

Prediction of Treatment Outcome in Patients With Chronic Hepatitis C: Significance of Baseline Parameters and Viral Dynamics During Therapy

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In patients with chronic hepatitis C virus (HCV) infection scheduled for a 48-week treatment period, premature discontinuation of treatment was previously recommended if HCV-RNA levels remained detectable at week 24 of therapy. Considering the number of side effects and treatment costs, measurement of initial viral decline during therapy may identify virologic nonresponse earlier than 24 weeks. We retrospectively analyzed 260 European patients treated with standard or pegylated interferon alfa (IFN- α) and ribavirin for 24 to 48 weeks. Early prediction of virologic response by HCV-RNA decline at weeks 4 and 12 (Versant Quantitative [branched DNA (bDNA) 3.0]; Bayer Diagnostics, Emeryville, CA; and Qualitative [transcription-mediated amplification (TMA)] HCV RNA assay; Bayer Diagnostics) as well as clinical, biochemical, virologic, and histologic baseline parameters were analyzed by logistic regression and receiver operating characteristic (ROC) curves. A viral load at treatment week 4 above 450,000 IU/mL and at week 12 above 30,000 IU/mL was 100% predictive for virologic nonresponse in all patients. From multivariate logistic regression analysis of all patients, independent predictors for sustained virologic response were: genotypes 2 and 3 ($P < .0001$), a low baseline gamma-glutamyltransferase (GGT) level ($P < .0001$), a high baseline alanine aminotransferase level ($P = .002$), and a low baseline viral load ($P = .04$). None of the latter 3 factors were predictive for sustained virologic response when analysis was restricted to the subgroup of genotypes 2- and 3-infected patients. In conclusion, virologic nonresponse can be predicted early at week 12 of treatment independent from the applied therapeutic regimen based on a cutoff level for HCV RNA of 30,000 IU/mL. This algorithm recognizes 53.7% of nonresponders previously identified at week 24 of treatment. (HEPATOLOGY 2003;37:600-609.)

Administration of pegylated interferons (IFN) and combination therapy with ribavirin improved considerably the virologic response rates in patients with chronic hepatitis C virus (HCV) infection.¹⁻³

On the basis of the results from pivotal studies^{4,5} and consensus conferences,^{6,7} recommendations for treatment duration were based on clinical, virologic, and histologic criteria. Previously, treatment was discontinued in virologic nonresponders on the basis of a positive qualitative HCV RNA at week 24 of standard combination therapy. More recently, it was suggested to (dis)continue antiviral therapy on the basis of a 2-log decline algorithm already after 12 weeks of treatment.^{8,9} The data for these recommendations were generated in pivotal trials using centralized laboratories.^{3,4,10} General application may be difficult due to the previous lack of standardization of HCV-RNA detection methods.¹¹⁻¹⁴ The HCV-RNA detection assay applied in several pivotal trials (Superquant; NGI, Los Angeles, CA) is not generally available outside the United States. Furthermore, current algorithms for treatment of chronic hepatitis C may have been influenced by marketing interests of pharmaceutical companies who designed and analyzed these multicenter trials. Moreover, these algorithms rely on treatments with certain schedules and generally may not be applicable to

Abbreviations: IFN, interferon; HCV, hepatitis C virus; bDNA, branched DNA; TMA, transcription-mediated amplification; ALT, alanine aminotransferase; ULN, upper limit of normal; GGT, gamma glutamyltransferase; ROC, receiver operating characteristics; CI, confidence interval; PPV, positive prediction value; NPV, negative prediction value.

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either standard IFN or peginterferon therapy with or without ribavirin.

Because of considerable side effects and treatment costs, it is highly desirable to identify virologic nonresponders as early as possible. Recently, it has been shown that virologic response to antiviral therapy is correlated with early viral clearance.¹⁵⁻²⁰ In the present study, we investigated the prognostic relevance of HCV-RNA viral load at baseline, week 4, and week 12 for virologic response to antiviral therapy in patients with chronic hepatitis C treated with different antiviral regimens. The aim of the study was to assess whether virologic response could be determined accurately early during therapy in patients treated with different regimens of (pegylated) IFN- α in combination with ribavirin for 24 to 48 weeks. On the basis of the obtained results, an algorithm for early decision to stop or to continue treatment is proposed. Furthermore, clinical, biochemical, virologic, and histologic pretreatment parameters were analyzed according to their relevance in predicting sustained virologic response.

Material and Methods

Patients. In the present study, individual data from 260 patients (mean age, 44 years; range, 19-72 years; 167 men; 93 women) with chronic hepatitis C were analyzed. Diagnosis of chronic HCV infection was based on elevated serum aminotransferase levels for at least 6 months, histologic examination, and the consistent detection of serum HCV RNA. The patients were anti-HCV positive, and negative for hepatitis B surface antigen and antibodies to human immunodeficiency virus 1 and 2. All patients had been enrolled in 7 different prospective studies and were treated in the hepatology outpatient clinics of the Universities of Berlin, Frankfurt, Kiel, and Munich between 1997 and 2001. To exclude any selection bias of patients only the complete cohorts of the prospective studies from each clinic were enrolled. Written informed consent was obtained from each patient, and the study was approved by the Ethics Committees of Medical Research in Berlin, Frankfurt, Kiel, and Munich in accordance with the 1975 Declaration of Helsinki.

All 260 patients received their first course of antiviral therapy and were treated for 24 to 48 weeks with IFN- α -2a or -2b or with pegylated IFN- α -2a or -2b in combination with ribavirin with the exception of 19 patients who were treated with pegylated IFN- α -2a alone (Table 1).

Virologic response in the prospective trials was assessed by a qualitative HCV-RNA assay with a lower sensitivity of 100 copies/mL (HCV Amplicor 2.0; Roche Diagnostics, Mannheim, Germany; or Superquant; NGI). Ac-

Table 1. Summary of the Different Treatment Protocols

Treatment*	IFN- α Dose	No. of Patients
Induction therapy	10 MU IFN- α -2b daily†	28
	9 MU IFN- α -2a daily‡	84
Pegylated IFN- α -2a	180 μ g once weekly	36
Pegylated IFN- α -2b	1.0-1.5 μ g/kg body weight once weekly	38
Standard IFN- α -2a or -2b	3 \times 3-6 MU IFN- α per week	74
Total		260

*All patients received ribavirin at a dose of 800 to 1,200 mg/d except 19 patients who received pegylated IFN- α -2a monotherapy.

†Induction with daily IFN- α dosing for 24 weeks followed by 3 \times 3 MU IFN- α per week. IFN- α dose was reduced to 5 MU daily after week 2 and to 3 MU daily after week 8.

‡Induction with daily IFN- α dosing for 8 weeks followed by 3 \times 3 MU IFN- α per week. IFN- α dose was reduced to 6 MU daily after week 2.

ording to the qualitative HCV-RNA results, patients were defined as (1) virologic nonresponders (HCV-RNA positive at treatment weeks 24 and 48, n = 82), (2) end-of-treatment responders with relapse (HCV-RNA negative at week 48, but positive thereafter, n = 82), or (3) as virologic sustained responders (HCV-RNA negative at the end of a 24-week follow-up period, n = 140). According to the respective study protocols, treatment was discontinued in 25 virologic nonresponders at week 24 of therapy based on a positive qualitative HCV-RNA test. Genotyping of HCV according to the classification of Simmonds et al.²¹ was performed by reverse hybridization assay (INNO LiPA HCV-II; Innogenetics, Gent, Belgium).

Liver biopsy specimens were fixed, paraffin-embedded, and stained. Hepatic inflammation (grade) and fibrosis (stage) was assessed by a semiquantitative histologic score.^{22,23} The amount of portal/periportal inflammatory activity, lobular inflammatory activity, and degenerative liver cell changes were graded by using a scale of 0 to 3 (0, absent; 0.5, minimal; 1, mild; 2, moderate; 3, severe). The degree of fibrosis was staged by using a scale of 0 to 4 (0, absent; 1, mild without septa; 2, moderate with few septa; 3, numerous septa without cirrhosis; 4, cirrhosis). Liver biopsy specimens were assessed by local pathologists of the University Clinics in Berlin, Frankfurt, Kiel, and Munich, respectively.

Retrospective Quantitative and Qualitative Measurement of HCV RNA. Stored serum samples (-80°C) from baseline as well as from weeks 4 and 12 during therapy were tested retrospectively by the third-generation branched DNA (bDNA) assay (Versant Quantitative HCV RNA 3.0 Assay, Bayer Diagnostics, Emeryville, CA). Serum samples were shipped on dry ice and retesting was performed in the laboratories of the University Clinics of Berlin and Frankfurt. In the third generation of this

bDNA-based, signal-amplification, nucleic acid probe assay, several modifications to enhance signal amplification and to reduce nonspecific binding in comparison with version 2.0 were introduced.²⁴ The bDNA version 3.0 is standardized for IU and the assay was reported linear over its entire dynamic range from the lower detection limit of 615 IU/mL up to 8 million IU/mL.²⁴

In 201 of the 260 patients an additional 500 μ L of stored serum samples were available for qualitative HCV-RNA testing at week 12 of therapy by using transcription-mediated amplification (TMA) assay (Versant Qualitative HCV RNA; Bayer Diagnostics). The lower detection limit of the TMA is reported to be 96% and 100% at 5 and 10 IU/mL, respectively. The clinical specificity is above 99.5%.²⁵ The test system has been described previously in detail.²⁶

Statistical Analysis. A database was created containing the following information: sex; age at baseline; body weight at baseline; body surface area at baseline; treatment regimen; virologic treatment outcome; fibrosis stage and activity grade at baseline liver biopsy; serum alanine aminotransferase (ALT) values at baseline (factor times upper limit of normal, ULN); serum gamma glutamyltransferase (GGT) values at baseline (factor times ULN); HCV-RNA viral load at baseline, week 4, and week 12; and HCV genotype (1, 2, 3, 4, and 6).

For the identification of favorable and unfavorable response factors among the baseline characteristics and the HCV-RNA levels at weeks 4 and 12 of therapy, we compared sustained virologic responders and nonsustained responders by Fisher exact test, χ^2 test, Mantel-Haenszel test, Mann-Whitney test, or univariate logistic regression. To determine cutoff levels of the quantitative factors with significant differences between the response groups, we also analyzed odds ratios and univariate receiver operating characteristics (ROC) showing sensitivity versus 1 – specificity of sustained virologic response for such levels. Here sensitivity describes the proportion of patients fulfilling the threshold from all patients with sustained virologic response whereas specificity describes the proportion of patients not fulfilling the threshold from all patients with nonsustained virologic response. For a balanced optimization of both sensitivity and specificity with a decision-theoretic approach, thresholds were obtained by maximizing specificity plus sensitivity. Multivariate logistic regression analysis was performed to select the independent prognostic parameters. For logistic regression analysis, log values of HCV-RNA viral load, GGT, and ALT were used.

If not stated otherwise all tests were 2-sided and *P* values lower than .05 were considered significant.

Table 2. Patient Characteristics at Baseline and Response to Treatment

	Patients With Chronic Hepatitis C (n = 260)
Age	44.5 \pm 0.7 (19–72)
Sex M/F (percentage men)	167/93 (64.2%)
Body weight (kg)	74.8 \pm 0.9 (47–125)
Body surface area (m ²)	1.87 \pm 0.01 (1.45–2.44)
ALT (U/L)	61.3 \pm 2.6 (20–224)
GGT (U/L)	40.0 \pm 2.8 (6–522)
HCV-RNA viral load at baseline (IU/mL)	1,024,000 \pm 82,000 (616–7,527,091)
HCV genotype	
Type 1	175 (67.3%)
Type 2	21 (8.1%)
Type 3	58 (22.2%)
Type 4	5 (1.9%)
Type 6	1 (0.4%)
Inflammation grade in liver histology	1.34 \pm 0.03 (0.5–3)
Fibrosis stage in liver histology	2.40 \pm 0.06 (0–4)
Number of patients with stage 3 or 4 fibrosis	123 (47.3%)
Virologic response to treatment	
Sustained response	140 (53.8%)
End-of-treatment response with relapse	38 (14.6%)
Nonresponse	82 (31.5%)

NOTE. Normal ranges: ALT <22 U/L for male and <17 U/L for female patients, GGT <28 U/L for male and <18 U/L for female patients. Data are expressed as mean \pm SEM (range) unless otherwise stated.

Results

From the 260 patients, 140 patients (53.8%) achieved a sustained virologic response, 38 patients (14.6%) relapsed after end of treatment, and 82 patients (31.5%) were virologic nonresponders. Baseline characteristics of all patients are summarized in Table 2.

Baseline Characteristics Associated With Sustained Virologic Response. Baseline characteristics significantly associated with sustained virologic response in all patients were genotypes 2 and 3 (*P* < .0001), a low baseline GGT level (*P* < .0001), and a young age (*P* = .03) by univariate comparisons of sustained and nonsustained virologic response with distribution-free statistical procedures. Sex, body weight, the stage of fibrosis, the grade of inflammatory activity in the liver, baseline HCV-RNA levels, and ALT levels were not significantly associated with sustained virologic response. Furthermore, no significant advantage of treatment with pegylated IFN- α or interferon induction therapy for sustained virologic response in comparison with standard IFN- α regimens could be observed. Results from univariate logistic regression analysis were similar but log baseline ALT levels became significant (*P* = .04), whereas the significance of age disappeared. From multivariate logistic regression analysis of

all patients, genotypes, 2 and 3 ($P < .0001$), a low baseline GGT level ($P < .0001$), a high baseline ALT level ($P = .002$), and a low baseline viral load ($P = 0.04$) were independent predictors for sustained virologic response.

When stratifying against genotype, a low baseline HCV level and low baseline GGT levels were significantly associated with response ($P = .02$ and $P = .0001$, respectively), whereas the significance of age disappeared for univariate comparison in genotype 1-, 4-, and 6-infected patients. For genotype 2- and 3-infected patients, only a low body surface area and a low body weight were associated significantly with sustained virologic response ($P = .005$ and $P = .04$, respectively). The same parameters were significant by univariate logistic regression analyses in genotype 1-, 4-, and 6-, and genotype 2- and 3-infected patients, respectively. For genotype 1-, 4-, and 6-infected patients by multivariate logistic regression a low baseline GGT level, a high baseline ALT level, and a low baseline viral load were independent predictors for sustained virologic response. None of these baseline parameters were significant by multivariate logistic regression analysis when restricting to genotype 2- and 3-infected patients. However, regression coefficients were comparable with genotype 1-, 4-, and 6-infected patients.

Prediction Levels. To identify suitable thresholds for baseline GGT levels, baseline ALT levels, baseline HCV-RNA viral load, and the HCV-RNA levels at weeks 4 and 12 of therapy, ROC curves for predicting sustained virologic response for all patients were calculated and specificity plus sensitivity was maximized.

Quantitative Factors at Baseline. The resulting threshold of baseline HCV-RNA levels for predicting virologic response was 130,000 IU/mL and achieved an odds ratio of 2.6 (95% confidence interval [CI], 1.4-5.0), and a positive prediction value (PPV) and a negative prediction value (NPV) of 71.7% and 50.7%, respectively (Table 3, Fig. 1A). Similarly, the threshold of baseline GGT of 0.93 times or less ULN (GGT \leq 16 U/L for

female and GGT \leq 26 U/L for male patients) was obtained and yielded an odds ratio of 5.7 (95% CI, 3.2-10.0), a PPV of 77.7%, and an NPV of 62.1% (Table 3, Fig. 1A). Baseline ALT of 3.18 times ULN or greater or ALT of 53 U/L or greater for female and ALT of 69 U/L or greater for male patients resulted in an odds ratio of 1.8 (95% CI, 1.1-3.1), a PPV of 63.6%, and an NPV of 51.2% (Table 3, Fig. 1A).

Based on the most significant baseline characteristics of HCV genotype, GGT, and viral load, a simple prediction rule was generated (Fig. 1B). For example, patients infected with genotypes 1, 4, and 6 had an overall sustained virologic response rate of 38.1%. In the subgroup of genotype 1-, 4-, and 6-infected patients with a baseline GGT of 16 U/L or less for female patients or of 26 U/L or less for male patients (≤ 0.93 ULN), sustained virologic response rates were 63.2% in comparison with 26.8% in patients with a high GGT level. In genotype 1-, 4-, and 6-infected patients with a low GGT level (≤ 0.93 ULN) and a baseline viral load above 130,000 IU/mL, sustained virologic response rates were 58.9% in comparison with 72.2% in patients with a baseline viremia lower or equal to 130,000 IU/mL. Similarly, individual sustained virologic response rates were calculated for the other subgroups stratified for HCV genotype, GGT level, and baseline viral load (Fig. 1B).

Stratification according to the different treatment schedules (standard interferon 3 times weekly vs. standard induction vs. peginterferon therapy) showed no significant differences for the prediction levels of quantitative factors at baseline.

Quantitative Factors During Therapy. For known quantification of the HCV-RNA level at weeks 4 and 12, baseline HCV-RNA, baseline ALT, and baseline GGT levels became insignificant for prediction of sustained virologic response in multivariate logistic regression analysis. By maximizing specificity plus sensitivity in the ROC analyses an HCV-RNA viral load of 750 IU/mL or less at week 4 of therapy was obtained for all patients and led to

Table 3. Independent Baseline Factors for Sustained Virologic Response (Logistic Regression Analysis)

		Odds Ratio (95 CI)	Sensitivity	Specificity	PPV	NPV
Viral load \leq 130,000 IU/mL	All patients	2.6 (1.4-5.0)	27.1%	87.5%	71.7%	50.7%
	Genotypes 1, 4, and 6	2.9 (1.3-6.1)	29.0%	87.5%	58.8%	66.7%
	Genotypes 2 and 3	2.4 (0.3-20.7)	25.4%	87.5%	94.7%	11.7%
GGT \leq 0.93 \times ULN	All patients	5.7 (3.2-10.0)	58.0%	80.5%	77.7%	62.1%
	Genotypes 1, 4, and 6	4.7 (2.4-9.1)	52.2%	81.1%	63.2%	73.2%
	Genotypes 2 and 3	4.4 (0.8-24.4)	63.8%	71.4%	95.7%	16.7%
ALT \geq 3.18 \times ULN	All patients	1.8 (1.1-3.1)	40.0%	73.3%	63.6%	51.2%
	Genotypes 1, 4, and 6	1.6 (0.8-3.0)	37.7%	72.3%	45.6%	65.3%
	Genotypes 2 and 3	5.1 (0.6-43.8)	42.3%	87.5%	96.8%	14.6%

NOTE. Thresholds were obtained from ROC analyses by maximizing specificity plus sensitivity.

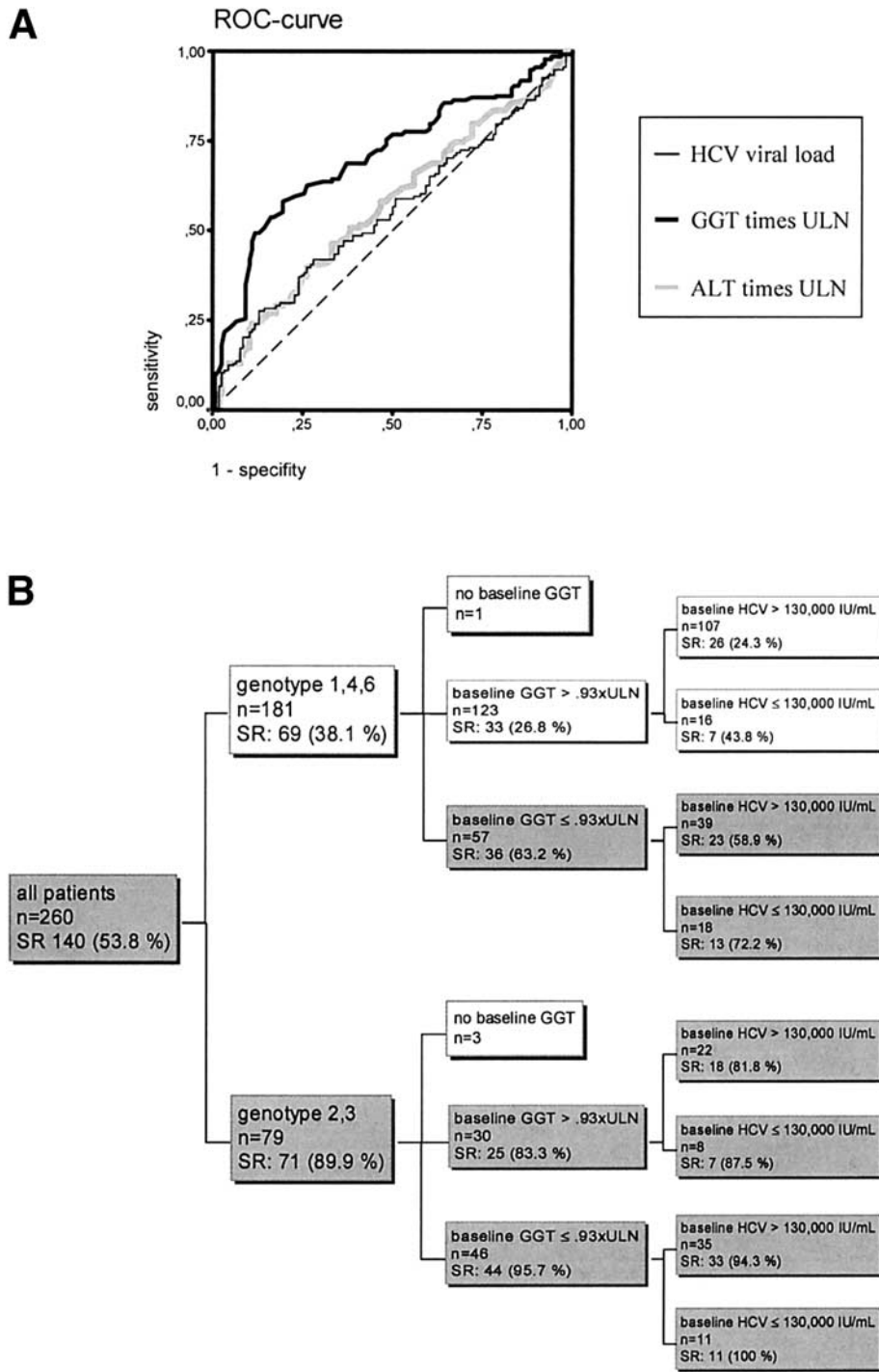


Fig. 1. (A) ROC curves of baseline HCV viral load (**thin black line**), of baseline GGT (times ULN, **thick black line**), and of baseline ALT (times ULN, **thick gray line**) of all 260 patients. (B) Rates of sustained virologic response for subgroups defined by classification to baseline parameters genotype, GGT level, and viral load (in the order of significance levels). Subgroups with a sustained virologic response rate above or equal to 50% are shaded.

an odds ratio of 16.8 (95% CI, 9.1-31.2), and a PPV and NPV of 80.3% and 80.5%, respectively. The maximum sensitivity and an optimal NPV of 100% was reached by using a cutoff level of 450,000 IU/mL for HCV-RNA viral load at week 4 because none of the patients with HCV-RNA concentrations at week 4 above 450,000 IU/mL achieved a virologic end-of-treatment response (Table 4, Fig. 2A). By applying this cutoff level at week 4,

treatment could be discontinued early in 14.6% of virologic nonresponders (12 of 82).

At week 12 the threshold calculated from the ROC curve was an HCV-RNA viral load of 615 IU/mL or less and yielded an odds ratio of 18.2 (95% CI, 8.2-40.5), and a PPV and NPV of 69.8% and 88.7%, respectively (Table 4). Maximizing the sensitivity and NPV to 100%, a cutoff level at 30,000 IU/mL for HCV viral load at week 12 was

Table 4. Viral Load Cutoff Values for Predicting Virologic Response During Therapy

		Odds Ratio (95 CI)	Sensitivity	Specificity	PPV	NPV
Week 4						
≤750 IU/mL*	All patients	16.8 (9.1-31.2)	84.3%	75.8%	80.3%	80.5%
≤450,000 IU/mL†	All patients	∞	100%	10.0%	56.5%	100%
Week 12						
≤10 IU/mL‡	All patients	33.3 (15.1-73.5)	88.9%	80.6%	84.2%	86.2%
≤615 IU/mL*	All patients	18.2 (8.2-40.5)	94.3%	52.5%	69.8%	88.7%
≤30,000 IU/mL‡	All patients	∞	100%	36.7%	64.8%	100%

*Cutoff level was obtained from maximizing relative risk.

†Cutoff level was obtained from maximizing sensitivity and NPV.

‡Analysis of 201 of 260 patients by qualitative assessment of HCV RNA (TMA).

obtained (Table 4, Fig. 2B). Because 44 of 82 patients with virologic nonresponse had a viral load above 30,000 IU/mL at week 12, treatment could be discontinued early in 53.7% of virologic nonresponders. Overall, applying thresholds on log declines instead of viral load itself lead to nearly as favorable results (Fig. 2A). Because 2 patients with a log decline of less than 2 (0.72 and 1.53) during the first 12 weeks achieved a sustained virologic response, a 100% sensitivity level would be below a 0.72 log decline.

Thresholds calculated at weeks 4 and 12 were not significantly different when analyses were based on patients with a certain treatment schedule (standard interferon 3 times weekly, standard induction, and peginterferon therapy).

Similar to the baseline characteristics, sustained virologic response rates were calculated for several subgroups based on genotype and viral load at weeks 4 and 12 (Fig. 2B). As an example, the rate of sustained virologic response in genotype 1-, 4-, and 6-infected patients with a viral load greater than 750 IU/mL at week 4 but below the bDNA detection limit of 615 IU/mL or less at week 12 is 25.6% (Fig. 2B).

In addition to quantitative measurements and for maximizing the positive prediction of sustained virologic response, a highly sensitive qualitative HCV-RNA assay (TMA) was performed for detection of HCV-RNA levels at week 12. When analyses were based on all patients the odds ratio was 33.3 (95% CI, 15.1-73.5) and the PPV and NPV were 84.2% and 86.2%, respectively (Table 4). When analyses were restricted to patients infected with either genotype 1, 4, or 6 or genotype 2 or 3, a PPV of 75.4% and 94.3%, respectively, was observed. Thus, by TMA testing at week 12 the highest positive prediction values were obtained. However, even with the highly sensitive TMA assay a 100% positive prediction of sustained virologic response was impossible.

In the prospective clinical trials, treatment was discontinued prematurely in only 11 of 260 patients. Because of the different study regimens with different IFN- α doses,

analyses for IFN- α dose reduction were not applicable. There was no significant difference between the number of patients with sustained virologic response with (21 of 42, 50.0%) or without (118 of 217, 54.4%) dose reduction of ribavirin before week 24 of treatment.

Discussion

An optimal way to manage antiviral therapy in patients with chronic hepatitis C would be to treat only those patients who will become sustained responders. However, positive prediction of sustained virologic response is still difficult, although different baseline predictors have been described: genotype 2 or 3, a baseline viral load less than 2 to 3.5 million copies/mL (581,000-1,017,000 IU/mL), no or only portal fibrosis, female gender, and age younger than 40 years.^{4,5} However, even in the most favorable population with all positive prediction characteristics the rates of sustained virologic response with standard combination therapy IFN- α plus ribavirin reached only 79%.⁵ Therefore, it was suggested to discontinue therapy early only in those patients who will definitely become virologic nonresponders. In several trials, all patients with detectable HCV-RNA levels in serum or plasma after 24 weeks of therapy became virologic nonresponders. Thus, it was proposed to treat all patients for 24 weeks and then to stop treatment in those individuals with detectable HCV-RNA levels. The data for this proposed algorithm were obtained in large multicenter studies and were based on combination therapy standard IFN- α 3 MU 3 times per week and ribavirin.^{4,10} In these pivotal trials, measurements of viral load were performed with an assay for HCV-RNA quantification (Superquant; NGI) not readily available outside the United States. Furthermore, these trials had been performed before standardization of HCV-RNA quantification according to the World Health Organization standard (IU/mL).²⁷

In the present study, patients treated with different regimens including standard IFN- α 3 times weekly,

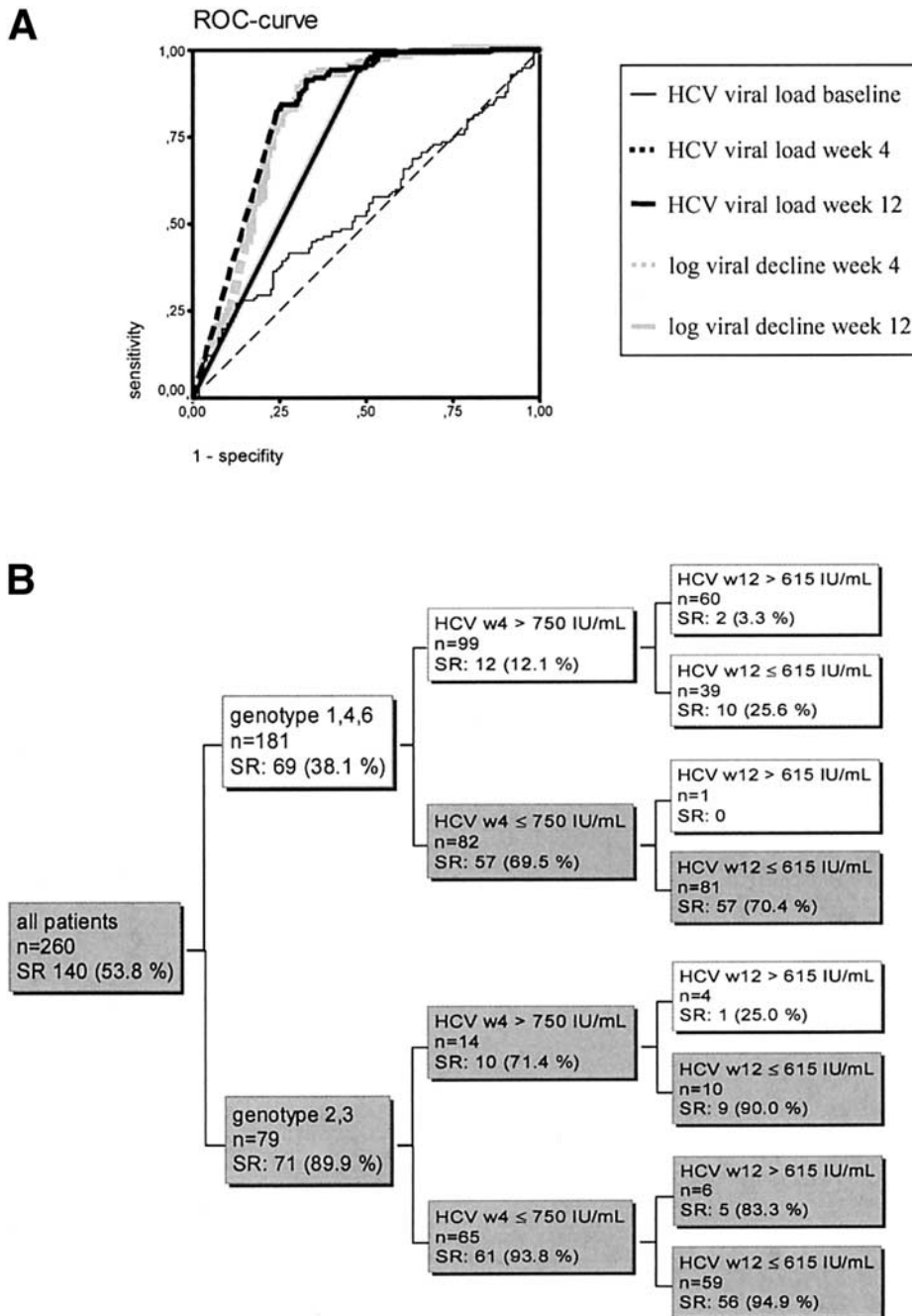


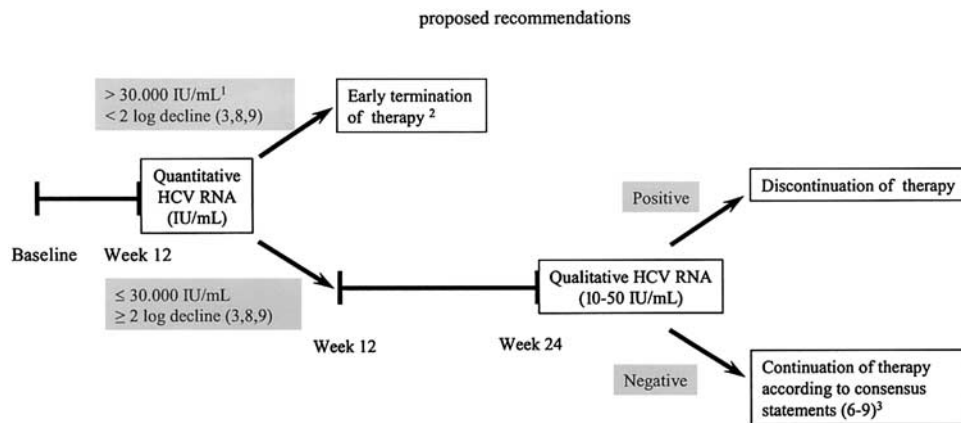
Fig. 2. (A) ROC curves of HCV viral load at baseline (**thin black line**), week 4 (**dashed thick black curve**), and week 12 (**thick black curve**), and of log viral decline at week 4 (**dashed thick gray curve**) and week 12 (**thick gray curve**) from all 260 patients. (B) Rates of sustained virologic response in genotype 1-, 4-, and 6- and genotype 2- and 3-infected patients further classified on the basis of viral load at weeks 4 and 12. Subgroups with a sustained virologic response rate above or equal to 50% are shaded.

IFN- α induction therapy, and treatment with pegylated IFN- α in combination with ribavirin were investigated. Our intention was to generate a more refined approach for treatment decision independent from different treatment schedules and independent from the data banks of the pharmaceutical industry.

Interestingly, of the 5 baseline predictors for sustained virologic response originally described by Poynard et al.,⁵ we only were able to confirm 2: genotypes 2 and 3 and a low baseline viremia. Although age was associated significantly with sustained virologic response, this significance disappeared after stratification for genotypes 1, 4, and 6

and genotypes 2 and 3. Age was a weak predictor in the studies by Poynard et al.⁵ and possibly was not confirmed in the present study because of a smaller study cohort. In the present study, sex was not confirmed as an independent baseline predictor. However, sex was analyzed together with body weight and recent studies already suggested that sex was not a predictive factor when body weight also was considered.^{1,2} The degree of fibrosis also was not confirmed in the present study to be an independent factor for virologic response. A possible explanation for this finding is that in other studies^{2,4,10} assessment of all liver biopsy specimens was performed by the same

Fig. 3. Proposed algorithm for management of patients with chronic hepatitis C during antiviral therapy. ¹Repeat if HCV viral load is between 30,000 and 35,000 IU/mL (two times standard deviation of bDNA interassay coefficient variation). ²A total of 53.7% of virologic nonresponders previously terminated at week 24 according to consensus conferences.^{6,7} ³Evaluation of treatment duration is currently performed in prospective trials.



pathologist, whereas in the present study liver biopsy specimens were investigated in a noncentralized manner by local pathologists. This may lead to significant inter-observer differences but reflects the realistic situation in clinical practice. As an additional baseline parameter, GGT levels were found to be correlated highly with sustained virologic response in all patients. Low GGT levels were described previously as predictors for virologic response²⁸ but were never pursued in the large pivotal trials. Furthermore, in the multivariate logistic regression analysis of the present study, pretreatment ALT levels were found to be correlated positively with sustained virologic response ($P = .002$). Similar to this finding a higher ALT quotient (baseline ALT levels divided by the ULN) was correlated with sustained virologic response in patients treated with peginterferon- α -2a.¹

The subgroup of genotype 2- and 3-infected patients had an overall sustained virologic response rate of 89.9%. This implied that it was statistically difficult to prove additional prediction factors and indeed none of the predictive factors for all patients could be confirmed when analysis was restricted to genotype 2- and 3-infected patients.

To define predictive parameters for virologic nonresponse, which allowed for early discontinuation of therapy, we retrospectively investigated the viral decline of HCV-RNA levels at weeks 4 and 12. Quantitative measurement of HCV-RNA levels was performed with the third-generation bDNA assay, which was standardized to IU/mL. This test is linear over the entire dynamic range from the lower detection limit of 615 IU/mL up to 8 million IU/mL and is available worldwide.^{24,27} For early termination of therapy in virologic nonresponders at week 4 a threshold of 450,000 IU/mL was obtained with an NPV of 100% because all patients with an HCV viral load above 450,000 IU/mL at week 4 became virologic nonresponders. The HCV viral load cutoff level for 100% NPV at week 12 to detect virologic nonresponders was

30,000 IU/mL. By this threshold, antiviral therapy could be discontinued early in approximately 50% of patients who were defined previously as virologic nonresponders at week 24.

Baseline parameters and HCV viral load thresholds at weeks 4 and 12 were not different between the different treatment regimens (standard IFN- α , induction therapy with standard IFN- α , or pegylated IFN- α). Thus, we generally would propose an algorithm for the management of patients during antiviral therapy as shown in Fig. 3. Application of the viral load threshold at week 4 (450,000 IU/mL) is not optimal because at this timepoint only a small group of virologic nonresponders (14.6%) could be identified. Thus, we suggest treating all patients for 12 weeks. At week 12 a quantitative measurement of HCV-RNA level by an assay standardized to IU/mL (Versant HCV Quantitative; HCV Amplicor Monitor; Superquant NGI) should be performed. In patients with a viral load above 30,000 IU/mL at week 12, treatment can be discontinued. For a maximum of confidence, HCV-RNA testing should be repeated for results within the coefficient variation of the test system. The coefficient of intra-assay (interassay) variation for the bDNA test was calculated to be 8.1% (9.1%) at 141,000 IU/mL and 5.5% (4.5%) at 15,000 IU/mL.²⁴ Therefore, based on a 2 times SD distance and the highest coefficient variation (9.1%), HCV-RNA quantification should be repeated in patients with HCV-RNA levels between 30,000 and 35,000 IU/mL. In patients with HCV-RNA levels lower than 30,000 IU/mL, therapy should be continued until week 24. Similar to the recommendations of previous consensus conferences, we would further suggest performing a qualitative assessment of HCV-RNA levels at week 24 and discontinuing therapy in HCV-RNA-positive patients. This is in concordance with the results of the pivotal trial of peginterferon- α -2b/ribavirin combination therapy in which 99% of patients with positive serum HCV-RNA levels at 24 weeks after initiation of therapy

did not achieve a sustained virologic response.² For HCV-RNA–negative patients at 24 weeks, treatment should be continued when longer treatment periods have been proven to be beneficial.⁵⁻⁹ Future studies have to determine the optimal overall treatment duration in patients with initial virologic response according to clinical, biochemical, and virologic parameters.

The algorithm for discontinuation of therapy at week 12 also could be based on a decline of less than 2 logs, instead of on an absolute value of above 30,000 IU/mL. Application of the 2-log decline was recommended recently by the United States (National Institutes of Health) as well as the French (Agence Nationale d'Accréditation et Évaluation en Santé) consensus statement for the management of hepatitis C.^{8,9} However, in the present study 2 patients with a log decline of less than 2 during the first 12 weeks achieved a sustained virologic response. This is in accordance with reports that the 2-log decline at week 12 only achieved an NPV of 97% to 98%.^{3,29,30} Thus, we favor a defined cutoff level at week 12 by an assay standardized to IU/mL for the decision of treatment (dis)continuation. Larger prospective trials using different HCV quantification assays are required for a direct comparison between the 2-log decline algorithm and the 12-week cutoff level of 30,000 IU/mL.

In 126 of 260 patients the week 12 data of HCV RNA data from the original prospective studies were available. The cutoff level at week 12 of 30,000 IU/mL for differentiation between patients with and without virologic response was confirmed with the original HCV-RNA quantification values after conversion from copies/mL to IU/mL. All patients with an original viral load of more than 30,000 IU/mL became virologic nonresponders. Only in one patient with a subsequent sustained virologic response was a value of 31,000 IU/mL obtained at week 12 of treatment. However, according to our recommendations, retesting should be performed in patients with a viral load between 30,000 and 35,000 IU/mL at week 12 to take the assay variability into account (Fig. 3).

In conclusion, HCV genotypes 2 and 3, a low baseline viral load, a low baseline GGT level, and a high baseline ALT level have been shown to be predictive independent baseline parameters for sustained virologic response. It should be noted that because of the high overall response rates in genotype 2- and 3-infected patients, no additional baseline parameter was predictive for sustained virologic response in this subgroup. Positive prediction of sustained virologic response during therapy remains disappointing on the basis of the data available. However, from the data of the present study, treatment can be discontinued in virologic nonresponders already at week 12

based on a viral load measurement above 30,000 IU/mL independent from the therapeutic regimen applied.

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