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Suppression of Methotrexate-Induced Elevations in the Serum Alanine Aminotransferase Level of Patients with Rheumatoid Arthritis Who Had Prior Hepatitis B Infection

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Abstract

Background: Hepatitis after the reactivation of hepatitis B virus (HBV) has been recognized serious in the patients with rheumatoid arthritis (RA) treated with biologics. **Objectives:** The objective of the present study was to search some common background which might be relevant to the host factors that provoke such a serious hepatitis. **Methods:** We retrospectively collected and analyzed all data of serum alanine aminotransferase (ALT) levels in selected patients with RA at random. **Results:** A significant association ($P < 0.001$) between methotrexate (MTX) therapy and elevated serum ALT level was found only in the anti-HBcAb-negative RA group. The mean serum ALT level was significantly higher ($P < 0.001$) in patients with than without MTX therapy in the anti-HBcAb-negative RA group, although no significant difference was found ($P = 0.73$) in the anti-HBcAb-positive RA group. In addition, the anti-HBcAb-positive RA patients showed significantly lower mean serum level of ALT ($P < 0.01$) than anti-HBcAb-negatives provided they are naïve with biologics. But no significant difference between anti-HBcAb-positives and -negatives in the mean serum level of ALT was found among patients experienced with biologics ($P < 0.8$). **Conclusions:** The anti-HBcAb-positive RA group showed the suppression of MTX-induced elevations in serum ALT level. However, this suppression was not found in patients experienced in the treatment with biologics, although it was preserved in those who had not experienced biologics. Failure of this suppressive mechanism of ALT in anti-HBcAb-positive RA patients treated with biologics could be possibly asso-

ciated with serious hepatitis after the reactivation of HBV infection.

Keywords

Rheumatoid Arthritis, *De Novo* Hepatitis B, MTX-Induced Liver Injury, ALT, Biological Agents

1. Introduction

Success in antiviral and remission-induction therapies for patients with rheumatoid arthritis (RA) who had prior hepatitis virus infection is an important clinical challenge to be addressed in clinical practice. Adequate methods for viral elimination and a sustained virological response have been established for the hepatitis C virus (HCV) [1]. When remission-induction therapy of RA is initiated, however, special caution should be paid to patients with RA who are hepatitis B virus (HBV) carriers or who have prior HBV infection. Serious acute hepatitis after the reactivation of HBV [2] [3] has been reported after treatment with biologics in patients with RA who are both positive for the anti-hepatitis B core antibody (anti-HBcAb) and negative for the hepatitis B surface antigen (HBsAg) and even positive for the hepatitis B surface antibody (anti-HBsAb) [4] [5].

Hepatitis viruses and host factors are mentioned as the factors that are involved in the development of *de novo* hepatitis in patients with prior HBV infection. In the present study, we used medical records to randomly extract patients who were positive for anti-HBcAb and retrospectively examined changes in serum alanine transferase (ALT) levels after the first medical examination at our clinic.

2. Materials and Methods

2.1. Subjects

The medical records of 107 patients with RA who had been tested for HBsAg (Lumipulse Presto® HBsAg; Fujirebio, Tokyo, Japan), anti-HBcAb (Architect® Anti-HBc II; ABBOTT Japan, Chiba, Japan), and anti-HCV-Ab (Lumipulse Presto Ortho HCV; Fujirebio) between July 1997 and December 2011 except the alcohol abusers and patients with nonalcoholic steatohepatitis, were examined, although a large number of patients were selected at random and underwent the examination of HBV-related markers in addition to periodical routine laboratory tests happened to be done from September till December 2011 according to the recommendation of Japan Rheumatism Assoc that the time to monitor them should be every four weeks to three months. We then retrospectively assessed 103 patients after excluding 4 patients along with anti-HCV-Ab-positivity. A total of 103 patients with RA (22 men and 81 women; mean age ± SD [range]: 67.9 ± 11.8 years [41 - 88] and 63.5 ± 12.3 [34 - 86] years, respectively), who were negative for HBsAg and anti-HCV-Ab, were categorized to the anti-HBcAb-positive and

-negative RA groups, respectively (Table 1). Sociodemographic and clinical characteristics of patients with and without anti-HBcAb are shown on Table 2. In all these 103 patients, an ALT kit (L-type ALT.J2; Wako Pure Chemical Industries, Osaka, Japan) was used to measure serum ALT levels. Serum ALT levels in their medical records at our institution were investigated to specify the maximum, minimum, and mean levels of serum ALT during individual clinical course within our Clinic. Subsequently, we extracted patients according to the history of presence or absence of treatment with biologics and to the presence or absence of this treatment at the time of reaching the maximum or minimum serum ALT level, and thus we attempted to compare biologic treatment-naive patients with patients who underwent the treatment when reaching the maximum or minimum serum ALT level.

A hepatitis episode was considered to have occurred when a serum ALT level greater than 40 IU/L persisted for at least 2 weeks in the anti-HBcAb-positive RA group and the anti-HBcAb-negative RA group.

Patients were treated with the following biologics: infliximab, adalimumab, tocilizumab, etanercept, and abatacept in 23, 8, 5, 5, and 3 patients, respectively. An elevation in serum ALT level was considered attributable to self-limited transient hepatitis regarded as the mild drug-induced one in all patients. Although the dose reduction of methotrexate (MTX) and the dose increment of folic acid were conducted occasionally, no patients required special treatment apart from ursodeoxycholic acid therapy. Real-time polymerase chain reaction, however, was not conducted for the HBV DNA.

Treatment with biologics was conducted according to the established protocols. Blood samples were obtained immediately prior to their administration. This study was approved by the ethics committee at Showa Inan General Hospital. Informed consent was not required for this study, because all patients were examined retrospectively based on unidentifiable personal information.

2.2. Statistical Analyses

Statistical analyzes were made according to unpaired Student's t-test, Welch's t-test, and the Mann-Whitney U-test [6] [7]. Associations were analyzed using

Table 1. Characteristics of patients with rheumatoid arthritis.

Immunity status		History of treatment with biologics	
	N = 107 n (%)	Present (n = 42) n (%)	Absent (n = 65) n (%)
HBcAb-positive/HCV-Ab (III)-positive	0	0	0
HBcAb-positive/HCV-Ab (III)-negative	23† (21.5)	9 (21.4)	14 (21.5)
HBcAb-negative/HCV-Ab (III)-positive	4† (3.7)	1 (2.4)	3 (4.6)
HBcAb-negative/HCV-Ab (III)-negative	80† (74.8)	32 (76.2)	48 (73.8)

Anti-HBcAb, anti-hepatitis B core antibody; anti-HCV-Ab (III), anti-hepatitis C virus antibody third-generation. †: Patients were negative for hepatitis B surface antigen.

Table 2. Comparison of the sociodemographic and clinical characteristics of the patients with and without HBcAb.

	HBcAb-positive	HBcAb-negative	*P* value
Patients (no.)	23	80	
Gender (female, %)	15 (65.2%)	67 (83.8%)	>0.05
Age (mean ± SD years)	68.65 ± 7.37	63.32 ± 13.16	<0.02
Disease duration (years)	10.08 ± 8.65	10.42 ± 9.72	> 0.80
Observation period (years)	5.54 ± 4.28	5.92 ± 4.80	>0.70
Stage (I/II/III/IV)	5/3/9/6	24/15/29/12	
DAS28-CRP	2.08 ± 0.73	2.12 ± 0.96	>0.80
RF positive	17/23 (73.9%)	55/80 (68.8%)	>0.50
RF (U/ml)	124.3 ± 203.4	127.9 ± 447.7	>0.90
ACPA positive	21/23 (91.3%)	58/80 (72.5%)	>0.05
ACPA (U/ml)	163.6 ± 104.2	124.1 ± 125.0	>0.10
Corticosteroids	8/23 (34.8%)	23/80 (28.8%)	>0.50
MTX	22/23 (95.6%)	78/80 (97.5%)	>0.90
AF	0/23	7/80 (8.8%)	>0.30
SASP	4/23 (17.4%)	16/80 (20.0%)	>0.50
Buc	4/23 (17.4%)	8/80 (10.0%)	>0.80

Mean ± standard deviation (SD), unless otherwise noted.
DAS28-CRP: disease activity score assessing 28 joints with CRP; RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibody; MTX: methotrexate; AF: auranofin; SASP: salazosulfapyridine; Buc: bucillamine.
Differences between the groups were determined by chi-squared test, independent t-test, or Welch's t-test.

the ϕ coefficient, significance level of which was examined according chi-square test. A two-tailed value of $P < 0.05$ was considered statistically significant.

3. Results

To study the association between elevated serum ALT level and MTX therapy, we first investigated whether patients were on MTX therapy when the maximum or minimum serum level of ALT was reached. Eighty patients (85.1%) were on MTX therapy when serum ALT levels reached the maximum, while 47 patients (50%) were on MTX therapy when ALT reached the minimum. These results suggest a significant association between elevated serum ALT level and MTX therapy ($P < 0.001$) (Table 3). This association was found not in the anti-HBcAb-positive RA group ($P < 0.8$) (Table 4) but in the anti-HBcAb-negative RA group ($P < 0.001$) (Table 5). Namely, changes in methotrexate-induced elevation in serum ALT level were found only in RA patients without prior HBV infection.

Next, we examined the maximum, minimum, and mean serum levels of ALT, regardless of treatment with biologics or MTX. Consequently, no significant differences were found in these levels between the anti-HBcAb-positive RA group and the anti-HBcAb-negative RA group (Table 6), although the minimum

Table 3. Associations between serum ALT levels and MTX therapy in patients with rheumatoid arthritis.

MTX therapy at the maximum/minimum serum level of ALT	Episodes that presented the maximum serum ALT level (n = 94)	Episodes that presented the minimum serum ALT level (n = 94)
Present	80	47
Absent	14	47

MTX, methotrexate; ALT, alanine aminotransferase $\varphi = 0.3749$, $\chi^2 = 26.427$, $P < 0.001$. Nine of 103 patients were excluded from statistical analyses because the number of measurements of serum ALT levels was three or less.

Table 4. Associations between serum ALT levels and MTX therapy in the RA patients with anti-HBcAb-positivity.

MTX therapy at the maximum/minimum serum level of ALT	Episodes that presented the maximum serum ALT level (n = 22)	Episodes that presented the minimum serum ALT level (n = 22)
Present	17	14
Absent	5	8

MTX, methotrexate; ALT, alanine aminotransferase; anti-HBcAb, anti-hepatitis B core antibody $\varphi = 0.1494$, $\chi^2 = 0.4367$ (Yate's correction), $P < 0.8$. Twenty-two of 23 anti-HBcAb-positive patients were selected for the retrospective study because four or more measured serum ALT levels were mandatory for the present study.

Table 5. Associations between serum ALT levels and MTX therapy in the RA patients with anti-HBcAb-negativity.

MTX therapy at the maximum/minimum serum level of ALT	Episodes that presented the maximum serum ALT level (n = 72)	Episodes that presented the minimum serum ALT level (n = 72)
Present	63	33
Absent	9	39

MTX, methotrexate; ALT, alanine aminotransferase; anti-HBcAb, anti-hepatitis B core antibody $\varphi = 0.4419$, $\chi^2 = 28.119$, $P < 0.001$. Seventy-two of 80 anti-HBcAb-negative patients were selected for the retrospective study because four or more measured serum ALT levels were mandatory for the present study.

Table 6. Serum ALT levels in anti-HBcAb-positive and -negative patients with rheumatoid arthritis.

Serum ALT level	Anti-HBcAb-positive (n = 22)	Anti-HBcAb-negative (n = 72)	*P* value
Maximum	56.04 ± 48.7	62.85 ± 33.4	<0.6†
Minimum	12.68 ± 4.5	10.93 ± 4.5	<0.2‡
Mean	23.25 ± 12.5	25.93 ± 10.1	<0.4‡

ALT, alanine aminotransferase, anti-HBcAb, anti-hepatitis B core antibody †: Welch's t-test; ‡: Unpaired t-test. Patients, who had three or less measured serum ALT levels, were excluded from statistical analyses.

serum ALT levels tended to be higher in the former group ($P < 0.2$). In addition, the hepatitis episodes tended to be less frequent in patients with RA who had prior HBV infection ($P < 0.2$) (Table 7). We conjectured that elevated serum

Table 7. Relationships between the hepatitis episode and the immunity status of anti-HBcAb in patients with rheumatoid arthritis.

Immunity status	N = 98	Episode†-positive patients (n = 63)			Episode†-negative patients (n = 35)		
	n (%)	n (%)	Bio (+)	Bio (−)	n (%)	Bio (+)	Bio (−)
HBcAb-positive	22 (100)	11 (50.0)	5	6	11 (50.0)	4	7
HBcAb-negative	76 (100)	52 (68.4)	22	30	24 (31.6)	10	14

Anti-HBcAb, anti-hepatitis B core antibody; ALT, alanine aminotransferase; Bio, history of treatment with biologics. †: A hepatitis episode was an elevation in serum ALT level ≥ 40 IU/L persisted for at least 2 weeks. Association between the hepatitis episode and anti-HBcAb (50.0% vs. 68.4%): $\chi^2 = 2.522$ ($P < 0.2$). Patients, who had less than two measured serum ALT levels, were excluded from statistical analyses.

ALT level was associated with MTX therapy in RA patients and that MTX-associated elevations in serum ALT level were suppressed in the anti-HBcAb-positive RA group. Therefore, clinicians need to give heed to the presence or absence of MTX therapy and to prior HBV infection that can be indicated by anti-HBc and/or -anti-HBs antibody along with anamnesis when interpreting changes in serum ALT levels in patients with RA.

Accordingly, to further evaluate changes in serum ALT levels, patients were examined based on the positivity or negativity for anti-HBcAb and on the presence or absence of MTX therapy with regard to the maximum, minimum, and mean serum levels of ALT (Figure 1).

Both the anti-HBcAb-positive and -negative RA groups showed significantly higher ($P < 0.05$ and $P < 0.01$, respectively) maximum ALT levels in the presence of MTX therapy against the absence of MTX therapy. Of note was the fact that the level of significance was greater in the anti-HBcAb-negative RA group. The anti-HBcAb-positive RA group showed no significant difference in the mean ALT level between presence and absence of MTX-administration ($P = 0.73$). However, only the anti-HBcAb-negative RA group showed a significant increase ($P < 0.001$) in the mean ALT level in the presence of MTX therapy, as compared with absence of MTX therapy. Namely, the suppression of the MTX-induced increase in serum ALT levels was confirmed in the anti-HBcAb-positive RA group, which was suggested from the fact that the increased serum ALT level found in the anti-HBcAb-negative RA group was lost to find in the anti-HBcAb-positive RA group in the presence of MTX therapy (Table 4 and Table 5).

On the other hand, neither the anti-HBcAb-positive group nor the anti-HBcAb-negative RA group showed a significant difference in the minimum ALT level between the presence and absence of MTX therapy. However, of note was the fact that the anti-HBcAb-positive RA group tended to show the high minimum ALT level ($P < 0.1$) in the presence of MTX therapy against the anti-HBcAb-negative counterpart (Figure 1).

To examine the effects of treatment with biologics on serum ALT levels, subsequently, patients with RA were categorized to two groups according to the presence or absence of history of treatment with biologics. Furthermore, only

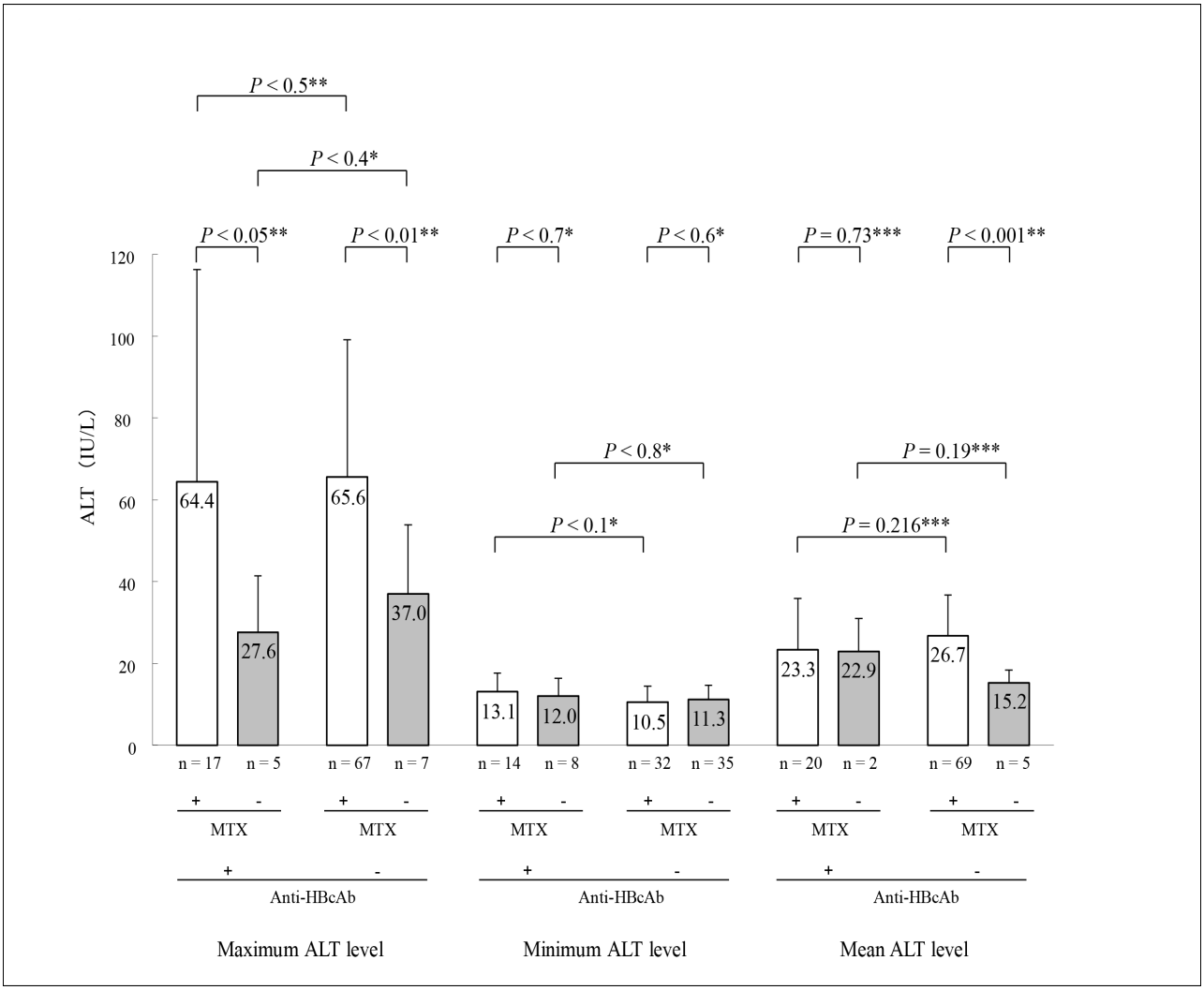

Figure 1. The maximum, minimum, and mean serum levels of ALT in patients with RA according to the positivity or negativity for anti-HBcAb and to the presence or absence of MTX therapy. *: Student's t-test; **: Welch's t-test; ***: The Mann-Whitney U-test. The bars represent standard deviations. ALT, alanine aminotransferase; anti-HBcAb, hepatitis B core antibody; MTX, methotrexate; RA, rheumatoid arthritis.

patients with RA, who had been on treatment with biologics when serum ALT levels reached the maximum or minimum, were extracted and compared with those who were neither on the treatment nor the history thereof (**Figure 2**). Consequently, no significant difference was found in serum ALT level between the anti-HBcAb-positive and -negative RA groups in the presence of treatment with biologics (**Figure 2**†). In the absence of treatment with biologics (**Figure 2**‡), however, the mean serum ALT level was significantly lower ($P < 0.01$) in the anti-HBcAb-positive group than in the anti-HBcAb-negative group. This result indicates the possibility that a significant difference ($P < 0.01$) in serum ALT level between the anti-HBcAb-positive and -negative RA groups was lost in the presence of treatment with biologics. Furthermore, no significant difference was found between the anti-HBcAb-positive and -negative RA groups with respect to background on the characteristics of MTX therapy (**Table 8**).

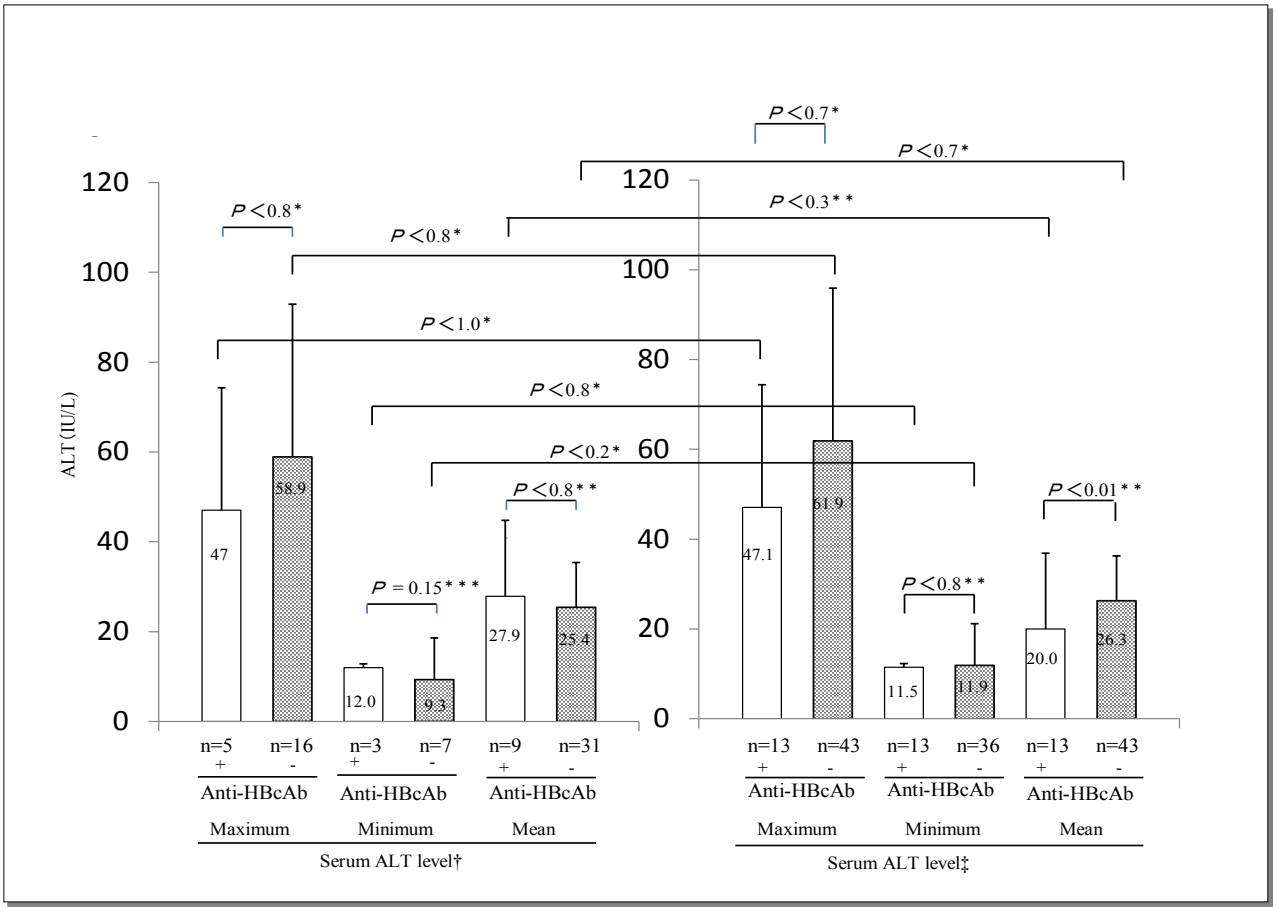

Figure 2. The maximum, minimum, and mean serum levels of ALT in patients with RA according to the positivity or negativity for anti-HBcAb and to the presence or absence of the history of treatment with biologics. *: Student's t-test; **: Welch's t-test; ***: The Mann-Whitney U-test. †: Presence of history of treatment with biologics; ‡: Absence of history of treatment with biologics. The bars represent standard deviations. RA, rheumatoid arthritis; ALT, alanine aminotransferase; anti-HBcAb, hepatitis B core antibody; MTX, methotrexate; RA, rheumatoid arthritis.

Table 8. Background on the characteristics of MTX therapy classified by immunity status of anti-HBcAb in patients with RA who have never been treated with biologics.

Immunity status	Characteristics of MTX therapy		
	Number of therapies	Duration (months)†	Accumulated dose (mg)†
HBcAb-positive(n = 14)	13	32.8 ± 46.8	2647.9 ± 2183.1
HBcAb-negative (n = 47)	46	27.1 ± 33.7	2149.4 ± 2534.0
P value	0.704‡	<0.8§	<0.5§

MTX, methotrexate; RA, rheumatoid arthritis; anti-HBcAb, anti-hepatitis B core antibody. †: Mean ± SD. ‡: Fisher's exact test. §: Welch's t-test.

4. Discussion

Serum ALT level is a well-known marker of hepatocyte injury [8] that is frequently examined in routine laboratory testing. The reference value at our institution ranges between 6 and 40 IU/L, and serum ALT levels can vary in the same

individual on different days. Serum ALT levels have been found in proper and constant ranges of variability in healthy individuals throughout the year [9]. Therefore, the maximum, minimum, and mean serum ALT levels measured at different time points during a defined period are considered appropriate as the representative scores of a given patient.

In this study, we retrospectively examined and compared these levels in RA patients to clarify their clinical relevance in relation to MTX therapy, prior HBV infection and treatment with biologics. One important finding in the present study was a significant association between elevated serum ALT level and MTX therapy ($\varphi = 0.3749$, $\chi^2 = 26.427$, $P < 0.001$) in patients with RA. This association was found in RA patients who had no history of prior HBV infection ($P < 0.001$), but not in those who had ($P < 0.8$). Furthermore, the anti-HBcAb-positive RA group tended to show a less number of hepatitis episodes ($P < 0.2$) as compared with the anti-HBcAb-negative RA group. Based on these findings, MTX-induced elevations in serum ALT level were suppressed in patients with RA who had prior HBV infection in contrast to those who had not. These findings indicate that the development of MTX-induced hepatotoxicity might be suppressed in patients with prior HBV infection.

Since no significant differences were found in serum ALT levels between RA patients who had prior HBV infection and patients who did not as a whole, they were subcategorized according to the presence or absence of MTX therapy (**Figure 1**). Consequently, significant elevations in the maximum ($P < 0.01$) and mean ($P < 0.001$) serum ALT levels were found in the anti-HBcAb-negative RA group in the presence of MTX therapy against the absence of MTX therapy. On the contrary, the anti-HBcAb-positive RA group in the presence of MTX therapy showed no significant elevations in the minimum($P < 0.7$) and mean($P = 0.73$) serum ALT level, although exhibited significantly less elevations ($P < 0.05$) in the maximum serum ALT level. Taken together, these findings based on the measured values of serum ALT levels suggest the presence of a suppressive mechanism on MTX-induced increases in serum ALT level in patients who had prior HB infection.

Hepatocyte apoptosis has been reported as a cardinal pathophysiological mechanism in MTX-induced hepatotoxicity from both histological [10] and biochemical viewpoints [11]. Accordingly, a suppressive mechanism on elevations in serum ALT level or a protection mechanism of hepatocytes, which was observed with RA patients who had HBV infection in the present study, possibly relies on the suppression of hepatocyte apoptosis. One of the reported suppressive mechanisms of apoptosis is the modulation of the Nrf2-NF-κB pathways [11] and might be involved in patients whose hepatocytes are infected with HBV and who do not present *de novo* hepatitis. Furthermore, HBV needs to prevent the apoptosis of host cells for its survival and spread in the liver [12]. We consider that our data provide clinical evidence to support the presence of the suggested suppressive mechanism on hepatocyte apoptosis in our patients.

On the other hand, the tendency for higher minimum serum ALT levels in RA patients who had prior HBV infection remains a mystery. One plausible mechanism is that the permeability of aged hepatocyte membranes increases along with the fact the expected viability of hepatocytes is extended by a suppressive mechanism on hepatocyte apoptosis, resulting in the persistence of the higher minimum serum ALT levels.

5. Conclusion

In conclusion, the suppression of MTX-induced increases in serum ALT levels was considered attributable to a suppressive mechanism on the apoptosis of HBV-infected hepatocytes. On the other hand, the suppression of an increase in mean serum ALT level was not found in anti-HBcAb-positive RA patients during treatment with biologics, which might suggest the failure (so to speak, "biologic-induced failure") of the suppressive mechanism on the apoptosis of HBV-infected hepatocytes. If this is true, the relevant failure would be one of the explanations about serious hepatitis that may develop subsequent to the reactivation of HBV due to treatment with biologics. Therefore, the elucidation of this mechanism by immuno-virological approaches potentially leads to the development of new categories of drugs (e.g., inhibitors of hepatocyte apoptosis) and therapeutic modalities (e.g., immunomodulation) for patients with hepatitis. Indeed, our outcomes are not conclusive because we don't have immuno-virological evidence relevant to this clinical phenomenon, and the patient population is just small. Therefore, a detailed immuno-virological study and a large-sized cohort study in other medical institutions are required to confirm our speculation.

Conflict of Interest

The authors have nothing to declare.

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