Nonresponder Patients with Hepatitis C Virus Genotype 2/3 Infection: A Question of Low Systemic Interferon Concentrations?

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Twelve of 303 per-protocol patients were nonresponders in a 12-week versus 24-week treatment study of hepatitis C virus (HCV) genotype 2/3 infection. The nonresponders had significantly lower interferon concentrations, as well as significantly greater mean age, body mass index, and viral load. Suboptimal drug concentrations may thus contribute to lack of response to therapy in patients with infection due to HCV genotype 2/3.

Approximately 50% of patients with chronic hepatitis C virus (HCV) infection due to genotype 1 virus and 20% of those with infection due to genotype 2/3 virus fail to achieve a sustained viral response (SVR) after treatment with pegylated interferon (peg-IFN) and ribavirin [1–3]. These patients can be divided into patients who experience relapse (who achieve undetectable HCV RNA while receiving therapy but experience relapse after the completion of treatment), patients with breakthrough infection, and patients who are nonresponders and never achieve undetectable HCV RNA levels.

Factors predictive of an unfavorable outcome of therapy include infection due to HCV of genotype other than 2/3, baseline viral load >400,000 IU/mL, age >40 years, liver fibrosis, incomplete treatment, high baseline plasma immune protein 10 (IP-10) levels, and African descent [2, 4–6]. The aim of this study was to further define factors associated with nonresponsiveness to therapy among patients with HCV genotype 2/3 infection, with special reference to the impact of IFN and ribavirin concentrations during treatment.

Patients, materials, and methods. Three hundred and eighty-two patients with HCV genotype 2/3 infection were included in the NORDynamiC trial and randomized at baseline to receive either 12 or 24 weeks of combination treatment with ribavirin (800 mg daily) and peg-IFN alfa-2a (180 μg once weekly), with the first dose administered by a study nurse and a patient diary for monitoring subsequent dosing. The primary end point was to compare the efficacy of 12 versus 24 weeks of treatment [7].

Three hundred and three patients were included in the per protocol analysis (ie, they received at least 80% of the target dose of peg-IFN, as well as at least 80% of the target dose of ribavirin for at least 80% of the treatment duration). End-of-treatment samples were available for 295 of these patients (10 Asian patients, 3 black patients, 281 white patients, and 1 Hispanic patient), 12 of whom never achieved undetectable HCV RNA levels in any sample throughout the study and were thus classified as nonresponders (9 patients in the 12-week study arm and 3 patients in the 24-week arm; 11 white patients and 1 Hispanic patient). None of the 12 nonresponders had any dose reduction of peg-IFN, and none reported missing any dose of peg-IFN.

Plasma concentration of IFN alfa-2a was measured at day 3, day 7 (ie, immediately before the second dose of peg-IFN), and day 29. All samples were collected using BD Vacutainer Plasma Preparation Tubes, frozen at −70°C, and subsequently analyzed at a central laboratory. Quantification was performed according to the manufacturer’s instructions (AMS Biotechnology; lower limit of detection, 400 pg/mL). Plasma samples were available from 361 patients on day 3, 357 on day 7, and 359 on day 29.

Serum antibodies to IFN-alfa were determined using quantitative sandwich enzyme-linked immunosorbent assay (Bender MedSystems Diagnostics) according to the manufacturer’s instructions. Through plasma ribavirin concentrations were measured at day 29 and at week 12 by use of solid-phase extraction and high-performance liquid chromatography (Merck-Hitachi).

Nonresponders were compared with responders using Mann-Whitney U test for the following variables: HCV RNA levels at
Figure 1.  
A, Box plots displaying the 10th, 25th, 50th, 75th, and 90th percentiles of the plasma concentrations of pegylated interferon (peg-IFN) \( \alpha-2a \) in pg/mL at treatment days 3, 7, and 29 among responders and nonresponders to combination therapy who had taken at least 80% of both peg-IFN and ribavirin for at least 80% of the planned treatment duration. \( P \) values were obtained using the Wilcoxon-Mann-Whitney \( U \) test. 

B, Correlation between body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) and plasma concentrations of peg-IFN \( \alpha-2a \) in pg/mL at treatment day 3 evaluated by use of Spearman’s rank correlation coefficient \( r_s \) test.

Written informed consent was obtained from each participating patient. Ethics committees in participating countries approved the study, which has been registered at the National Institutes of Health trial registry (ClinicalTrials.gov identifier: NCT00143000).

Results. One of 12 nonresponders achieved a \( <0.5 \log_{10} \) pg/mL decrease at week 12 and thus was classified as a null responder. Five of 12 nonresponders achieved a \( 0.5 \)–\( 2.0 \log_{10} \) pg/mL decrease, and the mean decrease (± standard deviation) for all nonresponders at week 12 was \( 3.3 \pm 2.6 \log_{10} \) pg/mL.

Nonresponders were older (median age, 51 vs 41 years; \( P = .02 \)), had higher BMI (median BMI, 29.4 vs 25.1; \( P = .001 \)), and had higher median baseline HCV RNA level (6.68 vs 6.23 \( \log_{10} \) IU/mL; \( P = .02 \)) than did responders. There was a nonsignificant trend towards a larger proportion liver biopsy findings indicating bridging fibrosis and cirrhosis among nonresponders, whereas no significant baseline differences regarding sex, route of infection, genotype distribution, ALT level, steatosis, baseline IP-10 level, alcohol consumption, or creatinine clearance were observed.

Nonresponders had lower concentrations of IFN at day 7 (median concentration, 550 vs 4850 pg/mL; \( P = .014 \)) and day 29 (median concentration, 6190 vs 9840 pg/mL; \( P = .002 \) by Mann-Whitney \( U \) test) (Figure 1A), with 2 of the 12 nonresponders at day 3, 6 of 12 at day 7, and 5 of 11 at day 29 having undetectable IFN levels. Both nonresponding patients who had undetectable concentrations of IFN at day 3 also had undetectable concentrations at days 7 and 29, and 5 of the 6 patients who had undetectable concentrations of IFN at day 7 also had undetectable concentrations at day 29. In contrast, 10 of 265 responding patients at day 3, 19 of 261 at day 7, and 6 of 265 at day 29 had concentrations of IFN below the level of detection. Four of 6 responding patients without detectable concentrations of IFN at day 29 had BMI ≥25, and 4 of 5 had
detectable levels at day 3. Samples from all patients were negative for antibodies to IFN. There was a nonsignificant trend towards lower concentrations of ribavirin at day 29 and week 12 among nonresponders, compared with concentrations among responders.

A multivariate logistic regression analysis to evaluate which of the explanatory variables, including IFN concentrations, was independently significant was not possible because of the low number of nonresponders. However, an association was noted between higher BMI and low plasma IFN concentrations ($r = -0.205; P < .001$) in the 12 nonresponsive patients 3 days after the first dose of IFN, which was administered under the supervision of a study nurse (Figure 1B). The association between BMI and IFN concentration was also significant on day 7 ($r = -0.282; P < .001$) and day 29 ($r = -0.112; P < .001$). No statistically significant association was noted between age or baseline viral load and IFN concentrations.

Patients with BMI $>$30 had lower IFN concentrations than did those with BMI $<$30 at day 3 (median IFN concentration, 5230 vs 6300 pg/mL; $P = .02$), day 7 (median concentration, 3530 vs. 4980 pg/mL; $P = .002$), and day 29 (median concentration, 7040 vs 10220 pg/mL; $P < .001$).

Discussion. A main finding in this study was that nonresponding patients with infection due to HCV genotype 2 or 3 had significantly lower systemic concentrations of IFN at day 7 and 29 after treatment onset. Similarly, a nonsignificant trend towards lower serum ribavirin concentrations at day 29 and week 12 was noted among nonresponders. Previous studies on the impact of IFN concentrations on outcome, which mostly have been conducted among patients with HCV genotype 1 infection, have yielded discordant results. Jen et al [8] reported that IFN concentrations during IFN monotherapy did not correlate with SVR. In contrast, Diago et al [9] reported that the peak serum concentration of IFN alfa-2a was significantly higher among patients with HCV genotype 1 infection who achieved SVR than it was in those who did not achieve SVR and that a higher induction dosage of peg-IFN alfa-2a (360 μg) for 12 weeks was associated with higher IFN concentrations and improved outcome. Similarly, Howell et al [10] reported that patients with HCV genotype 1 infection who achieved early virologic response had significantly higher plasma concentrations of IFN.

Our study is, to our knowledge, the first to identify low plasma IFN concentrations as a significant risk factor for nonresponsiveness to treatment in patients with HCV genotype 2 or 3 infection. The mechanisms underlying the lower drug concentrations in nonresponders in the present study remain to be elucidated. Early discontinuation or substantial dose reductions are not applicable, because only patients who had taken at least 80% of the prescribed doses of peg-IFN and ribavirin for at least 80% of the planned treatment duration were included in this study. Similarly poorer compliance among nonresponders is unlikely because of the use of a patient diary to monitor correct dosing, and poor injection technique cannot explain the lower concentrations measured at day 3 and 7, because a study nurse supervised the first dose. Moreover, antibodies to IFN could not be detected.

In our study, nonresponders had significantly higher BMI, and within the group of nonresponders, high BMI was strongly associated with lower IFN concentration. A higher BMI may reduce the bioavailability of IFN and ribavirin by deposition of drugs in adipose tissue or through altered metabolism. In analogy with several previous reports, we also found that greater age and higher pretreatment viral load adversely affected outcome.

In conclusion, patients with chronic HCV genotype 2/3 infection who were nonresponsive to combination therapy had significantly lower plasma concentrations of IFN, in addition to established risk factors, such as higher BMI, greater age, and higher baseline viral load. Future prospective studies are warranted to clarify whether older patients with high BMI and higher viral load may benefit from higher doses of IFN and ribavirin and whether monitoring of drug concentration during treatment is of benefit in patients with an insufficient response to antiviral therapy for HCV infection.

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