serum HBV DNA during immunosuppressive therapy and administer NAs should it be detected. The second measure is to administer NAs from the onset of immunosuppressive therapy. The third measure is to maintain circulating HBsAb titer using HB vaccines and/or HB immunoglobulins. Reports have suggested that regular evaluation of HBV DNA is effective in avoiding de novo hepatitis in patients treated with TNF-α inhibitors because HBV reactivation could be controlled by NAs when found at an early stage.[46,49] It is still unclear how often and for how long patients should be tested to detect HBV viremia. Prophylactic administration of NAs is also an option to preempt de novo hepatitis B due to TNF-α inhibitors because NAs are normally used to prevent reactivation in carrier patients. However, the issue of cost-efficiency versus relatively low incidence of de novo hepatitis B needs to be reconciled. Lastly, maintenance of circulating HBsAb titer using HB vaccines may be effective in responders since several studies[44,46] have shown that HBsAb titer decreases during TNF-α inhibitor therapy. As with HBV DNA monitoring and prophylactic NA administration, further studies are required to clarify the extent of HB vaccination effectiveness in preventing de novo hepatitis B due to TNF-α inhibitors.

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Original article

Serum interleukin (IL)-10 and IL-12 levels and *IL28B* gene polymorphisms: pretreatment prediction of treatment failure in chronic hepatitis C

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Background: Both *IL28B* gene polymorphisms and serum levels of interleukin (IL)-10, IL-12p40 and IL-18 have been reported to affect the outcome of natural and pegylated interferon and ribavirin-treated HCV infection.
Methods: To clarify their association and predictive value in treatment outcome of genotype 1 HCV-infected patients, we measured pretreatment serum IL-10, IL-12p40 and IL-18 levels using multiplex assays and determined *IL28B* gene polymorphisms (rs 8099917) in 52 cases with chronic hepatitis C.
Results: High baseline levels of IL-10 ($P<0.001$) and low levels of IL-12p40 ($P<0.001$) were significantly associated with a non-virological response (NVR) in our cohort. The *IL28B* polymorphism was tested and TT, TG or GG genotypes were found in 60%, 38% and 2% of patients, respectively, with corresponding NVR rates of 10%, 60% and 100% ($P<0.001$). Serum cytokine levels were significantly correlated with *IL28B* gene polymorphisms. When serum IL-10 levels were stratified at 5.0 pg/ml, NVR rates were 50% versus 0% ($P=0.004$) for the TT genotype and 87% versus 0% ($P=0.001$) for the TG or GG genotypes. Similarly, low IL-12p40 levels were associated with an NVR in patients with TG or GG genotypes ($P=0.006$). In multivariate analysis, high IL-10, low IL-12p40 and *IL28B* TG or GG genotypes were independently associated with an NVR.
Conclusions: Serum IL-10 and IL-12p40 levels in combination with *IL28B* genotype, especially G-allele carriage, are strong predictive markers of an NVR to HCV treatment with pegylated interferon and ribavirin.

Introduction

Chronic HCV infection often develops into chronic hepatitis leading to liver cirrhosis and/or hepatocellular carcinoma [1–3]. The successful eradication of HCV, defined as a sustained virological response (SVR), is therefore considered important. Despite recent advances, however, approximately 50% of patients with genotype 1 HCV infection do not achieve an SVR by conventional pegylated interferon (PEG-IFN) and ribavirin therapy [4,5].

It is considered beneficial to predict the response of patients with genotype 1 HCV and high viral load to PEG-IFN and ribavirin therapy before commencement of treatment because therapy can be long, costly and have many side effects. To date, many predictive factors have been reported for treatment response. Regarding viral factors, substitutions at core amino acids 70 and 91 [6] or the IFN sensitivity determining region (ISDR) have been reported [7]. Concerning host factors, Ge *et al.* [8] recently identified single nucleotide polymorphisms (SNPs) located 5′ to the *IL28B* gene that affect response to combination therapy using a genome-wide association study. Similarly, three other groups independently reported that these SNPs are associated with the effectiveness of combination treatment [9–11]. Thomas *et al.* [12] also reported that the same SNPs are associated with spontaneous clearance of HCV.

Interleukin (IL)-28A, IL-28B and IL-29 gene products belong to the IFN-λ family. These cytokines are functionally considered to be IFNs, but have been reported to be structurally related to the IL-10 family, which include IL-10, IL-22 and IL-26, and the

Table 1. Demographic and clinical characteristics of patients with chronic hepatitis C

Characteristic	All (*n*=52)	VR (*n*=36)	NVR (*n*=16)	*P*-value
Age, years	58 (17–74)	57 (17–72)	60 (45–74)	0.781
Male, *n* (%)	24 (46)	18 (50)	6 (38)	0.404
Body mass index, kg/m^2	23 (18–30)	24 (18–30)	22 (19–29)	0.115
White blood cell count, cells/μl	4,470 (1,980–7,890)	4,810 (1,980–7,890)	3,700 (2,270–5,180)	0.007
Haemoglobin, g/dl	14.7 (12–18)	15.0 (13–18)	14.1 (12–16)	0.094
Platelet count, 10^4/μl	17.5 (8–30)	17.9 (8–30)	16.7 (9–27)	0.420
ALT, IU/l	75 (22–389)	68 (24–389)	91 (22–357)	0.663
AST, IU/l	58 (20–288)	49 (20–218)	78 (25–288)	0.092
HCV RNA, 10^5 IU/ml	21 (1.1–>50)	20 (1.1–>50)	18 (2.9–>50)	0.469
Core aa 70 (Arg70/Gln70/ND), *n*	30/21/1	23/12/1	7/9/0	0.139
Core aa 91 (Leu91/Met91/ND), *n*	37/14/1	26/9/1	11/5/0	0.463
ISDR of NS5A (wild/mutant), *n*	44/8	29/7	15/1	0.218
rs8099917 allele (TT/TG/GG), *n*	31/20/1	28/8/0	3/12/1	<0.001

Data are mean (range) unless indicated otherwise. aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ISDR, interferon-sensitivity determining region; NVR, non-virological response; VR, virological response.

IFN-λ family. The ligand-binding chains for IL-22, IL-26 and IFN-λ are distinct from that used by IL-10. However, all of these cytokines use a common second chain, IL-10 receptor-2, to assemble their active receptor complexes. Thus, IL-10 receptor-2 is a shared component in at least four distinct class II cytokine-receptor complexes [13]. Although IL-10 was originally described as a cytokine synthesis inhibitory factor [14,15], recent studies have demonstrated that IL-10 produced by Th17 cells restrains the pathological effects of Th17 [16,17]. Furthermore, increased IL-10 levels are associated with a high risk of inefficient HCV clearance and resistance to IFN treatment [18–21]. Our recent study showed that low serum IL-10 levels as well as high IL-12p40 and IL-18 levels at baseline were independent predictive factors for a SVR to combination therapy [22]. Therefore, in the present study, we investigated the association between treatment outcome and the influence of *IL28B* genotype and serum cytokine levels in combination therapy.

Methods

Subjects

A total of 52 consecutive treatment-naive patients with genotype 1 chronic hepatitis C were included in this study. Diagnosis of chronic hepatitis C was based on the following criteria as reported previously [23]: presence of serum HCV antibodies and detectable viral RNA, absence of detectable hepatitis B surface antigen and antibody to HIV and exclusion of other causes of chronic liver disease. No patients had a history of or developed decompensated cirrhosis or hepatocellular carcinoma. The baseline characteristics of the patients are shown in Table 1.

Laboratory testing

Antibodies to HCV were measured in serum samples via third-generation enzyme-linked immunosorbent assays (EIA-3; Abbott Laboratories, North Chicago, IL, USA). Serum levels of HCV RNA were determined using the Cobas Amplicor assays (sensitivity 50 IU/ml; Roche Diagnostic Systems, Tokyo, Japan). HCV genotypes were determined using INNO-LiPA HCV II (Innogenetics, Gent, Belgium). All patients in our test cohort were infected with genotype 1b. Alanine aminotransferase, aspartate aminotransferase and other relevant biochemical tests were performed using standard methods [24].

Antiviral therapy and definition of treatment outcome

All patients received bodyweight-adjusted PEG-IFN-α2b (PegIntron; Schering–Plough KK, Tokyo, Japan; ≤45 kg, 60 μg/dose; 46–60 kg, 80 μg/dose; 61–75 kg, 100 μg/dose; 76–90 kg, 120 μg/dose; ≥91 kg, 150 μg/dose), and ribavirin (Rebetol; Schering–Plough KK; ≤60 kg, 600 mg/day; 61–80 kg, 800 mg/day; ≥81 kg, 1,000 mg/day) for 48 weeks.

The response to therapy categories were defined as follows: an SVR was defined as undetectable serum HCV RNA 24 weeks after completing therapy. Relapse was defined as a reappearance of serum HCV RNA after treatment in patients whose HCV RNA level was undetectable during or at the completion of therapy. A non-virological response (NVR) was defined as a decrease in HCV RNA of <2 log copies/ml at week 12 and detectable HCV RNA during the treatment course.

Detection of amino acid substitutions in the core and NS5A regions

Core region and ISDR were determined by direct sequencing after amplification by reverse transcription and PCR as reported previously [22]. Amino acids at

positions 70 and 91 of the core region identical to the reference sequence HCV-J D90208 [25] were considered wild type [6]. The number of amino acid substitutions in the ISDR was defined as in Enomoto *et al.* [7].

Detection of serum IL-10, IL-12p40 and IL-18

Serum IL-10, IL-12p40 and IL-18 were quantified using Luminex® Multiplex Cytokine Kits (Procarta Cytokine assay kit; Panomics Inc., Fremont, CA, USA) for serum samples obtained before the start of treatment as reported previously [22]. All collected samples were immediately stored at -70°C prior to testing.

Genotyping of *IL28B*

Genomic DNA was isolated from the whole blood of patients using QuickGene-800 (Fujifilm, Tokyo, Japan). The concentration of genomic DNA was adjusted to 10–15 ng/μl for the TaqMan SNP genotyping assay. Genotyping of *IL28B* SNP (rs 8099917) was performed with a TaqMan 5′ exonuclease assay using primers supplied by Applied Biosystems (Carlsbad, CA, USA). Probe fluorescence signals were detected with a TaqMan assay for real-time PCR (7500 Real Time PCR System; Applied Biosystems) according to the manufacturer's instructions.

The protocol of this study was approved by the ethics committee of Shinshu University School of Medicine and all patients provided written informed consent.

Statistical analyses

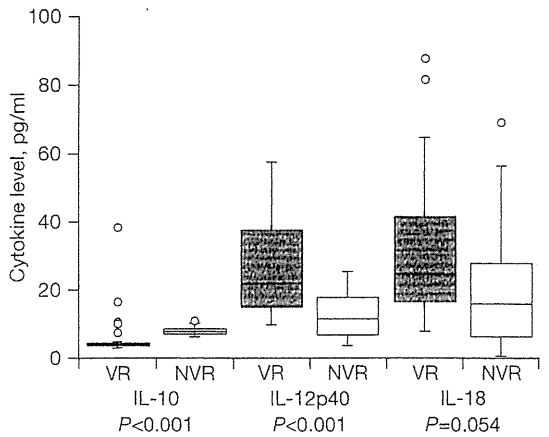
The Mann–Whitney U test was used to analyse continuous variables. The χ^2 test with Yate's correction was used for the analysis of categorical data. In cases where the number of subjects was <5, Fisher's exact test was used. A *P*-value of ≤0.05 was considered statistically significant. To predict treatment outcome, we analysed receiver operating characteristic (ROC) curves for serum levels of IL-10, IL-12p40 and IL-18. Optimal cutoff values were chosen as serum cytokine levels with the highest diagnostic accuracy, that is, when the sum of the false-negative and false-positive rates was minimized. The respective overall diagnostic values were expressed using the area under the curve (AUC). Multivariate analysis was performed using a logistic regression model with stepwise method. Statistical analyses were performed using PASW Statistics 18.0J (IBM, Tokyo, Japan).

Results

Treatment outcome in patients with chronic hepatitis C

Of the 52 patients receiving PEG-IFN and ribavirin therapy, 22 (42%) achieved an SVR. Among the 30 remaining patients, 14 had a relapse and 16 had an NVR. Before treatment, the median white blood cell count in the virological response group was significantly higher than that in the NVR group (Table 1). Haemoglobin value (15.4 versus 14.1 g/dl; *P*=0.021) was significantly higher in the SVR group compared to the NVR group as well. Substitutions in the ISDR and of aa70 and aa91 in the core region were not associated with treatment outcome.

Figure 1. Detection of serum cytokines related to treatment outcome



Boxes represent the IQR of the data, lines across the boxes indicate the median values and the hash marks above and below the boxes indicate the 90th and 10th percentiles, respectively for each group. Open circles indicate outliers. Serum interleukin (IL)-10, IL-12p40 and IL-18 levels were detected in 36 patients with a virological response (VR) and 16 patients without. NVR, non-virological response.

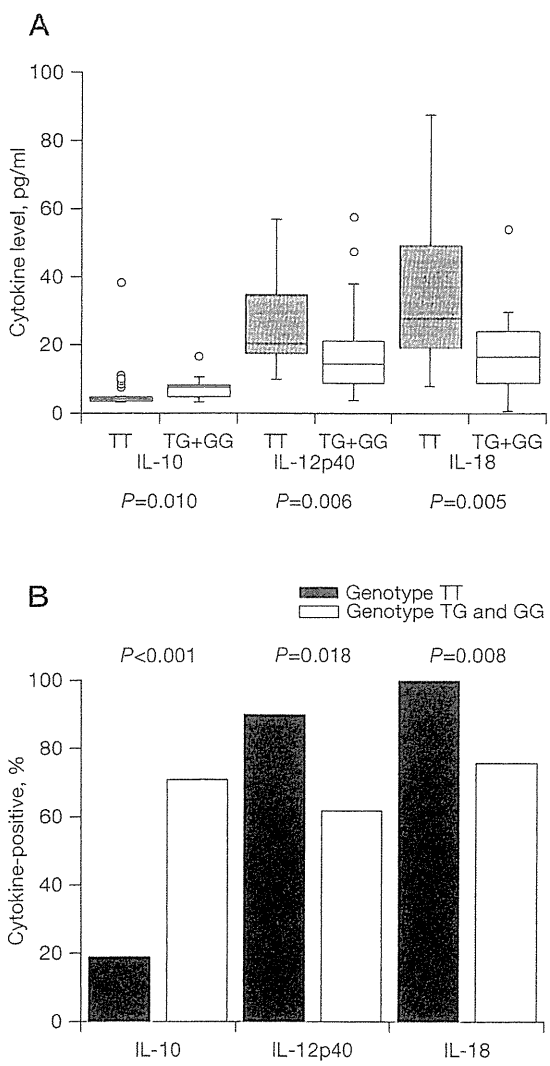
Effects of antiviral therapy on serum cytokine levels

Serum samples obtained prior to antiviral therapy were examined for the presence of IL-10, IL-12p40 and IL-18 by multiplex assays. NVR patients showed significantly higher baseline IL-10 concentrations (8.1 pg/ml) than virological responders (4.1 pg/ml; *P*<0.001; Figure 1). The median baseline serum levels of IL-12p40 (22.1 versus 11.7 pg/ml; *P*<0.001) and IL-18 (24.8 versus 16.0 pg/ml; *P*=0.054) were higher in patients who achieved a virological response than in those with an NVR (Figure 1). Furthermore, serum IL-10 level (4.0 versus 8.1 pg/ml; *P*<0.001) was significantly lower and serum IL-12p40 (25.0 versus 11.7 pg/ml; *P*<0.001) and IL-18 (31.6 versus 16.0 pg/ml; *P*=0.010) levels were significantly higher in the SVR group compared with the NVR group. We also analysed whether pretreatment serum cytokines were correlated with time to clearance of HCV RNA. Serum baseline IL-10 level was significantly lower in patients who eradicated HCV RNA 12 weeks after the start of treatment (*P*=0.002).

IL28B genotype and treatment outcome

Among the 52 patients studied for rs8099917, 31 had the TT genotype (60%), 20 had the TG genotype (38%) and 1 had the GG genotype (2%). Responses to combination therapy for rs8099917 are shown in Table 1. Overall SVR rates in patients with the TT genotype (16/31, 53%) and with the TG or GG genotypes (6/21, 29%) were not significantly different (P=0.09). However, NVR rates in patients with either TG or GG (13/21, 62%) were significantly higher than in those with TT only (3/31, 10%; P<0.001).

Figure 2. Serum cytokines related to *IL28B* gene polymorphisms



(A) Boxes represent the IQR of the data, lines across the boxes indicate the median values and the hash marks above and below the boxes indicate the 90th and 10th percentiles, respectively, for each group. Open circles indicate outliers. Serum interleukin (IL)-10, IL-12p40 and IL-18 were detected in 31 patients with the TT *IL28B* genotype and 16 patients with the TG or GG genotypes. (B) The prevalence of high serum IL-10, IL-12p40 and IL-18 levels in 31 patients with the TT *IL28B* genotype and in 16 patients with the TG or GG genotypes.

Association of *IL28B* genotype and serum cytokine levels

Median serum IL-10 levels were significantly higher in patients with the TG or GG genotypes (7.7 pg/ml) compared to those with TT (4.1 pg/ml; P=0.010; Figure 2A). Conversely, patients with TT had significantly higher median IL-12p40 (20.6 versus 14.5 pg/ml; P=0.006) and IL-18 (27.9 versus 16.6 pg/ml; P=0.005) levels than patients with TG or GG (Figure 2A).

ROC curve analyses were performed to determine the optimal threshold values of serum cytokines for predicting treatment outcome among the 16 NVR patients and 36 cases with a virological response in our cohort (Figure 3). The optimal threshold value of IL-10 was identical to the 5.0 pg/ml that we had reported in a prior study [22]. The cutoff values for IL-12p40 and IL-18 were 12.1 pg/ml and 6.4 pg/ml, respectively. The calculated AUC for IL-10, IL-12p40 and IL-18 was 0.89 (95% CI 0.77–0.96), 0.81 (95% CI 0.67–0.90) and 0.67 (95% CI 0.52–0.79), respectively, as shown in Figure 3.

The presence of high IL-10 levels (≥5.0 pg/ml) was significantly greater among patients with TG or GG genotypes (71%, 15 of 21) than among those with TT (19%, 6 of 31; P<0.001; Figure 2B). High IL-12p40 levels (≥12.1 pg/ml) were significantly less prevalent (P=0.018) among patients with TG or GG (62%, 13 of 21) than among those with TT (90%, 28 of 31). High IL-18 levels (≥6.5 pg/ml) were found in 100% (31 of 31) of patients with TT but only 76% (16 of 21) patients with TG or GG (P=0.008).

Predicting treatment outcome by serum cytokine levels in combination with *IL28B* genotype

The NVR prediction rate by serum IL-10 in combination with rs8099917 genotype is shown in Figure 4. In patients with TT, a significantly higher proportion of patients with high serum IL-10 levels (50%, 3 of 6) showed an NVR than patients with low IL-10 (0%, 0 of 25; P=0.004). Similarly, an NVR was significantly more likely in high versus low IL-10 levels (87%, 13 of 15 versus 0%, 0 of 6; P=0.001) in patients with TG or GG (Figure 4A).

NVR rates by serum IL-12p40 levels and IL-18 levels in combination with rs8099917 genotype are shown in Figure 4B and 4C. Among patients with the TT genotype, the NVR rate did not differ between low and high IL-12p40 levels (0% versus 11%; P=0.729) or IL-18 levels (0% versus 10%). In cases with TG or GG genotypes, the NVR rate was significantly higher for low IL-12p40 levels compared with high IL-12p40 levels (100% versus 38%; P=0.006). Patients with low serum

IL-18 had a higher NVR rate, but this difference was not statistically significant (100% versus 50%; P=0.063).

Factors associated with an NVR to PEG-IFN and ribavirin therapy

All factors found to be associated with an NVR were evaluated for independence in multivariate analysis. Genotype TG or GG (OR 10.43, 95% CI 1.73–62.96; P=0.011), serum IL-10 levels ≥5.0 pg/ml (OR 1.21, 95% CI 1.03–1.41; P=0.018) and IL-12p40 levels ≥17.4 mg/dl (OR 0.84, 95% CI 0.72–0.97; P=0.020) were all independent predictive factors of an NVR.

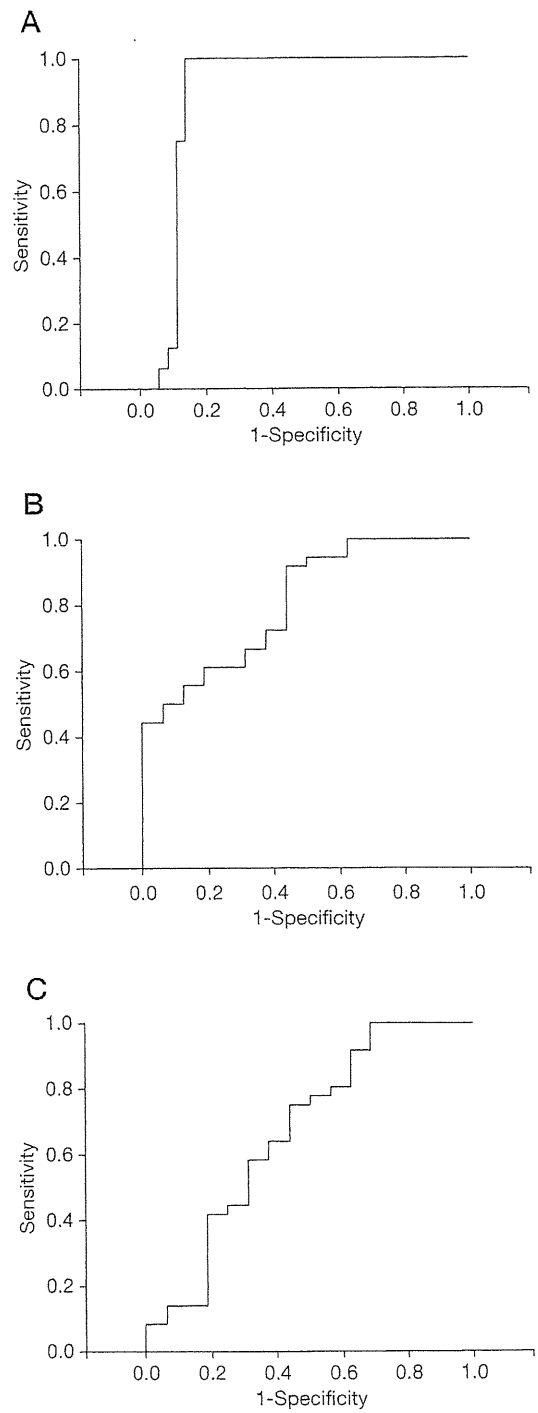
Discussion

This study examined the *IL28B* (rs8099917) genotype and serum levels of IL-10, IL-12p40 and IL-18 in patients with chronic hepatitis C to assess their predictive value in treatment outcome with PEG-IFN and ribavirin. The key findings were as follows: *IL28B* G-allele carriers were associated with an NVR to PEG-IFN and ribavirin therapy in patients infected with HCV genotype 1, consistent with recent findings; *IL28B* genotype was associated with baseline serum IL-10, IL-12p40 and IL-18 levels; in carriers of an *IL28B* G-allele, NVR rates were high (80–100%) and associated with increased IL-10 and decreased IL-12p40 and IL-18 levels, thus providing new predictive markers of an NVR in PEG-IFN and ribavirin therapy; and *IL28B* genotype, high serum IL-10 levels, and low serum IL-12p40 levels were all independent factors related to an NVR in multivariate analyses.

IL28B gene polymorphisms have recently been linked to the outcome of HCV infection during spontaneous and treatment-induced elimination of HCV [8–10,12]. In particular, carriage of a G-allele at the *IL28B* gene SNP (rs8099917) is associated with an NVR to PEG-IFN and ribavirin therapy in Japanese patients infected with HCV genotype 1 [10]. This finding was confirmed in our cohort with NVR rates of 62% with GT or GG genotypes versus 10% with TT genotypes (P<0.001). Therefore, detection of the *IL28B* genotype is a useful marker to predict the outcome of PEG-IFN and ribavirin therapy in patients with chronic hepatitis C. Data for *IL28B* SNP in healthy subjects were not available for this study.

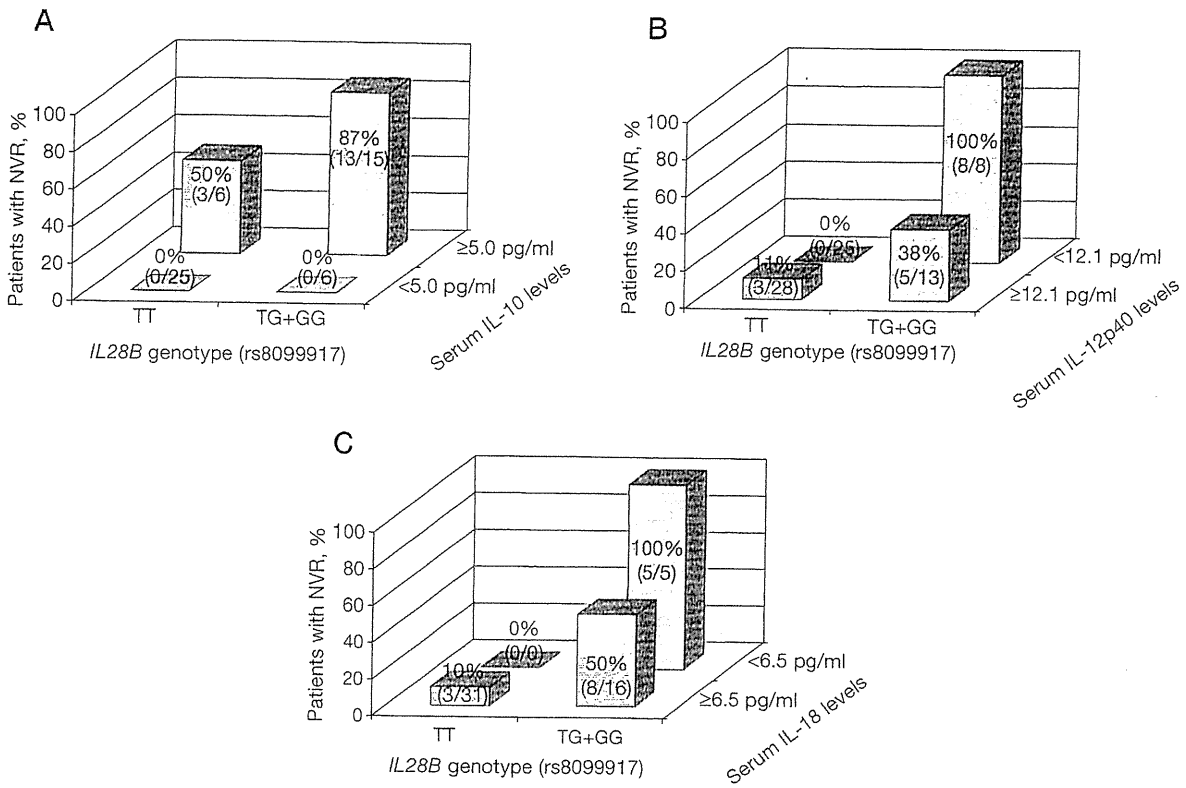
IFN-λ produces an antiviral state by triggering a cascade through the JAK-STAT pathway that up-regulates IFN-stimulated genes. *IL28B* binds to a distinct receptor that may up-regulate a different set of IFN-stimulated genes [26,27]; the precise role of IFN-λ in controlling multiple viral infections, including HCV, is currently under way. Further studies are also needed on how SNPs affect the function of *IL28B* and other cytokines.

Figure 3. Receiver-operating characteristic curves for serum cytokine levels on treatment outcome



The areas under the curve for (A) interleukin (IL)-10, (B) IL-12p40 and (C) IL-18 were 0.89, 0.81 and 0.67, respectively. All areas under the curve values were significantly higher than a 0.50 non-predictive value (P<0.01 for all comparisons). IL-10 was predictive of a non-response. IL-12p40 and IL-18 were predictive of a virological response.

Figure 4. Non-virological response rate determined by serum cytokine levels and *IL28B* gene genotype



The prevalence of a non-virological response (NVR) in patients with high or low serum (A) interleukin (IL)-10, (B) IL-12p40 and (C) IL-18 levels according to *IL28B* genotype.

A strong association between high IL-10, low IL-12p40 and low IL-18 levels and an NVR to PEG-IFN and ribavirin therapy was found in this study, which is consistent with previous studies [22,28–30]. In ROC curve analyses, AUCs were high, especially for IL-10 (AUC=0.89) and IL-12p40 (AUC=0.81), confirming that these cytokines are strong predictive markers for an NVR. This study showed a strong correlation between the *IL28B* genotype and serum IL-10, IL-12p40 and IL-18 levels at baseline. Most strikingly, all patients who had low pretreatment IL-10 levels achieved a virological response regardless of *IL28B* genotype. By contrast, among patients with high IL-10 levels (≥5.0 pg/ml), NVR rates were 87% in *IL28B* G-allele carriers and 50% for the *IL28B* TT genotype. Additionally, all *IL28B* G-allele carriers showed an NVR when pretreatment serum IL-12p40 and IL-18 levels were <12.1 pg/ml and <6.5 pg/ml, respectively. It is unclear how serum IL-10, IL-12p40 and IL-18 are associated with an NVR to antiviral therapy in patients with chronic hepatitis C. Although IL-10 was originally described as a cytokine synthesis inhibitory factor, recent studies have demonstrated that IL-10 produced by Th17 cells restrains the pathological effects of Th17 [31]. Production of IL-12p40 is directed towards the elimination of intracellular pathogens and viruses because IL-12p40 is a proinflammatory cytokine that promotes the differentiation of Th1 cells, suppresses Th2 function and amplifies the cytotoxicity of cytotoxic T-lymphocytes and natural killer cells [32]. Megjugorac *et al.* [33] reported that IL-29-treated plasmacytoid dendritic cells inhibiting production of IL-13, IFN-γ and IL-10 by allogeneic T-cells were consistent with a role for this cytokine in plasmacytoid dendritic cell maturation and activation. Very recently, another report has been published demonstrating that IL-29 enhances IL-12p40 by macrophages and that IL-29 pretreatment primes the activation of macrophages induced by IFN-γ [34]. However, the association between IL-28B and such cytokines has not been studied. To explain this relationship, further studies are needed to clarify whether a direct or indirect interaction exists between pretreatment levels of these cytokines and *IL28B* genotype.

Although other predictive factors of PEG-IFN and ribavirin therapy have been reported, including core amino acid 70 and 91 and ISDR mutations [6,7], no such significant associations were found here, possibly because of our study population size, which indicates that other factors may be more significant in predicting treatment outcome. However, multivariate analysis confirmed that *IL28B* G-allele, high IL-10 and low IL-12p40 levels were significant predictors of an NVR in patients with PEG-IFN and ribavirin therapy in this study. Hence, *IL28B* G-allele carriers combined with high IL-10 and/or low IL-12 may require alteration of treatment dose, duration, or regimen with a new antiviral drug.

In conclusion, serum IL-10, IL-12p40 and IL-18 levels are associated with *IL28B* genotype in patients with genotype 1 chronic hepatitis C. Pretreatment serum IL-10 and IL-12p40 levels with *IL28B* GT or GG genotypes are particularly useful for predicting an NVR to PEG-IFN and ribavirin therapy. The clinical significance of *IL28B* genotyping combined with baseline serum IL-10 and IL-12p40 levels to predict an NVR warrants further prospective validation.

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Disclosure statement

The authors declare no competing interests.

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Case Report

Early detection of interstitial pneumonia by monitoring KL-6 in a chronic hepatitis C patient undergoing pegylated interferon and ribavirin therapy

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A 58-year-old woman with chronic hepatitis C developed interstitial pneumonia (IP) while undergoing pegylated interferon (PEG IFN)-α-2a and ribavirin (RBV) therapy. Serum levels of sialylated carbohydrate antigen KL-6 (KL-6), a known marker of disease activity in fibrosing lung disorders, had been regularly measured once a month for early detection of IP, and had begun rising noticeably from 12 weeks to 540 U/mL at 33 weeks of treatment. On examination, remarkable fine crackles were detected by dorsal auscultation and bilateral ground-glass opacities and reticular shadows were depicted by computed tomography. The patient successfully recovered from her early-stage pneumonia by immediate discontinuation of therapy, which indicates that regular monitoring of serum KL-6 may be effective for avoidance of IP progression induced by PEG IFN and RBV therapy.

Key words: interstitial pneumonia, KL-6, pegylated interferon, ribavirin

INTRODUCTION

PEGYLATED INTERFERON (PEG IFN)-α-2a combined with ribavirin (RBV) has become one of the gold standards for hepatitis C virus (HCV) treatment.[1] However, side-effects are observed in almost 80% of patients receiving this therapy. Pulmonary toxicity in patients undergoing HCV treatment is rare, especially interstitial pneumonia (IP) induced by PEG IFN and RBV therapy. Sialylated carbohydrate antigen KL-6 (KL-6) is a mucinous high-molecular weight glycoprotein expressed on type 2 pneumonocytes that is a useful marker for the clinical diagnosis of interstitial lung diseases and the evaluation of disease activity.[2] It was reported that the sensitivity, specificity and diagnostic accuracy for KL-6 were 93.9%, 96.3% and 95.7%, respectively, for interstitial lung diseases.[3]

Herein, we describe a patient who avoided progression to severe IP induced by PEG IFN-α-2a and RBV therapy by regularly measuring serum levels of KL-6.

CASE REPORT

A 58-YEAR-OLD Japanese woman was referred to our hospital by her primary care physician for treatment of HCV likely stemming from a blood transfusion 33 years prior during childbirth. Her serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) had been consistently greater than 30 IU/L. Chronic hepatitis was histologically proven by liver tissue biopsy, which revealed scores for periportal bridging necrosis, intralobular degeneration and focal necrosis, and fibrosis of 1 each

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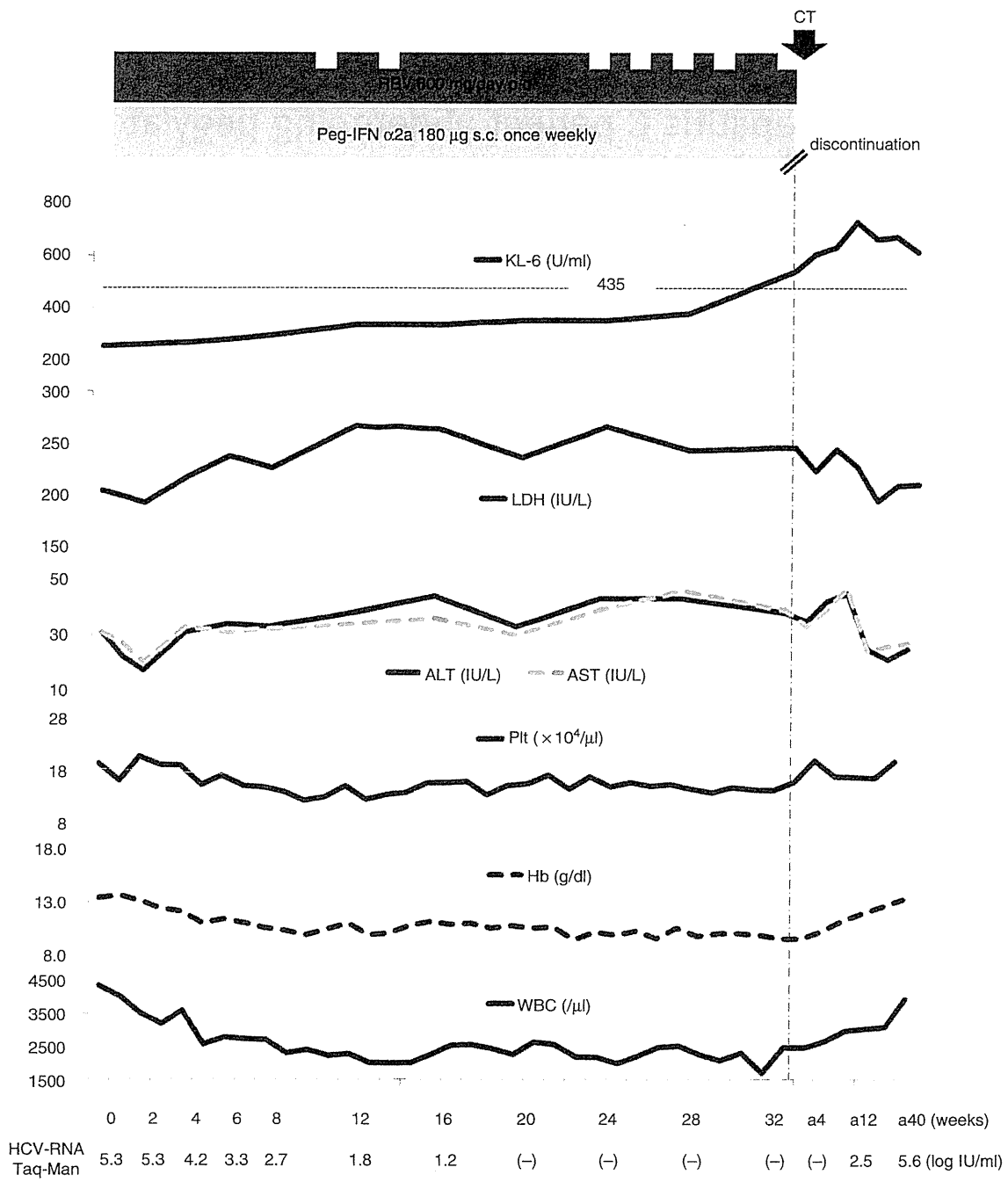

Figure 1 Clinical course of the present case. Figure shows the time course of white blood cell count (WBC), hemoglobin level (Hb), platelet count (Plt), serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase (LDH), serum level of KL-6 and serum hepatitis C virus (HCV) RNA by Taq-Man assay. Serum levels of C-reactive protein have been <0.03 mg/dL during the clinical course (data not shown). Shaded areas indicate the doses of pegylated interferon (PEG IFN)-α-2a and ribavirin (RBV). KL-6 began to rise noticeably from 12 weeks and reached 540 U/mL (upper limit of normal range, 435) after 33 weeks of combination therapy. Computed tomography (CT) performed at 33 weeks revealed findings compatible with interstitial pneumonia (IP) (Fig. 2a). PEG IFN and RBV treatment was immediately discontinued. The patient avoided development of severe IP without additional therapy.

Figure 2 (a) Computed tomography (CT) performed at 33 weeks of treatment with pegylated interferon (PEG IFN)-α-2a combined with ribavirin (RBV) shows bilateral ground-glass opacities (black arrow) and reticular shadows (black circles) located in the peripheral and dorsal areas of the lungs. (b) The shadows were diminished slightly 22 weeks after discontinuation of PEG IFN and RBV therapy. (c) The patient's prior pulmonary condition depicted by abdominal CT taken 2 years prior to treatment showed negligible reticular interstitial shadows at the base of the lungs, indicating that the patient might have had mild underlying chronic interstitial pneumonia.

and a portal inflammation score of 3, with 20% fatty deposition as assessed by the Knodell hepatitis activity index (HAI) classification.[4] Her single nucleotide polymorphism of interleukin 28Bat rs8099917[5,6] was G/G. She neither smoked nor habitually consumed alcohol. No history of pulmonary disease, frequent respiratory infection or autoimmune disease was noted.

The patient was started on combination treatment of regular doses of PEG IFN-α-2a 180 μg s.c. once weekly and RBV 600 mg/day p.o. (Chugai Pharmaceutical, Tokyo, Japan). Serum HCV RNA was decreased to an undetectable level by Taq-Man assay 20 weeks after the initiation of therapy (Fig. 1). She showed occasional mild hematopenia, resulting in periodic dose reductions of RBV to 400 mg/day (Fig. 1). Serum levels of KL-6 were 251 U/mL (normal range, 105–435) at treatment onset and were regularly measured once a month. KL-6 began to rise noticeably from 12 weeks and reached 540 U/mL after 33 weeks of therapy, at which time peripheral white blood cell count and serum levels of C-reactive protein and lactate dehydrogenase were 1690/μL with neutrophils of 900/μL, 0.03 mg/dL and 249 IU/L (normal range, 114–220), respectively. Although clinical symptoms of dry cough, fever and dyspnea were undetectable, fine crackles could be heard during auscultation of her back. Computed tomography (CT) revealed bilateral patchy ground-glass opacities around the dorsal area of the lungs (Fig. 2a). No evidence of congestive heart failure was demonstrated by cardiac ultrasonography. PEG IFN and RBV treatment was immediately discontinued due to suspicions of complicating IP. She was observed carefully without any further medication. Serum levels of KL-6 continued increasing until 4 weeks after cessation of therapy to 731 U/mL and then decreased gradually, but did not return to pretreatment levels at her final medical checkup 10 months after cessation of therapy. Accordingly, she was monitored by regular follow up at our outpatient clinic for further symptoms of IP and ultimately avoided development of severe IP. Serum HCV RNA became detectable by Taq-Man assay 12 weeks after discontinuation of PEG IFN and RBV therapy, but CT performed at 22 weeks after halting combination treatment depicted that the patchy ground-glass opacities had diminished slightly

Table 1 Published cases of interstitial pneumonia complicating pegylated interferon (PEG IFN) and ribavirin combination therapy in English-language published work

Case	Age (years)	Sex	Genotype	Fibrosis	Tobacco	Type of PEG IFN	PEG IFN (μg/week)	Ribavirin (mg/day)	Onset (weeks)	Clinical symptoms	Therapies	Result
1[8]	48	Female	2b	F3	N.D.	α2a	N.D.	N.D. + A	6	Fever, cough	Discontinuation and steroids	Resolved
2[9]	72	Male	N.D.	N.D.	N.D.	α2b	1.5/kg	800	16	Dyspnea	Discontinuation and steroids	Resolved
3[10]	49	Male	N.D.	Cirrhosis*	Former	α2b	150	1200	2	Cough, dyspnea	Discontinuation and steroids	Death§
4[11]	71	Female	2 and 4	N.D.	Never	α2a	180	800	6	Cough, shortness of breath	Discontinuation	Resolved
5[12]	51	Male	1a	F3※	N.D.	α2b	100	1200	5	Fever, dry cough, dyspnea	Discontinuation and steroids	Death #
6[13]	58	Female	1b	F3※	Never	α2b	1.5/kg	1000	12	Dyspnea	Discontinuation and	Resolved
						α2a	180	1000 + A	12	dyspnea	inhalation steroids	
7[14]	47	Female	2b	Cirrhosis**	Never	α2b	100	800	8	Dry cough, dyspnea	Discontinuation and steroids	Resolved
8[15]	43	Female	1b	Cirrhosis*	N.D.	α2b	120	800	48	Dyspnea, cough, fever	Discontinuation and steroids	Death$
9[16]	68	Male	1b	N.P.	N.D.	α2a	100	800	36	Exertional dyspnea	Discontinuation and steroids	Resolved
10[17]	51	Male	3	F2※	N.D.	α2b	150	800	4	Dry cough	Discontinuation and steroids	Resolved
Our case	58	Female	1b	F1	Never	α2a	180	600	33	None	Discontinuation	Resolved

*clinically diagnosed without histopathology; ※METAVIR score[21]; **histologically demonstrated as probable cirrhosis; §death from acute respiratory distress syndrome and multi-organ failure; $, death from progressive hypoxemia induced by interstitial pneumonia; $, death from acute cholestatic hepatitis.
+A, Amantadine; N.D., not described; N.P., not performed.

(Fig. 2b). Abdominal CT taken 2 years prior to therapy depicted negligible reticular interstitial shadows at the base of the lungs (Fig. 2c), indicating that the patient might have been complicated with mild chronic IP at treatment onset.

DISCUSSION

MANY SIDE-EFFECTS of PEG IFN and RBV therapy have been reported, such as hematological disorders, flu-like symptoms, neuropsychiatric disturbances, ophthalmological disorders, glucose metabolism disruption, autoimmune disease exacerbation, sarcoidosis, dermatological complications, hair loss and thyroid dysfunction; almost all of which can be managed with supportive care. It was also reported that respiratory tract symptoms, including a non-productive cough and shortness of breath, may occur,[7] and that the etiology of dyspnea and other respiratory symptoms is usually attributed to anemic severity. A total of 10 cases that were complicated with IP during combination therapy have been reported to date (Table 1).[8–17] Among them, no relationships with regards to age, sex or type of PEG IFN were apparent. In addition, IP arose at any stage of treatment or hepatic fibrosis, and was unrelated to present or former use of tobacco. Clinical symptoms included fever, cough and dyspnea in almost all cases, although these are non-specific as physical findings in patients with IP. However, crackles may be present despite the absence of abnormalities in chest X-rays; clinicians are advised to auscult the base of the lungs along the posterior axillary line when diagnosing for IP, as crackles may be audible in this location at disease onset, as presented in this case.

The mechanism of IP related to PEG IFN and RBV remains elusive, but is considered to be related to pathophysiological and immunomodulatory causes. One of the main contributing factors to IP is the direct toxicity of the HCV treatment to the lungs. Another possibility is indirect mechanisms acting via immunological pathways, such as T-cell abnormalities.[18] We also cannot exclude the involvement of HCV itself in the pathogenesis of IP induced by IFN therapy because no such reports have been found for patients treated for hepatitis B virus. IP associated with IFN monotherapy[19] or PEG IFN monotherapy[8] has been reported to date. However, RBV monotherapy has never been reported as the cause of IP because it is always given in conjunction with IFN or PEG IFN for treatment of HCV. Thus, PEG IFN, and not RBV, seems to have been the primary cause of IP.

Interstitial pneumonia developing during HCV treatment requires prompt detection and immediate discontinuation of PEG IFN and RBV therapy[8–17] due to a reported mortality rate of 7%.[17] Notably, three cases treated with PEG IFN and RBV died from associated IP despite being treated with corticosteroids after immediate IFN discontinuation.[10,12,15]

Serum KL-6 is a sensitive marker of disease activity in fibrosing lung disorders.[2,3] It was reported that KL-6 levels gradually increased from pretreatment levels when retrospectively measured every 12 weeks during a 48-week treatment course in chronic hepatitis C patients treated with PEG IFN and RBV therapy,[20] although no patients developed IP in the cohort. Nonetheless, changes in serum KL-6 may provide useful information to assess early suspicions of IP, especially if accompanied by other diagnostic findings such as clinical examination or CT. In this case, a progression to severe IP could be prevented by discontinuation of combination therapy through monthly monitoring of serum KL-6. Her continuously elevated serum KL-6 has necessitated regular follow up, and may be related to the persistent pulmonary shadows in chest CT.

In conclusion, clinicians should bear IP in mind as a complication during PEG IFN and RBV combination therapy. Measurement of serum KL-6 is advised to detect and avoid progression of IP at an early stage.

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Review Article

Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009

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Recently, much progress has been made in the field of hepatitis B, such as natural history of the disease in relation to the amount of hepatitis B virus (HBV) DNA, genotypes of HBV influencing the natural course and treatment effects, mutations of HBV influencing the severity of the disease and development of hepatocellular carcinoma, and antiviral treatment such as nucleos(t)ide analogues and pegylated interferon. To make the consensus for the diagnosis, management and treatment of hepatitis B, a meeting was held during 45th annual meeting of Japan Society of Hepatology (JSH) in June 2009. In the meeting, recommendations and informative statements were discussed on the following subjects: (i) natural history of HBV infection; (ii) clinical implication of HBV genotypes; (iii) HBV mutations and their potential impact on pathogenesis of HBV infection; (iv) indications for antiviral treatment of chronic hepatitis B; (v) nucleos(t)ide analogues for chronic hepatitis B; and (vi) interferon therapy for chronic hepatitis B. The presenters reviewed the data on these subjects and proposed the consensus statements and recommendations. These statements were discussed among the organizers and presenters, and were approved by the participants of the meeting. In the current report, the relevant data were reviewed and the 12 consensus statements and nine recommendations on chronic hepatitis B were described.

Key words: genotype, hepatitis B virus, interferon, mutation, natural history, nucleotide analogue

Hepatitis B virus (HBV) is one of the most distributed viruses which infect humankind. More than 3 billion people, one half of the world's population, have been exposed to HBV during their life.[1] Acute infection in adults is self-limited in general whereas infection during early childhood will develop into persistent chronic infection in most individuals.[2] More than 400 million people worldwide are chronically infected with HBV and are at risk of developing life-threatening complications including liver cirrhosis and hepatocellular carcinoma (HCC).[1] HBV is a major public health problem worldwide especially in East Asia and Africa. In Japan, approximately 1.5 million people are infected with HBV and it is one of the major causes of HCC and chronic hepatic failure. Other complications of HBV infection include fulminant hepatitis and acute liver failure.

The consensus meeting for diagnosis, management and treatment for hepatitis B was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M Kudo), where the recommendations and informative statements were discussed. Although the JSH consensus meeting of hepatitis B had been held four times so far, recommendations were hitherto published only in Japanese and this is the first report in English. Established

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information for pathogenesis and contributing factors for disease progression which was agreed by the organizers and presenters are shown as "consensus statements", and clinically useful consensus are shown as "recommendations". The quality of recommendations or informative statements are required to show a "level" (assessing strength or certainty) of evidence and "grading" of recommendations or assessment according to a standard reporting system of clinical guidelines.[3]

NATURAL HISTORY OF HBV INFECTION

AN EVALUATION OF studies on the natural history of HBV infection was done using the scoring system proposed by MacMahon *et al.*[4] in the present analysis because scoring systems for treatment studies cannot always be applied directly to those using natural history. The proposed scoring system consists of levels 1 (1a, 1b), 2 (2a, 2b, 2c), and 3. Level 1a is defined as a population-based longitudinal cohort study with a hepatitis B surface antigen (HBsAg) negative comparison group. Level 1b is identical to level 1a, but with no comparison group. Level 2a is defined as a clinic-based longitudinal cohort study, level 2b is a population-based or clinic-based cohort nested case–control study, and level 2c is a cross-sectional clinic-based study. Level 3 is defined as an observation study case series.

The natural history of chronic HBV infection can be classified into several phases based on levels of alanine aminotransferase (ALT), hepatitis B e-antigen (HBeAg) status, amounts of HBV DNA, and estimated immunological states.[4–9] A representative classification of these phases is shown in Table 1. In the immune tolerance phase, HBeAg is positive, serum levels of ALT are normal, histological activities of hepatitis are absent or minimal, and levels of HBV DNA are elevated. The immune tolerance phase is thought to occur most frequently in individuals who are infected through perinatal transmission, and this phase usually lasts until adolescence or young adulthood.[10–12]

The chronic hepatitis B phase is characterized by elevated ALT and HBV DNA levels. In this phase, the host's immune system recognizes HBV as being foreign and initiates an immune response that results in hepatitis. In cases who are HBeAg positive, active hepatitis can be prolonged and may result in cirrhosis. However, chronic hepatitis B eventually transitions into an inactive phase with a loss of HBeAg positivity in the majority of patients. Seroconversion to anti-HBe and the fall of serum HBV DNA to low levels result in the disappearance of disease activity, despite persisting HBsAg and low levels of HBV DNA.[13–16] Seroconversion rates range 7–16% per year according to reports with higher evidence levels (levels 1b, 2a).[16–19] Factors associated with seroconversion are age (level 1b),[20] ALT levels (level 1b), occurrence of acute exacerbation of hepatitis (level 1b),[19,21] and genotype (level 2c).[22,23]

The seroconversion of HBeAg results in the transition from hepatitis phase to inactive carrier phase, which is generally thought to be a benign course for HBV carrier, but sometimes hepatitis can be reactivated spontaneously.[24] Patients experiencing reactivation undergo another transition, with increases in HBV DNA and ALT levels and disease activity without reappearance of HBeAg.[24] This phase is referred to as HBeAg negative chronic hepatitis B. Occasional severe hepatitis B flare-ups with middle range HBV DNA levels (3–8 log copies/mL) occur in this phase.[8,25] HBeAg negative chronic hepatitis B is caused by mutant strains of HBV unable to produce HBeAg,[25,26] and tends to develop into cirrhosis and complicate HCC more than HBeAg positive chronic hepatitis B.[27–30]

Table 1 Phases in the natural history of HBV carriers (modified from [4])

Phase	Hepatitis	Blood			Liver
		DNA	HBeAg	HBsAg	cccDNA
Immune tolerance	–	8–11	+	+	+
HBeAg positive Chronic hepatitis	Usually Persistent	6–10	+	+	+
HBeAg negative Chronic hepatitis	Often Fluctuating	3–8	–	+	+
Inactive carrier	–	<4	–	+	+
Recovery	–	–	–	–	+

HBV DNA: log copies/mL. cccDNA, covalently close circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Many factors that are associated with the development of HCC have been reported so far. Higher age (level 1a), male sex (level 1a), presence of cirrhosis (level 2a) and familial cluster of carriers (level 2c) are reported as host factors.[31,32] Viral factors include high viral load (level 1b),[33–36] existence of pre-core and core promoter mutations (level 2a), genotype C and high ALT levels (level 1b). High viral load should be considered as a factor in patients over 35–40 years of age. Co-infection with hepatitis C virus, hepatitis D virus or HIV (level 2a), drinking habit (level 2c) and exposure to aflatoxin (level 2c) are reported as social and environmental factors.[37–39] Other lifestyle-related factors, such as smoking habit, obesity and complications from diabetes mellitus, have been documented as well.

Consensus 1
In patients with chronic hepatitis B, seroconversion of HBeAg usually results in the transition from hepatitis phase to inactive carrier phase, which generally has low HBV replication and normal ALT levels. However, reactivation of chronic hepatitis can spontaneously occur without the reappearance of HBeAg. At this point, active hepatitis continues and the risk of complicating cirrhosis and HCC is high in patients with HBeAg negative chronic hepatitis B. (Level 1b.)

In the inactive carrier phase, HBV replication is continuously suppressed as a result of predominantly host immunological pressure against HBV. Patients in the inactive carrier phase generally have a benign course because active hepatitis subsides and the risk of HCC decreases.[19,20,24,40] However, regular follow up is required because reactivation of HBV sometimes occurs spontaneously or as a result of immunosuppressive therapy.[19,24]

Hepatitis B surface antigen is known to fall to undetectable levels in some inactive carriers. This HBsAg negative phase, referred to as the recovery phase, has no hepatitis and a low risk of HCC. Still, caregivers must be aware that patients who are old or cirrhotic have a relatively higher risk of HCC.[41,42] Disappearance of HBsAg in the recovery phase does not indicate complete eradication of HBV because the HBV genome remains as covalently close circular DNA (cccDNA) in the nucleus of hepatocytes.

Consensus 2
2-1 HBV can not be completely eradicated using any currently existing treatment measures. (Level 2a.)
2-2 Patients in the inactive carrier or recovery phase have a benign clinical course. However, regular follow up of such patients is required because reactivation of hepatitis B and ensuing HCC can occur. (Level 1b, 2a.)

Clinicians have to consider two types of hepatitis B reactivation: one during the inactive carrier phase and the other in the recovery phase.[4] Both types of reactivation have been attributed with increasing incidence to strong immunosuppressive therapies. De novo hepatitis B, a reactivation of hepatitis B in the recovery phase, tends to develop into fulminant hepatitis, which has a very high mortality rate.[43–46] Thus, establishment of effective measures to prevent reactivation of hepatitis B is necessary.

Consensus 3
3-1 Reactivation of hepatitis B can occur during the inactive carrier or recovery phases and stems mainly from strong immunosuppressive treatment courses. (Level 2a.)
3-2 Recent advances in medical care have increased the use of immunosuppressive agents and thus the incidence of hepatitis B reactivation. (Level 2a.)
3-3 Reactivation of hepatitis B tends to develop into fulminant hepatitis. (Level 2a.)

Recommendation 1
In addition to the loss or seroconversion of HBeAg, a substantial decrease in HBV viral load and subsequent disappearance of hepatitis are the primary targets in the treatment of patients with chronic hepatitis B. (Level 1b.)

Recommendation 2
The main goals of HBV carrier treatment are patients in the inactive carrier and recovery phases. However, caregivers should be aware that reactivation of hepatitis B and complication of HCC can occur even in these benign phases. (Level 1b.)

Recommendation 3
Reactivation of hepatitis B due to immunosuppressive therapy tends to develop into severe hepatitis, thus requiring the establishment of effective preventative measures. (Level 2a.)