Hepatitis C, Iron, and Hemochromatosis Gene Mutations

A Meaningful Relationship or Simple Cohabitation?

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Since the discovery of hepatitis C and the subsequent use of interferon as treatment for chronic infections, hepatologists have been searching for criteria that might predict which patients are likely to benefit from therapy. For patients with an inadequate or no response to therapy, methods to improve or augment therapy have been sought. Factors generally considered predictive of a poor response to therapy include infection with genotype 1 virus, long duration of infection, high viral load, and fibrosis revealed on biopsy.[1,2] Most of these factors are not used generally to make decisions about treatment, however.[2] Hepatologists have been particularly ambiguous in their use of histologic features. Several studies indicate that fibrosis and piecemeal necrosis (interface hepatitis) are predictors of poor response to therapy, yet these same factors (particularly fibrosis) are used as arguments in favor of initiating interferon therapy (since fibrosis indicates that the patient is being harmed by the virus).[2] Another factor proposed by some as important in determining response to therapy and also as providing a potential way to improve therapy is iron accumulation in the liver.

The article in the current issue of the *Journal* by Pirisi et al[3] adds to a number of reports published during the last several years dealing with the issue of hepatitis C, iron deposition, and mutations of the hemochromatosis (*HFE*) gene.[3] The published works in this area vary markedly in the number of patients studied and the methods used, particularly differences in how iron content is assessed, making it difficult to compare the studies and to derive firm conclusions. The article by Pirisi et al[3] describes a moderately sized study that investigated both iron deposition and mutations in the *HFE* gene in a reasonably rigorous fashion. In particular, it reviewed the liver biopsy with an eye toward the pattern of deposition of iron within different cellular compartments of the liver rather than simply reporting overall iron levels, something that many articles on the subject do not do. It also directly addresses the issue of the relationship of mutations in the *HFE* gene with stage and grade of disease, as well as with iron accumulation, with some information provided on response to therapy.

So what are the relevant issues, and what were the findings of this study and others? The main issues involve the following: (1) whether iron deposition itself predicts response to therapy for hepatitis C, (2) whether this effect, if any, is the result of the iron or if the iron accumulation is the result of other factors (in particular fibrosis) that are more important predictive factors, and (3) whether removal of the iron improves the outcome of therapy.

For the first question, there is consensus (with a few exceptions[4]) that increased liver iron deposition, particularly as measured by histologic assessment, is correlated with a poor response to interferon therapy.[5-11] Most articles that provide data also report that iron accumulation is associated increased fibrosis.[5-7,12-15] The article by Pirisi et al[3] confirms these findings. The finding of excess iron in association with increased fibrosis suggests that iron might act as a cofactor increasing liver damage or, alternatively, that livers with preexisting fibrosis tend to accumulate iron or possibly that these are unrelated coincidental findings related to a third factor, such as duration of disease or age. Unfortunately, none of the articles in the literature address this topic directly. Perhaps the only way to address it would be with a series of sequential biopsies in individual patients to determine whether the iron deposition or the fibrosis comes first. Such a study may well never be done, and in many ways, the question is somewhat semantic from prognostic and therapeutic viewpoints. It is interesting that hepatitis C is not unique in

regard to the issue of iron and fibrosis; there are studies indicating almost identical findings for nonalcoholic steatohepatitis, although this is not a universal finding,[16-18] and iron also has been implicated as a cofactor in hepatitis B. It would seem that excess iron deposition is bad for the liver. Several articles reported that histologic iron deposition was associated with a poor response to therapy, even though the same patients did not show increased total liver iron levels by quantitative determinations.[7,8] In these studies, the histologic features of the liver generally demonstrated very small amounts of iron in endothelial or sinusoidal cells, which has been the general finding.[19] This would indicate that if there is a role for evaluating iron deposition, the tool for measurement should be histologic assessment rather than quantitative iron measurements.

The second relevant issue regards the cause of the iron deposition. In the past, the generally held view was that patients with viral hepatitis had iron deposition because of loss of iron from necrotic hepatocytes, which was picked up and concentrated by Kupffer cells, leaving a scattering of iron-laden Kupffer cells throughout the hepatocellular lobule.[20] These pigmented Kupffer cells then were considered a marker of recent hepatitis. With the discovery of the gene mutation responsible for most cases of hemochromatosis (the *HFE* gene), a new view has emerged that iron accumulation in these cases might be the result of mutations in the *HFE* gene, with a particular eye toward heterozygosity.[21] Although the view is intriguing, most available data are not definitive in this regard and, in fact, would suggest that the role of *HFE* mutations is minor.

Pirisi et al[3] found a statistical association of heterozygosity for the H63D mutation and portal iron deposition but concluded by multivariate analysis that there is no correlation between iron deposition and *HFE* mutation status, although the total number of patients manifesting mutations of the *HFE* gene, particularly those with the *C282Y* mutation, was quite small. This lack of association is not surprising, given the findings on liver biopsy that demonstrated that the iron is deposited in a fashion much more typical for secondary iron overload than for hereditary hemochromatosis, with most of the iron deposited in Kupffer cells and portal tracts, not in hepatocytes. On multivariate analysis, the only factor that correlated with iron deposition was inflammatory grade, although there also was an association on univariate analysis between stage and iron deposition, supporting the hypothesis that iron deposition is secondary to hepatocellular necrosis at the interface. Several other investigators have reported a correlation between iron deposition and *C282Y* mutations, but none describe the distribution of the iron.[12-14] Rather, grading systems are used that give only a numeric assessment of total iron staining. Of reports that mention the distribution of iron in hepatitis C (most of which do not report on *HFE* status), none report a predominantly hepatocellular distribution of iron as would be expected with hemochromatosis.[3,5-7,19] In addition, in the reports that demonstrate a correlation between the *C282Y* gene and iron deposition, the relationship is statistical but not absolute. There usually are as many or more patients with iron deposition without the mutation as there are with the mutation. In addition, several studies failed to find a correlation between *HFE* status and iron deposition.[13,22,23] This might mean that iron deposition in hepatitis C in general is independent of the *HFE* mutation status, although the presence of the *C282Y* gene may contribute to iron deposition in patients with the gene mutation. It would be most interesting to review the pattern of liver iron deposition in the patients with excess iron to compare those with the *C282Y* mutation and those without. As far as I know, however, such an analysis has not been reported, although there is at least 1 report that describes patients with hepatitis C and pure hepatocellular iron as might be expected in hemochromatosis.[19] In this latter report, however, there were only 2 patients with pure hepatocellular iron of 25 patients with iron overload, indicating that this probably is a rare event.

Another twist in the *HFE* mutation story is the reported association of the *C282Y* gene with increased liver fibrosis independent of iron deposition.[14] In fact, this was one of the findings of Pirisi et al.[3] Other authors dispute this finding.[10,23,24] It generally is thought that any effect of *HFE* mutations must be mediated by the pathway of increased iron deposition. It is somewhat difficult to come up with a mechanism outside of iron deposition for this mutation to be causing fibrosis.[16]

The final and probably the most clinically important issue is the question of the value of the finding of iron deposition in terms of clinical management of the patient with hepatitis C. Despite the reported correlation of iron deposition with a poorer response to interferon therapy, few studies have tested the efficacy of phlebotomy as an adjunct to treatment with interferon. Improvement of outcome would be a natural consequence if fibrosis were due to the iron deposition, and not vice versa. There are 2 possible mechanisms by which phlebotomy might be useful for patients with hepatitis C. In one, iron depletion might lead to less tissue injury and fibrosis but have no effect on clearance of virus from the liver. In the second, iron depletion would somehow increase the efficacy of interferon in eradicating the virus, perhaps because iron acts as a nutrient assisting in viral proliferation.

Several articles report that phlebotomy is associated with a decrease in serum transaminase levels in these patients.[25-27] Studies that used normalization of transaminase as the marker for complete response might be expected to find that phlebotomy results in improved

response to interferon. Although phlebotomy has decreased transaminase levels, there is no evidence that such improvements actually affect the long-term outcome for patients with hepatitis C. In theory, of course, transaminase is a marker for hepatocellular injury, and, hence, prolonged iron depletion might lead to a delay of the development of fibrosis despite persistence of the virus. Proving this would require a long-term randomized trial of long-term phlebotomy as an adjunct treatment and would take many years to complete, given the slow and variable progression of hepatitis C.

In regard to actual clearance of the virus, relatively few data are available for analysis. In 1 small prospective clinical trial of phlebotomy, there was no benefit to the patient in terms of clearance of the virus.[27] Unfortunately, this article included no patients who had serologic evidence of iron overload, the group that might be expected to benefit from iron reduction. Nevertheless, aspartate aminotransferase levels decreased with phlebotomy. In 1 other study, phlebotomy after failed interferon therapy (but while continuing the interferon) did not result in improvements of clearance of virus, although transaminase levels decreased.[26] This was true for a group selected because of clinical evidence of iron overload and in a second group not selected by iron levels.

There is also a study in hepatitis B using chelation therapy that found improved response to interferon in patients treated with deferoxamine.[28] Although the findings are intriguing, the dosages of deferoxamine given in this study seem inadequate to lead to iron depletion; therefore, the meaning of the findings this study is uncertain. Therefore, while there is evidence that phlebotomy leads to improvements in serum transaminase levels, there is no direct evidence that phlebotomy improves long-term outcome (although this hypotheses has not been tested rigorously) or leads to improved clearance of the virus.

While the significance of iron deposition continues to be an issue for debate among hepatologists, for the practicing pathologist the issue seems relatively simple. It would seem prudent to routinely perform iron stains for all patients with hepatitis C (a practice currently done in many laboratories on all liver biopsy specimens to rule out hemochromatosis) and to report iron by location of deposition (portal tract, hepatocyte, Kupffer cell) and by intensity. Occasional cases of concurrent hemochromatosis and hepatitis C will be detected by this method, and if the clinician wishes to use the information on iron deposition in the treatment of patients without hemochromatosis as an adjunct to therapy, the information would be available at no cost.

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