Factorial Analysis of Hepatitis B Virus Reactivation-Induced Hepatitis B Using JADER

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Hepatitis B caused by chemotherapy- and immunosuppression-associated hepatitis B virus reactivation is likely to become fulminant, and a high mortality rate has been reported. In this study, using the Japanese adverse drug event report database (JADER), factorial analysis of patients who developed hepatitis B as an adverse event was performed. The number of reported cases of hepatitis B during the survey period was 781 and 185 of them (24%) died. Rituximab and prednisolone were administered to many cases (233, 216 cases, respectively), and the reporting odds ratios were high (65.35, 13.40, respectively), suggesting their strong association with the development of hepatitis B. Regarding the onset time, rituximab-induced hepatitis B developed within one year after administration in 83%, being a high frequency. Prednisolone-induced hepatitis B developed even after one year in 36%. Since prednisolone is used to treat rheumatoid arthritis at a dose ≤10 mg/d, the patients were divided based on the prednisolone dose into the groups treated at >10 and ≤10 mg/d, and the onset time was investigated in each group. The median onset time was 113 and 330 d, respectively, showing a significant difference. On time-to-event analysis using the Weibull distribution, rituximab was classified as the early failure type, and prednisolone and methotrexate for rheumatoid arthritis were classified as the wear out failure type. These findings are important information which may lead to early discovery of and taking actions against hepatitis B being helpful for providing appropriate medical care.

Key words hepatitis B virus; Japanese Adverse Drug Event Report database; rituximab; prednisolone

In Japan, about 350–400 million people are estimated to be infected with hepatitis B virus (HBV), and the HBV carrier rate in Japan is reported to be 0.31%. An increase in the HBV DNA load in HBV carriers and previously infected patients by chemotherapy and immunosuppression is termed HBV reactivation, and this HBV reactivation has recently been problematic at clinical sites. Hui et al. reported that the incidence of fulminant hepatitis in 244 HBs antigen-negative malignant lymphoma patients treated with chemotherapy was significantly higher in the HBV reactivation-induced hepatitis group than in the sepsis-associated hepatitis group (37.5% vs. 2.5%, p<0.01), and Umemura et al. reported that 22% of patients with chemotherapy-associated HBV reactivation developed fulminant hepatitis and the mortality rate was 100%, showing that hepatitis caused by chemotherapy- and immunosuppression-associated HBV reactivation is likely to become fulminant and this is a fatal adverse event, for which countermeasures are essential for clinical practice. The frequency and timing of HBV reactivation may vary depending on the type of chemotherapy and immunosuppression, but these have not been fully clarified. Clarification of the tendency of development, timing, and onset age of adverse events may facilitate taking individual as well as rapid measures.

The Pharmaceutical and Medical Device Agency (PMDA) publishes the Japanese Adverse Drug Event Report database (JADER). Analysis of JADER may be useful to spread consciousness in medical care workers in addition to contributing to the selection of appropriate drugs and prevention and early discovery of adverse events. As such, contribution to the safety of medical care is strongly expected. Adverse events which occurred in 2004 and thereafter in Japan were accumulated in JADER. There are methods to calculate and quantify the safety signal to acquire beneficial information from JADER. Most of these are disequilibrium analyses relatively comparing the reporting frequency, and the reporting odds ratio (ROR) is the representative method with high sensitivity capable of detecting signals at an early stage with a few case reports. In addition, time information from drug administration to the development of adverse events is described in JADER, enabling time-to-event analysis using the Weibull distribution. In this study, using JADER, the sex, age, outcome, drug, and onset time were surveyed in patients who developed hepatitis B as an adverse event, and the ROR and Weibull distribution were calculated.

METHODS

Subjects JADER was downloaded from the PMDA website (http://www.pmda.go.jp) in November 2015, and data between April 2004 and March 2014 were used. Overlapping data of the same outcome from a drug with the same identification number were deleted. Cases in which hepatitis B, acute hepatitis B, chronic hepatitis B, hepatitis B antibody-positive, hepatitis B surface antigen-positive, hepatitis B virus test, hepatitis B virus test-positive, an increase in hepatitis B DNA, and hepatitis B DNA measurement-positive were described as adverse events were extracted as hepatitis B cases. The sex, age, drug suspected to have caused hepatitis B, outcome, and onset time were surveyed. Cases with no description of the sex, age, or outcome were regarded as unclear. Regarding the age, cases described as elderly and adults were also handled as unclear.

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When a drug was specified as a suspected drug and no other suspected drug or concomitant drug was described, the case was defined as having no concomitant drug.

The onset time was calculated by adding 1 to the time of appearance of the adverse event from initiation of administration. Cases with no description of the date of initiation of administration or development of the adverse event were excluded. Cases with description of only the year were also excluded. When the year and month were described but the day was not described, the day was assumed to be 15. Drug withdrawal for 7 d or shorter was handled as within the same data. When the drug was withdrawn for 8 d or longer, the data were handled as another case.

**Statistical Analysis**
The safety signal index, ROR, was calculated from the $2 \times 2$ contingency table\(^{2,9}\) (Table 1). When the lower limit of the 95% confidence interval (CI) exceeded 1, the drug was regarded as signal-positive.

The onset time in the groups treated with prednisolone at a dose $>10$ and $\leq 10$ mg/d was analyzed using the Mann–Whitney U test. Differences with $p<0.05$ were considered significant. Statistical analyses were performed using Excel 2010 (Microsoft Corporation, Redmond, WA, U.S.A.) with the add-in software Statcel 3.

Time-to-event analysis using the Weibull distribution was performed and the shape parameter $\beta$ was calculated.\(^{9-13}\) The Weibull distribution represents the failure rate distribution against time, and the parameter is estimated from the failure rate. In analysis of adverse reactions to drugs, the failure rate corresponds to the development of adverse reactions. When $\beta<1$, the incidence decreases with time, being classified as the early failure type, when $\beta=1$, adverse reaction develops at a constant pace, being classified as the random failure type, and when $\beta>1$, the incidence decreases with time, being classified as the wear out failure type. When the onset time of the adverse event was 1095 d (3 years) or longer after initiation of administration, calculation was performed with 1095 d as the onset time. Analysis was performed using JMP 11 (SAS Institute Inc., Cary, NC, U.S.A.).

**RESULTS**

**Outcome and Age of Patients Who Developed Hepatitis B**
The total number of cases registered in JADER was 562069 in November 2015, and the number of patients was 353397. Of these, 781 cases were hepatitis B, the number of patients was 768, 53% of them were male (407/768) and 45% (343/768) were female, and the sex was unclear in 2.3% (18/768). The outcome was remission in 28% (216/781), recovery in 25% (197/781), death in 24% (185/781), unrecovered in 5% (40/781), sequelae in 0.1% (1/781), and unclear in 18% (142/781). The outcomes are presented by age in Fig. 1. The incidence was the highest in their 60 s and it was 30% (235/781), followed by 24% (189/781) in their 50 s, and 19% (148/781) in their 70 s. The frequency of death was also the highest in their 60 s and it was 9.2% (72/781), followed by 6.0% (47/781) in their 50 s, and 4.6% (36/781) in their 70 s.

**Outcome and Onset Time of Hepatitis B Associated with Each Drug by Age**
Rituximab most frequently induced hepatitis B (234/781), followed by 220 cases induced by prednisolone, 109 cases induced by methotrexate, 108 cases induced by cyclophosphamide, 85 cases induced by vincristine, and 76 cases induced by doxorubicin. These 6 high-ranked drugs were reported in more than 70 cases. The outcome and onset time of hepatitis B in cases induced by

**Table 1. 2x2-Contingency Table**

<table>
<thead>
<tr>
<th></th>
<th>Target adverse events</th>
<th>Non-target adverse events</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Target drug</td>
<td>$n_{11}$</td>
<td>$n_{12}$</td>
<td>$n_{1+}$</td>
</tr>
<tr>
<td>Non-target drug</td>
<td>$n_{21}$</td>
<td>$n_{22}$</td>
<td>$n_{2+}$</td>
</tr>
<tr>
<td>Total</td>
<td>$n_{+1}$</td>
<td>$n_{+2}$</td>
<td>$n_{++}$</td>
</tr>
</tbody>
</table>

ROR$=\frac{n_{11}/n_{21}}{n_{12}/n_{22}}$.\(^{2,9}\)
these drugs are presented by age in Figs. 2 and 3, respectively. Figure 2a shows the outcomes of rituximab-induced cases by age. Patients in their 60, 70, and 50s accounted for 38% (90/234), 18% (52/234), and 16% (46/234), respectively, showing a high incidence in their 50–70s. The outcome was death in 40% (93/234), and the frequency of death was 46% in their 60s (41/90), being the highest. The date of initiation of administration and onset time were described in 145 of the 234 cases and the onset time was investigated in these cases. The most frequent onset time was 1–30 d after initiation of administration (26/145) and development slowly decreased thereafter (Fig. 3a).

Figure 2b shows the outcomes of prednisolone-induced cases by age. Patients in their 60, 50, and 70s accounted for 32% (70/220), 26% (58/220), and 23% (51/220), respectively, showing a high incidence in their 50–70s. Regarding the outcome, death accounted for 34% (75/220), and the highest frequency of death was 44% (33/75) noted in their 60s (41/90), being the highest. The onset time was investigated in 145 cases, and the frequency of onset time of 1–240 d after initiation of administration was high (Fig. 3b). In addition, 14 cases developed at 721 d or thereafter (Fig. 3c).

Figure 2c shows the outcomes of methotrexate-induced cases by age. Patients in their 50, 60, and 70s accounted for 33% (36/109), 28% (31/109), and 13% (28/220), respectively, showing a high incidence in their 50–70s. Regarding the outcome, death accounted for 32% (35/109), and the highest frequency of death was 34% (12/35) noted in their 70s. The onset time was investigated in 52 cases, and 19 cases developed at 721 d or thereafter (Fig. 3d).

Figure 2d shows the outcomes of cyclophosphamide-induced cases by age. Patients in their 60, 70, and 50s accounted for 38% (41/108), 27% (29/108), and 18% (19/108), respectively, showing a high incidence in their 50–70s. Regarding the outcome, death accounted for 39% (42/108), and the highest frequency of death was 50% (21/42) noted in their 60s. The onset time was investigated in 57 cases, and the frequency of onset time of 1–240 d after initiation of administration was high (Fig. 3d).

Figure 2e shows the outcomes of doxorubicin-induced cases by age. Patients in their 60, 70, and 50s accounted for 41% (31/76), 25% (19/76), and 22% (17/76), respectively, showing a high incidence in their 50–70s. Regarding the outcome, death accounted for 45% (34/76), and the highest frequency of death was 50% (17/34) noted in their 60s. The onset time was investigated in 35 cases, and the frequency of onset time of 1–240 d after initiation of administration was high (Fig. 3e).

Figure 2f shows the outcomes of vincristine-induced cases by age. Patients in their 50, 60, and 70s accounted for 36% (36/109), 28% (24/85), and 19% (16/85), respectively, showing a high incidence in their 50–70s. Regarding the outcome, death accounted for 45% (34/76), and the highest frequency of death was 50% (17/34) noted in their 60s. The onset time was investigated in 42 cases, and the frequency of onset time of 1–240 d after initiation of administration was high (Fig. 3f).
RORs of Hepatitis B Associated with the Drugs and Cancer Chemotherapy  
RORs of hepatitis B associated with the drugs are shown in Table 2. ROR of hepatitis B associated with rituximab was 65.35 (95% CI: 55.74–76.62); prednisolone, 13.40 (11.43–15.70); methotrexate, 5.16 (4.20–6.34); cyclophosphamide, 18.64 (15.16–22.92); doxorubicin, 16.61 (13.06–21.12); and vincristine, 23.35 (18.58–29.36); and signals were detected in all drugs.

RORs of hepatitis B associated with the drugs with no concomitant drug are shown in Table 3. Signals were detected in the following 4 drugs: rituximab, 89.09 (72.21–109.92); prednisolone, 11.71 (8.71–15.74); methotrexate, 3.42 (2.40–4.88); and doxorubicin, 3.57 (1.59–7.80).

To investigate the difference between the cancer chemotherapies, RORs of the disease associated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) therapy and CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) therapy excluding rituximab were calculated. These were 92.37 (68.80–75.19) and 9.93 (3.16–31.23), respectively, showing that signals were detected in both chemotherapies.

Daily Dose of Prednisolone and Onset Time of Hepatitis B  
The relationship between the daily dose of prednisolone and onset time of hepatitis B is shown in Fig. 4. Since prednisolone is administered at 10 mg/d or lower for rheumatoid arthritis, the cases were divided into those treated at a daily dose of ≤10 and >10 mg/d, and the onset time was statistically analyzed. The median onset time in the ≤10 and >10 mg/d groups were 330 d (minimum–maximum: 127–512 d) and 113 d (1–714 d), respectively, showing a significant difference.
When the daily dose was $>10\text{mg/d}$, 81% (25/31) of the cases developed within 200d, whereas when the daily dose was $\leq10\text{mg/d}$, 71% (5/7) developed at 200d or later. In addition, the duration of administration was short (within 5d) in 67% (22/31) in the $>10\text{mg/d}$ group.

**Reason for the Use of Methotrexate and Concomitant Drugs** The reasons for the use of methotrexate are shown in Fig. 5a. The use for rheumatoid arthritis accounted for 75% (82/109). Of the 82 rheumatoid arthritis cases, 55 were concomitantly treated with another drug. Drugs concomitantly administered with methotrexate in 3 or more cases are shown in Fig. 5b. Prednisolone was most frequently administered and accounted for 67% (37/55).

**Onset Time of Hepatitis B Induced by Each Drug Profiled Using the Weibull Distribution** The onset time of hepatitis B induced by each drug was profiled using the Weibull distribution. The results are shown in Table 4. The $\beta$ value of rituximab was 0.87 (95% CI: 0.76–0.98) being $\beta<1$, suggesting that the onset time of the adverse event was the early failure type. When the purpose of the use of prednisolone and methotrexate was to treat rheumatoid arthritis, $\beta$ was

<table>
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<th>Drugs</th>
<th>$\beta$</th>
<th>95%CI</th>
</tr>
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<tbody>
<tr>
<td>Rituximab</td>
<td>0.87</td>
<td>0.76–0.98</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>1.00</td>
<td>0.34–1.17</td>
</tr>
<tr>
<td>Prednisolone$^a$</td>
<td>1.80</td>
<td>1.16–2.63</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1.17</td>
<td>0.92–1.45</td>
</tr>
<tr>
<td>Methotrexate$^a$</td>
<td>1.64</td>
<td>1.23–2.13</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>0.92</td>
<td>0.75–1.11</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.05</td>
<td>0.81–1.38</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.98</td>
<td>0.77–1.22</td>
</tr>
</tbody>
</table>

$^a$ Used to treat rheumatoid arthritis.

![Fig. 4. Daily Dose of Prednisolone and Onset Time of Hepatitis B ($n=38$)](image1)

![Fig. 5. (a) Reason for the Use of Methotrexate ($n=109$) and (b) Drugs Concomitantly Used with Methotrexate for Treatment of Rheumatoid Arthritis ($n=55$)](image2)
1.80 (1.16–2.63) and 1.64 (1.23–2.13), respectively, being \( \beta > 1 \), suggesting that the onset time of the adverse events was the wear out failure type.

**DISCUSSION**

Hepatitis caused by chemotherapy- and immunosuppression-associated HBV reactivation is likely to become fulminant, being a fatal adverse event.\(^2\)\(^-\)\(^4\) In this survey, death from hepatitis B accounted for 24%. In a questionnaire survey performed by Umemura et al., the HBV reactivation-associated mortality rate was 22%,\(^6\) being consistent with our finding.

Yeo et al. reported that R-CHOP or CHOP therapy was performed in 80 patients with HBs antigen-negative diffuse large B-cell lymphoma, and HBV reactivation occurred in 5 patients only in the R-CHOP therapy group.\(^1\)\(^4\) Similarly, ROR was much higher in R-CHOP-treated than in CHOP-treated cases in our study, suggesting a strong association between rituximab and the development of hepatitis B. Actually, many cases of rituximab-associated hepatitis B have been reported.\(^1\)\(^5\)\(^-\)\(^9\)

When ROR of hepatitis B associated with each drug (Table 2) was compared with that in the absence of a concomitant drug, as shown in Table 3, no signal was detected in cyclophosphamide or vincristine, suggesting that drugs concomitantly administered in R-CHOP therapy, such as rituximab, increased the number of hepatitis B cases to the high rank. RORs of rituximab and prednisolone were higher than those of the other drugs in Table 3, suggesting that these drugs are more strongly associated with hepatitis B development than the other drugs. Hui et al. performed systemic chemotherapy in 244 HBs antigen-negative malignant lymphoma patients and observed development of HBV reactivation-induced hepatitis in 8.\(^5\) In their study, the frequency of HBV reactivation by a rituximab/prednisolone combination regimen was 12.2% whereas that by other regimens was 1.0%, and the rituximab/ prednisolone combination regimen was a risk factor on multivariate analysis. It has been reported that reduction of CD20-positive B cells by rituximab causes functional failure of antigen-presenting cells, which reactsivate HBV.\(^3\) Steroid was reported to promote HBV replication and increase HBV DNA production by binding to glucocorticoid-responsive element of HBV.\(^2\)\(^0\) Although the mechanism of HBV reactivation differs between rituximab and prednisolone, since both drugs activate HBV, rituximab/prednisolone combination regimens may be a risk factor of hepatitis B.

The hepatitis B onset time was 1–240 d after initiation of administration of rituximab, cyclophosphamide, doxorubicin, and vincristine or a high frequency of the development continued until 270 d (Figs. 3a, d–f). The incidence was high at 1–240 d and after 721 d in prednisolone-treated cases (Fig. 2b), and the incidence rose after 721 d in methotrexate-treated cases (Fig. 2b). For chemotherapies including rituximab and prednisolone, such as R-CHOP therapy, monitoring of HBV DNA from 1 to 240 d or at 270 d is necessary. The Guidelines for the Management of Hepatitis B Virus Infection recommend once-a-month monitoring of HBV DNA during treatment and for at least one year after completion of treatment when chemotherapy including rituximab and steroid is performed.\(^2\)\(^1\) This is consistent with our results. But, the incidence (8 cases) rose after 721 d in rituximab-treated cases (Fig. 3a). It may be desirable to continue HBV DNA monitoring periodically after one year.

Hepatitis B developed even after 721 d in cases treated with prednisolone and methotrexate, for which long-term HBV monitoring is necessary. Prednisolone is used to treat rheumatoid arthritis at a dose of \( \leq 10 \) mg/d. Thus, the cases were divided into groups treated at \( \leq 10 \) and \( > 10 \) mg/d and compared. The onset time was significantly delayed in the \( \leq 10 \) mg/d group compared with that in the \( > 10 \) mg/d group \(( p < 0.05)\). When the drug was administered at a dose \( > 10 \) mg/d for a short period, hepatitis B developed within 200 d in 94% (29/31), whereas it developed after 200 d when the drug was administered at \( \leq 10 \) mg/d for a prolonged period in 71% (5/7) (Fig. 4). Methotrexate was administered to treat rheumatoid arthritis in patients who developed hepatitis B (Fig. 5a) and prednisolone was most frequently administered concomitantly in treatment of rheumatoid arthritis (Fig. 5b), suggesting that prednisolone- and methotrexate-associated hepatitis B development after 721 d was related to the long-term treatment for rheumatoid arthritis. The Guidelines for the Management of Hepatitis B Virus Infection specify that when steroid is used for rheumatoid arthritis patients, it is desirable to monitor HBV-DNA once a month after initiation of immunosuppressive therapy and for at least 6 months after change of the treatment content, and the interval and duration are to be investigated after 6 months in consideration of the treatment content.\(^2\)\(^0\) However, our survey clarified that hepatitis B developed even after 6 months when prednisolone was administered at \( \leq 10 \) mg/d to rheumatoid arthritis patients for a prolonged period. Therefore, it may be desirable to continue HBV DNA monitoring periodically after 6 months when prednisolone is administered for long time.

The usefulness of the Weibull distribution for profiling the onset time of drug-related adverse events has been reported.\(^5\)\(^-\)\(^13\) On time-to-event analysis using the Weibull distribution, rituximab showed the profile of the early failure type (Table 4). Accordingly, it is particularly necessary to pay attention early after initiation of administration. In contrast, prednisolone and methotrexate used for rheumatoid arthritis showed the profile of the wear out failure type (Table 4), suggesting that long-term continuous monitoring is necessary when prednisolone and methotrexate are administered to treat rheumatoid arthritis.

The incidence of rituximab-, cyclophosphamide-, doxorubicin-, and vincristine-associated hepatitis B was the highest in their 60s, followed by their 70s and then 50s (Figs. 2a, d–f). The incidence of prednisolone-associated development was the highest in their 60s, followed by their 50s and then 70s (Fig. 2b), and that of methotrexate-associated development was the highest in their 50s followed by their 60s and then 70s (Fig. 2c). HBV DNA monitoring should be selectively performed in the order of the 60, 70, and 50 s in patients treated with chemotherapy including rituximab and prednisolone, such as R-CHOP therapy, and in the order of the 50, 60, and 70 s in patients treated with methotrexate/prednisolone combination therapy for rheumatoid arthritis.

Analysis of JADER is a superior means to detect unknown serious adverse events, but it has limitation because adverse events are spontaneously reported. Under-reporting, a lack of detailed clinical information, excess reports due to an influence of safety information, and an increase in reports at the
time of launch are known. Information acquired from JADER should be carefully interpreted. In this study, no significant differences were observed in the number of patients with rituximab, prednisolone, methotrexate, cyclophosphamide, vincristine, and doxorubicin-induced hepatitis B each year before and after provision of safety information data.

This study clarified the incidence and onset time of hepatitis B associated with each drug. These findings are important information leading to the early discovery of and taking early measures against hepatitis B and may help in providing appropriate medical care.

**Conflict of Interest** The authors declare no conflict of interest.

**REFERENCES**