Iron Overload, Wild-type HFE Gene

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Key Concepts

• In hemochromatosis, ferritin levels are representative of tissue iron stores
• About 85% of typical hemochromatosis patients are C282Y homozygotes
• Patients with iron overload may be HFE-negative (wt/wt)
• Patients with various liver diseases may have secondary iron overload and be HFE-negative
• Normal ferritin levels may be higher than what we have considered in the past

It is well accepted that serum ferritin levels are proportionate to tissue iron stores. This is particularly so in uncomplicated patients with hereditary hemochromatosis (HH). This direct relationship of iron stores to ferritin has been known for over 40 years (see Figure 1).1

When evaluating a patient with iron overload, genetic testing (HFE mutation analysis) has become the standard of care and is routinely available. About 85% to 90% of typical HH patients are homozygous for the principal mutation in HFE, C282Y. Accordingly, about 10% to 15% of patients with iron overload are negative for mutations in HFE. These patients can frequently present a diagnostic and therapeutic conundrum.

Patients who are found to have iron overload (elevated ferritin level, with or without an elevated transferrin saturation) should be evaluated with HFE mutation analysis. If negative, there are two principal explanations that should be considered by the gastroenterologist/hepatologist.

1. Presence of an inherited iron-loading disease other than HFE-linked to HH
2. Presence of secondary iron overload in association with another liver disease

Presence of an Inherited Iron-loading Disease Other Than HFE-linked HH

There are several other inherited forms of iron overload which are classified as non-HFE-related HH.2 These include juvenile hemochromatosis, and iron overload resulting from mutations in the genes for hepcidin, transferrin receptor 2 (TFR2), and ferroportin. Juvenile HH is characterized by rapid iron accumulation. Mutations in two different genes have been shown to cause forms of juvenile HH. The more common mutation occurs in the HJV gene on chromosome 1q; this gene encodes a protein called hemojuvelin.

Mutations in the hepcidin gene (HAMP) also produce a form of juvenile HH; hepcidin is a hepatic peptide that acts to down-regulate iron absorption. Mutations in the gene TFR2 produce an autosomal recessive form of HH that is clinically similar to HFE-related HH. How these TFR2 mutations result in iron overload is not yet known; they possibly cause abnormal iron sensing by hepatocytes, the
predominant site of TFR2 expression. A rare autosomal form of HH results from two categories of mutations in the gene for the iron transporter ferroportin. "Loss-of-function" mutations decrease the cell surface localization of ferroportin, thereby reducing its ability to export iron. The result is iron deposition primarily in macrophages, and this disorder is sometimes termed ferroportin disease. The second category includes “gain-of-function” ferroportin mutations that abolish hepcidin-induced ferroportin internalization and degradation; the distribution of excess iron is similar to that in HFE-related HH, primarily within parenchymal cells. Undoubtedly, there are other mutations in genes affecting proteins of iron homeostasis that have not yet been discovered/described.

Presence of Secondary Iron Overload in Association With Another Liver Disease

The principal liver conditions with secondary iron overload are seen in patients with alcoholic liver disease (ALD), chronic hepatitis C (CHC), and nonalcoholic steatohepatitis (NASH). These three hepatic disorders account for the majority of liver disease seen in the United States. Several clinical studies have shown that approximately 50% of patients with ALD, CHC, and NASH have abnormalities in serum iron studies. Usually this is an elevation in serum ferritin alone, but an elevated transferrin saturation can occasionally be seen as well. When liver biopsy is performed, increased iron deposits are seen, usually in a panlobular distribution with iron in both hepatocytes and sinusoidal lining cells (Kupffer cells). Hepatic iron concentrations may be slightly to moderately increased. When HFE mutations have been evaluated in patients with alcoholic liver disease, there has been no increased incidence of either C282Y or H63D compared with control populations. Furthermore, there was no increase in HFE mutations in patients with alcoholic liver disease.
disease who had an increased amount of fibrosis. Thus, the abnormal iron studies frequently seen in patients with ALD are most likely due to an effect of alcohol on iron absorption or to unknown mechanisms.

In chronic hepatitis C, the relationship of abnormal iron studies and elevated hepatic iron concentration with a response to interferon treatment has been known for several years. When HFE mutation analysis has been investigated in patients with chronic hepatitis C, the frequency of C282Y and H63D has been equivalent to that of control populations. Most studies have shown that, when HFE mutations are present, they correlate with increased iron stores seen histologically and some studies have shown a synergistic effect with the development of fibrosis. At present, it is recommended that HFE mutation analysis be performed when abnormal iron studies are seen in patients with chronic hepatitis C. Also, iron stains are typically performed on liver biopsy samples when biopsies are done for grading and staging of chronic hepatitis C (see Figures 2).

If iron stores are significantly increased, it is reasonable to perform therapeutic phlebotomy to deplete excess iron stores before initiating antiviral therapy (see Figure 3 & 4).

In patients with NASH, several studies have provided conflicting results. Some have shown an increase in HFE mutations and others have shown no difference from control populations. One study of NASH patients who were HFE-negative showed a reduction in elevated liver enzymes and improvement in parameters of insulin resistance in patients treated by phlebotomy to produce near iron deficiency. These observations suggest an interaction between expression of fatty liver disease and iron metabolism (Figure 5 & 6).

In patients without liver disease who have iron overload and are HFE-negative, liver biopsy should be performed (see Figure 7). If, as expected, significant iron overload is found, then phlebotomy should be initiated.

Table 1. Examples of HFE-negative patients with iron overload

<table>
<thead>
<tr>
<th>Liver Disease</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Ferritin (ng/mL)</th>
<th>TS (ng/mL)</th>
<th>ALT IU/mL</th>
<th>AST IU/mL</th>
<th>HFE</th>
<th>Liver Biopsy</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD</td>
<td>58</td>
<td>M</td>
<td>1,027</td>
<td>65</td>
<td>65</td>
<td>75</td>
<td>wt/wt</td>
<td>Cirrhosis 2+iron</td>
<td>Abstinence</td>
</tr>
<tr>
<td>None</td>
<td>52</td>
<td>F</td>
<td>2,553</td>
<td>32</td>
<td>30</td>
<td>26</td>
<td>wt/wt</td>
<td>Cirrhosis 4+iron</td>
<td>Phlebotomy</td>
</tr>
<tr>
<td>None</td>
<td>32</td>
<td>M</td>
<td>3243</td>
<td>72</td>
<td>33</td>
<td>18</td>
<td>wt/wt</td>
<td>HJV mutation 4+iron</td>
<td></td>
</tr>
<tr>
<td>NASH</td>
<td>46</td>
<td>M</td>
<td>846</td>
<td>32</td>
<td>75</td>
<td>65</td>
<td>wt/wt</td>
<td>NASH 2+iron</td>
<td>Wt loss, exercise +/-phlebotomy</td>
</tr>
<tr>
<td>HCV</td>
<td>38</td>
<td>M</td>
<td>730</td>
<td>40</td>
<td>110</td>
<td>90</td>
<td>wt/wt</td>
<td>Grade 2, stage 2, HCV 3+iron</td>
<td>Antiviral therapy +/-phlebotomy</td>
</tr>
</tbody>
</table>

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Finally, a recent report by Olynyk and colleagues in Perth, has suggested that the normal range of ferritin should be increased. This may be due to an effect of sub-clinical NAFLD on ferritin expression in the absence of iron overload.

Examples of HFE-negative patients with iron overload are shown in Table 1.

When patients with iron overload (elevated ferritin) are encountered, HFE mutation analysis should be performed. If HFE testing is negative, liver biopsy is recommended in most patients to assess the degree of iron overload. Patients with secondary iron overload with either ALD, CHC, or NASH, should be managed as usual and if the histologic iron grading is high, phlebotomy should be performed.

References