Table 1 Comparison of clinical and virological backgrounds between patients with and without relapse of hepatitis at initiation and discontinuation of nucleos(t)ide analogs (NAs)

Background	Non-relapse patients ($n = 32$)	Relapse patients ($n = 53$)	*P*-value
At initiation of NAs			
Age (years)†	47 (17–75)	48 (26–74)	>0.2
Gender (M : F)	23:9	32:21	>0.2
ALT (IU/L)†	183 (9–1182)	187 (20–2052)	>0.2
Genotype (A : B : C : UD)	1:2:21:8	0:3:44:6	0.193
HBeAg (positive)‡	11 (34%)	16 (30%)	>0.2
HBV DNA			
Amplicor assay (log copies/mL)†	6.2 (<2.6–>7.6)	6.5 (<2.6–>7.6)	0.099
HBsAg (log IU/mL)†	2.7 (0.1–4.3)	3.3 (1.6–3.9)	0.018
HBcrAg (log U/mL)†	5.2 (<3.0–>6.8)	5.6 (<3.0–>6.8)	>0.2
At discontinuation of NAs			
Age (years)†	50 (21–78)	49 (26–79)	>0.2
NAs (LVD : LVD+ADV : ETV : ADV)	28:1:3:0	50:0:2:1	>0.2
Duration of NA treatment (months)†	36 (4–129)	17 (4–84)	0.007
Follow-up period after discontinuation of NAs (months)†	45 (6–123)	12 (1–111)	0.002
ALT (IU/L)†	16 (7–38)	20 (9–65)	0.002
HBV DNA			
Amplicor assay (log copies/mL)†	<2.6 (<2.6–2.9)	<2.6 (<2.6–2.9)	>0.2
TaqMan assay (negative signal)‡	5 (23%) ($n = 22$)	3 (14%) ($n = 21$)	>0.2
TaqMan assay (negative or positive signal)‡	13 (59%) ($n = 22$)	13 (62%) ($n = 21$)	>0.2
HBsAg (log IU/ml)†	2.0 (<–1.5–4.3)	3.1 (0.6–4.0)	0.001
HBcrAg (log IU/mL)†	3.4 (<3.0–4.9)	4.3 (<3.0–>6.8)	0.003

†Data are expressed as the median (range)
‡Data are expressed as a positive number (%)
ADV; adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LVD, lamivudine; UD, undetermined.

patients with a negative detection signal or a positive signal also did not vary significantly. The follow-up period after discontinuation of NAs was significantly shorter in patients with relapse than in those without because formal follow-up ended once patients relapsed. The median period of follow-up was 45 months in patients without relapse.

Multivariate analyses revealed that a shorter duration of NA treatment and higher levels of HBsAg and HBcrAg at discontinuation were significantly associated with the occurrence of hepatitis relapse (Table 2). The cut-off values that showed the highest significance by ROC analysis were 1.9 log IU/mL for HBsAg (AUC = 0.707, $P = 0.001$), 4.0 log U/mL for HBcrAg (AUC = 0.692, $P = 0.003$), and 16 months (AUC = 0.674, $P = 0.007$) for treatment duration.

Model for predicting relapse of hepatitis using levels of HBsAg and HBcrAg

The existence of a second cut-off value was suggested by ROC analysis for both of HBsAg (2.9 log IU/mL) and HBcrAg (3.0 log IU/mL) to discriminate between

Table 2 Multivariate analysis of factors associated with relapse of hepatitis after discontinuation of nucleos(t)ide analogs (NAs)

Factor	Hazard ratio	95%CI	*P*-value
HBsAg at discontinuation ≥ 1.9 log IU/mL	5.21	1.87–14.55	0.002
HBcrAg at discontinuation ≥ 4.0 log U/mL	2.20	1.25–3.87	0.006
Duration of NA treatment ≥ 16 months	0.54	0.31–0.93	0.027

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

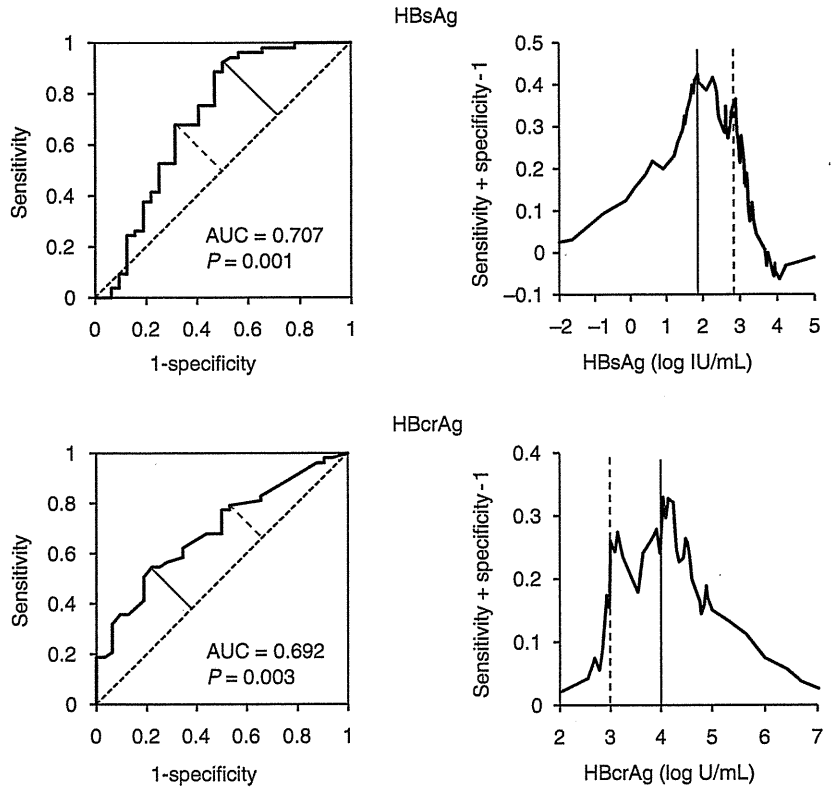

Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

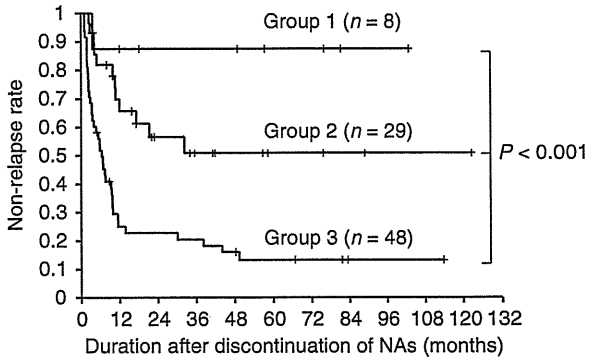

Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleo-s(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

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Review Article

Risk of hepatitis B reactivation in patients treated with tumor necrosis factor-α inhibitors

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The use of tumor necrosis factor-α (TNF-α) inhibitors has been increasing especially in patients with rheumatoid arthritis (RA). As TNF-α inhibitors are strongly immunosuppressive, the occurrence of hepatitis B virus (HBV) reactivation has recently been observed. Reports suggest a higher risk of complicating HBV reactivation in carriers who are treated with TNF-α inhibitors. Therefore, HBV carriers are recommended to undergo prophylactic administration of nucleos(t)ide analogs (NAs). Our literary analysis uncovered several characteristics of de novo hepatitis B due to TNF-α inhibitors. First, the time between the start of TNF-α inhibitors and the occurrence of de novo hepatitis was longer than one year. Second, patients were usually treated with additional non-biologic agents, which also had immunosuppressive effects. Third, the disease could be fatal. Fourth, several types of TNF-α inhibitors exhibited a risk of developing de novo hepatitis. Although the incidence of de novo hepatitis B varied among reports (0–5%/year), it is suggested that patients with prior HBV infection are at risk of developing de novo hepatitis due to TNF-α inhibitors. Many reports maintain that regular measurement of HBV DNA is effective in preventing de novo hepatitis. Prophylactic administration of NAs is also considered useful to avoid de novo hepatitis, although the issue of cost-effectiveness needs to be addressed. Lastly, whereas maintenance of circulating anti-HBs titer using HB vaccines may be effective in responders to prevent de novo hepatitis, further studies are required to clarify the utility of HB vaccination.

Key words: hepatitis B, nucleos(t)ide analog, de novo hepatitis B, reactivation, rheumatoid arthritis, tumor necrosis factor-α inhibitor

INTRODUCTION

APPROXIMATELY 3 BILLION people have been exposed to the hepatitis B virus (HBV), and there are an estimated 350 million chronic carriers worldwide.[1,2] HBV infection is usually detected by the presence of hepatitis B surface antigen (HBsAg) in the serum, and clearance of HBsAg is generally considered as an indication of hepatitis B resolution. However, recent studies have shown that HBV replication persists at low levels in the liver and peripheral blood mononuclear cells for decades, even in HBsAg-negative patients with resolved HBV infection.[3–5] In such patients, HBV replication is suppressed by immune responses to HBV, for instance specific cytotoxic T lymphocyte-mediated responses.[3]

Hepatitis B virus reactivation in patients with resolved HBV infection has been reported in increasing numbers as the number of patients undergoing strong immunosuppressive therapy grows worldwide for malignant neoplasms, autoimmune disorders, and following transplantation for prevention of rejection. In patients like these with resolved HBV infection, reactivation of hepatitis B is recognized as de novo hepatitis B, which can lead to fulminant hepatic failure and often death.[6,7] Thus, de novo hepatitis B is becoming a well-recognized severe complication of immunosuppressive therapy that should be prevented.[6,8]

The risk of developing de novo hepatitis B varies among immunosuppressive therapies; it is as high as 14–20% in patients who receive hematopoietic stem cell transplantation and as low as 1–3% in those who undergo conventional chemotherapies.[9–13] The introduction of rituximab, a genetically engineered chimeric anti-CD20 monoclonal antibody,[14,15] in the treatment of

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CD20+ B-cell non-Hodgkin's lymphoma increased the risk of de novo hepatitis B. Hui *et al.*[16] analyzed the occurrence of de novo hepatitis B in patients who were treated for lymphoma and reported that its risk was significantly higher in patients who received rituximab and steroids (12%) than in other patients (1%). Similarly, Yeo *et al.*[17] reported that the risk of de novo hepatitis B was significantly higher in patients who were treated with chemotherapy including rituximab (24%) than in those treated with chemotherapy only (0%). Because the introduction of rituximab increased the risk of de novo hepatitis B considerably in lymphoma patients, the need to examine the occurrence of HBV reactivation has emerged when a new agent that suppresses host immune responses is introduced.

Tumor necrosis factor-α is a crucial pro-inflammatory and immunoregulatory cytokine in the pathogenesis of various inflammatory and autoimmune conditions. Inhibitors of TNF-α have recently been introduced in treatments for various kinds of autoimmune and inflammatory disorders, including rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, and Crohn's disease. TNF-α inhibitors have revolutionized the therapeutic approaches and treatment paradigms for these patients. However, their optimal use requires consideration of possible adverse effects; increased risks of tuberculosis and other infections are a major concern in TNF-α treatment.[18] Complicating tuberculosis is considered to be caused by reactivation of latent tuberculosis.[19] A similar reactivation of HBV has also been reported, which leads to de novo hepatitis B and possibly fulminant hepatic failure and death. In the present review article, we summarize reports regarding reactivation of hepatitis B due to TNF-α inhibitors to clarify its characteristics and occurrence (Table 1).

Table 1 Summary of references regarding reactivation hepatitis due to tumor necrosis factor-α inhibitors

Category/Reference	Publication type
Case report and review in HBV carriers	
25. Ostuni P, *et al.* Ann Rheum Dis. 2003	Case report
26. Carroll MB, *et al.* Clin Rheumatol. 2010	Review
27. Kuroda T, *et al.* Rheumatol Int. 2010	Case report & review
28. Verhelst X, *et al.* Eur J Gastroenterol Hepatol. 2010	Case report & review
29. Pyrpasopoulou A, *et al.* Rheumatol Int. 2011	Case report
30. Esteve M, *et al.* Gut. 2004	Case report
31. Ojiro K, *et al.* J Gastroenterol. 2008	Case report
34. Wendling D, *et al.* Joint Bone Spine. 2009	Case report
Risk and prevention in HBV carriers	
32. Zingarelli S, *et al.* Reumatismo. 2008	Original
33. Kalyoncu U, *et al.* Rheumatol Int. 2009	Original
35. Vassilopoulos D, *et al.* Ann Rheum Dis. 2010	Original
36. Lan JL, *et al.* Ann Rheum Dis. 2011	Original
37. Calabrese LH, *et al.* Ann Rheum Dis. 2006	Review
Case report of de novo hepatitis B	
40. Madonia S, *et al.* Inflamm Bowel Dis. 2007	Case report
41. Matsumoto T, *et al.* Liver Int. 2010	Case report
42. Montiel PM, *et al.* Liver Int. 2008	Case report
43. Zingarelli S, *et al.* J Rheumatol. 2009	Case report
Risk of de novo hepatitis B	
18. Takeuchi T, *et al.* Ann Rheum Dis. 2008	Original
44. Charpin C, *et al.* Arthritis Res Ther. 2009	Original
45. Caporali R, *et al.* Arthritis Care Res (Hoboken). 2010	Original
46. Tamori A, *et al.* J Gastroenterol. 2011	Original
47. Mori S. Mod Rheumatol. 2011	Original
48. Kim YJ, *et al.* J Rheumatol. 2010	Original
49. Urata Y, *et al.* Mod Rheumatol. 2011	Original

REACTIVATION OF HEPATITIS IN HBV CARRIERS

THE MAJORITY OF patients with a confirmed diagnosis of RA use disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, but the rate of biologic agent use is rising rapidly.[20,21] Since both methotrexate[22–24] and biologic agents carry the danger of HBV reactivation, the advent of new biologic agents, such as TNF-α inhibitors, has increased this risk. Patients with RA who developed reactivation of hepatitis B due to TNF-α inhibitors were first reported in 2003.[25–29] Because these cases had been HBV carriers prior to starting TNF-α inhibitors, the authors recommended preliminary serological tests for HBV infection.

Caroll *et al.* conducted a systemic literature review on HBV reactivation in carriers who were treated with TNF-α inhibitors for RA and reported that reactivation was seen in six (17%) of 35 patients.[26] They concluded that clinicians prescribing TNF-α inhibitors to HBsAg-positive patients should consider prophylactic antiviral therapy and close monitoring for any clinical or sero-

logical evidence of hepatitis. Reactivation of hepatitis B was also reported in patients with Crohn's disease who were treated with TNF-α inhibitors,[30,31] and thus reactivation became considered to be drug dependent and not disease dependent.

Prophylaxis using nucleos(t)ide analogs (NAs) has been reported to be effective in preventing the occurrence of hepatitis reactivation in HBV carriers.[32-36] Vassilopoulos *et al.*[35] administered lamivudine in 14 HBV carriers with RA who were treated with TNF-α inhibitors and showed that reactivation of hepatitis B did not occur in any patient except one. The appearance of lamivudine resistance was considered to be the cause of reactivation in this exceptional patient, and so the authors concluded that TNF-α inhibitors represented a safe option for patients with chronic HBV infection when combined with NAs. Zingarelli *et al.*[32] reported 20 patients with RA who were treated with DMARDs and/or TNF-α inhibitors. Prophylaxis and therapy with lamivudine were performed in patients with a high risk of HBV reactivation, and no cases of viral reactivation were observed. Thus, it is likely that prophylaxis using NAs may prevent the occurrence of hepatitis reactivation in HBV carriers who are treated with TNF-α inhibitors. Indeed, Calabrese *et al.*[37] recommended that all HBsAg-positive patients be started on prophylactic anti-viral drugs before receiving immunosuppressive therapy. However, long-term follow-up studies in large groups of patients are required to ensure the safety of prophylaxis with NAs.

Descriptions of HBV reactivation due to TNF-α inhibitors in the guidelines of rheumatologist associations several years ago tended to be brief and passive. It was described that TNF-α inhibitor therapy should be avoided in patients with hepatitis B infection until more definitive data were available in the 2005 guidelines of The British Society for Rheumatology.[38] In the 2007 Japanese guidelines,[39] it was advised that TNF-α inhibitors should be avoided in patients with HBV infection. However, if the potential benefits of treatment with TNF-α inhibitors exceeded the risk of reactivation, such therapy could be pursued provided that patients were pre-treated with lamivudine.

RISK OF DE NOVO HEPATITIS B

ALTHOUGH IT HAS become clear that HBsAg-positive patients are prone to developing HBV reactivation during TNF-α inhibitor therapy, little is known about the occurrence of de novo hepatitis B. Several cases of de novo hepatitis B due to TNF-α inhibitors have been reported recently.[40-43] Mondonia *et al.*[40] reported a 41-year-old woman with Crohn's disease who developed de novo hepatitis B after having been treated with prednisolone for 13 years and infliximab for 3 years. The hepatitis subsided with lamivudine administration. Montiel *et al.*[42] described a 73-year-old man with ankylosing spondylitis who developed de novo hepatitis 15 months after starting etanercept. The patient had also undergone treatment with prednisolone for 23 years. Although etanercept was discontinued when the hepatitis occurred, it could be re-started with concurrent lamivudine administration. Matsumoto *et al.*[41] reported a 71-year-old woman with RA who developed de novo hepatitis 22 months after starting treatment with infliximab, methotrexate, and prednisolone. Although entecavir was given when hepatitis occurred, the patient died of hepatic failure. Such case reports reveal several characteristics of de novo hepatitis B due to TNF-α inhibitors. First, the duration between the start of the drugs and the occurrence of de novo hepatitis was at least one year. Second, patients were treated not only with TNF-α inhibitors, but also with DMARDs and prednisolone, which themselves had immunosuppressive effects. Third, there was a risk of death from de novo hepatitis. Fourth, several kinds of TNF-α inhibitors appeared able to cause de novo hepatitis.

The incidence of HBV reactivation from occult HBV infection and ensuing de novo hepatitis B due to TNF-α inhibitor therapy in patients with RA has been reported by several groups. Charpin *et al.*[44] followed 21 patients with RA who were HBsAg-negative and hepatitis B core antibody (HBcAb)-positive before starting TNF-α inhibitors, and found that no patient developed HBV reactivation during a mean follow-up period of 27.2 months. They concluded that TNF-α inhibitor therapy was likely safe in patients with a past hepatitis B serological pattern. However, they also suggested that such patients required HBV virological follow-up, especially those with a low HBs antibody (HBsAb) titer at baseline because HBsAb decreased significantly during therapy. Caporali *et al.*[45] followed 67 patients with RA who also had HBV markers of past HBV infection, and found no elevations of HBV DNA in sera or appearances of HBsAg during a mean follow-up period of 42.5 months. Of the 67 patients, 23 were treated with infliximab, 23 with etanercept, and 19 with adalimumab. Almost all patients underwent methotrexate (51 patients) and/or prednisolone (43 patients) administration in addition to TNF-α inhibitors. Tamori *et al.*[46] followed 50 patients with RA who were positive for HBcAb for a mean period of 23 months. All patients were treated with immunosuppressive agents such as

methotrexate, prednisolone, and/or TNF-α inhibitors for more than one year. HBV reactivation was observed in two of five patients with HBsAg, compared with only in one of the remaining 45 patients without it. Therefore, HBV reactivation leading to de novo hepatitis B was observed in 2% (1%/year) of patients. It should be noted that the lone HBsAg-negative reactivation patient had been treated with methotrexate but not with TNF-α inhibitors. Mori[47] performed a cross-sectional analysis of 239 patients with RA who were treated with biological and/or non-biological agents, among whom 60 were found to have HBV markers indicating earlier HBV infection. Of these, two were signal-positive for serum HBV DNA but without ALT elevation or HBsAg positivity: one patient was treated with tacrolimus, prednisolone, and methotrexate, and the other was treated with adalimumab, prednisolone, and methotrexate. Whereas HBV DNA level in the former patient increased and HBsAg and HBeAg became weakly positive after 10 weeks, the latter patient became HBV DNA-negative without additional anti-viral therapy. The authors also concluded that biological and non-biological agents are relatively safe in RA patients with past HBV infection. Thus, these studies suggested that the occurrence of de novo hepatitis B was rare in RA patients who were treated with TNF-α inhibitors in addition to DMARDs over the medium term. A large-scale post-marketing surveillance study was carried out in Japan to determine the safety profile of infliximab in patients with RA.[18] All patients with RA who were treated with infliximab were prospectively monitored for any adverse events for a period of 6 months after the initiation of infliximab. No cases of de novo hepatitis B were found. Although the follow-up period was short, the number of patients enrolled was over 5000. This report indicated that de novo hepatitis B due to TNF-α inhibitors would be very rare over the short-term as well.

In contrast to the abovementioned reports, several studies have suggested a relatively high incidence of de novo hepatitis B due to TNF-α inhibitor therapy. Kim *et al.*[48] followed 266 patients with RA who were treated with TNF-α inhibitors and analyzed the occurrence of clinically significant (over two times higher than normal range) and persistent (two or more incidences) alanine aminotransferase (ALT) elevation in relation to HBV markers. Elevation of ALT was significantly more frequent in patients with HBcAb (HBsAg negative) than in those without (16% vs. 6%, $P = 0.009$). In multiple logistic regression analysis controlling for various potential confounding factors, such as methotrexate, nonsteroidal anti-inflammatory drugs, and type of TNF-α inhibitor, only potential occult HBV infection was identified as a significant risk factor for ALT elevation, suggesting a close association between HBcAb-positivity and ALT elevation during TNF-α inhibitor therapy in RA patients. However, it cannot be confirmed whether ALT elevations in that study were indeed caused by reactivation of occult HBV because HBV DNA was not measured along with ALT. Urata *et al.*[49] prospectively followed 135 patients with RA who had HBV markers suggesting past HBV infection for 12 months. The cohort was treated with biological and/or non-biological anti-rheumatic agents and followed for a total mean period of approximately 20 months, including the period before follow-up. Serum HBV DNA was measured every 3 months during the study period, and revealed that HBV reactivation occurred in seven patients (5%/year). HBV reactivation was significantly associated with use of TNF-α inhibitors with a hazard ratio of 10.9 ($P = 0.008$). This study suggested that careful monitoring of HBV DNA level is required in RA patients with resolved hepatitis B when receiving anti-rheumatic agents, especially biologic ones.

In Japan, HBV reactivation rates tend to differ regionally. A study from Aomori prefecture[49] in the northern part of Japan reported a relatively higher rate of de novo hepatitis stemming from TNF-α inhibitors than studies from Osaka[46] and Kumamoto[47] prefectures in the central and southern parts of Japan, respectively. It is speculated that these differences are attributed to variations in HBV genotype distribution; whereas genotype B is predominant in the former area, genotype C is more frequent in the latter areas.[50] Further studies are required to address this phenomenon.

In light of the above findings, it is evident that RA patients with past HBV infection who are treated with anti-rheumatic agents are at risk of developing HBV reactivation and ensuing de novo hepatitis B, especially those being treated with anti-rheumatic agents, such as TNF-α inhibitors, for an extended time. Spontaneous remission of HBV reactivation was observed in one of the two patients reported by Mori[47] and two of the seven patients reported by Urata *et al.*,[49] and so it should be noted that HBV reactivation does not necessarily result in the occurrence of de novo hepatitis B.

PROPHYLACTIC MEASURES FOR DE NOVO HEPATITIS B

THREE MEASURES ARE generally used to prevent de novo hepatitis B due to immunosuppressive therapy.[7] The first measure is to regularly check for

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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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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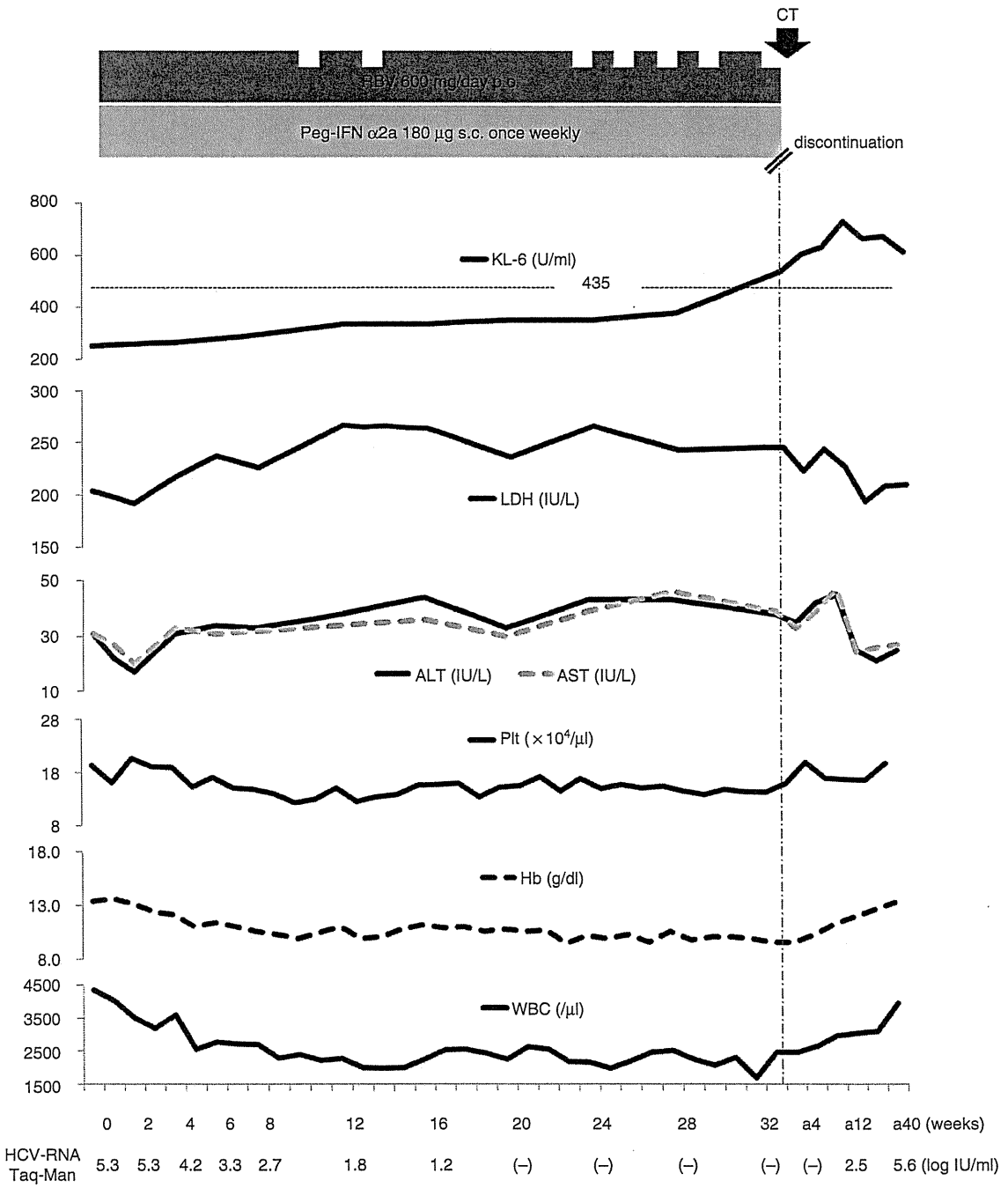

Figure 1 Clinical course of the present case. Figure shows the time course of white blood cell count (WBC), hemoglobin level (Hb), platelet count (Plt), serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase (LDH), serum level of KL-6 and serum hepatitis 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.

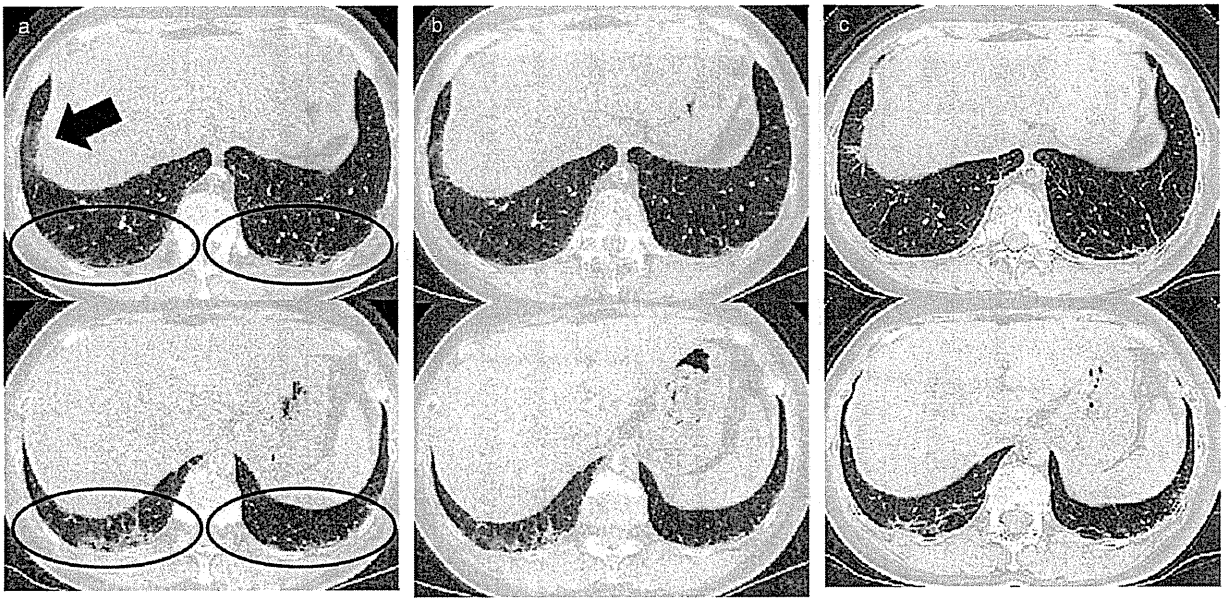

Figure 2 (a) Computed tomography (CT) performed at 33 weeks of treatment with pegylated interferon (PEG IFN)-α-2a combined with ribavirin (RBV) shows bilateral ground-glass opacities (black arrow) and reticular shadows (black circles) located in the peripheral and dorsal areas of the lungs. (b) The shadows 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 α2a	1.5/kg 180	1000 1000 + A	12 12	Dyspnea dyspnea	Discontinuation and inhalation steroids	Resolved
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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Genome-wide association study identified *ITPA/DDRGK1* variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C

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Hematologic abnormalities during current therapy with pegylated interferon and ribavirin (PEG-IFN/RBV) for chronic hepatitis C (CHC) often necessitate dose reduction and premature withdrawal from therapy. The aim of this study was to identify host factors associated with IFN-induced thrombocytopenia by genome-wide association study (GWAS). In the GWAS stage using 900K single-nucleotide polymorphism (SNP) microarrays, 303 Japanese CHC patients treated with PEG-IFN/RBV therapy were genotyped. One SNP (rs11697186) located on *DDRGK1* gene on chromosome 20 showed strong associations in the minor-allele-dominant model with the decrease of platelet counts in response to PEG-IFN/RBV therapy [$P = 8.17 \times 10^{-9}$; odds ratio (OR) = 4.6]. These associations were replicated in another sample set ($n = 391$) and the combined *P*-values reached 5.29×10^{-17} (OR = 4.5). Fine mapping with 22 SNPs around *DDRGK1* and *ITPA* genes showed that rs11697186 at the GWAS stage had a strong linkage disequilibrium with rs1127354, known as a functional variant in the *ITPA* gene. The

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