Table 1. Hepatocellular Distribution of ^{111}In–AGP and ^{111}In–rAGP

	Uptake Per Cell Population[a] (% of Total Recovered)	
	AGP	rAGP
Parenchymal cell	99.4 ± 4.31	95.0 ± 2.12
Non-parenchymal cell		
Kupffer cell	0.62 ± 0.11	2.79 ± 0.21
Liver endothelial cell	ND	2.18 ± 0.11

[a]Each value represents the means ± SD of three independent experiments.
ND, not detected.

DISCUSSION

We recently found that the native AGP purified from human blood was mainly incorporated into the parenchymal cells of mice livers via a receptor-mediated pathway.[14] As mentioned in the introduction section, it has been proposed that a cell surface protein interacts with AGP in several cells, including hepatocytes, but, so far, all recognize the glycan chains of AGP such as ASGPR,[18–21] and there is no experimental evidence in support of the existence of proteins that recognize the peptide moiety of AGP. However, the latter type of proteins in liver have been found in other members of the lipocalin family, such as

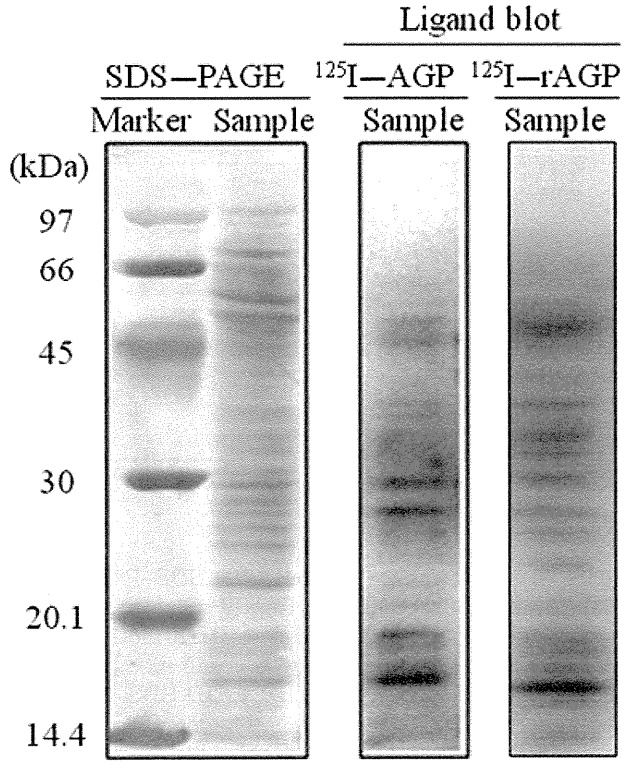

Figure 2. Ligand blotting of ^{111}In–AGP and ^{111}In–rAGP using a crude membrane fraction of mice primary hepatocytes. Each lane represents a molecular weight marker (lane 1), CBB-stained crude membrane fraction of mice primary hepatocytes (lane 2), and radioactivities of the crude membrane fraction of mice hepatocytes that had interacted with ^{111}In–AGP (lane 3) or ^{111}In–rAGP (lane 4).

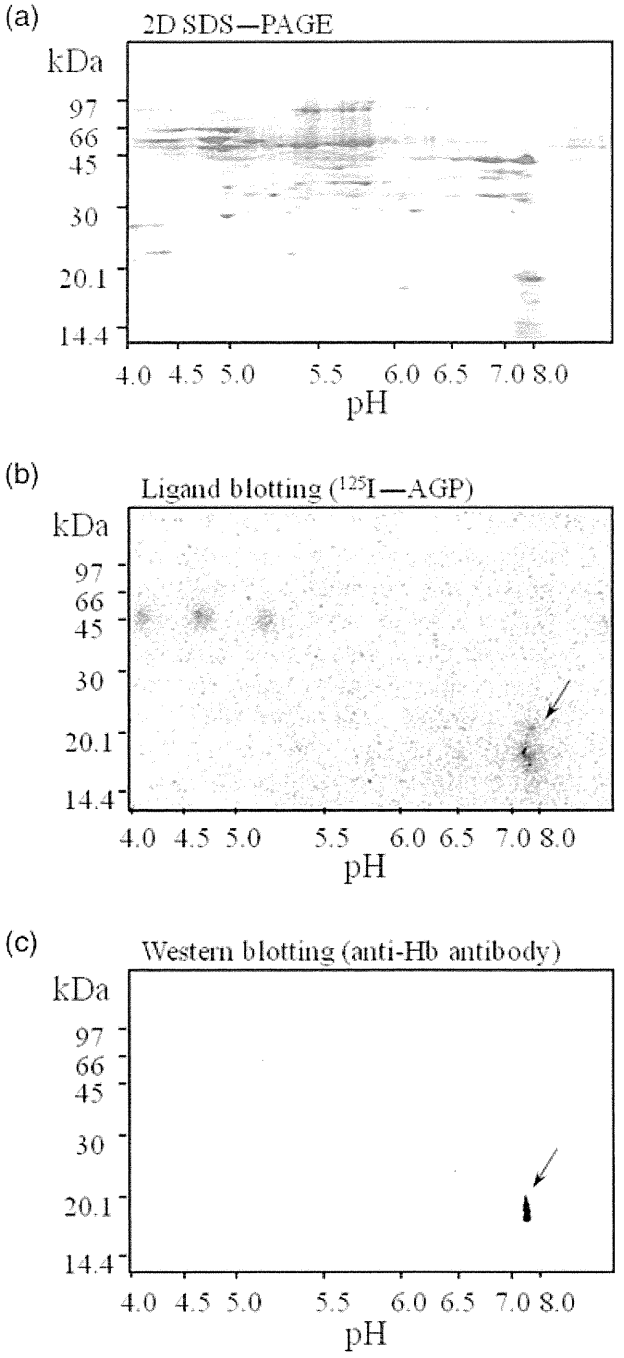

Figure 3. (a) Two-dimensional (2D) SDS–PAGE of crude membrane fraction of mice hepatocyte, (b) ligand blot analysis with ^{111}In–AGP, and (c) western blotting analysis using anti-AGP antibody. A crude membrane fraction of mice hepatocytes was subjected to 2D SDS–PAGE and then transferred to a PVDF membrane. The membrane fraction was stained with coomassie brilliant blue (a) and the radioactivity that interacted with ^{111}In–AGP was detected (b). A western blotting analysis was performed using an anti-hemoglobin antibody (c). An arrow indicates the isolated and identified protein bands as described in Table 2.

Table 2. MALDI–TOF-MS Analysis of Tryptic Peptide Digest of the 16 kDa Protein

Observed Mass (*m/z*)	Theoretical Mass (*m/z*)	Sequence Position	Matched Sequence
1126.63	1126.5640	96–104	LHVDPENFR
1274.81	1274.7256	31–40	LLVVYPWTQR
1294.71	1294.6424	121–132	DFTPAAQAAFQK
1757.02	1756.9228	67–132	VITAFNDGLNHLDSLK
1981.08	1980.9008	41–59	YFDSFGDLSSASAIMGNAK
2212.34	2208.0641	41–61	YFDSFGDLSSASAIMGNAKVK

retinol-binding protein and α_1-microglobulin.[27] These findings lead to the possibility that AGP also interacts with a cell surface protein that recognizes the peptide portion of AGP.

To demonstrate this, we initially compared the pharmacokinetics between AGP and rAGP and the effect of glycan chains on the uptake of AGP by mice livers. Our results clearly showed that both AGPs are mainly incorporated into the mouse liver and that the uptake of rAGP was suppressed in the presence of excess of AGP (Fig. 1). In addition, the hepatic distribution and uptake clearance of rAGP was higher than the corresponding values for AGP. These findings strongly indicate that AGP is incorporated into the liver via a specific route that recognizes a peptide moiety of AGP, and the highly sialylated glycan chains of AGP, which occupied approximately 40% of the total molecular weight, possibly interferes with the interaction with HBB due to steric hindrance or charge repulsion. In fact, it was shown that the affinity of erythropoietin for its receptor is inversely related to the sialylation of erythropoietin carbohydrate.[28] In addition, increasing the sialic acid-containing carbohydrate content in erythropoietin leads to a molecule with long circulating half-life.[29]

The results from a ligand blotting analysis with a crude membrane fraction from mice hepatocytes indicate that a 16 kDa protein interacts with AGP and rAGP, and the protein was identified as HBB by MALDI–TOF-MS and western blotting analysis. This is very interesting because, as of this writing, only haptoglobin has been shown to be a protein that interacts with hemoglobin. In addition, due to the identical results between AGP and rAGP, a specific sequence in the peptide moiety of AGP could interact with HBB on the plasma membrane of mice hepatocytes. However, based on the present limited data, it is difficult to specify this specific sequence of the AGP molecule. Site-directed mutagenesis studies of AGP are currently ongoing with the goal of identifying the sequence that interacts with HBB. Moreover, the fact that the interaction between HBB and the peptide portion of AGP was also observed in a crude membrane fraction of HepG2 cells excludes the possibility

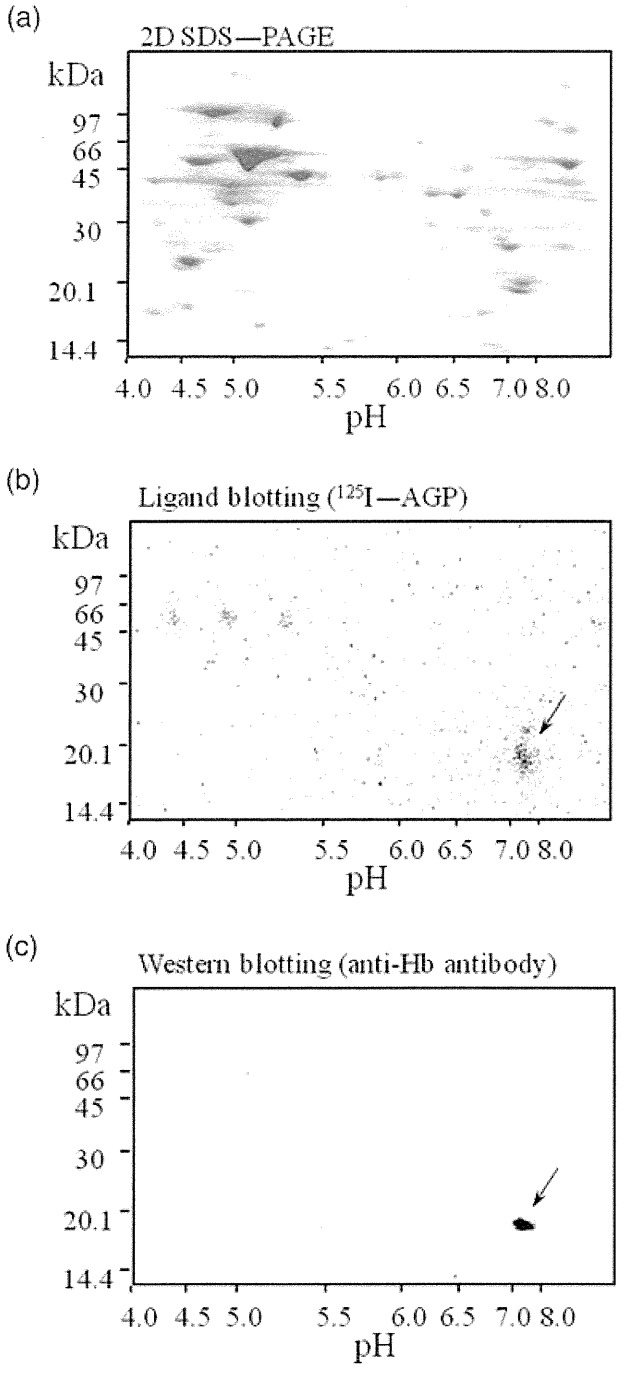

Figure 4. (a) Two-dimensional (2D) SDS–PAGE of the crude membrane fraction from HepG2 cells, (b) ligand blot analysis with ^{111}In–AGP, and (c) western blotting analysis using an anti-AGP antibody. A crude membrane fraction of HepG2 cells was subjected to 2D SDS–PAGE and transferred to a PVDF membrane. The membrane fraction was stained with coomassie brilliant blue (a) and the radioactivity that interacted with ^{111}In–AGP was detected (b). A western blotting analysis was performed using an anti-hemoglobin antibody (c).

that the HBB detected in hepatocytes is derived from a blood-derived contaminant and suggests that AGP is also incorporated into human hepatocytes.

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The present pharmacokinetic analysis shows that rAGP is distributed to the kidney, in addition to the liver, and is also excreted in urine. The molecular weight of rAGP is about 20 kDa, and rAGP lacks the number of negative charges derived from the glycan chains of AGP. Therefore, rAGP may be filtered through the glomerulus and a part of the filtered rAGP may be reabsorbed by the renal cells. However, the presence of an excess of AGP suppressed the uptake of rAGP to kidney and the excretion of rAGP in urine, although the uptake of AGP by the kidney and its excretion in urine are minimal. Therefore, AGP might inhibit the filtration of rAGP by masking the glomerulus. Indeed, Haraldsson and Jeansson reported that AGP suppressed the filtration of albumin via interaction with the glomerulus.[30] In addition, the same research group reported that AGP is one of the major components of the glomerular endothelial cell coat, which is essential for glomerular filtration.[31] To clarify this issue, further investigation will be necessary because the recovered radioactivity in urine after the administration of ^{111}In–rAGP might contain both the intact protein and its degradation products.

On the basis of the results obtained in this study, we conclude that HBB can be the binding protein for AGP in liver parenchymal cells and that it recognizes a peptide moiety of AGP. In addition, the glycan chains of AGP possibly hinder its interaction with HBB. To our knowledge, this report is the first finding regarding a peptide that recognizes AGP that is located on the cell surface. The binding to HBB may be involved in the hepatocellular uptake of AGP and subsequently may contribute to the clarification of the biological functions of AGP in the liver.

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shortcut though impairs the analysis in many different ways, limiting our full understanding of the phenomenon being modelled and ultimately our ability to accurately assess 'value for money' beyond the simple 'average'. This paper explores the value of access to individual patient data for cost-effectiveness modelling, structuring the discussion of the topic around three interrelated questions. First, what benefits can access to IPD bring to cost-effectiveness modelling? Second, what are the challenges for the simultaneous statistically synthesis of AD plus IPD to derive input parameters for a cost-effectiveness model? Third, what is the value of access to IPD compared to AD for cost-effectiveness modelling? Using two different case studies, the above questions will be addressed and discussed in the context of the debate around CEA of individualised treatment decisions.

DISEASE-SPECIFIC STUDIES

GASTROINTESTINAL DISORDERS - Clinical Outcomes Studies

PGI1

THE EFFECTIVENESS AND TOLERABILITY OF COMBINED TREATMENT WITH PEGINTERFERON ALPHA-2A OR ALPHA-2B AND RIBAVIRIN IN THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C: RESULTS BASED ON THE NATIONWIDE HEPATITIS REGISTRY IN JAPAN

Shimbo T, Miyaki K, Song Y, Masaki N, Study Group Developing Nationwide Database of Hepatitis Japan
National Center for Global Health and Medicine, Shinjuku-ku, Tokyo, Japan

OBJECTIVES: When comparing combined therapy with peginterferon alpha-2a or alpha-2b and ribavirin to treat chronic hepatitis C (CHC), the results of clinical trials, observational studies, and meta-analyses have been inconsistent. Their effectiveness and tolerability were investigated using the nationwide database of chronic hepatitis patients who received interferon therapy in Japan. **METHODS:** The proportion with a sustained virologic response (SVR) and the dropout rate due to adverse events (AEs) were compared between alpha-2a and alpha-2b. All patients also received ribavirin. Multivariate logistic regression was conducted with adjustment for age, sex, platelet counts, ALT, viral load, genotype, and whether the patient was treatment-naïve, which are associated with effectiveness and tolerability. **RESULTS:** By December 2011, the database included 7820 patients. CHC patients treated with either alpha-2a (n=1737) or alpha-2b (n=4495) were analyzed. The mean (SD) age was 58.1 (10.4) years, and 3131 (50.2%) were female. In total, 2503 (41.0%) patients had a platelet count <150x103, 2503 (40.5%) had ALT > 60 IU/L, and 5765 (93.2%) had a high viral load. The numbers with genotype 1, 2, and 3 were 4291 (69.2%), 1838 (29.6%), and 76 (1.2%), respectively. Overall, 4434 (71.2%) patients were treatment-naïve. SVR was achieved in 53.5% (95% CI: 51.1-55.9%) with alpha-2a and 61.6% (95% CI: 60.2-63.1%) with alpha-2b (p<0.001). The dropout rate due to any AEs was 10.3% (95%CI: 8.9-11.8%) and 9.3% (95%CI: 8.5-10.2%) for alpha-2a and alpha-2b, respectively (p=0.226). After adjustment for possible confounders, no differences in effectiveness or tolerability were observed between the therapies, and the odds ratio of alpha-2a for SVR was 0.97 (95% CI: 0.86-1.10), and its odds ratio for dropout due to any AEs was 0.96 (95% CI: 0.79-1.17). There was no significant interaction of genotype and therapy. **CONCLUSIONS:** Alpha-2a and alpha-2b in combination with ribavirin showed comparable effectiveness and tolerability in clinical settings.

PGI2

INFLIXIMAB REDUCES THE RISK OF SURGICAL INTERVENTIONS AND HOSPITALIZATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Costa J[1], Alarcão J[1], Caldeira D[2], Borges M[1], Vaz Carneiro A[1]
[1]*Center for Evidence Based Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal,* [2]*Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal*

OBJECTIVES: In addition to the pharmacological efficacy of infliximab therapy in inflammatory bowel disease (IBD), it is also important to evaluate its impact on other health outcomes, particularly in the rate of surgical interventions and hospitalizations, which have high economic burden and are believed to represent a marker of IBD severity. We aimed to estimate the impact of infliximab in these outcomes in patients with IBD. **METHODS:** Systematic review and meta-analysis of experimental (clinical trials) and observational studies comparing infliximab with any other control group in IBD. Studies were identified by searching Medline and Cochrane from inception to April 2012. Search results and studies characteristics were assessed independently. Subgroup analyses were done according to IBD type: Crohn disease (CD) and ulcerative colitis (UC). Pooled estimates were performed separately for clinical trials and observational studies. Odds ratios (OR) and 95% confidence intervals (CI) were derived by random-effects meta-analysis. Heterogeneity was assessed with I^2 test. **RESULTS:** Nine trials and 9 observational studies were included. Infliximab significantly decreased risk of gastrointestinal surgery in experimental studies (OR 0.36; 95%CI: 0.18-0.71), both in DC (OR 0.25; 95%CI: 0.10-0.63) and UC (OR 0:55; 95%CI: 0.40-0.76). In absolute terms, there was a 9% reduction in the rate of surgery (95%CI: 1-19%). Observational studies also showed a reduced risk of surgery, which was significant in the case of CD (OR 0.42; 95% CI: 0.22 to 0.78). Infliximab significantly reduced the risk of hospitalization, both in experimental (OR 0.48; 95%CI: 0.34-0.66) and observational (OR 0:38; 95%CI: 0.24-0.58) studies, with a decrease of 9% in hospitalization rate (95%CI: 5-14%). Mean duration of hospitalization was shorten by 4.2 days (95%CI: 1.9-6.5) in infliximab treated patients. **CONCLUSIONS:** Based on the best available evidence, infliximab therapy is associated with a reduced risk of gastrointestinal surgery and hospitalization rates in patients with IBD.

PGI3

TREATMENT OF CHRONIC HEPATITIS C GENOTYPE 1 IN POLAND – REAL-LIFE DATA

Kaczor MP[1], Pawlik D[2], Wójcik R[2], Rolka M[2], Maniszewska-Weyher I[3], Tronczynski K[3]
[1]*Jagiellonian University Medical College, Kraków, Poland,* [2]*Aestimo s.c., Kraków, Poland,* [3]*Janssen-Cilag Polska, Warsaw, Poland*

OBJECTIVES: To describe health outcomes, the course of treatment and the demographic and clinical characteristics of HCV adult patients infected with genotype 1 receiving interferon-alfa+ribavirin therapy in Poland. **METHODS:** A retrospective analysis of anonymous data of patients treated in the HCV therapeutic programme of the National Health Fund was performed. Data was gathered from three medical centres and included demographic and clinical characteristics (sex, age, body weight, initial HCV RNA level, disease staging and grading) as well as treatment course (first line/retreatment, posology, treatment duration, outcomes and discontinuations). **RESULTS:** A total of 813 HCV genotype 1 adult patients' records [586 treatment-naïve (N) and 227 treatment-experienced (E)] were included in the analysis. 55% were male (N: 53%, E: 60%), mean age at the beginning of therapy was 48 (SD:13) years. Mean body weight was 68,0 (SD:11,8) kg in females and 82,4 (SD:12,3) kg in males. Mean initial HCV RNA was 5,9 (SD:0,8) $\log_{10}$IU/mL and 46% of patients had HCV RNA<800 000 IU/mL. A total of 85% records included data on disease staging (Sheuer 0-2: 67%; stage 3: 19%; stage 4: 14%). 96% of patients received pegylated interferons (pegylated interferon-alfa2a: 54%), 97% with ribavirin. A total of 15% of patients discontinued therapy prematurely (N: 14%, E: 18%) after a mean of 6 months, and mean treatment duration was 44 weeks for all patients. Overall SVR (sustained viral response) was achieved in 42% of patients (N: 45%, E: 33%). Among treatment-naïve patients not fully responding to therapy, 41% had relapse, 21% were partial responders and 38% were null-responders. **CONCLUSIONS:** The real-life results of HCV genotype 1 treatment, with SVR rates below 50% in treatment naïve patients, are unsatisfactory, especially when in Poland the prevalence of this difficult-to-treat genotype is one of the most highest in Europe. Forthcoming triple therapy with HCV protease inhibitors are promising and anticipated options for these patients.

PGI4

EVALUATION OF THE EFFICACY AND INCONTINENCE RATE OF BIOMATERIALS IN COMPARISON TO CONSERVATIVE AND OTHER INTERVENTIONAL THERAPIES IN TREATMENT OF PERIANAL FISTULA. A META-ANALYSIS

Mirfazaelian H[1], Nikfar S[2], Derakhshani S[3], Abdollahi M[1]
[1]*Tehran University of Medical Sciences, Tehran, Iran,* [2]*Tehran University of Medical Sciences, Tehran, Tehran, Iran,* [3]*Parsian Hospital, Tehran, Iran*

OBJECTIVES: This meta-analysis of randomized controlled trials was conducted to evaluate the efficacy and incontinence rate of biomaterials (fibrin glue and fibrin plug) in comparison to conservative and other interventional therapy in the treatment of perianal fistula. **METHODS:** PubMed, Embase, Scopus, Google Scholar, and Web of Science were searched for clinical trial studies investigated the effects of biomaterials in the treatment of fistul in-ano. Clinical response and incontinence were the key outcomes of interest. Data were searched from the time period of 1966 through June 2012. **RESULTS:** Eight randomized placebo-controlled clinical trials that met our criteria (six comparing biomaterial with conservative treatment and two with other interventions) were included in the analysis. Pooling of data showed biomaterials effectiveness in comparison to other interventions was non significant with relative risk (RR) of 1.23 (95% CI of 0.31-4.84, P= 0.77). The RR for biomaterials comparing with conservative was non significant (RR= 0.73 with 95% CI = 0.31-0.89, P= 0.096). The incontinence rate RR in biomaterials and intervention was also non significant with RR of 0.35 (95% CI = 0.05-2.28, P = 0.27). **CONCLUSIONS:** This meta-analysis demonstrates that the effectiveness of biomaterials and conservative treatment was not different. The biomaterials in comparison to other interventional therapies did not show any difference in regard to effectiveness and also incontinence rate.

PGI5

EFFECTIVENESS AND SAFETY OF ANTACIDS IN PREGNANT WOMEN SUFFERING FROM GERD/HYPERACIDITY SYMPTOMS

Donde S
Pfizer India, Mumbai, India

OBJECTIVES: Symptoms of gastro-oesophageal reflux disease are estimated to occur in 30-50% of pregnancies, with the incidence approaching 80% in some populations. Indian studies have shown prevalence ranging upto 50 % . As with many other conditions in pregnancy, medical therapy with pharmaceutical agents is a concern, as the potential teratogenicity of medications is not well known. Though prevalence numbers are high, many patients have mild and infrequent symptoms, which often respond to lifestyle and dietary modifications. However, some patients report very severe symptoms of hyperacidity affecting their Quality of Life which need treatment. The safety of H2 Receptor antagonists and PPIs in pregnancy is not well established. Antacids could be a good option as their systemic toxicity is low and safety profile is enhanced. **METHODS:** Data has been collected from practicing gynaecologists in a hospital located in Southern India . 50 female patients suffering from heartburn and/or dyspepsia as symptoms of hyperacidity and GERD were treated with antacids containing aluminium hydroxide, magnesium hydroxide and dimethicone. The patients were prescribed antacids for atleast 7 days and the effectiveness and safety profile of antacids was studied. The patients were followed up after one week and the response along with adverse effects were documented. **RESULTS:** The effectiveness was achieved in 85 % of women who took only antacids atleast for 1 week. In 10 % of patients, Proton Pump Inhibitor was also added to achieve the desired response. Apart from 2 cases of mild diarrhea, no other significant side effects were noted. **CONCLUSIONS:** Antacids containing aluminium hydroxide , magnesium hydroxide and dimethicone can be a good therapy option

Hepatology Research

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Original Article

Serum RANTES level influences the response to pegylated interferon and ribavirin therapy in chronic hepatitis C

Kazuki Komase,[1] Shinya Maekawa,[1,2] Mika Miura,[1] Ryota Sueki,[1] Makoto Kadokura,[1] Hiroko Shindo,[1] Kuniaki Shindo,[1] Fumitake Amemiya,[1] Yasuhiro Nakayama,[1] Taisuke Inoue,[1,2] Minoru Sakamoto,[1] Atsuya Yamashita,[3] Kohji Moriishi[3] and Nobuyuki Enomoto[1]

[1]*First Department of Medicine, Faculty of Medicine,* [2]*Department of Advanced Medicine for Liver Diseases, Faculty of Medicine, and* [3]*Department of Microbiology, University of Yamanashi, Yamanashi, Japan*

Aim: Prediction of treatment responses to pegylated interferon (PEG IFN) plus ribavirin (RBV) therapy is uncertain for genotype 1b chronic hepatitis C.

Methods: In this study, 96 patients were investigated for the correlation between 36 pretreatment serum chemokine/cytokine levels and PEG IFN/RBV treatment efficacy by a sandwich enzyme-linked immunoassay (ELISA) and a bead array.

Results: First, chemokines/cytokines were measured semi-quantitatively by sandwich ELISA in 31 randomly-selected patients and the serum regulated on activation normal T-cell expressed and secreted (RANTES) level was found to be significantly higher in the sustained virological response (SVR) group than the non-SVR group ($P = 0.048$). Precise RANTES measurement in all 96 patients using a bead array confirmed this correlation ($P = 0.002$). However, the genetic RANTES haplotype was not significantly related to the serum level. The serum RANTES level was extracted by multivariate analysis (odds ratio = 4.09, 95% confidence interval = 1.02–16.5, $P = 0.048$) as an independent variable contributing to SVR.

Conclusion: The serum RANTES level is an important determinant influencing the virological response to PEG IFN/RBV therapy in chronic hepatitis C.

Key words: hepatitis C virus, pegylated interferon plus ribavirin therapy, RANTES

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a major cause of chronic liver disease worldwide and persistent infection may lead to liver cirrhosis and hepatocellular carcinoma.[1] Therapy leading to HCV eradication is the only treatment with proven efficacy in decreasing the occurrence of hepatocellular carcinoma.[2] Recently, treatment with telaprevir, a non-structural (NS)3/4A protease inhibitor, combined with pegylated interferon (PEG IFN) and ribavirin (RBV), increased the rates of sustained viral response (SVR) up to 64–75%[3,4] compared to the SVR rate of approximately 50% for the previous PEG IFN/RBV therapy. However, it has become evident that genotype 1-infected patients with a null response to previous PEG IFN/RBV therapy have poor responses to PEG IFN/RBV/telaprevir,[5] with an SVR rate as low as approximately 30%, illustrating the difficulty in treating patients infected with genotype 1 HCV. Therefore, precise and accurate prediction of the viral response to PEG IFN/RBV therapy remains an important issue.

Treatment resistance is attributed to various factors associated with the virus and host. Viral factors, such as amino acid (a.a.) sequence variation in the core and NS5A regions, have been investigated extensively for their contribution to the outcome of IFN-based therapy,[6,7] including PEG IFN/RBV therapy. On the other hand, host factors such as African-American race, older age, being obese, the presence of cirrhosis and

Correspondence: Dr Shinya Maekawa, First Department of Medicine, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan. Email: maekawa@yamanashi.ac.jp

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steatosis, and insulin resistance have been reported to be associated with treatment resistance.[8–11] Especially, single nucleotide polymorphisms (SNP) near the interleukin (*IL*)-28B gene, including rs12979860 and rs8099917, have been reported to have a significant correlation with the response to IFN-based therapy.[12,13] However, even with inclusion of these factors, prediction of the treatment response in chronic HCV infection remains uncertain at present.

Chemokines are a group of small, exogenously secreted cytokines that modulate the migration of leukocytes to sites of tissue damage and inflammation in a variety of infectious and autoimmune diseases.[14] In chronic HCV infection, chemokines such as *RANTES* (regulated on activation normal T-cell expressed and secreted), macrophage inflammatory protein (*MIP*)-1α, *MIP-1β* and interferon-γ inducible protein 10 kDa (*IP-10*) are elevated and considered to play crucial roles in inflammatory processes and viral elimination, as well as the transition from innate to adaptive immunity.[14,15] Upregulation of several serum chemokines, such as eotaxin, *IP-10* and *RANTES* also has been reported in HCV infection, possibly reflecting hepatic inflammation.[16] Considering the roles of chemokines/cytokines in establishing chronic hepatitis, it is possible that these chemokines also affect the response to antiviral therapy, and actually several chemokines as interleukin (*IL*)-8, *IL-10*, *MIP-1β*, *RANTES* or *IP-10* have been investigated previously for their association with the treatment response.[16–20] However, the importance of those chemokines has not been established yet and, moreover, these studies did not characterize in detail these chemokines in association with other factors, including *IL-28B* influencing the response to therapy.

In this study, we explored extensively the association of 36 serum cytokines/chemokines and the treatment response, with detailed information of host and virus, to predict better the treatment response to PEG IFN and RBV therapy in genotype 1b HCV infection. Because the pretreatment serum *RANTES* level was found to be correlated significantly with the response, we analyzed further the association between the serum level of *RANTES* and the genomic SNP.

METHODS

Patients

NINETY-SIX CONSECUTIVE PATIENTS with genotype 1b HCV and receiving PEG IFN/RBV therapy between 2004 and 2010 at Yamanashi University Hospital were recruited retrospectively into the study. All patients received the standard therapy according to the treatment protocol of PEG IFN/RBV therapy for Japanese patients, established by a hepatitis study group of the Ministry of Health, Labor and Welfare, Japan (PEG IFN-α-2b 1.5 μg/kg bodyweight, once weekly s.c., and RBV 600–800 mg daily p.o. for 48 weeks).[21] All patients enrolled fulfilled the following criteria: (i) negative for hepatitis B surface antigen; (ii) no other forms of hepatitis, such as primary biliary cirrhosis, autoimmune liver disease or alcoholic liver disease; (iii) not co-infected with HIV; and (iv) a signed consent was obtained for the study protocol that had been approved by the Human Ethics Review Committee of Yamanashi University Hospital. The study was approved by the ethics committees of University of Yamanashi, and the study protocol conformed to the ethical guidelines of the 2008 Declaration of Helsinki.

Definition of treatment outcome

An SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Relapse was defined as reappearance of detectable HCV RNA levels following discontinuation of treatment. Null response was defined as less than 2 log decrease of the baseline HCV RNA levels after 12 weeks of treatment. Based on this definition, when patients were classified according to the achievement of SVR, patients with relapse or null response were classified as non-SVR.

Serum cytokine measurement

Sandwich enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected before initiation of treatment and were stored at −80°C until use. Semiquantitation of serum cytokines was performed using the Proteome Profiler Human Cytokine Array Kit Panel A (R&D Systems, Minneapolis, CA, USA) according to the manufacturer's instructions. The kit consists of a nitrocellulose membrane containing 36 different anti-cytokine antibodies (anti-C5a, anti-CD154, anti-G-CSF, anti-GM-CSF, anti-CXCL1, anti-CCL1, anti-sICAM-1, anti-IFN-γ, anti-IL-1α, anti-IL-1β, anti-IL-1ra, anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-8, anti-IL-10, anti-IL-12p70, anti-IL-13, anti-IL-16, anti-IL-17, anti-IL-17E, anti-IL-23, anti-IL-27, anti-IL-32α, anti-IP-10, anti-CXCL11, anti-CCL2, anti-MIF, anti-CCL3, anti-CCL4, anti-PAI-1, anti-RANTES, anti-CXCL12, anti-TNF-α, anti-sTREM-1), spotted in duplicate. Serum samples were diluted and mixed with a cocktail of biotinylated detection antibodies. The sample/antibody mixture

was then incubated with the membrane. Any cytokine/detection antibody complex present was bound to the membrane by its cognate immobilized capture antibody. Following washing to remove unbound material, streptavidin-horseradish peroxidase and chemiluminescent detection reagents (ECL Western Blotting Analysis System; GE Healthcare, Buckinghamshire, UK) were added sequentially. Arrays were scanned using a LAS-3000 mini-luminescent image analyzer (Fujifilm, Tokyo, Japan) and were quantified for the densities using Multi Gauge ver. 3.0 software (Fujifilm). Concentrations of cytokines and chemokines were expressed as their signal intensity ratios relative to that of the positive control spotted on the same membrane.

Bead array

Precise serum concentrations of regulated on *RANTES* were measured using the Luminex Bio-Plex system (Bio-Rad, Hercules, CA, USA) and the Procarta Cytokine Assay Kit (Panomics, Fremont, CA, USA) in a 96-well plate ELISA-based format according to the manufacturers' recommendations. The sensitivity of the assays is greater than 10 pg/mL cytokine. Serum and standards were incubated with a mixture of the Luminex antibody-conjugated beads for 30 min with constant shaking. After washing, the detection antibodies and substrates were added and incubated for another 30 min. Fluorescent signals were collected and data expressed, using internal standards, in pg/mL as the mean of two individual experiments carried out in duplicate.

Viral core and interferon sensitivity-determining region (ISDR) sequence determination by direct sequencing

Hepatitis C virus RNA extraction from serum samples, complementary DNA synthesis and amplification by two-step nested polymerase chain reaction (PCR) were carried out using specific primers for the HCV core and ISDR. PCR amplicons were sequenced directly by Big Dye Terminator ver. 3.1 (ABI, Tokyo, Japan) with universal M13 forward and reverse primers using an ABI prism 3130 sequencer (ABI). The sequence files generated were assembled using Vector NTI software (Invitrogen, Tokyo, Japan) and base-calling errors were corrected following inspection of the chromatogram.

SNP typing of the *RANTES* and *IL-28B* genes

Genomic DNA of the patients was extracted from peripheral blood using a blood DNA extraction kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol. The allele typing of each DNA sample was performed by real-time PCR with a model 7500 (ABI) using FAM-labeled SNP primers for the loci rs2107538, rs2280788, rs2280789, rs4796120 and rs3817655 (ABI) for *RANTES* and the locus rs8099917 (ABI) for IL-28B.

Statistical analysis

Student's *t*-test and Mann–Whitney *U*-test were used to analyze continuous variables, as appropriate. Fisher's exact test was used for the analysis of categorical variables. Receiver–operator curve (ROC) analyses were performed to establish cut-off values for serum cytokine concentration. The optimum cut-off was defined as the value that maximized the area under the ROC. Spearman's correlation coefficient (R) was calculated to clarify the strength of relationship between the pretreatment serum cytokine concentrations and clinical parameters. Variables that achieved statistical significance ($P < 0.05$) in univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. The odds ratios and 95% confidence intervals also were calculated. Data were analyzed using Ekuseru-Toukei 2008 (SSRI, Tokyo, Japan). The haplotype block among rs2107538, rs2280788, rs2280789, rs4796120 and rs3817655 variants was analyzed using SNPAlyze software ver. 8.0 (Dynacom, Chiba, Japan). $P < 0.05$ was considered significant.

RESULTS

Semiquantitative measurement of pretreatment serum cytokines in 31 randomly-selected patients

AT FIRST, TO identify cytokines/chemokines related to the treatment responses to PEG IFN/RBV therapy, semiquantitative measurement of the serum concentrations of 36 comprehensive cytokines/chemokines was performed by sandwich ELISA method by randomly selected patients. Next, to further confirm the result, cytokines showing the associations with the response were measured more precisely by bead array method in all patients.

In the first analysis, 31 patients were randomly selected from the 96 patients. The clinical characteristics of these 31 patients at the start of the therapy are shown in Table 1. Significant differences in the clinical backgrounds between those who did and those who did not

Table 1 Baseline characteristics of the 31 patients analyzed using the sandwich ELISA method

Factor	SVR ($n = 20$)	Non-SVR ($n = 11$)	*P*-value
Age (years)	52 ± 11†	57 ± 10	0.25‡
Sex (male : female)	11:9	6:5	0.64§
Bodyweight (kg)	60.9 ± 9.6†	61.9 ± 13.9	0.81‡
Body mass index (kg/m^2)	22.6 (18.9–31.3)¶	22.7 (17.5–26.8)	0.87††
History of IFN therapy (%)	30	36	0.78§
ALT (IU/L)	130 ± 100†	75 ± 35	0.09‡
AST (IU/L)	76 (22–331)¶	64 (24–178)	0.73††
γ-GTP (IU/L)	40 (12–289)	52 (24–137)	0.17††
Albumin (g/dL)	4.1 (3.7–4.5)	4.0 (3.0–4.7)	0.46††
Total cholesterol (mg/dL)	170 ± 24†	149 ± 33	0.06‡
HbA1c (%)	5.3 ± 0.5	5.3 ± 0.6	0.95‡
Creatinine (mg/dL)	0.71 ± 0.15	0.68 ± 0.15	0.54‡
WBC count (/μL)	4561 ± 1631	4056 ± 1277	0.38‡
Neutrophil count (/μL)	2130 (820–4200)¶	1500 (800–2700)	0.02††
Hemoglobin (g/dL)	14.5 ± 1.0†	13.8 ± 1.6	0.15‡
Platelet count (×10^{-4}/μL)	16.4 ± 5.4	12.2 ± 3.9	0.03‡
α-Fetoprotein (ng/mL)	4.6 (1.4–28.9)¶	22.3 (11.4–79.7)	0.00005††
HCV RNA (KIU/mL)	1520 ± 1079†	2146 ± 899	0.11‡
Fibrosis (F1/F2/F3/F4)‡‡	14/1/1/2	3/2/2/3	0.02††
Activity (A1/A2/A3)‡‡	12/5/1	3/5/2	0.06††

†Mean ± standard deviation.
‡Student's *t*-test.
§Fisher's exact probability test.
¶Median (range).
††Mann–Whitney *U*-test.
‡‡SVR, $n = 18$; non-SVR, $n = 10$.
Activity, the score of activity in liver biopsies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELISA, enzyme-linked immunoassay; fibrosis, the score of fibrosis in liver biopsies; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; SVR, sustained virological response; WBC, white blood cell; γ-GTP, γ-glutamyl transpeptidase.

achieve SVR were neutrophil counts, platelet counts, serum α-fetoprotein levels and the score of fibrosis in liver biopsies. Table 2 shows the difference in the cytokine/chemokine expression between the SVR and the non-SVR group. Because some cytokines/chemokines were below the measurement limit of the ELISA kit, as shown in Table 1, those cytokine/chemokines were not studied further. As shown here, the *RANTES* level was significantly higher in the SVR group than the non-SVR group ($P = 0.048$).

Precise measurement of serum *RANTES* in all 96 patients

Because the semiquantitative measurement of pretreatment serum *RANTES* levels in 31 randomly selected patients demonstrated their significant correlation with the SVR, we determined the precise serum RANTES levels in all 96 patients using the bead array method and investigated the correlation between those concentrations and the treatment outcome. The clinical characteristics of the 96 patients are shown in Table 3. Significant differences were seen between those with and without SVR in platelet count, viral loads and the liver fibrosis score, but there was no apparent difference in the total doses of PEG IFN and RBV. As shown in Figure 1, the distribution of serum *RANTES* levels in each treatment response differed significantly; the median serum *RANTES* level in the SVR group was significantly higher than that in the non-SVR group. Successive ROC analysis confirmed a significant association of the serum *RANTES* level with SVR, and the cut-off value of 3400 pg/mL to be most appropriate (Table 4). Using the cut-off value of 3400 pg/mL, 50.9% sensitivity, 79.5% specificity, 78.4% positive predictive value and 52.5% negative predictive value (area under the ROC, 0.643) were obtained for the prediction of SVR by serum *RANTES* level.

Table 2 Difference in cytokine and chemokine expression between the SVR group and the non-SVR group in the 31 patients

Cytokine/chemokine	SVR ($n = 20$)	Non-SVR ($n = 11$)	*P*-value
RANTES	4.99 (0.25–8.32)†	1.24 (0.17–8.01)	0.048‡
MIF	1.31 (0.06–3.31)†	0.45 (0.08–2.67)	0.0630
IL-1ra	0.09 (0.00–3.30)†	0.07 (0.00–2.05)	0.2300
PAI-1	3.10 (0.35–7.34)†	2.73 (0.46–8.42)	0.3900
sICAM-1	3.18 (0.37–8.33)†	2.78 (0.74–10.3)	0.4800
IL-23	0.08 (0.01–0.78)†	0.07 (0.00–0.38)	0.5900
IL-27	0.05 (0.02–0.18)†	0.05 (0.00–0.23)	0.6500
IL-6	0.08 (0.01–3.22)†	0.10 (0.00–1.36)	0.7100
C5a	0.21 (0.01–2.72)†	0.12 (0.00–1.67)	0.7700
IFN-γ	0.07 (0.02–0.31)†	0.08 (0.00–0.40)	0.8000
CCL4	0.04 (0.01–3.08)†	0.05 (0.00–0.69)	0.8400
IL-32α	0.04 (0.00–0.71)†	0.07 (0.00–0.20)	0.9000
IL-8	0.16 (0.05–2.61)†	0.17 (0.03–2.21)	0.9300
IL-1α			N.A.
IL-1β			N.A.
IL-2			N.A.
IL-4			N.A.
IL-5			N.A.
IL-10			N.A.
IL-12 p70			N.A.
IL-13			N.A.
IL-16			N.A.
IL-17			N.A.
IL-17E			N.A.
CCL1			N.A.
CCL2			N.A.
CCL3			N.A.
CXCL1			N.A.
CXCL11			N.A.
CXCL12			N.A.
CD154			N.A.
G-CSF			N.A.
GM-CSF			N.A.
IP-10			N.A.
TNF-α			N.A.
sTREM-1			N.A.

†Median (range).
‡Mann–Whitney *U*-test.
N.A., not available; SVR, sustained virological response.

Correlation between serum *RANTES* level and clinical parameters

Spearman's correlation coefficients between the pre-treatment serum *RANTES* level and clinical parameters in all 96 patients are shown in Table 5. As a result, a significant negative correlation with aspartate aminotransferase level and a significant positive correlation with platelet count were found, while no significant correlation was observed in other clinical parameters.

Univariate and multivariate analysis of factors related to SVR

Univariate and multivariate analyses were performed successively in order to clarify the factors related to SVR. The viral factors included in the analysis were the ISDR and core a.a. 70 and 91, along with the host factor, IL-28B SNP. Those factors, conventional clinical background factors and serum *RANTES* levels were subjected to univariate and multivariate analysis. In the univariate

Table 3 Baseline characteristics of all patients analyzed using the bead array method ($n = 96$)

Factor	SVR ($n = 57$)	Non SVR ($n = 39$)	*P*-value
Age (years)	53 ± 10†	57 ± 8	0.08‡
Sex (male : female)	34:23	23:16	0.56§
Bodyweight (kg)	60.6 ± 10.5†	57.8 ± 7.8	0.17‡
Body- mass index (kg/m^2)	22.9 ± 2.8	22.1 ± 2.2	0.15‡
History of IFN therapy (%)	25	28	0.74§
ALT (IU/L)	68 (19–413)¶	64 (20–215)	0.25††
AST (IU/L)	58 (21–331)	62 (21–178)	0.80††
γ-GTP (IU/L)	37 (11–289)	50 (13–167)	0.12††
Albumin (g/dL)	4.1 ± 0.3†	4.1 ± 0.4	0.93‡
Total cholesterol (mg/dL)	166 ± 30	158 ± 31	0.25‡
HbA1c (%)	5.2 (4.7–6.6)¶	5.3 (4.5–7.4)	0.47††
Creatinine (mg/dL)	0.72 ± 0.15†	0.69 ± 0.16	0.39††
WBC count (/μL)	4497 ± 1247	4501 ± 1281	0.99‡
Neutrophil count (/μL)	2243 ± 857	2144 ± 825	0.57‡
Hemoglobin (g/dL)	14.1 ± 1.2	14.2 ± 1.2	0.87‡
Platelet count (×10^{-4}/μL)	15.1 (7–29)¶	13.2 (6.9–19.7)	0.03††
α-Fetoprotein (ng/mL)	4.8 (1.3–137.1)	9.0 (1.4–79.7)	0.05††
HCV RNA (KIU/mL)	1300 (100–5000)	2400 (620–5000)	0.0002‡
Fibrosis (F1/F2/F3/F4)‡‡	35/6/5/6	11/13/5/6	0.006††
Activity (A1/A2/A3)‡‡	27/18/7	12/20/3	0.26††
PEG IFN dose (%)	92 (40–113)¶	73 (27–147)	0.23††
RBV dose (%)	97 (44–147)	100 (33–135)	0.38††

†Mean ± standard deviation.
‡Student's *t*-test.
§Fisher's exact probability test.
¶Median (range).
††Mann–Whitney's *U*-test.
‡‡SVR, $n = 52$; non-SVR, $n = 35$.
Activity, the score of activity in liver biopsies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; fibrosis, the score of fibrosis in liver biopsies; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; WBC, white blood cell; γ-GTP, γ-glutamyl transpeptidase.

analysis, significant differences were observed for the ISDR mutation, core a.a. 70, viral loads, platelet counts, IL-28B SNP and serum *RANTES* levels. When multivariate analysis was carried out with these factors, the serum RANTES level was extracted as an independent factor related to SVR (Table 6).

RANTES haplotyping and serum *RANTES* level

Because a high serum *RANTES* level was an independent factor predicting SVR, we sought to examine further the role of the RANTES gene and tried to clarify the association of the SNP of the gene with the serum levels. First, we determined how many and which SNP in the *RANTES* gene should be investigated to represent all *RANTES* haplotypes found in the Japanese population. Reference to the HapMap project database (http://snp.cshl.org) made it clear that the information from five unique SNP was required to determine the majority of haplotypes found in the Japanese population. Therefore, to determine the *RANTES* haplotype of each patient, we investigated these five SNP in the 65 of the 96 patients available for the haplotype analysis. The *RANTES* haplotypes were finally divided into three types (named R1, R2 and R3 for convenience), as shown in Figure 2(a). However, the *RANTES* gene haplotype and serum RANTES level did not show any clear correlation (Fig. 2b).

DISCUSSION

FROM THE ANALYSIS of 36 cytokine and chemokine species, we discovered that a high pretreatment serum *RANTES* level was significantly related to SVR

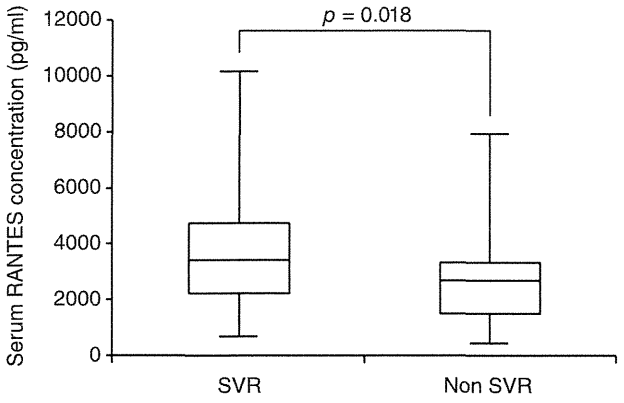


Figure 1 Difference in serum *RANTES* levels between the sustained virological response (SVR) group and the non-SVR group. Ninety-six patients who underwent the standard therapy for 48 weeks were analyzed for serum *RANTES* level using the bead array method. They were divided into the SVR ($n = 57$) and non-SVR groups ($n = 39$) and their serum *RANTES* levels compared. Box and whisker plots show the distributions of serum *RANTES* levels for the SVR and non-SVR groups. The boxes represent the 25th to 75th percentile and horizontal lines within the box show the median values. The ends of the whiskers show the minimum and maximum values of all the data. *P*-values were obtained using Mann–Whitney's *U*-test.

following PEG IFN/RBV combination therapy of patients infected with genotype 1b HCV. In particular, a high serum *RANTES* level was an independent factor contributing to SVR in the multivariate analysis, even among other treatment-restricting factors as the HCV ISDR, core a.a. 70, viral loads, platelets or IL-28B SNP. On the other hand, a systematic haplotyping study did not reveal any correlation between the *RANTES* haplotype and serum *RANTES* level.

RANTES, also known as CC-chemokine ligand (CCL)5, is classified as a chemotactic T-helper (Th)1-type chemokine.[23] In chronic hepatitis C, *RANTES* is significantly upregulated in the infected liver, and considered to play a role in recruiting T cells to portal and periportal regions, regulating liver inflammation and innate and adaptive immunity through interactions with CC-chemokine receptor (*CCR*)5, *CCR1* and *CCR3* expressed on activated T cells.[24] The serum *RANTES* level is significantly upregulated in the early stages of fibrosis in chronic hepatitis and its upregulation becomes weaker in advanced chronic disease.[14] HCV-encoded proteins are considered to affect *RANTES* production, for example, exposure of peripheral blood mononuclear cells to the HCV envelope 2 (E2) protein induces the release of *RANTES*,[25] the HCV NS3/4A proteins suppress *RANTES* promoter activity[26] and the HCV core protein may either induce or inhibit the expression of *RANTES* in various cell types.[27] A recent *in vitro* study has shown that human hepatoma cells secrete *RANTES* via the Toll-like receptor (*TLR*)3-mediated recognition of HCV dsRNA and activation of the nuclear factor (*NF*)-*kB* pathway, suggesting that the hepatocytes themselves may serve as the source of *RANTES*.[15]

In this study, we showed the close association between the serum *RANTES* level and SVR in the PEG IFN/RBV combination therapy by analyzing 31 randomly selected, primary test patients and then all 96 patients. In addition to the association with SVR, we also searched the association between *RANTES* and the initial viral response because SVR could be influenced by the initial viral dynamics, and revealed that complete early viral response (HCV RNA negative at 12 weeks

Table 4 Association between the serum *RANTES* level and SVR rate in all 96 patients analyzed using the bead array method

Cytokine/ chemokine	Serum concentration	SVR rate	*P*-value
RANTES	≥3400 pg/mL†	78% (29/37)	0.002‡
	<3400 pg/mL†	47% (28/59)	

†A cut-off value of 3400 pg/mL was determined by receiver–operator curve analysis in all 96 patients.
‡Fisher's exact probability test.
SVR, sustained virological response.

Table 5 Spearman's correlation coefficient (*R*) between the pretreatment serum *RANTES* level and clinical parameters ($n = 96$)

Clinical parameters	Serum *RANTES* level	
	R	*P*-value
Platelet count	−0.30	0.0025
Aspartate aminotransferase	−0.24	0.0200
White blood cell	−0.15	0.1600
Total cholesterol	−0.11	0.2700
Alanine aminotransferase	−0.088	0.3900
α-Fetoprotein	−0.088	0.4100
Neutrophil count	−0.064	0.5400
Hemoglobin A1c	−0.056	0.6300
γ-Glutamyl transpeptidase	−0.047	0.6500
Albumin	−0.021	0.7900
Hemoglobin	−0.025	0.8000
Creatinine	−0.00098	0.9900

Table 6 Factors associated with SVR analyzed by univariate and multivariate analysis

Characteristic	Subcategory	Univariate analysis			Multivariate analysis		
		Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Platelet count		1.13	1.03–1.25	0.012	1.20	1.00–1.41	0.042
IL-28B SNP	T/T or not	16.0	3.37–76.2	0.0005	9.48	1.40–64.3	0.02
RANTES	≥3400†	4.01	1.58–10.2	0.0036	4.09	1.02–16.5	0.048
Viral loads		0.99	0.99–0.99	0.0012	0.99	0.99–1.00	0.51
ISDR mutation	≥2	21.7	2.76–170	0.0034	28.2	2.05–388	0.013
Core a.a. 70	R or not	2.52	1.03–6.20	0.044	3.19	0.73–13.9	0.12

†The cut-off value of 3400 pg/mL was determined by receiver–operator curve analysis in all 96 patients.
a.a., amino acids; CI, confidence interval; IL, interleukin; ISDR, interferon sensitivity-determining region; R, arginine; SNP, single nucleotide polymorphisms.

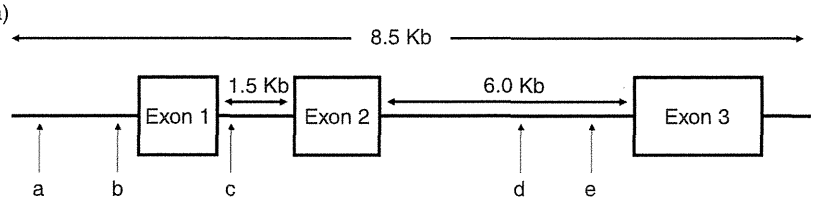
after commencement of therapy) was also significantly correlated with high pretreatment serum *RANTES* level ($P = 0.015$, data not shown). Moreover, we could also show that high serum *RANTES* levels correlated with the clinical background factors low alanine aminotransferase values and high platelet counts, suggesting that the patients with high *RANTES* levels have less severe hepatitis. A previous study also showed a tendency for correlation between the serum *RANTES* level and SVR in PEG IFN/RBV therapy, but this correlation did not reach significance.[16] Although the reason for this discrepancy is not known, we speculate that a difference in drug dosage may have contributed. In our study, most of the patients received a sufficient dose of both PEG IFN and RBV, as shown in Table 3. However, the previous study lacks information regarding drug dosage, suggesting that the study group comprised a heterogeneous population.

Then, what is the mechanism of the association between high serum *RANTES* levels and high SVR? Because *RANTES* is a chemotactic Th1-type chemokine, it may be speculated that a high serum *RANTES* level reflects activation and preservation of the Th1-type immune responses needed to suppress viral replication and so enhances viral elimination by PEG IFN/RBV therapy. Although it is also possible that a high *RANTES* level could be simply a reflection of early stages of the disease, we suggest that it could have a more direct role in achieving SVR, because multivariate analysis extracted a high serum *RANTES* level as a variable contributing to SVR independently of the platelet count, which reflects the stage of disease. Importantly, our result also demonstrated that the serum *RANTES* level was a factor contributing to SVR independently of other treatment-restricting factors, including the *IL-28B* SNP and the viral factors of NS5A and core. This independent contribution of a high serum *RANTES* level among other variables indicates its importance and potency in improving the prediction of the treatment efficacy.

Concerning the association between the serum *RANTES* level and *RANTES* haplotype, we could not find a significant correlation in the HCV-infected patients, although there was a tendency that patients with the R3 haplotype had higher serum *RANTES* levels. In patients with coronary artery disease and type 1 diabetes mellitus, and in healthy volunteers, the serum *RANTES* level has been reported to correlate with the *RANTES* gene SNP. Specifically, those patients and healthy volunteers with the A allele in the *RANTES* promoter polymorphism at position −403 (rs2107538) had lower serum *RANTES* levels than those with the G allele.[28,29] On the other hand, in previous reports of chronic hepatitis C, no evident correlation was reported between the *RANTES* SNP at position −403 (rs2107538) and serum *RANTES* level.[30] In this study, through more systematic haplotyping analysis based upon the HapMap Database, we tried to determine the correlation between the serum *RANTES* level and the *RANTES* gene SNP in chronic hepatitis C in more detail. However, we could not find any association and the result shows that the serum *RANTES* level is not primarily determined by the *RANTES* haplotype in chronic hepatitis C. The result seems strange at first, however, it is understandable considering that *RANTES* expression is modulated by multiple factors in chronic hepatitis C, including viral components and the stage of liver disease, as described before.

However, there are some limitations in our study. Namely, the number of investigated patients was rather small, and included patients for the analysis were limited to those with genotype 1b HCV infection. Therefore, it is considered that additional independent studies including the analysis of other genotypes would

(a)



RANTES haplotype	a	b	c	d	e	Haplotype frequency
	rs2107538	rs2280788	rs2280789	rs4796120	rs3817655	
R1	G	C	T	A	A	0.599
R2	A	C	C	G	T	0.197
R3	A	G	C	A	T	0.189

(b)

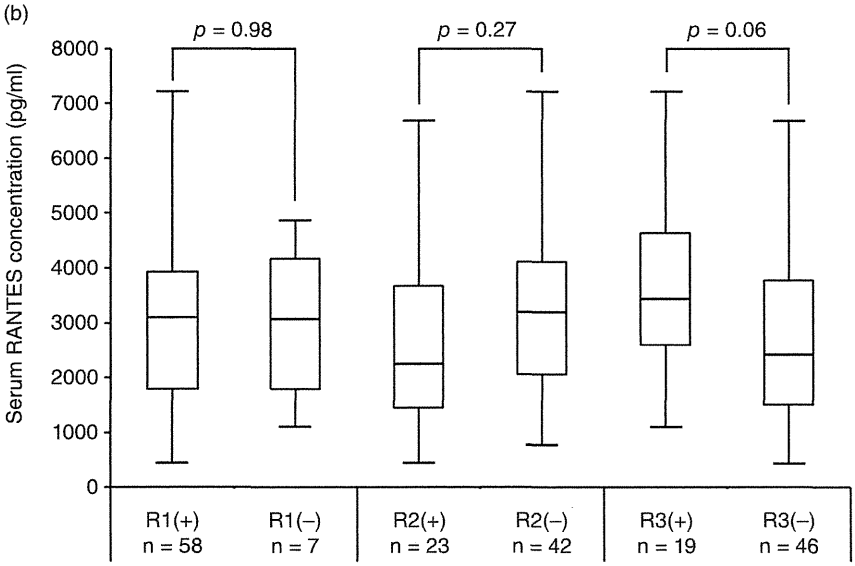


Figure 2 *RANTES* haplotypes and serum *RANTES* level. (a) *RANTES* haplotypes in the patients studied. The human *RANTES* gene spans 8.5 kb on chromosome 17q11-q12 and has the characteristic three exon and two intron organization of the CC chemokine family.[22] Exons are shown as open boxes while introns are shown as solid lines. Five single nucleotide polymorphisms (SNP) (rs2107538/rs2280788/rs2280789/rs4796120/rs3817655) were selected on the basis of data from the HapMap project (http://snp.cshl.org) to obtain complete coverage of the *RANTES* gene in the Japanese population. The locations of SNP variants are indicated by arrows. After the analysis of five *RANTES* SNP in 65 hepatitis C virus patients, haplotypes were determined using SNPAlyze software ver. 8.0 (Dynacom, Chiba, Japan) and divided into three groups on the basis of linkage disequilibrium. These were designated R1, R2 and R3 on the basis of haplotype frequency. (b) Serum *RANTES* level and *RANTES* haplotype. The correlation between serum the *RANTES* level and *RANTES* haplotype was investigated. Box and whisker plots shows distributions of serum *RANTES* levels for the haplotypes R1(+), R1(−), R2(+), R2(−), R3(+) and R3(−). The boxes represent the 25th to 75th percentile and horizontal lines within the boxes show the median values. The ends of the whiskers show the minimum and maximum values of all the data. *P*-values were obtained using Mann–Whitney's *U*-test. R1(+), the patients with the R1 haplotype; R1(−), the patients with a non-R1 haplotype; R2(+), the patients with the R2 haplotype; R2(−), the patients with a non-R2 haplotype; R3(+), the patients with the R3 haplotype; R3(−), the patients with a non-R3 haplotype.

further clarify the correlation. On the other hand, we could not show an association of pretreatment cytokines/chemokine concentrations with the treatment response to PEG IFN/RBV therapy for the other 35 cytokine and chemokine species investigated in this study. Recently, the serum level of *IP-10* was reported to be strongly associated with the response to PEG IFN/RBV therapy and baseline *IP-10* levels were elevated in patients infected with HCV genotype 1 or 4 who did not achieve an SVR after completion of interferon therapy.[19,20] In our study, however, *IP-10* was not extracted as a molecule associated with treatment responses. Actually, due to the measurement limit of the ELISA kit used, several cytokines and chemokines, including *IP-10*, were undetectable in this study, as shown in Table 2, raising the possibility that some cytokines and chemokines associated with SVR were not extracted. Therefore, our study cannot exclude the possibility of other cytokine/chemokines making a contribution to treatment efficacy.

In conclusion, we found that a high pretreatment serum *RANTES* level was related to the efficacy of PEG IFN/RBV therapy in genotype 1b HCV, independent of other treatment-restricting factors, and prediction of treatment outcome could be improved with the measurement of the pretreatment serum *RANTES* level.

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Correlation between Pretreatment Viral Sequences and the Emergence of Lamivudine Resistance in Hepatitis B Virus Infection.

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Correlation between Pretreatment Viral Sequences and the Emergence of Lamivudine Resistance in Hepatitis B Virus Infection.

Ryota Sueki[‡], Shinya Maekawa[I], Mika Miura, Makoto Kadokura, Kazuki Komase, Hiroko Shindo, Asuka Kanayama, Takako Ohmori, Kuniaki Shindo, Fumitake Amemiya, Yasuhiro Nakayama, Tomoyoshi Uetake, Taisuke Inoue, Minoru Sakamoto, Nobuyuki Enomoto

Sueki[‡] and Maekawa[I] contributed equally to the work.

First Department of Internal Medicine, Faculty of Medicine, University of Yamanashi,

1110 Shimokato, Chuo, Yamanashi 409-3898, JAPAN

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Corresponding author:

Shinya Maekawa, M.D., Ph.D.

First Department of Internal Medicine, Faculty of Medicine, University of Yamanashi,

1110 Shimokato, Chuo, Yamanashi 409-3898, JAPAN

Tel: +81-55-273-9584

Fax: +81-5-5273-6748

E-mail: maekawa@yamanashi.ac.jp

The emergence of amino acid or nucleotide substitutions leads to lamivudine resistance in Hepatitis B virus (HBV) infected patients. The aim of this study was to investigate whether viral sequences help predict the emergence of lamivudine resistance. The study subjects comprised 59 consecutive patients infected with HBV treated with daily therapy of 100 mg lamivudine. Among those, 32 patients with adequate pretreatment serum preservation were investigated for the correlation between viral amino acid substitutions and the appearance of lamivudine resistance with consideration of clinical background by determining dominant HBV full open reading frames. Viral resistance to lamivudine emerged in 28 of 59 patients (47%) in a median period of 2.45 years. Sequence comparisons of HBV genomes between patients who later developed lamivudine resistance and patients who did not revealed the existence of significant differences between the two groups in the pre-S1 84 (P=0.042), pre-S2 1 (P = 0.017) and 22 (P=0.015), and polymerase tp 95 (P=0.046), judged by a log-rank test. Viral sequence analyses revealed the presence of amino acid substitutions in HBV pre-S1 and pre-S2 that may be associated with the emergence of lamivudine resistance during chronic HBV infection.