The effect of iron depletion on chronic hepatitis C virus infection

Massimo Franchini · Giovanni Targher · Franco Capra · Martina Montagnana · Giuseppe Lippi

Abstract Increasing evidence exists that iron overload, a common finding in chronic hepatitis C virus (HCV) infection, plays an important role in the pathophysiology of this disease. The mechanisms by which iron excess induces liver damage along with the benefit of iron depletion via phlebotomy on biochemical and histological outcomes in patients with chronic HCV infection have been discussed in this review. Finally, we focus on the effect of iron reduction on the rate of response to interferon antiviral therapy.

Keywords Iron · Phlebotomy · HCV · IFN · Therapy

Introduction

Hepatitis C virus (HCV) infection, which affects nearly 2% of the human population, is a major cause of liver disease worldwide. Following acute HCV infection, a chronic state is established in as many as 80% of infected individuals. Although many subjects carrying the virus remain asymptomatic, chronicity is often accompanied by altered liver function and progressive liver disease and culminates in cirrhosis or hepatocellular carcinoma in up to 20% of infected individuals [1].

Mild-to-moderate iron overload is a common finding among patients with chronic HCV infection; indeed, up to 30–40% of them may show increased serum transferrin-iron saturation and serum ferritin or increased hepatic iron concentration [2, 3]. On the other hand, elevated iron indices have been correlated with a progression of the liver disease and a decreased response to antiviral therapy [4–9]. The association between iron overload and chronic HCV infection, along with the effect of iron depletion on the course of chronic HCV infection and the response to antiviral therapy, has been addressed in this review.

Sources

We first performed an electronic search on MEDLINE, EMBASE, SCOPUS, and OVID databases without temporal limits using different combinations of the following keywords: “phlebotomy,” “iron depletion,” “iron overload,” “iron reduction,” “hepatitis C virus infection,” “HCV,” “chronic hepatitis C,” “antiviral therapy,” “interferon,” and “ribavirin.” In addition, the bibliographic references of all retrieved studies and reviews were assessed for additional reports of clinical trials. Only full-
text articles published in English were considered in this review.

Iron overload and chronic HCV infection

The mechanism of hepatotoxicity of iron accumulation in chronic HCV infection is still unclear. The deposition of iron predominantly within Kupffer cells and portal macrophages, the correlation between hepatic necroinflammatory activity and iron accumulation, and the reduction of hepatic iron content following response to interferon (IFN) therapy suggest that hepatic iron overload is the result of hepatocyte necrosis, which leads to release of ferritin from hepatocytes and subsequent uptake by macrophages [10–12]. However, another possible mechanism to explain the elevated iron stores could be the increased intestinal iron absorption. Indeed, recent investigations have found decreased levels of hepcidin (a peptide hormone produced in the liver that has an inhibitory effect on iron absorption) and increased levels of transferrin receptor 2 (which is located on the hepatocyte membrane and is involved in the uptake of iron by hepatocytes) in chronic HCV infection [13]. Thus, the resulting effect of these abnormalities could be an increased delivery of iron to hepatocytes from macrophage iron stores and intestinal mucosa.

Excess iron increases the formation of reactive oxygen species leading to lipid peroxidation, damage to protein and DNA, and thereby to cell membranes and genomic damage. Reactive oxygen species, which include hydroxyl radicals, may cause hepatic stellate cell activation and proliferation and upregulate synthesis of smooth muscle actin and collagen, thus contributing to hepatic fibrogenesis [11, 14, 15]. In vitro studies also suggest that iron deposition in hepatocytes enhances HCV replication, thus facilitating the viral infection in the liver [16]. Moreover, these hydroxyl radicals are known to generate pro-mutagenic bases, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), which have been implicated in spontaneous DNA mutagenesis and carcinogenesis [12].

Finally, hemochromatosis gene mutations could play a role in the pathogenesis of iron overload among patients with chronic HCV infection [17]. Indeed, after the initial observation by Smith and colleagues [18] that HCV-infected patients who were carriers of C282Y mutation had higher serum ferritin levels than patients homozygous for the wild type of the HFE gene, several other reports have found that heterozygous HFE mutations are more frequent and are associated with increased iron storage among patients with chronic HCV infection [19–24]. However, studies examining the relationship between HFE mutations and disease progression in chronic HCV infection have given conflicting results. Indeed, while some studies have found a positive correlation between HFE mutations and the severity of liver disease [19, 22, 25–30], others failed to find such association [31–36]. Methodological- or population-based differences among studies could account for these discrepant findings [37]. For instance, Tung and colleagues [19] studied the liver histology in 316 patients with chronic HCV infection at various stages and found that the presence of HFE mutations was independently associated with iron loading and advanced fibrosis in patients with compensated liver disease. In contrast, Thorburn and colleagues [31] performed liver biopsies in 164 chronically HCV-infected patients and observed that the carriage of HFE mutation did not have a role in the iron accumulation or the progression of liver disease.

The effect of iron depletion on liver status in chronic HCV infection

Several groups have evaluated the effects of iron reduction on chronic HCV infection [38–46]. The majority of the studies performed phlebotomies of 400–500 ml of whole blood every 1 or 2 weeks until the development of an iron-deficient anemia. Hayashi and colleagues [39] first reported that iron reduction performed by repeated venesection led to normalization of serum alanine aminotransferase (ALT) levels in 5 of the 10 patients. However, serum ALT levels significantly decreased in all patients (from 152 ± 49 to 55 ± 32 U/L, \( P < 0.001 \)). According to a report by Piperno and colleagues [41], serum ALT levels significantly improved in 32 iron-depleted patients with chronic HCV infection. Similar results were subsequently reported by other groups with phlebotomy alone [42–45]. However, no significant reduction in serum HCV RNA levels was observed [42, 46]. The long-term effect of phlebotomy on biochemical and histological parameters of chronic HCV infection was addressed by Yano and colleagues in 25 patients undergoing a 5-year maintenance phlebotomy program [47]. Interestingly, the authors found that the mean serum ALT levels decreased significantly during the initial phlebotomy program (from 117 to 75 U/L, \( P < 0.05 \)) and this improvement persisted during the study period. Furthermore, phlebotomies were able to prevent the progression of liver histology because the severity of liver fibrosis (staging score) decreased from 2.3 to 1.7 (\( P < 0.05 \)) in the iron-reduction group, whereas the mean values increased from 1.7 at baseline to 2.0 at the end of follow-up in controls (\( P = \text{NS} \)). Likewise, the severity of inflammation (grading score) remained unchanged in the study group (1.8 vs. 2.0, \( P = \text{NS} \)) but progressed in the control group (2.0 vs. 2.9, \( P < 0.005 \)). Thus, the authors concluded that long-term maintenance of iron depletion is a safe and effective alternative to IFN treatment and could be
particularly indicated for those patients who do not respond to antiviral therapy or cannot tolerate such drugs.

Recently, Alexander and colleagues [48] found that iron depletion was associated with a biochemical response in 22% of patients who did not respond to IFN monotherapy and that, among patients with serum ALT normalization, there was a significant reduction of serum markers of liver fibrosis (procollagen III peptide). Kaito and colleagues [49] found that iron-reduction therapy by phlebotomy significantly reduced lipid peroxidation and oxidative stress, which mediate the deleterious effect of iron overload on the liver.

Finally, other groups have demonstrated that the association of a low-iron diet to phlebotomy has an additional effect in removing iron-induced oxidative stress [50, 51]. Indeed, in a study conducted by Kato and colleagues [51], 34 patients with chronic HCV infection unresponsive to IFN therapy were maintained in an iron-depleted state with phlebotomy and a low-iron diet for 6 years. This therapy was associated with a high rate of biochemical response (65%), improvement in liver histology, and reduction in hepatic levels of 8-OHdG, a marker of oxidant stress. In a recent cohort study, the same authors demonstrated that long-term phlebotomy with a low-iron diet therapy reduced the risk of progression of chronic HCV infection to hepatocellular carcinoma [52].

Table 1 summarizes the results of the most important studies on the effect of iron depletion by phlebotomy on chronic HCV infection.

| Author [ref] | Number of patients | Characteristics | Results |
|--------------|--------------------|-----------------|---------|
| Hayashi et al. [40] | 40 | 40 naïve | Phlebotomy significantly reduced mean ALT levels |
| Sartori et al. [42] | 24 | 12 IFN NR, 12 naïve | Reduction of ALT levels and inflammatory grading score, suppression of the progression of staging score for fibrosis |
| Yano et al. [43] | 33 | NI | Phlebotomy significantly reduced ALT |
| Tanaka et al. [44] | 22 | NI | Phlebotomy significantly reduced ALT, AST, and α-fetoprotein levels |
| Yano et al. [47] | 25 | 22 IFN NR, 3 naïve | Reduction of ALT levels, improvement of liver inflammation, and suppression of the progression of liver fibrosis |
| Alexander et al. [48] | 18 | 18 NR | Biochemical response was accompanied by a reduction of markers of fibrogenesis |

**Abbreviations:** IFN, interferon; NR, nonresponders; NI, not indicated; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
study (5 million units daily) could account for the high rate of virologic response observed. Fong and colleagues [59] observed a higher rate of sustained virologic responses in naı̈ve patients treated with phlebotomy before and during IFN treatment than those receiving IFN alone (29% vs. 5%). In a similar trial, Fontana and colleagues [60] showed that iron reduction via therapeutic phlebotomy improved the virologic and histologic response to IFN therapy. Recently, Desai et al. [61] performed a meta-analysis of six prospective randomized controlled trials and concluded that phlebotomy improves the response to IFN in patients with chronic HCV infection. Table 2 summarizes the most important studies analyzing the effect of iron depletion and IFN in the treatment of chronic HCV infection.

Conclusions

On the whole, the literature data suggest that iron depletion via phlebotomy improves biochemical and histological outcomes in patients with chronic HCV infection. In addition, a number of studies have documented that combining iron depletion with IFN monotherapy may improve the rate of virologic responses in previously untreated and nonresponder patients.

Of note, recent investigations have found that hepatic iron concentration does not influence the response to antiviral therapy with IFN plus ribavirin [62, 63]. In addition, an iron-deficiency anemia due to repeated phlebotomies could be a risk factor for unsuccessful outcome of the antiviral treatment because of dose reduction of ribavirin. Thus, it will be interesting to see the results of future randomized controlled trials assessing the efficacy of combining iron-depletion therapy with pegylated IFN and ribavirin in chronic HCV infection.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.
2. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology 1992;102:2108–2113.
3. Bonkowsky HL. Iron as a comorbid factor in chronic viral hepatitis. Am J Gastroenterol 2002;97:1–4.
4. Bonkovsky HL, Banner BF, Rothman AL. Iron and chronic viral hepatitis. Hepatology 1997;25:759–768.
5. Fujita N, Sugimoto R, Urawa N, Araki J, Misufi R, Yamamoto M, et al. Hepatic iron accumulation is associated with disease progression and resistance to interferon/ribavirin combination therapy in chronic hepatitis C. J Gastroenterol Hepatol 2007;22:1886–1893.
6. Sherrington CA, Olynyk JK. Iron as a cofactor in chronic hepatitis C infection. Liver 2002;22:187–189.
7. Distante S, Bioro K, Hellum KB, Myrvang B, Berg JP, Skaug K, et al. Raised serum ferritin predicts non-response to interferon and ribavirin treatment in patients with chronic hepatitis C infection. Liver 2002;22:269–275.
8. van Thiel DH, Friedlander L, Fagioli S, Wright HI, Irish W, Gavaler JS. Response to interferon alpha therapy is influenced by the iron content of the liver. J Hepatol 1994;20:410–415.
27. Gehrke SG, Stremmel W, Mathes I, Riedel HD, Bents K, Kallinowski B. Hemochromatosis, transferrin receptor gene polymorphisms in chronic hepatitis C: impact on iron status, liver injury and HCV genotype. J Mol Med 2003;81:780–787.

28. Geier A, Reugels M, Weiskirchen R, Wasmuth HE, Dietrich CG, Siewert E, et al. Common heterozygous hemochromatosis gene mutations are risk factors for inflammation and fibrosis in chronic hepatitis C. Liver Int 2004;24:285–294.

29. Mah YH, Kao JH, Liu CJ, Chen CL, Chen PJ, Lai MY, et al. Prevalence and clinical implications of HFE gene mutations (C282Y and H63D) in patients with chronic hepatitis B and C in Taiwan. Liver Int 2005;25:214–219.

30. Corengia C, Galimberti S, Bovo G, Vergani A, Arosio C, Mariani R, et al. Iron accumulation in chronic hepatitis C: relation of hepatic iron distribution, HFE genotype, and disease course. Am J Clin Pathol 2005;124:846–853.

31. Thorburn D, Curry G, Spooner R, Spence E, Oien K, Halls D, et al. The role of iron and haemochromatosis gene mutations in the progression of liver disease in chronic hepatitis C. Gut 2002;50:248–252.

32. Negro F, Samii K, Rubbia-Brandt L, Quadri R, Male PJ, Zarski JP, et al. Hemochromatosis gene mutations in chronic hepatitis C patients with and without liver siderosis. J Med Virol 2000;60:21–27.

33. Kohler HH, Stremmel W, Mathes I, Riedel HD, Bents K, Kallinowski B. Hemochromatosis, transferrin receptor gene polymorphisms in chronic hepatitis C: impact on iron status, liver injury and HCV genotype. J Mol Med 2003;81:780–787.

34. Lal P, Scott CA, Avellini C, Toniotto P, Fracis C, Soardo G, et al. Iron deposition and progression of disease in chronic hepatitis C: role of interface hepatitis, portal inflammation, and HFE gene mutations. J Med Virol 2000;60:21–27.

35. Smith BC, Gorve J, Guzail MA, Day CP, Daly AK, Burt AD, et al. Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. Hepatology 1998;27:1695–1699.

36. Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdle KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124:318–326.

37. Kazemi-Shirazi L, Datz C, Maier-Dobersberger T, Kaserer K, Hackl F, Polli C, et al. The relation of iron status and hemochromatosis gene mutations in patients with chronic hepatitis C. Gastroenterology 1999;116:127–134.

38. Piperno A, Vergani A, Malosio I, Parma L, Fossati L, Ricci A, et al. Hepatic iron overload in patients with chronic viral hepatitis: role of HFE gene mutations. Hepatology 1998;28:1105–1109.

39. Bonkovsky HL. Therapy of hepatitis C: other options. Hepatology 1999;30:143S–151S.

40. Ioannou GN, Tung BY, Kowdle KV. Iron in hepatitis C: villain or innocent bystander? Semin Gastroenterol Dis 2002;13:95–108.

41. Pirisi M, Scott CA, Avellini C, Tomio P, Francis C, Sorado G, et al. Iron deposition and progression of disease in chronic hepatitis C: role of interface hepatitis, portal inflammation, and HFE gene mutations. J Med Virol 2000;60:21–27.
46. Herrera JL. Iron depletion is not effective in inducing a virologic response in patients with chronic hepatitis C who failed to respond to interferon therapy. Am J Gastroenterol 1999;94:3571–3575.

47. Yano M, Hayashi H, Wakisawa S, Sanae F, Takikawa T, Shiono Y, et al. Long-term effects of phlebotomy on biochemical and histological parameters of chronic hepatitis C. Am J Gastroenterol 2002;97:133–137.

48. Alexander J, Tung BY, Croghan A, Kowdle KY. Effect of iron depletion on serum markers of fibrogenesis, oxidative stress, serum liver enzymes in chronic hepatitis C: results of a pilot study. Liver Int 2007;27:268–273.

49. Kaito M, Iwasa M, Kobayashi Y, Fujita N, Tanaka H, Gabazza EC, et al. Iron reduction therapy by phlebotomy reduces lipid peroxidation and oxidative stress in patients with chronic hepatitis C. J Gastroenterol 2006;41:921–922.

50. Kimura F, Hayashi H, Yano M, Yoshioka K, Matsumura T, Fukuda T, et al. Additional effect of low iron diet on iron reduction therapy by phlebotomy for chronic hepatitis C. Hepatology 2005;52:563–566.

51. Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, et al. Normalization of elevated hepatic 8-hydroxy-2′-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001;61:8697–8702.

52. Kato J, Miyanishi K, Kobune M, Nakamura T, Takada K, Takimoto R, et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol 2007;42:830–836.

53. Guyader D, Boucher E, Andre P, Even C, Cottereau J, Bianchi A, et al. A pilot study of iron depletion as adjuvant therapy in chronic hepatitis C patients not responding to interferon. Am J Gastroenterol 1999;94:1696–1699.

54. Tsai NCS, Zuckerman E, Han SH, Goad K, Redeker AG, Fong TL. Effect of iron depletion on long-term response to interferon-alpha in patients with chronic hepatitis C who previously did not respond to interferon therapy. Am J Gastroenterol 1997;92:1831–1834.

55. Di Bisceglie AM, Bonkovsky HL, Chopra S, Flamm S, Reddy RK, Grace N, et al. Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology 2000;32:135–138.

56. Fargion S, Fracanzani AL, Rossini A, Borzio M, Riggio O, Belloni G, et al. Iron reduction and sustained response to interferon-alpha therapy in patients with chronic hepatitis C: results of an Italian multicenter randomized study. Am J Gastroenterol 2002;97:1204–1210.

57. Carlo C, Daniela P, Giancarlo C. Iron depletion and response to interferon in chronic hepatitis C. Hepato-gastroenterology 2003;50:1467–1471.

58. Van Thiel DH, Friedlander L, Molloy PJ, Kania RJ, Fagiuloi S, Wright HI, et al. Retreatment of hepatitis C interferon non-responders with larger doses of interferon with and without phlebotomy. Hepato-gastroenterology 1996;43:1557–1561.

59. Fong TL, Han SH, Tsai NC, Morgan TR, Mizokami M, Qian D, et al. A pilot randomized, controlled trial of the effect of iron depletion on long-term response to alpha-interferon in patients with chronic hepatitis C. J Hepatol 1998;28:369–374.

60. Fontana RJ, Israel J, LeClair P, Banner BF, Tortorelli K, Grace N, et al. Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. Hepatology 2000;31:730–736.

61. Desai TK, Jamil LH, Balasubramanian M, Koff R, Bonkovsky HL. Phlebotomy improves therapeutic response to interferon in patients with chronic hepatitis C: a meta-analysis of six prospective randomized controlled trials. Dig Dis Sci 2008;53:815–822.

62. Hofer H, Osterreicher C, Jessner W, Penz M, Steinidl-Munda P, Wrba F, et al. Hepatic iron concentration does not predict response to standard and pegylated-IFN/ribavirin therapy in patients with chronic hepatitis C. J Hepatol 2004;40:1018–1022.

63. Pianko S, McHutchison JG, Gordon SC, Heaton S, Goodman ZD, Patel K, et al. Hepatic iron concentration does not influence response to therapy with interferon plus ribavirin in chronic HCV infection. J Interf Cytokine Res 2002;22:483–489.