Table 3 Distributions of combined score assigned to patients in the study cohort and the validation cohort

	Study cohort (n = 190)			Validation cohort (n = 31)		
	SVR	Non-SVR	SVR rate (%)	SVR (n = 9)	Non-SVR (n = 22)	SVR rate (%)
Age (years)	58 (48–66)	64 (57–68)		58 (55–64)	61 (53–68)	
Gender (M/F)	41/33	58/58		5/4	10/12	
BMI (kg/m^2)	22.0 (20.6–25.2)	23.1 (20.9–25.2)		22.6 (21.5–23.8)	23.0 (21.0–24.7)	
score: 1/0	43/31	46/70		4/5	6/16	
rs8099917						
score: 1/0	64/10	73/43		6/3	10/12	
PAI-1 (pg/mL)	20 302	17 639		25 688	17 728	
	(15 564–26 746)	(13 122–21 278)		(22 185–30 591)	(15 422–26 666)	
Score: 1/0	24/50	11/105		5/4	7/15	
Combined score						
0	3	19	13.6	1	6	14.3
1	18	66	21.4	3	10	23.1
2	46	29	61.3	3	5	37.5
3	7	2	77.8	2	1	66.7
0–3	74	116	38.9	9	22	29.0

For categorical data, the number of patients in each category is shown.
For continuous data, the median and 25th–75th percentile are displayed.

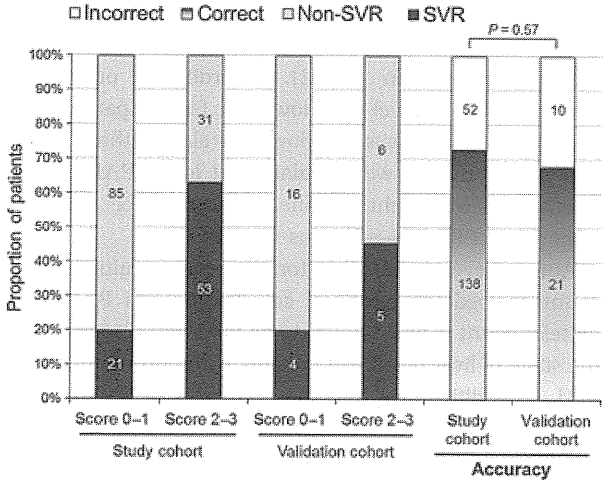

Fig. 1 Associations between combined score and treatment outcome. The accuracy of the prediction score with cut-off of <2 for SVR versus non-SVR in each cohort is also displayed. Number in bar indicates the number of patients. The accuracies of two cohorts are compared using the chi-squared test, and were not significantly different.

tendency in the validation cohort: the proportion of patients assigned 1 point was higher in the SVR group than in the non-SVR group for all three factors, and a higher combined score predicted higher SVR rate (Table 3). As shown in Fig. 1, the SVR rate of patients who scored 2 or 3 was more than twice as high as that of patients who scored 0 or 1 (45.5% and 20.0%, respectively). The accuracy of the prediction score in this validation cohort (21/31, 68%) was slightly reduced compared to that in the study cohort (73%), but the difference was not significant (P = 0.57). In the validation cohort, ROC_AUC was 0.68 and specificity, sensitivity, and PPV and NPV for SVR were 73%, 56%, 46% and 80%, respectively. Specificity and NPV had the same values between the two cohorts, but sensitivity and PPV were reduced in the validation cohort (−16% and −17%, respectively).

Subsequently, we also predicted SVR using RVR, which is well known as an important predictor [22–24]. The data of HCV-negativity at 4 weeks were available in 161 patients. Of 161 patients, only 40 patients (24.8%) showed RVR. Of 40 patients with RVR, 29 patients achieved SVR. As a result, prediction using RVR showed higher PPV and specificity than our score (PPV, 0.73 *vs* 0.63; specificity, 0.89 *vs* 0.73) but conversely, prediction using RVR showed lower NPV and sensitivity (NPV, 0.71 *vs* 0.80; sensitivity, 0.45 *vs* 0.72). Finally, the accuracy of the prediction using RVR was 0.71, which was similar to ours; 0.73 in the study cohort and 0.68 in the validation cohort. In our cohort of Japanese patients infected with HCV-1b with high viral load, overall SVR rate was poor (83/221 = 37.6%) and prediction of SVR was not sufficient even if using RVR. Therefore use of not only a predictor during therapy such as RVR but also a pretreatment predictor such as our score may be useful to predict SVR.

Correlation between PAI-1 level and other clinical data

We examined the correlations between the most significant predictor, PAI-1 level, and all of the other clinical data analyzed in the study cohort. As shown in Table 4, six

Table 4 Spearman correlation between PAI-1 level and other clinical data

	n	*R*	*P* value
Age (years)	190	-0.23	0.0013
Gender (Male)	190	0.20	0.0067
WBC (/mm^3)	189	0.22	0.0028
Platelet count (×10^4/mm^3)	190	0.38	1.8×10^{-7}
Triglyceride (mg/dL)	138	0.24	0.0043
Fibrosis stage (0–1/2/3/4)	155	-0.20	0.012

Only factors with correlation coefficient (r) ≥0.2 or ≤−0.2 are shown.

factors showed significant correlation with PAI-1 level ($r \geq 0.2$ or ≤ -0.2, $P < 0.05$). Platelet count showed the strongest correlation ($r = 0.38$, $P = 1.8 \times 10^{-7}$) and fibrosis stage was inversely correlated (Fig. 2a and b, respectively), suggesting the close association between PAI-1 and liver fibrosis. Age was also inversely correlated ($r = -0.23$) and, in addition, male gender, leukocyte count, and triglycerides were significantly correlated with PAI-1 level.

DISCUSSION

In this study, we found that pretreatment serum PAI-1 level was a novel independent predictive marker for the response to PEG-IFN-α-2b plus RBV therapy in patients with hepatitis C, which has not been reported before. We propose a simple and noninvasive prediction score for SVR consisting of rs8099917, BMI and PAI-1 level. The accuracy of this score was confirmed using an independent validation cohort. We also found that serum PAI-1 level was correlated with a number of other clinical factors such as platelet count, fibrosis stage, age, gender, leukocyte count and triglyceride level.

PAI-1 is the primary physiological inhibitor of tissue-type plasminogen activator and urokinase-like plasminogen activator and inhibits both fibrinolysis and proteolysis [25]. Circulating PAI-1 levels in humans are increased in obesity and the insulin resistance syndrome [14]. Adipose tissue produces and secretes a large number of hormones, cytokines, and proteins that affect glucose homeostasis and insulin sensitivity, including tumour necrosis factor-alpha, PAI-1, leptin, resistin, and adiponectin [14,15]. Among the adipocytokines that we examined, PAI-1 was identified as an independent predictor for SVR, but the mechanism by which PAI-1 affects treatment outcome of combination therapy for chronic hepatitis C patients is unclear.

One possible mechanism is suggested by an interesting report postulating that PAI-1 activates the Janus kinase (Jak)-signal transducer and activator of transcription (STAT) signalling system and that this activation is mediated by the low density lipoprotein receptor-related protein [26]. IFNs are known to activate the Jak-STAT signalling system and induce the transcription of IFN-stimulated genes (ISGs), which are essential for the induction of an antiviral state [27,28]. Based on these findings, PAI-1 may affect SVR via this signalling pathway through activation in a similar way to IFNs. However, other reports demonstrate that up-regulation of ISGs in the liver prior to treatment confers a poor treatment outcome [29,30].

On the other hand, PAI-1 level was correlated with both age and gender in our study. It was reported that response to combination therapy is poorer in older female patients with hepatitis C in Japan [31,32]. According to our findings (Table 4), PAI-1 level was lower in female patients than male patients and was also lower in elderly than younger patients. Therefore, we speculate that lower PAI-1 level in elderly females might be one of reasons for their poor response to PEG-IFN-α-2b plus RBV therapy.

Among several clinical factors that we examined, platelet count showed the strongest correlation with PAI-1 level. Platelet count is well known to be inversely correlated with the stage of liver fibrosis in patients with chronic hepatitis C [33]. In addition, we also observed an inverse correlation between PAI-1 level and fibrosis stage. PAI-1 assumed to positively correlate with fibrosis due to its inhibitive effect on fibrinolysis, but actually we found the opposite result. Some

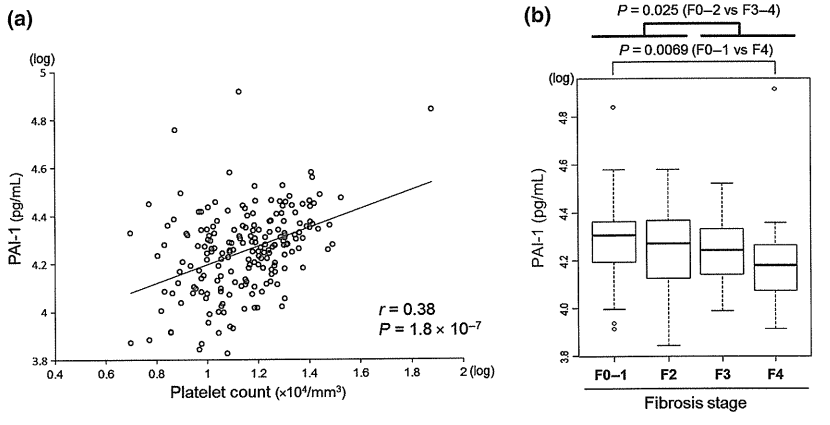

Fig. 2 The correlations between PAI-1 level and platelet count as well as fibrosis stage. (a) PAI-1 level and platelet count are significantly correlated using Spearman's tests. The solid line represents a least squares best fit line. (b) PAI-1 level is inversely correlated with fibrosis stage. The box indicates the inter-quartile range (25% and 75%) and the line within the box represents the median. *P* values were calculated by Mann–Whitney *U*-test.

reports suggest a positive correlation between PAI-1 and hepatic fibrosis using animal models, such as the bile duct ligation model [34,35], but recently von Montfort *et al.* reported that PAI-1 plays a protective role in hepatic fibrosis using another, more severe, model of hepatic fibrosis caused by carbon tetrachloride (CCl_4) [36]. They showed that hepatic mRNA expression of collagen Iα1 was elevated in PAI-1-deficient mice after CCl_4 exposure and also that livers from PAI-1-deficient mice have an impaired regenerative response to injury, in part via a mechanism involving impaired hepatocyte growth factor (HGF) maturation or via impaired HGF signalling via p38 phosphorylation. The role of PAI-1 in hepatic fibrosis is still controversial, but here we suggest a clinically protective role of PAI-1.

In this study, we identified pretreatment serum PAI-1 level as an independent predictor for SVR and suggest a scoring method for predicting outcome of combination therapy. Interestingly, this prediction score consists of one SNP and two factors that are strongly associated with metabolic syndrome. In addition to lower BMI and higher PAI-1 level, higher triglyceride and lower fasting blood sugar were associated with SVR in univariate analysis (Table 2), and PAI-1 level is correlated with triglyceride level (Table 4). These results suggest that metabolic factors have strong effects and close connections to treatment outcome in HCV patients. It may be worth investigating whether intervention in such metabolic disorders improves treatment outcome. In the near future, direct-acting antiviral agents such as protease inhibitors will become available [37,38]. As such drugs are very powerful, the predictive ability of PAI-1 might become less significant. However, PEG-IFN plus RBV combination therapy will still be used to treat non-genotype 1b infected patients. Accordingly, the predictive value of PAI-1 should be assessed in such patients. Functional studies using animal models have suggested a role for PAI-1 in liver fibrosis [34–36], and should be investigated further with regard to therapy.

In conclusion, measurement of pretreatment PAI-1 level as well as rs8099917 genotype could be useful in planning an individualized treatment strategy against HCV.

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DISCLOSURES

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Original Article

Follow up of the 987 blood donors found with hepatitis C virus infection over 9–18 years

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Aim: To follow up blood donors found with hepatitis C virus (HCV) infection, to improve the outcome by antiviral treatments.

Methods: Between 1991 and 2001, 3377 of the 1 925 860 donors (0.18%) were found to have HCV infection at the Hiroshima Red Cross Blood Center in Japan. Of them, 987 were able to be followed regularly over 9–18 years until 2009, and received antiviral treatments as required.

Results: At the start, chronic hepatitis was diagnosed in 541 (54.8%), cirrhosis in five (0.5%) and hepatocellular carcinoma (HCC) in one (0.1%), whereas the remaining 439 (44.5%) had persistently normal aminotransferase levels (PNAL). Hospital visits were terminated voluntarily in 24.3% within the first year, 46.8% by 10 years and 50.9% by 17 years. Liver disease improved in 178 (18.0%), remained stable in 606 (61.4%) and aggravated in 170 (17.2%). Of the 541 donors with chronic hepatitis, HCC developed in 28 (5.2%) and cirrhosis in 11 (2.0%), whereas HCV infection was cleared in 107 (19.8%) by antiviral treatments. In addition, HCV infection resolved in 54 of the 439 donors (12.3%) with PNAL after they had developed chronic hepatitis and received treatments. In donors with chronic hepatitis, the cumulative incidence of HCC was 4.1% at 10 years. By multivariate analysis, age and diagnosis of chronic hepatitis at the entry were found to be independent risk factors for the development of HCC.

Conclusion: Individuals with undiagnosed HCV infection need to be identified and receive medical care. They have to be motivated to merit from this health-care program.

Key words: alanine aminotransferase, chronic hepatitis C, hepatitis C virus, hepatocellular carcinoma, interferon, natural history

INTRODUCTION

OVER THE WORLD, an estimated 130–170 million people are infected with hepatitis C virus (HCV),[1] and most of them are unaware of their HCV infection. Because HCV infection evolves insidiously, it takes decades before overt liver disease develops, such as decompensated cirrhosis and hepatocellular carcinoma (HCC).[2] Hence, it is necessary to identify individuals with undiagnosed HCV infection, and provide them with medical care for clearing HCV and preventing severe liver disease developing in them.

To make plans for management of individuals with undiagnosed HCV infection, it needs to be established to what extent liver disease has progressed in them, and how it advances over the long run, with or without medical interventions. Blood donation offers a unique opportunity to pursue such an undertaking, because it can identify HCV infections that have not been diagnosed previously.[3–9]

In 1991, when screening for antibody to HCV (anti-HCV) was introduced, a program was launched by the Hiroshima Hepatitis Study Group in Japan to identify HCV carriers at the blood donation. Liver disease was

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diagnosed in the donors infected with HCV, so as to provide them with medical care to prevent the development of serious complications. Initially, 1019 blood donors with HCV infection were enrolled in the program, and the outcome was reported for 408 (40.0%) of them who had been followed for 5 years or longer by the year 2003.[10]

Here, we report the outcome of liver disease in 987 (96.9%) of them over 18 years of follow up. Various issues came up during the study, and they would need to be taken into consideration for improving health-care of the population with undiagnosed HCV infection hidden in the society. Further, the obtained results are hoped to lessen the national burden for management of hepatitis C that is expected to increase substantially in the foreseeable future.

METHODS

Study population

BETWEEN AUGUST 1991 and November 2001, 1 925 860 individuals wished to donate blood at the Japanese Red Cross (JRC) Hiroshima Blood Center, and 3377 (0.18%) were found to have HCV infection with high-titered anti-HCV and HCV RNA in the serum. They were informed of their HCV infection, and recommended to consult hepatology specialists in the 20 institutions constituting the Hiroshima Hepatitis Study Group: Hiroshima City Asa Hospital, Akitsu Prefectural Hospital, Hiroshima Prefectural Hospital, Kure City Medical Association Hospital, Kure Kyosai Hospital, Hiroshima University Hospital, KKR Hiroshima Kinen Hospital, Hiroshima City Hospital, Hiroshima Red Cross Hospital, Hiroshima General Hospital, Hiroshima JR Hospital, National Hospital Organization Kure Medical Center, National Hospital Organization Fukuyama Medical Center, Mitsubishi Mihara Hospital, Shobara Red Cross Hospital, Chugoku Rousai Hospital, Chuden Hospital, Nippon Kokan Fukuyama Hospital, Onomichi General Hospital and National Hospital Organization West Medical Center. Serving there as hepatology specialists were: Keiji Tsuji, M.D., Toshio Miura, M.D., Mikiya Kitamoto, M.D., Norihiko Katayama, M.D., Shuji Yamaguchi, M.D., Shoichi Tanakahshi, M.D., Hideaki Kodama, M.D., Yasuyuki Araki, M.D., Yasuyuki Aisaka, M.D., Kunio Ishida, M.D., Keitaro Yamashina, M.D., Hiroshi Kouno, M.D., Toshihiko Kaneyoshi, M.D., Kazushi Teramen, M.D., Kouji Kamada, M.D., Takashi Moriya, M.D., Hiroto Ishihara, M.D., Tomoo Yoshida, M.D., and Makoto Obayashi, M.D.

Of the 3377 donors infected with HCV, who were advised to undergo medical examination, 1097 (32.5%) visited liver clinics. The initial diagnosis was established in 1019 of them (30.2% of the total), and the date of initial visit, the date of birth and the baseline liver disease were filed in the computer. They were recommended to take regular check-ups, and receive antiviral treatment as required. The results of 408 carriers (40.0%), who had been followed for 5 years or longer, were reported in 2007.[10] Since then, they had been followed for an additional 6 years. The present study compiled all the data that had been accumulated on them over 18 years from 1991 to 2009, in an attempt to portray the outcome of undiagnosed HCV infection, and evaluate the efficacy of preventing liver disease by antiviral treatments. The 32 donors with normal aminotransferase levels at the initial diagnosis were excluded, because they visited clinics only once, and therefore the diagnosis of liver disease was not established in them. The remaining 987 donors entered the present study.

The study design conformed to the Declaration of Helsinki, and was approved by the Ethic Committees of Hiroshima University. Informed consent was obtained from each blood donor who was infected with HCV.

Data collection

A questionnaire form was distributed among hepatology specialists in the 20 institutions of the Hiroshima Hepatitis Study Group. They were asked to log the following: (i) initial diagnosis; (ii) compliance to regular visits; (iii) changes in liver disease over time; (iv) treatments with interferon (IFN); and (v) development of HCC. These data were made anonymous for the personal identification of any participant, and analyzed collectively.

Diagnosis of liver disease

Four clinical states were classified. They were: (i) persistently normal aminotransferase levels (PNAL); (ii) chronic hepatitis; (iii) cirrhosis; and (iv) HCC. PNAL was judged by: (i) values of alanine aminotransferase (ALT) within normal limits (≤40 IU/L) twice or more within 6 months at least 2 months apart; (ii) normal platelet counts ($\geq 150 \times 10^3/mm^3$); (iii) lack of abnormal findings in those examined by imaging modalities; and (iv) no pathological findings in the liver biopsy for those who received it. Each attending specialist was asked his/her comprehensive opinion on the absence of liver disease in the HCV-infected donor with PNAL. The

diagnosis of chronic hepatitis, cirrhosis and HCC was left to the judgment of the attending hepatology specialist, who took into consideration the results of biochemical, imaging and other tests. The specialist made the decision as to whether his/her patient should receive IFN-based treatment or would better be followed regularly without treatment.

Markers of HCV infection

Hepatitis C virus RNA was determined by reverse-transcription polymerase chain reaction (RT–PCR) with primers deduced from conserved sequences in the 5′-non-coding region of the genome, irrespective of genotypes.[11] Genotypes of HCV were determined by RT–PCR with type-specific primers[12]

Genotypes of the interleukin 28B (*IL28B*) gene

The three donors who cleared HCV spontaneously were examined for *IL28B* genotypes.[13–15] The genomic DNA was extracted from the serum by RT–PCR, and polymorphisms of the *IL28B* gene at rs12979860 and rs8099917 were determined by the direct sequencing.

IFN-based treatments

Interferon was administrated according to the regular protocol at the discretion of the attending doctor and with the agreement of his/her patient. Types of IFN were natural IFN-α, recombinant IFN-α2 and -α2b until 2002. Natural IFN or recombinant IFN-α2a or -α2b at a daily dose of 6–9 million units (MU) was administrated during the initial 2 weeks, followed by 3 MU three times per week until 24 weeks after the start of IFN (total dose, 300–342 MU). After 2002, ribavirin was combined with recombinant IFN. After 2005, the standard-of-care therapy was implemented with pegylated-IFN-α2b (PEG-Intron; Shering Plough, Kenilworth, NJ) s.c. at a median dose of 1.5 μg/kg [range, 1.3–2.0 μg/kg]) once a week until 48 weeks, together with a daily dose of 600–1000 mg ribavirin (Rebetol [600–1000 mg]; Shering Plough) that was adjusted by the bodyweight. Sustained virological response (SVR) to IFN was diagnosed by the clearance of HCV RNA from serum 24 weeks after the treatment completion and thereafter.

Statistical analyses

Means and proportions were compared between groups by Student's *t*-test and χ^2-test or Fisher's exact test, respectively. For comparison of the frequency of the response to IFN (SVR and non-SVR) and the lack of IFN treatments in blood donors in whom liver disease was improved, unchanged and aggravated, post-hoc pairwise comparisons were carried out using the χ^2-test, and the *P*-value was adjusted by the method of Bonferroni. The Kaplan–Meier's life-table analysis and Cox proportional hazards models were employed in assessing the risk of developing chronic hepatitis or HCC with reference to sex, age and diagnosis at the entry, as well as the response to IFN-based treatments, utilizing JMP ver. 9 software (SAS Institute, Cary, NC, USA). All *P*-values were two-tailed, and those less than 0.05 were considered statistically significant.

RESULTS

Compliance with the follow up of blood donors who had HCV infection

FIGURE 1 ILLUSTRATES the compliance with follow up and shifting status of blood donors with HCV over 18 years. Notably, 24.3% of them were lost to follow up within the first year. Dropouts increased to 46.8% at 10 years and reached 51.6% at 15 years. Exclusive of the 52 blood donors, who entered the study in the first year and were followed for 18 years, only approximately 20% of donors with HCV infection complied with observation between 14 and 17 years. HCV was cleared in 17.4% of them over 17 years, including 8.3% who complied with observation and 9.1% who were lost to follow up.

Liver disease in the 987 blood donors with HCV infection

Clinical states of the 987 blood donors with HCV infection, at the time of donation, are summarized in Table 1. Cirrhosis had developed in five (0.5%) and HCC in one (0.1%) already. Chronic hepatitis was diagnosed in 541 (54.8%) of them, leaving only 439 donors (44.5%) who had PNAL. Acute hepatitis was diagnosed in a single donor infected with HCV-2a, who had been negative for HCV RNA at the previous donation. He developed chronic hepatitis 9 months later, and achieved SVR to antiviral treatment 1 year thereafter. Chronic hepatitis was more frequent in men than women (65.7% vs 45.2%, $P < 0.001$). Conversely, women possessed PNAL more often than men (54.4% vs 33.3%, $P < 0.001$). Liver biopsy was performed in

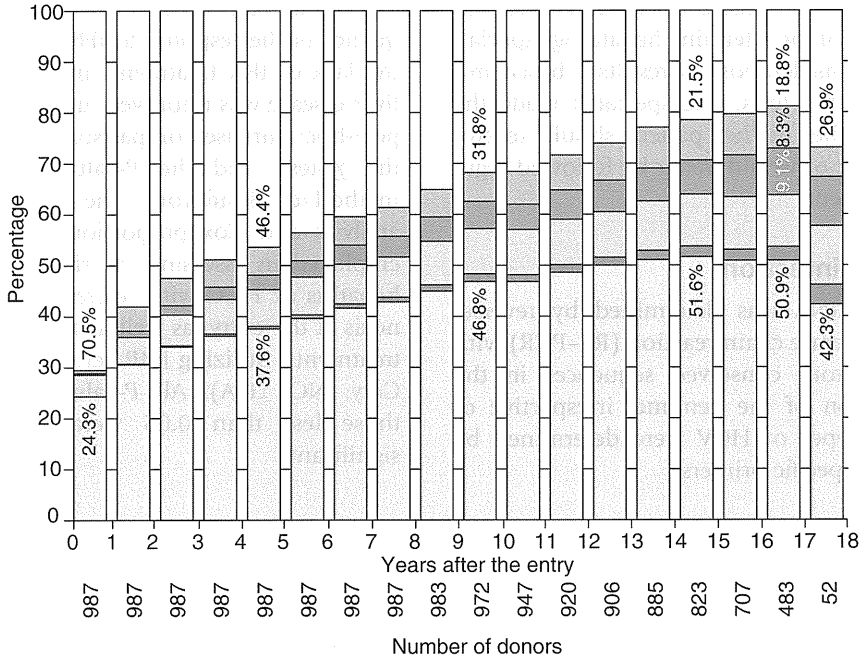

Figure 1 Follow up of the 987 donors with hepatitis C virus (HCV) infection over 18 years. □, on observation (HCV kept); ▨, on observation (HCV cleared); ▨, lost to follow up (HCV cleared); □, transferred (HCV kept); ■, deceased (HCV kept); □, lost to follow up (HCV kept).

356 (36.1%) of the 987 donors, and 393 (39.8%) received IFN-based treatments. Among the 709 donors, for whom genotyping was feasible, genotype 1b was the most prevalent both in men (67.6%) and women (65.7%).

Evolution of liver disease in the 987 donors with HCV during the follow up

Figure 2 depicts the outcome of 987 blood donors with HCV infection during an average follow-up period

Table 1 Clinical states of the 987 blood donors found with HCV infection at the donation

Features	Total ($n = 987$)	Men ($n = 463$)	Women ($n = 524$)	Differences Men vs. Women
Persistently normal aminotransferase levels	439 (44.5%)	154 (33.3%)	285 (54.4%)	$P < 0.001$
Chronic hepatitis	541 (54.8%)	304 (65.7%)	237 (45.2%)	$P < 0.001$
Cirrhosis	5 (0.5%)	3 (0.7%)	2 (0.4%)	$P = 0.670$
Hepatocellular carcinoma	1 (0.1%)	1 (0.2%)	0	$P = 0.470$
Acute hepatitis	1 (0.1%)	1 (0.2%)	0	$P = 0.470$
Age at the entry	45.1 ± 11.3	43.2 ± 11.1	46.8 ± 11.2	$P < 0.001$
Observation period (years)	7.3 ± 6.6	6.8 ± 6.4	7.7 ± 6.8	$P = 0.024$
Biopsy	356 (36.1%)	181 (39.1%)	177 (33.8%)	$P = 0.096$
IFN-based therapy	393 (39.8%)	190 (41.0%)	203 (38.7%)	$P = 0.503$
SVR	166 (42.2%)	84 (44.2%)	82 (40.4%)	$P = 0.507$
Genotypes identified	709 (71.8%)	339 (73.2%)	370 (70.6%)	$P = 0.402$
Genotype 1b	472 (66.6%)	229 (67.6%)	243 (65.7%)	$P = 0.366$
Genotype 2a	153 (21.6%)	71 (20.9%)	82 (22.2%)	$P = 0.962$
Genotype 2b	68 (9.6%)	35 (10.3%)	33 (8.9%)	$P = 0.512$
Mixed Genotype	16 (2.3%)	4 (1.2%)	12 (3.2%)	$P = 0.129$

SVR, sustained virological response.

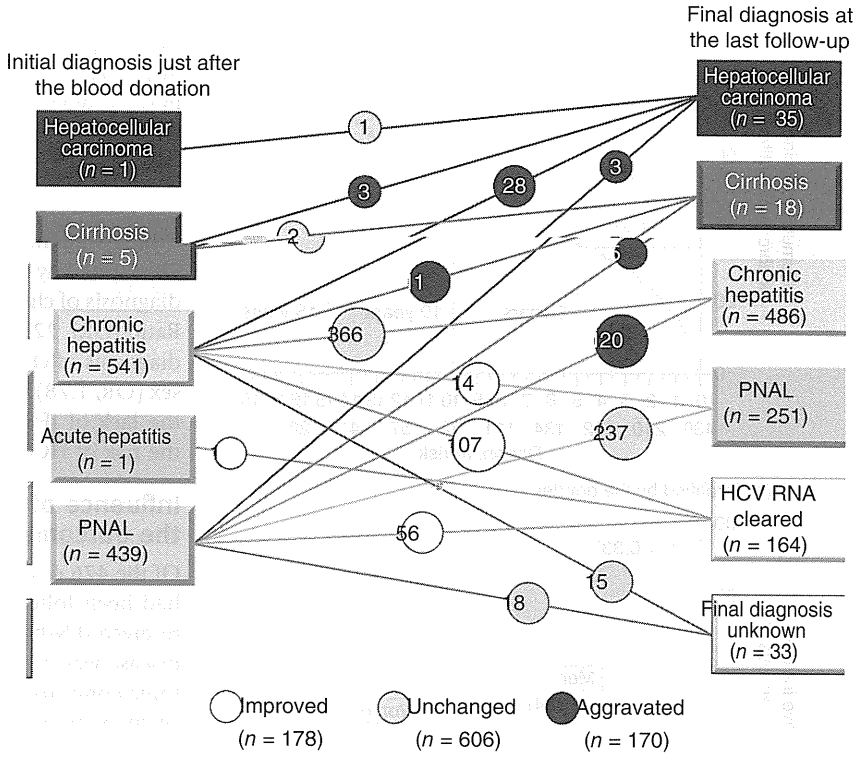

Figure 2 Outcome of hepatitis C virus (HCV) donors with HCV infection. ○, improved ($n = 178$); ◎, unchanged ($n = 606$); ●, aggravated ($n = 170$). PNAL, persistently normal aminotransferase levels.

of 7.3 ± 6.6 years; they were stratified by the initial diagnosis. Liver disease improved in 178 (18.0%), and remained stable in 606 (61.4%), whereas it worsened in the remaining 170 (17.2%). HCV infection was cleared by antiviral treatments in 107 of the 541 donors (19.8%) with chronic hepatitis. Chronic hepatitis developed in 120 of the 439 donors (27.3%) with PNAL. Of them, HCV infection was cleared in 54 by treatments given after they had developed chronic hepatitis, whereas it resolved spontaneously in two. Cirrhosis occurred in 16 donors (1.6%), including 11 with chronic hepatitis and five with PNAL at entry. HCC developed in 34 donors (3.4%), and in three of them, it was detected at 1, 4 and 7 years, respectively, after they had cleared HCV infection by antiviral treatments.

Cumulative incidence of chronic hepatitis in donors with PNAL

Figure 3 shows the cumulative incidence of chronic hepatitis in the 439 donors with PNAL. Chronic hepatitis developed in 36.2% by 5 years, 51.5% by 10 years and 72.9% by 15 years, with an annual incidence of 4.9–7.2% (Fig. 3a). Men tended to develop chronic hepatitis more frequently than women (Fig. 3b). Figure 3c compares the development of chronic hepatitis in donors with PNAL classified into four age groups. Chronic hepatitis developed in the group aged 40–49 years at entry, more frequently than in those aged 39 years or younger or 50 years or older, although the difference fell short of being significant ($P = 0.089$). The cumulative incidence of chronic hepatitis reached 66.3% by 10 years in the group aged 40–49 years at entry.

Development of HCC in donors with chronic hepatitis

Overall, 680 donors (68.9%) were diagnosed with chronic hepatitis either at entry or during this study, and they were followed for the development HCC. The cumulative incidence of HCC was 9.0% by 15 years after they had been diagnosed with chronic hepatitis (Fig. 4a). HCC developed comparably frequently in men and women (Fig. 4b). It developed more frequently ($P < 0.001$) in the donors in whom chronic hepatitis had been diagnosed at ages 60 years or older than 59 years or younger (Fig. 4c). HCC occurred less frequently ($P = 0.037$) in responders than non-responders to IFN, or the donors who did not receive IFN (Fig. 4d).

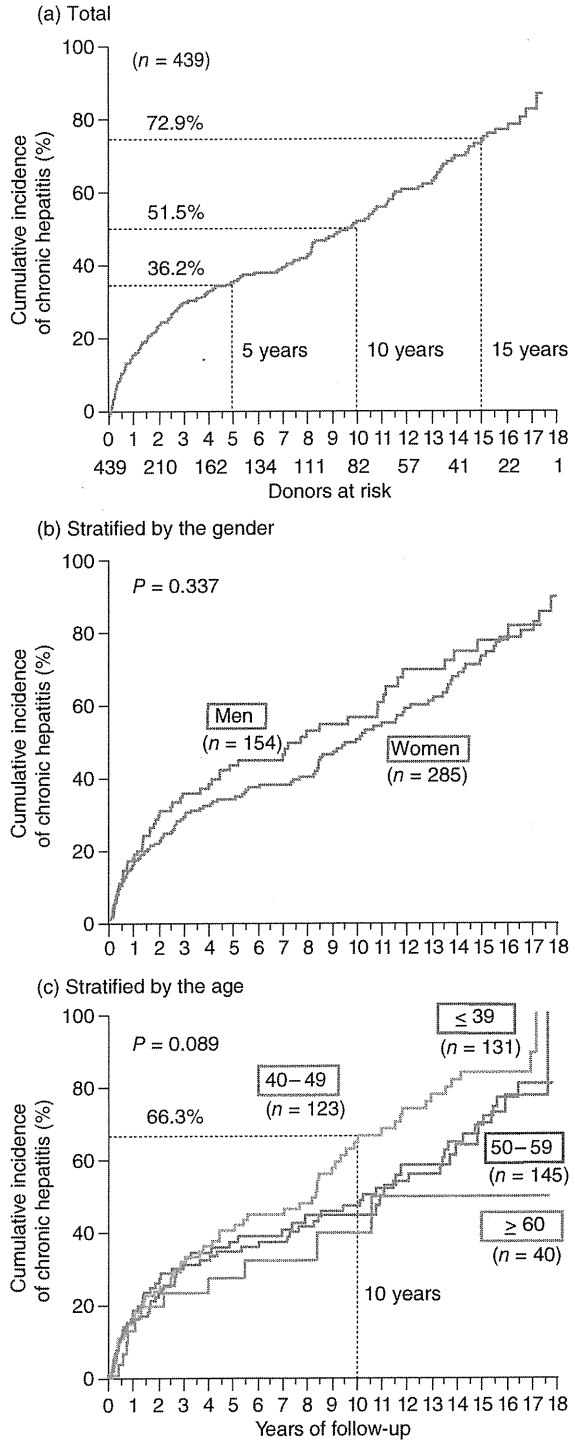

Figure 3 Cumulative incidence of chronic hepatitis in the 439 donors with persistently normal aminotransferase levels (PNAL). Development of chronic hepatitis is depicted in the total donors with PNAL (a), those stratified by sex (b), and those in different age groups (c). (b) Men, ($n = 154$); Women, ($n = 285$); (c) ≤39, ($n = 131$); 40–49, ($n = 123$); 50–59, ($n = 145$); ≥60

Independent risk factors for the development of HCC were sorted by multivariate analysis (Table 2). Age at the diagnosis of chronic hepatitis was the highest risk (Odds Ratio [OR], 22.70 for ≥60 years), followed by the initial diagnosis of chronic hepatitis (OR, 6.52) and the male sex (OR, 1.78). Failure to gain SVR to IFN (OR, 2.11), or the lack of IFN-based treatment (OR, 2.06), increased the risk of HCC, as well.

Influence of the response to IFN on the outcome of donors

Of the 476 donors with PNAL or chronic hepatitis, who had been followed for 5 years or longer, 280 (58.8%) received IFN-based treatments, and the outcome of liver disease was evaluated with reference to the response in three combinations, namely, SVR versus non-SVR, SVR versus without IFN, and non-SVR versus without IFN (Table 3). Of the 50 patients with improvement in liver disease, SVR was more frequent than non-SVR and without IFN ($P < 0.001$ for both). Of the 281 patients in whom liver disease did not change, SVR was more frequent than without IFN ($P < 0.001$), and non-SVR was more frequent than without IFN ($P < 0.01$). There were no differences among the frequency of SVR, non-SVR and without IFN in the 145 patients in whom liver disease aggravated.

Spontaneous clearance of HCV infection

Hepatitis C virus RNA was cleared from the serum in three donors who had not received antiviral treatments (Table 4). HCV RNA disappeared 6, 15 and 15 years, respectively, after they had been found with HCV infection at the blood donation. Two of them had PNAL and the remaining one had chronic hepatitis at entry. HCV genotypes were able to be determined in two, and they were 2a and 2b, respectively; neither of them was infected with the genotype 1b that is most prevalent and detected in 82% of Japanese blood donors.[12] All three donors possessed CC at rs12979860 and TT at rs8099917 in the *IL28B* gene, which increase the response to IFN in hepatitis C patients,[13–15] and promote the spontaneous clearance of HCV infection.[16] Among

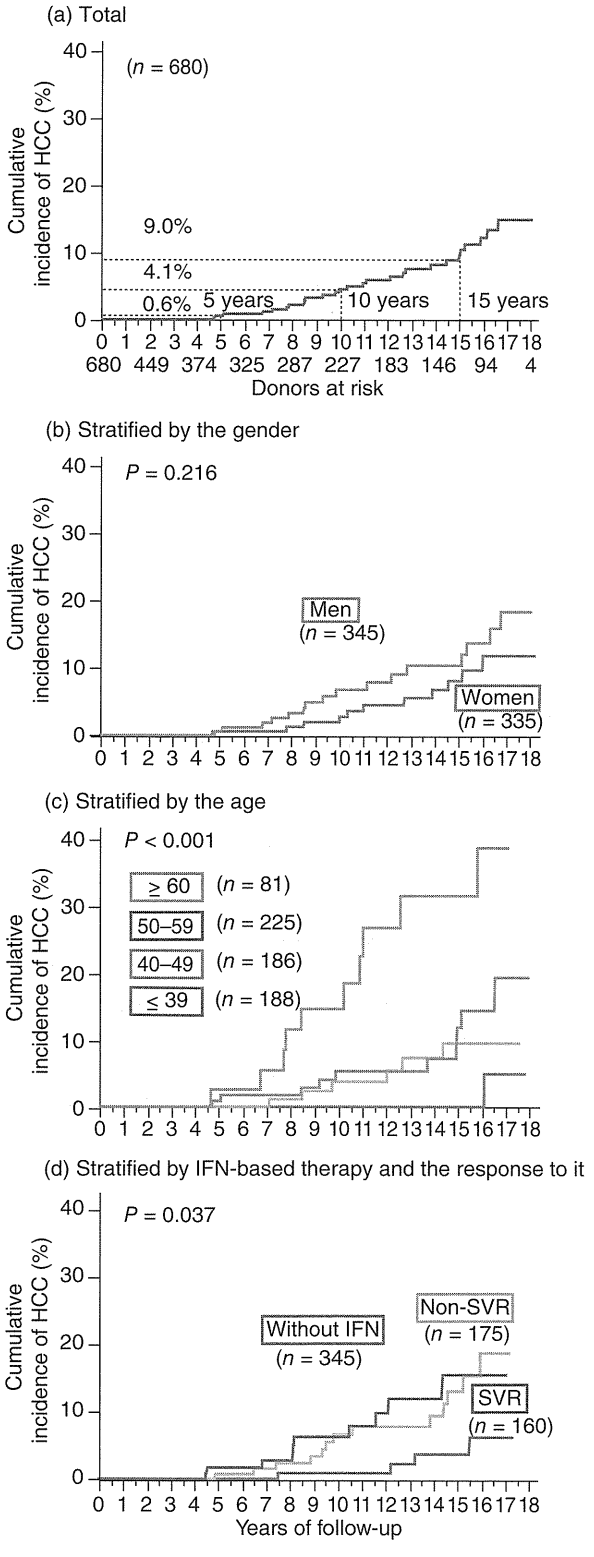

Figure 4 Cumulative incidence of hepatocellular carcinoma (HCC) in donors with chronic hepatitis. Development of HCC is portrayed in the 680 donors since the day of diagnosis with chronic hepatitis (start point) till the day of diagnosis with HCC (end-point) (a). Influence of sex (b), age (c) and interferon (IFN)-based treatments (d) is evaluated, also. (b) Men, ($n = 345$); Women, ($n = 335$); (c) ≥60, ($n = 81$); 50–59, ($n = 225$); 40–49, ($n = 186$); ≤39, ($n = 188$); (d) Non-SVR, ($n = 175$); Without IFN, ($n = 345$) SVR, ($n = 160$). SVR, sustained virological response.

the 316 donors with PNAL who did not receive IFN treatment, HCV was cleared spontaneously in only two, at the rate of $13.0/10^4$ person-years (95% confidence interval, $1.6–47.1/10^4$ person-years).

DISCUSSION

A LONG-TERM prospective study was performed on blood donors who were found to have HCV infection. For 18 years (1991–2009), 987 donors with HCV infection were followed up for an average of 7.3 years. The entry to this study was started in 1991 when an anti-HCV test was introduced to blood screening, and terminated in 2000 when merely a few donors were found to have HCV infection at the Hiroshima Red Cross Blood Center annually.[17] Despite our initial expectation, only one-third (32.5%) of blood donors with HCV infection visited liver clinics, even though they were advised to do so strongly. Another distressing issue was the low compliance with the study (Fig. 1). Within the first year after the entry, 24.3% of donors ceased to visit liver clinics. The rate of dropouts increased gradually to 46.8% over 10 years; it plateaued thereafter and stayed at 50.9% over 17 years. Thus, approximately one-half of blood donors with HCV infection were unable to receive any possible benefit of this study. It is not known whether these dropouts differed from the followed donors in the outcome of HCV infection and response to IFN-based treatments. Therefore, there remain possible biases in the results obtained only in the followed donors in the present study. We can say that the symptoms at the onset were almost the same among donors because they were well enough to give someone their blood regardless of whether they consulted later or not. It is of utmost importance to motivate blood donors with HCV infection to visit liver clinics, and encourage early dropouts to stay in follow up, to gain the full effect of screening blood donors.

Over 17 years of follow up, IFN-based treatments cleared HCV infection in 17.4%. Restricted to the

Table 2 Independent risk factors for the development of hepatocellular carcinoma in the 680 donors with chronic hepatitis

Factors	*n*	Odds ratio	95% CI	Differences
Sex				
Female	335	1.00		
Male	345	1.78	0.81–4.04	$P = 0.148$
Age (years) at the diagnosis of CH				
≤39	188	1.00		
40–49	186	5.32	0.90–100.94	$P = 0.068$
50–59	225	8.63	1.59–160.31	$P = 0.009$
≥60	81	22.70	4.14–424.53	$P < 0.001$
Initial diagnosis				
PNAL	161	1.00	7.94	
CH	519	6.52	1.36–116.95	$P = 0.014$
IFN treatment				
SVR	160	1.00		
Non-SVR	176	2.11	0.71–7.73	$P = 0.187$
Without	344	2.06	0.62–7.94	$P = 0.237$

CH, chronic hepatitis; CI, confidence interval; IFN, interferon; PNAL, persistently normal aminotransferase levels; SVR, sustained virological response.

donors who stayed on the surveillance (49.1%), 39.8% were helped by antiviral treatments. A high SVR of 42.2% was gained by blood donors who received IFN-based treatment. A similarly high SVR (42%) has been reported in blood donors with minimal to moderate liver disease who had received IFN monotherapy.[18] This would underscore the need for identifying people with undiagnosed HCV infection, and provide them with treatments as required. In this study, IFN-based treatments were offered to blood donors who presented with chronic hepatitis or those with PNAL who had developed chronic hepatitis. Since the standard-of-care therapy with pegylated IFN and ribavirin was initiated in the early 2000s, it has been indicated to HCV-infected individuals with PNAL and achieved an excellent efficacy.[19,20] If the indication of the combination therapy would have been extended to blood donors with PNAL in this study, a further gain may have been brought about in the clearance of HCV.

Sustained virological response was accomplished by the 166 donors with chronic hepatitis or PNAL who received IFN-based treatments. Spontaneous clearance of HCV without IFN treatment occurred in two of the 316 donors with PNAL at entry, at a rate of $13.0/10^4$ (95% confidence interval, $1.6–47.1/10^4$) person-years. Notably, they all possessed CC at rs12979860 and TT at rs8099917 in the *IL28B* gene, which improve the response to IFN,[13–15] and accelerate spontaneous clearance of HCV.[16]

Table 3 Clinical outcomes of the 280 donors with and the 196 without interferon-based treatment who had been followed for longer than 5 years

	Liver disease		
	Improved ($n = 50$)	Unchanged ($n = 281$)	Aggravated ($n = 145$)
Treatments			
With IFN			
SVR	42 (84.0%)[a]	62 (22.1%)[d]	34 (23.4%)
Non-SVR	1 (2.0%)[b]	85 (30.2%)[e]	56 (38.6%)
Without IFN	7 (14.0%)[c]	134 (47.7%)[f]	55 (37.9%)

Improved: a vs b, $P < 0.001$; a vs c, $P < 0.001$ (comparison was made by the post-hoc test with Bonferroni's adjustment).
Unchanged: d vs f, $P < 0.001$; e vs f, $P < 0.01$.
IFN, interferon; SVR, sustained virological response.

Table 4 Blood donors who resolved HCV infection spontaneously

Case No.	Sex	Age (years) at		Diagnosis at		Genotype	*IL28B* genotypes	
		Entry	HCV loss	Entry	HCV loss		rs12979860	rs8099917
1	Male	64	70	PNAL	Normal	2a	CC	TT
2	Female	43	58	PNAL	Normal	ND	CC	TT
3	Male	54	69	CH	LC/HCC	2b	CC	TT

CH, chronic hepatitis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LC, cirrhosis; ND, not determined due to low HCV RNA titers; PNAL, persistently normal aminotransferase levels.

Although blood donors with HCV infection were healthy, both subjectively and objectively, many had developed chronic hepatitis with the prevalence higher in men than women (65.7% vs 45.2%, $P < 0.001$). This would reflect the progression of hepatitis C that is more rapid in men than women.[21] Cirrhosis had developed in five of the 987 donors (0.5%) with HCV, and HCC accompanied by chronic hepatitis in one (0.1%) at entry. Donors with PNAL, defined by ALT below the upper limit of normal (≤40 U/L) at least twice during 6 months, accounted only for 44.5%. Development of significant liver disease in donors found to have HCV infection has been reported from various countries,[3–9] but a long-term follow up on a large scale was rarely performed. During a follow up for 7 years of 118 blood donors with HCV infection, 52.5% dropped out.[22]

Such a failure is ascribable to the lack of motivation of individuals with asymptomatic HCV infection who are forced to bear substantial economic and psychological burdens.[23] In Japan, 807 903 individuals are estimated to have undiagnosed HCV infection, corresponding to 0.63% of the total population.[24] The national campaign for screening undiagnosed HCV infections in the Japanese aged 40 years or older was started in 2002.[25] Although approximately one half of those infected with HCV visited hepatology specialists, only a minor portion of them (12–15% of those with HCV) have received IFN-based treatments (unpubl. obss.).

During the follow up of 7.3 ± 6.6 years, liver disease improved in 178 donors (18.0%), stayed unchanged in 606 (61.4%) and aggravated in 170 (17.2%). Although 237 of the 439 donors (54.0%) with PNAL kept normal ALT levels during a follow up of 7.3 years, the cumulative incidence of chronic hepatitis increased almost linearly over 15 years, from 36.2% at 5 years, to 51.5% at 10 years, and to 72.9% at 15 years (Fig. 3). Thus, chronic hepatitis developed in 7–8% of blood donors with PNAL yearly, at a rate comparable with that estimated by the Markov model based on the transition probability.[26]

Among 680 donors with chronic hepatitis at entry or in whom it was diagnosed during the follow up, HCC developed in 27 (4.0%). It increased steadily over time, and reached 4.1% and 9.0% at 10 and 15 years, respectively (Fig. 4). As expected, HCC developed more rapidly in the donors aged 60 years or older than 40–59 years; the development was the least frequent in the donors aged 39 years or younger. HCC developed less frequently in the donors with SVR to IFN-based treatments than those with no treatments or the lack of SVR. Independent factors for the development of HCC were age (OR, 22.70 for ≥60 years), initial diagnosis of chronic hepatitis (OR, 6.52), male sex (OR, 1.78) and response to IFN-based treatments (OR, 2.11 for non-responders and 2.06 for donors without IFN). These risk factors for HCC are in accord with those in previous reports.[27,28] The response to IFN-based treatments did not influence the risk for HCC (OR, 2.11 for non-responders [$P = 0.187$] and 2.06 for donors without IFN [$P = 0.237$]). In view of the influence of SVR on the risk of HCC observed in the Kaplan–Maier analysis (Fig. 4d), it would be expected to gain significant difference in the risk for HCC in the multivariate analysis by studying patients in larger scales. It has to be noted that HCC developed in three donors 1, 4 and 7 years, respectively, after they had achieved SVR to antiviral treatments. This underscores the need for continuing the follow up of donors with chronic hepatitis who have achieved SVR.

In conclusion, this study has demonstrated a wide range of liver disease, low compliance with follow up and high efficacy to IFN-based treatments in blood donors found with HCV infection. The results reported herein are hoped to help in coping with many undiagnosed HCV infections over the world, which are predicted to increase in the foreseeable future.[29] Efforts

along this line would improve the health-care of people with undiagnosed HCV infection, and decrease social and economic burdens on the nation.

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Research Article

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Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan

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Background & Aims: To evaluate the efficacy and safety of telaprevir in combination with peginterferon-α2b (PEG-IFN) and ribavirin (RBV) in patients with chronic hepatitis C.

Methods: In a multi-center randomized clinical trial in Japan, on patients infected with HCV of genotype 1, 126 patients were assigned to telaprevir for 12 weeks along with PEG-IFN and RBV for 24 weeks (Group A), while 63 to PEG-IFN and RBV for 48 weeks (Group B).

Results: HCV RNA disappeared more swiftly in patients in Group A than B, and the frequency of patients without detectable HCV RNA at week 4 (rapid virological response (RVR)) was higher in Group A than B (84.0% *vs.* 4.8%, p <0.0001). Grade 3 and 4 skin disorders, including Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms, as well as Grade 3 anemia (<8.0 g/dl), occurred more frequently in Group A than B (skin disorders, 11.9% *vs.* 4.8%; anemia, 11.1% *vs.* 0.0%). The total RBV dose was smaller in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). Despite these drawbacks, sustained virological response (SVR) was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020).

Conclusions: Although the triple therapy with telaprevir-based regimen for 24 weeks resulted in more adverse events and less total RBV dose than PEG-IFN and RBV for 48 weeks, it was able to achieve higher SVR within shorter duration by carefully monitoring adverse events and modifying the RBV dose as required.

Keywords: Telaprevir; Chronic hepatitis C; Peginterferon; Ribavirin; Sustained virological response; Genotypes.

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Abbreviations: PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; SOC, standard of care; DAA, direct acting antiviral.

Introduction

Over the world, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1]. Most individuals with persistent HCV infection can fulfill the life expectancy, while about 30% of them develop life-threatening liver disease such as decompensated cirrhosis and hepatocellular carcinoma [2,3].

Currently, interferon (IFN) is the only antiviral drug capable of terminating HCV infection. The present standard-of-care (SOC) therapy for patients infected with HCV of genotype 1, the most prevalent genotype over the world, is peginterferon (PEG-IFN) combined with ribavirin (RBV) for 48 weeks. However, sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after the completion of therapy, can be achieved in only 42–52% of the patients [4–6]. To cope with this grim situation, a number of direct acting antivirals (DAAs) have been designed and developed, represented by NS3/4A protease inhibitors and NS5B polymerase or NS5A inhibitors [7]. Among them, telaprevir has shown promising results, when combined with PEG-IFN and RBV, in the phase 2 [8,9] and 3 clinical trials [10,11], by improving SVR to ~70% in patients infected with HCV-1.

Previous trials with the triple therapy were conducted in Europe and the United States, respectively. Hence, Asians were under-represented, accounting only for 1.6–2.1% of studied patients, and distributions of genotypes 1a (44–67%) and 1b (27–55%) varied widely [8–10]. In view of ethnic differences in response to IFN-based treatments [12,13], as well as profiles of resistance to telaprevir difference between genotypes 1a and 1b [14], a multicenter, randomized, and treatment-controlled clinical trial was conducted for comparison of therapeutic efficacy between the triple therapy and SOC in patients infected with HCV-1b in Japan.

Patients and methods

Patients

From November 2008 through August 2010, 220 patients, who were infected with HCV-1 and had not received antiviral treatments before, were recruited at 41 institutions in Japan. They joined the study for finding differences in the

Table 1. Baseline characteristics of patients.

Features[a]	Group A: T12PR24 (n = 126)	Group B: PR48 (n = 63)
Men (%)	66 (52.4%)	33 (52.4%)
Age (years)	53.0 (20-65)	55.0 (20-65)
Weight (kg)	60.2 (40.7-87.5)	64.1 (42.1-84.9)
BMI (kg/m^2)	22.6 (16.2-31.1)	23.3 (17.9-30.8)
Hemoglobin (g/dl)	14.3 (12.1-17.1)	14.5 (12.3-17.5)
White blood cells (/mm^3)	5300 (2900-10,670)	5130 (2950-11,050)
Platelets (x10^4/mm^3)	19.2 (9.0-36.2)	20.2 (8.7-37.0)
ALT (IU/L)	36.5 (12-252)	45.0 (18-259)
AST (IU/L)	34.0 (18-170)	38.0 (17-142)
Total bilirubin (mg/dl)	0.70 (0.3-1.9)	0.80 (0.4-1.8)
Total cholesterol (mg/dl)	182 (111-299)	180 (116-263)
HCV RNA ($\log_{10}$ IU/ml)	6.7 (5.1-7.5)	6.9 (5.1-7.4)
HCV genotypes		
1a	2 (1.6%)	0 (0.0%)
1b	124 (98.4%)	63 (100.0%)

[a]Values are the median with the range in parentheses, or number with the percentage in parentheses.

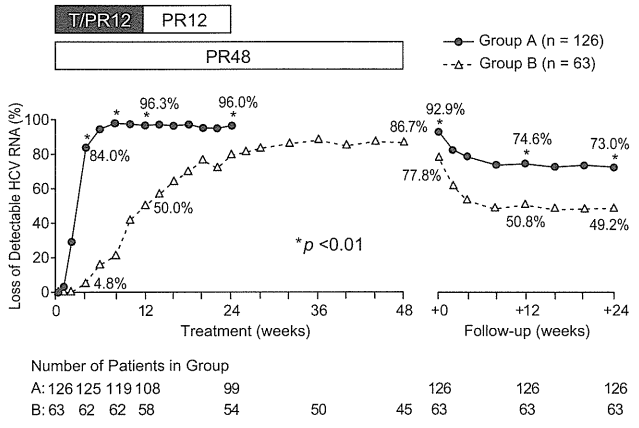

Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 differences. The number of patients at each time point is indicated below the graph.

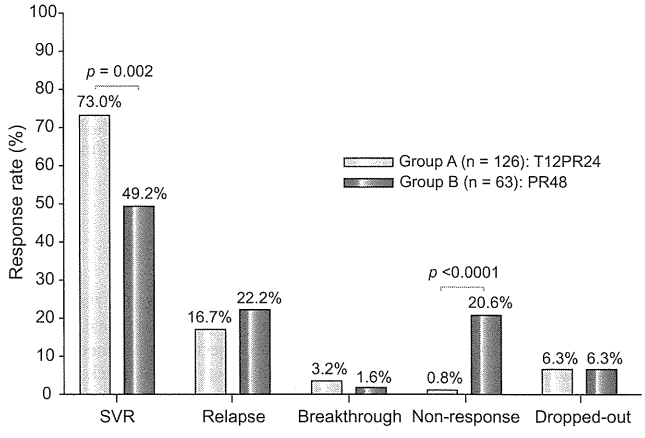

Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment period; non-response, HCV RNA continuously detectable in serum during treatment period.

treatment response and adverse events between the triple therapy involving telaprevir, PEG-IFN and RBV, and SOC with PEG-IFN and RBV. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of each institution. Each patient gave a written informed consent before participating in this study.

Study design

This prospective, multi-center, and randomized study was planned on Japanese patients with chronic hepatitis C who met inclusion and did not meet exclusion criteria. Main inclusion criteria were: (a) diagnosed with chronic hepatitis C, and had not received antiviral treatments before; (b) infected with HCV-1 confirmed by the sequence analysis in the NS5B region; (c) had HCV RNA levels $\geqslant$5.0 $\log_{10}$ IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (d) Japanese aged from 20 to 65 years at the entry; (e) had the body weight between >40 and $\leqslant$120 kg; (f) were not pregnant and capable of contraception till 24 weeks after the treatment; and (g) agreed on the admission for 15 days since the treatment start. Main exclusion criteria were: (a) decompensated liver cirrhosis; (b) hepatitis B surface antigen; (c) hepatocellular carcinoma or other malignancy, or its history; (d) autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (e) depression or schizophrenia, or its history, or history of suicide attempts; (f) chronic renal disease or creatinine clearance $\leqslant$50 ml/min at the baseline; (g) hemoglobin <12 g/dl, neutrophil counts <1500/mm^3 or platelet counts <100,000/mm^3 at the baseline; and (h) pregnancy in progress or planned during the study period of either partner.

Patients were randomly assigned to either of the following two treatment groups in a 2:1 ratio, with stratification to balance sex and age: (1) the triple therapy with telaprevir, PEG-IFN, and RBV for 12 weeks, followed by PEG-IFN and RBV for an additional 12 weeks (Group A: T12PR24); and (2) SOC with PEG-IFN and RBV for 48 weeks (Group B: PR48). After the treatment was completed or discontinued, they were followed for $\geqslant$24 weeks for SVR evaluation. Patients were followed regularly for subjective symptoms and objective signs, as well as blood

Table 2. Comparison of SVR stratified by demographic and virological factors as well as discontinuation of study drugs between two groups with different therapeutic regimens.

	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
Gender			
Men	50/66 (75.8%)	18/33 (54.5%)	0.0400
Women	42/60 (70.0%)	13/30 (43.3%)	0.0214
Age (years)			
≤49	35/41 (85.4%)	13/21 (61.9%)	0.0543
≥50	57/85 (67.1%)	18/42 (42.9%)	0.0125
HCV RNA (log_{10} IU/ml)			
≥7	18/26 (69.2%)	5/18 (27.8%)	0.0132
<7	74/100 (74.0%)	26/45 (57.8%)	0.0556
Discontinuation of study drugs			
Not discontinued	66/79 (83.5%)	27/46 (58.7%)	0.0030
All drugs discontinued	14/27 (51.9%)	4/17 (23.5%)	0.1143

counts and chemistry. HCV RNA levels were monitored at day −28, days 1 (pre-dose), 2, and 3, weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 (both groups), as well as weeks 26, 28, 32, 36, 40, and 48 (Group B), during the treatment period; they were monitored at weeks 2, 4, 8, 12, 16, 20, and 24 in the follow-up period (both groups).

HCV RNA and genotypes

HCV RNA was quantified using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log_{10} IU/ml, and samples with no HCV RNA detected were reported as: <1.2 log_{10} IU/ml (no HCV RNA detectable). Genotypes of HCV were determined by direct sequencing followed by phylogenetic analysis of the NS5B region [15].

Antiviral treatments

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) 750 mg was administered three times a day at an 8-h interval (q8h) after each meal. Peginterferon-α2b (PegIntron®, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.250–1.739 μg/kg) once a week. Ribavirin (Rebetol®, MSD, Tokyo, Japan) 200–600 mg was administered after breakfast and dinner. The daily dose of RBV was adjusted to the body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ∼≤80 kg; and 1000 mg for >80 kg.

RBV dose was diminished by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased <12 g/dl, and by extra 200 mg when it lowered <10 g/dl. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dl at baseline or in those in whom it decreased by 1 g/dl within a week and below 13 g/dl. Dose modification of RBV in Group B was conducted in accordance with SOC. PEG-IFN dose was reduced to one half, when leukocyte counts decreased <1500/mm^3, neutrophil counts <750/mm^3 or platelet counts <8 × 10^4/mm^3; PEG-IFN was discontinued when they decreased <1000/mm^3, 500/mm^3 or 5 × 10^4/mm^3, respectively. The triple therapy was discontinued or interrupted when hemoglobin decreased <8.5 g/dl. In patients whose hemoglobin increased ≥8.5 g/dl within 2 weeks after the interruption, treatment was resumed with PEG-IFN and RBV 200 mg. The reduction of telaprevir dose was not permitted.

Statistical analysis

SVR was evaluated in the full analysis set. The difference in SVR between Groups A and B with the 2-sided 95% confidence interval (CI) was calculated with the adjustment for sex and age, and *p* value was evaluated by the Wald-test. Continuous variables between groups were compared by the Mann–Whitney test (*U*-test), and categorical variables by the Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC), and a *p* value <0.05 was considered significant.

Results

Patient cohorts

Of the 220 Japanese patients from whom an informed consent was obtained, 31 (14.1%) were found not eligible for the study entry. The remaining 189 patients were randomly assigned to T12PR24 (Group A [n = 126]) or PR48 (Group B [n = 63]). Overall, 114 out of the 126 (90.0%) patients in Group A and 54 out of the 63 (85.7%) in Group B completed the full study period. Table 1 compares baseline characteristics of studied patients in Groups A and B. There were no differences in demographic characters, hematology, biochemistry, or virology between the two groups of patients.

Loss of HCV RNA during treatment

Dynamics of HCV RNA during treatment was much different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, p <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Time courses of the loss of HCV RNA are compared in Fig. 1. The loss of HCV RNA increased constantly, sharply, and swiftly in Group A. By contrast, in Group B, it gradually increased during the first 24 weeks of treatment. Rapid virological response at 4 weeks (RVR) occurred more frequently in Group A than B (84.0% *vs.* 4.8%, p <0.0001). HCV RNA was undetectable in >90% of patients in Group A, while it stayed undetectable in <80% of patients in Group B at the start of follow-up. After treatment completion, HCV RNA re-appeared in patients in both Groups A and B (16.7% *vs.* 22.2%, p = 0.4272).

Responses to treatments

Fig. 2 compares treatment responses between Groups A and B. SVR was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020). By contrast, non-response was less frequent in Group A than B (0.8% *vs.* 20.6%, p <0.0001). The difference in SVR between Groups A and B, adjusted for sex and age, was 23.8% (95% CI: 9.4–38.2%, p = 0.0012, Wald-test).

Table 3. Adverse events developing in more than 15% of patients in either Groups A or B.

	A: T12PR24 (n = 126)		B: PR48 (n = 63)	
Anemia	115	(91.3%)	46	(73.0%)
Pyrexia	98	(77.8%)	46	(73.0%)
Leukocytopenia	86	(68.3%)	46	(73.0%)
Thrombocytopenia	81	(64.3%)	23	(36.5%)
Malaise	73	(57.9%)	30	(47.6%)
Serum uric acid increased	65	(51.6%)	5	(7.9%)
Serum hyaluronic acid increased	64	(50.8%)	25	(39.7%)
Alopecia	51	(40.5%)	29	(46.0%)
Headache	48	(38.1%)	32	(50.8%)
Skin rashes	48	(38.1%)	18	(28.6%)
Anorexia	42	(33.3%)	17	(27.0%)
Insomnia	40	(31.7%)	17	(27.0%)
Vomiting	37	(29.4%)	9	(14.3%)
Drug eruption	37	(29.4%)	2	(3.2%)
Arthralgia	36	(28.6%)	15	(23.8%)
Serum triglycerides increased	36	(28.6%)	11	(17.5%)
Dysgeusia	34	(27.0%)	10	(15.9%)
Diarrhoea	34	(27.0%)	19	(30.2%)
Nausea	32	(25.4%)	7	(11.1%)
Serum creatinine increased	32	(25.4%)	0	
Erythema at the injection site	33	(26.2%)	21	(33.3%)
Reactions at the injection site	29	(23.0%)	16	(25.4%)
Stomatitis	24	(19.0%)	12	(19.0%)
Abdominal discomfort	23	(18.3%)	12	(19.0%)
Pruritus	23	(18.3%)	13	(20.6%)
Nasopharyngitis	23	(18.3%)	18	(28.6%)
Influenza-like symptoms	22	(17.5%)	16	(25.4%)
Serum bilirubin increased	22	(17.5%)	13	(20.6%)
Back pain	21	(16.7%)	12	(19.0%)
Hyperuricemia	20	(15.9%)	2	(3.2%)
Serum phosphorus decreased	16	(12.7%)	13	(20.6%)
Constipation	14	(11.1%)	13	(20.6%)
Erythema	9	(7.1%)	13	(20.6%)

Factors influencing the treatment response are compared in Table 2. SVR was higher in Group A than B, irrespective of different genders, age ranges, or HCV RNA loads. Of note, SVR in women in Group A was higher than that in Group B (70.0% *vs.* 43.3%, $p = 0.0214$). Likewise, SVR in patients $\geqslant 50$ years was higher in Group A than B (67.1% *vs.* 42.9%, $p = 0.0125$), and that in patients with high HCV RNA loads ($\geqslant 7$ $\log_{10}$ IU/ml) at the baseline was higher in Group A than B (69.2% *vs.* 27.8%, $p = 0.0132$).

Adverse events

Adverse events occurred in all patients in both Groups A and B. Adverse events with a frequency >15% in either group are listed in Table 3. Of them, frequencies of anemia, thrombocytopenia, malaise, and elevated serum levels of uric acid as well as hyaluronic acid were >10% higher in Group A than B. Most of them were mild, and severe and serious adverse events occurred in small proportions of patients (9.5% and 11.9% in Group A, respectively, and 9.5% and 9.5% in Group B). All drugs were discontinued due to adverse events comparatively frequently in Groups A and B (16.7% and 22.2%, respectively), and telaprevir alone in 19.0% of patients in Group A. The total dose of RBV was less in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). Doses of antiviral treatments were reduced or discontinued in some patients with moderate to severe adverse events, patients were taken care of by specialists, and received specific therapies when necessary. Eventually, all patients recovered from adverse events.

Hematological disorders

Anemia occurred in 91.3% and 73.0% of patients in Groups A and B, respectively. Table 4 compares the severity of anemia between Groups A and B. Combined, Grade 1 and 2 anemia developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Grade 3 anemia occurred in 11.1% in Group A only. During the follow-up, hemoglobin increased both in Groups A and B, and returned to pretreatment levels 12 weeks after the completion of therapy and thereafter (Fig. 3A). Platelet counts decreased more extensively in Group A than B (Fig. 3B). They rebounded after the completion of therapy, and then returned to pretreatment values. Decreases in neutrophil counts were milder in Group A than B (Fig. 3C). Both in Groups A and B, neutrophils started to increase immediately after the treatment completion, and returned to pretreatment levels within 12 weeks.

Skin disorders

Skin disorders were monitored at every hospital visit for severity and extent, and they were categorized into four Grades (Table 4). When skin disorders of Grades 2–4 occurred, the attendant physician was instructed to consult with a dermatologist in each institution for the diagnosis and specific cares, and telaprevir was discontinued, while PEG-IFN and RBV were reduced or discontinued, as required. Skin disorders were mainly rash, drug eruptions, and erythema. They occurred comparably frequently in Groups A and B (89.7% and 84.1%, respectively). Most skin disorders were mild and categorized into Grade 1 in 75.4% and 76.2% of patients in Groups A and B, respectively. Combined, skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). Due to skin disorders, at least one drug was discontinued in merely 9.5% and 3.2% of patients in Groups A and B, respectively, and most skin disorders were controllable by anti-histamine and/or steroid ointments.

Serious skin disorders developed in three patients in Group A, but none in Group B. Stevens–Johnson syndrome occurred in one patient 35 days after the treatment start, and led to the discontinuation of treatment. Erythema spread widely in the trunk (Fig. 4A), as well as limbs and the face. Erosion of oral mucosae, epidermal detachment, conjunctival redness, high fever to reach 39.3 °C, and lymphadenopathy were also noted. Histopathology showed the epidermal necrosis, satellite-cell necrosis, and perivascular dermatitis with infiltration of lymphocytes, neutrophils, and eosinophils in the superficial dermis (Fig. 4B). The patient was admitted and received steroids intravenously, and recovered completely within 9 weeks. Drug rash with eosinophilia and

Table 4. Decreases in hemoglobin levels and skin disorders according to the grade.

Grade	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
A Hemoglobin levels			
Grade 1 (9.5- <11.0 g/dl)	50 (39.7%)	32 (50.8%)	0.1631
Grade 2 (8.0- <9.5 g/dl)	34 (27.0%)	11 (17.5%)	0.2043
Grade 3 (<8.0 g/dl)	14 (11.1%)	0	0.0055
Total	98 (77.8%)	43 (68.3%)	0.1613
B Skin disorders			
Grade 1[a]	95 (75.4%)	48 (76.2%)	1.0000
Grade 2[b]	44 (34.9%)	12 (19.0%)	0.0282
Grade 3[c]	13 (10.3%)	3 (4.8%)	0.2709
Grade 4[d]	2 (1.6%)	0 (0.0%)	0.5532
Any grade	113 (89.7%)	53 (84.1%)	0.3451

[a]Localized skin lesions.
[b]Diffuse or multiple skin lesions.
[c]Skin lesions covering >50% of the body surface or rashes with some characteristics such as bullae, ulceration of mucous membrane, epidermal detachment, target lesion or significant systemic signs.
[d]Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS) were categorized in Grade 4.

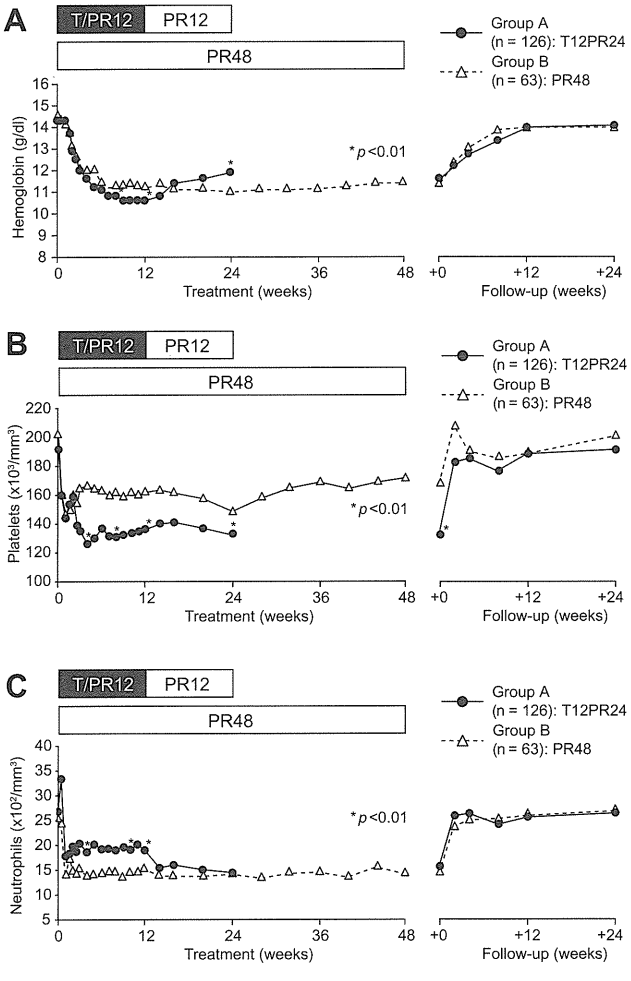

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and at weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 difference.

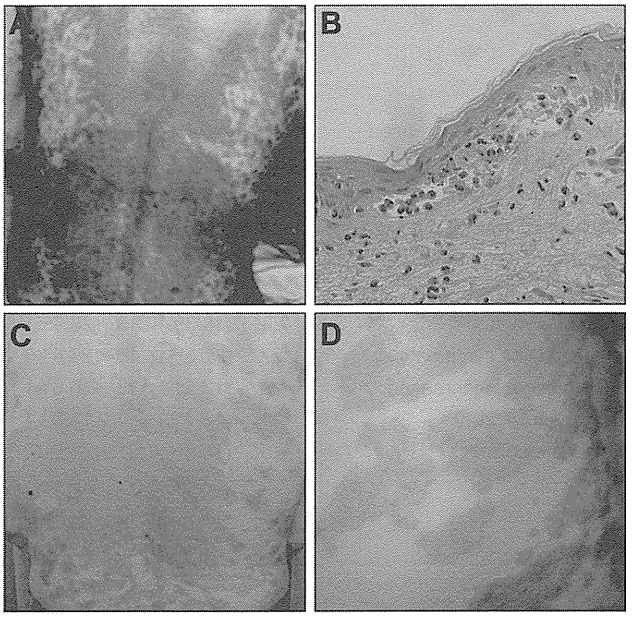

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are shown.

systemic symptoms (DRESS or drug-induced hypersensitivity syndrome) occurred in another patient. Fresh red erythema appeared on the whole body, and fresh red-colored target lesions (up to 3–4 cm in diameter) were also observed (Fig. 4C and D). Edema in the face, lymphadenopathy, fever up to 39.7 °C, and erosion of oral mucosae were noted, also. Maximum levels of white blood cells, eosinophils, and atypical lymphocytes were 46,300/mm^3, 45.7%, and 23.3%, respectively. Titers of IgG antibody to human herpes virus 6 were ×160 (29 days after the onset) and ×2560 (57 days). The remaining patient developed erythema multiforme. These two patients received steroids orally and recovered completely within 14 weeks.