Iron Reduction Before and During Interferon Therapy of Chronic Hepatitis C: Results of a Multicenter, Randomized, Controlled Trial

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Patients with chronic hepatitis C and low serum and hepatic iron stores may have an improved response to interferon (IFN). We tested whether iron reduction before and during IFN therapy would lead to an improved sustained biochemical and virological response compared with IFN alone. Eighty-two previously untreated patients with chronic hepatitis C were randomized to either: group A IFN-α2b 3 MU 3 times per week for 6 months, or group B iron reduction before and during IFN-α2b 3 MU 3 times per week for 6 months. Group B patients had lower mean serum alanine transaminase (ALT) levels than group A patients during treatment and follow-up. Group B patients had significantly lower mean hepatitis C virus (HCV)-RNA levels at treatment weeks 4 and 12 (P < .05). Serum HCV RNA was undetectable at the end of treatment in 15 group B patients compared with 7 group A patients (P = .03); 7 group B patients and 3 group A patients had persistently undetectable serum HCV RNA 24 weeks after the end of therapy (P = .20). Paired pre- and posttreatment liver biopsies in 18 group B patients demonstrated significant improvements in 2 of the 3 inflammation scores of the Knodell histological activity index (P < .05). No changes occurred in the paired biopsies from 15 group A patients. We conclude that iron reduction via therapeutic phlebotomy improves the end-of-treatment virological and histological response to short-term IFN therapy. Additional studies are needed to determine if iron reduction in combination with higher doses or longer duration of IFN may be of benefit. (HEPATOLOGY 2000;31:730-736.)

Chronic hepatitis C is a leading cause of liver-related morbidity and mortality in the United States and throughout the world. Despite recent progress in treating chronic hepatitis C with interferon (IFN) or IFN and ribavirin, most North American patients still do not experience a sustained virological response with these therapies. Furthermore, these treatments are associated with unpleasant side effects leading to dose reduction in as many as 26%, and early discontinuation of therapy in as many as 21%, of treated patients. Improved and better-tolerated approaches to the management of chronic hepatitis C are needed.

Lesser amounts of hepatic fibrosis, younger patient age, female gender, infection with non–genotype 1 viral strains, and lower levels of serum hepatitis C virus (HCV) RNA at baseline are associated with an improved likelihood of response to IFN. In addition, patients with lower levels of serum ferritin, transferrin saturation, and hepatic iron have been reported to have an improved response to IFN. Several studies have demonstrated that iron reduction via therapeutic phlebotomy leads to improvements in serum aminotransferase levels in patients with chronic hepatitis C. In addition, iron reduction before IFN therapy in small pilot studies has been shown to improve the response to IFN in previous nonresponders with chronic hepatitis C. The aim of this multicenter, randomized, controlled trial was to determine if iron reduction before and during IFN therapy of previously untreated patients with chronic hepatitis C would lead to an improved biochemical and virological response compared with IFN alone.

PATIENTS AND METHODS

Patient Population. Previously untreated patients with chronic hepatitis C without histopathological evidence of bridging fibrosis or cirrhosis were recruited for participation in this randomized, controlled trial between October 1995 and June 1998. Subjects with bridging fibrosis or cirrhosis at baseline were excluded because of their known lower rates of response to IFN and greater likelihood of having increased hepatic iron content, which could have biased the results of the study. All subjects were adult (≥18 years of age) outpatients with detectable anti-HCV antibody and elevated serum alanine transaminase (ALT) levels (>1.3 times the upper limit of normal for >2 months). Other potential causes of chronic hepatitis were uniformly excluded by reviewing the serum ceruloplasmin, antinuclear antibody, smooth muscle antibody, antimitochondrial

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antibody, serum α1-antitrypsin, hepatitis B surface antigen, and serum iron studies, as well as the liver biopsy. We also excluded subjects with serious underlying medical conditions, contraindica-
tions to IFN therapy, human immunodeficiency virus infection, active drug or alcohol use, evidence of leukopenia (<3,000/μL), thrombocytopenia (<70,000/μL), anemia (hemoglobin <12.0 g/dL), or iron deficiency (baseline serum ferritin <15 ng/mL or transferrin saturation <15%). Written informed consent was obtained from all participants. The study protocol and consent forms were approved by the Institutional Review Boards of the 7 participating centers (University of Massachusetts, University of Michigan, Hartford Hospital, Faulkner Hospital, SUNY Syracuse, Lahey Clinic, Mt. Sinai Medical Center). Eighty-two subjects were entered into the trial.

**Study Design and Treatment Regimens.** Subjects were randomized to receive either treatment A, IFN-α2b (Intron-A; Schering-Plough Inc., Kenilworth, NJ) 3 MU 3 times per week for 6 months, or treatment B, iron reduction before and during IFN-α2b 3 MU 3 times per week for 6 months. The randomization was performed at UM ass, the coordinating Center; a stratified randomization scheme was used to assure that, for every 4 patients enrolled by each participating center, 2 would be in treatment A and 2 in treatment B. All subjects underwent standard laboratory and clinical monitoring at treatment weeks −1, 0, 2, 4, 8, 12, 16, 20, 24, and during posttherapy follow-up at weeks 4, 8, 12, and 24. Subjects with persistently abnormal serum ALT levels and detectable HCV RNA levels at treatment week 12 were classified as nonresponders and were offered the opportunity to discontinue participation in this trial so that they could pursue other treatment options.

Subjects randomized to treatment B underwent iron reduction via therapeutic phlebotomy of 400 to 500 mL of whole blood using standard techniques before and during IFN therapy. Initial phlebotomies were performed every 1 to 2 weeks until mild iron deficiency anemia had developed, defined by 2 of the following 3 laboratory criteria: prephlebotomy hematoctrit <35%, serum ferritin <10 ng/mL, serum transferrin saturation <10%. During IFN therapy, additional phlebotomies were performed if the hematocrit exceeded 35% and either the serum ferritin exceeded 15 ng/mL or serum transferrin saturation was greater than 15%.

Serum samples for quantitative HCV RNA were obtained at enrollment, weeks 4, 12, and 24 of treatment, and week 24 of follow-up. HCV RNA testing using quantitative reverse-transcription polymerase chain reaction assay with a lower limit of detection of 100 copies/mL (Superquant25) and HCV genotyping were performed at a central reference laboratory (National Genetics Institute, Culver City, CA).22,23 HFE gene mutational analysis of 27 patients was performed at UM ass using previously described methods.23

**Study End-points.** The primary end-point of this study was the absence of detectable HCV RNA in serum 6 months' posttreatment (SVR). Secondary end-points of the study included the absence of detectable HCV RNA at the end of therapy (ETVR), a biochemical response at the end of treatment (ETBR), and a biochemical response at the end of follow-up (SRB). A biochemical response was defined as a decrease in serum ALT level into the normal range (usually <40 U/L).

**Liver Biopsy.** An adequate (>1.0 cm total length) pretreatment liver biopsy obtained within 12 months of enrollment was required of all subjects. Whenever possible, we also obtained posttreatment biopsies at or near the end of treatment. Coded pretreatment (n = 72) and posttreatment liver biopsies (n = 33) were reviewed by a single pathologist (B.F.B.) who was unaware of the treatment assignment, clinical outcome, or sequence of the liver biopsies. Posttreatment liver biopsies were obtained within 3 weeks of the completion of therapy in most patients. Hepatic inflammation and fibrosis were scored using the Knodell histological activity index (HAI).24 Sections of liver tissue were also stained for iron. The degree of iron staining was semiquantitatively graded using previously described methods.23 In addition, the percentage of portal triads with iron-positive cells was determined.18

**Measurements of Lipid Peroxidation.** Lipid peroxidation was assessed in 48 patients in whom concentrations of 8-epi prostaglandin F2α (BEPGF2) and malondialdehyde in plasma samples were measured at enrollment, weeks 4, 8, 12, and 24 of treatment, and weeks 4, 12, and 24 of posttreatment follow-up. Measurements of BEPGF2 were made using an immunoassay kit following the manufacturer's instructions (Oxford Biomedical Research, Oxford, MI). Concentrations of malondialdehyde were measured by a commercial colorimetric assay (Bioxytech LPO-586; Oxis International, Portland, OR).

**Data Management and Statistical Analyses.** Relevant data were recorded on appropriate forms. The forms were sent to UM ass, where the data were reviewed for accuracy, and a centralized database was established. Initial analyses showed that results were distributed normally. Therefore, parametric statistical procedures were used for subsequent analyses (χ², Student t, paired t tests). All analyses were performed on an intention-to-treat basis. To investigate correlations between baseline characteristics and response to treatment, ANOVA procedures were used, including both univariate and step-wise multivariate regression analyses. Analyses were aided by SAS statistical software (SAS Institute, Cary, NC). P < .05 was considered statistically significant.

**RESULTS**

The clinical, laboratory, and demographic features of the patients in the 2 treatment groups at enrollment were comparable (Table 1). However, by chance, black patients were more often randomized to receive treatment A, while other nonwhite patients were more often randomized to treatment B.

**Serum HCV RNA Levels.** The mean serum HCV RNA levels decreased in both groups during treatment (Fig. 1). Subjects receiving iron reduction + IFN had significantly lower mean HCV RNA levels at treatment weeks 4 (0.81 × 10¹⁰ ± 0.25 vs. 1.71 × 10¹⁰ ± 0.37) and 12 (0.78 × 10¹⁰ ± 0.25 vs. 1.58 × 10¹⁰ ± 0.35) (P < .05). At the end of treatment, HCV RNA was undetectable in 7 of the 41 patients who received treatment A compared with 15 of the 40 patients who received treatment B (P = .03) (Table 2). Among patients who had undetectable HCV RNA at the end of treatment, the levels were already undetectable at week 4 in 2 of 7 (28%) treatment A patients compared with 11 of 15 (73%) treatment B patients (P = .029).

Serum HCV RNA remained undetectable at the end of follow-up in 3 of the patients who had received treatment A compared with 7 who had received treatment B (P = .20). In patients with a sustained virological response, HCV RNA had become undetectable at week 4 in 1 of 3 (33%) treatment A

| TABLE 1. Clinical Characteristics of Study Patients at Enrollment |
|------------------|------------------|------------------|
|                  | Treatment A     | Treatment B      |
|                  | IFN             | Iron Red + IFN   |
|                  | (n = 42)        | (n = 40)         |
| Male (%)         | 26 (62%)        | 23 (58%)         | .69 |
| White (%)        | 34 (81%)        | 33 (82%)         |    |
| Black (%)        | 7 (17%)         | 1 (3%)           | .018 |
| Other (%)        | 1 (2%)          | 6 (15%)          |    |
| Age (yr)         | 43.0 ± 1.26     | 42.6 ± 1.17      | .82 |
| Serum ALT (U/L)  | 132 ± 15        | 110 ± 8.6        | .20 |
| Serum ferritin (ng/mL) | 253 ± 55 | 235 ± 27         | .77 |
| Transferrin saturation (%) | 33.6 ± 2.12 | 34.6 ± 2.3       | .75 |
| Serum HCV RNA (cc/mL × 10⁻⁶) | 2.7 ± 0.33 | 2.9 ± 0.31 | .64 |
| HCV genotype 1 (%) | 32/40 (80%) | 27/38 (71%) | .21 |

*Two-tailed Fisher's exact test for continuous variables; χ² for dichotomous variables. Results are numbers of patients (%) or mean ± SE.
patients compared with 7 of 7 (100%) treatment B patients (P = .054).

**Serum ALT Levels.** In all group B patients in whom data were available (n = 22), serum ALT levels decreased during initial iron reduction before IFN therapy (Fig. 2). Patients who received treatment B had consistently lower serum ALT levels than those who received treatment A during treatment and follow-up. Serum ALT levels were normal at the end of treatment in 20 of the 42 patients treated with IFN alone and 25 of the 40 patients treated with iron reduction + IFN (P = .14). Serum ALT levels remained normal at the end of follow-up in 6 of the patients who received IFN alone and 11 of the patients who received iron reduction + IFN (P = .14). However, serum HCV RNA levels remained detectable after treatment despite persistently normal serum ALT levels in 3 of the patients who received treatment A and 4 of the patients who received treatment B. Similar discrepancies between the sustained virological and biochemical responses have been noted previously.26-28

**Liver Histopathology.** Liver histopathology before treatment was available for review in 35 (83%) treatment A patients and 37 (92%) treatment B patients. The mean pretreatment total HAI and inflammation scores were not significantly different in the 2 groups (data not shown). However, the mean (±SE) pretreatment fibrosis score was lower in group B patients (0.9 ± 0.1 fibrosis units) than in group A patients (1.5 ± 0.2 fibrosis units) (P < .05). There were no appreciable differences before treatment in the presence or degree of stainable iron in sinusoidal endothelial cells in lobular or portal areas in the 2 treatment groups (data not shown). Posttreatment liver biopsies were available in 15 (36%) group A patients and 18 (45%) group B patients. Paired comparisons of the pretreatment and posttreatment biopsies revealed no significant change in the total HAI scores or inflammation or fibrosis subscale scores in the patients receiving IFN alone. In contrast, significant decreases in 2 of the 3 inflammation subscale scores (interface hepatitis and lobular hepatitis) were observed in patients receiving treatment B, while the fibrosis subscale and total HAI score remained unchanged (Table 3).

**Serum Iron Studies.** The mean baseline serum ferritin and transferrin saturation levels were comparable in the 2 treatment groups (Table 1). As expected, the mean pretreatment group B patients (9.0 ± 0.53 vs. 5.7 ± 0.83; P = .001) and the mean volume of blood removed (4.3 ± 0.3 vs. 2.8 ± 0.4 L; P = .003) to achieve initial iron reduction were both greater in men compared with women. In addition, men required a greater number of phlebotomy sessions (1.6 vs. 0.6; P = .01) and blood removal (0.7 ± 0.1 vs. 0.3 ± 0.1 L; P = .01) than women to maintain a state of iron depletion during IFN therapy.

**Correlation of Pretreatment Characteristics With Response.** We tested the following clinical variables as univariate correlates of SVR and SBR in all of the treated patients: gender, ethnic background, HCV genotype, baseline serum ALT, ferritin, transferrin saturation, HCV-RNA level, and total HAI score. Only the pretreatment transferrin saturation level inversely correlated with SVR (P = .03), while female gender showed a trend toward a significant correlation with SBR (P = .07). There were no significant correlations between ethnic background, HCV genotype, pretreatment serum ferritin, HCV-RNA level, serum ALT, or total HAI score with either SVR or

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**TABLE 2. End-of-Treatment and Sustained Biochemical and Virological Response Rates**

<table>
<thead>
<tr>
<th></th>
<th>Treatment A IFN (n = 42)</th>
<th>Treatment B Iron Red + IFN (n = 60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study completion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. completing (%)</td>
<td>30 (71%)</td>
<td>28 (70%)</td>
<td>.94</td>
</tr>
<tr>
<td>No. withdrawn (%)</td>
<td>12 (29%)</td>
<td>12 (30%)</td>
<td>.90</td>
</tr>
<tr>
<td>End of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical response (%)</td>
<td>20 (48%)</td>
<td>25 (62%)</td>
<td>.14</td>
</tr>
<tr>
<td>Virological response (%)</td>
<td>7 (17%)</td>
<td>15 (37%)</td>
<td>.03</td>
</tr>
<tr>
<td>End of follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical response (%)</td>
<td>6 (14%)</td>
<td>11 (27%)</td>
<td>.14</td>
</tr>
<tr>
<td>Virological response (%)</td>
<td>3 (7%)</td>
<td>7 (17%)</td>
<td>.20</td>
</tr>
</tbody>
</table>

NOTE. Biochemical response was defined as serum ALT less than the upper limit of normal (usually < 40 u/L); virological response was defined as a lack of detectable HCV RNA (<100 cc/mL).
she did not experience a virological or biochemical response. Despite the removal of 5.5 L of whole blood followed by IFN, saturation was 36%, and HCV genotype was 2b. There was no significant change.

Her baseline serum ferritin level was 143 ng/mL, transferrin was measured to iron reduction. Reanalysis of our data omitting blacks, who according to recent results have been noted previously (and confirmed in this study) in 30% to 40% of patients with chronic hepatitis C. Whether increased serum or hepatic iron may potentiate the cytotoxic effects of hepatitis C via activation of iron-dependent lipid peroxidation or alteration of immune-mediated responses in the liver is unknown. Because serum ferritin levels correlate with aminotransferase levels and the amount of hepatic iron accumulation correlates with the degree of inflammation and liver biopsy, hepatocytolysis with uptake of iron by hepatic reticuloendothelial cells may be major sources of serum and hepatic iron in chronic hepatitis C.

In group A patients, there was a significant correlation between SBR and non-1 viral genotype (P = .04) and a trend for women to achieve SBR more often than men (P = .07). Among group B patients, there were significant inverse correlations between SBR and baseline serum HCV RNA levels (P = .009) and serum transferrin saturation levels (P = .046). In group B patients, an inverse correlation between SVR was significant for baseline transferrin saturation (P = .01), and there was a trend toward inverse correlations with baseline HCV RNA levels (P = .072) and HAI scores (P = .099). In group B patients, there was also a significant inverse correlation between the total available body iron stores at baseline and SVR (P = .02). The total available body iron stores were estimated from the total amount of blood removed to achieve initial iron reduction. Reanalysis of our data omitting blacks, who according to recent results have poorer response to IFN therapy, did not significantly change the outcomes of the regression analyses (data not shown).

**Study Withdrawal.** The same number of patients in each treatment group (12 in A, 12 in B) withdrew from the study prematurely. In 10 (77%) group A patients and 7 (58%) group B patients, premature withdrawal was a result of nonresponse at week 12. Other reasons for withdrawal included: moving away (1 in B), noncompliance (1 in B), and other reasons (2 in A, 1 in B). Two subjects receiving treatment B developed severe fatigue at weeks 1 and 8 of IFN therapy and were withdrawn from the study. One subject originally randomized to iron reduction + IFN had poor venous access precluding phlebotomy and was treated with IFN alone.

**HFE Mutations.** Serum samples for HFE genotyping were available in 27 patients (9 in group A and 18 in group B). Eighteen of the tested patients demonstrated the wild-type genotype at both the H63D and C282Y positions. Two patients were heterozygous for the C282Y mutation, and 6 patients were heterozygous for the H63D mutation. A 37-year-old white female was homozygous for the H63D mutation. Her baseline serum ferritin level was 143 ng/mL, transferrin saturation was 36%, and HCV genotype was 2b. There was only trace iron staining in her pretreatment liver biopsy. Despite the removal of 5.5 L of whole blood followed by IFN, she did not experience a virological or biochemical response and she withdrew from the study at week 12.

**Lipid Peroxidation.** There were no significant effects of therapy on the plasma levels of 8-epiPGF2α or malondialdehyde (data not shown).

**DISCUSSION**

IFN is recommended for chronic hepatitis C patients with abnormal serum ALT levels and moderate hepatitis on liver biopsy. In addition to hepatitis C viral genotype and hepatic fibrosis, lower levels of serum ferritin, transferrin saturation, and hepatic iron have been associated with an improved response to IFN. In particular, the presence of stainable iron in sinusoidal endothelial cells and/or portal tracts has been correlated with a lower response to IFN. Elevated serum ferritin and transferrin saturation levels have been noted previously (and confirmed in this study) in 30% to 40% of patients with chronic hepatitis C. Whether increased serum or hepatic iron may potentiate the cytotoxic effects of hepatitis C via activation of iron-dependent lipid peroxidation or alteration of immune-mediated responses in the liver is unknown.

Because serum ferritin levels correlate with aminotransferase levels and the amount of hepatic iron accumulation correlates with the degree of inflammation on liver biopsy, hepatocytolysis with uptake of iron by hepatic reticuloendothelial cells may be major sources of serum and hepatic iron in chronic hepatitis C.

Emerging evidence suggests that reduction of serum and hepatic iron stores via therapeutic phlebotomy may lead to improved biochemical and histological outcomes in patients with chronic hepatitis C. Therapeutic phlebotomy in patients with chronic hepatitis C consistently improves serum aminotransferase levels and more recently has been shown to improve liver histology. Phlebotomy before IFN therapy has also been shown to improve the response to retreatment with IFN in previous nonresponders. Therefore, we hypothesized that iron reduction + IFN may lead to an improved response in previously untreated patients with chronic hepatitis C compared with IFN alone.

In this study, patients who received iron reduction + IFN had consistently lower mean serum ALT levels during both treatment and follow-up. In addition, patients receiving iron reduction + IFN had lower mean serum HCV-RNA levels during treatment and demonstrated a trend toward earlier and improved sustained virological response compared with patients receiving IFN alone. Paired comparison of pre- and posttreatment liver biopsies revealed no significant change in the total HAI or component scores in the patients treated with IFN alone. However, significant decreases in 2 of the 3 component scores of inflammation were noted in the iron reduction + IFN patients. Therefore, the results of this study demonstrate that iron reduction + IFN is associated with more frequent and rapid suppression of HCV RNA during treatment and significant improvements in liver histology compared with IFN alone. However, the improvements in SBR and SVR with iron reduction + IFN failed to reach statistical significance at the 5% level.

**Stepwise Multiple Regression Analysis.** It is hypothesized that iron reduction + IFN may lead to an improved response in previously untreated patients with chronic hepatitis C compared with IFN alone. In the 42 patients receiving...
IFN alone, women and those infected with non-1 HCV genotypes were more likely to achieve an SBR as reported in other studies. The lack of a clear association between HCV genotype and SVR may be the result of the small number of patients achieving viral eradication in both treatment groups. In the 40 patients receiving iron reduction + IFN, SVR was inversely correlated with baseline serum transferrin saturation and the total available body iron stores. These findings suggest that baseline serum and total body iron stores are important predictors of response to IFN therapy, and that patients with low serum and hepatic iron stores may particularly benefit from iron reduction + IFN therapy. These results may also help explain why women with chronic hepatitis C, who have lower levels of iron at baseline, have better responses to IFN than men.

The patients entered into this multicenter, randomized, controlled trial had comparable pretreatment clinical and laboratory characteristics (Table 1). However, through the vagaries of randomization, black patients and those with higher pretreatment fibrosis scores were more likely to be randomized to treatment A. Both of these factors have been associated with a poorer response to IFN and may have contributed to a lower sustained virological response in group A patients compared with group B patients. However, at the time that this study was designed, there were no data to suggest that blacks may have a poorer response to IFN than whites. As a result, stratified randomization based on ethnic background was not performed. In addition, in our regression analyses, neither patient race nor baseline hepatic histopathology was correlated with outcome regardless of the treatment received. In addition, when we reanalyzed our data omitting blacks, the results did not significantly change.

Higher doses or a longer duration of IFN therapy might have led to an improved SVR in patients receiving iron reduction + IFN. At the time that this study was designed and initiated, however, IFN (3 MU 3 times per week for 6 months) was the recommended dose and duration of therapy for previously untreated patients with chronic hepatitis C. Since then, we have learned that a longer duration of IFN (i.e., 12 vs. 6 months) is associated with a greater sustained biochemical and virological response. In addition, other recent studies suggest that higher doses of IFN, as well as daily administration of IFN, lead to a more rapid suppression of HCV RNA levels than standard doses of IFN. In one study of previous nonresponders to IFN alone, iron reduction before and during retreatment with high doses of daily IFN (5 MU per day for 6 months) led to a SVR of 6 of 15 (40%) compared with 2 of 15 patients (13%) retreated with 5 MU per day for 6 months without iron reduction. Therefore, a greater benefit from iron reduction + IFN might have been achieved if higher doses or a longer duration of IFN had been used.

Another study, similar in design to ours, comparing the potential benefit of iron reduction + short-term IFN with IFN alone in 38 previously untreated patients was recently reported by Fong et al. The 17 patients treated with iron reduction + IFN demonstrated a trend toward improved SVR compared with the 21 patients receiving IFN alone (29% vs. 5%; P = .07). Another study from Italy also demonstrated an improved response to iron reduction + IFN compared with IFN alone in 103 patients with noncirrhotic chronic hepatitis C. The similar results obtained in these 3 studies suggest that iron reduction + IFN may be superior to IFN alone, but a larger sample size may be needed to demonstrate a statistically significant difference. Other recent data also suggest that long-term maintenance of hepatic iron reduction via therapeutic phlebotomy in chronic hepatitis C decreases the incidence of hepatic decompensation and hepatocellular carcinoma. Thus, iron reduction may be beneficial in hepatitis C even though it does not lead to viral eradication.

Therapeutic phlebotomy before and during IFN was well tolerated in our study, with only 2 (5%) patients reporting adverse events that required early discontinuation. The overall adverse-event rate was comparable in the 2 treatment groups. The low number of therapeutic phlebotomy sessions required to achieve mild iron deficiency anemia suggests that the increase in hepatic iron content in these noncirrhotic patients with hepatitis C was absent or mild as previously reported. Semiquantitative analysis of the lobular and portal distribution of iron in pretreatment liver biopsies also demonstrated low hepatic iron levels (data not shown). Although serum ferritin and transferrin saturation levels were elevated in 33% and 20%, respectively, of study subjects before treatment, HFE mutational analysis demonstrated that none of the 27 subjects tested had a genotype associated with the genetic hemochromatosis phenotype (C282Y homozygote or compound heterozygote, C282Y/H63D). The frequencies of heterozygosity for the C282Y and H63D mutations observed in our patients were comparable with those reported in other studies of patients with chronic hepatitis C, other forms of chronic liver disease, and normal controls. One patient was homozygous for the H63D mutation, but her hepatic iron content was not increased. It remains unclear if H63D homozygotes are at risk of developing phenotypic hemochromatosis.

No significant differences in the plasma levels of lipid peroxidation markers were noted during treatment or follow-up in the 2 treatment groups (data not shown). Although we are uncertain as to the reasons for a lack of an effect of treatment on measures of lipid peroxidation, it is possible that the assays used were insufficiently sensitive and precise to detect changes caused by iron reduction and/or IFN. In addition, it is possible that the increases caused by chronic hepatitis C may have overshadowed the effects of the therapies used.

In summary, our results demonstrate that iron reduction before and during IFN therapy is well tolerated and associated with greater improvements in end-of-treatment virological and histological response rates than IFN alone. However, perhaps as a result of the limited number of patients enrolled and the low doses and short duration of IFN therapy used, the SVR and SBR with iron reduction + IFN was not significantly greater than that seen with IFN alone. Nonetheless, this study and others suggest a role for iron as a comorbid factor in mediating liver injury in chronic hepatitis C. We conclude that iron reduction + IFN is a safe and potentially promising means of treating patients with chronic hepatitis C. However, large, randomized, controlled trials using iron reduction with higher doses or longer duration of IFN therapy will be needed before iron reduction + IFN can be recommended as an established means of treating patients with chronic hepatitis C. When newer approaches to manage-
ment of chronic hepatitis C are developed (e.g., helicase or RNA polymerase inhibitors), it may be worthwhile assessing iron reduction as an adjunctive therapy.

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