Table 3 Univariate and multivariate analyses of variables associated with thrombocytopenia (PLT < 1.3 × 10^5/ul) in HD + HCV patients

Variables	Odds ratio	95% CI	*P* value
Univariate analysis			
Age (years)			
<60	1.0		
≥60	0.616	0.247–1.534	0.298
Sex			
Female	1.0		
Male	1.273	0.518–3.129	0.599
Duration of HD (years)			
<10	1.0		
≥10	1.321	0.555–3.141	0.529
Follow-up period (months)			
<55	1.0		
≥55	1.057	0.445–2.515	0.899
History of diabetes mellitus			
−	1.0		
+	1.426	0.557–3.646	0.459
Serotype			
I	1.0		
II	1.051	0.384–2.871	0.923
AST (IU/L)			
<30	1.0		
≥30	3.4	0.676–17.103	0.138
ALT (IU/L)			
<20	1.0		
≥20	2.686	1.083–6.662	0.033
GGT (IU/L)			
<50	1.0		
≥50	4.333	1.235–15.206	0.022
TC (mg/dl)			
<150	1.0		
≥150	0.727	0.27–1.958	0.528
Multivariate analysis			
ALT (IU/L)			
<20	1.0		
≥20	1.972	0.665–5.847	0.221
GGT (IU/L)			
<50	1.0		
≥50	3.305	0.876–12.467	0.078

Abbreviations as in Table 1

$P = 0.018$) by multivariate analysis using two variables including average ALT levels and GGT at baseline. The average ALT levels were also associated with decreased PLT (OR 4.470; 95% CI, 1.571–12.719; $P = 0.005$) by multivariate analysis using average ALT levels and sex. These results indicate that the clinical course of ALT levels is associated with thrombocytopenia and a decrease in PLT in patients with HCV.

Demographics of HD patients with HCV who were treated with UDCA

We enrolled 16 HD patients with HCV who were treated with 300 mg/day UDCA orally for more than 3 months in August 2008, and compared these patients (UDCA group) to 84 HD patients with HCV who were not treated with UDCA (non-UDCA group). The UDCA group and non-UDCA group showed similar demographics in regard to age, sex, HCV RNA levels, distribution of HCV serotype, GGT and PLT. The UDCA group, however, had a shorter duration of dialysis and higher AST and ALT levels just before UDCA administration compared to those in the non-UDCA group in May 2008 (Table 5).

Efficacy of UDCA in HD patients with HCV

After administering UDCA, percent of ALT and AST significantly decreased after one month and remained constant up to 6 months compared to the non-UDCA group (Fig. 2). Percent of GGT also significantly decreased after 2 months of UDCA treatment compared to the non-UDCA group. In addition, ALT, AST and GGT levels significantly decreased after UDCA treatment compared to levels before treatment, but PLT did not change during the 6 months of UDCA treatment (Fig. 2). In contrast, serum AST, ALT, GGT and PLT in the non-UDCA group did not change during the 6-month period from May 2008 to November 2008.

Discussion

Our study indicated that HD patients persistently infected with HCV are at risk for thrombocytopenia (less than 1.3 × 10^5/μL) and a decrease in PLT (more than 20%), although the exact dates of HCV infection were not clear in our study population. In addition, the basal or clinical course of ALT levels appears to predict decreased PLT or thrombocytopenia in patients with HCV. In this study population, the prevalence [243 anti-HCV positive among 2539 HD patients (9.6%)] and age distribution (average age was 63 years old) of anti-HCV antibody-positive subjects and the frequency of the HCV serotype I (74%) were similar to previous reports on HD patients with HCV in Japan [24–26], suggesting that the clinical course of anti-HCV-positive subjects in this study reflects those in Japan as a whole.

It is known that patients on HD often have thrombocytopenia [27], and there is a negative correlation between the dialysis period and PLTs [27, 28]. It was also reported that megakaryocytes are produced at lower levels in the bone marrow [28], platelets are destroyed due to the

Table 4 Baseline characteristics of four groups of HD patients with HCV according to the clinical course of average ALT levels

Average ALT	A; ALT < 15	B; 15 ≤ ALT < 20	C; 20 ≤ ALT < 30	D; 30 < ALT	*P* value
Number	30	19	18	17	
Age (years)	67.8 ± 10.8	60.8 ± 10.6	64.0 ± 9.7	63.1 ± 8.7	0.105
Sex male/female	11/19	15/4	14/4	14/3	0.001
Duration of dialysis (years)	14.4 ± 10.7	14.2 ± 9.2	12.8 ± 8.8	11.7 ± 9.1	0.945
Follow-up period (months)	53.2 ± 14.3	55.4 ± 16.4	64.2 ± 16.0	57.5 ± 16.3	0.290
HCV-RNA (Log IU/mL)	4.9 ± 1.6	4.8 ± 1.3	5.2 ± 1.2	4.8 ± 1.4	0.774
HCV Serotype (I/II/undetermined)	21/7/2	13/6/0	13/4/1	12/4/1	0.949
History of diabetes mellitus (-)/(+)	23/7	12/7	12/6	10/7	0.592
AST (IU/L)	15.0 ± 4.7	19.8 ± 8.6	22.8 ± 9.8	24.9 ± 8.0	<0.001
ALT (IU/L)	10.4 ± 4.1	19.3 ± 6.8	22.3 ± 8.0	27.8 ± 7.9	<0.001
GGT (IU/L)	21.3 ± 15.2	34.8 ± 22.1	81.2 ± 71.2	48.5 ± 35.2	<0.001
TC (mg/dl)	149.7 ± 31.4	152.3 ± 46.1	154.9 ± 37.0	161.2 ± 57.2	0.970
PLT (× 10^5/μl)	1.62 ± 0.55	1.62 ± 0.61	1.46 ± 0.42	1.64 ± 0.51	0.764

Abbreviations as in Table 1

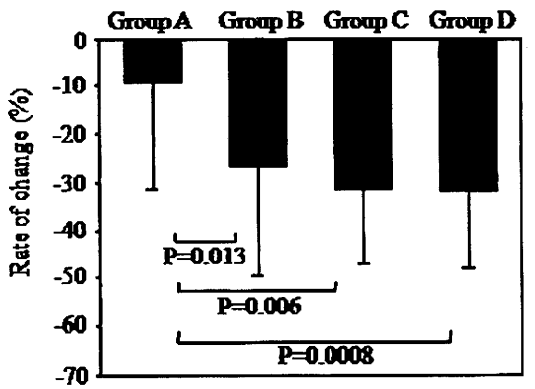

Fig. 1 Comparison of the rate of change in platelet counts by average alanine aminotransferase (ALT) levels during the follow-up period. Group A, average ALT < 15; Group B, 15 ≤ average ALT < 20; Group C, 20 ≤ average ALT < 30; Group D, 30 ≤ average ALT

Table 5 Demographics of HD patients with HCV who were treated with UDCA

	UDCA[a]	Non-UDCA[b]	*P* value
Number	16	84	
Age (years)	66.4 ± 8.6	69.2 ± 10.2	0.261
Sex male/female	9/7	54/30	0.743
Duration of dialysis (years)	6.5 ± 6.6	18.2 ± 9.9	<0.001
HCV-RNA	4.1 ± 2.6	4.9 ± 1.4	0.918
Serotype (I/II/undetermined)	12/4/0	59/21/4	0.669
AST (IU/L)	30.2 ± 24.2	19.2 ± 10.2	0.008
ALT (IU/L)	25.3 ± 16.9	17.1 ± 9.9	0.004
GGT (IU/L)	32.3 ± 23.4	41.4 ± 39.1	0.793
PLT (× 10^5/μl)	1.55 ± 0.56	1.39 ± 0.56	0.577

Abbreviations as in Table 1

[a] Data was obtained at just before the treatment period

[b] Data was obtained in May 2008

appearance of the anti-platelet antibodies [28, 29] and uremic materials reduce the effects of hemopoietic cells [30]. In our study, PLT in HD patients without HCV was significantly decreased after 62.2 months (−5.3%). However, PLT decreased even more dramatically in HD patients with HCV after 56.7 months (−22.4%) compared to patients without HCV. In addition, persistent HCV infection was independently associated with thrombocytopenia and a decrease in PLT in HD patients by a multivariate analysis, but dialysis period was not associated with those. Although the data regarding liver histology and serum markers of hepatic fibrosis were lacking in our study, it has also been reported that severe hepatic fibrosis is associated with thrombocytopenia in HCV carriers with end-stage renal disease [19]. These results suggest that thrombocytopenia is more associated with HCV viremia than with the HD procedure or dialysis period in HD patients.

Hepatocellular carcinoma (HCC) and hepatic failure are critical complications in HCV patients, even in those undergoing HD [10, 31]. These complications occur more frequently in patients with advanced hepatic fibrosis [32, 33]. It has been reported that hepatic fibrosis can be predicted by thrombocytopenia in chronic hepatitis C patients with or without HD [19, 34]. In addition, hepatitis is usually assessed by ALT levels, and changes in ALT levels have been shown to be the most important factor that affects hepatic fibrosis in chronic hepatitis C patients without HD [11, 12]. In this study, we showed that basal ALT levels are associated with thrombocytopenia by a univariate analysis and with decreased PLT by a multivariate analysis. The clinical

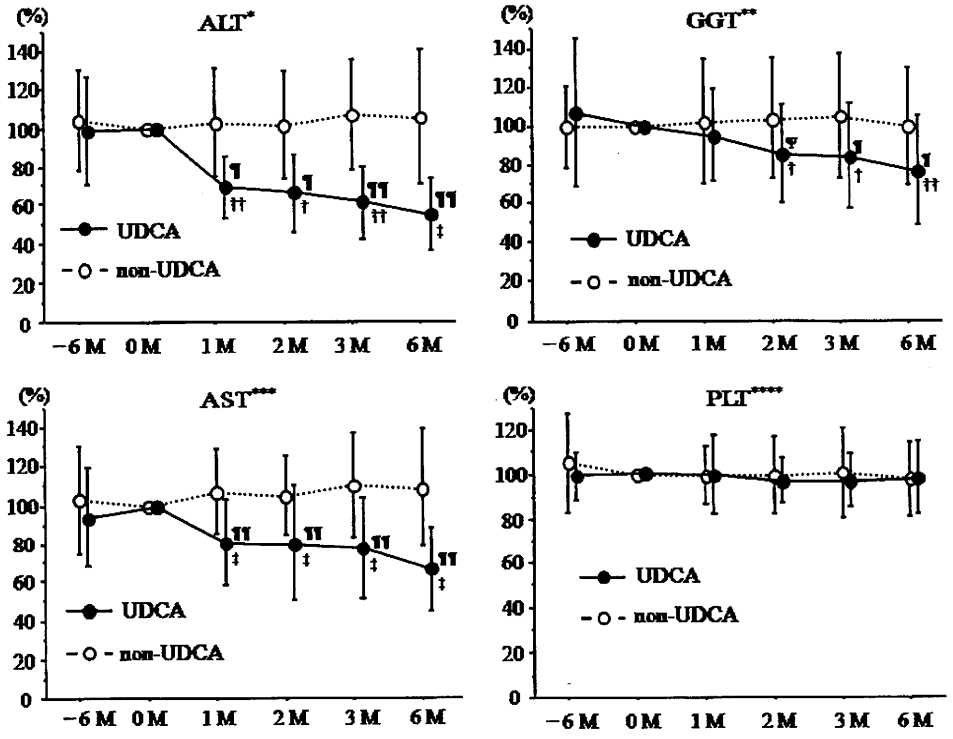

Fig. 2 Efficacy of ursodeoxycholic acid (UDCA) in hemodialysis (HD) patients with hepatitis C virus. Percent of ALT, AST, GGT and PLT in the UDCA group ($n = 16$) 6 months (−6 M) or just (0 M) before and during the treatment period [1, 2, 3 or 6 months (M)] compared to patients in the non-UDCA group ($n = 84$ excluding 6 M) in December 2007 (−6 M), May 2008 (0 M), June (1 M), July (2 M), August (3 M) or November 2008 (6 M, $n = 82$; two patients died before November 2008). Data are expressed as mean ± standard deviation. Closed (*black*) and open circles indicate the UDCA group and non-UDCA group, respectively. The percent of ALT was calculated according to the formula: %ALT = (ALT[−6 M, 0 M, 1 M, 2 M, 3 M or 6 M]/ALT[0 M]) × 100. *ALT* alanine aminotransferase, *AST* asparate aminotransferase, *GGT* gamma-glutamyl transpeptidase, *PLT* platelet count. $^{\Psi}P < 0.05$ (UDCA vs. non-UDCA). $^{\P}P < 0.01$ (UDCA vs. non-UDCA), $^{\P\P}P < 0.001$ (UDCA vs. non-UDCA), $^{\dagger}P < 0.05$ (vs. 0 M), $^{\dagger\dagger}P < 0.01$ (vs. 0 M), $^{\ddagger}P < 0.001$ (vs. 0 M)

course of ALT is also associated with these clinical changes. These results indicate that ALT is an important predictor of thrombocytopenia which should be associated with hepatic fibrosis in HD patients with HCV. In contrast, serum ALT levels are significantly lower in chronic hepatitis C patients on HD than in chronic hepatitis C patients with normal renal function [19]. It was reported that a vitamin B6 deficiency [35], uremic toxins [36], or ultraviolet-absorbing materials [37] are associated with low ALT levels in HD patients. Furthermore, ALT levels have been reported to predict liver disease-related deaths in HD patients, even when ALT levels are in the normal range [38, 39]. Our study also revealed that both patients with abnormal ALT levels (Group D) and normal ALT levels close to the ULN (Groups B and C) had a significant decrease in PLT compared to patients with low ALT levels (Group A). These findings suggest that ALT levels can be used to assess liver damage in HD patients with HCV, although the normal range of ALT should be determined in those patients with HCV in a large cohort study or by liver biopsy.

HCV carriers with persistently normal ALT (PNALT) are more often females than chronic hepatitis C patients with abnormal ALT [40]. This distinction is likely due to lifestyle differences such as alcohol consumption [40], hormonal factors [41] or lower serum iron levels [42]. Although the normal range of ALT in HD patients with HCV may be different compared to the range in HCV carriers with normal renal function, our study demonstrated that females are more likely to have lower ALT levels, even in HD patients (Table 4). This difference in sex may also affect the decrease in PLT. In contrast, the frequency of serotype II, which is reportedly higher in PNALT patients than in chronic hepatitis C patients with abnormal ALT [43], was not different between the four groups in this study (Table 4). A further analysis of the factors associated with elevated ALT levels in those patients with HCV is required.

Interferon therapy has been shown to improve hepatic fibrosis [44] and to reduce the occurrence of HCC in chronic hepatitis C patients with normal renal function. Compared to untreated patients, the risk of HCC after interferon treatment in patients who did not achieve a virological response was shown to be 0.20, 0.36 and 0.91 in chronic hepatitis C patients whose ALT levels were

normal, moderately elevated (less than twice the upper normal limit) and highly elevated, respectively [45]. These results indicate that ALT might predict the mortality of patients with liver-related diseases who have or have not received interferon treatment. Although lower serum ALT levels decreased the risk of HCC, biochemical and virological responses were limited [20, 46] and HD was one of the factors associated with patients who did not respond to interferon and ribavirin treatment [21, 22]. Other therapies that lower serum ALT levels but do not involve interferon-based treatment need to be investigated. Recently, it has been established that UDCA up to 900 mg/day dose-dependently improves biochemical indices such as serum ALT, GGT and bilirubin [23]. Although UDCA seems to lower serum ALT levels, the risk of liver fibrosis, and possibly the incidence of hepatocellular carcinoma, liver histology, serum hepatic fibrosis markers and prognosis (including the incidence of HCC) should also be evaluated over a long time period in HCV carriers with or without HD.

Our study had several limitations; a small number of patients was simply treated with UDCA as routine care, selection of patients depended on each physician and then the data collected retrospectively after a specified duration of therapy. However, this study showed that UDCA effectively had reduced serum ALT, AST and GGT levels in HD patients with HCV. Interestingly, UDCA decreased ALT levels even in patients with normal ALT levels less than 30 IU/L (data not shown). Therefore, HCV patients with normal ALT levels should also be considered for the indication of treatment.

Although the patients in this study were treated with 300 mg/day UDCA, it has also been reported that a 600 mg/day dose of UDCA more effectively decreases ALT and AST levels than a 150 mg/day dose in chronic hepatitis C patients with normal renal function [23]. In addition, PLT did not change during UDCA treatment. Future studies need to investigate the dose-dependent effects of UDCA on ALT levels and prospective double-blind UDCA treatment over a long period in HD patients with HCV.

In conclusion, HCV viremia and ALT levels at basal conditions and during the clinical course of disease were associated with thrombocytopenia and decreased PLT in HD patients. We recommend that HCV carriers on HD who have ALT levels greater than 15 IU/mL be considered for treatment. In addition, UDCA should be considered for HD patients who have chronic hepatitis due to HCV infection but cannot receive interferon-based therapy.

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B型肝炎再活性化による劇症肝炎の現状と対策

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今月のテーマ●B型肝炎ウイルス再活性化の問題点とその対策

B型肝炎再活性化による劇症肝炎の現状と対策

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要旨：近年，強力な免疫抑制・化学療法の普及によりB型肝炎ウイルスの再増殖によるHBV再活性化肝炎が増加している．また，従来から知られている非活動性キャリアからの再活性化に加え，既往感染者からの再活性化の報告が増え，*de novo* B型肝炎とよばれている．*de novo* B型肝炎は劇症化率が高く，劇症化例の予後は極めて不良である．HBV再活性化対策では免疫抑制・化学療法前にHBVキャリアだけでなく，既往感染者をスクリーニングすることが重要である．前者は治療開始前に，後者は治療中または治療終了後にHBV DNAが陽転化した時点で速やかに核酸アナログ製剤を使用する必要がある．

索引用語：HBV再活性化，*de novo* B型肝炎，劇症肝炎，リツキシマブ，核酸アナログ製剤

はじめに

肝機能正常の非活動性B型肝炎ウイルス（HBV）キャリアにおいて，自然経過もしくは免疫抑制剤の使用が誘因となり，HBVの再増殖により肝炎が発症することがあり，HBV再活性化（reactivation）と呼ばれる．HBV再活性化はHBV DNAの上昇とそれに引き続く急激なALTの上昇が特徴である[1)2)]．最近は化学療法や移植医療の進歩により，多様な抗癌剤や免疫抑制剤を使用する機会が増え，HBVが再活性化する事例が増加している．また，HBs抗原が陰性化した既往感染者からの再活性化もみられるようになり，*de novo* B型肝炎と呼ばれ，注目されている．

HBV再活性化は重症化しやすく肝不全に至ることがあるだけでなく，現疾患に対する治療の中断により予後に重大な影響を与える．本稿では*de novo* B型肝炎を含むHBV再活性化の病態とそれによる劇症肝炎の現状と対策について述べる．

I　非活動性HBVキャリアにおける再活性化

1．HBV再活性化の機序

HBV再活性化の多くは血液悪性腫瘍や固形癌にステロイドを含む抗癌剤やリツキシマブを使用した場合[3)4)]，自己免疫性疾患にステロイドやインフリキシマブを使用した場合など[5)6)]，宿主の免疫機能の低下を契機に発症する．また，HIV感染症[7)]，肝臓移植をはじめとする臓器移植後や骨髄移植後などでもみられる[8)~11)]．

HBVは母子感染や幼児期の水平感染でキャリア化し，免疫寛容期を経て思春期に肝炎期に入り，HBe抗原/HBe抗体のセロコンバージョンとともに，HBV DNA量が減少し，肝炎のない非活動性キャリア期に移行する．このような非活動性のキャリアではHBV DNA量10^4copies/ml未満の微量なウイルス増殖がみられるが，最近のリアルタイムPCR法など高感度の測定系で，その検出が可能となった．HBV DNA量の低下は，

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Current status and prevention of fulminant hepatitis due to hepatitis B reactivation

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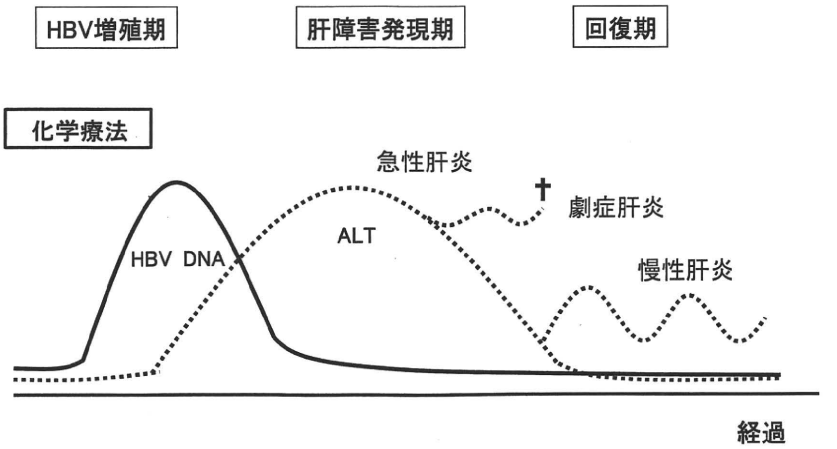

Figure 1. HBV 再活性化の臨床経過.

ウイルス自体の増殖能の低下によるものとは限らず，宿主の免疫学的圧力によりもたらされたものである．したがって抗癌剤や免疫抑制剤により，この防御機構が破たんするとHBVは容易に増殖を開始する．

一般にHBV再活性化は ①HBVの増殖 ②肝障害の出現 ③回復の3段階からなる（Figure 1）．HBVの増殖は必ず肝障害の出現に先行し，その間には時間的ずれ（range 1〜11週）がある．まず，薬剤による免疫細胞の消失あるいは機能低下により，HBVが再増殖し感染細胞が拡大する．免疫機能が低下したままでは肝炎はおこりにくいが，薬剤の中止によりCTL（細胞障害性Tリンパ球）を中心とする免疫系が再構築された際には，短期間に感染細胞が広範に破壊され，重症肝炎が惹起される．感染細胞が排除され，免疫学的均衡が回復すると再び肝炎は鎮静化するが，慢性肝炎に移行することもあり，時に肝炎は致死的になる．

2. HBV再活性化の実態

HBV再活性化は，宿主の免疫機能，治療による免疫抑制の程度およびHBVの感染状態により影響される．HBV再活性化の危険因子として，男性，若年，ステロイド治療などが報告されているが[12]，治療前のHBV DNA量が多いことが最も重要な因子である[13]．ステロイド（グルココルチコイド）を使用するレジメンではHBV再活性化のリスクが増加するが，これはHBVゲノム内にglucocorticoid responsive elementがあり，ステロイドが直接的にウイルスを増殖させるためと考えられている[14]．悪性リンパ腫に対するシクロフォスファミド，ドキソルビシン，ビンクリスチン，プレドニゾロンを使用する化学療法では，HBs抗原陽性者の48%が再活性化し，7.4%に肝不全が惹起されたと報告されている[12]．HBe抗体陽性キャリアではpre C変異，BCP変異を持つウイルスが再活性化する可能性があり，肝炎を発症すると劇症化しやすいため注意が必要である[15][16]．

HBs抗原陽性キャリアに対する免疫抑制・化学療法の際には，従来より治療前に核酸アナログの予防投与が推奨されている．Lauらは30名のHBs抗原陽性の悪性リンパ腫患者に化学療法を施行する際にラミブジンの予防投与群と非投与群に分け比較試験を行った[17]．非投与群の53%にHBV再活性化がみられたのに対し投与群では1例もHBV再活性化はみられず，ラミブジン予防投与の有用性が検証された．

II HBV既往感染者における再活性化 —*de novo* B型肝炎

1. 既往感染者のHBVマーカー

成人でHBVに初感染すると急性肝炎がおこるが，通常は1〜3カ月の経過で肝炎は沈静化する．肝炎の回復期ではHBs抗原は陰性化し，HBs抗体が陽転化する．またHBc抗体は感染1カ月後

より陽転化し，長期間陽性のままである．このようなHBs抗原陰性で，HBc抗体ないしはHBs抗体が陽性の状態は既往感染と診断される．またHBVキャリアの感染晩期にも同様な状態がみられる．HBVの既往感染者はわが国では約20%存在すると推測されている．

これまでHBV既往感染者は，ウイルスが排除され，臨床的には治癒の状態と考えられてきた．しかし既往感染例においてもHBVはcccDNA (covalentlt closed circular DNA) として肝細胞核内に存在し，HBV DNAの複製が長期間持続していることが明らかになった[18][19]．このようなHBc抗体ないしHBs抗体が陽性の既往感染者において，免疫能の低下により惹起されるHBV再活性化を*de novo* B型肝炎と呼んでいる[20][21]．肝臓移植では，HBs抗原陰性かつHBc抗体陽性の肝臓を移植したレシピエントの33%から78%にHBV感染がみられる[8][9]．また，血液疾患における同種骨髄移植においても*de novo* B型肝炎は約50%と比較的高率に報告されている[10][11]．HBs抗原陰性であるが，血液中あるいは肝臓中にHBV DNAが少量検出される状態はOccult HBV infection（潜伏性HBV感染）と呼ばれるが，これらの多くはHBc抗体ないしはHBs抗体が陽性である[20]．

2. 既往感染者からのHBV再活性化

従来より化学療法後のHBV再活性化の中でHBs抗原陰性例の一部においても少数ながらHBV再活性化がおこることが知られていた[12]．しかし近年，強力な免疫抑制・化学療法を契機にHBs抗原陰性かつHBc抗体ないしHBs抗体陽性例からのHBV再活性化，すなわち*de novo* B型肝炎例が増加している[21][22]．*de novo* B型肝炎は特に悪性リンパ腫患者にリツキシマブとステロイドを併用した場合に高率にみられる．リツキシマブによる*de novo* B型肝炎は2001年にdervite らにより最初に報告された[4]．その後，Huiらは HBs抗原陰性の悪性リンパ腫244例を対象に，前向きコホートで*de novo* B型肝炎の発症率を検討した．それによると*de novo* B型肝炎の発症頻度は，リツキシマブとステロイド併用化学療法では12.2%（6/49例）であったのに対し，併用していない場合では1.0%（2/195例）であり，リツキシマブとステロイド併用が*de novo* B型肝炎発症のリスク因子であった[22]．リツキシマブは抗ヒトCD20ヒト・マウスキメラ抗体からなるモノクローナル抗体で，CD20を発現する正常および腫瘍性B細胞を減少させる．これにより，液性免疫能の低下の他に抗原特異的CTLを誘導するB細胞の抗原提示機能も障害されると考えられる．リツキシマブ投与中には顕著にHBs抗体の低下が見られることからも，B細胞系に対する免疫抑制がHBV再活性化の誘因になっているものと思われる．

3. *De novo* B型肝炎の臨床像

De novo B型肝炎の臨床的特徴は，以下のとおりである．①化学療法終了後，HBV DNAの上昇がおこる（中央値12週）②HBV DNAの上昇から中央値10週でHBs抗原が陽転化し，その後中央値9.5週で肝炎が発症する（Figure 2）[22]．すなわち，HBV DNA上昇から肝炎発症までに中央値18.5週の時間的ずれがみられる．厚生労働省研究班では，過去5年間のB型急性肝炎1184例と*de novo* B型肝炎55例の全国調査結果を報告している[23]．それによると*de novo* B型肝炎は急性B型肝炎と比べ，発症年齢が高く，トランスアミナーゼのピーク値は900IU/Lと低いものの総ビリルビン値，HBV DNA量は有意に高値であった．劇症化率は*de novo* B型肝炎27%に対し急性肝炎で7%であり，また劇症化例の死亡率も*de novo* B型肝炎100%に対し急性肝炎44%と，いずれも*de novo* B型肝炎が有意に高率であった．劇症化した*de novo* B型肝炎5例はいずれもリツキシマブとステロイド併用の化学療法によるものあった．

III HBV再活性化による劇症肝炎の実態

厚生労働省研究班による劇症肝炎の全国調査では，HBV再活性化肝炎がB型例に占める割合は，2004年の8%（2/26例）から2008年は35%（8/23例）と増加している．さらにHBV再活性化肝炎のうち，発症前のHBs抗原陰性のいわゆる*de novo* B型肝炎と推測される症例は48%（14/

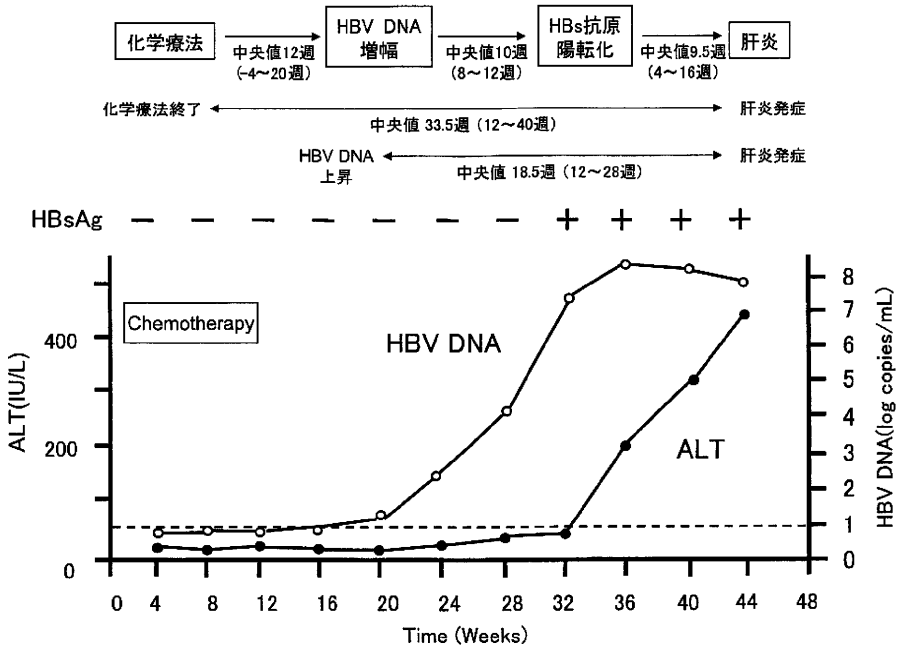

Figure 2. *De novo* B型肝炎の臨床パターン（文献22より引用改変）.

29例）を占めている[24]．HBs抗原陽性キャリアからの再活性化では，基礎疾患として悪性リンパ腫が最も多いが，乳癌などの固形癌や慢性関節リウマチなどの膠原病などもみられる．前治療としては，ステロイドが最も多い．一方，*de novo* B型肝炎はほとんどが，悪性リンパ腫に対するリツキシマブとステロイドの併用療法が原因である．臨床背景では*de novo* B型肝炎は高齢者が多く，病型は劇症肝炎亜急性型を呈し，全例が核酸アナログで治療されていたが救命例はなかった（Table 1）．*de novo* B型肝炎は発症時のトランスアミナーゼはあまり高くなく，治療薬による肝障害との鑑別も困難であり，診断が遅れる一因になっていると思われる．また，肝炎発症後の核酸アナログの治療効果も不良であり，その病態は不明である．

IV HBV再活性化対策

近年のHBV再活性化肝炎の増加をうけて，厚生労働省の「難治性の肝・胆道疾患に関する調査研究」班劇症肝炎分科会と「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班が合同で，HBV再活性化に対するガイドラインを作成した[25]．現在，本ガイドラインを検証する前向きコホート研究が実施されている．免疫抑制・化学療法前のスクリーニングで重要な点は，従来のHBs抗原検査だけではなくHBc抗体およびHBs抗体検査をできるだけ感度のよい方法で実施することである．以下にガイドラインの骨子を述べる．

1. HBV再活性化対策ガイドライン

1) HBVキャリア

HBs抗原陽性例に対しては，さらにHBe抗原，HBe抗体，HBV DNA定量検査を実施し，感染状態を詳しく把握する必要がある．HBV DNA量が高値であるほど再活性化のリスクは高い．HBs抗原陽性例ではHBe抗原，HBe抗体，HBV DNA定量検査の結果にかかわらず治療開始前に核酸アナログ製剤の予防投与を行う．核酸アナログは最も耐性株出現率の低いエンテカビル投与が推奨される．核酸アナログにより血中HBV DNA量が低下するまでには日数を要するため，HBV DNA量が多い症例などでは，できるだけ

Table 1. HBV 再活性化による劇症肝炎（2004～2008年）

	HBV キャリア (n = 15)	*de novo* B 型肝炎 (n = 14)
年齢（Median（range）歳）	59（29～80）	66（48～76）
性別（男/女）	9/6	8/6
病型（急性/亜急性/LOHF）	3/4/8	0/14/0
転帰（生存/死亡/肝移植）	2/12/1	0/14/0
基礎疾患		
悪性リンパ腫	7（46.7%）	11（78.6%）
その他の血液悪性腫瘍	1	2
血液以外の悪性腫瘍	2	1
膠原病（リウマチなど）	4	
その他	1	
前治療		
リツキシマブ	5（33.3%）	11（78.6%）
ステロイド	11（73.3%）	11（78.6%）
末梢血幹細胞移植		1
肝炎の治療		
核酸アナログ	15（100%）	14（100%）

早期に投与を開始するのが望ましい．

2）HBV 既往感染者

HBs 抗原陰性で HBc 抗体ないし HBs 抗体陽性の場合は更に HBV DNA 定量検査を実施する．HBV DNA 定量検査が陽性であれば核酸アナログの予防投与を開始する．HBV DNA が検出感度以下で陰性の場合は，治療中および治療終了後に HBV DNA を月1回モニタリングする．HBV DNA の測定には現在最も検出感度の高い，リアルタイム PCR 法での測定がのぞましい．経過観察中に HBV DNA が陽性化した時点で直ちに核酸アナログの投与を開始する．HBV DNA のモニタリングは治療中だけでなく治療終了後も12カ月間は継続する．HBs 抗原陰性で HBc 抗体，HBs 抗体いずれも陰性の場合は通常の対応とするが，患者が既に免疫抑制あるいは化学療法後で免疫抑制状態にある場合には抗体が検出されないことがあり，HBV DNA 定量検査まで測定することが望ましい．

核酸アナログ投与終了に関する明確な基準はないが，HBs 抗原陽性例では使用する各核酸アナログの投与終了基準に準ずる．HBs 抗原陰性，HBc 抗体ないし HBs 抗体陽性例では治療終了後も12カ月間は投与を継続し，この継続期間中に ALT と HBV DNA が持続陰性化している場合は投与終了の検討も可能である．ただし，核酸アナログ予防投与終了6～8カ月後にウイルス血症の再出現および重症肝炎の発症も報告されており[26]，投与終了後も更に12カ月間は厳重な経過観察が必要である．

2. HBV 再活性化に関連する薬剤

HBV 再活性化は血液悪性疾患以外の膠原病やアレルギー性疾患に対する免疫抑制療法でもみられるが，その頻度は低い[27]．少量のステロイドやアザチオプリンでは一般的にはみられないが，メソトレキセートの長期投与ではまれに報告例がある[28]．またステロイドと抗 TNF-α 抗体（インフリキシマブ）併用による HBV 再活性化の報告がみられる[6]．Crohn 病，慢性関節リウマチ，強直性脊椎炎に対するインフリキシマブ使用により重篤な HBV 再活性化や，少数ながら *de novo* B 型肝炎の報告もみられる[29)~31)]．現在，わが国では HBV 感染者に対するインフリキシマブ，エタネルセプト，アダリムマブの使用に際しては HBV

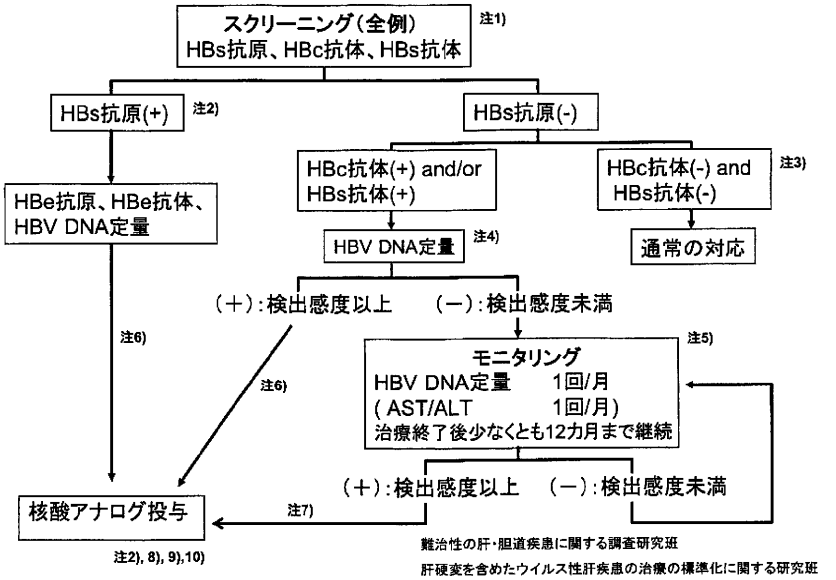

Figure 3. 免疫抑制・化学療法により発症するB型肝炎対策ガイドライン*.
*血液悪性疾患に対する強力な免疫抑制・化学療法中あるいは終了後に，HBs抗原陽性あるいはHBs抗原陰性例の一部に，HBV再活性化によりB型肝炎が発症し，その中には劇症化する症例があり，注意が必要である．その他の疾患においても治療によるHBV再活性化のリスクを考慮して対応する必要がある．また，ここで推奨する核酸アナログ予防投与のエビデンスはなく，劇症化予防効果を完全に保証するものではない．
注1) CLIA法で測定することが望ましい． 注2) HBs抗原陽性例は肝臓専門医にコンサルトすること．すべての症例で核酸アナログ投与にあたっては肝臓専門医にコンサルトするのが望ましい． 注3) 初回治療時にHBc抗体，HBs抗体未測定の再治療例では抗体価が低下している場合があり，HBV DNA定量検査などによる精査が望ましい． 注4) PCR法およびリアルタイムPCR法により実施する．より検出感度の高いリアルタイムPCR法が望ましい． 注5) リツキシマブ・ステロイド使用例，造血細胞移植例はHBV再活性化の高リスクであり，注意が必要である．フルダラビンは強力な免疫抑制作用を有するが，HBV再活性化のリスクは不明であり，今後注意が必要である． 注6) 免疫抑制・化学療法を開始する前，できるだけ早期に投与を開始するのが望ましい． 注7) 免疫抑制・化学療法中はHBV DNA定量検査が検出感度以上になった時点で直ちに投与を開始する． 注8) 核酸アナログはエンテカビルの使用を推奨する． 注9) 下記の条件を満たす場合には核酸アナログ投与の終了を検討して良い．スクリーニング時にHBs抗原（+）例ではB型慢性肝炎における核酸アナログ投与終了基準を満たす場合．スクリーニング時にHBc抗体（+）and/or HBs抗体（+）例では，(1) 免疫抑制・化学療法終了後，少なくとも12カ月間は投与を継続すること．(2) この継続期間中にALT（GPT）が正常化していること．(ただしHBV以外にALT異常の原因がある場合は除く) (3) この継続期間中にHBV DNAが持続陰性化していること． 注10) 核酸アナログ投与終了後12カ月間は厳重に経過観察する．経過観察方法は各核酸アナログの使用上の注意に基づく．経過観察中にHBV DNA定量検査が検出感度以上になった時点で直ちに投与を再開する．

再活性化に注意することが喚起されている．これまでにHBV再活性化が報告されている薬剤はTable 2のとおりである[32)33)]．今後も生物学的製剤，分子標的治療薬など新規の免疫抑制剤や抗癌剤の登場が予想され，HBV再活性化には十分注意が必要である．

おわりに

HBV再活性化は発症すれば危険な病態だが，

Table 2. B型肝炎再活性化に関連する薬剤

コルチコステロイド	プレドニゾロン，デキサメタゾン，メチルプレドニゾロン
抗腫瘍抗生物質	ドキソルビシン，エピルビシン，ダウノルビシン，ブレオマイシン（BLM），マイトマイシン-C（MMC），アクチノマイシン-D（ACT-D）
植物アルカロイド	ビンクリスチン（VCR），ビンブラスチン（VLB）
アルキル化薬	シクロホスファミド（CPA），イホスファミド（IFM），クロラムブシル，カルボプラチン（CBDCA），シスプラチン（CDDP），プロカルバジン（PCZ）
代謝拮抗薬	シタラビン（SPAC），アザウリジン，フルオロウラシル（5-FU），ゲムシタビン，メルカプトプリン（6-MP），メソトレキセート（MTX），チオグアニン
その他	L-アスパラギナーゼ（L-ASP），コラスパーゼ，フォリニックアシッド，プロカルバジン，ドセタキセル，エトポシド（VP-16），フルダラビン，インターフェロン（IFN）
分子標的治療薬	リツキシマブ（anti-CD20），アレムツズマブ（anti-CD52），インフリキシマブ（anti-TNF），メシル酸イマチニブ

文献32）33）より引用改変．

発症前に核酸アナログを投与できればその予防が可能である．個々の症例においてHBV再活性化およびその劇症化のリスクを正確に予測することは困難だが，スクリーニングによりHBV再活性化高リスク群を治療前に把握し，適切な時期に核酸アナログを使用することが，HBV再活性化対策において最も重要である．

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＜速　報＞

B型急性肝炎におけるHBs抗原陽性持続期間の検討

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はじめに：Genotype AによるB型急性肝炎が全国的に急速に広がってきている．Genotype Aによる急性肝炎は慢性化しやすいことが示唆されている．しかしながら，Genotype間でのHBs抗原陽性持続期間の差異に関しては十分な検討は行われていない．今回この点に関して検討した．

対象と方法：対象は1994年から2010年にかけて首都圏3施設で経験した急性肝炎188例である．188例中HBs抗原の消失まで経過観察が可能であった88例（核酸アナログを使用した症例は含まれていない）に関しては，HBs抗原，HBe抗原，ALTを経時的に測定して経過を調べた．HBs抗原測定は各施設および各genotypeともにALT正常化までは1～2週間毎，ALT正常化後はおよそ1カ月毎に検査を行った．HBs抗原の測定はCLIA法（化学発光免疫測定法），ジェノタイプ（GT）判定はジェノタイプ特異的プローブアッセイ（スマイテストHBVジェノタイプ判定キット：ゲノムサイエンス社）を用いて行った．

結果：対象188例のうち174例でGTが決定できた．GTA 92例（53%），GTB 20例（11%），GTC 60例（34%），GTD 1例（1%），GTF 1例（1%）であった．GTAの症例の割合は1994年から徐々に増加し，2010年にはB型急性肝炎の症例の82%（9/11）がGTAによるものであった．

HBs抗原の消失まで経過を観察できた88症例におけるHBs抗原陽性持続期間を（Fig. 1）に示す．HBs抗原消失までの期間は平均1.5±2.2カ月（GTA 2.8±2.6カ月，GTB 1.0±0.6カ月，GTC 1.7±1.5カ月）であった（GTA vs GTB；$p<0.01$，GTA vs GTC；$p<0.05$）．GTAでは47例中14例（29.8%）が3カ月以上HBs抗原陽性を持続した．また3例ではHBs抗原陽性が6カ月以上持続し，そのうち2例は後にHBs抗原が消失し1例は慢性化した．また，ALT正常化までに要した期間は平均2.7±2.94カ月（GTA 3.3±4.2カ月，GTB 2.1±1.1カ月，GTC 2.2±1.1カ月）であった（GT間に有意差なし）．

発症時のALT値と臨床経過，発症時のHBe抗原の有無と臨床経過の間には一定の傾向は認められなかった．

考察：GTA HBVは男性同性間性交渉及び風俗店での性交渉により急速に拡大していることを我々は既に報告してきた[1)]．本邦におけるHBVキャリアでのGT分布に関する2回の全国調査からはGTAに感染したキャリアが増えていることも示されている[2)3)]．

GTAの症例は今回報告した通り，HBs抗原陽性の時期が長く，慢性化例の報告もあることから二次感染を起こす可能性が高い．従って治癒即ちHBs抗原の消失を確認する必要がある．ところが若年のB型急性肝炎の症例，殊にGTAに感染した症例はHBs抗原陽性のまま通院を打ち切る傾向がある．自覚症状が消失してしまうこと，慢性肝炎が最終的に肝硬変/肝細胞癌に至り得る疾患であることに対する理解が不十分であること，経済的に余裕がないことなどがその主な理由と思われる．このような例が感染を拡大させている可能性があり，他のSTD同様啓発活動が重要である．我々も通院を中断した例に対しては，書面で通院の勧告を行っているが，来院しない例が大部分である．このような通院中断例を通じた感染拡大を防止する意味でも，ユニバーサルHBワクチンの導入を真剣に検討する時期に来ていると思われる．

結論：GTAの症例ではHBs抗原陽性が他のGTに比べて長期にわたり持続する．HBVの感染拡大を防止するためにもユニバーサルHBワクチンの導入を検討すべきである．

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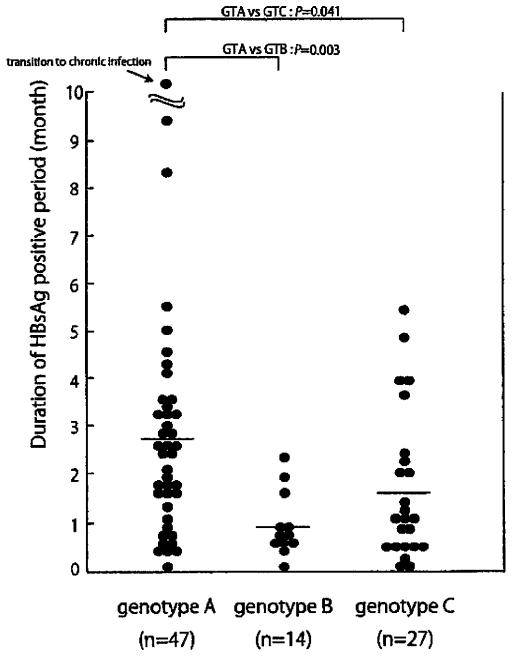

Fig. 1 Duration of HBs antigenemia in patients with acute HBV infection. The duration is longer in patients with genotype A than those with genotype B or C.

索引用語：B型肝炎ウイルス，遺伝子型，
ユニバーサルワクチン

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英文要旨

Duration of HBs antigenemia in patients with acute hepatitis B

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Recently distribution of hepatitis B virus (HBV) genotypes (GT) in the patients with acute HBV infection has been changing. It has been suggested that acute hepatitis caused by GTA HBV becomes chronic more often than that by other genotypes. We studied HBsAg-positive period in 88 patients with various HBV genotypes. HBsAg-positive period in GTA HBV is longer than that in GTB and GTC. HBsAg-positive period exceeded 6 months in 3 of 47 patients with GTA HBV. One of the three patients became chronic. GTA HBV, which is detected more than half of the patients, is related to prolonged or chronic outcome. Universal HBV vaccination program for the prevention of HBV infection should be launched in the near future.

Key words: hepatitis B virus, genotype, universal vaccination

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ORIGINAL ARTICLE

Pegylated interferon α-2b plus ribavirin for older patients with chronic hepatitis C

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Abstract

AIM: To analyze the efficacy and safety of a combination therapy of pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) in older Japanese patients (65 years or older) infected with hepatitis C virus (HCV).

METHODS: This multicenter study included 938 patients with HCV genotype 1 who received 1.5 μg/kg per week PEG-IFN α-2b plus RBV 600-1000 mg/d for 48 wk and 313 HCV genotype 2 patients who received this treatment for 24 wk.

RESULTS: At 24 wk after the end of combination therapy, the overall sustained virological response (SVR) for genotypes 1 and 2 were 40.7% and 79.6%, respectively. The SVR rate decreased significantly with age in each genotype, and was markedly reduced in genotype 1 ($P < 0.001$). Moreover, the SVR was significantly higher in patients with genotype 1 who were less than 65 years (47.3% of 685) than in those 65 years or older (22.9% of 253) ($P < 0.001$) and was higher in patients with genotype 2 who were less than 65 years (82.9% of 252) than in those 65 years or older (65.6% of 61) ($P = 0.004$). When patients received a dosage at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target dosage of RBV, the SVR rate significantly increased to 66.5% in patients less than 65 years and to 45.2% in those 65 years or older ($P <$

0.001). Adverse effects resulted in treatment discontinuation more often in patients with genotype 1 (14.4%) than in patients with genotype 2 (7.3%), especially by patients 65 years or older (24.1%).

CONCLUSION: PEG-IFN α-2b plus RBV treatment was effective in chronic hepatitis C patients 65 years or older who completed treatment with at least the minimum acceptable treatment dosage.

Key words: Hepatitis C virus; Gerontology; Pegylated interferon; Ribavirin

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, affecting 170 million individuals worldwide[1]. It is well known that patients with chronic hepatitis C eventually develop hepatocellular carcinoma (HCC)[2]. Previous studies have made clear that interferon (IFN) treatment is effective for eliminating HCV[3,4] and that it significantly reduces the progression of liver fibrosis and the risk of HCC[5,6]. Antiviral treatment for chronic hepatitis C has greatly improved, and the combination treatment of pegylated (PEG)-IFN α-2b plus ribavirin (RBV) has been approved and recommended in Japan since 2004, as the first choice for chronic hepatitis C. This combination treatment attained a sustained virological response (SVR) rate of 50%-60% for genotype 1 in the United States and Europe[7]. However, SVR was relatively low (42.4%) in Japan[8], where chronic hepatitis C patients are older, indicating that older patients did not respond well to IFN treatment[9]. Moreover, the combination treatment was associated with more adverse effects than IFN monotherapy[7,10]. Older patients who have decreased cardiovascular, pulmonary and renal function have a higher incidence of adverse effects than younger patients. The rate of discontinuation due to adverse effects was reported to be significantly higher in patients aged 65 years or more than in those less than 65 years[11]. Older patients with HCV infection are at risk for progressive liver disease. It was reported that clearance of HCV after IFN therapy significantly reduces the incidence of HCC and death in older chronic hepatitis C patients[6,12]. Ikeda *et al*[13] demonstrated that IFN treatment is needed for 65-70-year-old patients with chronic hepatitis C to prevent the occurrence of HCC. We also consider older patients to be acceptable candidates for antiviral treatment to prevent the development of HCC, and previously reported that monotherapy with natural IFN α was not effective in older patients[9]. Therefore, in an attempt to ameliorate these problems, we decided to treat older patients with a combination of PEG-IFN plus RBV therapy.

Little data concerning the response and safety of this combination treatment in a large number of older patients with chronic HCV infection has been published. A multicenter study of the efficacy and safety of antiviral treatments for Japanese patients with chronic liver disease, the Kyushu University Liver Disease Study (KULDS), was launched in 2003[8,14]. The present prospective study was carried out to analyze the efficacy and safety of the combination treatment of PEG-IFN α-2b plus RBV in older patients.

MATERIALS AND METHODS

Patients

Treatment of chronic hepatitis C with a combination of PEG-IFN α-2b plus RBV was accepted by the Japanese Ministry of Health in October, 2004. We used this combination treatment from December 2004 to July 2008, and enrolled chronic hepatitis C patients with exclusion criteria which included: (1) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by bleeding, high-risk esophageal varices, history of gastrointestinal bleeding, ascites, encephalopathy, or HCC; (2) hemoglobin level < 11.5 g/L, white blood cell count < 3×10^9/L, and platelet count < 50×10^9/L; (3) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or HIV positive); (4) excessive active alcohol consumption > 60 g/d or drug abuse; (5) severe psychiatric disease; or (6) antiviral or corticosteroid treatment within 12 mo prior to enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan. We have treated 2270 Japanese patients aged 18 years or older with PEG-IFN α-2b plus RBV. All patients who were positive for both antibody to HCV and HCV RNA for over 6 mo were enrolled in KULDS. Three months before the start of treatment and every 3 mo during the treatment period, each patient was tested for α-fetoprotein (AFP) and had an abdominal ultrasonographic examination. If an abnormal AFP level of 40 ng/mL and/or focal lesions on ultrasonographic examination were found at any testing, further testing for HCC was carried out, which included dynamic computed tomography, and angiography. Patients confirmed to have HCC within 3 mo after starting treatment were excluded from this study (n = 14). Of 2270 patients, 1021 were currently under combination treatment or we were not yet able to judge the effect of the combination treatment. This left the data of 1251 patients (938 with genotype 1 and 313 with genotype 2) available for analysis.

Table 1 Characteristics of 938 chronic hepatitis C genotype 1 patients treated with a combination of pegylated interferon plus ribavirin according to age (mean ± SD)

	Group A (age < 65 yr) (*n* = 685)	Group B (age ≥ 65 yr) (*n* = 253)	*P*-value
Age (yr)	53.1 ± 8.9	68.6 ± 3.1	< 0.001
Male/female	374/311	122/131	0.090
Body mass index (kg/m^2)	23.7 ± 3.3	22.8 ± 2.7	< 0.001
Prior IFN monotherapy, *n* (%)	163 (23.8)	76 (30.0)	0.052
Prior combined IFN plus RBV treatment, *n* (%)	51 (7.4)	20 (7.9)	< 0.001
Alanine aminotransferase (IU/L)	80.2 ± 62.0	67.9 ± 46.6	0.004
γ-glutamyltranspeptidase (IU/L)	60.2 ± 56.6	57.1 ± 49.2	0.708
Albumin (g/dL)	4.1 ± 0.4	4.0 ± 0.4	< 0.001
White blood cell count (/mm^3)	5200.0 ± 1476.7	4756.3 ± 1458.9	< 0.001
Hemoglobin (g/dL)	14.1 ± 1.4	13. 5 ± 1.4	< 0.001
Platelet count (10^9/L)	16.6 ± 5.3	15.0 ± 5.2	< 0.001
Creatinine (mg/dL)	0.7 ± 0.6	0.8 ± 1.4	0.107
Creatinine clearance (mL/min)	105.5 ± 28.7	75.8 ± 17.5	< 0.001
Serum HCV-RNA level (kIU/mL)	1776.1 ± 1500.0	1986.9 ± 1604.5	0.125
Histological fibrosis			0.008
F0/F1/F2/F3/F4	36/155/121/61/30	9/46/49/31/17	

IFN: Interferon; RBV: Ribavirin; HCV: Hepatitis C virus.

Table 2 Characteristics of 313 chronic hepatitis C genotype 2 patients treated with a combination of pegylated interferon plus ribavirin according to age (mean ± SD)

	Group C (age < 65 yr) (*n* = 252)	Group D (age ≥ 65 yr) (*n* = 61)	*P*-value
Age (yr)	47.7 ± 10.4	69.2 ± 3.4	< 0.001
Male/female	124/128	28/33	0.671
Body mass index (kg/m^2)	23.1 ± 3.5	22.8 ± 2.9	0.577
Prior IFN monotherapy, *n* (%)	47 (18.7)	16 (26.2)	< 0.001
Prior combined IFN plus RBV treatment, *n* (%)	5 (2.0)	4 (6.6)	0.056
Alanine aminotransferase (IU/L)	79.9 ± 78.7	68.9 ± 52.9	0.821
γ-glutamyltranspeptidase (IU/L)	55.8 ± 64.7	44.3 ± 34.7	0.937
Albumin (g/dL)	4.2 ± 0.4	3.9 ± 0.5	< 0.001
White blood cell count (/mm^3)	5276.3 ± 1636.3	4958.0 ± 1495.6	0.005
Hemoglobin (g/dL)	14.1 ± 1.4	13.4 ± 1.3	< 0.001
Platelet count (10^9/L)	18.9 ± 6.3	15.6 ± 4.7	< 0.001
Creatinine (mg/dL)	0.8 ± 1.5	0.7 ± 0.2	0.581
Creatinine clearance (mL/min)	112.1 ± 31.4	74.6 ± 17.2	< 0.001
Serum HCV-RNA level (kIU/mL)	1588.3 ± 1628.7	1195.4 ± 1645.5	0.038
Histological fibrosis			< 0.001
F0/F1/F2/F3/F4	30/77/39/10/10	1/21/9/2/12	

IFN: Interferon; RBV: Ribavirin; HCV: Hepatitis C virus.

Informed consent was obtained from all patients before enrollment in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

Table 1 (genotype 1) and Table 2 (genotype 2) show the baseline characteristics of the enrolled patients, who were further classified into four groups according to age and genotype status: group A, genotype 1 aged less than 65 years (*n* = 685); group B, genotype 1 aged 65 years or older (*n* = 253); group C, genotype 2 aged less than 65 years (*n* = 252); and group D, genotype 2 aged 65 or older (*n* = 61). In group B, body mass index, prior combined IFN plus RBV treatment, alanine aminotransferase, albumin, white blood cell count, hemoglobin, platelet count, and creatinine clearance calculated using the Modification of Diet in Renal Disease equation[15] were significantly lower than in group A ($P < 0.010$). In group D, albumin, hemoglobin, platelet count, creatinine clearance and serum HCV RNA level were significantly lower than in group C ($P < 0.010$). The percentage of patients with platelet counts below 10 × 10^{10}/L was significantly higher in group B (36 of 253, 14.2%) than in group A (56 of 685, 8.2%) ($P = 0.006$), however, there was no significant difference between group C (16 of 252, 6.3%) and group D (7 of 61, 11.5%).

Liver histology

Liver biopsy was performed in 555 patients (59.2%) with genotype 1 and 209 patients (66.8%) with genotype 2. The other patients refused liver biopsy. Fibrosis was staged on a 0-4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Liver fibrosis was more advanced in group B than in group A

and was more advanced in group D than in group C (P = 0.008, $P < 0.001$, respectively).

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous PEG-IFN α-2b (PegIntron, Schering-Plough, Osaka, Japan), in combination with RBV (Rebetol, Schering-Plough), which was given orally at a daily dose of 600-1000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60-80 kg, and 1000 mg for those weighing 80 kg or over). The length of treatment was 48 wk for patients with HCV genotype 1 and 24 wk for patients with genotype 2. The above duration and dosage are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to less than 100 g/L. In such cases, a reduction in the dose of RBV was required. Patients aged 65 years or older had a significantly higher frequency of RBV dose reduction during the treatment period than those aged less than 65 years old (HCV genotype 1: group A *vs* group B, 41.2% *vs* 49.0%, $P = 0.032$, genotype 2: group C *vs* group D, 28.6% *vs* 54.1%, $P < 0.001$). Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease in white blood cell and platelet counts. In such cases, a reduction in the dosage of PEG-IFN α-2b was required. Both PEG-IFN α-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9/L, and 25×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic disorders developed, continuation of treatment was judged not to be possible by the attending physician, or if the patient desired discontinuation of treatment.

Determination of baseline HCV RNA level and HCV genotype

The pretreatment, baseline, serum HCV RNA level was measured by a quantitative HCV RNA polymerase chain reaction (PCR) assay (COBAS Amplicor HCV Monitor Test v 2.0 using the 10-fold dilution method; Roche Diagnostics, Tokyo, Japan), which has a lower limit of quantitation of 5000 IU (13 500 copies)/mL (5 kIU/mL) and an outer limit of quantitation of 5 100 000 IU/mL (5100 kIU/mL). The HCV genotype was determined by type-specific primers of the core region of the HCV genome. The protocol for genotyping was carried out as previously described[3].

Efficacy of treatment

End of treatment (EOT) response and SVR were defined as serum HCV RNA undetectable at the end of treatment and at 24-wk follow-up after the end of treatment, respectively. EOT response and SVR were defined as non-detectable HCV-RNA as measured by qualitative COBAS Amplicor HCV Monitor Test v 2.0, with the results labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL). The analysis of EOT and SVR was performed on an intention-to-treat basis.

Statistical analysis

Continuous data are expressed as mean ± SD. The statistics were carried out using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The χ^2 test, Fisher's exact test and Kruskal-Wallis test were used to determine the differences in baseline clinical characteristics, safety, efficacy of the combination therapy, adherence to the total dose, and the association between the adherence and SVR. Logistic regression analysis was used to identify the association between age and SVR. A $P < 0.05$ was considered significant.

RESULTS

EOT response rate by intention-to-treat analysis

Among patients with genotype 1, the EOT response rate was significantly higher in group A (497 of 685, 72.5%) than in group B (129 of 253, 45.0%) ($P < 0.001$). Among patients with genotype 2, there was no significant difference between groups C (239 of 252, 94.8%) and D (55 of 61, 90.1%).

SVR rate by intention-to-treat analysis

Of 1251 patients, 631 (50.4%) achieved SVR in the intention-to-treat analysis. The SVR rate was significantly higher for genotype 2 (249 of 313, 79.6%) than for genotype 1 patients (382 of 938, 40.7%) ($P < 0.001$). Among patients with genotype 1, the SVR rate was significantly higher in group A (324 of 685, 47.3%) than in group B (58 of 253, 22.9%) ($P < 0.001$). Among patients with genotype 2, SVR was also significantly higher in group C (209 of 252, 82.9%) than in group D (40 of 61, 65.6%) ($P = 0.004$). The rate of SVR was significantly higher for females (113 of 128, 88.3%) than for males (96 of 124, 77.4%) in group C only (Figure 1). Furthermore, we analyzed whether or not the SVR rate differed according to the age at which the combination treatment of PEG-IFN α-2b plus RBV was started. The results showed that the SVR rate decreased significantly with age for both genotype 1 and 2. SVR was achieved by 5.6%-26.3% of genotype 1 patients aged 70 years or older, and by 57.1%-100% of genotype 2 patients aged 70 years or older (Figure 2).

We previously reported a minimum acceptable dose of at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target dosage of RBV for the successful treatment of Japanese patients with genotype 1[8]. Therefore, we analyzed the SVR rates in patients with genotype 1 by the dosage they actually received during treatment (a total dose of at least 80% or more of PEG-IFN α-2b and 60% or more of RBV) (Table 3). The number who received at least this minimum acceptable dosage during treatment were 278 (40.6%) of 685 patients in group A and 62 (24.5%) of 253 in group B, significantly lower in group B than in group A ($P < 0.001$). Compared with patients who received less than the minimum acceptable dosage, in patients who received at least this minimum dosage, the SVR rates increased from 34.2% to 66.5% in group A patients and from 15.7% to 45.2%