



Title	Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions
Author(s)	Suda, Goki; Yamamoto, Yoshiya; Nagasaka, Astushi; Furuya, Ken; Kudo, Mineo; Chuganji, Yoshimichi; Tsukuda, Yoko; Tsunematsu, Seiji; Sato, Fumiyuki; Terasita, Katsumi; Nakai, Masato; Horimoto, Hiromasa; Sho, Takuya; Natsuizaka, Mitsuteru; Ogawa, Kouji; Ohnishi, Shunsuke; Chuma, Makoto; Fujita, Yasuyuki; Abe, Riichiro; Taniguchi, Miki; Nakagawa, Mina; Asahina, Yasuhiro; Sakamoto, Naoya
Citation	Hepatology research, 45(8), 837-845 https://doi.org/10.1111/hepr.12421
Issue Date	2015-08
Doc URL	http://hdl.handle.net/2115/62582
Rights	This is the peer reviewed version of the following article: Suda, G., Yamamoto, Y., Nagasaka, A., Furuya, K., Kudo, M., Chuganji, Y., Tsukuda, Y., Tsunematsu, S., Sato, F., Terasita, K., Nakai, M., Horimoto, H., Sho, T., Natsuizaka, M., Ogawa, K., Ohnishi, S., Chuma, M., Fujita, Y., Abe, R., Taniguchi, M., Nakagawa, M., Asahina, Y., Sakamoto, N., (2015) Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions. Hepatol Res, 45: 837–845., which has been published in final form at http://doi.org/10.1111/hepr.12421 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	article (author version)
File Information	manuscript.pdf



[Instructions for use](#)

Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions

Goki Suda¹, Yoshiya Yamamoto², Astushi Nagasaka³, Ken Furuya⁴, Mineo Kudo⁵, Chuganji Yoshimichi⁶, Yoko Tsukuda¹, Seiji Tsunematsu¹, Fumiyuki Sato¹, Katsumi Terasita¹, Masato Nakai¹, Hiromasa Horimoto¹, Takuya Sho¹, Mitsuteru Natsuizka¹, Kouji Ogawa¹, Shunsuke Ohnishi¹, Makoto Chuma¹, Yasuyuki Fujita⁷, Riichiro Abe⁷, Miki Taniguchi⁸, Mina Nakagawa⁸, Yasuhiro Asahina⁸, and Naoya Sakamoto¹ for the NORTE Study Group

¹ Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University, Hokkaido, Japan

² Hakodate City Hospital, Hokkaido, Japan

³ Sapporo City General Hospital, Hokkaido, Japan

⁴ Hokkaido Social Insurance Hospital, Hokkaido, Japan

⁵ Sapporo Hokuyu Hospital, Hokkaido, Japan

⁶ Tokyo Metropolitan Bokuto Hospital, Tokyo, Japan

⁷ Department of Dermatology, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

⁸ Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

*Correspondence to:

Goki Suda, M.D., Ph.D.

Department of Gastroenterology and Hepatology/Graduate School of Medicine,

Hokkaido University

North 15, West 7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan

Phone: +81 11-716-1161; Fax: +81 11-706-7867; Email:

gsudgast@pop.med.hokudai.ac.jp

Abbreviations

DAAs, direct-acting antivirals; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; HCV, hepatitis C virus; CHC, chronic hepatitis C; Peg-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SJS, Stevens—Johnson syndrome; SVR, sustained virological response; TPV, telaprevir; TEN, toxic epidermal necrolysis

Financial disclosure

The authors declare that they have nothing to disclose regarding funding from the industry or conflicts of interest with respect to the manuscript.

Abstract

Background: Telaprevir-based therapy for chronic hepatitis C patients is effective; however, the high prevalence of dermatological reactions is an outstanding issue. The mechanism and characteristics of such adverse reactions are unclear; moreover, predictive factors remain unknown. Granulysin was recently reported to be upregulated in the blisters of patients with Stevens–Johnson syndrome (SJS). Therefore, we investigated the risk factors for severe telaprevir-induced dermatological reactions as well as the association between serum granulysin levels and the severity of such reactions.

Methods: A total of 89 patients who received telaprevir-based therapy and had complete clinical information were analyzed. We analyzed the associations between dermatological reactions and clinical factors. Next, we investigated the time-dependent changes in serum granulysin levels in 5 and 14 patients with grade3 and non-grade3 dermatological reactions.

Results: Of the 89 patients, 57 patients had dermatological reactions, including 9 patients with grade3. Univariate analysis revealed that grade3 dermatological reactions were significantly associated with male sex. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Three patients with grade3 dermatological reaction had severe systemic manifestations including SJS, drug-induced hypersensitivity syndrome, and systemic lymphoid swelling and high-grade fever; all were hospitalized. Importantly, among the 3 patients, 2 patients' serum granulysin levels exceeded 8ng/mL at onset and symptoms deteriorated within 6 days.

Conclusions: Male patients are at high risk for severe telaprevir-induced dermatological

reactions. Moreover, serum granulysin levels are significantly associated with the severity of dermatological reactions and might be a predictive factor in patients treated with telaprevir-based therapy

Keywords: HCV, Telaprevir, granulysin, TEN, DIHS

Introduction

Hepatitis C is a major pathogen causing liver cirrhosis and hepatocellular carcinoma worldwide. Until recently, standard therapies for chronic hepatitis C virus (HCV) genotype 1 infection were based on the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV); these combination therapies yield a sustained virological response (SVR) rate of ~50% (1). Several classes of novel direct-acting antivirals (DAAs) were recently developed and tested in clinical trials. Two first-generation HCV NS3/4A protease inhibitors, boceprevir (2, 3), and telaprevir (4-6), have been approved for the treatment of genotype 1 HCV infection. The inclusion of these agents in HCV treatment regimens has led to large improvements in treatment success rates.

Telaprevir, the first DAA, is administered in combination with PEG-IFN and RBV for 24 weeks, resulting in SVR rates up to 70–80% (4, 6-8). Although the telaprevir combination regimen is highly effective, the high frequency and severity of adverse events are outstanding issues limiting its use. Dermatological reactions are particularly prevalent, developing in 56–84.6% of patients treated with telaprevir, PEG-IFN, and RBV combination therapy (9, 10). Moreover, the prevalence of severe dermatological reactions including Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome (DIHS) are substantially higher in patients treated with telaprevir-based therapy than PEG-IFN and RBV combination therapy (8, 10). McHutchison et al. reported that 7% of patients treated with telaprevir, PEG-IFN, and RBV combination therapy discontinued therapy because of rash or pruritus in contrast to only 1% of patients treated with PEG-IFN and RBV (8). In some patients, serious skin reactions persist even after stopping all drugs

(10). However, the pathogenesis and clinical predictors of these adverse reactions are poorly understood.

Granulysin is a 15-kDa cationic cytolytic protein released by cytotoxic T lymphocytes and natural killer cells that induces apoptosis in target cells and has antimicrobial activities (11). Serum levels of granulysin are elevated in primary virus infections including Epstein–Barr virus and parvovirus B19 (12). It was recently reported that serum granulysin levels are significantly elevated in patients with several types of severe dermatological lesions including SJS/TEN, which is the characteristic serious adverse event in telaprevir-containing regimens(13) (14).

Accordingly, the present study determined the risk factors for severe dermatological reactions in patients receiving telaprevir, PEG-IFN, and RBV combination therapy as well as the association between serum levels of granulysin and severe dermatological reactions.

Methods

Patients and methods

In this retrospective case-control study, at Hokkaido University Hospital and associated hospitals in the NORTE STUDY group, between December 2011 and November 2013, a total of 123 patients positive for HCV genotype 1 with high serum HCV RNA titer (>5 log IU/mL) received PEG-IFN, RBV, and telaprevir combination therapy. Patients were excluded if they required hemodialysis or had a positive test result for serum hepatitis B surface antigen, co-infection with other HCV genotypes or HIV, evidence of autoimmune hepatitis or alcoholic hepatitis, or malignancy. Serum granulysin levels were analyzed in 5 healthy volunteers with no HCV, HIV, or hepatitis B virus infection or any inflammatory diseases.

Written Informed consent according to the process approved by the hospital's ethics committee was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of each participating hospital.

Study design and treatment regimen

Telaprevir 500 or 750 mg was typically administered every 8 hours after meals for 12 weeks. PEG-IFN-a-2b (Peg-Intron, MSD, Tokyo, Japan) 1.5 IU/kg was administered subcutaneously once per week for 24 weeks. RBV (Rebetol, MSD) was administered for 24 weeks in two-divided dairy doses according to body weight: 600, 800, and 1,000 mg for patients with body weight <60 , 60–80, and >80 kg, respectively. The doses of PEG-IFN-a-2b, RBV, and telaprevir were reduced at the attending physician's discretion on the basis of hemoglobin levels, decreased white blood cell or

platelet count, or adverse events.

During treatment, patients were assessed as outpatient at weeks 1, 2, 4, 6, and 8 and then every 4 weeks thereafter for the duration of treatment. Physical examinations and blood tests were performed at all time points.

Outcomes

The primary endpoint was SVR, which was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. The secondary endpoints were end-of-treatment virological responses (HCV RNA undetectable in serum) and rapid virological response (RVR), which was defined as serum HCV RNA undetectable at 4 weeks after the start of treatment. Dermatological reactions were classified according to severity in the same manner as in phase III trials in Japan (10).

Serum granulysin measurement

To evaluate serum granulysin levels in chronic hepatitis C, we first measured serum granulysin levels in 5 healthy volunteers and compared them with those of 20 chronic hepatitis C patients before treatment. Serum granulysin levels were measured at the onset of dermatological reactions (within 3 days of onset); if the symptoms worsened, the time when worsening occurred was adopted. Meanwhile, in patients with no dermatological reactions, the highest serum granulysin level during treatment was adopted.

Serum granulysin levels were measured by a sandwich-enzyme-linked immunosorbent assay as described previously (12, 14, 15). Briefly, plates coated with 5 mg/mL mouse antibody against human granulysin, RB1 antibody, were washed with

phosphate-buffered saline containing 0.1% Tween-20. Next, they were blocked with 10% fetal bovine serum in washing buffer at room temperature for 2 hours. The samples and standards (Recombinant Granulysin, R&D Systems, Minneapolis, MN, USA) were incubated for 2 hours at room temperature. Next, they were reacted with 0.1 mg/mL biotinylated another mouse antibody against human granulysin, RC8 antibody. The plates were subsequently treated with horseradish peroxidase-conjugated streptavidin (Roche Diagnostics, Basel, Switzerland). The plates were then incubated with tetramethylbenzidine substrate (Sigma, St. Louis, MO, USA), and 1 M sulfuric acid was then added. The optical density was measured at 450 nm using a microplate reader

Diagnosis of dermatological reactions

Dermatological reactions were investigated throughout the 24-week administration period in the telaprevir-based combination therapy. Dermatological reactions were classified according to severity as follows. Grade 1 was defined as involvement of <50% of the body surface and no evidence of systemic symptoms. Grade 2 was defined as involvement of <50% of the body surface but with multiple or diffuse lesions or rashes with characteristic mild systemic symptoms or mucous membrane involvement with no ulceration/erosion. Grade 3 was defined as a generalized rash involving >50% of the body surface or a rash with any new significant systemic symptoms and considered to be related to the onset and/or progression of the rash. Life-threatening reactions included SJS, TEN, drug rash with eosinophilia and systemic symptoms (DRESS)/DIHS, erythema multiforme, and other life-threatening symptoms or patients presenting with features of serious disease.

When adverse skin reactions were detected, the attending physician classified

the degree of severity and referred the patients to a dermatologist as needed. In principal, when grade 3 dermatological reactions occurred, the attending physician referred the patient to a dermatologist and discontinued telaprevir. When severe dermatological reactions including SJS/TEN and DRESS/DIHS were suspected, all drugs were discontinued immediately. SJS/TEN and DIHS were diagnosed by skin biopsy and according to disease criteria, respectively.

Statistical analysis

Categorical and continuous variables were analyzed by the χ^2 test and the unpaired Mann–Whitney *U*-test, respectively. All *P*-values were two-tailed, and the level of significance was set at $P < 0.05$. Multivariate logistic regression analysis with stepwise forward selection included variables showing $P < 0.05$ in univariate analyses.

The association between dermatological reactions and serum granulysin levels were evaluated by one-way analysis of variance followed by the Tukey honestly significant different test. All statistical analyses were performed using SPSS version 21.0 (IBM Japan, Tokyo, Japan)

Results

Patients.

We included 123 CHC patients who received telaprevir based triple therapy. Of these, 89 patients who had proper information of dermatological adverse events were included. The base line characteristic of patients is shown in **Table1**.

Of these 89 patients, time dependent changes of serum granulysin concentrations were measured in 20 patients who have had conserved serum, at least, at the pre-treatment point, one and two weeks after commencement of therapy, one and two month after commencement of therapy, the onset point of dermatological adverse reaction and the worsening point if symptom have become worse.

Among 89 patients, 64% (57/89) developed dermatological reactions, including 9 with grade 3 reactions (**Table 2**). The characteristics of dermatological reactions by grade are shown in **Table 2**. Non-grade 3 dermatological reactions tended to occur early during treatment compared to grade 3 dermatological reactions.

Association between dermatological reactions and treatment outcomes

First, we determined whether dermatological reactions were associated with final treatment outcomes. Univariate analyses identified baseline white blood cell and platelet counts, RVR, and non-grade 3 dermatological reactions significantly associated with SVR (**Table 3**). Among 9 patients with grade3 dermatological reactions, 3 patients discontinued of all treatment and 6 patients discontinued of telaprevir administration, SVR was achieved 0/3 (0%) and 2/6 (33%) respectively.

Multivariate analysis showed that RVR and non-grade 3 dermatological reactions were significantly associated with SVR (**Table 3**).

Analysis of risk factors for telaprevir-induced dermatological reactions

Next, we analyzed the association between severe (i.e., grade 3) dermatological reactions and clinical parameters (**Table 4**). Univariate analysis showed that only sex was significantly associated with the grade 3 dermatological reactions ($P = 0.03$).

Serum granulysin levels in healthy subjects and chronic hepatitis C patients

As shown in **Figure 1**, serum granulysin levels did not differ significantly between healthy volunteers and chronic hepatitis C patients. Next, we evaluated the association between the severity of dermatological reactions and serum peak granulysin levels in 20 patients including 5, 4, 5, 6 with grade 1, grade 2, grade 3, and no dermatological events, respectively. One-way analysis of variance showed that serum granulysin level was significantly associated with the severity of dermatological reactions ($P = 0.036$); in addition, the Tukey honestly significant difference test revealed that the serum granulysin levels of patients with grade 3 dermatological reactions were significantly higher than those of patients with grade 1 or no dermatological reactions (both $P < 0.05$, **Figure 2**).

Time-dependent changes in serum granulysin levels

We investigated the time-dependent changes in serum granulysin levels in 5 and 15 patients with grade 3 and non-grade 3 dermatological reactions, respectively (**Figure 3a, b**). Serum granulysin levels of patients with non-grade 3 dermatological reactions never exceeded more than 10ng/ml. Of the 5 patients with grade 3 reactions, 3 had severe systemic manifestations that necessitated hospital admission: 1 each had SJS,

DIHS, and systemic lymphoid swelling and high fever ($>39^{\circ}\text{C}$). All patients with grade 3 dermatological reactions with systemic manifestations had peak serum granulysin levels exceeding 10ng/mL; importantly, the serum granulysin levels of 2 patients already exceeding 8 ng/mL at the onset of the reactions and worsened within six days

Discussion

The present study demonstrates a significant association between telaprevir-induced dermatological reactions and elevated serum granulysin levels for the first time. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Thus, the results indicate that serum granulysin level seems to be a useful predictor of telaprevir-induced dermatological reactions. Because the emergence of grade 3 dermatological reactions was significantly associated with non-SVR (**Table 3**), probably associated with high rate of treatment discontinuation, it is important to predict dermatological events in the early stage to achieve good treatment outcomes.

Recent genome-wide association studies have identified that genetic polymorphisms around the IL28B gene locus significantly associated with the outcome of PEG-IFN and RBV combination therapy in HCV patients. Thus, PEG-IFN and RBV combination therapy is ineffective in a subset of HCV-infected patients who have IL28B TG or GG genotypes, limiting the use of this therapy (16). Therefore, novel drugs with different anti-viral mechanisms were required. Accordingly, DAAs were developed; they are mainly classified as NS3/4A protease inhibitors, or NS5B or NS5A inhibitors (17). The NS3/4A serine protease inhibitor telaprevir, in combination with PEG-IFN and RBV, has demonstrated the most promising results (6-8). However, adverse events, especially severe dermatological reactions, develop more frequently in patients treated with telaprevir than those treated with only PEG-IFN and RBV.

Little is known about the mechanisms of telaprevir-induced dermatological reactions. Reactions develop in patients treated with PEG-IFN and RBV combination therapy (18, 19) as well as telaprevir monotherapy (20, 21). It should be noted that the

dermatological reactions in telaprevir monotherapy or PEG-IFN and RBV therapy alone are generally mild (7, 8, 20). However, dermatological reactions in telaprevir and PEG-IFN/RBV combination therapy may be severe, indicating a synergistic effect. Severe dermatological events including SJS/TEN and DIHS have been reported in telaprevir-based triple therapy; these are life threatening, and fatal cases have been reported.

The onset of grade 3 dermatological reactions tended to be later than non-grade 3 reactions, the same as in the study of Torii et al. (10). Taken together with the finding that male sex is a clinical risk factor, the results indicate that late-onset dermatological reactions in male patients treated with telaprevir-based triple therapy require more attention.

Roujeau et al. analyzed the risk factors for telaprevir-induced eczematous dermatitis and report that the incidence of telaprevir-related dermatitis was significantly higher age >45 years, body mass index <30 (kg/m²), Caucasian ethnicity, and treatment-naïve status (9). While they analyzed the risk factors for telaprevir-induced eczematous dermatitis, the present study focused on the risk factors for severe telaprevir-induced dermatological reactions, because such reactions can affect treatment outcome (**Table 2**) and can be fatal. As mentioned above, male sex was significantly associated with grade 3 dermatological reactions. Sex is reported to be associated with the prevalence of some kinds of severe drug-induced dermatological events although the underlying mechanism remains unknown (22).

Fujita et al. report that serum granulysin levels are significantly elevated in SJS/TEN patients and thus might be good predictive factor (14). Therefore, we hypothesized that in telaprevir-based triple therapy for chronic hepatitis C patients,

serum granulysin levels are associated with the severity of dermatological reactions and might thus be a predictive biomarker. However, Ogawa et al. report that serum granulysin levels also increase as a result of primary virus infections such as Epstein–Barr virus or parvovirus B19 (12). Thus, it remains unclear whether and how chronic viral infections, especially HCV, affect serum granulysin levels. In the present study, we compared serum granulysin levels between healthy volunteers and chronic hepatitis C patients; the results show that chronic HCV infection was not associated with serum granulysin levels (**Figure 1**).

Chung et al. have reported that granulysin is the most highly expressed cytotoxic molecule in blisters of SJS/TEN and that massive keratinocyte death was induced by granulysin (11). Fujita et al. reported that serum granulysin levels increased in early stage of SJS/TEN caused by drug including carbamazepine, imatinib and phenytoin(14). Taken together with our results, we speculate that granulysin may be involved in the pathogenesis of early stage of telaprevir-mediated dermatological adverse reactions possibly through induction of keratinocyte death.

Of 5 patients with grade 3 reactions, 2 patients without severe systemic manifestations did not elevate serum granulysin more than 10ng/ml or did not elevate before symptom worsen. On the contrary, 3 patients with severe systemic manifestations had peak serum granulysin levels exceeding 10ng/mL and the serum granulysin levels of 2 patients already exceeding 8ng/ml **at onset and within 6 days**, symptoms worsen. Therefore serum granulysin tests might predict grade3 dermatological adverse reaction with systemic manifestations. **Furthermore if serum granulysin levels elevate more than 8ng/mL, more attention should be paid.**

In Western countries, the prevalence of dermatological reactions in patients

treated with telaprevir-based and PEG-IFN/RBV therapy are reported to be approximately 55% and 33%, respectively(9, 23); meanwhile, in Japanese patients, the respective rates are 74.9% and 58.7%. Moreover, approximately 4% and 9.0% of patients in Western and Japanese patients develop grade 3 reactions, respectively (10); this is almost the same as that in the present study (10%). The difference may be due to genetic or ethnic variation. Therefore, genome-wide association studies may have identified a gene locus associated with telaprevir-induced severe dermatological reactions.

A limitation of this study is that the number of patients with grade3 dermatological reactions is relatively small. However, the serum granulysin levels of patients with grade3 dermatological reactions were significantly higher than those of other patients. And in two of the three patients with severe dermatological reactions, the serum granulysin level elevated before symptoms worsen, these would be novel findings. Further study would be required.

Triple therapy with the second-generation protease inhibitor simeprevir is reported to result in a similar prevalence of adverse reactions as PEG-IFN and RBV combination therapy (24, 25). However, simeprevir is not approved worldwide. Although simeprevir-based triple therapy is effective, but only 36–53% of prior non-responders achieve SVR (24). Shimada et al. recently reported that by extending PEG-IFN and RBV therapy from 24 to 48 weeks, telaprevir-based triple therapy improves the SVR to up to 68% in prior null responders (26). Thus, telaprevir is a therapeutic option for prior null responders.

In conclusion, the present study suggests that male sex is a significant risk factor for severe telaprevir-induced dermatological reactions. In addition, serum

granulysin levels are significantly associated with the severity of dermatological reactions and thus might be a good predictor of severe dermatological reactions with systemic manifestations in patients treated with telaprevir-based triple therapy.

Acknowledgments

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Japan Society for the Promotion of Science, and the Ministry of Health, Labour and Welfare of Japan.

The authors would like to thank all patients and their families as well as the investigators and staff of the 22 participating institutions. The principal investigators of the NORTE study sites are listed below in alphabetical order: Junichi Yoshida (Sapporo Social Insurance General Hospital), Atsushi Nagasaka (Sapporo City General Hospital), Akira Fuzinaga, Manabu Onodera (Abashiri-Kosei General Hospital), Hideaki Kikuchi, Tomofumi Atarashi (Obihiro-Kosei General Hospital), Ken Furuya (Hokkaido Social Insurance Hospital), Yukio Oohara, Sousi Kimura (National Hospital Organization Hokkaido Medical Center), Takuto Miyagihima (Kushiro Rosai Hospital), Takashi Meguro (Hokkaido Gastroenterology Hospital), Akiyoshi Saga (Aiiku Hospital), Mineo Kudou (Sapporo Hokuyu Hospital), Jun Konno (Hakodate General Central Hospital), Kenichi Kumagaya (Hakodate Medical Association Hospital), Nobuaki Akakura (Sapporo Medical Center NTT EC), Tomoe Kobayashi (Tomakomai City Hospital), Uebayashi Minoru (Japanese Red Cross Kitami Hospital), Hiroshi Katou (Iwamizawa Municipal General Hospital), Yasuyuki Kunieda (Wakkanai City Hospital), Miki Tateyama (Tomakomai Nissho Hospital), Munenori Okamoto (Sapporo Century Hospital), Izumi Tunematsu (Touei hospital), and Chuganji Yoshimichi (Tokyo Metropolitan Bokuto Hospital)

References

1. Sakamoto N, Nakagawa M, Tanaka Y, Sekine-Osajima Y, Ueyama M, Kurosaki M, Nishida N, et al. Association of IL28B Variants With Response to Pegylated-Interferon Alpha Plus Ribavirin Combination Therapy Reveals Intersubgenotypic Differences Between Genotypes 2a and 2b. *Journal of Medical Virology* 2011;83:871-878.
2. Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;364:1195-1206.
3. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011;364:1207-1217.
4. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;364:2417-2428.
5. Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011;365:1014-1024.
6. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;364:2405-2416.
7. Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naïve patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012;56:78-84.
8. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
9. Roujeau JC, Mockenhaupt M, Tahan SR, Henshaw J, Martin EC, Harding M, van Baelen B, et al. Telaprevir-related dermatitis. *JAMA Dermatol* 2013;149:152-158.
10. Torii H, Sueki H, Kumada H, Sakurai Y, Aoki K, Yamada I, Ohtsuki M. Dermatological side-effects of telaprevir-based triple therapy for chronic hepatitis C in phase III trials in Japan. *J Dermatol* 2013;40:587-595.
11. Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, Chin SW, et al. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008;14:1343-1350.
12. Ogawa K, Takamori Y, Suzuki K, Nagasawa M, Takano S, Kasahara Y, Nakamura

Y, et al. Granulysin in human serum as a marker of cell-mediated immunity. *Eur J Immunol* 2003;33:1925-1933.

13. Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H. Granulysin as a marker for early diagnosis of the Stevens-Johnson syndrome. *Ann Intern Med* 2009;151:514-515.

14. Fujita Y, Yoshioka N, Abe R, Murata J, Hoshina D, Mae H, Shimizu H. Rapid immunochromatographic test for serum granulysin is useful for the prediction of Stevens-Johnson syndrome and toxic epidermal necrolysis. *J Am Acad Dermatol* 2011;65:65-68.

15. Saigusa S, Ichikura T, Tsujimoto H, Sugasawa H, Majima T, Kawarabayashi N, Chochi K, et al. Serum granulysin level as a novel prognostic marker in patients with gastric carcinoma. *J Gastroenterol Hepatol* 2007;22:1322-1327.

16. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41:1105-1109.

17. Aghemo A, De Francesco R. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. *Hepatology* 2013;58:428-438.

18. Lubbe J, Kerl K, Negro F, Saurat JH. Clinical and immunological features of hepatitis C treatment-associated dermatitis in 36 prospective cases. *Br J Dermatol* 2005;153:1088-1090.

19. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, 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.

20. Yamada I, Suzuki F, Kamiya N, Aoki K, Sakurai Y, Kano M, Matsui H, et al. Safety, pharmacokinetics and resistant variants of telaprevir alone for 12 weeks in hepatitis C virus genotype 1b infection. *J Viral Hepat* 2012;19:e112-119.

21. Toyota J, Ozeki I, Karino Y, Asahina Y, Izumi N, Takahashi S, Kawakami Y, et al. Virological response and safety of 24-week telaprevir alone in Japanese patients infected with hepatitis C virus subtype 1b. *J Viral Hepat* 2013;20:167-173.

22. Bersoff-Matcha SJ, Miller WC, Aberg JA, van Der Horst C, Hamrick Jr HJ, Powderly WG, Mundy LM. Sex differences in nevirapine rash. *Clin Infect Dis* 2001;32:124-129.

23. Cacoub P, Bourliere M, Lubbe J, Dupin N, Buggisch P, Dusheiko G, Hezode C, et al. Dermatological side effects of hepatitis C and its treatment: patient management in the era of direct-acting antivirals. *J Hepatol* 2012;56:455-463.

24. Izumi N, Hayashi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, et al. Once-daily simeprevir with peginterferon and ribavirin for treatment-experienced HCV genotype 1-infected patients in Japan: the CONCERTO-2 and CONCERTO-3 studies. *J Gastroenterol* 2014.
25. Hayashi N, Seto C, Kato M, Komada Y, Goto S. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naive hepatitis C genotype 1-infected patients in Japan: the DRAGON study. *J Gastroenterol* 2014;49:138-147.
26. Shimada N, Tsubota A, Atsukawa M, Abe H, Ide T, Takaguchi K, Chuganji Y, et al. A 48-week telaprevir-based triple combination therapy improves sustained virological response rate in previous non-responders to peginterferon and ribavirin with genotype 1b chronic hepatitis C: A multicenter study. *Hepatol Res* 2014.

Figure Legends

Figure 1. Serum granulysin levels of healthy volunteers and chronic hepatitis C patients

Serum granulysin levels were compared between 5 healthy volunteers and untreated 20 chronic hepatitis C patients. $P < 0.05$, Mann–Whitney *U*-test.

Figure 2. Association between dermatological adverse reaction severity and serum granulysin level

Serum granulysin levels were measured at the onset of dermatological reactions (i.e., within 3 days of onset); if the symptoms worsened, the time of worsening was adopted. In patients with no dermatological events, the highest serum granulysin level during treatment was adopted. $P < 0.05$, one-way analysis of variance.

Figure 3. Association between time-dependent changes in serum granulysin levels and severe telaprevir-induced dermatological adverse reactions.

(A) Time-dependent changes in serum granulysin levels patients with non-grade 3 dermatological reactions (3, 5, and 6 with grade 2, grade 1, and no reactions, respectively). The dash line, gray line and black line indicates grade 2, grade 1 and no reaction patients respectively. (B) Time-dependent changes in serum granulysin levels of 5 patients with grade 3 dermatological events. The dashed line indicates patients with severe systemic manifestations. Arrowheads indicate the onset of dermatological events and asterisk indicate the onset of grade3 dermatological events.

Table 1 Baseline characteristics of the participating patients

Total number	89
HCV genotype 1b (1b/others)	89/0
Age (years) ^a	60.0 (19–73)
Sex (male/female)	48/41
Body weight (kg) ^a	63.0 (32–97)
Baseline white blood cell count (/μL) ^a	4800 (1500–9800)
Baseline hemoglobin level (g/dL) ^a	13.5 (9.9–16.7)
Baseline platelet count (×10 ³) ^a	15.9 (6.6–86)
Baseline ALT level (IU/L) ^a	40 (15–300)
Baseline HCV RNA level (log ₁₀ IU/mL) ^a	6.5 (3.2–7.6)
Initial telaprevir dose (1500 mg/2250 mg)	20/89
Initial Peg-IFN dose (1.5 μg/kg/<1.5 μg/kg)	775/14
Initial RBV dose (mg/kg) ^a	9.8 (2.2–15.5)
IL28 B gene (rs8099917) (TT/non-TT/ ND)	51/22/16
HCV 70 core mutation (wild/mutant/ND)	43/24/22
Previous treatment (naive/relapse/NVR)	40/38/11

HCV: hepatitis C virus, IL28B: interleukin 28B, Peg-IFN: pegylated interferon, RBV: ribavirin, ALT :alanine transaminase

^a Data are shown as median (range) values.

Table 2 Characteristics of the patients with each dermatological adverse event grade

	No	Age ^a	Sex (male/female)	Initial telaprevir dose (2250/1500)	Onset of DAR ^a (days)
No DAR	32	61 (28–72)	15/17	26/6	
Grade 1	32	58 (19–73)	15/17	24/8	7 (3–50)
Grade 2	16	61 (44–73)	10/6	12/4	3.5 (1–56)
Grade 3	9	61 (48–65)	8/1	9/1	22 (1–60)

DAR: Dermatological adverse reaction

^a Data are shown as median (range) values.

Table 3 Comparison of the clinical and laboratory characteristics of the patients with HCV infection based on therapeutic response

All patients	SVR	Non-SVR	Univariate analysis	Multivariate analysis		
n = 89	n = 68	n = 21	p value	OR	95% CI	p value
Age (years) ^a	60 (19–73)	62 (28–73)	0.402			
Sex (male/female)	37/31	11/10	0.870			
Body weight (kg) ^a	62 (39–97)	64 (32–87)	0.761			
Baseline white blood cells (/μL) ^a	5135 (1500–9800)	4200 (2490–7200)	0.048	0.492	(0.121–1.993)	0.320
Baseline hemoglobin level (g/dL) ^a	13.5 (10.5–16.7)	12.1 (9.9–15.4)	0.862			
Baseline platelet count (×10 ³) ^a	16.7 (6.6–31.5)	12.8 (7.2–86)	0.025	0.388	(0.093–1.614)	0.193
Baseline ALT level (IU/L) ^a	37(15–300)	53 (23–159)	0.070			
Baseline HCV RNA level (log ₁₀ IU/mL) ^a	6.7 (3.2–7.6)	6.4 (5.7–7.3)	0.812			
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.7 (0.5–0.9)	0.433			
Initial telaprevir dose (1500 mg/2250 mg)	52/16	17/4	0.460			
Initial Peg-IFN dose (1.5 μg/kg/<1.5 μg/kg)	58/10	17/4	0.430			
Initial RBV dose (mg/kg) ^a	9.9 (2.2–15.5)	9.5 (4.4–12.5)	0.546			
IL28 B gene						
(rs8099917) (TT/non-TT/ND)	43/15/10	8/7/6	0.107			
Core 70aa mutation (wild/mutant/ND)	36/16/16	7/8/6	0.108			
Previous treatment (naive/relapse/NVR)	34/28/6	6/10/5	0.095			
Rapid virologic response (+/–)	60/8	10/11	<0.001	10.89	(2.838–41.83)	0.001
Grade 3 DAR (–/+)	66/2	14/7	<0.001	27.44	(3.718–202.5)	0.001

HCV: hepatitis C virus, IL28B: interleukin 28B, ITPA: inosine triphosphatase, Peg-IFN: pegylated interferon, RBV: ribavirin,

ALT :alanine transaminase, NVR: nonvirological response , DAR: Dermatological adverse reaction, CI: confidence interval

^a Data are shown as median (range) values.

Table 4 Comparison of the clinical and laboratory characteristics of the patients based on the presence or absence of at least a grade 3 dermatological adverse event

All patients	Non-GT3	GT3	Univariate analysis
n = 89	n = 80	n = 9	p value
Age (years) ^a	60 (19–73)	61 (48–65)	0.453
Sex (male/female)	40/40	8/1	0.027
Body weight (kg) ^a	62 (32–97)	64 (51–87)	0.593
Baseline white blood cell count (/μL) ^a	4900 (1500–9800)	4700 (3000–7000)	0.876
Baseline hemoglobin level (g/dL) ^a	13.5 (9.9–16.7)	14.4 (12.1–15.4)	0.196
Baseline platelet count (×10 ³) ^a	16.0 (6.6–86.0)	13.5 (10.4–22.5)	0.605
Baseline ALT level (IU/L) ^a	40 (15–300)	37 (23–87)	0.765
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.8 (0.6–0.9)	0.123
Baseline HCV RNA level (log ₁₀ IU/mL) ^a	6.6 (3.2–7.6)	6.4 (5.7–7.1)	0.465
Initial telaprevir dose (1500 mg/2250 mg)	62/18	7/2	0.675
Initial telaprevir/body weight (mg/kg)	33.7 (20–71.4)	30.0 (23.6–44.1)	0.563
Initial Peg-IFN dose (1.5 μg/kg/<1.5 μg/kg)	66/14	9/0	0.198
Initial RBV dose (mg/kg) ^a	9.7 (2.2–15.5)	10.7 (7.7–12.9)	0.161
IL28 B gene (rs8099917) (TT/non-TT/ND)	47/19/14	4/3/2	0.353
Core 70aa mutation (wild/mutant/ND)	38/22/20	5/2/2	0.511
Previous treatment (naive/relapse/NVR)	35/36/9	5/2/2	0.972
Onset of dermatological AE (days)	5 (1–75)	22 (1–60)	0.352

HCV: hepatitis C virus, IL28B: interleukin 28B, Peg-IFN: pegylated interferon, RBV: ribavirin, ALT :alanine transaminase, AE :adverse event

^a Data are shown as median (range) values.

Figure 1. Serum granulysin levels of healthy volunteers and chronic hepatitis C patients

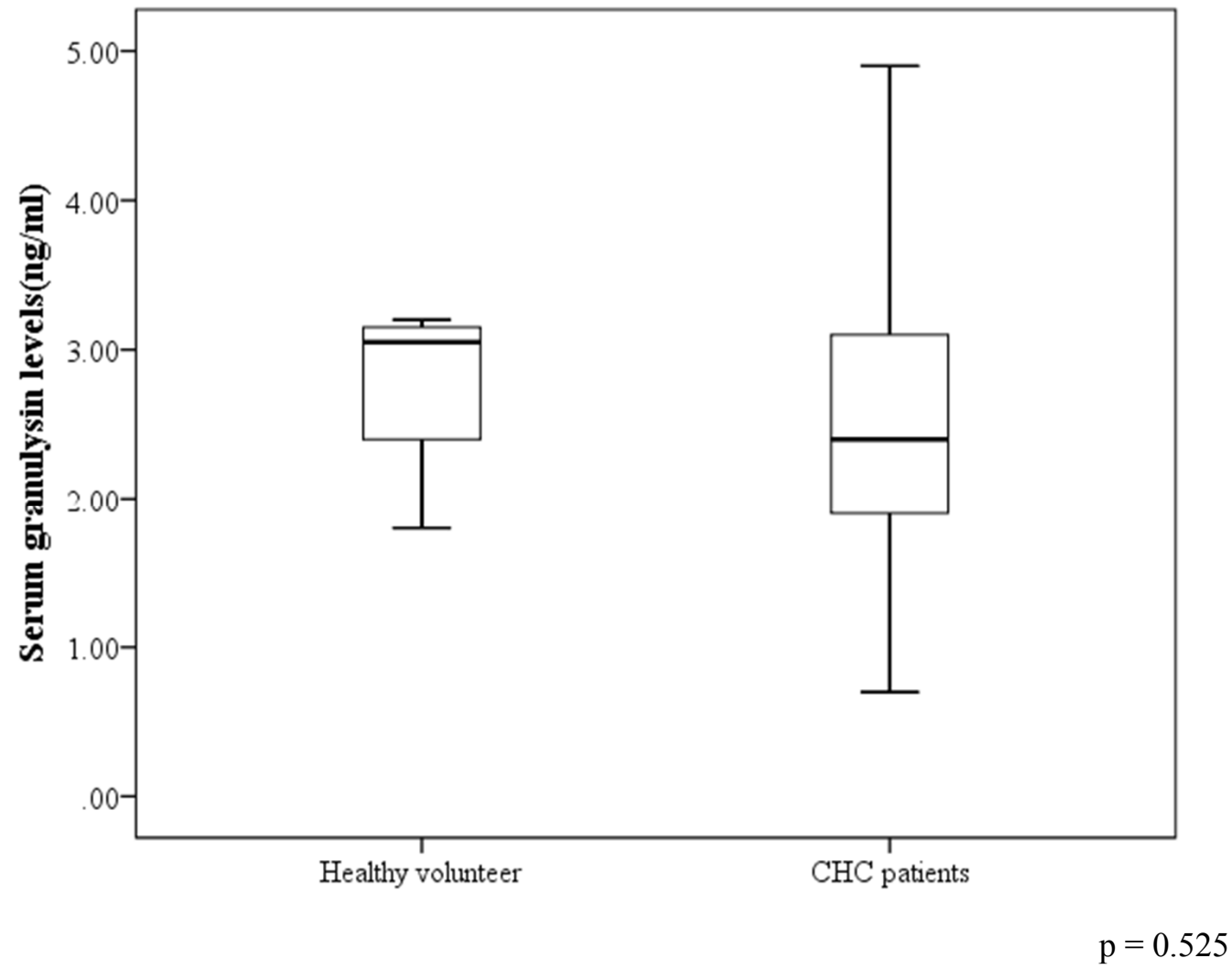
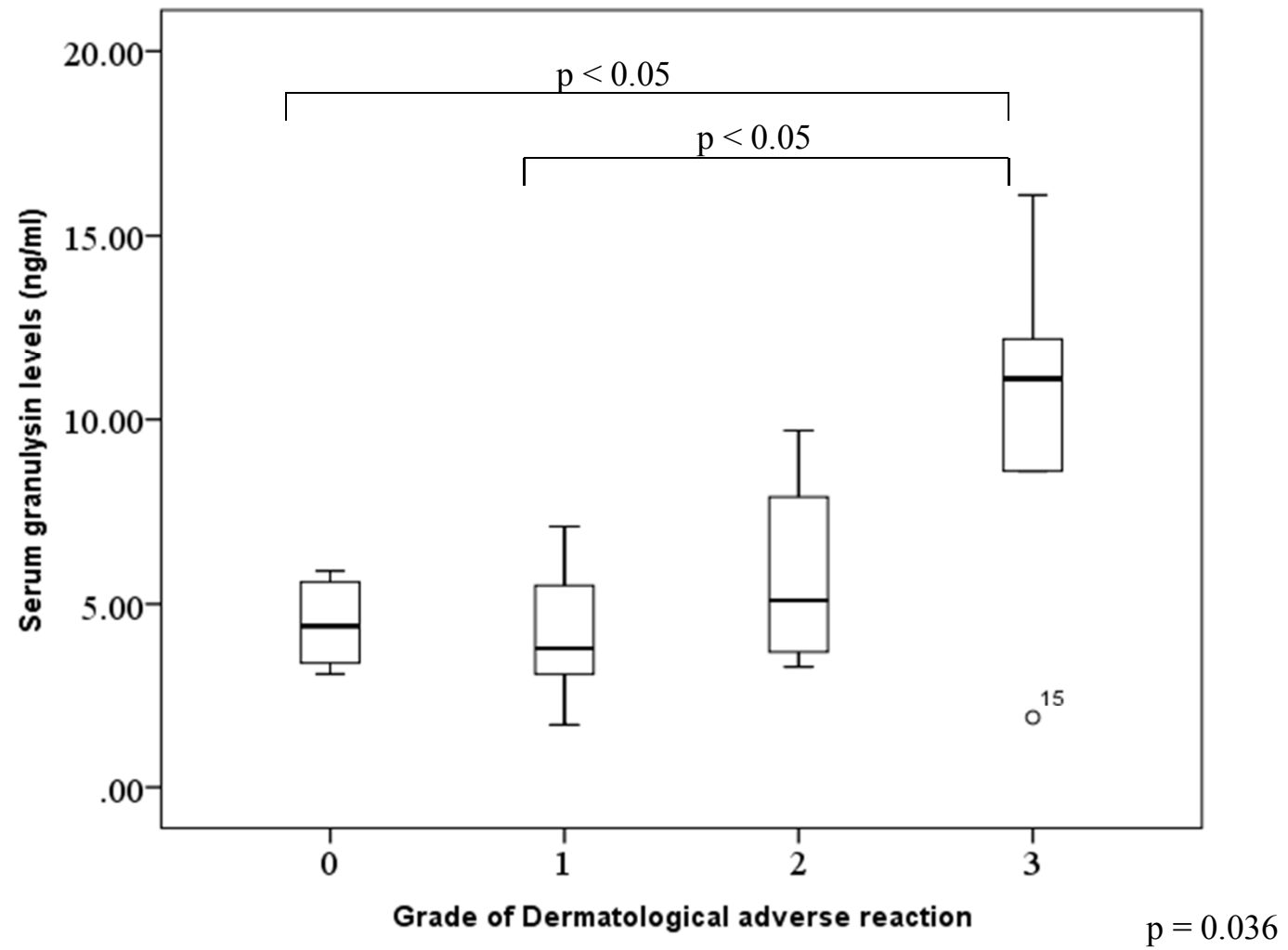


Figure2

Association between dermatological adverse reaction and serum granulysin level



A.

