8例中1例(12.5%)に新たな所見を認め、Sonazoidでは8例中3例(37.5%)に新たな結節が検出された。

4. 症例

①症例1 肝細胞癌(図1)

存在部位S5、最大径14mmの中分化型肝細胞癌である。上段にSonazoid、下段にLevovistの造影超音波像を示す。Sonazoidの投与量は推奨量の1/2、Focusは60mm、MI値を0.34に設定した。

血管相早期においてSonazoid(PS-low)はLevovist(ADF)に比し細かな血管影が描出され、血管相後期においてもLevovistに劣ることなく良好な染影像が得られた。また、後血管相では、Levovist(ADF)は結節内に染影効果が残り周囲とほぼ同等となったがSonazoid(PS-low)では周囲に比し低い染影像として描出された。

②症例2 転移性肝癌(図2)

存在部位S7、最大径15mmの胃癌を原発巣とする転移性肝癌である。Sonazoidの投与量は推奨量の1/2、Focusは40mm、MI値を0.23に設定した。

血管相早期においてLevovist(ADF)でもring enhancementは確認できるが、Sonazoid(PS-low)の描出能が優っている。血管相後期において両者ともに結節はやや淡いhypovascularに描出された。後血管相では、Sonazoid(PS-low)とLevovist(PS-high)とも主結節は欠損像として描出され、周囲に新たな転移巣が描出された。

③症例3 肝血管腫(図3)

存在部位はS6肝表面、最大径約30mmの血管腫である。本症例はSonazoidの投与量を推奨量とした症例で、Focusは50mm、MI値を0.22に設定した。

血管相にて造影剤が周辺から中心に向かって徐々に染影されていく像(fill in)が認められる。同所見は、Sonazoid(PS-low)がLevovist(ADF)よりリアルタイムに描出された。後血管相ではSonazoid(PS-low)とLevovist(ADF)ともにあきらかな欠損像は認められなかった。

④症例4 転移性肝癌の存在診断(図4)

本症例は症例2と同一症例である。Sonazoid静注10分後(PS-low)の全肝検索によりB-modeにて描出されなかった約2mm前後の結節が、後血管相にて多数の欠損像として描出され、胃癌転移巣の存在診断の把握に有用であった。

考察

肝腫瘤性病変の各疾患における特徴的な血流動態を詳細に把握することは、鑑

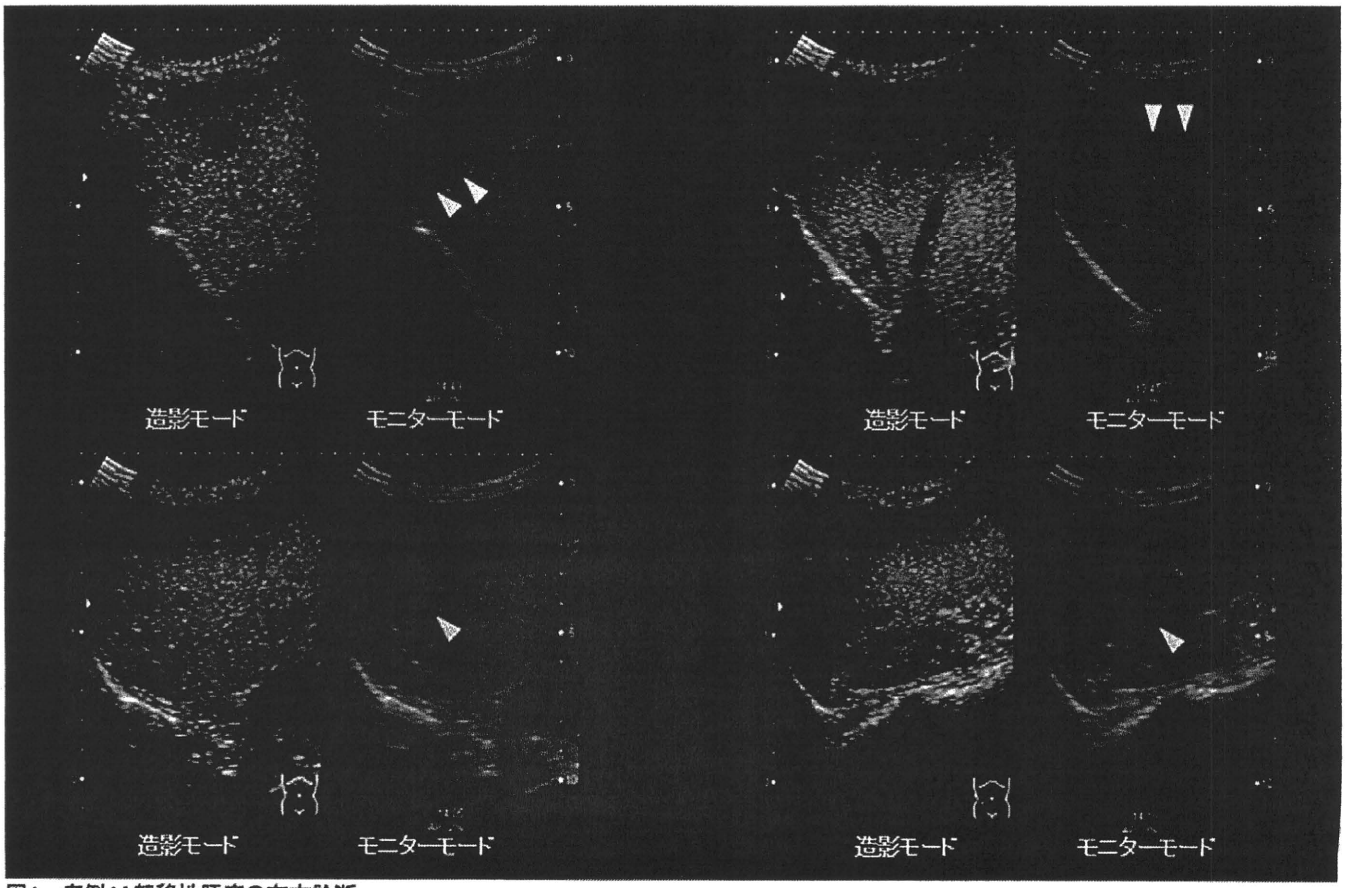

図4 症例4:転移性肝癌の存在診断

別診断に役立ち、より確実な治療方針を決定することが可能となる。これまで超音波を用いた肝腫瘤性病変の血流評価はカラードプラ法や動注US Angiography[1]が用いられ高い評価を得てきた。しかし後者は血管造影時に観血的に行なう必要があり、侵襲性が高く繰り返し行なうことは不可能であった。そこで経静脈性造影検査の要望が高まり1999年、Levovistが発売され、その有用性と診断能の高さから肝腫瘤性病変に対して広く使用されてきた。

そして今回、次世代超音波造影剤としてSonazoidが第一三共株式会社より発売された。今回我々は、Sonazoidを使用していく上で必要な条件として投与量、撮像モードの違い、後血管相における存在診断能についてLevovistと比較をしてみた。その結果、投与量に関しては肝細胞癌・転移性肝癌は推奨量では腫瘍血管および腫瘍濃染は短期間に生ずるため推奨量の1/2量が適量であった。これはSonazoidは、Levovistとは異なりbubbleの多くが破壊されないために腫瘍に多量にかつ早期に造影剤が到達するためと考えられた。一方、多量の造影剤を必要とする血管腫では推奨量のほうがfill inなどがより明瞭となったが、推奨量の1/2量においてもその描出能が劣ることはなく、最終的に投与量は1/2量が適量であると考えた。

撮像モードの違いは、その方法の感度の違いが乖離の理由があったと考えられ、両者をPSで評価したところ、その間には大きな差はなく、ほぼ同様の評価が得られた。従ってSonazoidにおける後血管相の画像評価は、今までにLevovist (PS-high)にて確立されたカテゴリーをそのまま応用することが可能であると考えられた。

また、腫瘍の存在診断能の向上は造影剤を用いることにより飛躍的に向上した。今までB-modeにて検出不可能であった腫瘍が、後血管相で欠損像として描出され、腫瘍の大きさや広がりを明瞭に把握でき非常に有用であった。しかしながらLevovistを使用した場合、bubbleが崩壊するため一度しかスキャンしかできず入念なる腫瘍の検出は録画した画像で再検討するしかなかった。しかし、Sonazoidは共振を主体とした超音波造影剤のため、繰り返しのスキャンによる詳細な検索が可能となり、以前に比べてより小さな腫瘍がより多く検出可能となった。

結語

Sonazoidによる造影超音波検査の造影効果をLevovistと比較した結果、投与量は推奨の1/2量が適量であり、今後Sonazoidは肝腫瘤性病変の血流動体の把握に非常に有効であると考えられた。

＜文献＞
1) Matsuda Y et al: Hepatic tumors: US contrast enhancement with CO_2 microbubbles. Radiology 161(3): 701-705, 1986

Original Paper

Intervirology

Intervirology 2008;51:21–25
DOI: 10.1159/000118792

Received: October 16, 2007
Accepted after revision: January 2, 2008
Published online: February 29, 2008

Prevalence of Hepatitis E Virus IgG Antibody in Japanese Patients with Hemophilia

Hidenori Toyoda[a] Takashi Honda[b] Kazuhiko Hayashi[b] Yoshiaki Katano[b] Hidemi Goto[b] Takashi Kumada[a] Kazuaki Takahashi[d] Natsumi Abe[d] Shunji Mishiro[d] Junki Takamatsu[c]

[a]Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Departments of [b]Gastroenterology and [c]Transfusion Medicine, Nagoya University School of Medicine, Nagoya, and [d]Department of Medical Science, Toshiba General Hospital, Tokyo, Japan

Key Words
Hemophilia · Hepatitis E virus · Transfusion transmission

Abstract
Objective: We investigated the prevalence of antibody against hepatitis E virus (HEV) in Japanese patients with hemophilia. ***Methods:*** IgG antibody against HEV was measured in serum of 80 Japanese patients with hemophilia by enzyme-linked immunosorbent assay. The prevalence of HEV antibody was compared with the reported prevalence of HEV antibody in Japanese patients undergoing hemodialysis and in Japanese healthy blood donors. Characteristics of patients and coinfection with other transfusion-transmissible viruses were compared in patients with and without HEV antibody. ***Results:*** Anti-HEV IgG antibody was detected in 13 of 80 patients (16.3%). The prevalence was far higher than that reported in Japanese blood donors (3.7%) and was higher than that in Japanese patients undergoing hemodialysis (9.4%). The patients with HEV antibody were significantly older than those without. HEV antibody was not detected in patients <20 years of age and in patients who had received only virus-inactivated coagulation factors. No association was observed between positivity for anti-HEV antibody and severity of hemophilia or coinfection with other parenterally transmissible viruses. ***Conclusion:*** Our results suggest that the parenteral transmission of HEV may have occurred in Japanese patients with hemophilia via non-virus-inactivated coagulation factors.

Introduction

Infection with hepatitis E virus (HEV), which can cause acute hepatitis E, is an important public health concern in many developing countries, where sanitation is suboptimal; large epidemics of hepatitis E have been reported from Asia, Africa, and Latin America [1]. Although only sporadic cases of acute hepatitis E have been reported in many industrialized countries including the United States, Europe, and Japan [1–5], some healthy individuals in industrialized countries are seropositive for HEV antibodies [6, 7].

A relatively recent report [8] described a patient who was infected with HEV via transfused blood from a vol-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/08/0511–0021$24.50/0

Accessible online at:
www.karger.com/int

Hidenori Toyoda, MD, PhD
Department of Gastroenterology, Ogaki Municipal Hospital
4-86 Minaminokawa
Ogaki 503-8502 (Japan)
Tel. +81 584 81 3341, Fax +81 584 75 5715, E-Mail tkumada@he.mirai.ne.jp

untary blood donor, and the potential risk of posttransfusion hepatitis E even in non- or low-endemic countries including Japan was suggested. The parenteral route of HEV transmission, however, remains controversial; some studies have suggested parenteral transmission of HEV, but others have shown no parenteral transmission of this virus [9–20]. Patients with hemophilia are at high risk of infection by transfusion-transmissible viruses due to the frequent use of coagulation factors. High rates of infection by hepatitis C virus (HCV), human immunodeficiency virus (HIV), and GB virus C (GBV-C) have been reported in patients with hemophilia [21–26]. If HEV infection could also have occurred in patients with hemophilia via coagulation factors, the prevalence of seropositivity for HEV antibodies would be high.

We previously investigated the prevalence of IgG antibody against hepatitis A virus (HAV) in Japanese patients with hemophilia [26] and reported a higher prevalence of HAV antibody in Japanese patients with hemophilia in comparison with normal Japanese subjects, suggesting the association between the use of coagulation factor and HAV infection. As for HEV, that is the other hepatitis virus with nonparenteral transmission, we did not investigate its prevalence in hemophilia patients because it had been considered to be rare in Japan. However, it was reported that zoonotic food-borne transmission of HEV to humans sometimes occurs; HEV infection is not so rare in Japan. In the present study, we evaluated the prevalence of antibody against HEV in Japanese patients with hemophilia to investigate the possibility of parenteral transmission of HEV by means of coagulation factors.

Patients and Methods

Patients

Eighty Japanese patients with hemophilia were involved in the study. These patients were selected from among 188 hemophilia patients who were followed up at Nagoya University Hospital and because they had sufficient stored serum samples after 2003. The patient group comprised 80 males, 59 with hemophilia A and 21 with hemophilia B. Fifty-one patients had received both non-virus-inactivated and virus-inactivated coagulation factors, and 29 had received only virus-inactivated coagulation factors. Fifty-four patients had received both domestic and imported coagulation factors that had been manufactured in the United States or in Europe, and 26 had received only domestic coagulation factors. Patients were 39.0 ± 14.4 years of age. No patient had a history of travel abroad. Written informed consent was obtained from all patients before serum samples were obtained. The study was approved by the University Ethics Committee and carried out in compliance with the Helsinki Declaration.

Serologic and Virologic Analyses of HAV, Hepatitis B Virus (HBV), HCV, GBV-C, HIV, and HEV

HAV antibody was measured in serum samples with a commercially available enzyme immunoassay kit (Dainabot, Tokyo, Japan) according to the manufacturer's instructions. HBV surface antigen (HBsAg), HBV surface antibody (HBsAb), and HBV core antibody (HBcAb) were measured with Architect HBsAg QT, Architect HBs, and Architect HBc, respectively (all Abbott Japan, Tokyo). HCV was analyzed by HCV antibody assay (third-generation assay kit; Dainabot), and HCV RNA was analyzed with the Amplicor HCV test, version 2.0 (Roche Diagnostics, Branchburg, N.J., USA). GBV-C RNA was measured by RT-PCR with nested primers deduced from conserved blocks in the 5′-untranslated region by a method described previously [27]. HIV1 infection was confirmed by anti-HIV1 antibody detection achieved with a particle agglutination test (Serodia-HIV; Fuji Rebio, Tokyo, Japan). IgG antibody against HEV was measured in serum by enzyme-linked immunosorbent assay as described by Li et al. [28].

Statistical Analysis

Differences in the proportion of patients with and without HEV antibody were analyzed by χ^2 test. Differences in quantitative variables were analyzed by Mann-Whitney U test. All p values were derived from two-tailed tests, and $p < 0.05$ was accepted as statistically significant.

Results

IgG antibody against HEV was detected in 13 of the 80 patients (16.3%) with hemophilia. The clinical characteristics of patients with and without HEV antibody are shown in table 1. The patients in whom HEV antibody was detected were significantly older than those in whom HEV antibody was not detected (46.9 ± 17.9 vs. 37.4 ± 13.1 years, $p = 0.0346$). No patient <21 years of age had HEV antibody. All patients with HEV antibody had started coagulation factor therapy before 1985. No patient who had received only virus-inactivated coagulation factors was positive for HEV antibody, whereas 13 of 51 patients (25.5%) who had received non-virus-inactivated coagulation factors were positive for HEV antibody. In contrast, HEV antibody was detected in similar percentages of patients who received only domestic and those who had received both domestic and imported coagulation factors (15.4 vs. 16.7%).

The prevalences of HAV, HBV, HCV, HIV, and GBV-C in patients with and without HEV antibody are shown in table 2. No differences were observed in the prevalence rates of these viruses between the two groups.

Table 1. Characteristics of the patients with and without HEV antibody

	HEV antibody positive (n = 13)	HEV antibody negative (n = 67)	p
Age, years (mean ± SD)[a]	46.8 ± 17.1	36.0 ± 13.5	0.0345
Type of hemophilia			
A	9 (15.3)	50 (84.7)	0.9519
B	4 (19.0)	17 (81.0)	
Severity of hemophilia			
Mild	3 (23.1)	10 (76.9)	0.6476
Moderate	0	2 (100)	
Severe	10 (15.4)	55 (84.6)	
Coagulation factors			
Virus-inactivated only	0	29 (100)	
Both non-virus-inactivated and virus-inactivated	13 (25.5)	38 (74.5)	0.0079
Domestic	4 (15.4)	22 (84.6)	
Both domestic and imported	9 (16.7)	45 (83.3)	0.8842

Numbers (and percentages) of patients are shown unless otherwise indicated.

[a] Age at the time of measurement of HEV antibody.

Table 2. Prevalence rates of other transfusion-transmissible viruses in patients with and without HEV antibody

Positive for	HEV antibody positive (n = 13)	HEV antibody negative (n = 67)	p
HAV IgG antibody	6 (46.2)	15 (22.4)	0.1505
HBV surface antigen	1 (7.7)	0	0.3572
HBV surface antibody	8 (61.5)	40 (59.7)	0.9015
HBV core antibody	10 (76.9)	52 (77.6)	0.9566
HCV antibody	13 (100.0)	63 (94.2)	0.8348
HCV RNA	13 (100.0)	63 (94.2)	0.8348
HIV1 antibody	5 (38.5)	27 (40.3)	0.9015
GBV-C RNA[a]	4 (44.4)	12 (30.8)	0.6949

Numbers (and percentages) of patients are shown.

[a] Among 48 patients in whom GBV-C RNA was measured (9 with HEV antibody and 39 without HEV antibody).

Table 3. Reported prevalence rates of HEV antibody in patients with hemophilia and in patients undergoing hemodialysis

Authors (year of publication)	Country	Prevalence (%) of patients with HEV antibody
Patients with hemophilia		
Mannucci et al. [12] (1994)	Italy	0/60 (0)
Barzilai et al. [13] (1995)	Israel	16/188 (8.5)
Klarmann et al. [14] (1995)	Germany	1/37 (2.7)
Zaaijer et al. [15] (1995)	Netherlands	4/296 (1.4)
Buffet et al. [16] (1996)	France	5/63 (7.9)
Our study (2007)	Japan	13/80 (16.3)
Patients undergoing hemodialysis		
Courtney et al. [10] (1994)	Ireland	0/45 (0)
Halfon et al. [11] (1994)	France	16/147 (10.9)
Psichogiou et al. [17] (1996)	Greece	27/420 (6.4)
Fabrizi et al. [18] (1997)	Italy	6/204 (2.9)
Mitsui et al. [20] (2004)	Japan	39/416 (9.4)

Table 4. Prevalence rates of HEV antibody among patients with hemophilia, patients on hemodialysis, and healthy blood donors in Japan

Age, years	Patients with hemophilia (n = 80)	Patients undergoing hemodialysis (n = 416) [20]	Healthy blood donors (n = 5,343) [29]
≤19	0/6		7/812 (0.9)
20–29	1/18 (5.6)	1/33 (3.0)[a]	19/1,043 (1.8)
30–39	5/24 (20.8)		28/1,146 (2.4)
40–49	2/17 (11.8)	3/40 (7.5)	53/966 (5.5)
50–59	3/10 (30.0)	10/109 (9.2)	54/744 (7.3)
≥60	2/5 (40.0)	25/234 (10.7)	39/632 (6.2)

Numbers (and percentages) of patients are shown.

The age range was 16–84 years in patients with hemophilia, 23–91 years in patients undergoing hemodialysis, and 16–69 years in healthy blood donors.

[a] Patients with an age range of 23–39 years.

Discussion

Whether HEV is transmitted parenterally remains controversial (table 3); the existence of transfusion transmission of HEV is still unclear. A high prevalence of anti-HEV antibody was reportedly observed among hemodialysis patients, the majority of whom had a history of blood transfusions [11, 20]. However, other investigators found only a few HEV antibody-positive patients in larger groups of hemodialysis patients [10, 17, 18]. In industrialized countries, the prevalence of positivity for HEV antibody in patients with hemophilia seems to differ between countries [12–16], whereas a higher prevalence in patients with hemophilia than in volunteer blood donors has been reported in nonindustrialized countries [19].

This is the first report that investigated the prevalence of HEV antibody in hemophilia patients in Japan. The prevalence of HEV antibody in our study patients (16.3%) was higher than that previously reported in Japanese blood donors (3.7%) [29] and in Japanese patients undergoing hemodialysis (9.4%) [20] (table 4). The gradual increase in the prevalence of HEV antibody between healthy blood donors, patients undergoing hemodialysis, and patients with hemophilia suggests a possible role of parenteral transmission of HEV. Because coagulation factors that are currently used in Japan are very unlikely to contain IgG antibodies [30, 31], the HEV antibodies that were detected in our patients cannot have been passively acquired from recently used coagulation factors.

The absence of HEV antibody in patients <20 years of age and in patients who had received only virus-inactivated coagulation factors suggests that HEV infection might have occurred by means of non-virus-inactivated coagulation factors that had been used before the mid-1980s. In contrast, we did not find a difference in the prevalence of HEV antibody between patients who received only domestic coagulation factors and those who had a history of using imported coagulation factors, unlike the difference in the prevalence of HIV or HCV genotype 1a infections between Japanese hemophilia patients with and without the use of imported coagulation factors [23]. Recent studies have indicated that hepatitis E is a zoonosis [2, 4, 32–39], and it has been shown that zoonotic food-borne transmission of HEV to humans may play an important role in the occurrence of HEV infection in Japan [38–41]. In addition, silent viremia due to HEV, i.e., the presence of HEV in the bloodstream but without acute hepatitis, has been reported [19, 42, 43]. HEV infection, therefore, may not be rare in Japan [44, 45]. Contamination of domestic, non-virus-inactivated coagulation factors by HEV is, therefore, not unlikely, and the use of domestic coagulation factors could have caused HEV infection in Japanese patients with hemophilia. In addition, the use of coagulation factors that had been manufactured from plasma of individuals from an area where HEV infection is endemic could have caused HEV infections in our patients.

We found no association of HEV antibody with infection by transfusion-transmissible viruses (HBV, HCV, HIV, and GBV-C) or with the presence of HAV antibody. It was difficult to elucidate the characteristics of patients with HEV antibody on the basis of the coinfecting viruses because of the high prevalence of infection by these viruses.

The marked differences between countries in the prevalence of HEV antibody in patients with hemophilia, which sparks controversy over the possibility of parenteral transmission of HEV, might be due to differences in the origins and methods of manufacturing coagulation factors, especially during the period in which non-virus-inactivated coagulation factors were used. Unfortunately, the prevalence of HEV antibodies in volunteer blood donors in the various countries has not been reported, and the origins of the plasma used for manufacturing coagulation factors in these countries are unknown. It is well known that the prevalence of HEV varies widely throughout the world. Plasma of individuals from high-prevalence areas might have had more chance of contamination during the period when non-virus-inactivated coagulation factors were used.

In summary, the high prevalence of HEV antibody in our patients with hemophilia suggests the possibility of a parenteral route of HEV transmission. However, the coagulation factors used in our patients are not now available for examination, so we cannot prove that the coagulation factors used in Japan were contaminated by HEV. Further studies are needed to clarify whether HEV has been transmitted by means of coagulation factors. Tracing the origin of the plasma used for coagulation factors would be helpful in understanding the association between the prevalence of HEV antibody and the use of coagulation factors in patients with hemophilia.

References

1 Purcell RH, Emerson SU: Hepatitis E virus; in Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, Straus SE (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 3051–3061.

2 Harrison TJ: Hepatitis E virus – an update. Liver 1999;19:171–176.

3 Schlauder GG, Mushahwar IK: Genetic heterogeneity of hepatitis E virus. J Med Virol 2001;65:282–292.

4 Smith JL: A review of hepatitis E virus. J Food Protect 2001;64:572–586.

5 Okamoto H, Takahashi M, Nishizawa T: Features of hepatitis E virus infection in Japan. Intern Med 2003;42:1065–1071.

6 Mast EE, Kuramoto IK, Favorov MO, Schoening VR, Burkholder BT, Shapiro CN, Holland PV: Prevalence of and risk factors for antibody to hepatitis E virus seroreactivity among blood donors in Northern California. J Infect Dis 1997;176:34–40.

7 Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, Purcell RH: Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 1997;35:1244–1247.

8 Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, Takahashi K, Mishiro S, Imai M, Takeda N, Ikeda H: Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004;44:934–940.
9 Wang CH, Flehmig B, Moeckli R: Transmission of hepatitis E virus by transfusion? Lancet 1993;341:825–826.
10 Courtney MG, O'Mahoney M, Albloushi S, Sachithanandan S, Walshe J, Carmody M, Donoghue J, Parfrey N, Shattock AG, Fielding J: Hepatitis E virus antibody prevalence. Lancet 1994;344:1166.
11 Halfon P, Ouzan D, Chanas M, Khiri H, Feryn JM, Mangin L, Masseyef MF, Salvadori JM: High prevalence of hepatitis E virus antibody in haemodialysis patients. Lancet 1994;344:746.
12 Mannucci PM, Gringeri A, Santagostino E, Romano L, Zanetti A: Low risk of transmission of hepatitis E virus by large-pool coagulation factor concentrates. Lancet 1994;343: 597–598.
13 Barzilai A, Schulman S, Karetnyi YV, Favorov MO, Levin E, Mendelson E, Weiss P, Fields HA, Varon D, Martinowitz U: Hepatitis E virus infection in hemophiliacs. J Med Virol 1995;46:153–156.
14 Klarmann D, Kreuz W, Kornhuber B: Low prevalence of hepatitis E virus antibodies in hepatitis C virus-positive patients with coagulation disorders. Transfusion 1995;35: 969–970.
15 Zaaijer HL, Mauser-Bunschoten EP, ten Veen JH, Kapprell HP, Kok M, van den Berg HM, Lelie PN: Hepatitis E virus antibodies among patients with hemophilia, blood donors, and hepatitis patients. J Med Virol 1995;46:244–246.
16 Buffet C, Laurent-Puig P, Chandot S, Laurian Y, Charpentier B, Briantais MJ, Dussaix E: A high hepatitis E virus seroprevalence among renal transplantation and haemophilia patient populations. J Hepatol 1996;24:122–125.
17 Psichogiou M, Vaindirli E, Tzala E, Voudiclari S, Boletis J, Vosnidis G, Moutafis S, Skoutelis G, Hadjiconstantinou V, Troonen H, Hatzakis A: Hepatitis E virus (HEV) infection in haemodialysis patients. The Multicentre Haemodialysis Cohort Study on Viral Hepatitis. Nephrol Dial Transplant 1996; 11:1093–1095.
18 Fabrizi F, Lunghi G, Bacchini G, Corti M, Pagano A, Locatelli F: Hepatitis E virus infection in haemodialysis patients: a seroepidemiological survey. Nephrol Dial Transplant 1997;12:133–136.
19 Arankalle VA, Chobe LP: Hepatitis E virus: can it be transmitted parenterally? J Viral Hepat 1999;6:161–164.
20 Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H: Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004;74:563–572.
21 Inagaki M, Miura T, Taki M, Saito N, Yamada K, Unuma T, Tsuda F, Miyakawa Y, Mayumi M: Infection with hepatitis delta and human immunodeficiency viruses among hemophiliacs in Japan. Transfusion 1988;28: 18–20.
22 Kashiwagi S, Hayashi J, Ikematsu H, Noguchi A, Ikeda K, Kishida K, Shirakawa M, Takenaka A, Mori R: Prevalence of human immunodeficiency virus (HIV) infection among hemophiliacs in Fukuoka, Japan. Microbiol Immunol 1988;32:851–855.
23 Isobe K, Imoto M, Fukuda Y, Koyama Y, Nakano I, Hayakawa T, Takamatsu J: Hepatitis C virus infection and genotypes in Japanese hemophiliacs. Liver 1995;15:131–134.
24 Makris M, Preston FE, Rosendaal FR, Underwood JCE, Rice KM, Triger DR: The natural history of chronic hepatitis C in haemophiliacs. Br J Haematol 1996;94:746–752.
25 Darby SC, Ewart DW, Giangrande PLF, Spooner RJD, Rizza CR, Dusheiko GM, Lee CA, Ludlam CA, Preston FE: Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. Lancet 1997;350:1425–1431.
26 Hayashi K, Fukuda Y, Nakano I, Katano Y, Nagano K, Yokozaki S, Hayakawa T, Toyoda H, Takamatsu J: Infection of hepatitis A virus in Japanese haemophiliacs. J Infect 2001; 42:1–4.
27 Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, Saito H, Okamoto H: GB virus C/hepatitis G virus isolates in Japanese haemophiliacs and their origins. Thromb Haemost 1998;80:242–245.
28 Li TC, Zhang J, Shinzawa H, Ishibashi M, Sata M, Mast EE, Kim K, Miyamura T, Takeda N: Empty virus-like particle-based enzyme-linked immunosorbent assay for antibodies to hepatitis E virus. J Med Virol 2000; 62:327–333.
29 Fukuda S, Sunaga J, Saito N, Fujimura K, Itoh Y, Sasaki M, Tsuda F, Takahashi M, Nishizawa T, Okamoto H: Prevalence of antibodies to hepatitis E virus among Japanese blood donors: identification of three blood donors infected with a genotype 3 hepatitis E virus. J Med Virol 2004;73:554–561.
30 Mannhalter JW, Ahmad R, Leibl H, Göttlicher J, Wolf HM, Eibl MM: Comparable modulation of human monocyte functions by commercial factor VIII concentrates of varying purity. Blood 1988;71:1662–1668.
31 Wadsworth C, Hanson LA, Kjellman H, Söderström T, Blombäck M: Some characteristics of aggregates of IgG and plasma proteins in heat-treated factor VIII concentrates. Blut 1989;58:133–141.
32 Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU: A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA 1997;94:9860–9865.
33 Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU: Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol 1998;72:9714–9721.
34 Erker JC, Desai SM, Schlauder GG, Dawson GJ, Mushahwar IK: A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. J Gen Virol 1999;80: 681–690.
35 Halbur PG, Kasorndorkbua C, Gilbert C, Guenette D, Potters MB, Purcell RH, Emerson SU, Meng XJ: Comparative pathogenesis of infection of pigs with hepatitis E viruses recovered from a pig and a human. J Clin Microbiol 2001;39:918–923.
36 Okamoto H, Takahashi M, Nishizawa T, Fukai K, Muramatsu U, Yoshikawa A: Analysis of the complete genome of indigenous swine hepatitis E virus isolated in Japan. Biochem Biophys Res Commun 2001;289:929–936.
37 Nishizawa T, Takahashi M, Mizuo H, Miyajima H, Gotanda Y, Okamoto H: Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99% identity over the entire genome. J Gen Virol 2003;84:1245–1251.
38 Tei S, Kitajima N, Takahashi K, Mishiro S: Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362:371–373.
39 Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H: Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 2003;84:2351–2357.
40 Matsuda H, Okada K, Takahashi K, Mishiro S: Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. J Infect Dis 2003;188:944.
41 Tamada Y, Yano K, Yatsuhashi H, Inoue O, Mawatari F, Ishibashi H: Consumption of wild boar linked to cases of hepatitis E. J Hepatol 2004;40:869–870.
42 Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK: Hepatitis E virus transmission to a volunteer. Lancet 1993; 341:149–150.
43 Mitsui T, Tsukamoto Y, Suzuki S, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Tsatsralt-Od B, Nishizawa T, Okamoto H: Serological and molecular studies on subclinical hepatitis E virus infection using periodic serum samples obtained from healthy individuals. J Med Virol 2005;76:526–533.
44 Ding X, Li TC, Hayashi S, Masaki N, Tran TTH, Hirano M, Yamaguchi M, Usui M, Takeda N, Abe K: Present state of hepatitis E virus epidemiology in Tokyo, Japan. Hepatol Res 2003;27:169–173.
45 Tanaka E, Matsumoto A, Takeda N, Li TC, Umemura T, Yoshizawa K, Miyakawa Y, Miyamura T, Kiyosawa K: Age-specific antibody to hepatitis E virus has remained constant during the past 20 years in Japan. J Viral Hepat 2005;12:439–442.

Journal of Viral Hepatitis, 2008, 15, 651–658 doi:10.1111/j.1365-2893.2008.01004.x

Correlation of serum ribavirin concentration with pretreatment renal function estimates in patients with chronic hepatitis C receiving combination antiviral therapy with peginterferon and ribavirin

H. Toyoda,[1] T. Kumada,[1] S. Kiriyama,[1] Y. Sone,[1] M. Tanikawa,[1] Y. Hisanaga,[1] A. Kanamori,[1] H. Atsumi,[1] S. Nakano,[1] T. Arakawa,[1] T. Honda,[2] K. Hayashi,[2] Y. Katano[2] and H. Goto[2] [1]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki; and* [2]*Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Received December 2007; accepted for publication February 2008

SUMMARY. Serum ribavirin concentration is an important factor in antiviral therapy in combination with peginterferon (PEG-IFN) and ribavirin for patients with chronic hepatitis C in terms of both beneficial and adverse effects. We evaluated whether the serum ribavirin concentration can be predicted on the basis of renal function estimates. Serum creatinine and cystatin C concentrations were measured at the start of treatment in a total of 148 patients with chronic hepatitis C who underwent combination PEG-IFN and ribavirin therapy. Creatinine clearance (CrCl) and total clearance of ribavirin (CL/F) were calculated on the basis of the serum creatinine level. The glomerular filtration rate was calculated with two different formulae on the basis of the serum cystatin C level. These values were compared with serum ribavirin concentrations 4 weeks after the start of therapy. The cystatin C level increased with the progression of liver fibrosis, whereas the creatinine level was constant regardless of the degree of liver fibrosis. Significant correlation was not observed between the serum ribavirin concentration and serum creatinine level, cystatin C level, or calculated renal function estimates. However, significant correlation was found between the serum ribavirin concentration and CrCl and CL/F in patients who were given ribavirin >800 mg/day. Overall, renal function estimates do not correlate with the serum ribavirin concentration in Japanese patients with chronic hepatitis C who undergo combination PEG-IFN and ribavirin therapy. Serum creatinine-based renal function estimates might be predictive for the serum ribavirin concentration only in patients with a daily ribavirin intake of 800 mg or more.

Keywords: chronic hepatitis C, creatinine, cystatin C, renal function, ribavirin.

INTRODUCTION

Combination interferon (IFN) and ribavirin therapy is the current standard antiviral therapy for chronic hepatitis C. The addition of ribavirin to IFN-α substantially increases the sustained virological response rate when compared with that of IFN-α alone among treatment-naïve [1–3] and nonresponding or relapsed IFN-α-experienced patients [4–6]. Recently, the combination of peginterferon (PEG-IFN)-α and ribavirin has been shown to be superior to IFN-α and ribavirin with a high sustained virological response rate [7,8].

Abbreviations: CrCl, creatinine clearance; CL/F, total clearance of ribavirin; HCV, hepatitis C virus; GFR, glomerular filtration rate.

Correspondence: Hidenori Toyoda, Department of Gastroenterology, Ogaki Municipal Hospital, 4-86, Minaminokawa, Ogaki, Gifu, 503-8502, Japan. E-mail: tkumada@he.mirai.ne.jp

Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a synthetic guanosine nucleoside analogue, inhibits the replication of a wide range of RNA and DNA viruses, including those of the flavivirus family, of which hepatitis C virus (HCV) is a member [9]. Although ribavirin improves the antiviral effect of IFN against HCV infection, ribavirin accumulates in red blood cells, and haemolytic anaemia is one of the main adverse effects of ribavirin therapy. Therefore, maintaining an appropriate ribavirin concentration is important for providing a beneficial antiviral effect while avoiding or reducing the ribavirin-induced anaemia [10,11].

Ribavirin is normally eliminated by renal filtration [9]. Several studies have shown the importance of renal function for maintaining an appropriate serum ribavirin concentration during combination antiviral therapy with PEG-IFN and ribavirin for chronic hepatitis C [12,13]. Therefore, renal

function estimates can help to predict the serum ribavirin concentration in patients during treatment.

In the present study, we analyzed the correlation between pretreatment renal function estimates and the serum ribavirin concentration during treatment, and we evaluated renal function markers as pretreatment predictors of the serum ribavirin concentration.

PATIENTS AND METHODS

Patients

A total of 148 patients with chronic hepatitis C undergoing combination therapy with PEG-IFN-α and ribavirin were enrolled in the study. The clinical characteristics of the study patients are shown in Table 1. Patients included 85 women and 63 men with a mean age of 58.3 ± 10.2 years. Liver histology was evaluated by examining liver specimens obtained by fine-needle biopsy prior to antiviral therapy. METAVIR activity scores were A0 in 3 patients, A1 in 84, A2 in 42 and A3 in 11, and METAVIR fibrosis stages were F0 in 13 patients, F1 in 70, F2 in 38 and F3 in 19 [14]. Liver biopsy was not carried out in eight patients. One hundred and three patients were infected with HCV genotype 1b, 31 patients were infected with HCV genotype 2a and the other 14 patients were infected with HCV genotype 2b. The pretreatment HCV RNA concentration was shown to be $1747 \pm 1281 \times 10^3$ IU/mL by quantitative PCR assay (Amplicor GT-HCV Monitor, Version 2.0; Roche Molecular Systems, Pleasanton, CA, USA). Eighty-seven patients had no history of IFN therapy (naïve cases) and the other 61 patients had previous IFN therapy (retreatment cases).

The daily dose of ribavirin was adjusted by patient's body weight in accordance with the manufacturer's recommendations. Patients weighing ≤60 kg were given 600 mg of ribavirin per day, those weighing >60 and ≤80 kg were given 800 mg of ribavirin per day, and those weighing >80 kg were given 1000 mg of ribavirin per day. Patients weighing ≤45 kg were given 60 μg of PEG-IFN-α 2b (Schering-Plough, Osaka, Japan) once a week, those weighing >45 and ≤60 kg were given 80 μg, those weighing >60 and ≤75 kg were given 100 μg, those weighing >75 and ≤90 kg were given 120 μg and those weighing >90 kg were given 150 μg. Eighty-four patients were given 600 mg of ribavirin per day, 58 patients were given 800 mg per day and 6 patients were given 1000 mg per day. No patient had the daily dose of ribavirin reduced during the first 4 weeks of therapy.

Measurement of serum renal function markers and evaluation of renal function

Serum creatinine and cystatin C were measured from the same serum samples. The serum creatinine level (mg/dL) was measured on the Dimension Clinical Chemistry System (Dade Behring, Marburg, Germany) with a commercially available assay. The serum cystatin C level (mg/L) was analyzed by a fully automated latex-enhanced immunonephelometric method (N Latex Cystatin C assay on the Nephelometer II System, Dade Behring).

Table 1 Clinical characteristics of the study patients (n = 148)

Age (years)	58.3 ± 10.2
Sex (female/male)	85 (57.4)/63 (42.6)
Body weight (kg)	58.7 ± 10.0
History of interferon therapy (naïve/retreatment)	87 (58.8)/61 (41.2)
History of transfusion (−/+)	119 (80.4)/29 (19.6)
Alanine aminotransferase (IU/L)	56.7 ± 48.1
Aspartate aminotransferase (IU/L)	50.2 ± 41.0
Gamma-glutamyl transpeptidase (IU)	50.2 ± 68.2
Alkaline phosphatase (IU/L)	265.4 ± 103.4
Albumin (g/dL)	4.21 ± 0.32
Total bilirubin (mg/dL)	0.68 ± 0.27
White blood cell counts (/μL)	5062 ± 1319
Haemoglobin (g/dL)	14.0 ± 1.3
Platelet counts (×103/μL)	17.6 ± 5.6
Liver histology-activity (A0/A1/A2/A3)	3 (2.1)/84 (60.0)/42 (30.0)/11 (7.9)*
Liver histology-fibrosis (F0/F1/F2/F3)	13 (9.3)/70 (50.0)/38 (27.1)/19 (13.6)*
HCV genotype (1b/2a/2b)	103 (69.6)/31 (20.9)/14 (9.5)
HCV RNA concentration (×103 IU/mL)	1747 ± 1281
Daily dose of ribavirin (600 mg/800 mg/1000 mg)	84 (56.8)/58 (39.2)/6 (4.0)

HCV, hepatitis C virus. Percentages are shown in parentheses.
*Liver biopsy was not done in eight patients

Renal function estimates were calculated on the basis of serum creatinine and cystatin C levels and patient's age, sex and body weight. Creatinine clearance (CrCl, mL/min) and total body clearance of ribavirin (CL/F, L/h) were calculated from the serum creatinine level by means of the Cockcroft–Gault formula [15]:

$$\text{CrCl} = (140 - \text{age}) \times \text{body weight} \times \text{sex}/72 \times \text{serum creatinine}$$

where CrCl is in mL/min, body weight is in kg, sex = 1 for men and 0.85 for women and serum creatinine is in mg/dL; and the Kamar formula [13] is given as follows:

$$\text{CL/F} = 32.3 \times \text{body weight} \times [1 - (0.0094 \times \text{age})] \times [1 - (0.42 \times \text{sex})]/\text{serum creatinine},$$

where CL/F is in L/h, body weight is in kg, sex = 0 for men and 1 for women, and serum creatinine is in μmol/L. Serum creatinine (mg/dL) was converted to serum creatinine (μmol/L) by the following formula:

$$\text{Serum creatinine}(\mu\text{mol/L}) = \text{Serum creatinine(mg/dL)} /113.12 \times 10,000$$

The glomerular filtration rate (GFR, mL/min/1.73 m^2) was calculated from the serum cystatin C level by means of the Hoek formula [16]:

$$\text{GFR} = -4.32 + 80.35 \times 1/\text{serum cystatin C}$$

and the Larsson formula [17] is given as follows:

$$\text{GFR} = 77.239 \times (\text{serum cystatin C})^{-1.262}$$

where GFR is in mL/min/1.73 m^2 and serum cystatin C is in mg/L.

Measurement of the serum ribavirin concentration

Serum ribavirin concentrations were analyzed in serum samples obtained at 4 weeks after the start of ribavirin administration, because the serum ribavirin concentration reportedly reaches a plateau 4 weeks after the start of ribavirin intake [18,19]. Serum ribavirin concentrations (ng/mL) were determined by means of a high-performance liquid chromatography method which has been previously reported [20].

Statistical analyses

Data are shown as mean ± SD, unless otherwise indicated. Differences in quantitative values between two groups were analyzed by the Mann–Whitney *U*-test. Correlation between values was tested by the Spearman rank correlation coefficient. All *P*-values were two-tailed and $P < 0.05$ was accepted as statistically significant.

The study protocol was approved by the hospital ethics committee and was carried out in compliance with the Helsinki declaration.

RESULTS

Concentrations of serum creatinine and cystatin C and renal function

The median serum creatinine level was 0.62 mg/dL (range 0.30–1.30) and the median serum cystatin C level was 0.82 mg/L (range 0.60–1.43). These two values were well-correlated ($P < 0.0001$). The changes in serum creatinine and serum cystatin C levels are shown in Fig. 1 according to the grade of liver fibrosis evaluated by examining biopsy specimens. The serum creatinine level was constant regardless of the grade of liver fibrosis (0.65 ± 0.12 mg/dL in F0, 0.67 ± 0.15 mg/dL in F1, 0.65 ± 0.11 mg/dL in F2 and 0.61 ± 0.16 mg/dL in F3). In contrast, the serum cystatin C level increased with the degree of liver fibrosis (0.74 ± 0.11 mg/L in F0, 0.84 ± 0.15 mg/L in F1, 0.86 ± 0.14 mg/L in F2 and 0.94 ± 0.17 mg/L in F3; for F0 *vs* F1, $P = 0.0175$; for F0 *vs* F2, $P = 0.0006$; for F0 *vs*

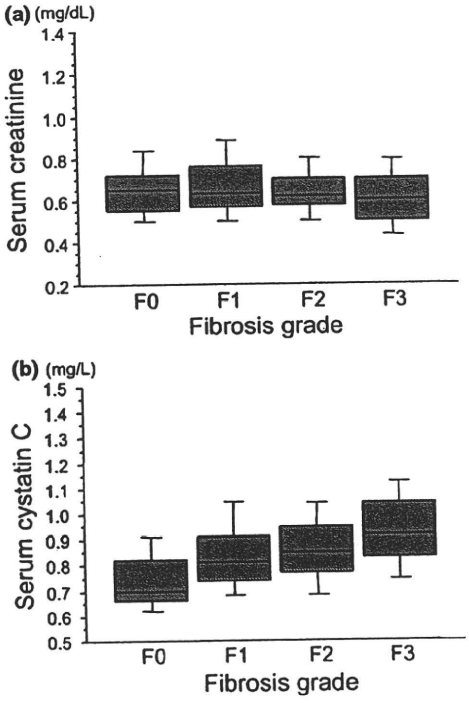

Fig. 1 Pretreatment serum creatinine and cystatin C levels in relation to histologic liver fibrosis grade ($n = 140$). Whereas the serum creatinine level is constant regardless of the grade of liver fibrosis, the serum cystatin C level increases with the degree of liver fibrosis (for F0 *vs* F1, $P = 0.0175$; for F0 *vs* F2, $P = 0.0006$; for F0 *vs* F3, $P = 0.0007$; for F1 *vs* F3, $P = 0.0128$; for F2 *vs* F3d, $P = 0.0482$).

F3, $P = 0.0007$; for F1 *vs* F3, $P = 0.0128$; for F2 *vs* F3, $P = 0.0482$).

The renal function estimates were calculated from serum creatinine and serum cystatin C levels by applying patient's age, sex and body weight to the appropriate formula. The median CrCl was 95.7 mL/min (range 49.7–209.7), calculated from the serum creatinine level according to the Cockcroft–Gault formula. The median CL/F was 11.7 L/h (range 2.5–56.9), calculated from the serum creatinine level according to the Kamar formula. The GFR calculated from the serum cystatin C level was 93.7 mL/min/1.73 m^2 (range 51.9–129.6) according to the Hoek formula and 99.2 mL/min/1.73 m^2 (range 49.2–147.2) according to the Larsson formula.

Correlation between the serum ribavirin concentration and renal function markers

The median serum ribavirin concentration was 2260 ng/mL (range 961–4394). Correlation between serum creatinine-based renal function estimates and the serum ribavirin concentration and between serum cystatin C-based renal function estimates and the serum ribavirin concentration are shown in Fig. 2. Significant correlation was not found between the serum ribavirin concentration and renal function estimates, with the exception of a mild correlation with creatinine-based CL/F ($P = 0.0240$).

We evaluated correlations by dividing patients into two groups according to the daily dose of ribavirin: 84 patients who were given 600 mg of ribavirin daily (i.e., patients with body weight of ≤60 kg) and 64 patients who were given 800 mg or more of ribavirin daily (i.e., patients with body weight of >60 kg). In patients who were given 600 mg of ribavirin daily, we found no correlation between creatinin- or cystatin C-based renal function estimates and the serum ribavirin concentration. In contrast, we found significant correlations between creatinine-based CrCl and CL/F and the serum ribavirin concentration in patients who were given 800 mg or more of ribavirin daily (column A of Fig. 3; $P = 0.0059$ for CrCl and $P = 0.0082$ for CL/F). We found no correlation when cystatin C-based renal function estimates were compared with serum ribavirin concentration (column B of Fig. 3; $P = 0.2322$ for serum cystatin C, for GFR by the Hoek formula, and for GFR by the Larsson formula).

DISCUSSION

In the present study, we investigated correlation between serum ribavirin concentration during its plateau phase and renal function estimates. For renal function estimates, we measured the serum creatinine level and calculated CrCl and CL/F based on this value, and we measured the serum cystatin C level and the calculated GFR based on this value.

Cystatin C is a non-glycosylated cationic protein of 13.3 kDa belonging to the cystatin superfamily of cysteine protease inhibitors [21,22]. Recent studies have shown that measuring cystatin C allows detection of renal impairment, earlier than measuring serum creatinine does [23,24]. An increase in the serum cystatin C level preceding an increase in the serum creatinine level has been reported in patients with cirrhosis [25–29], in patients with diabetes [30] and in elderly persons without chronic kidney disease [31]. Use of the cystatin C level has been reported for prediction of the concentration and for dose adjustment of several drugs including digoxin, amikacin, gentamicin, tobramycin and vancomycin [32,33].

In the present study, serum cystatin C and cystatin C-based GFR did not correlate with the serum ribavirin concentration. Therefore, cystatin C-based renal function estimates cannot predict the serum ribavirin concentration in patients with chronic hepatitis C who undergo combination therapy with PEG-IFN and ribavirin. The increase in the cystatin C level with the progression of liver fibrosis, which was observed in our study patients and has previously been reported [34,35], might be one of the reasons for the lack of association between the serum cystatin C level or cystatin C-based GFR and serum ribavirin concentration, despite reports that cystatin C is a sensitive indicator of early renal impairment and that ribavirin is eliminated by renal filtration.

Bruchfeld *et al.* previously reported a significant link between renal function evaluated by CrCl and serum ribavirin concentration in Swedish patients with chronic hepatitis C [12]. In contrast, we failed to find a significant correlation between serum creatinine-based renal function and serum ribavirin concentration in our overall study patients. The body weight of patients between these two studies was largely different: 77 ± 16 kg in patients of the Swedish study and 58.7 ± 10.0 kg in patients of our study. In addition, a large number of patients (56.8%) from our study were given 600 mg of ribavirin daily, whereas all patients were given ≥800 mg of ribavirin daily in the Swedish study. These differences might account for the conflicting result between the study by Bruchfeld *et al.* and the present study. Indeed, we found significant correlation between serum creatinine-based CrCl and CL/F and serum ribavirin concentration in patients who were given ≥800 mg of ribavirin daily (patients with a body weight of >60 kg). Because of the lower body weight of Asian persons including the Japanese, in comparison to Western and African persons, larger percentages of Asian patients have a body weight of ≤60 kg, and 600 mg of ribavirin is given. However, in Western countries, most of the patients start combination therapy with a ribavirin dose of 800 mg or more. Serum creatinine-based CrCl and CL/F can, therefore, be more useful and predictive in Western countries. Further studies are needed to confirm creatinine-based CrCl and CL/F as predictors of serum ribavirin

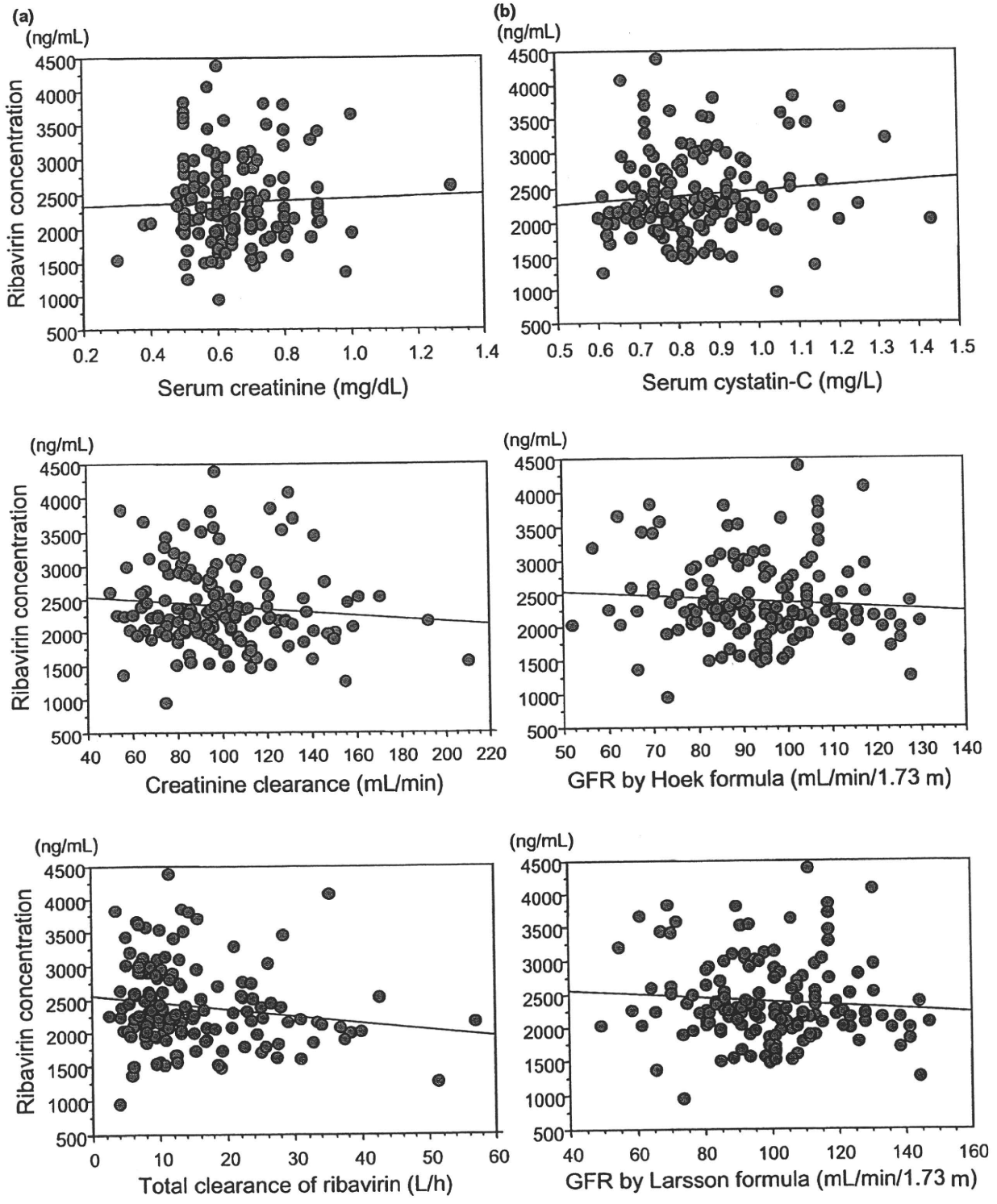

Fig. 2 Correlation between the serum ribavirin concentration 4 weeks after the start of combination therapy and pretreatment serum creatinine and creatinine-based renal function (CrCl and total clearance of ribavirin) (a), and pretreatment serum cystatin C and cystatin C-based renal function (GFR by the Hoek formula and by the Larsson formula) (b). No significant correlation was observed except for the correlation between ribavirin concentration and total clearance of ribavirin (for serum creatinine *vs* ribavirin concentration, $P = 0.9102$; for CrCl *vs* ribavirin concentration, $P = 0.1416$; for total clearance of ribavirin *vs* ribavirin concentration, $P = 0.0240$, for serum cystatin C *vs* ribavirin concentration, GFR by the Hoek formula *vs* ribavirin concentration and GFR by the Larsson formula *vs* ribavirin concentration, $P = 0.2122$).

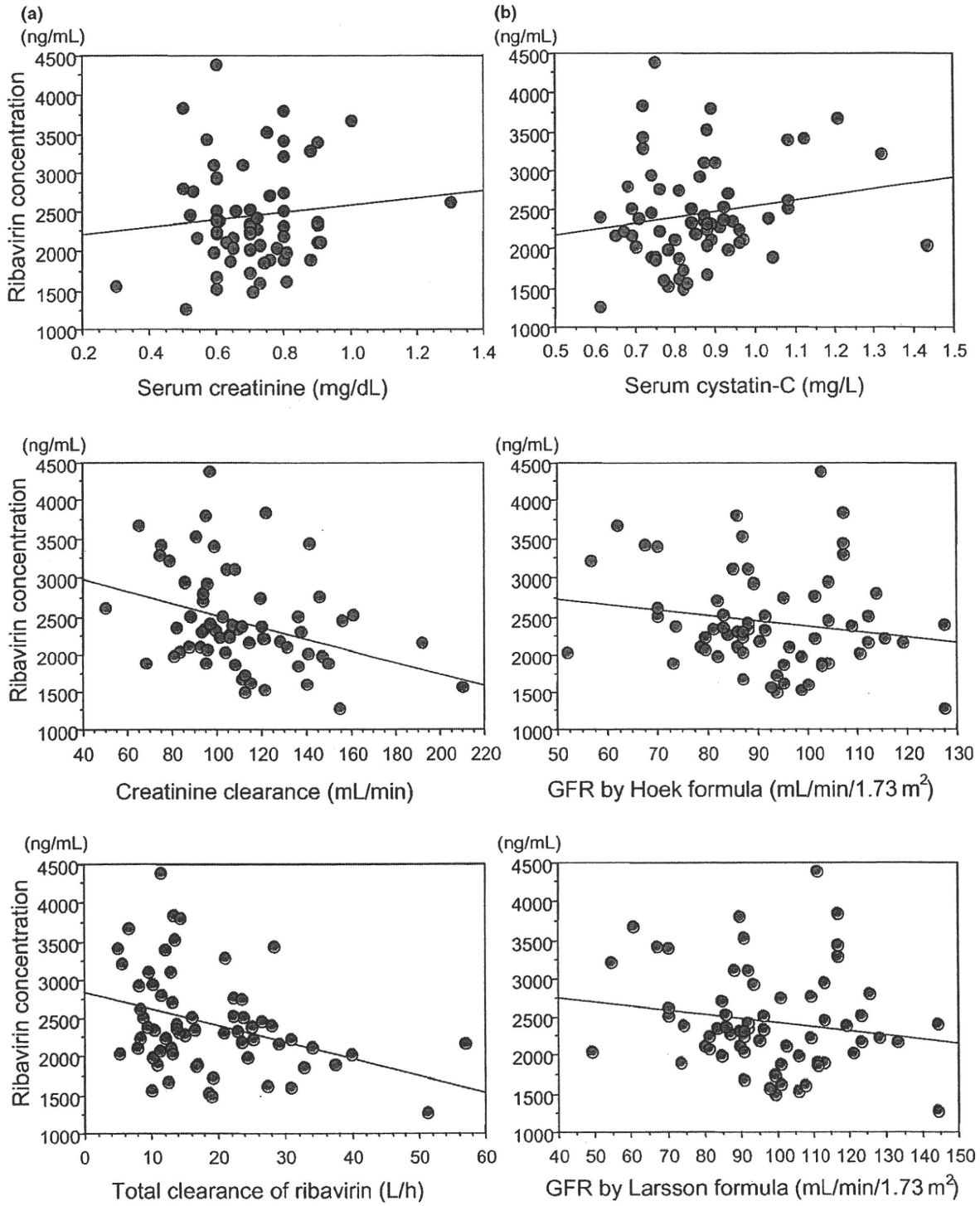

Fig. 3 Correlation between the serum ribavirin concentration 4 weeks after the start of combination therapy and pretreatment serum creatinine and creatinine-based renal function (CrCl and total clearance of ribavirin) (a), and pretreatment serum cystatin C and cystatin C-based renal function (GFR by the Hoek formula and by the Larsson formula) (b) in patients with a daily ribavirin intake of ≥800 mg. Significant correlation was observed between the ribavirin concentration and CrCl and between the ribavirin concentration and total clearance of ribavirin (for serum creatinine *vs* ribavirin concentration, $P = 0.9186$; for CrCl *vs* ribavirin concentration, $P = 0.0059$; for total clearance of ribavirin *vs* ribavirin concentration, $P = 0.0082$). In contrast, no significant correlation was observed between the ribavirin concentration and any of serum cystatin C-based renal function markers (for serum cystatin C *vs* ribavirin concentration, GFR by the Hoek formula *vs* ribavirin concentration and GFR by the Larsson formula *vs* ribavirin concentration, $P = 0.2322$).

concentrations in Asian patients who were given ≥800 mg of ribavirin daily.

In summary, renal function estimates did not correlate with the serum ribavirin concentration in patients with chronic hepatitis C undergoing combination therapy with PEG-IFN and ribavirin. However, serum creatinine-based renal function estimates might be predictive of the serum ribavirin concentration for patients taking 800 mg or more of ribavirin daily. Recent studies have shown that dose adjustment of ribavirin according to renal function improves the antiviral effect and reduces adverse effects [36,37]. Further studies are needed to find better predictors of the ribavirin concentration during combination antiviral therapy for chronic hepatitis C to allow for individualized adjustment of the ribavirin dose aimed at a greater antiviral effect and fewer adverse effects.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1. Correlation between pretreatment serum creatinine and serum cystatin C levels in 148 patients with chronic hepatitis C undergoing combination antiviral therapy with peginterferon and ribavirin ($P < 0.0001$).

Figure S2. Correlation between serum ribavirin concentration 4 weeks after the start of combination therapy and creatinine- and cystatin C-based renal function in patients who were given 600 mg of ribavirin.

Please note: Blackwell Publishing are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

1 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998; 339: 1485–1492.

2 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; 352: 1426–1432.

3 Reichard O, Norkrans G, Fryden A *et al.* Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. *Lancet* 1998; 351: 83–87.

4 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *N Engl J Med* 1998; 339: 1493–1499.

5 Schvarcz R, Ando Y, Sonnerborg A, Weiland O. Combination treatment with interferon alfa-2b and ribavirin for chronic hepatitis C in patients who have failed to achieve sustained response to interferon alone: Swedish experience. *J Hepatol* 1995; 23(Suppl. 2): 17–21.

6 Barbaro G, Di Lorenzo G, Belloni G *et al.* Interferon alfa-2B and ribavirin in combination for patients with chronic hepatitis C who failed to respond to, or relapsed after, interferon alpha therapy: a randomized trial. *Am J Med* 1999; 107: 112–118.

7 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–965.

8 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.

9 Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. *Rev Infect Dis* 1990; 12: 1139–1146.

10 Lindahl K, Schvarcz R, Bruchfeld A, Stahle L. Evidence that plasma concentration rather than dose per kilogram body weight predicts ribavirin-induced anaemia. *J Viral Hepat* 2004; 11: 84–87.

11 Arase Y, Ikeda K, Tsubota A *et al.* Significance of serum ribavirin concentration in combination therapy of interferon and ribavirin for chronic hepatitis C. *Intervirology* 2005; 48: 138–144.

12 Bruchfeld A, Lindahl K, Schvarcz R, Stahle L. Dosage of ribavirin in patients with hepatitis C should be based on renal function: a population pharmacokinetic analysis. *Ther Drug Monit* 2002; 24: 701–708.

13 Kamar N, Chatelut E, Manolis E, Lafont T, Izopet J, Rostaing L. Ribavirin pharmacokinetics in renal and liver transplant patients: evidence that it depends on renal function. *Am J Kidney Dis* 2004; 43: 140–146.

14 The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; 20: 15–20.

15 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41.

16 Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant* 2003; 18: 2024–2031.

17 Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. *Scand J Clin Lab Invest* 2004; 64: 25–30.

18 Tsubota A, Hirose Y, Izumi N, Kumada H. Pharmacokinetics of ribavirin in combined interferon-alpha 2b and ribavirin therapy for chronic hepatitis C virus infection. *Br J Clin Pharmacol* 2003; 55: 360–367.

19 Uchida M, Hamada A, Yamasaki M, Fujiyama S, Sasaki Y, Saito H. Assessment of adverse reactions and pharmacokinetics of ribavirin in combination with interferon alpha-2b in patients with chronic hepatitis C. *Drug Metab Pharmacokinet* 2004; 19: 438–443.

20 Svensson J-O, Bruchfeld A, Schvarcz R, Stahle L. Determination of ribavirin in serum using highly selective

solid-phase extraction and high-performance liquid chromatography. *Ther Drug Monit* 2000; 22: 215–218.
21 Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A. Cystatin C as a marker of GFR -history, indications, and future research. *Clin Biochem* 2005; 38: 1–8.
22 Newman DJ, Cystatin C. *Ann Clin Biochem* 2002; 39: 89–104.
23 Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function – a review. *Clin Chem Lab Med* 1999; 37: 389–395.
24 Coll E, Botey A, Alvarez L *et al.* Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis* 2000; 36: 29–34.
25 Woitas RP, Stoffel-Wagner B, Flommersfeld S *et al.* Correlation of serum concentrations of cystatin C and creatinine to inulin clearance in liver cirrhosis. *Clin Chem* 2000; 46: 712–715.
26 Demirtas S, Bozbas A, Akbay A, Yavuz Y, Karaca L. Diagnostic value of serum cystatin C for evaluation of hepatorenal syndrome. *Clin Chim Acta* 2001; 311: 81–89.
27 Gerbes AL, Gulberg V, Bilzer M, Vogeser M. Evaluation of serum cystatin C concentration as a marker of renal function in patients with cirrhosis of the liver. *Gut* 2002; 50: 106–110.
28 Randers E, Ivarsen P, Erlandsen EJ *et al.* Plasma cystatin C as a marker of renal function in patients with liver cirrhosis. *Scand J Clin Lab Invest* 2002; 62: 129–134.
29 Orlando R, Mussap M, Plebani M *et al.* Diagnostic value of plasma cystatin C as a glomerular filtration marker in decompensated liver cirrhosis. *Clin Chem* 2002; 48: 850–858.
30 MacIsaac RJ, Tsalamandris C, Thomas MC *et al.* Estimating glomerular filtration rate in diabetes: a comparison of cystatin-C- and creatinine-based methods. *Diabetologia* 2006; 49: 1686–1689.
31 Shlipak MG, Katz R, Sarnak MJ *et al.* Cystatin C and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease. *Ann Intern Med* 2006; 145: 237–246.
32 Nakamura T, Ioroi T, Sakaeda T *et al.* Serum cystatin C levels to predict serum concentration of digoxin in Japanese patients. *Int J Med Sci* 2006; 3: 92–96.
33 Hermida J, Tutor JC. Serum cystatin C for the prediction of glomerular filtration rate with regard to the dose adjustment of amikacin, gentamicin, tobramycin, and vancomycin. *Ther Drug Monit* 2006; 28: 326–331.
34 Takeuchi M, Fukuda Y, Nakano I, Katano Y, Hayakawa T. Elevation of serum cystatin C concentrations in patients with chronic liver disease. *Eur J Gastroenterol Hepatol* 2001; 13: 951–955.
35 Chu S-C, Wang C-P, Chang Y-H *et al.* Increased cystatin C serum concentrations in patients with hepatic diseases of various severities. *Clin Chim Acta* 2004; 341: 133–138.
36 Maeda Y, Kiribayashi Y, Moriya T *et al.* Dosage adjustment of ribavirin based on renal function in Japanese patients with chronic hepatitis C. *Ther Drug Monit* 2004; 26: 9–15.
37 Karino Y, Toyota J, Akutsu N *et al.* Total clearance of ribavirin (CL/F) based ribavirin dosage improves the safety and effect of interferon and ribavirin combination therapy for chronic hepatitis C. *Hepatology* 2005; 42 (S1): 650A [Abstract].

肝臓　49巻4号　171–173（2008）

＜速　報＞

Peginterferon alpha-2b＋ribavirin 併用療法の効果判定における COBAS AmpliPre/COBAS TaqMan HCV test の有用性

熊田　卓*　豊田　秀徳　桐山　勢生　曽根　康博　谷川　誠
久永　康宏　金森　明　渥美　裕之　中野　聡　荒川　恭宏

緒言：Peginterferon＋ribavirin 併用療法の出現で難治とされてきた genotype 1 型高 virus 量の症例でも約 50% に SVR（sustained virological response）が得られる．従来の Amplicor HCV v2.0（Amplicor 定性，Roche Diagnostics，東京）の限界は 50 IU/ml で，1 型高 virus 量では治療開始から 12 週までの陰性化が重要である．今回高感度 HCV 検出系である COBAS AmpliPre/COBAS TaqMan HCV test（TaqMan HCV，Roche Diagnostics，東京）と Amplicor 定性の治療効果予測における有用性を比較したので報告する．

対象および方法：対象は HCV RNA 陽性 C 型慢性肝炎に peginterferon alpha-2b＋ribavirin 併用療法を施行し，投与終了 24 週後の HCV RNA が確認された 175 例（1 型 117 例 48 週治療，2 型 58 例 24 週治療）である．男性 94 例，女性 81 例で，年齢中央値は 59 歳（21～74 歳），投与前のウイルス量中央値は 1500 KIU/ml（11～9100 KIU/ml）であった．なお，今回は 12 週以上治療継続例を対象とした．

TaqMan HCV の測定は治療開始後 4 週，8 週，12 週の 3 ポイントで行った．TaqMan HCV の測定方法は既報に従い測定し判定した[1)2)]．

結果：1）1 型と 2 型の SVR 率はそれぞれ 117 例中

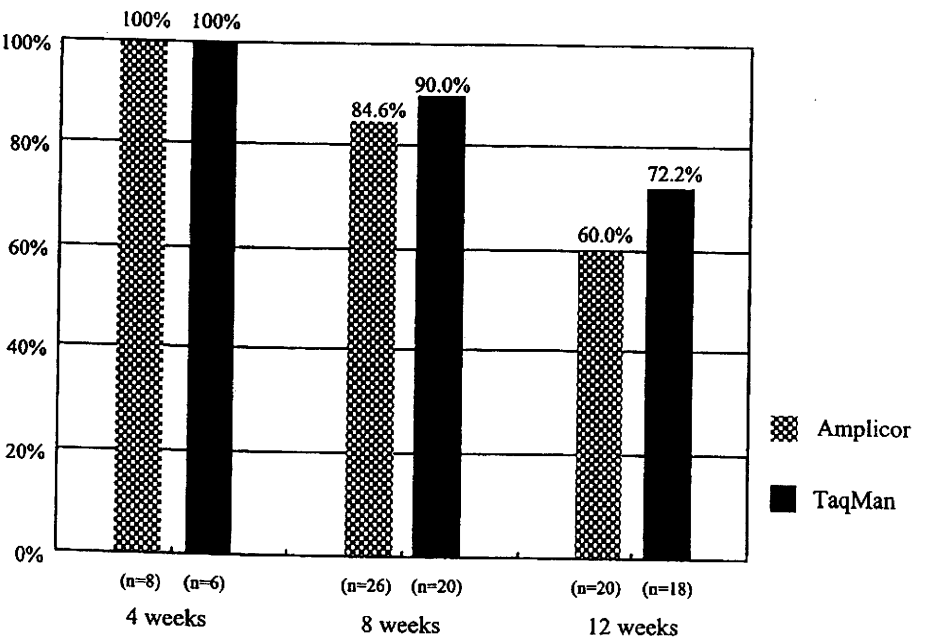

Fig. 1 Sustained virological response (SVR) at 4, 8, and 12 weeks by Amplicor HCV v2.0 or COBAS AmpliPre/COBAS TaqMan HCV test in patients with genotype 1 treated with peginterferon α-2b plus ribavirin.

大垣市民病院消化器科
*Corresponding author: hosp3@omh.ogaki.gifu.jp
＜受付日2008年2月2日＞＜採択日2008年3月14日＞

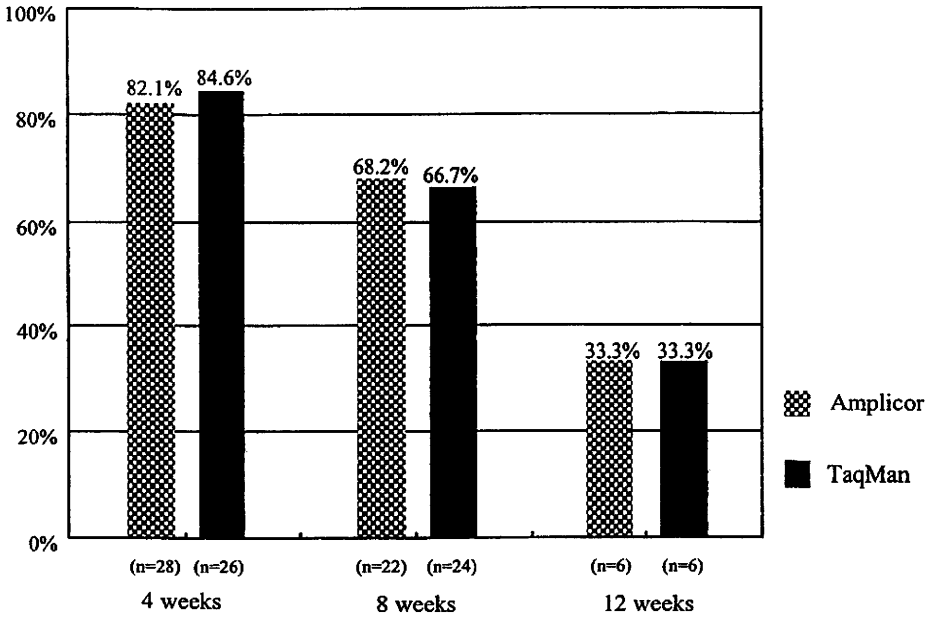

Fig. 2

47例40.2%，58例中40例69.0%であった．2)治療経過と陰性化率は，1型はAmplicor定性とTaqMan HCVの4週，8週，12週の時点の陰性化はそれぞれ5.8%と6.8%，29.1%と22.2%，46.2%と37.6%であった．一方2型はAmplicor定性とTaqMan HCVの4週，8週，12週の時点の陰性化はそれぞれ48.3%と44.8%，86.2%と86.2%，96.6%と96.6%であった．3)陰性化時期とSVR率は，1型はAmplicor定性とTaqMan HCVの4週，8週，12週陰性化例のSVR率はそれぞれ100%と100%，84.6%と90.0%，60.0%と72.2%であった(Fig. 1)．一方2型はAmplicor定性とTaqMan HCVの4週，8週，12週陰性化例のSVR率はそれぞれ82.1%と84.6%，68.2%と66.7%，33.3%と33.3%であった．(Fig. 2)

考案：SVRの予測には血中HCV RNA陰性化時期が重要な因子となる．1型高virus量で高いSVR率を得るには4週までの陰性化が望まれるが約10%しか認められない．次の目標が12週までの陰性化で70%前後のSVR率が得られる．しかし問題なのは8週陰性化例と12週陰性化例のSVR率の違いである[3]．今回の検討でも8週陰性化例は84.6%，12週陰性化例は60.0%のSVR率で後者は決して高いとは言えなかった．これに対してTaqMan HCVによる8週陰性化例と12週陰性化例のSVR率はそれぞれ90.0%と72.2%であった．12週にAmplicor定性で陰性にもかかわらずTaqMan HCVで陽性の症例が10例あり，これらが24週までに陰性化例すれば，ガイドラインからは72週の投与が望ましい症例となる．一方，2型に関してはAmplicor定性とTaqMan HCVと陰性化時期の間の差は認められなかった．以上の結果から，1型においてはTaqMan HCVを測定することにより，今後投与期間の再検討が必要になる可能性がある．高感度HCV検出系であるTaqMan HCVによりHCV RNAの血中動態を見ることで，正確に症例に応じた投与計画の設定が可能となることが期待される．

索引用語：
HCV RNA,
AmpliPrep/COBAS Taq Man HCV Test,
peginterferon + ribavirin

文献：1）熊田博光，豊田成司，泉　並木，他．HCV RNA定量キット，コバスTaqMan HCV「オート」の検討—TaqMan HCV「オート」に関する共同研究：中間報告—．医学と薬学　2007；58：595—605　2）狩野吉康，赤池　潤，山崎　克，他．HCV RNA測定試薬コバスTaqMan HCV「オート」およびHBV DNA測定試薬コバスTaqMan HBV「オート」における既存測定法との比較．医学と薬学　2007；58：137—149　3）Mangia A, Minerva N, Bacca D, et al. In pts who clear HCV RNA at Week

12, SVR is higher after 72 than after 48 weeks tx: results of a randomized controlled trial (RCT). Program and abstracts of the 42nd Annual Meeting of the European Association for the Study of the Liver; April 11—15, 2007; Barcelona, Spain. Abstract 7

英文要旨

Clinical utility of COBAS AmpliPre/COBAS TaqMan HCV test for evaluating the efficacy of peginterferon plus ribavirin therapy

Takashi Kumada*, Hidenori Toyoda, Seiki Kiriyama, Yasuhiro Sone, Makoto Tanigawa, Yasuhiro Hisanaga, Akira Kanamori, Hiroyuki Atsumi, Satosi Nakano, Takahiro Arakawa

We evaluated the performance characteristics of Amplicor HCV v2.0 (Amplicor HCV) and COBAS AmpliPre/COBAS TaqMan HCV test (TaqMan HCV) for predicting sustained virological response (SVR) at 4, 8, and 12 weeks in patients treated with peginterferon plus ribavirin therapy. In genotype 1, the percentage of patients having SVR with undetectable HCV RNA by TaqMan HCV at 8 and 12 weeks is higher than that by Amplicor HCV (90.0% and 72.2% vs 84.6% and 60.0%). However, in genotype 2, there was no difference between Amplicor HCV and TaqMan HCV. We believe TaqMan HCV is useful for determining the duration of treatment with peginterferon plus ribavirin therapy, especially in genotype 1.

Key words:
HCV RNA,
COBAS AmpliPre/COBAS TaqMan HCV test,
peginterferon + ribavirin

Kanzo 2008; 49: 171—173

Department of Gastroenterology, Ogaki Municipal Hospital, Gifu, Japan
*Corresponding author: hosp3@omh.ogaki.gifu.jp

●特集2●

RFA：経皮的ラジオ波焼灼治療

超音波造影剤ソナゾイドにおけるRFAの治療効果判定の有用性

大垣市民病院 診療検査科形態診断室[*1]／同 消化器科[*2]

乙部克彦[*1]／熊田 卓[*2]

はじめに

造影超音波検査（contrast enhancement ultrasound：CEUS）による肝腫瘤性病変の質的診断や肝細胞癌（hepatocellular carcinoma：HCC）に対する治療効果判定の有用性が多く報告されている[1-6]。当院でも、2000～2005年の6年間にレボビストを用いたCEUSを2,945件施行した。肝腫瘤性病変の質的診断が61.6%（1,814件）に対し、HCCの治療効果判定が29%（749件）と造影検査の1/3を占め、治療効果判定に対するCEUSの重要性は高いといえる。

現在、HCCに対する局所療法の主流は経皮的ラジオ波熱凝固療法（radiofrequency ablation: RFA）[7,8]で、当院ではRFAの治療効果判定にCEUSとダイナミックMRI（magnetic resonance image）を用いている。

以前、われわれはレボビストを用いたRFAの治療効果判定に対するCEUSの有用性を報告した[6]。しかし、レボビストは高音圧間歇送信によりマイクロバブルを崩壊させながら血流動態を評価するためリアルタイム性に乏しく、また造影時間が短いという欠点があり、レボビスト造影下でのRFAは困難であった。

一方、2007年1月に発売された第2世代造影剤ソナゾイドは、中音圧にてマイクロバブルを共振させ非線形信号を持続的に得るため、リアルタイムに血流動態を評価することが可能である。特にソナゾイド注入後10分以降の後血管相では、全肝をsweep scanで何度も評価することができ、腫瘍の存在診断の向上が期待されている。

また、工藤らが考案したdefect re-perfusion imaging[9]は、後血管相でクッパー細胞が欠落している領域をdefect像として検出し、そこへ再度ソナゾイドを注入することにより、defect内の血流の有無を評価するものである。これを用いることにより、HCCの診断や治療効果判定、治療支援など幅広く応用することが可能である。

本稿では、RFA治療の現状とソナゾイドを用いたRFA治療後の効果判定の成績を報告し、RFAに対するCEUSの有用性について述べる。

ソナゾイドを用いたRFA後の効果判定の成績

1）CEUSによる治療効果判定の実際

CEUSによる効果判定は、血管相（静注開始より15～60秒）にて腫瘍染影の有無とsafety marginの判定が重要となる。一般的に効果判定は血管相にて腫瘍染影の有無を評価している場合が多く[10,11]、当院では、術前CEUSの腫瘍染影が消失し、腫瘍よりひと回り大きなdefect像が確認されれば治療効果ありとしている。Safety marginの有無については、Bモードでは治療後の変化により腫瘍の境界は不明瞭となり、腫瘍の同定が困難となる場合がほとんどで、safety marginの判定は一般的にCT（computed tomography）で行われているのが現状である[12]。

CEUS上問題となる点は、RFA後の焼灼部と周囲肝実質との間に炎症性の変化（hyperemia）が発生し、血管相では焼灼部辺縁にリング状の染影として確認され（**図1、2**）、辺縁の一部にしかみられない場合もあり、腫瘍の残存部と見間違えることである。後血管相では、hyperemia部や腫瘍の残存部も焼灼部と同様にdefect像を呈する場合がほとんどで、後血管相でも評価することは困難である。Defect re-perfusion imaging[9]も腫瘍残存の有無の評価に有効な手法であるが、hyperemiaと腫瘍残存部を完全に鑑別できない。最終的には、RFA前のCEUSによる腫瘍サイズや存在位置を把握し、RFA後のCEUSも同一な断面画像で評価することが望ましく、効果判定の精度の向上にもつながる。

ソナゾイドの登場により、造影下による肝動脈化学塞栓療法（transcatheter arterial chemoembolization: TACE）の治療不良の腫瘍残存部に対しての追加治療も可能となった（**図3**）。また、治療対象の結節がBモードで描出不明瞭な結節に対しても、造影下で腫瘍染影の範囲を同定してからの治療も可能となっている（**図4**）。

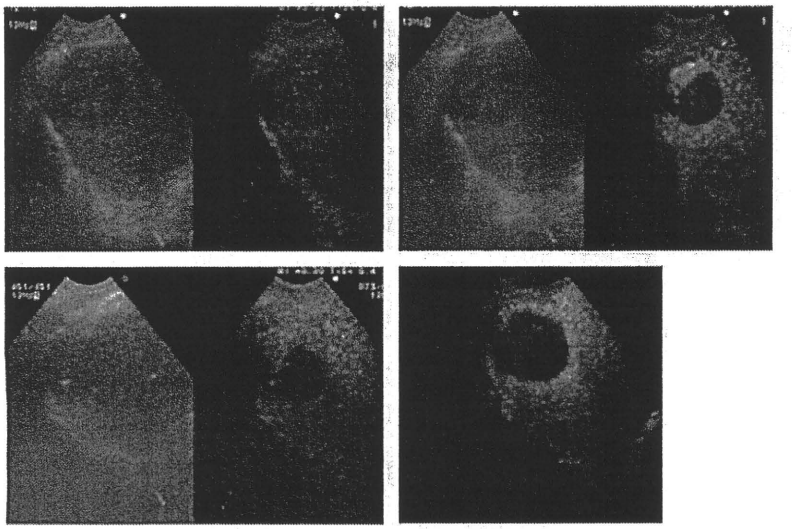

図3a | 図3b
図3c | 図3d

図3　76歳男性、S8/44mm/TAE後
a：RFA直前のBモードでは腫瘍残存の位置は同定できない。
b：RFA直前のCEUS血管相で残存部の腫瘍染影が確認できる。
c：ソナゾイドによる造影下にて腫瘍残存部を見ながら穿刺針を挿入し、治療を施行。
d：RFA後のCEUSでは腫瘍染影が消失。

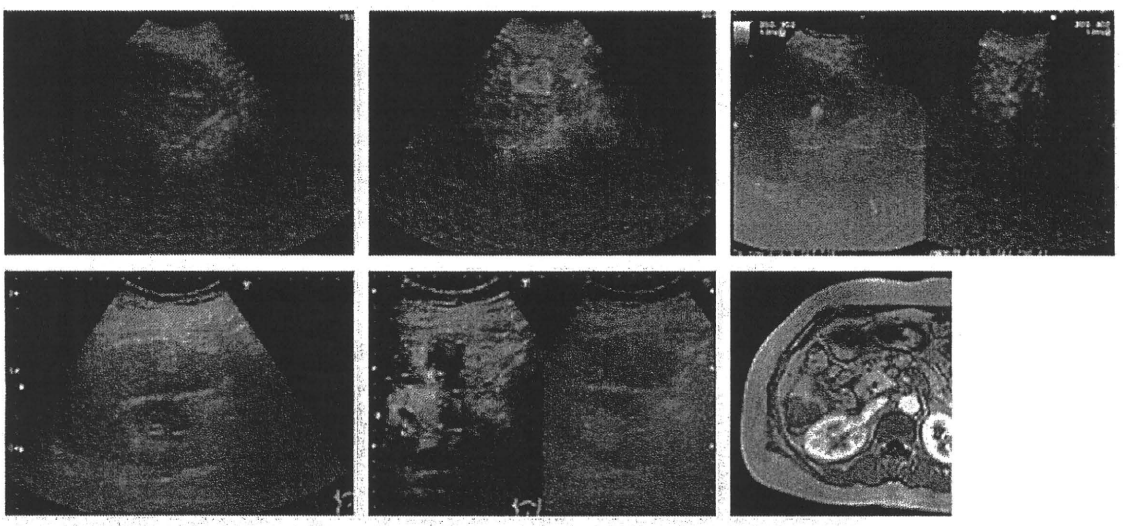

図4a | 図4b | 図4c
図4d | 図4e | 図4f

図4　69歳女性、S6/18mm
a：造影前のBモードでは腫瘍の存在は同定できない。
b：RFA直前のCEUS血管相で腫瘍染影が確認できる。
c：ソナゾイドによる造影下にて穿刺針を挿入しRFAを施行。
d：RFA後3日後の結節は高エコー結節として描出される。
e：CEUS（defect re-perfusion）の血管相で腫瘍染影が消失している。
f：MRI動脈相、RFA後2週間後の効果判定にて腫瘍濃染が消失している。

2）RFA効果判定におけるCEUSとダイナミックMRIとの比較

対象は2007年1月～2008年3月までRFAが施行されたHCC121症例、126結節中ソナゾイドによるCEUSとダイナミックMRIの両者にて治療効果判定が行われた34結節である。男女比28：6、平均年齢69.4歳（53～84歳）、平均腫瘍径15.4mm（7～44mm）、平均腫瘍存在深部62.9mm（30～100mm）であった。CEUSはRFA後2日～8日に、ダイナミックMRIは1ヵ月以内に行った。

CEUSおよびダイナミックMRIにより治療効果判定がなされた34結節は、全例腫瘍染影が消失し、効