52% of patients.[5–7] Moreover, response rates are influenced by patient factors such as sex, age and ethnicity,[8–10] as well as virological factors such as genotype and viral load.[11] SVR rates remain unsatisfactorily low (22%) in women aged 50 years or more who are infected with HCV genotype 1 in Japan.[12] Hence, there is a pressing need to improve the efficacy of antiviral treatment in such patients.

Recently, a new class of drugs, with a mechanism based on inhibition of the NS3/NS4 protease of the HCV polyprotein, has been investigated for the treatment of chronic hepatitis C. Of the drugs in this class, telaprevir has been selected as a clinical candidate for further development.[13] Telaprevir combined with PEG IFN and RBV has shown potent antiviral activity in phase II[14,15] and III clinical trials;[16,17] SVR rates of

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approximately 70% have been reported in patients infected with HCV-1. Similarly, in Japan, a phase III study was conducted in patients with HCV-1 to compare the efficacy and safety of the telaprevir regimen with those of the current SOC in treatment-naïve patients,[18] and to assess the efficacy and safety of the telaprevir regimen in relapsers and non-responders after previous IFN-based therapy.[19] However, the high efficacy was offset by treatment-induced anemia: early hemoglobin levels during triple therapy decreased by up to 4 g/dL, whereas decreases with SOC were not higher than 3.0 g/dL.[14,15] Additionally, we have previously reported that the factors associated with decreases in hemoglobin levels during triple therapy included female sex and age of more than 50 years.[20] Japanese patients infected with HCV genotype 1b with high viral loads are, on average, much older than Western patients infected with the same genotype, owing to a widespread HCV infection that occurred in Japan approximately 20 years ago.[21] Therefore, we considered that triple therapy would be highly effective when combined with careful monitoring of hemoglobin levels and prompt modification of RBV dose.

Consequently, in this study, we evaluated the effectiveness and safety of telaprevir-based triple therapy, administrated at an initial telaprevir dose of 2250 or 1500 mg/day, in the retrospective matched control study of 120 Japanese patients with chronic HCV-1 infection with high viral loads.

METHODS

Patients

FROM DECEMBER 2008 to August 2012, 204 patients with chronic hepatitis C were recruited to receive triple therapy with telaprevir, PEG IFN and RBV for 24 weeks at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. All patients had the following characteristics: (i) positive for HCV RNA genotype 1 and antibody to HCV (anti-HCV), absence of co-infection with HCV of other genotypes; (ii) negative for hepatitis B surface antigen; (iii) HCV RNA levels of 5.0 log IU/mL or more as determined with the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) platelet counts of more than $80 \times 10^3/mm^3$ without cirrhosis diagnosed by ultrasonography; (v) not pregnant or lactating; (vi) total previous alcohol intake of less than 500 kg; (vii) absence of HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; and (viii) absence of antiviral or immunosuppressive treatment during the previous 3 months.

Patients were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of the triple therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval of the institution's human research committee.

Study design

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administrated at the dose of 2250 (750 mg three times daily) or 1500 mg/day (750 mg twice daily). We selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment from the telaprevir 2250 and 1500 mg/day groups (Table 1), because 204 patients had many differences in baseline characteristics in both groups. PEG IFN-α-2b (PEG-Intron; Schering Plough, Kenilworth, NJ, USA) was injected s.c. at a median dose of 1.5 μg/kg (range, 1.1–1.8) once a week. RBV (Rebetol; Schering Plough) was administrated at 200–1000 mg/day; RBV dose of 600 mg/day (for bodyweight ≤60 kg), 800 mg/day (for bodyweight >60 to ≤80 kg) or 1000 mg/day (for bodyweight >80 kg) in principle. Since November 2011, the initial dose of RBV was reduced by 200 mg in cases of female sex, aged 66 years or older, hemoglobin level of less than 13 g/dL, bodyweight of less than 45 kg or platelet counts of less than $150 \times 10^3/mm^3$ at baseline by the judgment of the physician. All participating patients received these three drugs for the initial 12 weeks, followed by PEG IFN and RBV for an additional 12 weeks. All patients were followed up for at least 24 weeks after the last dose of study drugs to assess SVR.

Doses of telaprevir, PEG IFN and RBV were reduced or their administration discontinued as required, based on the reduction of hemoglobin levels; reduction of white blood cell, neutrophil or platelet counts; or the development of adverse events. Thus, the total dose of each drug administrated during the 12–24 weeks was calculated as the ratio of the actual administrated total dose to the anticipated total dose of each drug; these ratios provided adherence measures for telaprevir, PEG IFN and RBV.

HCV RNA measurements

Blood samples were obtained at weeks 1, 2, 4, 6, 8, 12, 16, 20 and 24 after initiation of treatment and at week 24 after completion of treatment, and routine biochemical

Table 1 Baseline characteristics of the patients infected with genotype 1 HCV who received triple therapy with pegylated interferon, ribavirin and TVR

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Sex (male/female)	30/30	30/30	Matched
Age (years)	60 (53–63)	62 (56–64)	Matched
Body mass index (kg/m^2)	22.1 (20.4–24.0)	22.7 (20.1–24.8)	0.278
IL28B genotype (rs8099917) TT/TG + GG	40/20	54/6	0.003
ITPA genotype (rs12979860) CC/CA + AA	44/16	36/23	0.175
Hemoglobin (g/dL)	14.3 (13.5–15.2)	14.2 (13.0–14.8)	0.223
Platelets (×104/μL)	17.6 (14.9–21.0)	16.9 (13.8–19.9)	0.227
Albumin (g/dL)	3.8 (3.7–4.0)	3.8 (3.7–4.1)	0.404
Alanine aminotransferase (IU/L)	35 (25–49)	37 (25–58)	0.437
γ-Glutamyltransferase (IU/L)	29 (18–49)	22 (17–39)	0.230
Creatinine (mg/dL)	0.7 (0.6–0.8)	0.6 (0.6–0.7)	0.333
Uric acid (mg/dL)	5.6 (4.9–6.5)	5.5 (4.7–6.3)	0.487
α-Fetoprotein (μg/L)	4 (3–7)	5 (3–8)	0.740
HCV RNA (log10 IU/mL)	6.8 (6.4–7.0)	6.7 (6.3–7.0)	0.551
Core a.a. 70 (wild/mutant)	38/22	45/15	0.235
Core a.a. 91 (wild/mutant)	28/32	36/24	0.200
Previous IFN-based treatment			
Naïve/relapsed/null response	23/25/12	23/25/12	Matched

Values are number with percentage in parentheses or median with interquartile range in parentheses.
a.a., amino acid; HCV, hepatitis C virus; IFN, interferon; TVR, telaprevir.

and hematological tests were performed. The antiviral effects were assessed by measuring plasma HCV RNA levels using the COBAS TaqMan HCV test. The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL; undetectable samples were defined as negative.

Detection of amino acid substitutions in the core of HCV-1b

Amino acid (a.a.) substitutions in the HCV core region were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. Core a.a. substitutions at positions 70 and 91 (core 70 and 91, respectively) were determined according to the methods of our previous reports.[22,23]

Determination of IL28B and ITPA genotypes

ITPA (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped using the Invader assay, TaqMan assay or direct sequencing, as described.[24,25]

Statistical analyses

Non-parametric tests, including the χ^2-test, Fisher's exact test, Mann–Whitney *U*-test and Kruskal–Wallis tests, were used to analyze differences in the baseline clinical profiles of patients. Kaplan–Meier analysis and the log-rank test were applied to estimate and compare serum HCV RNA elimination rates between the groups. $P < 0.05$ by two-tailed test was considered statistically significant. All analyses were performed using SPSS software version 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the 120 patients are listed in Table 1. There were no significant differences in the baseline characteristics between the telaprevir 2250 mg/day group and 1500 mg/day group, except for *IL28B* genotypes. Patients receiving telaprevir 1500 mg/day had a significantly higher incidence of TT in *IL28B* genotypes than did those receiving 2250 mg/day.

Initial drug doses, drug adherence and discontinuation rate up to 12 weeks

Patients receiving telaprevir 1500 mg/day had a significantly lower initial telaprevir dose and initial RBV dose than those receiving 2250 mg/day (Table 2). Telaprevir adherence was significantly lower in the 1500 mg/day

Table 2 Initial drug doses, drug adherence up to 24 weeks and discontinuation rates up to 12 weeks

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Initial TVR dose (mg/kg per day)	38.1 (33.6–45.1)	25.6 (22.5–29.6)	<0.001
TVR adherence up to 12 weeks (%)	100 (75–100)	67 (65–67)	<0.001
Discontinuation of TVR	15 (25.0%)	6 (10.0%)	0.053
Discontinuation of TVR due to anemia	12 (20%)	3 (5%)	0.025
Initial PEG IFN dose (μg/kg per week)	1.5 (1.4–1.6)	1.5 (1.4–1.6)	0.706
PEG IFN adherence up to 24 weeks (%)	100 (85–100)	100 (89–100)	0.062
Initial RBV dose (mg/kg per day)	11.6 (10.6–12.8)	9.9 (7.9–11.3)	<0.001
RBV adherence up to 24 weeks (%)	51 (41–61)	59 (46–68)	0.090
Discontinuation of all drugs up to 12 weeks	5 (8.3%)	1 (1.7%)	0.207

Values are number with percentage in parentheses or median with interquartile range in parentheses.
PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir.

group than in the 2250 mg/day group, while there were no differences in adherence for the other two drugs. Although there were no significant differences between the groups in the rates of discontinuation of telaprevir or all drugs up to 12 weeks, the rates of discontinuation of telaprevir due to anemia in the 1500 mg/day group were significantly lower than in 2250 mg/day group.

Loss of serum HCV RNA according to *IL28B* genotypes

Figure 1 compares the on-treatment virological response over the first 12 weeks for the telaprevir 2250 and 1500 mg/day groups according to *IL28B* genotypes, respectively, because there were significant differences in distribution of *IL28B* genotypes between both groups.

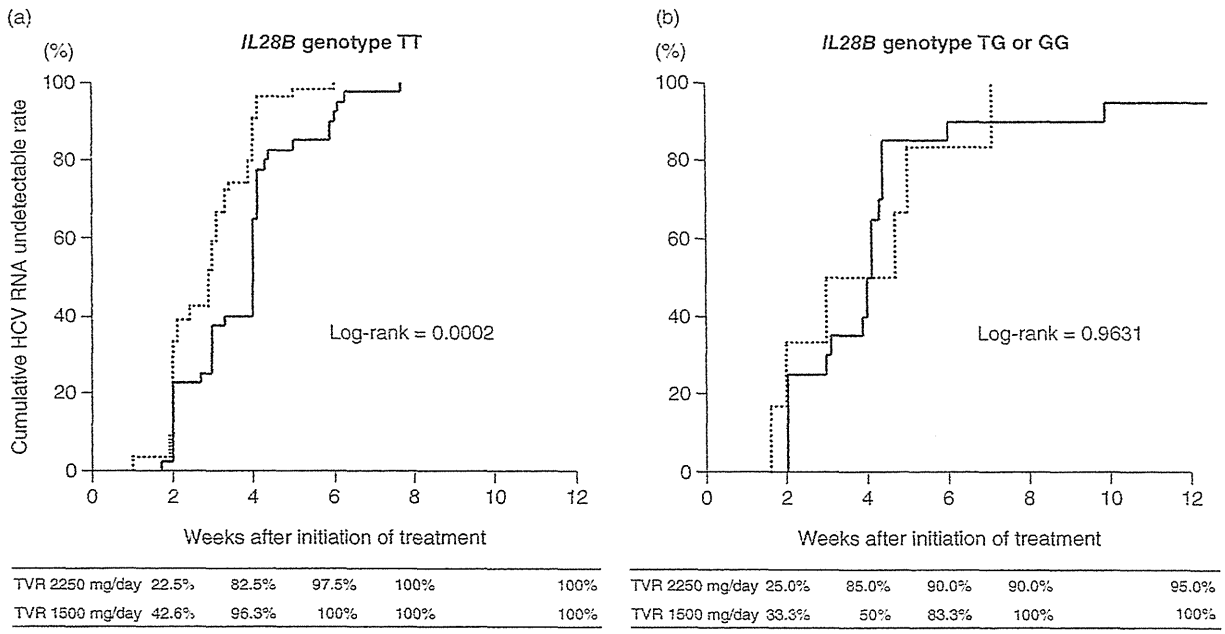

Figure 1 Cumulative rate of undetectable hepatitis C virus (HCV) RNA during triple therapy with pegylated interferon, ribavirin and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. (a) *IL28B* genotype TT, (b) *IL28B* genotype TG or GG. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

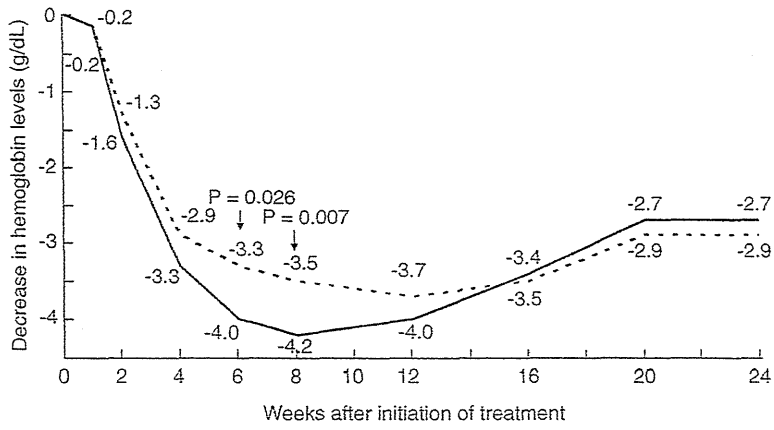

Figure 2 Decreases in hemoglobin levels during triple therapy with pegylated interferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. Each time point in this figure corresponds to median values. Patients evaluated at each time point are indicated below, with the number of patients who discontinued TVR (continued PEG IFN and RBV) in parentheses. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

Number of patients (TVR withdrawn)								
2250 mg/day	60	60	60 (1)	59 (4)	55 (10)	55	55	55
1500 mg/day	60	60	60 (1)	59 (2)	59 (3)	59	59	59

Triple therapy suppressed HCV RNA levels quickly and effectively in both groups. In the 2250 and 1500 mg/day groups of *IL28B* genotype TT, HCV RNA became undetectable in 22.5% and 42.6% of patients at 2 weeks, 82.5% and 96.3% at 4 weeks, and 100% and 100% at 8 weeks, respectively (Fig. 1a). The early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group in *IL28B* genotype TT (log-rank test = 0.0002).

In the subgroups of *IL28B* genotype non-TT patients receiving telaprevir 2250 and 1500 mg/day, HCV RNA became undetectable in 25.0% and 33.3% of patients at 2 weeks, 85.0% and 50% at 4 weeks, 90.0% and 100% at 8 weeks, and 95.0% and 100% at 12 weeks, respectively. The virological responses during the first 12 weeks in this subgroup of patients did not significantly differ between the telaprevir 2250 and 1500 mg/day groups (log-rank test = 0.9631, Fig. 1b).

Safety

Figure 2 shows the decreases in hemoglobin levels in telaprevir 2250 and 1500 mg/day recipients. Data from six patients were omitted (five receiving telaprevir 2250 mg/day and one receiving 1500 mg/day) because treatment was withdrawn between 8 and 12 weeks after initiation. Telaprevir was discontinued in 15 of the 60 (25.0%) patients receiving telaprevir 2250 mg/day (one at week 6, four at week 8 and 10 at week 12) and six of the 60 (10.0%) receiving 1500 mg/day (one at week 6, two at week 8 and three at week 12). Hemoglobin decreased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 6 (−4.0 [−6.7 to −1.2] vs −3.3 [−5.2 to 0.2] g/dL, $P = 0.026$) and week 8 (−4.2 [−7.7 to −1.3] vs −3.5 [−6.9 to −1.3] g/dL, $P = 0.007$).

Skin disorder frequency was comparable between the telaprevir 2250 mg/day group and 1500 mg/day group (81.7% and 75%, respectively). However, skin disorders of grades 2–3 occurred more frequently in the telaprevir 2250 mg/day group than in the 1500 mg/day group (55% vs 35%, $P = 0.043$).

With respect to renal dysfunction, increases in serum creatinine (sCR) levels during therapy were not significantly different between both groups. However, blood uric acid levels increased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 1 (1.3 [−1.6 to 4.8] vs 0.9 [−2.1 to 4.3] g/dL, $P = 0.015$), week 2 (1.2 [−2.3 to 4.1] vs 0.5 [−2.3 to 2.7] g/dL, $P = 0.004$), week 4 (1.6 [−1.1 to 5.5] vs 0.7 [−2.4 to 3.8] g/dL, $P < 0.001$), week 6 (1.6 [−1.7 to 4.8] vs 0.5 [−3.5 to 3.6] g/dL, $P < 0.001$) and week 8 (1.1 [−3.6 to −4.9] vs 0.7 [−1.6 to 3.7] g/dL, $P = 0.029$).

Predictive factors associated with SVR

The overall SVR rate was 83% (169/204) in our hospital. SVR was accomplished in 106 (88%) of 120 patients selected for this study, including 50 of 60 (83%) patients in the telaprevir 2250 mg/day and 56 of 60 (93%) patients in telaprevir 1500 mg/day groups (Fig. 3).

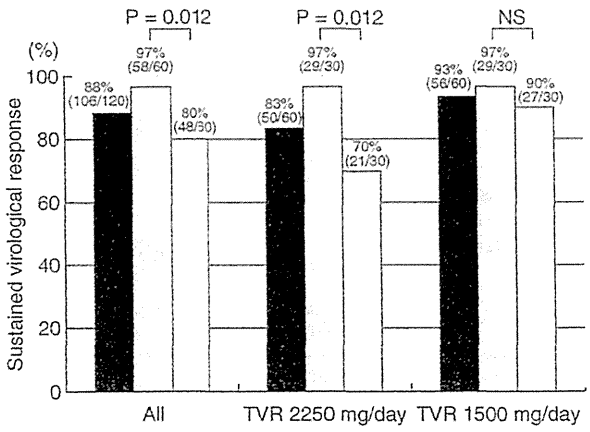

Figure 3 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared among all patients (men and women), TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (■) Total, (□) male, (□) female.

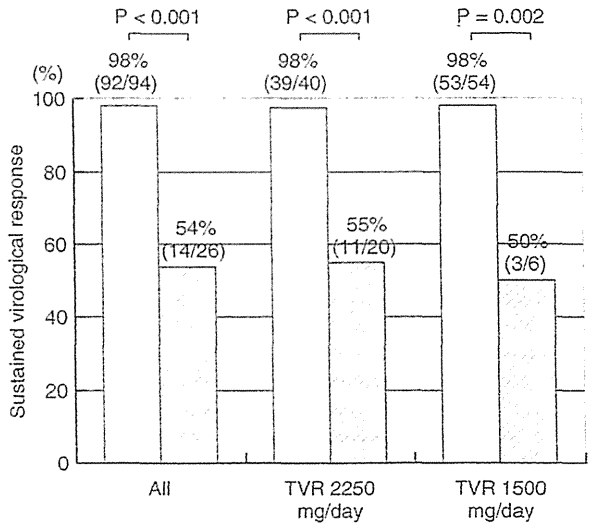

Figure 4 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared between *IL28B* (rs8099917) genotype TT and TG/GG in all patients, TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (□) TT, (□) TG or GG.

Significant univariate predictors for SVR included male sex, *IL28B* genotype TT, and HCV core a.a. 70 wild type, except for null response to prior treatment, initial telaprevir dose of 37.5 mg/kg per day or more, telaprevir dosing period of 10 weeks or more, 100% PEG IFN adherence up to 24 weeks, PEG IFN adherence up to 12 weeks of 80% or more, RBV adherence up to 12 weeks of 50% of more, γ-glutamyltransferase of 35 IU/mL or less, and sCr of 0.6 mg/dL or more ($P < 0.05$). Of these, male sex (odds ratio [OR] = 13.7; $P = 0.028$) and *IL28B* genotype TT (OR = 44.4; $P = 4.47 \times 10^{-5}$) were identified as significant independent predictors for SVR (Table 3).

Therefore, we assessed the SVR rate of triple therapy according to sex and *IL28B* genotype. SVR was much less frequent in women than in men (48/60 [80%] vs 58/60 [97%], $P = 0.0012$, Fig. 3). Especially, in the telaprevir 2250 mg/day group, there were significant differences between men and women (29/30 [97%] vs 21/30 [70%], $P = 0.0012$). However, there were no differences between men and women in the telaprevir 1500 mg/day group (29/30 [97%] and 27/30 [90%], respectively).

Patients with *IL28B* genotype TT were significantly more likely to achieve SVR (92/94 [98%] vs 14/26 [54%], $P < 0.001$, Fig. 4), compared with patients with TG or GG genotypes. There were significant differences between *IL28B* genotype TT and non-TT in both the telaprevir 2250 and 1500 mg/day groups (39/40 [98%] vs 11/20 [55%], $P < 0.001$ and 53/54 [98%] vs 3/6 [50%], $P = 0.002$, respectively).

Table 3 Multivariate analysis of factors associated with sustained virological response of TVR, pegylated interferon and ribavirin triple therapy in Japanese patients infected with HCV

Factor	Category	Odds ratio (95% CI)	*P*-value
Sex	1; female	1	
	2; male	13.7 (1.33–141.2)	0.028
IL28B genotype (rs8099917)	1; TG or GG	1	
	2; TT	44.4 (7.18–274.2)	4.47×10^{-5}

CI, confidence interval; HCV, hepatitis C virus; TVR, telaprevir.