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ORIGINAL ARTICLE

Ultra-Rapid Virological Response, Young Age, Low γ-GT/ALT-Ratio, and Absence of Steatosis Identify a Subgroup of HCV Genotype 3 Patients Who Achieve SVR with IFN-α_{2a} Monotherapy

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Abstract
Background and Aims The standard treatment regimen for chronic HCV genotype 3 (HCV-G3) hepatitis consists of PEGylated interferon-α (IFN-α) and ribavirin at varying doses ranging from 400 to 1,200 mg and results in response rates of 80%. However, this therapy has substantial side-effects including anemia, is teratogenic, and costly. To reduce the side-effects of therapy, the role of monotherapy consisting of only IFN-α was investigated.
Methods A retrospective analysis of individual therapy courses of HCV-G3-infected patients who were treated with IFN-α_{2a} monotherapy or a combination therapy with attention to the treatment outcome and the presence of IL28B rs12979860 and IL28B rs8099917 single-nucleotide polymorphism genotypes was performed. Conventional prognostic features in each case were assessed as well.
Results In the study, 15/30 (50%) of patients treated with IFN-α_{2a} monotherapy and 32/36 (89%) treated with combination therapy achieved a sustained virological response (SVR). In addition, 7/11 (64%) of those treated initially with monotherapy and subsequently with combination therapy achieved an SVR. An "ultra-rapid" virological response occurring within 2 weeks of initiation of therapy ($p = 0.005$), young age (<40; $p < 0.001$) and low initial γ-GT/ALT-ratio ($p = 0.03$) were associated with a SVR to IFN-α_{2a} monotherapy. An SVR in those treated with combination therapy was found to be associated with a rapid virological response (RVR) ($p = 0.03$). The absence of histologic steatosis was associated with SVR in all patient groups ($p = 0.01$). Therapy duration (24 vs. 48 weeks) did not affect the SVR in either group. As expected, combination therapy resulted in more hematological side-effects than did monotherapy.
Conclusions An "ultra-rapid" virological response, young age, low initial γ-GT/ALT-ratio and absence of steatosis were each associated with an SVR in those receiving IFN-α_{2a} monotherapy. Therefore, monotherapy in these patients should still be discussed independently of the existence of the IL28B polymorphisms.

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Keywords Chronic hepatitis C virus infection · Treatment · Monotherapy · Ribavirin

Introduction

In the 1990s, chronic hepatitis C virus (HCV) infection began to be treated with an interferon alpha (IFN-α) monotherapy. Dependent on HCV genotype, viral load, IFN-α dose, and treatment duration, sustained virological response (SVR) rates of up to 55% could be achieved in patients with HCV non-1 genotypes [1]. Patients with HCV genotype 3 (HCV-G3) infection had SVR rates of 38% [2] to 67% [3] dependent of IFN-α dose. Studies that compared conventional IFN-α monotherapy to PEGylated interferon alpha (PEG-IFN-α) monotherapy achieved SVR rates of 28–46 and 38–68%, respectively [4, 5]. Furthermore, studies that compared the efficacy of the combination therapy of PEG-IFN-α or IFN-α and ribavirin (RBV) to monotherapy used IFN-α dose of 3 million units three times per week. SVR rates of patients with HCV-G3 were thus 21–32% [6, 7] for IFN-α and 38–45% for PEG-IFN-α [5, 8]. In subpopulations of HCV-G3 patients with low baseline viral load, 58% of those who were treated with PEG-IFN-α achieved SVR [8]. However, currently the standard treatment for patients with HCV-G3 is a combination therapy of PEG-IFN-α/RBV for 24 weeks attaining SVR rates of up to 80% [9]. Current recommendations for treatment of HCV genotype 2 or 3-infected patients are based on the result of a trial that compared 24–48 weeks of treatment with PEG-IFN-α/RBV [10]. Patients with HCV-G3 were treated with a schedule (RBV: 800 mg/day or 1,000/1,200 mg/day) similar to that of HCV genotype 1. A subsequent study of chronic HCV genotype 2 or 3-infected patients with PEG-IFN-α and 800 mg/day RBV but reduced duration [11] showed that SVR rates in a subpopulation of patients with low virus load and rapid virological response (RVR) were similar. Dalgard et al. [12] treated patients with chronic HCV-infected genotype 2 or 3 for 14 weeks with PEG-IFN-α and RBV 800 to 1,400 mg/day. SVR rates after 14 weeks of treatment were achieved more frequently among subtype 3a patients with low viral load and absence of bridging fibrosis/cirrhosis. A further study compared reduced doses of RBV (400 and 800 mg/day) for 24 weeks with equivalent outcomes in patients infected with HCV-G3 [13].

Interestingly, comparison of combination therapy with IFN-α monotherapy in patients with early virological response in HCV genotype 2 or 3 showed no significant differences with regard to the outcome [14]. Furthermore, two published studies showed acceptable SVR rates for patients with HCV genotype 2 or 3 after treatment with IFN-α monotherapy [15, 16]. Patients with virological response between 7 days and 2 weeks have the greatest chance of achieving an SVR.

Since the combination therapy with RBV increases the rate of side-effects, the discontinuation rate is more frequent [17]. The combination therapy is associated with a range of treatment-limiting serious adverse events in 4–9% in patients with HCV genotype 2 or 3 [11]. Patients' hemoglobin concentration decreases under combination therapy with RBV significantly [18]. RBV-related anemia is an increased risk in patients with impaired renal dysfunction [19]. Because of the teratogenicity and the accumulation of RBV, contraception is strictly essential during and 6 months after therapy.

For these reasons, the current strategy for the treatment of chronic HCV infection is to individualize the treatment duration guided to HCV genotype and on-treatment viral response. Since the efficacy of therapy significantly depends on the virus genotype and on the host's clinical, biochemical, and genetic background [20, 21], new therapeutic regimens have to be considered. Host genetic predictors for a successful therapy have been identified upstream of the interleukin 28B (IL28B)/IFN-λ3 gene. One single-nucleotide polymorphism (SNP), IL28B rs12979860, was independently and highly associated with SVR in patients with HCV genotype 1 [22]. In HCV genotype 2b patients who were treated with combination therapy, IL28B SNP rs8099917 was an independent factor for SVR, and, conversely, patients with HCV genotype 2a treated with IFN-α monotherapy, IL28B SNP rs8099917 was also an independent factor for SVR [23]. In patients with HCV-G3 treated with the combination therapy both IL28B-SNPs (rs12979860 and rs8099917) were not associated with SVR but with early virological response [24]. The impact of these two SNPs regarding combination therapy or IFN-α monotherapy has not been reported. Two subgroups of patients with HCV-G3 at our center who were treated either with combination therapy or IFN-α_{2a} monotherapy provided the opportunity to assess the impact of the SNP rs12979860 and rs8099917 for SVR in addition to other clinical prognostic markers.

Materials and Methods

Patients

Sixty-six patients with chronic hepatitis C genotype 3 who were HCV-RNA-positive for more than 6 months were treated between 1994 and 2010. Thirty were treated with IFN-α2a monotherapy and 36 were treated with the standard combination therapy consisting of IFN-α and RBV. Eleven of 30 (50%) patients initially treated with IFN-α2a monotherapy did not achieve an SVR and were

subsequently retreated with combination therapy. Disease chronicity was defined histopathologically by using established criteria [25]. In the patients who refused liver biopsy, chronicity was documented by longitudinal observation and the presence of advanced clinical liver disease. Inclusion criteria consisted of the following: (1) chronic hepatitis C by clinical criteria and/or histopathology, (2) HCV-antibody and HCV-RNA positivity, (3) genotype 3 infection. The exclusion criteria were decompensated liver disease, poorly controlled diabetes, clinically active autoimmune disorders, an elevated serum alpha-fetoprotein concentration, active alcohol or injection drug abuse, a history of major psychiatric disease, anemia (hemoglobin < 120 g/l), a reduced white blood cell (WBC) count ($<3{,}000/\mu l$) or thrombocytopenia ($<50{,}000/\mu l$), and pregnancy. In addition, the presence of active hepatitis A virus, human immunodeficiency virus, hepatitis B virus, cytomegalovirus, or an Epstein–Barr virus infection utilizing upon conventional laboratory diagnostic tests resulted in exclusion.

The study was approved by the local ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed written consent was obtained from all subjects.

Laboratory Procedures

Detection of Serum HCV-Specific RNA by RT-PCR

RNA was extracted from serum samples (140 µl) using the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. One-fifth of the extracted material was subjected to a nested RT-PCR procedure as described [26].

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 (PBMCs) through the use of the QIAamp DNA Mini Kit following the manufacturer's blood and body fluid spin protocol (Qiagen). The concentration and the purity of the DNA isolated from PBMCs were determined photometrically by reading the absorbance levels at 260 and 280 nm. The integrity of genomic DNA was ascertained through electrophoresis using a 0.6% agarose gel.

Single-Nucleotide Polymorphism Genotyping by 5′-Nuclease Assay

Genomic DNA (5 ng derived from PBMCs or an aliquot corresponding to 16.7 µl serum) was amplified in a total volume of 20 µl by real-time PCR using the TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) and 36 µmol/l at each primer in each case [IL28B rs rs12979860: forward, 5′-GCCTGTCGTGTAC TGAACCA-3′; reverse 5′-GCGCGGAGTGCAATTCA AC-3′]. Allelic discrimination was achieved by adding 8 µmol/l differentially fluorescent dye-labeled allele-specific minor groove binder (MGB) probes (IL28B: VIC, 5′-TGGTTCGCGCCTTC-3′; FAM, 5′-CTGGTTCACGCC TTC-3′). IL28B rs8099917 was genotyped using a predesigned TaqMan SNP Genotyping Assay (Applied Biosystems; assay ID C__11710096_10). Reactions and analyses were carried out in the sequence detection system ABI prism step one plus (Applied Biosystems) according to the manufacturer's instructions.

Treatment Regimens

IFN-α2a 3-6 Million Units (MU) (Roferon-A, F. Hoffmann-La Roche, Basel, Switzerland) was administered either daily or three times a week in those receiving monotherapy. PEG-IFN-α was administered once a week to those receiving combination therapy. Treatment was for 24 or 48 weeks, as treatment follow-up period of 48 weeks after the end of treatment (EOT) was utilized to define an SVR. All patients with an SVR were undetectable for HCV-RNA in serum 24 weeks and 48 weeks after the EOT. An "ultra-rapid" virological response was defined as serum HCV-RNA negativity at week 2. RVR was defined as undetectable serum HCV-RNA at week 4 of therapy.

Monitoring Procedures

Patients were assessed in an outpatient setting at weeks 2, 4, 6, 8, and every 4th week thereafter during treatment. Follow-up assessments were made at week 24 and 48 after EOT. Clinical examinations consisting of blood cell counts and routine biochemical tests were conducted at each clinic visit. Laboratory values were normalized by dividing the measurement by the upper limit of normal used as a continuous variable. HCV-RNA by RT-PCR was assessed at time zero and at week 2, 4, 12, 24, 36, and 48 during the 48-week treatment regimen and at time zero and weeks 2, 4, 12, and 24 for patients treated for 24 weeks. During the follow-up period, the presence of HCV-RNA in serum was assessed at week 24 and 48 after the EOT.

Histological Evaluation

Before starting therapy, liver biopsies for histological evaluation were taken from 51 (77%) patients. Sections (5–10 μm) from formalin-fixed and paraffin-embedded blocks were stained with hematoxylin and eosin (H&E), trichrome, and Prussian blue. Necroinflammatory activity (grading: score 1–3) and structural alterations (staging: scored 0–4) were scored separately according to Desmet et al. [27]. Other characteristic lesions of hepatitis C, such as the degree of steatosis (score 0–3) were reported and graded as described previously [25].

Statistical Analyses

Continuous and categorical variables were compared between the two groups by Wilcoxon Mann–Whitney test, χ^2 test and Fisher's exact test. A p value less than 0.05 was considered to be statistically significant. Hardy–Weinberg equilibrium calculations cited at http://ihg.gsf.de/cgi-bin/hw/hwl.pl were used as well. All statistical analyses were performed using the free available statistic program R cited at http://www.r-project.org.

Results

Baseline Characteristics of the Patients

Data on 66 chronic HCV-infected patients (17 females, 49 males, mean age of 49 years, ranging from 23 to 61 years) were evaluated retrospectively with regard to the outcome of antiviral therapies and with regard to pretreatment serum enzymatic activities (AST, ALT, and γ-GT) and with regard to pre- and on-treatment hematological parameters (hemoglobin (Hb), and platelet and white blood cell (WBC) counts). Thirty (45%) naive patients had IFN-α_{2a} monotherapy, 36 (55%) naive patients had definitive combination therapy, and 11 (37%) therapy experienced patients with initial IFN-α_{2a} who were non-virological responder (non-SVR) were treated with combination therapy. Table 1 summarizes baseline demographic, biochemical, and virological characteristics of all patients with regard to different therapy regime. The two groups did not differ with regard to gender, age, HCV genotype, normalized AST, ALT, γ-GT activities, and hematological parameters nor IL28B genotype distributions or γ-GT/ALT ratio.

Genotyping of the two SNPs under investigation, IL28B rs12979860 and IL28B rs8099917, revealed distributions and minor allele frequencies (MAF) close to that given for Caucasians in public databases (IL28B rs12979860 CC:CT:TT 33:29:4 MAF 0.280, IL28B rs8099917 TT:TG:GG 49:16:1 MAF 0.136). For the patients as a whole, no deviation from Hardy–Weinberg equilibrium was found (IL28B rs12979860 $p = 0.29$ and IL28B rs8099917 $p = 0.06$).

Table 1 Baseline characteristics of chronic HCV-G3-infected patients provided with an IFN-α_{2a} mono- or combination therapy

	IFN-α_{2a} mono-therapy ($n = 30$)	Combination therapy ($n = 36$)	p value
Gender (female/male)	10/20	7/29	0.32[a]
Mean age (years ± SD)	50.4 ± 9.5	47.8 ± 11.9	0.46[b]
HCV subtype (3a/3c)	22/8	26/10	0.99[a]
AST-N (mean ± SD)	1.18 ± 0.80	1.63 ± 1.25	0.07[b]
ALT-N (mean ± SD)	1.81 ± 1.27	2.46 ± 2.03	0.10[b]
γ-GT-N (mean ± SD)	0.81 ± 0.63	0.86 ± 0.74	0.33[b]
Hb-N (mean ± SD)	1.21 ± 0.09	1.16 ± 0.08	0.53[b]
Platelet × 10^3/μl (mean ± SD)	198 ± 48	215 ± 53	0.13[b]
WBC × 10^3/μl (mean ± SD)	6.69 ± 1.64	7.15 ± 1.88	0.24[b]
IL28B rs12979860 (CC:CT:TT)	13:14:3 (43%:47%:10%)	20:15:1 (55%:42%:3%)	0.37[c]
IL28B rs8099917 (TT:TG:GG)	19:10:1 (63%:33%:4%)	30:6:0 (83%:17%:0%)	0.14[c]
Low γ-GT/ALT-ratio	18 (60%)	28 (78%)	0.32[a]

N normalized

[a] Fisher's exact test

[b] Wilcoxon Mann–Whitney test

[c] χ^2 test

Characteristics of Patients Treated with IFN-α_{2a} Monotherapy

Fifteen of 30 (50%) patients treated with IFN-α_{2a} achieved SVR. Factors found to be significantly associated with response were young age under 40 ($p < 0.001$), "ultra-rapid" virological response within 2 weeks ($p = 0.005$), and low γ-GT/ALT-ratio ($p = 0.03$). Neither gender, HCV subtype, pre-treatment normalized AST, ALT, γ-GT levels, normalized hematological values, IL28B rs12979860 or IL28B rs8099917 genotypes nor therapy duration (24 weeks vs. 48 weeks) were found to be related to treatment outcome (Table 2).

Table 2 Comparison of pre- and on-treatment characteristics of chronic HCV-G3-infected patients treated with an IFN-α_{2a} monotherapy with regard to therapy outcome

	+SVR ($n = 15$)	−SVR ($n = 15$)	p value
Gender (female/male)	6/9	4/11	0.69[a]
Mean age (years ± SD)	38.4 ± 7.8	50.1 ± 9.5	<0.001[b]
HCV subtype (3a/3c)	11/4	11/4	1[a]
AST-N (mean ± SD)	0.97 ± 0.74	0.90 ± 0.41	0.52[b]
ALT-N (mean ± SD)	1.69 ± 1.66	1.33 ± 0.78	0.79[b]
γ-GT-N (mean ± SD)	0.74 ± 0.82	0.58 ± 0.44	0.84[b]
Hb-N (mean ± SD)	1.22 ± 0.11	1.19 ± 0.07	0.93[b]
Platelet × 10^3/µl (mean ± SD)	192 ± 45	203 ± 47	0.60[b]
WBC × 10^3/µl (mean ± SD)	6.12 ± 1.67	6.91 ± 1.42	0.08[b]
IL28B rs12979860 (CC:CT:TT)	6:9:0 (40%:60%:0%)	7:5:3 (47%:33%:20%)	0.12[c]
IL28B rs8099917 (TT:TG:GG)	11:4:0 (73%:27%:0%)	10:4:1 (67%:27%:6%)	0.48[c]
HCV neg. 2 week (ultra-rapid virological response)	12 (80%)	4 (27%)	0.005[a]
Low γ-GT/ALT	12 (80%)	6 (40%)	0.03[a]
Therapy duration/regime (weeks)			0.5[a]
24	6 (40%)	5 (33%)	
48	9 (60%)	10 (67%)	
IFN-α_{2a} (dose)			
24 weeks (9–21 Mio/week)	0	4 (27%)	
24 weeks (18–42 Mio/week)	6 (40%)	1 (6%)	
48 weeks (9–42 Mio/week)	8 (54%)	10 (67%)	
48 weeks (PEG-IFN-α 180 µg/week)	1 (6%)	0	

N normalized

[a] Fisher's exact test

[b] Wilcoxon Mann–Whitney test

[c] χ^2 test

Characteristics of Patients Treated with Combination Therapy

Thirty-nine of forty-seven (83%) patients with combination therapy achieved SVR. Only RVR ($p = 0.03$) as an on-treatment factor was associated with SVR, while neither gender, age, HCV subtype, pre-treatment normalized AST, ALT, γ-GT and pre-treatment, hematological values, therapy duration, nor IL28B rs12979860 or IL28B rs8099917 genotypes were associated with antiviral treatment outcome (Table 3). Evaluated hematological values until 12 weeks differed significantly with regard to response. Patients who developed anemia and thrombocytopenia within 8 weeks and leucopenia within 12 weeks during combination therapy showed association to SVR (data not shown). Patients under combination therapy also showed the development of pancytopenia within 12 weeks under the combination therapy.

Comparison of Hematological Values with Regard to Mono- or Combination Therapy

Further examinations were conducted to evaluate pre- and on-treatment hematological factors in terms of therapy regime. Hb levels of patients with monotherapy decreased but development of anemia could not be observed. Both therapy regimes caused thrombocytopenia and leucopenia but the combination therapy had a more pronounced effect. In comparison to monotherapy patients, under a combination therapy, pancytopenia developed (Table 4), but only decrease of Hb-N within 4 weeks and development of anemia at week 8 and 12 was significantly associated with combination therapy.

Evaluation of Histological Examinations for Outcome

Prior to treatment, 51 patients underwent histological examinations. These results were evaluated with regard to outcome independently of therapy regime. Only absence of steatosis ($p = 0.01$) was found to be associated with SVR, while neither the degree of hepatitis activity nor fibrosis stage were observed to be related to response (Table 5).

Discussion

In this retrospective uncontrolled and monocentric study, the experience of 66 consecutive patients infected with genotype 3 HCV who received either IFN-α_{2a} monotherapy

Table 3 Comparison of pre- and on-treatment characteristics of chronic HCV-G3-infected patients treated with a combination therapy with regard to therapy outcome

	+SVR ($n = 39$)	−SVR ($n = 8$)	p value
Gender (female/male)	9/30	2/6	0.73[a]
Mean age (years ± SD)	49.3 ± 12.1	47.6 ± 7.5	0.15[b]
HCV subtype (3a/3c)	29/10	6/2	0.67[a]
AST-N (mean ± SD)	1.43 ± 1.19	1.64 ± 1.04	0.1[b]
ALT-N (mean ± SD)	2.35 ± 1.99	1.41 ± 0.96	0.23[b]
γ-GT-N (mean ± SD)	0.74 ± 0.59	0.91 ± 1.08	0.35[b]
Hb-N (mean ± SD)	1.18 ± 0.08	1.17 ± 0.09	0.25[b]
Platelet × 10^3/μl (mean ± SD)	214 ± 56	205 ± 38	0.42[b]
WBC × 10^3/μl (mean ± SD)	7.15 ± 1.85	6.81 ± 1.75	0.31[b]
IL28B rs12979860 (CC:CT:TT)	20:16:3 (51%:41%:8%)	4:3:1 (50%:38%:12%)	0.9[c]
IL28B rs8099917 (TT:TG:GG)	29:9:1 (74%:23%:3%)	7:1:0 (88%:12%:0%)	0.48[c]
RVR	31 (80%)	3 (38%)	0.03[a]
Low γ-GT/ALT-ratio	26 (74%)	4 (50%)	0.21[a]
Therapy duration/regime (weeks)			0.66[a]
24	33 (85%)	7 (88%)	
48	6 (15%)	1 (12%)	

N normalized

[a] Fisher's exact test

[b] Wilcoxon Mann–Whitney test

[c] χ^2 test

($n = 19$) or combination therapy consisting of PEG-IFN-α and RBV ($n = 36$) or both ($n = 11$), is reported. The patients were treated for either 24 or 48 weeks. Six months after the EOT, 54/67 patients (82%) had achieved a SVR; 15/30 (50%) responded to IFN-α_{2a} monotherapy while 32/36 (89%) responded to combination therapy with an additional 11 of having initially received monotherapy achieved a SVR with combination therapy. Factors associated with an SVR whether assessed pre- or on-treatment with IFN-α_{2a} monotherapy were age (<40), the development of "ultra-rapid" virological response occurring within 2 weeks of the initiation of therapy and a low initial γ-GT/ALT-ratio. Factors associated with an SVR with the combination therapy were an RVR, decreases of Hb, WBC count, platelet count, and the degree of steatosis. The nadir Hb value on combination therapy was significantly lower than that of IFN-α_{2a} monotherapy group.

Table 4 Comparison of hematological side-effects of IFN-α_{2a} monotherapy and combination therapy

	IFN-α_{2a} monotherapy ($n = 30$)	Combination therapy ($n = 36$)	p value
Hb-N (mean ± SD)			
Pre-treatment	1.21 (±0.09)	1.16 (±0.08)	0.53
4 weeks	1.12 (±0.11)	1.02 (±0.11)	0.04
8 weeks	1.07 (±0.11)	0.95 (±0.12)	0.005
12 weeks	1.03 (±0.11)	0.91 (±0.11)	0.004
Platelet × 10^3/μl (mean ± SD)			
Pre-treatment	198 (±48)	216 (±53)	0.12
4 weeks	157 (±45)	157 (±55)	0.96
8 weeks	146 (±43)	130 (±46)	0.09
12 weeks	142 (±45)	125 (±50)	0.08
WBC × 10^3/μl (mean ± SD)			
Pre-treatment	6.69 (±1.64)	7.22 (±1.88)	0.24
4 weeks	4.23 (±1.73)	4.43 (±1.85)	0.86
8 weeks	4.00 (±1.52)	4.09 (±3.78)	0.24
12 weeks	3.91 (±1.53)	3.20 (±1.16)	0.06

Wilcoxon Mann–Whitney test was applied; *N* normalized

Table 5 Histological features of chronic HCV-G3-infected with regard to outcome *n* (%)

	+SVR ($n = 40$)	−SVR ($n = 11$)	p value
Hepatitis			ns
Mild	32 (80%)	8 (73%)	
Moderate	7 (18%)	2 (18%)	
Severe	1 (2%)	1(9%)	
Fibrosis			ns
Absent	25 (63%)	5 (46%)	
Mild	11 (27%)	3 (27%)	
Moderate	0	0	
Marked	0	2 (18%)	
Cirrhosis	4 (10%)	1 (9%)	
Steatosis			0.01
Absent	20 (50%)	1 (9%)	
Mild	12 (30%)	6 (55%)	
Moderate	7 (18%)	4 (36%)	
Marked	1 (2%)	0	

χ^2 test was applied to compare SVR versus non-SVR with regard to hepatitis (mild vs. moderate or severe), to fibrosis (absent vs. mild, moderate, marked or cirrhosis), and to steatosis (absent vs. mild, moderate or marked)

In chronically HCV-infected patients, SVR rates range up to 80% among patients infected with genotype 2 or 3 [9]. The therapeutic options for individuals with a genotype

3 infection are expanding. Recently, several studies have proposed new strategies for treatment of these patients. These include shortened duration of therapy [28, 29], a reduced dose of RBV and duration of therapy [30], and IFN-α_{2a} monotherapy [16, 31]. These options for individuals infected with genotype 3 are of great interest because combination therapy has many side-effects, are teratogenic, and costly. Moreover, many individuals with a chronic HCV infection are of childbearing age and some have renal disease that makes the use of RBV difficult, if not impossible. As a result, individualized treatment options need to be considered and should be based upon recognized predictive factors for a SVR.

In recent years, many different factors predictive for a successful therapeutic outcome have been described. In addition to the HCV genotype [10], host-specific clinical, laboratory, and genetic factors [22, 26, 32–34] have been recognized. Pre-treatment laboratory factors such as γ-GT/ALT-ratio [26, 34], AST to platelet ratio index (APRI) [24, 35], or clinical and histological factors [36] should also be considered in making a therapeutic decision.

In 2009, three different genome-wide association studies showed that various polymorphisms in the region of IL28B on chromosome 19 are highly associated with response in chronic HCV-genotype-1-infected patients after having sustained treatment with PEG-IFN-α and RBV therapy [22, 37, 38]. These polymorphisms of IL28B were associated with response in treatment-naive and non-responders of genotype-1-infected patients. Yu et al. [39] and Sakamoto et al. [40] investigated the role of IL28B in chronic HCV-genotype-2-infected patients after treatment with PEG-IFN-α and RBV. While Yu et al. [39] reported that the IL28B polymorphism did not predict a SVR but a RVR in HCV genotype 2 patients, Sakamoto et al. [40] reported that patients infected with genotype 2b with the major allele of IL28B showed increased SVR rates. Recently, Moghaddam et al. [24] examined the predictive ability of the IL28B polymorphism (rs12979860 and rs8099917) in patients with chronic HCV-G3 after treatment with combination therapy. They reported that these two SNPs are not predictive for a SVR. These results could be confirmed by Scherzer et al. [41] who suggested an association between IL28B polymorphisms and the early virological response to the application of a PEG-IFN-α and RBV therapy, but no effect was observed on SVR rates in HCV-G3 patients. In this study, we examined the predictive ability of the mentioned IL28B polymorphisms above with regard to monotherapy, but since the sample size of both groups are small, the statistical analysis of IL28B genetic distribution does not have enough power to reveal a significant outcome. However, rather, the findings of either a ultra rapid or RVR [24, 41] or a reduction in Hb [18] during therapy are associated with a SVR.

One of the reasons for the low response to standard IFN-α is its short half-life, which leads to wide fluctuations in the plasma concentration of the drug during the treatment period. Because HCV has a high rate of turnover [42], an intermittent increase in viral load can be observed in patients treated with standard IFN-α on treatment-free days [43]. Despite these disadvantages, Bjoro et al. [44] reported that in patients with genotype 2b/3a, low viral load and without cirrhosis the SVR rate could be increased by IFN-α induction (6 MU IFN-α_{2a} daily for 4 weeks) up to 61%. Akuta et al. [45] and Yokosuka et al. [46] reported SVR rates of 68 and 84% in selected groups of chronic HCV-genotype-2 treated with induction IFN-α monotherapy. In these two studies, 6-10 MU IFN-α was administered daily for 2–4 weeks. The addition of a polyethylene glycol molecule to interferon produces a biologically active molecule with a longer half-life. This characteristic allows a more convenient dosing, i.e., once a week. In case of only applying PEG-IFN-α to patients with chronic HCV (all genotypes) for 48 weeks, the SVR rate was approximately twice (39%) as high as the rate of having applied standard IFN-α to the patients [5]. The subgroup analysis of the prospective randomized study by Fried et al. [8] showed that all HCV-G3 patients achieved SVR rates of 45% after PEG-IFN-α monotherapy. Another prospective randomized study by Angelico et al. [14] showed that selected groups of patients with HCV-genotype 2/3 had a SVR rate of 67% through the application of PEG-IFN-α therapy. Based upon these data, in patients infected with HCV-G3 who are young (<40 years), have a low viral load, histologically mild disease, and a low initial γ-GT/ALT-ratio, monotherapy with IFN-α is effective in 50% of the individuals treated. As mentioned above, considerable data on the use of PEG-IFN-α for the treatment of HCV-infected patients are available in the literature [8, 9]. In contrast, daily dosing of IFN-α consisting of 3 MU for 24 weeks has been reported to achieve a 66.7% SVR rate. The patients who achieved an SVR had an initial virological response after only 2 weeks of IFN-α_{2a} monotherapy [16]. These data are in accordance with the present results and suggest that the adverse effects of RBV can be avoided in a subgroup of patients with HCV-G3 who show an "ultra-rapid" virological response within 2 weeks of the start of IFN-α treatment. Those who do not achieve an "ultra-rapid" virological response can have RBV added to their therapy to enhance the likelihood of their achieving an SVR. This approach would eliminate the potential teratogenic effects of RBV in patients, reduce the severity of the anemia occurring during therapy, and reduce the cost of therapy in those receiving monotherapy who achieve an "ultra-rapid" virological response.

It needs to be recognized that important limitations to the present report exist: (1) It is a retrospective study,

which was placed on (2) a single center, (3), which included only a small sample size referring to each group, and (4) with different therapy regimes within the groups. This study contains an explorative analysis for further suggestions of different therapy regimes for chronic HCV-G3 patients. In order to examine the hypothesis of this retrospective study, a prospective trail should be initiated that should include the suggested criteria by starting PEG-IFN-α_{2a} monotherapy in selected patients.

In conclusion, patients, who are under 40 years, having a low viral load and a low initial γ-GT/ALT-ratio, absence of fibrosis/cirrhosis and steatosis, and who develop an on-treatment "ultra-rapid" virological response can achieve an SVR of 50%. This subgroup of patients may benefit from a treatment regimen consisting of PEG-IFN-α monotherapy.

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