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# High Predictability of a Sustained Virological Response (87%) in Chronic Hepatitis C Virus Genotype 1 Infection Treatment by Combined IL28B Genotype Analysis and γ-Glutamyltransferase/Alanine Aminotransferase Ratio: A Retrospective Single-Center Study

A. Amanzada[a] A.D. Goralczyk[a] S. Schneider[b] F. Moriconi[a] A. Lindhorst[a] S. Mihm[a] D.H. Van Thiel[c] G. Ramadori[a]

[a]Division of Gastroenterology and Endocrinology, University Medical Center Göttingen, and [b]Department of Medical Statistics, Georg-August-University Göttingen, Göttingen, Germany; [c]Division of Liver Disease, Rush University Medical Center, Chicago, Ill., USA

**Key Words**
Chronic hepatitis C · Ribavirin · Pegylated interferon · IL28B genotype · γ-Glutamyltransferase · Alanine aminotransferase · Predictive factors

**Abstract**
***Background:*** Chronic hepatitis C virus genotype 1 (HCV-G1) infection is treated with pegylated interferon-α and ribavirin. Predictive factors for treatment success are even more important now as direct-acting antiviral agents are available. ***Methods:*** Clinical and laboratory parameters were analyzed by uni- and multivariate statistical means in 264 patients with HCV-G1 infections with regard to treatment outcome. ***Results:*** The overall sustained virological response (SVR) rate was 44%. Univariate analyses revealed SVRs to be associated with age, high alanine aminotransferase (ALT) and low γ-glutamyltransferase (γ-GT) serum activities, a low pretreatment γ-GT/ALT ratio, rapid virological response (RVR), and absence of steatosis. Multivariate analyses unveiled IL28B rs12979860 genotype (CC vs. CT: OR = 2.8, CI: 1.5–4.9, p = 0.001; CC vs. TT: OR = 7.1, CI: 3.1–16.7, p < 0.001), low pretreatment γ-GT/ALT ratio (OR = 2.5, CI: 1.7–3.3, p < 0.001), age (OR = 0.96, CI: 0.94–0.98, p = 0.001) and RVR (OR = 4.18, CI: 2.85–8.65, p < 0.001) to be significantly related to treatment outcome. Patients with the IL28B rs12979860 CC genotype and a low pretreatment γ-GT/ALT ratio achieved the highest rate of a SVR with the highest predictive values (OR = 26.7, 95% CI: 10–71.1, p < 0.0001). ***Conclusion:*** The pretreatment γ-GT/ALT ratio significantly enhances the predictability of the IL28B genotype. Employing this combination will help to identify patients who will most likely benefit from an interferon-α-based combination therapy in a nontriaged ordinary setting.



## Introduction

Hepatitis C virus (HCV) is a positive-stranded RNA virus that chronically infects approximately 3% of the world population [1]. Infection with HCV induces innate and adaptive immune responses that achieve permanent

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Prof. Dr. med. G. Ramadori
Department of Gastroenterology and Endocrinology
Georg-August-University, Robert-Koch-Strasse 40
DE–37075 Göttingen (Germany)
Tel. +49 551 396301, E-Mail gramado@med.uni-goettingen.de

control of HCV in 20–50% of the infected individuals [2, 3]. A failure to clear HCV leads to the development of the clinical complications of chronic hepatitis C. Chronic infection occurs in the majority (55–85%) of individuals infected [4]. As a result, HCV-associated liver disease leading to cirrhosis is still the most common indication for liver transplantation in Western countries [5]. Chronic HCV infection is also a major risk factor for the development of hepatocellular carcinoma [1].

The current standard combination therapy with pegylated interferon-α and ribavirin (PEG-IFN-α/RBV) fails to produce a sustained viral response (SVR) in half of the individuals with chronic HCV genotype 1 (HCV-G1) infection [6, 7]. SVR relies on both viral and host factors [8]. In recent years, various predictive factors for the therapeutic outcome in chronic HCV-G1 infection have been described. These include demographic, virological, laboratory, histological and genetic factors. In all large prospective studies, younger age has been associated significantly with a SVR when assessed by uni- or multivariate analyses [6, 7]. In contrast to age, female gender was a significant factor for SVR in univariate analyses only in both PEG-IFN-α and RBV registration trials [6, 7]. Essentially, HCV baseline viral load, HCV genotype and rapid virological response (RVR) were found to be the most important predictors of a SVR [6, 7, 9, 10].

Dichotomous data exist with regard to baseline alanine aminotransferase (ALT) serum activity. In two prospective trials, serum ALT activity was not related to SVR. In contrast, in retrospective studies using multivariate multinomial logistic regression analysis, high ALT activity was associated with treatment outcome [7, 11, 12]. In addition, low pretreatment serum γ-glutamyltransferase (γ-GT) activity was found to be significantly and independently related to SVR in multivariate regression analyses [12, 13]. A low pretreatment serum γ-GT/ALT ratio was recognized as a simple predictive factor for therapeutic outcome in individuals infected with either genotype 1 or 3 both in retro- and prospective analyses [14, 15]. These two laboratory parameters have also been described as being associated with a successful therapeutic outcome in patients treated with triple therapies [16]. Moreover, multivariate regression analyses have identified histological features as the absence of cirrhosis and the absence of steatosis to be predictive for HCV eradication [17–19].

More recently, several genome-wide association studies have identified genetic variations within the intergenic region of interleukin 28B (IL28B), which is associated both with spontaneous HCV clearance [20] and SVR to antiviral therapy in HCV-G1-infected individuals [21–24]. Moreover, total PEG-IFN-α dose has been associated with treatment outcome in multivariate analysis [25]. Akuta et al. [26] reported that an amino acid substitution in the HCV core region and the genetic variation T/G of IL28B rs8099917 predict the response to triple therapy in individuals with HCV-G1 infection. With respect to a widening spectrum of available direct-acting antiviral agents, reliable predictive factors appear to be even more important in making a therapeutic decision for an IFN-α/RBV-based therapy versus the newer more expensive triple drug therapeutic regimens.

To address this issue, uni- and multivariate analyses for treatment outcome were performed including the host genetic variation (rs12979860) within the IL28B gene and demographical, laboratory, virological and histological parameters, as well as the pretreatment ratio of serum γ-GT/ALT as potential predictive tools to identify chronic HCV-G1-infected individuals who are likely to obtain an SVR in response to an IFN-α/PEG-IFN-α and RBV combination therapy.

## Patients and Methods

*Patients and Study Design*

A total of 264 mainly Caucasian (97%) patients with a chronic HCV-G1 infection (positive for HCV-RNA for more than 6 months) were included in this study and had their records reviewed. Their disease chronicity was confirmed histopathologically utilizing established criteria [27]. The patients, who refused liver biopsy, had their disease chronicity characterized by longitudinal observation in combination with clinical parameters and noninvasive imaging studies. All 264 patients were treated by the Department of Gastroenterology and Endocrinology, University Medical Centre of Göttingen, Germany. Patients with an active hepatitis B virus or human immunodeficiency virus infection, or who continued alcohol abuse or were receiving immunosuppressive medications were excluded. All patients gave written informed consent to participate in the study in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and the Ethical Committee of the University Medical Center. Additionally, written informed consent was obtained from the patients to perform the IL28B genetic testing. The patients were treated with either recombinant IFN-$\alpha_{2a}$ or recombinant IFN-$\alpha_{2b}$ at an initial dose of $3 \times 10^6$ IU three times per week in combination with weight-based RBV (1,000 or 1,200 mg per day) or PEG-IFN-$\alpha_{2b}$ at a dose of 1.5 μg/kg body weight in combination with weight-based RBV (800–1,400 mg per day) or 180 μg PEG-IFN-$\alpha_{2a}$ in combination with weight-based RBV (1,000 or 1,200 mg per day). Depending on the individual's tolerance and response parameters, both the dose and duration were adjusted appropriately. Initial laboratory responses were defined by the normalization of the serum aspartate aminotransferase and ALT activities. Serum HCV-RNA was monitored monthly. RVR was defined as the elim-

ination of viral RNA (<12 IU of viral genomes per ml serum) during the first 4 weeks of therapy. Successful treatment was defined as an SVR (the primary endpoint with HCV RNA below <12 IU of viral genomes per ml serum) 24 weeks after the end of treatment. Enzymatic activities of serum ALT and γ-GT were analyzed by utilizing the automated systems of the central laboratory of the Department of Clinical Chemistry of the University Medical Center Göttingen.

*Histopathological Evaluation*

Liver biopsies were obtained from 201 patients (76%) before the beginning of therapy. Sections (5–10 mm) from formalin-fixed and paraffin-embedded liver tissue samples were stained with hematoxylin-eosin, trichrome, and Prussian blue reaction [28], and evaluated on their necroinflammatory changes (grading) and architectural alterations (staging). Other lesions typical for hepatitis C, such as the presence of steatosis and its degree, were assessed [27].

*Detection of Serum HCV-Specific RNA by RT-PCR*

Serum RNA was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) starting with a volume of 140 μl in accordance with the supplier's spin protocol. One-third of the final eluate was then reversely transcribed and subjected to a highly sensitive nested PCR which has been previously described [29] and/or for quantitative analysis using Abbott RealTime HCV assay with a detection limit of 12 IU/ml.

*Determination of HCV Genotypes*

In HCV RNA-positive sera, virus genotyping was performed using the Innolipa HCV II line probe assay (Innogenetics, Ghent, Belgium).

*Isolation of Genomic DNA*

Genomic DNA was purified from peripheral blood mononuclear cells through the use of the QIAamp DNA Mini Kit following the manufacturer's blood and body fluid spin protocol (Qiagen). The concentration and purity of the isolated DNA was determined photometrically by the absorbance levels at 260 and 280 nm. The integrity of the genomic DNA was ascertained by electrophoresis using a 0.6% agarose gel. When peripheral blood mononuclear cells were not available for analysis, genomic DNA was purified from 2 ml of serum using the QIAamp DNA Blood Midi Kit.

*IL28B Single Nucleotide Polymorphisms Genotyping*

Genomic DNA (5 ng derived from peripheral blood mononuclear cells or an aliquot corresponding to 16.7 μl serum) was amplified in a total volume of 20 μl in real-time PCR using the TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) and 36 μmol/l of each primer in each case (IL28B rs12979860: forward, 5′-GCCTGTCGTGTACTGAACCA-3′; reverse 5′-GCGCGGAGTGCAATTCAAC-3′). Allelic discrimination was achieved by adding 8 μmol/l of differentially fluorescent dye-labeled allele-specific minor groove binder probes (VIC, 5′-TGGTTCGCGCCTTC-3′; FAM, 5′-CTGGTTCACGCCTTC-3′). Reactions and analyses were carried out in the sequence detection system ABI Prism Step One Plus (Applied Biosystems) according to the manufacturer's instructions.

*Statistical Analyses*

Baseline characteristics obtained before starting treatment were identified. The predictive value of the γ-GT/ALT ratio was analyzed for its receiver operating characteristics (ROC) to find the most accurate threshold. The ROC curve is a plot of sensitivity versus (1 – specificity) for all possible cutoff values of the variable being studied. The most commonly used index of accuracy is the area under the ROC (AUROC) curve with values close to 1.0 indicating a high diagnostic accuracy. Dichotomous data (e.g. gender, RVR) were analyzed using Pearson's $\chi^2$ test and continuous data were analyzed by a Mann-Whitney U test. Descriptive analyses of patient's characteristic data and baseline values are reported as median values and range unless otherwise stated. All statistical analyses were performed with the R language and environment for statistical computing (R Development Core Team: R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2011; ISBN 3-900051-07-0; http://www.R-project.org/). Multivariate associations between predictor variables and treatment outcome were modeled using logistic regression models. Variables showing associations with treatment outcome in univariate analysis (significance level <0.10) were included in multivariate logistic regression models. Variables were then eliminated in a step-wise fashion based on the likelihood ratio test and the Akaike information criterion. In general, results were regarded as significant with $p < 0.05$.

## Results

*Baseline Characteristics*

A total of 264 chronic HCV-G1-infected patients were included in this analysis (table 1). Their median age was 51 years (range: 23–70). Most of the patients were Caucasians (97%). Patients were infected with either HCV subtype 1a (30%) or 1b (66%), with a few coinfected with both genotype 1 subtypes (4%). A histological evaluation of a pretreatment liver biopsy was available for 76% of the patients. Nine percent (19/201) of the patients had cirrhosis. Thirty-seven percent (23/63) of the patients without liver biopsy showed indirect signs of bridging fibrosis/cirrhosis by abdominal ultrasound and thrombocytopenia. The majority (54%) of the patient population had been treated at least one time or repeatedly with PEG-IFN-$\alpha_{2a}$, 35% with PEG-IFN-$\alpha_{2b}$ and 11% with standard IFN-α, respectively. Fifty-nine percent of the entire cohort had never received therapy before.

Overall, the genotype distribution at IL28B rs12979860 and the minor allele frequencies in hepatitis C patients were found to be statistically indistinguishable from that of a liver-healthy control group of an ethnically matched population (table 2). For the patients as a whole, no deviations from Hardy-Weinberg equilibrium were identified (IL28B rs12979860, $p = 0.37$).

**Table 1.** Patient characteristics (n = 264)

| | |
|---|---|
| Sex | |
| Male | 153 (58) |
| Female | 111 (42) |
| Age, years | 51 (23–70) |
| Ethnicity | |
| Caucasian | 255 (97) |
| Non-Caucasian | 9 (3) |
| HCV subgenotype | |
| 1a | 79 (30) |
| 1b | 174 (66) |
| 1a+b | 11 (4) |
| Hepatitis activity[a] | |
| Mild | 129 (64) |
| Moderate | 64 (32) |
| Severe | 8 (4) |
| Fibrosis[a] | |
| Absent | 101 (50) |
| Mild | 59 (29) |
| Moderate | 10 (5) |
| Marked | 12 (6) |
| Cirrhosis | 19 (10) |
| Steatosis[a] | |
| Absent | 136 (68) |
| Mild | 52 (26) |
| Moderate | 9 (4) |
| Marked | 4 (2) |
| ALT, U/l | 42 (4–618) |
| γ-GT, U/l | 37 (4–481) |
| γ-GT/ALT ratio | 0.83 (0.1–6) |
| Hb, g/dl | 14.9 (12–18) |
| Platelets, $\times 0^3/\mu l$ | 228 (96–483) |
| White blood cells, $\times 0^3/\mu l$ | 6.8 (4–17) |

Data are given as n (%) or medians (range).
[a] 201 patients underwent histological evaluation.

**Table 2.** IL28B genotype and allele frequencies in HCV-G1-infected patients and noninfected controls

| | Hepatitis C (n = 264) | Control[a] (n = 202) | OR (95% CI) |
|---|---|---|---|
| rs12979860 | | | 0.94 (0.6–1.4)[b] |
| CC | 93 (35) | 74 (37) | |
| CT | 123 (47) | 101 (50) | |
| TT | 48 (18) | 27 (13) | |
| MAF | 0.414 | 0.381 | |

Values are given as n (%) unless otherwise indicated. MAF = Minor allele frequency.
[a] Mangia et al. [34].
[b] Calculated from CC vs. CT and TT.

*Therapeutic Outcome with Regard to Clinical, Laboratory and Virological Factors*

The overall SVR rate of chronic HCV-G1-infected patients was 44%. Seventy-five percent of the patients with an SVR had an RVR. Among parameters potentially related to SVR in chronic HCV-G1 infection, age at initiation of therapy, but not gender, was an important host factor associated with successful treatment outcome (table 3). Younger patients (<50 years) were more likely to respond than older ones. This demographical factor was highly associated with SVR both in univariate (OR = 0.95, 95% CI: 0.93–0.97, $p < 0.001$) and in multivariate analysis (OR = 0.96, 95% CI: 0.94–0.98, p = 0.001; tables 3, 4). The rate of achieving an SVR in patients with genotype 1a or 1b did not differ. Higher [54 (7–618) U/l] pretreatment ALT serum activity (OR = 2.56, 95% CI: 1.69–3.85, p = 0.002) and a lower pretreatment γ-GT activity [29 (4–481) U/l] were associated with a greater likelihood of a SVR (OR = 0.85, 95% CI: 0.59–1.20, p = 0.004; table 3) in univariate analyses. In contrast to pretreatment Hb level, white blood cell and platelet count RVR was highly associated with a SVR in both uni- (OR = 5.18, 95% CI: 3.16–9.00, $p < 0.0001$) and multivariate analysis (OR = 4.18, 95% CI: 2.85–8.65, $p < 0.001$; table 4).

*Therapeutic Outcome with Regard to IL28B Genotype and Pretreatment Serum γ-GT/ALT Ratio*

As shown in figure 1a, 63% of IL28B rs12979860 C homozygotes, 37% of heterozygotes and 25% of T homozygotes achieved a SVR. IL28B C homozygotes showed an OR of 2.9 (95% CI: 1.7–5.1, p = 0.0002) or 5.2 (95% CI: 2.4–11.3, $p < 0.0001$) compared to heterozygotes or T homozygotes to achieve a SVR. This finding persisted using multivariate analysis (CC vs. CT: OR = 2.8, 95% CI: 1.5–4.9, p = 0.001; CC vs. TT: OR = 7.1, 95% CI: 3.1–16.7, $p < 0.001$; table 4). The ratio of γ-GT/ALT was found to be more closely related to SVR than serum activities themselves. Specifically, univariate (OR = 2.33, 95% CI: 1.78–3.70, $p < 0.0001$) and multivariate analyses (OR = 2.5, 95% CI: 1.7 to 3.3, $p < 0.001$) revealed that the pretreatment serum γ-GT/ALT ratio was highly associated with treatment outcome (tables 3, 4). Sensitivity, specificity, and positive and negative predictive values (PPV and NPV) for the desirable genotypes and low pretreatment γ-GT/ALT ratio are reported in table 5. Using a cutoff value of 0.70, sensitivity, specificity, PPV and NPV of the pretreatment γ-GT/ALT ratio was greater than that of the IL28B genotype (table 5). The accuracy index for the pretreatment serum γ-GT/ALT ratio was calculated to be 0.75 (fig. 2). Utilizing both parameters, the SVR increased

**Table 3.** Patient characteristics with regard to therapeutic outcome

| Characteristics | SVR (n = 117) | Relapse (n = 44) | NVR (n = 103) | p |
|---|---|---|---|---|
| Male sex | 76 (65) | 26 (59) | 51 (50) | 0.07 |
| Age, years | 47 (23–67) | 51 (34–63) | 57 (29–70) | <0.001 |
| HCV subgenotype | | | | |
| 1a | 36 (31) | 11 (25) | 32 (31) | 0.60 |
| 1b | 75 (64) | 30 (68) | 69 (67) | |
| 1a+b | 6 (5) | 3 (7) | 2 (2) | |
| ALT, U/l | 54 (7–618) | 33 (12–160) | 40 (4–199) | 0.002 |
| γ-GT, U/l | 29 (4–481) | 36 (4–200) | 47 (5–345) | 0.0004 |
| γ-GT/ALT ratio, | 0.5 (0.1–6.3) | 0.9 (0.1–3.9) | 1.3 (0.2–5.5) | <0.0001 |
| Hb, g/dl | 15.2 (12–18) | 15.2 (13–17) | 14.8 (12–18) | 0.20 |
| White blood cells, $\times 10^3/\mu l$ | 6.9 (4–17) | 6.5 (4–13) | 6.8 (4–13) | 0.16 |
| Platelets, $\times 10^3/\mu l$ | 234 (102–459) | 222 (120–368) | 217 (96–483) | 0.23 |
| RVR | 88 (75) | 17 (39) | 0 | <0.0001 |

Data are given as n (%) or medians (range).

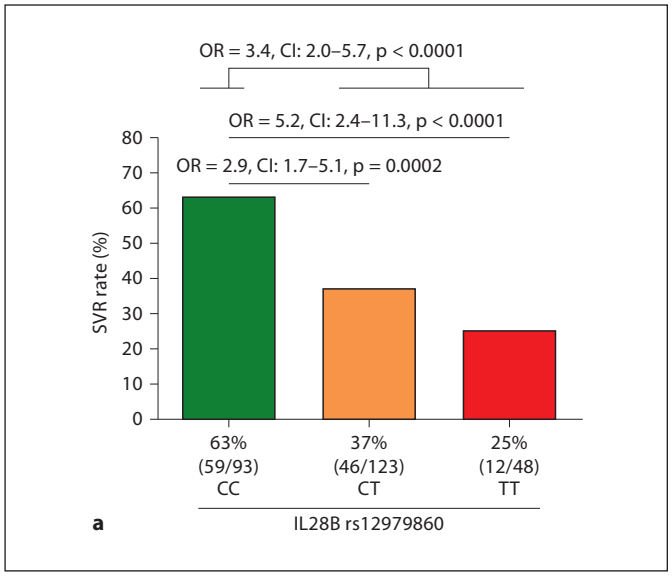
**Table 4.** Uni- and multivariate logistic regression analysis of factors predictive for SVR

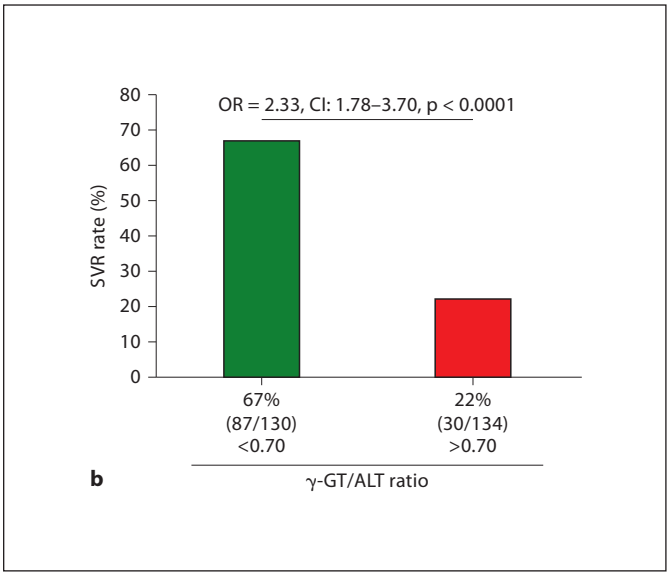
| Characteristics | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | p | OR (95% CI) | p |
| Male sex | 0.99 (0.72–1.36) | 0.07 | 1.00 (0.96–1.04) | 0.18 |
| Age | 0.95 (0.93–0.97) | <0.001 | 0.96 (0.94–0.98) | 0.001 |
| γ-GT/ALT ratio | 2.33 (1.78–3.70) | <0.0001 | 2.5 (1.7–3.3) | <0.001 |
| RVR | 5.18 (3.16–9.00) | <0.0001 | 4.18 (2.85–8.65) | <0.001 |
| IL28B rs12979860, CC vs. CT | 2.9 (1.7–5.1) | 0.0002 | 2.8 (1.5–4.9) | 0.001 |
| IL28B rs12979860, CC vs. TT | 5.2 (2.4–11.3) | <0.0001 | 7.1 (3.1–16.7) | <0.001 |

from 63% (fig. 1a) among IL28B rs12979860 C homozygotes to 87% among patients with a favorable IL28B rs12979860 genotype and a pretreatment serum γ-GT/ALT ratio below 0.7 (fig. 1c), while the SVR in those with a pretreatment γ-GT/ALT ratio <0.70 was 67% (fig. 1b). C homozygotes with a low pretreatment γ-GT/ALT ratio had an OR of 4.8 (95% CI: 1.8–12.5, p = 0.01) and 7.7 (95% CI: 2.4–24.5, p = 0.0008) compared to heterozygotes or T homozygotes, respectively. C homozygotes of IL28B with low pretreatment γ-GT/ALT ratio showed an OR of 26.6 (95% CI: 10–71.1, $p < 0.0001$) compared to CT and TT genotypes of IL28B.

*Therapeutic Outcome with Regard to Histological Findings and PEG-IFN-α Subtype*

Among patients with a histological evaluation of liver, 46% (93/201) revealed a SVR while 17% (33/201) relapsed and 37% (75/201) had either a partial or a null response. The number of patients without steatosis was significantly higher among those who experienced a SVR [80% (74/93)] than among patients who either relapsed [61% (20/33)] or failed to respond [56% (42/75); OR = 1.19, 95% CI: 0.85–1.68, p = 0.005)]. Absence of steatosis, but not the level of hepatitis activity (p = 0.59) or stage of fibrosis (p = 0.08), was associated with a successful treatment outcome. Inclusion of patients without liver biopsy but with indirect signs of cirrhosis in the statistical analysis revealed that the presence of bridging fibrosis or cirrhosis was associated with nonresponse (p = 0.0002; table 6). Treatment-naive HCV-G1-infected patients treated with PEG-IFN-$\alpha_{2a}$ showed a higher SVR rate (60 vs. 38%, p = 0.04) than with PEG-IFN-$\alpha_{2b}$ (data not shown).

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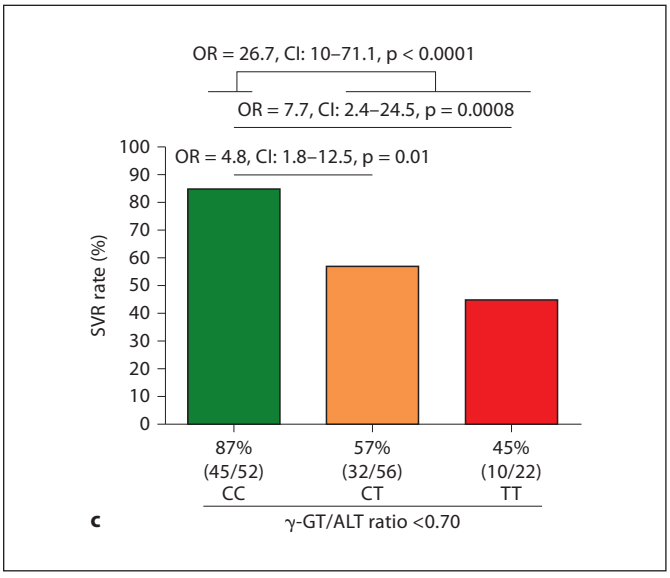


**Fig. 1.** Antiviral treatment outcome in chronic HCV-G1 infected patients with regard to IL28B rs12979860 genotype and pretreatment serum γ-GT/ALT ratio. **a** SVR rates of chronic hepatitis C patients with regard to IL28B rs12979860 genotypes. **b** SVR rates of chronic HCV-infected patients according to their pretreatment serum γ-GT/ALT ratio (cutoff value: <0.70). **c** SVR rates to antiviral combination therapy in hepatitis C patients when both IL28B rs12979860 genotypes and pretreatment low-serum γ-GT/ALT ratio (with a cutoff value of 0.70) were considered.

## Discussion

The major results of this study are: (1) in univariate analysis, IL28B genotype, younger age, ALT and γ-GT levels, pretreatment ratio of γ-GT/ALT, absence of steatosis, and RVR are associated with an SVR; (2) in uni- and multivariate analysis, IL28B genotype, pretreatment ratio of γ-GT/ALT, younger age and RVR are significantly associated with an SVR; (3) pretreatment ratio of γ-GT/ALT with a cutoff value of 0.70 showed higher sensitivity, specificity, PPV and NPV than IL28B rs12979860 genotype, and (4) pretreatment ratio of γ-GT/ALT and IL28B

**Table 5.** Comparison of performance of IL28B rs12979860 CC genotypes and low γ-GT/ALT ratios for predicting SVR

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| Low γ-GT/ALT ratio (cutoff value 0.70) | 78 | 67 | 71 | 74 |
| IL28B rs12979860 genotype CC | 77 | 50 | 66 | 63 |

Data are expressed as percentages.

**Table 6.** Baseline histological features in the 201 patients infected with HCV-G1 biopsied with regard to outcome

| Parameter | All patients (n = 201) | Group | | | p |
|---|---|---|---|---|---|
| | | SVR (n = 93) | relapser (n = 33) | NVR (n = 75) | |
| Steatosis | | | | | 0.005 |
| Absent | 136 (68) | 74 (80) | 20 (61) | 42 (56) | |
| Mild | 52 (26) | 17 (18) | 11 (33) | 24 (32) | |
| Moderate | 9 (4) | 2 (2) | 2 (6) | 5 (7) | |
| Marked | 4 (2) | 0 (0) | 0 (0) | 4 (5) | |
| Fibrosis | | | | | 0.08[a] |
| Absent | 101 (50) | 52 (56) | 19 (58) | 30 (40) | |
| Mild | 59 (29) | 28 (30) | 4 (12) | 27 (36) | |
| Moderate | 10 (5) | 2 (2) | 2 (6) | 6 (8) | |
| Marked | 12 (6) | 3 (3) | 4 (12) | 5 (7) | |
| Cirrhosis | 19 (10) | 8 (9) | 4 (12) | 7 (9) | |
| Hepatitis activity | | | | | 0.59 |
| Mild | 129 (64) | 65 (70) | 20 (61) | 44 (59) | |
| Moderate | 64 (32) | 25 (27) | 12 (36) | 27 (36) | |
| Severe | 8 (4) | 3 (3) | 1 (3) | 4 (5) | |

Data given as n (%). [a] Inclusion of patients without liver biopsy but with indirect ultrasound signs of cirrhosis and thrombocytopenia in the statistical analysis showed a significant association of the stage of fibrosis with treatment outcome (p = 0.0002).

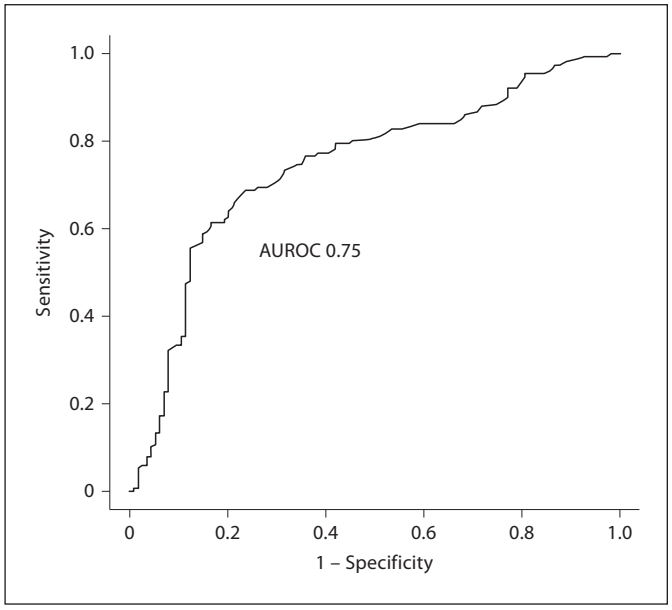


**Fig. 2.** ROC analysis of the γ-GT/ALT ratio revealed an AUROC of 0.75 for a cutoff value of 0.70.

genotype complement each other in predicting therapeutic outcome to dual combination therapy with an 87% SVR rate in the respective favorable group.

Currently, there are multiple factors for predicting an SVR in patients chronically infected with HCV-G1. However, with the approval of new direct-acting antiviral agents that are used in combination with PEG-IFN-α and RBV, either at a fixed dose or dosed according to the body weight, the pivotal clinical trials have shown that approximately 25–35% of treatment-naive patients, as well as 50–60% of those who have previously failed PEG-IFN-α and RBV therapy, fail to achieve an SVR with these new agents (triple combination). Nonetheless, the therapy options for individuals with a chronic HCV-G1 infection are widening [30–32]. To offer these patients the best possible therapy, various predictive factors need be assessed before initiating therapy. These factors include demographics, virology, host allelic variation of IL28B, laboratory baseline levels of ALT and γ-GT, and histological features such as the stage of fibrosis and degree of steatosis. Younger age as a demographic predictive factor is associated with treatment outcome in several prospective and retrospective studies in both univariate analysis and multivariate analysis [6, 7]. In both univariate and multivariate analyses, RVR has been shown to be the most

powerful virological factor for predicting an SVR [9]. Presently, the most powerful genetic predictors in genome-wide association studies are the IL28B genotypes [21–23]. Univariate and multivariate analyses have revealed that these genetic factors are highly associated with treatment outcome in individuals with a chronic HCV-G1 infection. Importantly, these studies evaluating the predictive value of the IL28B single nucleotide polymorphisms were performed on highly selected patients and the reported SVR rate for these with a favorable genotype was 60 or 80% [21, 33]. The present finding of a 63% SVR rate for IL28B rs12979860 C homozygotes in an unselected ordinary chronic HCV-G1 cohort may reflect the expected therapeutic outcome for patients with the preferred genotype more realistically.

Laboratory parameters such as the level of ALT and γ-GT are associated with treatment outcome after antiviral therapy irrespective of receiving either dual or triple therapy combinations [7, 11–13, 16]. None of these studies, however, reported a cutoff value for these two laboratory factors that define a successful treatment outcome. In the present study, the pretreatment γ-GT/ALT ratio with a statistically determined cutoff value of 0.70 was found to be associated with an SVR. Mihm et al. [14] analyzed this ratio retrospectively in 1996, and more recently in a prospective manner in 1999 in chronic HCV-infected individuals with either a genotype 1 or 3 infection [15]. Both studies included only patients treated with IFN-α monotherapy. The current retrospective analysis of 264 patients with chronic HCV-G1 confirms that the finding of the ratio of γ-GT/ALT is an important predictive factor for an SVR when individuals are treated with a combination of PEG-IFN-α and RBV. The present analysis has identified a cutoff value of 0.70 utilizing ROC analysis with a maximum Youden index for genotype 1 patients with regard to the achievement of an SVR. The Youden index describes the cutoff value having both a maximum value for sensitivity and specificity. Moreover, the present study documents the finding of a higher sensitivity, specificity, PPV and NPV for this ratio than those reported for the IL28B single nucleotide polymorphisms with an AUROC of 0.75.

The frequency of more than trivial steatosis in chronic hepatitis C ranges between 40 and 80% [19, 35]. In several studies, it has been suggested that HCV is responsible for the steatosis [36] and that steatosis upregulates hepatocyte CD95/Fas and increases apoptosis [37]. Wedemeyer et al. [37] could show that adiponectin protected hepatoma cell lines from induction of apoptosis. According to the literature, γ-GT levels are also known as a predictor of the presence of steatosis [11, 38]. In two large studies, the absence of steatosis was associated strongly with an SVR in multivariate analyses [18, 19]. In this study, the stage of fibrosis was not associated with treatment outcome. This could be due to two reasons. First, the number of patients [9% (19/201)] with histological presence of cirrhosis might be too small to achieve a statistical significance. Second, 37% (23/63) of the patients without liver biopsy had indirect signs of cirrhosis in ultrasound. Inclusion of these patients in the statistical analysis revealed that bridging fibrosis or cirrhosis was significantly associated with nonresponse.

With respect to specific therapeutic regimens, two recently published prospective trials compared the efficacy of PEG-IFN-$\alpha_{2a}$ and PEG-IFN-$\alpha_{2b}$ in treatment-naive patients and reported significantly greater SVR rates with PEG-IFN-$\alpha_{2a}$, with rates as high as 69% [39, 40]. These observations are clinically important as triple therapy increases viral response rates, but at the cost of additional side effects. A response rate of 44% in genotype 1 patients irrespective of their therapeutic regimens in this study was observed. This response rate is in agreement with the reported prospective pivotal studies of antiviral combination therapy [6, 7]. Patients treated with the new direct antiviral agents achieved up to a 75% SVR. Combining the IL28B genotype with the pretreatment serum γ-GT/ALT ratio identifies individuals who achieve an SVR rate as high as 87% with two-agent combination therapy. The use of both the ratio of γ-GT/ALT and IL28B single nucleotide polymorphism factors enhances the predictability of antiviral combination therapy. In several studies using both uni- and multivariate analysis, a significant association with treatment outcome using the γ-GT level and the serum ALT for predicting an SVR has been shown [11–13]. In contrast to the reported studies, the present investigation focused on the role of the pretreatment γ-GT/ALT ratio. In multivariate analyses, IL28B allelic variation, low pretreatment γ-GT/ALT ratio, younger age and RVR are highly associated with treatment outcome. With data for these factors, the best choice for antiviral treatment can be identified as either a combination therapy consisting of PEG-IFN-α and RBV, or triple therapy with the addition of a direct-acting agent.

In conclusion, the genetic variation of IL28B identifies patients who probably will benefit from antiviral treatment. Pretreatment serum γ-GT/ALT ratio amends the IL28B genotype in predicting an SVR by augmenting the SVR rate to 87% in a nontriaged ordinary cohort. Importantly, the pretreatment serum γ-GT/ALT ratio is a reliable, simple and inexpensive parameter to raise in essentially all clinical centers.

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## Disclosure Statement

None of the authors have a conflict of interest to declare.

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Digestion

# Erratum

In figure 1c of the article by Amanzada et al. [2012;86:218–227] entitled 'High predictability of a sustained virological response (87%) in chronic hepatitis C virus genotype 1 infection treatment by combined IL28B genotype analysis and γ-glutamyltransferase/alanine aminotransferase ratio: a retrospective single-center study' an error occurred.

Different from what is stated in the Results section on page 222, left column, line 9, the text should read: 'C homozygotes of IL28B with low pretreatment γ-GT/ALT ratio showed an OR of 5.5 (95% CI: 2.2–13.7, p = 0.0001) compared to CT and TT genotypes with low γ-GT/ALT ratio. C homozygotes of IL28B with low pretreatment γ-GT/ALT ratio showed an OR of 26.6 (95% CI: 10–71.1, p < 0.0001) compared to CT and TT genotypes with high (>0.70) pretreatment γ-GT/ALT ratio (fig. 1c).' Figure 1c should read as follows: