Molecular pathogenesis and clinical consequences of iron overload in liver cirrhosis

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BACKGROUND: The liver, as the main iron storage compartment and the place of hepcidin synthesis, is the central organ involved in maintaining iron homeostasis in the body. Excessive accumulation of iron is an important risk factor in liver disease progression to cirrhosis and hepatocellular carcinoma. Here, we review the literature on the molecular pathogenesis of iron overload and its clinical consequences in chronic liver diseases.

DATA SOURCES: PubMed was searched for English-language articles on molecular genesis of primary and secondary iron overload, as well as on their association with liver disease progression. We have also included literature on adjuvant therapeutic interventions aiming to alleviate detrimental effects of excessive body iron load in liver cirrhosis.

RESULTS: Excess of free, unbound iron induces oxidative stress, increases cell sensitivity to other detrimental factors, and can directly affect cellular signaling pathways, resulting in accelerated liver disease progression. Diagnosis of liver cirrhosis is, in turn, often associated with the identification of a pathological accumulation of iron, even in the absence of genetic background of hereditary hemochromatosis. Iron depletion and adjuvant therapy with antioxidants are shown to cause significant improvement of liver functions in patients with iron overload. Phlebotomy can have beneficial effects on liver histology in patients with excessive iron accumulation combined with compensated liver cirrhosis of different etiology.

CONCLUSION: Excessive accumulation of body iron in liver cirrhosis is an important predictor of liver failure and available data suggest that it can be considered as target for adjuvant therapy in this condition.

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KEY WORDS: liver cirrhosis; fibrosis; iron overload; hepatocellular carcinoma; hereditary hemochromatosis

Introduction

Iron is an essential micronutrient for basic metabolic reactions in living organisms. Iron as a component of many molecules participates in basic metabolic reactions in living organisms. As a non-heme iron it is embedded in the iron-sulfur (Fe-S) centers of enzymes which regulate the expression of many genes encoding enzymes involved in the proliferation and differentiation of cells (protein kinase C-β, isozyme5, acid phosphatase, and p21). High redox potential of iron implicates its toxicity associated with the presence of iron ions in different oxidation states (II to VI), mostly in the form of divalent or trivalent ions (Fe²⁺ or Fe³⁺ respectively). Disturbed iron balance leads to an overproduction of toxic reactive oxygen species (ROS), increased oxidative stress, peroxidation of the lipid membrane, protein and DNA resulting in damage to cellular structures which triggers the mechanisms of cell death, i.e. necrosis or apoptosis.

Iron accumulates in many tissues (lung, kidney, heart, pancreas, and endocrine glands), but the bone marrow and liver remain the main reservoirs. Since the liver is an iron storage compartment and site of hepcidin synthesis, it is involved in maintaining iron homeostasis in the body and rapidly shows pathology in a case of excessive accumulation of iron. The risk of progression of liver fibrosis and development of cirrhosis finally with symptoms of liver failure is associated with duration of the exposure to excess iron in hepatocytes. Liver iron
overload is also significantly correlated with an increased risk of developing hepatocellular carcinoma (HCC). On the other hand, the diagnosis of liver cirrhosis is attributed to signs of pathological accumulation of iron with relatively frequent presence of stainable liver iron. It has been shown in one-third of explanted cirrhotic livers, and in 10% of cases the intensity of iron overload corresponded to the diagnostic criteria for hereditary hemochromatosis (HH). Advancements in knowledge of the molecular mechanisms involved in the control of iron metabolism in the human body have been made in the past 15 years, and have contributed to charting new directions of research into the pathogenesis of iron accumulation in liver cirrhosis, its clinical consequences, and possible therapies.

The role of the liver in the regulation of iron uptake
Hepatocytes serve as a major storage site of iron and are the predominant cells that synthesize hepcidin. Hepcidin, the “iron hormone”, regulates iron transport out of the cell and plays a role as an important controller of iron content in the extracellular space. Iron in forms of both bound and unbound to transferrin (NTBI: non-transferrin bound iron) is captured by hepatocytes. Transferrin receptors (TfR) 1 and 2, present on the membrane of hepatocytes and showing only a slight degree of similarity, are involved in the uptake of transferrin-bound iron. TfR2 expression in hepatocytes is higher compared to TfR1 and its role becomes clearly predominant with an increasing amount of iron in the blood. Only TfR1 synthesis is controlled by iron regulatory proteins (IRPs) and iron responsive elements (IREs), and depends on iron content in the cell. Even when the TfR1 transcript is degraded and its synthesis inhibited in a case of increasing intracellular iron content, the intrahepatocytic system of iron uptake mediated by TfR2 is still possible. According to one of the hypotheses TfR2 acts as an iron sensor through the interaction with hemochromatosis protein (HFE) and signaling pathways for transcription factors involved in synthesis of hepcidin. This is why the liver plays such a significant role in protecting against the toxicity of iron and is significantly involved in the pathogenesis of excessive accumulation of iron, i.e. in HH.

NTBI is a highly toxic form of iron that appears in the serum with a drastic increase in transferrin saturation and when iron binding capacity is exceeded. It is particularly hazardous because of the ease of generating ROS. The quick clearance of NTBI from the serum is possible by transporting it into hepatocytes through divalent metal transporter 1 (DMT1). In hepatocytes, iron is incorporated into the ferritin molecule. That compensates for cell damage and preserves iron bioavailability. With increased demand for iron in the organism (e.g. intensified erythropoiesis), it is released from the complex with ferritin and transported out of the cell through the molecular mechanism which has been not fully understood. “Iron-poor” ferritin is also present in the serum and its concentration increases in case of tissue iron overload. Therefore serum ferritin serves as a useful clinical marker of pathological iron accumulation. According to recent discoveries ferritin may be a part of cellular protection under conditions of stress and inflammation. Ferritin synthesis is also regulated by the inflammatory response and activity of cytokines (TNF-α, IL-1α, IL-1β and IL-6), oxidants, growth factors (IGF-1), and hormones [T3, thyrotropin-releasing hormone (TRH) and insulin]. Recently, Meyron-Holtz et al gathered data confirming that ferritin may be polarized in epithelial barrier cells and play a role also as an iron transporter, including export out of the cell.

Molecular pathogenesis of iron overload
The role of hepcidin in the regulation of iron uptake, transport and storage
Although hepcidin was discovered as an antibacterial peptide, soon it was demonstrated that the expression of hepcidin mRNA is strongly associated with liver iron content. Nicolas et al observed massive iron overload after inactivation of the gene encoding for mouse hepcidin (HAMP). In turn, hypochromic life-threatening anemia caused by iron deficiency was observed in transgenic mice with HAMP gene overexpression. In humans, a defect in the synthesis of hepcidin in carriers of HAMP gene mutations causes excessive accumulation of iron, revealed at a young age in the form of multi-organ damage and classified as juvenile HH type 2A. This is an autosomal recessive disease, where the rapid progression of iron overload is accompanied by severe organ dysfunction, especially the heart and endocrine glands. Studies using cellular models have led to the determination of the hepcidin mechanism of action. The basis of its activity is direct binding to ferroportin (Fpn), which acts as a membrane receptor for hepcidin. The strongest expression of Fpn in mammals is observed in cells which are the most important for systemic iron homeostasis: enterocytes, hepatocytes, macrophages of the reticuloendothelial system, and syncytiotrophoblasts of the placenta. The activity of Fpn depends on the content of iron in the cell and is mediated by IRP-IRE binding. As a result of the attachment of hepcidin to the Fpn molecule, Fpn is endocytosed and degraded in lysosomes. Conse-
Iron in cirrhosis

Judging the release of iron from macrophages is blocked and iron absorption from the gastrointestinal tract into the blood is reduced due to the inhibition of iron excretion from the basolateral enterocyte membrane. These changes in iron turnover lead to a decrease in the concentration of iron in the blood.

Signaling pathways which regulate the transcription of hepcidin are strictly controlled. Hepcidin expression is stimulated by inflammation through STAT3 signaling dependent on IL-6 and an increased iron content. The transcriptional regulation of hepcidin depends also on another pathway, which is sensitive to the iron concentration and includes the activation of bone morphogenetic protein (BMP), mainly BMP6 signaling with subsequent phosphorylation of intracellular Smad proteins. BMPs belong to the TGF-β family of peptide growth factors and form a complex with membrane receptors (BMPR), which induces the activation of the signaling pathway involving the transcription of Smad proteins, especially Smad4. Smad proteins translocate into the nucleus of the cell where they affect the transcription of specific genes and activate the expression of HAMP, Smad6, Smad7, and Id1. The activity of the BMP/Smad system is controlled by regulatory proteins (repulsive guidance molecules; RGMs) that function as co-receptors modulating the binding of BMP with BMPR. One of such cell-specific co-receptor that modulates hepatic BMP/Smad signaling is hemojuvelin (HJV). In 2004, an HJV mutant was described in patients with severe juvenile hemochromatosis (HH type 2B). BMP-related signaling pathways seem to be involved in the modulation of HFE-dependent hepcidin expression as well.

Hepcidin expression is also controlled by hypoxia and mechanisms involved in induction of erythropoiesis. According to data published by Chaston et al., hypoxia could repress hepcidin expression through inhibition of BMP/Smad signaling. Recently Sonnweber et al. showed that a novel regulatory pathway associated with platelet derived growth factor (PDGF)-BB is responsible for down-regulation of hepatic hepcidin expression in hypoxia.

The role of HFE protein in the regulation of iron homeostasis

The HFE protein is the product of a gene located on the short arm of chromosome 6 (6p21.3) which was first cloned in 1996. A missense HFE gene mutation involving the exchange of cysteine for tyrosine, i.e. C282Y (Cys282Tyr), has been identified in over 80% of clinically diagnosed HH patients, so called HFE or classical HH, type 1. The HFE protein consists of 343 amino acids forming a heavy chain, three extracellular domains (α1, α2, and α3), transmembrane domain and a short cytoplasmic domain.

It is now understood that a mutation in the gene encoding the HFE protein leads to a reduction in hepcidin expression in the liver, inadequate for the iron stores in the body. However, the complete mechanism of action has not been completely described. HFE appears to mediate the cellular uptake of transferrin-bound iron by interacting with TfR, although alone it has no iron-binding capacity. The distinctive structure of the HFE protein allows binding to the α1 and α2 domains of TfR1 protein and the α3 domain of β2-microglobulin (β2M). As binding sites on TfR1 for HFE and holotransferrin overlap, HFE protein plays a role in the competitive inhibition of the attachment of iron-saturated transferrin to TfR1.

Gao et al. found that the interaction between HFE and TfR2 is necessary for proper transcriptional regulation of hepcidin in response to holotransferrin. It was suggested that an increase in transferrin saturation and binding of holotransferrin to TfR1 displaces the HFE protein and at the same time stabilizes TfR2 to form the complex Tf/TfR2/HFE. Calzolari et al. showed that the binding of HFE with TfR2 induced hepcidin expression by activating the kinase cascade through Erk1, Erk2 and p38 mitogen activated protein kinase. However, the role of the HFE/TfR2 interaction in the induction of hepcidin is still debated. Although the interaction of overexpressed HFE and TfR2 has been found in tissue culture, recent in vivo studies on transgenic mice and observations in cell lines have challenged the above thesis. It is now even postulated that HFE and TfR2 may influence hepcidin regulation independently.

Regardless of these findings, a key hypothesis regarding the impact of HFE/TfR on the optimal functioning of the BMP/Smad signaling pathway in the activation of hepcidin expression has been endorsed. When studying liver sections from people diagnosed with HH type 1, no correlation was found between the expression of HAMP, Smad7, and Id1 and the severity of iron accumulation in tissue. This observation was not confirmed in patients with iron deposits free of the HFE mutation. D’Alessio et al. observed the interaction between HFE, TfR2, and HJV in iron sensing, and found that HFE, TfR2, and HJV form a multi-protein membrane complex on the surface of hepatocytes to regulate hepcidin expression.

Recently, Wu et al. proposed a novel molecular model of the interaction of HFE with BMP/Smad signaling. They showed that, in cultured hepatocytes and mouse liver, HFE binds to the BMP type I receptor, thereby inhibiting activin receptor like kinase-3 (ALK3) ubiquitination and proteasomal degradation and increasing ALK3 protein expression and accumulation on the cell surface. In the presence of the two HFE
mutations C282Y and H63D, regulation of ALK3 protein ubiquitination was different and the final increase in expression of both: ALK3 cell surface and hepcidin.\textsuperscript{[40]}

Homozygous C282Y alleles have the strongest impact on the excessive accumulation of iron in patients with HH. In carriers with both mutated alleles, a significant transformation of the spatial structure of the HFE protein occurs. It has been suggested that the disruption of a disulfide bridge within the α3 domain may interfere with β2M protein binding and prevent the normal interaction of HFE with TfR1.\textsuperscript{[51]} In the Caucasian population, the C282Y mutation at a single allele is detected at a frequency ranging from 1:12 to 1:20, and at both alleles at 1:200 to 1:400. Among Afro-Americans, the mutation is recognized much less frequently, at a ratio of 1:4000.\textsuperscript{[41]} In the European population, the frequency of the C282Y mutation is variable and ranges from 0% in southern Europe to 12.5% in Ireland.\textsuperscript{[42]} The presence of the mutation is not definitely and unambiguously correlated with disease, as not all individuals who are homozygous for C282Y develop symptoms. Homozygous C282Y mutations have been detected in 60% to 96% of patients presenting typical symptoms of HH, among inhabitants of Europe, North America, and Australia.\textsuperscript{[43]} The risk determination of phenotypic expression is very controversial, because the research results are significantly variable. Beutler et al, by comparing the frequency of co-occurrence of the symptoms of HH with HFE gene mutations, suggested only a 1% chance for the phenotypic manifestation.\textsuperscript{[44]} Observations conducted among residents of Europe, USA, Canada, Australia, and New Zealand indicated the prevalence of iron accumulation symptoms in 38% to 50%, and the possibility of developing multi-organ pathology caused by iron storage in 10% to 33% of C282Y homozygotes.\textsuperscript{[45-47]} The risk of a disturbance in iron metabolism is much higher for males (28% vs 1%).\textsuperscript{[48]} The reason for a lack of phenotypic expression is not clearly defined, and extensive research is being conducted to identify other genes and factors that increase the risk of disturbances in iron homeostasis like male gender, hepatotropic virus infections, alcohol consumption, or a co-existing metabolic syndrome.\textsuperscript{[31, 49]} Recent discoveries indicate new gene variants which seem to modify clinical course of HFE-HH. Among them a novel variant in the glyceronephosphate O-acyltransferase (GNPAT) is mentioned. Knockdown of its expression in human hepatoma cells led to downregulation of hepcidin expression. Also the coinheritance of GNPAT.p.D519G in some male C282Y homozygotes was associated with severe iron overload.\textsuperscript{[50]}

In approximately 4% of HFE-HH patients, a mixed heterozygocity for the C282Y mutation, in combination with the H63D mutation (His63Asp, i.e. the replacement of histidine with asparagine) has been detected.\textsuperscript{[51]} Unlike the C282Y mutant protein, no H63D mutant-specific aberrations in the synthesis, intracellular transport, expression, or binding to β2M have been revealed.\textsuperscript{[31]} Based on the results of these molecular studies, it was initially assumed that the H63D mutation alone does not lead to pathological iron accumulation. However, the higher frequency of H63D allele detection in the population of Caucasian patients with clinical symptoms of HH when compared with the general population points to the potential involvement of this polymorphism in the pathophysiology of iron storage.\textsuperscript{[31]} The expected risk of pathological accumulation of iron with different degrees of severity in mixed heterozygotes is estimated to vary from 1% to 2%.\textsuperscript{[52]} The H63D mutation itself occurs in Caucasian populations with an incidence of 15% to 20%.\textsuperscript{[31]} In homo- and heterozygous carriers, mild symptoms of iron storage can manifest, mainly in the form of disturbances in biochemical parameters of iron metabolism.\textsuperscript{[53]} The significance of these additional mutations as possibly pathogenic has been raised, as they may affect the risk of developing cirrhosis and HCC in the case of co-existing hepatitis virus infections and excessive alcohol consumption.\textsuperscript{[54-56]} On the other hand, according to different observations, simple heterozygotes for either the C282Y or H63D HFE gene did not present evident iron overload. Morbidity for both HFE simple heterozygote groups was similar to that of HFE wild-type participants.\textsuperscript{[57]}

Other HFE polymorphisms have been rarely reported, and they are usually detected in patients with the typical clinical symptoms of HH, with no classical HFE HH genotype confirmed.\textsuperscript{[42]} Some of them may lead to significant dysfunction of the HFE protein.\textsuperscript{[58]}

The clinical consequences of TfR2 dysfunction, which result from TfR2 gene mutations, are classified as HH type 3. It is clinically similar to HFE-HH and shares the same inheritance pattern but manifests at an earlier age.\textsuperscript{[9]}

Excessive accumulation of iron dependent on variations in the gene encoding Fpn

Fpn is the only well-described cellular transporter of iron.\textsuperscript{[20, 59]} It is assumed that the spatial structure of Fpn consists of 9 or 10 transmembrane helices. Pathogenic mutations in the gene encoding Solute Carrier family 11, member A3 (SLC11A3) appear to be associated with autosomal dominant HH. They cause the second most common genetic iron overload disorder after classical HFE-HH, called HH type 4.\textsuperscript{[60, 61]} The molecular consequences of mutations at different locations are divided into two groups. The Fpn mutations A77D, D157G,
V162del, N174I, Q182H, Q248H, G323V, and G490D impact on the ability of the protein to reach the cell surface, lead to the loss of Fpn activity and decreased cellular iron export. Retention of iron occurs in macrophages responsible for recovering iron from old erythrocytes and clinically manifests itself as tissue iron accumulation, hyperferritinemia and low serum transferrin saturation. The decreased iron content in the extracellular space is parallel to the reduction of transferrin saturation in the circulatory system, which becomes a signal to intensify iron absorption from the intestinal tract. The described pathology has been defined as ferroportin disease. The second group of mutations (N144H, Y64N, C326Y/S, S338R and Y501C) is associated with increased activity of ferroportin due to the resistance of ferroportin to inhibition by hepcidin or by decreased hepcidin binding ability or impaired internalization. Insensitivity to hepcidin results in an excessive iron absorption from the gastrointestinal tract and a decrease in the amount of this metal in macrophages. All this leads to an increase in the amount of iron in plasma. Clinically, it is called a non-classical Fpn disease characterized by hyperferritinemia, elevated or normal transferrin saturation, and increased iron accumulation, mainly in hepatocytes, which what makes its clinical picture similar to that observed in other types of HH.[63]

As a very rare clinical observation, Japanese authors have described a single-point dominant mutation, A49U, in the IRE at the 5’ end of the mRNA of the H-subunit of ferritin members of one family. They all presented symptoms of non-HFE iron overload with excessive accumulation of iron, increased transferrin saturation, and high serum ferritin concentrations.[64, 65]

### Primary and secondary iron overload

Pathological accumulation of iron in the body can generally be divided into primary, i.e. genetically determined (Table 1), or secondary, resulting from other genetic or acquired diseases (Table 2). HH, called primary or in-

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**Table 1. Summary of primary iron overload disorders**

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Mechanism</th>
<th>Fe indices*</th>
<th>Hepcidin#</th>
<th>Liver Fe%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HFE (HLA-H)</td>
<td>AR</td>
<td>Low hepcidin</td>
<td>↑SF/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>2A</td>
<td>Hemojuvelin HJV</td>
<td>AR</td>
<td>Low hepcidin</td>
<td>↑SF/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>2B</td>
<td>Haptenin HAMP</td>
<td>AR</td>
<td>No functional hepcidin</td>
<td>↑SF/↑TS</td>
<td>No or inactive</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>Transferrin receptor 2 (TrR2)</td>
<td>AR</td>
<td>Low hepcidin</td>
<td>↑SF/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>4 Classical (Ferroportin disease)</td>
<td>Ferroportin SLC40A1</td>
<td>AD</td>
<td>Loss of FPN activity</td>
<td>↑SF/↑TS</td>
<td>↑</td>
<td>M</td>
</tr>
<tr>
<td>4 Non-classical (Ferroportin disease)</td>
<td>Ferroportin SLC40A1</td>
<td>AD</td>
<td>FPN active but resistant to hepcidin-induced internalization</td>
<td>↑SF/↑TS</td>
<td>↑</td>
<td>H</td>
</tr>
</tbody>
</table>

*: arrows ↑↓↔ indicate increase, decrease and normal levels, respectively; #: liver iron loading in hepatocytes (H) and/or macrophages (M). AR/AD: autosomal recessive/dominant; SF: serum ferritin; TS: transferrin saturation.

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**Table 2. Summary of secondary iron overload disorders**

<table>
<thead>
<tr>
<th>Underlying disorder</th>
<th>Mechanism</th>
<th>Fe indices*</th>
<th>Hepcidin#</th>
<th>Liver Fe%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological disorders</td>
<td>Transfusional iron overload, inhibition of hepcidin synthesis</td>
<td>↑SF/↓TS</td>
<td>↓</td>
<td>M</td>
</tr>
<tr>
<td>Thalassemia syndromes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate kinase deficiency</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sickle cell anemia</td>
<td></td>
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<tr>
<td>Hereditary spherocytosis</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Myelodysplastic syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>Oxidative stress and chronic low-grade inflammation</td>
<td>↑SF/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>ALD</td>
<td>cause changes in levels of iron regulatory proteins</td>
<td>↑SF/↑TS</td>
<td>↑</td>
<td>H/M</td>
</tr>
<tr>
<td>NAFLD</td>
<td></td>
<td>↑SF/↑TS</td>
<td>↑</td>
<td>H/M</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Impaired release of Tf bound Fe from cells due to deficiency in multicopper ferroxidase, ceruloplasmin</td>
<td>↑SF/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>Aceruloplasminemia</td>
<td>Oxidative stress due to accumulation of porphyrins in cells decreases hepcidin expression</td>
<td>↑FS/↑TS</td>
<td>↓</td>
<td>H</td>
</tr>
<tr>
<td>Porphyria cutanea tarda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: arrows ↑↓↔ indicate increase, decrease and normal levels, respectively; #: liver iron loading in hepatocytes (H) and/or macrophages (M). ALD: alcoholic liver disease; NAFLD: non-alcoholic fatty liver disease; Tf: transferrin; SF: serum ferritin; TS: transferrin saturation.
born, is one of the most common genetic metabolic diseases, with a prevalence in Caucasian population ranging from 3 to 8 cases per 1000 individuals.\textsuperscript{[66]} It is defined as a set of pathological symptoms accompanying iron accumulation in the parenchymal cells of many organs, including liver, pancreas, heart, gonads, and the pituitary. The most common causes of death diagnosed in patients with HH include liver cirrhosis, HCC, diabetes and cardiovascular failure caused by myocardial iron deposition.\textsuperscript{[67]} Hemochromatosis is caused by mutation(s) in genes whose protein products regulate iron homeostasis (Table 1).\textsuperscript{[68]}

Secondary iron overload develops in the course of many hematological disorders such as thalassemia syndromes, pyruvate kinase deficiency, hereditary spherocytosis, and myelodysplastic syndrome. Many of these diseases are associated with a need for frequent blood transfusions, which causes an excessive body iron supply. Additionally, impaired erythropoiesis leads to hypoxia which stimulates the synthesis of erythropoietin, type II transmembrane serine proteinase, and growth differentiation factor 15. These molecules, in turn, suppress hepcidin synthesis, leading to even more profound body iron accumulation.\textsuperscript{[69-71]} Aceruloplasminemia, hypotransferrinemia, and DMT1 deficiency are genetic disorders with an autosomal recessive pattern of inheritance which result in secondary hemosiderosis due to ineffective transport of iron. The mutation underlying aceruloplasminemia is localized in the ceruloplasmin gene, encoding for a multicopper ferroxidase that is synthesized in hepatocytes and is required for iron mobilization in cells. Deficiency of ceruloplasmin results in iron deposition associated with retinal degeneration, diabetes mellitus, and neurological symptoms accompanied with hyperferritinemia.\textsuperscript{[72]} Lack of transferrin production in patients with transferrinemia or the presence of non-functional DMT1 protein are associated with severe microcytic anemia, and an adaptive increase in iron absorption, which leads to massive liver iron loading.\textsuperscript{[73, 74]} Other factors associated with secondary iron accumulation include chronic liver diseases such as infection with hepatitis C virus (HCV),\textsuperscript{[75]} alcoholic liver disease,\textsuperscript{[76, 77]} non-alcoholic fatty liver disease (NAFLD),\textsuperscript{[78]} and porphyria cutanea tarda (Table 2).\textsuperscript{[79]} Chronic infection with HCV and NAFLD are among the major causes of liver disease and a serious risk factors for cirrhosis and HCC.\textsuperscript{[80]} Secondary iron overload frequently diagnosed in NAFLD or chronic hepatitis C (CHC) is a significant comorbid factor and it associates with disease progression.\textsuperscript{[81, 82]} The molecular mechanisms underlying dysregulation of iron metabolism has not been completely elucidated. The question why, in the same clinical background, some patients develop secondary iron overload and others do not still remains to be answered. It is known that some genetic factors may be involved.\textsuperscript{[83-86]} Also the benefits of application of iron depletion as an adjuvant therapy in these diseases have been extensively discussed.\textsuperscript{[87, 88]}

Iron overload in NAFLD

The pathogenesis of NAFLD is considered a multifactorial process. Unhealthy lifestyle leads to changes in gut microflora, abnormal endocrine function of adipose tissue and disturbances in glucose and fat metabolism in the liver. As a result, a chronic, low-grade inflammation develops which causes further damage leading to liver disease progression to steatohepatitis.\textsuperscript{[89]} Excessive body iron accumulation which is found in about 30% of patients with NAFLD has been termed dysmetabolic iron overload syndrome (DIOS). DIOS is defined as a co-existence of hepatic steatosis with mild to moderate iron deposition in liver biopsies and elevated serum ferritin level with nearly normal transferrin saturation in patients with insulin resistance.\textsuperscript{[90]} However, the reasons why only a subset of patients with NAFLD shows alterations in iron parameters is at present unclear.

Elevated serum ferritin concentration has been found as an independent predictor of advanced hepatic fibrosis in patients with NAFLD, and is also associated with hepatic iron deposition, lobular inflammation, steatosis and hepatocyte ballooning.\textsuperscript{[82, 91]} Parenchymal accumulation of iron in NAFLD was associated with higher risk of a fibrosis stage greater than 1 (OR=1.7; 95% CI: 1.2-2.3).\textsuperscript{[83]} Iron depletion therapy of patients with NAFLD and hyperferritinemia resulted in the improvement of liver histology and a decrease of activity of liver enzymes.\textsuperscript{[92]} In NAFLD multiple factors contribute to development of disturbances in body iron metabolism. Unhealthy, imbalanced diet, rich in free fatty acids (FFA) and monosugars, is considered one of the major risk factors for disease development and progression. It was shown that FFA can specifically bind to ferritin and thereby modulate iron uptake and release by this protein. Binding of intracellular FFA to ferritin would enhance iron mineralization as well as prevent iron release, and probably affect downstream iron signaling.\textsuperscript{[93]} Indeed, recently Dongiovanni and colleagues found that a diet rich in FFA directly affects iron metabolism by inducing expression of IRP1 and TFR1 leading to intracellular accumulation of iron.\textsuperscript{[94]} Diet-induced hepatic iron overload in mice appeared prior to the onset on liver steatosis and insulin resistance.\textsuperscript{[95]} Accumulation of iron in the livers of rats fed a high-fat diet was associated with oxidative stress and increased hepatic production of TNF-α, IL-6 and leptin.\textsuperscript{[96]} This diet-induced increase in hepatocyte
Iron in cirrhosis

Iron overload in CHC

Elevation of total body iron stores is diagnosed in up to 40% cases of CHC and in 50% of patients with both CHC and HCC. Viral infection interferes with cell iron metabolism and iron, in turn, modulates HCV proliferation. Filebeen and colleagues showed a reduction of intracellular Fe levels, reduced iron uptake and increased iron release capacity in Huh7 cell lines stably expressing a subgenomic HCV replicon. They also found a decreased expression of ceruloplasmin and TfR1 as well as an elevated Fpn mRNA levels in these replicon cells. Higher expression of Fpn was also found in the duodenum and the liver of CHC patients. Additionally, viral infection upregulates TfR2 expression and mRNA level of TfR2 decreases significantly in parallel with a decline in the liver iron content after a successful eradication of HCV. Recently Namba and colleagues found a downregulated expression of iron-sensing protein, F-box and leucine-rich repeat protein 5 in CHC patients in comparison to control despite their significantly higher levels of serum iron and ferritin. This effect was associated with an increased iron deposition.

HCV infection increases intracellular generation of ROS by directly affecting mitochondrial metabolism and elevating Nox protein expression. ROS can affect iron metabolism, for example by upregulation of TfR1 expression and iron uptake by the cell or activation of IRP1. HCV-mediated oxidative stress was also found to decrease hepcidin expression in hepatoma cells. It has been suggested that this effect is responsible for secondary iron overload observed in CHC. Down-regulation of hepcidin by HCV core protein involved ROS generation and a displacement of C/EBPα and STAT3 proteins from the hepcidin promoter, while core+/ARFP protein repressed hepcidin transcription via the activator protein 1 binding site. Also patients with CHC display a decreased hepcidin expression in serum and in the liver in comparison to uninfected controls. After the successful antiviral treatment patients show significantly higher hepatic iron values in comparison with nonresponders to the therapy. Kohjima et al found that lower expression of hepcidin and Fpn in untreated CHC patients associates with SVR after PEG-IFN and ribavirin therapy but other studies seem to question the predictive value of hepcidin on therapeutic outcome.

Iron inhibits HCV RNA polymerase NS5B and treatment of hepatoma cells with iron donor suppresses sub-cellular replication of HCV. Also viral replication is blocked in Huh7 cells by siRNA-mediated knockdown of hepcidin. Treatment with iron donor and the inhibition of hepcidin both upregulate intracellular ferritin which, and not free intracellular iron, was postulated to influence viral replication. This in vitro effect of iron on HCV is confusing in the light of clinical data suggesting a clear association of excess hepatic or serum iron with disease progression and treatment outcome in CHC. There is still much discussion on the relevance of these findings for HCV infection management.

Iron is an important factor in liver disease progression to cirrhosis

Liver fibrogenesis is a complex and dynamic tissue and cellular process, occurring in the course of chronic liver disease and leading to a progressive accumulation of extracellular matrix (ECM) components in an attempt to limit the consequences of hepatic damage. Progression of hepatic fibrosis leading to the formation of regenerative nodules of liver parenchyma results in disruption of lobular architecture, impairment of the fine vascular structure, development of portal hypertension, and loss of metabolic liver function with the risk of variceal bleeding, encephalopathy, and kidney insufficiency. Liver cirrhosis also poses a significant risk factor for the development of HCC and represents the most common non-neoplastic cause of death in Europe and USA.

The molecular mechanisms of the stimulatory effect of iron overload on the development of fibrosis and cirrhosis are not clear. It is known that intracellular iron catalyzes the formation of several ROS, which promote lipid peroxidation and protein damage. This sensitizes hepatocytes to other stresses, finally leading to mitochondrial dysfunction and apoptosis resulting in degenerative changes in liver tissue (ballooning hepatocytes,
Apgoptotic hepatocytes are subsequently phagocytosed by Kupffer cells, which become activated and synthesize a wide range of pro-inflammatory and pro-fibrotic factors including TNF-α, IL-1, IL-10, IFN-γ, TGF-β1, PDGF, bFGF, MCP-1, and ROS. Activation of Kupffer cells can also occur through the direct action of excessive iron accumulation in these cells. Soluble factors produced by activated Kupffer cells as well as by injured hepatocytes lead to the recruitment and infiltration of macrophages and lymphocytes. Inflammatory infiltration localized in portal and periportal areas is correlated with the intensity of fibrosis in HH patients. Stimulated Kupffer cells and damaged hepatocytes also activate hepatic stellate cells (HSCs), which is considered a crucial step in the initiation of fibrogenesis. HSCs, comprising 13%-15% of all liver cells, reside within the space of Disse and are considered a main source of collagen and other components of ECM in normal and fibrotic liver. Activated HSCs trans-differentiate into myofibroblast-like hepatic stellate cells (HSC-MFs). HSC-MFs, together with other types of myofibroblasts (MF), originating from different types of cells, are highly proliferative and synthesize the components of ECM as well as several growth and immunomodulatory factors, and thus are able to sustain and perpetuate fibrosis, chronic inflammation, and angiogenesis in the liver (Fig.). Iron can directly activate the process of fibrogenesis in the liver as HSCs have been found to be activated by ferritin or transferrin, which both bind to their high-affinity receptors on the cell surface. Additionally, HSC activation in HH patients increases with hepatic iron concentration; this process can be reversed by iron removal by phlebotomy. Clinical consequences of iron overload in liver cirrhosis

Iron overload and risk of infections and liver failure

The relationship between disturbances in body iron homeostasis and the function of the immune system is a notable issue in the context of clinical repercussions. Infections are common reasons for clinical deterioration and decompensation in liver cirrhosis. In post-transplant medical care, infectious complications may influence survival as negative prognostic factors. Thus, excessive accumulation of iron in liver cirrhosis is perceived as a systemic pathology and warrants a thorough diagnosis and treatment, also taking into account the post-transplantation prognosis. Cellular disorders of iron metabolism, theoretically, may affect the normal stimulation of macrophage differentiation and proliferation of lymphocyte subpopulations, and increase their susceptibility to oxidative stress associated with an increased risk of DNA damage. Lowering the content of iron in phagocytic cells, which is observed with the down-regulation of hepcidin, inhibits the translation of pro-inflammatory cytokines such as TNF-α and IL-6. Also,
Iron in cirrhosis

dendritic cell differentiation, their involvement in antigen presentation, and the induction of specific immune cell populations correlate with a decrease in TfR1 expression and the activity of Fpn. Mice with homozygous disruption of the Hfe gene, resulting in low hepcidin and high expression of macrophage Fpn, appear to be a good model for research focused on the impact of decreased intramacrophage iron on the TLR4-activated inflammatory response. Impairment of TLR4 signaling leading to abnormal cytokine production may result in greater susceptibility to some bacterial infections.

There are some interesting observations in carriers of C282Y homozygotes which may partially explain increased susceptibility to bacterial and some viral infections in patients with liver cirrhosis and iron overload. Some studies in patients with HFE-HH show a significant reduction in total lymphocytes, especially in the population of CD8 lymphocytes. Based on studies in rodents, some authors suggest that excessive accumulation of iron under the conditions of the experiment did not alter the number of cells, but affected the mechanisms of cytokine regulation. However, in cases of excessive iron accumulation, the number of NK cells and CD8 cells in liver tissue is decreased. Porto et al showed a significant correlation between an increased CD4/CD8 ratio in the blood with the severity of iron storage and liver fibrosis. It has even been suggested that this indicator could be a predictive factor in determining the severity of the illness. Undoubtedly, the impact of iron overload on innate or acquired immunity requires further research, based both on experimental and clinical observations, particularly in view of the rapid progress in studies on cellular iron metabolism.

Clinical observations confirm that the serum concentration of ferritin, considered not only as an acute phase protein, but also an indicator of iron overload in patients with cirrhosis, appears to be associated with markers of liver insufficiency. In other research, serum ferritin was described as an independent predictor of mortality and liver-related clinical events in patients awaiting liver transplantation. Moreover, in this study, the authors showed that patients with elevated serum ferritin had more intense iron accumulation in explanted livers. Maiwall et al found the same correlation between higher serum ferritin and early mortality in patients with decompensated cirrhosis, excluding from the study subjects with secondary iron overload. In turn, Abu Rajab et al demonstrated that marked hepatic hemosiderosis secondary to cirrhosis may be associated with the risk of extrahepatic complications, especially pancreatic and cardiac, due to an increased body iron content. Dysregulation of iron homeostasis with elevated serum parameters of iron metabolism and low hepcidin is associated with acute-on-chronic liver failure and multi-organ insufficiency. Maras et al even suggested that the measurement of plasma transferrin saturation could serve as a predictor of poor outcome and early mortality in this group of patients. The results from the National Transplant Registry indicate that hepatic iron overload may be associated with decreased survival after liver transplantation.

Thus, it seems important for the clinical prognosis to establish both the diagnostic rules for accurate assessment of the iron content in liver cirrhosis and possible methods of safe iron removal therapy. It has been reported in patients who were qualified for liver transplantation that iron reduction with preoperative venesection reduced the risk of cardiac and infection complications postoperatively.

Iron and HCC

HCC is the sixth most common cancer in the world and the leading cause of death from malignancy. Liver cirrhosis is the primary risk factor for developing HCC as it is diagnosed in 70%-90% of all cases of HCC worldwide. The microenvironment of a cirrhotic liver, with cytokines, growth factors, and products of oxidative stress released as a result of activation of HSC and Kupffer cells, can stimulate neoplastic transformation. Also, the inhibition of the hepatocyte proliferation rate, observed in cirrhosis, can facilitate clonal expansion of preneoplastic cells in the liver and thus lead to HCC development.

Hepatic iron overload is linked with an increased risk of HCC in patients with cirrhosis of different etiology. HCC occurs in approximately 20% of cirrhotic HH patients and is one of the most common causes of death in non-treated HH. In patients with cirrhosis without a determined background, the presence of iron deposition in liver regenerative nodules has been associated with the presence of HCC. Elevated serum transferrin saturation correlates with an increased incidence of cirrhosis and HCC, especially in patients with high alcohol consumption. The prevalence of both iron overload and HCC was found to be high in cirrhotic patients with viral hepatitis C or hepatitis B, but relatively low in patients with biliary cirrhosis. The critical role of the iron-overload state in the development of HCC was shown in patients with CHC. A combination of phlebotomies and low-iron diet significantly reduced the level of hepatic oxidant markers and was suggested to lower the risk of HCC.

Excessive hepatic iron can cause neoplastic transformation in the presence of cirrhosis or it can be directly
hepatocarcinogenic. Exogenous iron administration is associated with enhanced tumor development and growth in HCC cell lines and murine models.\textsuperscript{174-176} Additionally, HCC has been documented to occur in iron-overloaded patients in the absence of cirrhosis, albeit rarely.\textsuperscript{177} The majority of diagnosed HCC cases, however, develop as a consequence of cirrhosis, and iron overload accelerates the progression of the disease.

Free, unbound iron catalyzes the formation of highly toxic ROS. The accumulation of ROS-mediated DNA damage can facilitate the progression of chronic liver injury to HCC. This process of cell immortalization is accompanied by the induction of telomerase activity and overexpression of pro-oncogenic microRNA miR-92.\textsuperscript{178} Oxidative stress in HCC diagnosed in HH patients has also been found to be associated with an increased mutation rate in the p53 tumor suppressor. In fact both, genetic instability and p53 inactivation play critical and cooperative roles in the progression of HCC.\textsuperscript{179} The background of cirrhotic liver with chronic cycles of hepatocyte death and regeneration further stimulates disease progression. Iron overload can also act synergistically with other HCC risk factors such as aflatoxin B1 or alcohol, and increase the mutagenesis rate.\textsuperscript{180, 181} Chronic severe iron overload resulting in damage to hepatocytes may lead to the epigenetic abnormalities found in HCC. In patients with HH, aberrant hypermethylation has been detected in pre-neoplastic liver tissue. Among the tested genes, the tumor suppressors RASSF1A and p16-CDKN2A, and the cell-cycle regulator cyclin D2 exhibited differential methylation, albeit to a different extent (55%, 45%, and 32%, respectively). Moreover, changes in methylation patterns were observed in the liver tissue adjacent to HCC.\textsuperscript{182} In a rodent model of chronic iron overload, both tumor suppressive as well as tumor-promoting mechanisms were found to be activated in liver tissue. Among the pro-carcinogenic mechanisms, a decrease in p53 levels and increases in p52, p46, and β-catenin levels were found, while protective responses included an increase in C/EBPα and a decrease in Yes-associated protein. In this study, no tumors were observed in iron-loaded rats; the authors suspected that this was due to the high resistance of rat models to the development of cirrhosis in response to iron overload, which contrasts with observations made in humans and eliminates this important risk factor for HCC.\textsuperscript{183}

Nitric oxide (NO) produced by cytokine-stimulated macrophages and hepatocytes can inhibit the proliferation of tumor cells. The non-heme iron content is a key factor modulating the effect of NO on cell viability.\textsuperscript{184} Finally, excess iron may prevent efficient removal of arising tumor cells by influencing immune system functions, since exogenous iron reduces the tumoricidal activity of macrophages,\textsuperscript{185} inhibits lymphocyte proliferation, and modulates the balance between different populations of T cells.\textsuperscript{154, 186}

The development of HCC is associated with changes in the expression of iron regulatory genes, as the mRNA levels of HAMP, ceruloplasmin, transferrin, and TfR2 are reduced while TfR1 expression is elevated in HCC.\textsuperscript{187} Interestingly, HAMP expression is downregulated in cancerous tissue regardless of the degree of tumor differentiation and the serum concentration of hepcidin-25.\textsuperscript{188} Boyault et al identified 16 genes, among them the HAMP gene, whose expression could correctly distinguish between different subgroups of HCC.\textsuperscript{189} Hepcidin suppression may be linked with decreased p53 activity, frequently observed in HCC.\textsuperscript{190} The promoter region of HAMP gene contains a putative p53 response element and its expression is induced by the activation of p53.\textsuperscript{190}

**Targeting iron in treatment of liver cirrhosis**

**Iron depletion therapy**

Reduction of body iron stores in the early stages of hepatic fibrosis can slow the disease progression and thereby improve survival. Phlebotomy is a safe, cheap and easy to perform iron depletion therapy in patients with iron overload. It was proven to be especially effective in HH patients with fibrosis where it leads to normalization of ALT levels, alleviation of some of the associated symptoms of HH such as abdominal pain, malaise, fatigue, skin pigmentation and insulin resistance. However, other clinical features of HH such as arthropathy and hypogonadism respond poorly to iron depletion or are not responsive at all.\textsuperscript{191} Among 185 HH patients treated with phlebotomy 42 (23%) had a documented decrease in liver fibrosis stage during a 14-year observation period.\textsuperscript{193}

Phlebotomy can also be useful in reversing liver fibrosis in a certain types of secondary iron overload. Venesection resulted in a significant reduction in serum procollagen III, a marker of fibrogenesis, in 22% of CHC patients who previously did not respond to IFN monotherapy.\textsuperscript{194} Long-term phlebotomy (56±28 months) improved liver function tests as well as histopathology of the liver in 15 out of 30 patients with CHC.\textsuperscript{195} Likewise the severity of liver fibrosis decreased significantly during a 5-year phlebotomy maintenance program of CHC patients in comparison to IFN treated, non-responsive control CHC group. At the same time the intensity of liver inflammation remained unchanged as compared to significant progression observed in the controls.\textsuperscript{196} The
Iron in cirrhosis

results of phase II clinical trial on effect of phlebotomy in 31 NAFLD patients suggest that this treatment may improve the overall liver histology (measured as NAFLD activity score).^{197}

Once cirrhosis has developed, iron depletion therapy is generally considered ineffective in the reversal of the fibrosis process. Cirrhotic HH patients have an increased risk of HCC and death regardless of treatment.^{191} It is generally accepted, that for patients with liver failure due to cirrhosis or HCC in cirrhotic liver, an orthotopic liver transplantation is the only solution.^{191} However, there are some reports on successful therapy by venesection in cirrhotic patients with a diagnosed primary or secondary iron overload. Falize and colleagues reported a regression of advanced fibrosis, defined as a decrease of at least 2 units on a METAVIR scale, during a 2-year period in 17 among 36 HH patients treated with phlebotomy. In this study a subgroup of 23 patients with histological evidence of cirrhosis, 8 subjects presented regression of fibrosis. In this study, authors also defined serum gamma-globulins, prothrombin activity, and platelet count as biochemical parameters which can predict regression of the fibrosis.^{141} What was important, they recruited patients with stable liver function, without severe abnormalities in mean values of serum albumin concentration, prothrombin activity which could indicate liver failure. Phlebotomy was shown to improve liver function and thrombocytopenia, a frequent hematological complication of advanced fibrosis, in cirrhotic patients with iron overload.^{200} Given this data and the safety of phlebotomy, this treatment should be considered as an important adjuvant therapy in patients with advanced fibrosis, even liver cirrhosis. But in cirrhotic patients an especial attention should be paid to exponents of good liver function. Advanced portal hypertension, unstable erythropoiesis are obvious contraindications to this form of therapy.

Indications for therapeutic phlebotomy should take into account not only serum markers of iron overload: elevated iron and ferritin concentrations, transferrin saturation but if possible also the intensity of tissue iron accumulation. The use of histopathological examination to assess liver iron deposits semiquantitatively or quantitative spectrophotometric analysis of iron content in liver biopsy specimens is limited. In case of cirrhosis biopsy samples are not sufficiently representative due to advanced fibrosis with nodular transformation of liver parenchyma. MR imaging appeared to be useful diagnostic procedure to detect liver iron overload in liver cirrhosis.^{201} Another method of iron removal, alternative to phlebotomy, hypothetically safer in cirrhotic patients is erythrocytapheresis. The costs of the procedure are the same as with phlebotomy, and because a larger amount of iron can be removed fewer sessions of erythrocytapheresis are required.^{88} Generally patients are treated until near-iron depletion is reached that is ferritin concentration 50 ng/mL or lower and serum transferrin saturation below 45%. The subsequent phlebotomy sessions (usually once per 2-3 months) aim to maintain these levels.^{88, 197, 200, 202}

For patients who cannot tolerate phlebotomy because of anemia or other comorbidities such as cardiac dysfunction, iron chelation therapy could be considered. Among licensed iron chelators deferoxamine must be administered by intravenous or subcutaneous injections, while deferiprone and deferasirox can be given orally.^{203} Numerous clinical trials showed that these drugs decrease organ iron toxicity in patients with severe iron overload and dramatically improve their survival. Therefore, despite their unexplained low iron chelation activity in some patients and various side effect (such as agranulocytosis, neutropenia, arthropathy) chelators are the drugs of choice when there is no other iron removal therapy applicable.^{204, 205}

Just as phlebotomy, iron chelation therapy can improve liver functions. For example early trials showed that deferoxamine prevented progression of hepatic fibrosis in children.^{206} Treatment with deferiprone resulted in a reversal of liver fibrosis in 15 out of 17 patients with β-thalassemia with iron overload during a 3-year period.^{207} Deugnier et al observed an improvement in liver fibrosis in iron-overloaded β-thalassemia patients treated with deferasirox for at least 3 years. Liver fibrosis was reversed or stabilized in 83% of 219 patients. Interestingly, this effect was independent of a reduction in the concentration of hepatic iron concentration.^{208} Treatment with deferasirox inhibited hepatic fibrogenesis in a rat model of non-alcoholic steatohepatitis.^{208} However, in a mutant mouse model of liver fibrosis, apart from reduction of expression of fibrogenic genes, no effect was observed.^{210} The reason for this inconsistency might be due to the differences in doses and treatment periods applied in these studies and further research is needed to elucidate the potential antifibrotic activity of deferasirox. Moreover, expanded clinical observational studies are needed to study the risk of fungal infections associated with interaction between iron chelators and iron-uptaking siderophores of zygomycetes (invasive fungi). Unlike deferoxamine, oral iron chelators deferiprone and deferasirox probably may present antifungal activity.^{211}

Reduction of iron-related oxidative damage

Liver diseases is closely linked with oxidative stress and many antioxidants were shown to exhibit hepatopro-
Protective activity in animal models of liver injury. Considering important role of iron in mediating oxidative damage it is interesting that a number of natural antioxidants also possess iron-chelating properties. For example curcuminoids from Curcuma longa can bind ferric and ferrous ions and were found to decrease oxidative stress and serum NTBI levels in β-thalassemia patients. Polyphenols from green tea exhibited hepatoprotective effect on iron-loaded hepatocytes, and decreased serum iron indices and lipid peroxidation in iron-loaded mice. Drinking tea with meals reduced dietary iron absorption in patients with metabolic syndrome as well as the frequency of phlebotomies needed for the management of patients with HH. Importantly, green tea extract inhibited or delayed hepatic iron deposition in regularly iron-loaded thalassemic mice. Another well known natural polyphenol, resveratrol, was found to protect the liver from iron-mediated injury. Although resveratrol did not affect the degree of hepatic iron-overload in livers of mouse models of primary and secondary iron overload, it showed significant antiinflammatory, antioxidant, antipoptotic and antifibrotic activity. Silymarin from Silybum marianum is a complex mixture of phenolic compounds with a recognized hepatoprotective, immunomodulatory and antioxidant properties. Silymarin, and one of its flavonolignan constituents, silybin, bind Fe(III) with high affinity, and have shown to reduce body iron indices in patients with CHC. A number of clinical trials reported significant decrease of serum iron indices in β-thalassemia patients treated with a combination of silymarin with conventional iron-chelators, in comparison with chelator alone. In HFE-HH patients silymarin reduced dietary iron absorption of non-heme iron by over 40%. Although many antioxidants prevent hepatic damage, reduce inflammation and oxidative stress markers, they are very often much less effective for treatment of already established liver disease. So far beneficial effects of antioxidants application for patients with liver cirrhosis have not been unequivocally confirmed, although some studies reported a significant decrease of liver-related mortality rates in treatment groups. According to ClinicalTrials.gov several antioxidants were recently tested in clinical trials as complementary therapeutics in patients with hepatic fibrosis and cirrhosis. However, no study addressed so far any potential beneficial effects of antioxidants with iron chelating properties on patients with liver cirrhosis with elevated body iron stores. It remains to be studied if these compounds could be specifically useful in this group of patients. Nevertheless application of adjuvant therapy with antioxidants in cirrhotic patients should be taken with caution as degree of liver damage associates with changes in bioavailability of some compounds and adverse effects of particular antioxidants on liver function have also been reported.

Conclusion
Disturbances in body iron balance occur commonly in liver cirrhosis. They may be of genetic origin like in HH, which is one of the most common genetic metabolic diseases of humans. Iron overload is an important factor in liver disease progression to cirrhosis. In turn, the diagnosis of liver cirrhosis is often attributed to signs of pathological accumulation of iron, even in the absence of genetic abnormalities. Very few research studies have been devoted to the analysis of changes in the expression of iron regulatory proteins in liver cirrhosis. Possible complex disturbances in iron homeostasis may be a consequence of both genetic factors and morphological changes in the liver, including the disruption of lobular architecture, progressive fibrosis, and loss of parenchyma. As iron overload in liver cirrhosis is suggested to associate with the risk of liver failure, severe infections, pancreatic and cardiac complications, and the development of HCC, further studies on the role of iron in advanced liver disease are needed. Better knowledge of the mechanisms associated with disturbed iron homeostasis and its clinical consequences is desirable to implement treatment for iron overload in liver cirrhosis and improve the prognosis in this condition.

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