bjh review

How we manage patients with hereditary haemochromatosis

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Summary

A number of disorders cause iron overload: some are of genetic origin, such as hereditary haemochromatosis, while others are acquired, for instance due to repeated transfusions. This article reviews the treatment options for hereditary haemochromatosis, with special attention to the use of erythrocytapheresis. In general, therapy is based on the removal of excess body iron, for which ferritin levels are used to monitor the effectiveness of treatment. For many decades phlebotomy has been widely accepted as the standard treatment. Recent publications suggest that erythrocytapheresis, as a more individualized treatment, can provide a good balance between effectiveness, tolerability and costs. Other treatments like oral chelators and proton pomp inhibitors, which are used in selected patients, create the possibility to further individualize treatment of hereditary haemochromatosis. In the future, hepcidin-targeted therapy could provide a more fundamental approach to treatment.

Keywords: hereditary haemochromatosis, treatment, phlebotomy, erythrocytapheresis.

Hereditary haemochromatosis (HH) is a term used to describe a group of genetic disorders characterized by increased iron absorption. This may lead to a progressive accumulation of iron in tissues and organs, resulting in impairment of organ structure and function, especially of the liver, pancreas, heart, pituitary gland and, probably, joints. The prevailing mechanism in most types of HH is deficiency of hepcidin (also termed HAMP), originally identified as an antimicrobial peptide (Park *et al*, 2001) and then shown to play a major role in iron homeostasis (Pigeon *et al*, 2001; Ganz, 2003). Hepcidin is synthesised mainly in hepatocytes and controls plasma iron concentration by binding to ferroportin (also termed SLC40A1), the only known cellular iron exporter. After binding, ferroportin is degraded, reducing both intestinal absorption of iron from enterocytes and iron release from hepatocytes and macrophages. Increased plasma iron or cellular iron stores, as well as inflammation, generate a negative feedback that leads to a restriction of iron release into plasma and blockade of dietary iron absorption, through increased hepcidin production. Both hypoxia and increased erythroid demand generate a positive feedback, leading to increased flow of iron into plasma mediated by a decreased hepcidin production. The effects of inflammation, hypoxia and erythroid activity on hepcidin production are independent of the normal homeostatic feedback mediated by iron.

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HH classification

Four main types of HH can be distinguished according to which of the proteins involved in iron homeostasis is affected.

Type 1, also called *HFE*- related HH, caused by mutations in the *HFE* gene, is the most frequent form of genetic iron overload and results in a decreased production of hepcidin. The most common causal mutation is a G to A transition at nucleotide 845 of the *HFE* gene, resulting in cysteine to tyrosine substitution at amino acid 282, referred as p.C282Y (Type 1a) (Powell *et al*, 2016). This form is mainly present in populations of north European origin where one in 200–300 individuals are homozygous for this mutation. Although the prevalence of homozygosity for the p.C282Y mutation is high, the penetrance of disease is relatively low. Between 1–33% of the homozygotes develop clinical manifestations related to iron overload (Allen *et al*, 2008; Pietrangelo, 2010). Such a wide range is caused by the different ways in which homozygotes are identified, e.g. in population- or patient-based studies, as well as by the influence of other genetic and non-genetic factors, e.g. certain bone morphogenic protein (BMP) 2 variants (Milet *et al*, 2007) and alcohol consumption (Fletcher *et al*, 2002). Allen *et al* (2008) found gender differences, reporting much lower penetrance in females versus males (1·2% vs. 28·4%). This has been attributed to physiological iron loss during menstruation and pregnancies and an antioxidant effect of oestrogen. With the discovery of hepcidin, however, the lower penetrance in females has also been attributed to naturally occurring higher hepcidin levels as this was observed in female mice (Krijt *et al*, 2004), and in C282Y homozygous women with a body mass index ≥28 (Desgrippes *et al*, 2013).

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Another known genetic subtype is the p.H63D mutation, which does not cause a significant iron overload, but may act as cofactor for iron overload phenotypic expression (Moirand *et al*, 1999; Kanwar & Kowdley, 2013), mostly in combination with p.C282Y, classified as compound heterozygosity (p.C282Y/p.H63D) (Type 1b) (Powell *et al*, 2016) with a prevalence of 2% in populations of north European origin. Only 0·5–2·0% of people with this specific type of haemochromatosis develop clinical symptoms of iron overload. Porto *et al* (2016) concluded that this genotype is insufficient to result in haemochromatosis and that these patients need additional risk factors for iron overload or liver disease. HFE genotype type 1c, e.g. p.S65C, etc., does not substantially affect the phenotype (Powell *et al*, 2016). However, there are sparse clinical and epidemiological data available on this genotype.

The other HH genotypes are not related to the *HFE* gene and have a very low prevalence. Type 2 HH is associated with mutations in the haemojuvelin gene, *HFE2* (Type 2A) or the hepcidin gene, *HAMP* (Type 2B) respectively. As in type 1 HH, hepcidin deficiency is the prevailing mechanism. Type 2 HH is the most severe form, occurring in younger individuals and therefore called juvenile haemochromatosis.

Type 3 HH is associated with mutations in the transferrin receptor 2 gene (*TFR2*), causing hepcidin deficiency.

Ferroportin disease, or type 4A HH, is the only autosomal dominant form and is associated with mutations in the ferroportin gene (*SLC40A1*). In this type the production of hepcidin is normal, but the export function of ferroportin is lost. This leads to intracellular iron retention with low levels of plasma iron as well as low levels of transferrin saturation (TS). The spleen is the most affected organ in type 4A HH, because of high ferroportin activity at macrophage level (Schimanski *et al*, 2005; Brissot, 2016). In type 4B the hepcidin concentration is also normal, but the receptor function of ferroportin is altered, with similar consequences for iron accumulation as in type 1 HH. The *SLC40A1* mutation in type 4B leads to insensitivity of ferroportin to hepcidin, resulting in excessive iron efflux from cells to plasma (Drakesmith *et al*, 2005).

Another form of a very rare and serious inherited iron overload disease is aceruloplasminaemia, caused by the absence of the feroxidase enzyme ceruloplasmin, resulting in iron accumulation in most organs, including the central nervous system.

Iron overload mechanisms

Hepcidin production is decreased in types 1, 2 and 3 HH. Hepcidin deficiency allows hyperabsorption of dietary iron into plasma from duodenal enterocytes and increased release of recycled iron from splenic and liver macrophages and hepatocytes. This results in a chronic elevation of plasma iron, which exceeds the iron-binding capacity of circulating transferrin and results in the presence of iron in a non-transferrin-bound form (NTBI). Various parenchymal cells, especially in the liver, pancreas and heart, avidly take up NTBI. A special NTBI component, called labile plasma iron (LPI), is involved in the Haber-Weis and Fenton reactions, leading to production of reactive oxygen species in the cells, resulting in tissue oxidative damage and vital organ dysfunction.

Diagnosis

An increase in serum ferritin (SF) concentration with a concomitant increase in the TS is suggestive for HH. When TS is low or normal, iron overload might be based on type 4A HH, although this pattern is most commonly seen as part of an acute phase response. TS levels ≥75% are highly suggestive for the presence of highly toxic LPI (Brissot, 2016).

In published cohorts approximately 77–78% of men and 47–52% of women, homozygous for the p.C282Y mutation had elevated SF and TS at baseline and 37% of males and 3% of females had SF > 1000 μg/l at baseline (Adams *et al*, 2008; Allen *et al*, 2008). The predicted probability for SF to increase to >1000 μg/l after 12 years with baseline SF between 300–1000 μg/l was 13–35% in males and 16–22% in females (Allen *et al*, 2008).

In patients with consistently elevated SF and increased TS, after excluding haematological or inflammatory diseases, genetic tests should be performed. The first step in populations of north European origin is testing for the p.C282Y mutation (Porto *et al*, 2016). Testing for the p.H63D mutation can be considered as an optional complementary test. Genetic testing for other variants of HH is indicated in patients that test negative for p.C282Y homozygosity but have proven iron overload, demonstrated by magnetic resonance imaging (MRI) or liver biopsy. For patients that are homozygous for p.C282Y, genetic screening is indicated in their adult first-degree relatives (Porto *et al*, 2016).

A liver biopsy is no longer required to confirm the diagnosis of HH or to assess iron load, but is still recommended to stage the degree of fibrosis when SF is >1000 μg/l (Bacon *et al*, 2011). However, transient elastography, having a specificity of about 80%, has become a suitable non-invasive alternative for liver biopsy (Legros *et al*, 2015). Currently, MRI techniques are used to evaluate body iron excess before treatment (Gandon *et al*, 2004), and are probably accurate enough to rule out iron overload in the liver and other organs (Sarigianni *et al*, 2014). To date, it is not clear whether MRI can be used to control the result of iron depletion after treatment.

Prospective studies and standardized MRI protocols are needed to determine the role of MRI in the management of HH.

Clinical manifestations

The manifestation of *HFE* HH, usually occurring in middle-aged patients, is diverse because iron deposition can occur in multiple tissues and may vary from only genetic abnormalities (genotype) through biochemical abnormalities, such as

increased SF and TS (biochemical phenotype) to severe organ damage (clinical phenotype). The traditional HH stigmata of bronze diabetes, arthropathy and liver cirrhosis were found in early reports before the discovery of the *HFE* gene. Nowadays chronic fatigue, joint pains, abdominal complaints and moderately elevated transaminases are typical early clinical features (Swinkels *et al*, 2002; Janssen & Swinkels, 2009). The most common sign on physical examination is hepatomegaly (Pietrangelo, 2010).

Organ damage

The principal target of iron overload is the liver. Iron is initially preferentially stored in hepatocytes and, with severe overload (SF > 1000 μg/l), also in bile ductular and Kupffer cells. Iron-induced activation of stellate cells may stimulate fibrogenesis and cause cirrhosis (Barton *et al*, 2010), ultimately even leading to hepatocellular carcinoma (HCC). Concomitant diseases, such as alcoholic liver disease, non-alcoholic fatty liver disease and chronic viral hepatitis, which can be present in up to 15% of HH patients, will increase the risk of liver injury and development of cirrhosis (Barton, 2013). Cirrhosis and HCC are the major causes of death in patients with untreated HH. The quantity of iron stored in the liver is the major known predictor of cirrhosis. However, recent cohort data showed that, in some patients, cirrhosis may already occur at lower levels of iron overload, while other individuals with severe iron overload do not have cirrhosis (Barton, 2013), indicating that other factors besides iron overload may be responsible for the development of cirrhosis and HCC.

Other complications of advanced HH are (i) diabetes, caused by iron-overload in the pancreas, (ii) cardiomyopathy and arrhythmias caused by heart siderosis, (iii) impotence and hypogonadotropic hypogonadism caused by iron overload in the pituitary gland and testes, and (iv) destructive arthropathy/arthritis with primary involvement of the 2nd and 3rd metacarpophalangeal joints, interphalangeal joints, hips and knees.

Some manifestations, such as weakness, skin pigmentations and hepatic fibrosis, may regress with appropriate treatment while others, such as cirrhosis, cardiomyopathy, diabetes and arthropathy, are irreversible, although certain aspects of these complications might be improved with treatment (increased liver enzymes, daily insulin requirements) (Bomford & Williams, 1976; Falize *et al*, 2006). Early diagnosis and treatment are therefore important if morbidity and mortality are to be reduced.

Treatment

Once HH is diagnosed, therapy is straightforward and effective, based on the removal of excess of body iron. The treatment consists of two phases, the depletion phase with the goal of lowering SF to target values, and the maintenance treatment with the goal of maintaining stable target SF values.

Despite the absence of randomized controlled trials (RCTs), the benefit of iron depletion has been well documented and established by case series from clinically diagnosed HH patients. Life expectancy of HH patients on therapy equals that of the non-HH population when this disorder is diagnosed before the onset of cirrhosis and diabetes (Bomford & Williams, 1976; Niederau *et al*, 1996; Milman *et al*, 2001; Wojcik *et al*, 2002).

Given that about 90% of all HH cases are associated with homozygosity for the p.C282Y mutation (Burke *et al*, 2000), this article will focus on the treatment of type 1 HH.

Timing: when should treatment be started?

After the diagnosis of HH has been established, either surveillance or treatment is recommended according to the European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) guidelines (European Association for the Study of the liver (EASL), 2010; Bacon *et al*, 2011). Where the SF level is within the normal range, yearly follow-up is advised. Where the SF level is elevated, pre-emptive treatment is recommended to reduce SF to target levels. This includes treatment of asymptomatic individuals with homozygous HH and increased iron stores. As there are no studies providing evidence-based data about optimal timing to start treatment, all current recommendations are based on expert opinion.

Whether patients with mild hyperferritinaemia (SF 300–1000 μg/l) need treatment is currently under debate. Bardou-Jacquet *et al* (2015) followed 1085 p.C282Y homozygotes who were treated according to current recommendations. The subgroup with mild hyperferritinaemia (SF 300–1000 μg/l) treated with iron depletion had a better survival than the general population due to a decreased cardiovascular and extrahepatic cancer-related mortality. In treated patients only those with SF higher than 2000 μg/l had increased mortality compared to the general population, mainly related to liver disease. These results suggest a beneficial effect of early and sustained management of patients with even mild iron excess, in contrast to earlier studies (Gurrin *et al*, 2008; Allen *et al*, 2010) that advise surveillance instead of therapy. However, the results reported by Bardou-Jacquet *et al* (2015) cannot clarify whether treatment of HH patients with mild hyperferritinaemia favours surveillance, because HH patients were compared to the general population. A randomized controlled study comparing either surveillance or treatment of HH patients with mild hyperferritinaemia may give better insight into the optimal SF level to start iron removal treatment.

What are target SF levels for depletion phase?

According to the 2010 EASL guideline for *HFE* HH, the target level of SF is less than 50 μg/l (European Association for

the Study of the liver (EASL), 2010). However, new data from the literature suggest that excessive iron depletion down to iron-deficient levels may be counter-productive and may lead to increased intestinal iron absorption through hepcidin suppression caused by lowering SF levels (Piperno *et al*, 2007; Van Dijk *et al*, 2008; Girelli *et al*, 2011). It has long been known (Lynch *et al*,1989) that iron absorption is increased at lower SF levels: 42% versus 12% when the mean SF levels were 14 and 538 μg/l respectively.

The Current Practice Guidelines developed by the AASLD advises a target SF level between 50–100 μg/l (Bacon *et al*, 2011). Although several national guidelines still mention a target SF level of less than 50 μg/l, most experts now state that SF levels close to 50 μg/l are the optimal target level (Kanwar & Kowdley, 2013; Brissot, 2016).

What are target SF levels for maintenance phase?

The same target SF levels as for the depletion phase (50–100 μg/l) are recommended for maintenance therapy. Some experts recommend surveillance rather than treatment for patients with SF below the upper limit of normal value, given that non-haem absorption is markedly increased at lower levels of iron stores leading to more frequent maintenance treatments (Van Dijk *et al*, 2008). More data are needed to confirm this recommendation.

Methods for iron depletion

Phlebotomy

Since 1950, phlebotomy has been the most widely accepted treatment for HH (Davis & Arrowsmith, 1950). The effect of the treatment is simple: repeated withdrawal of red blood cells (RBCs) reduces the iron that is present in haemoglobin (Hb). This stimulates erythropoiesis and thereby mobilises iron stored in organs and in this way eliminates excessive stored iron.

Treatment regime in the depletion phase. Once weekly, if tolerated, on average 450–500 ml of whole blood (7 ml/kg), containing 200–250 mg of iron is removed until SF is decreased to target levels (European Association for the Study of the liver (EASL), 2010; Bacon *et al*, 2011; Adams & Barton, 2010). Although the SF decreases by an average 30 μg/l per phlebotomy, the total number of procedures needed is highly variable and depends mainly on iron reserve status (Harrison & Bacon, 2003). In one study (Adams, 1998), of 77 HH patients with a mean SF of 2·554 μg/l, the mean duration of phlebotomy therapy was 1·4 years (range 0·44–3·6 years) with a treatment frequency of once every 1·38 weeks. McDonnell *et al* (1999), reported that the mean duration of treatment in the depletion phase was 14 months with a total number of procedures between 28 and 43.

Treatment regime in the maintenance phase. In the maintenance phase the phlebotomy frequency is reduced to 2–6/year. After initial therapy not all patients re-accumulate iron at the same speed, as shown in a study of Adams *et al* (1993), in which 21 homozygous HH patients were followed for a mean of 4·0 years. Manet *et al* (2013) point to the iron reabsorption index, expressed as milligrams of iron removed per day of treatment, as a potential valuable phenotypic indicator for daily iron over-absorption, which may guide the clinician in balancing phlebotomy interval and volume. Recently Verhaegh *et al* (2016) suggested that modified iron avidity index (IAI) might be a fairly good predictor at identifying those patients that need more than 3 phlebotomies per year. The calculated IAI consists of SF at diagnosis divided by age at diagnosis minus 20 when male or plus 20 when female. However, the correlation between IAI and number of phlebotomies was only moderate and the usefulness of IAI has to be confirmed in prospectively conducted studies.

How should treatment be monitored?. The SF level, known to be directly related to total body iron (1 μg/l of SF corresponded to 7·5 mg of body iron) (Basset *et al*, 1984), is used to monitor effectiveness of treatment. The frequency of measurements depends upon SF values: every 3 months in the initial stage of depletion treatment and more frequently as SF approaches the normal range (European Association for the Study of the liver (EASL), 2010; Bacon *et al*, 2011). In the maintenance phase of treatment the frequency of SF measurement is decreased to once every 6 months.

It should be taken into account that the SF test suffers from low specificity, as elevated SF values can be the result of a range of inflammatory, and neoplastic conditions. However in the group of patients with a secure diagnosis of HH, it is a sensitive and specific marker to monitor therapy, taking into account that in certain situations increased SF may result from other causes.

Regular Hb measurements should be performed before each phlebotomy, to avoid reducing the Hb to <80% of the starting value. When anaemia is present, phlebotomy should be postponed until the anaemia has resolved.

Side effects of phlebotomy treatment. The most common side effects of phlebotomy treatment are fatigue, fainting, pain at the venous access site, haematomas and anaemia (Table I). Overall, 52% of patients during the induction phase of treatment and 37% in the maintenance phase report negative experiences related to treatment and 16% of patients would even decide to stop phlebotomy treatment if alternative options became available (Brissot *et al*, 2011).

Patient compliance with phlebotomy treatment was evaluated in a study by Hicken *et al* (2003). During the depletion phase 76% of patients complied with weekly (33%) or bi-weekly (43%) schedules. In the first year of maintenance therapy 84% of patients complied with therapy; subsequent compliance decreased by 6·8% annually.

Table I. Treatment of Hereditary Haemochromatosis: comparison between phlebotomy and personalised erythrocytapheresis.

Phlebotomy Regime	Volume removed	Advantages	Disadvantages	Compliance	Adverse effects
• Once weekly in depletion phase • Once every 2–6 month in the maintenance phase	• 450–500 ml whole blood	• Widely available • Broad experience • Safe • Inexpensive	• Frequent visits	• Depletion phase: good • Maintenance phase: good	• Transient hypovolaemia • Fatigue • Pain at the venous access site • Haematomas

Personalised erythrocytapheresis Regime	Volume removed	Advantages	Disadvantages	Compliance	Adverse effects
• Once every 2–3 weeks in the depletion phase • Once every 4–18 month in the maintenance phase	• Individually determined, between 300–1000 ml RBCs	• Most effective • Safe • Excellent tolerability • Return of valuable blood components • Possibility of compensation of removed volume • Reduction of adverse events	• Requires special equipment and trained staff	• Depletion phase: excellent-good • Maintenance phase: excellent-good	• Citrate reactions • Mild dizziness • Transient hypovolaemia • Pain at the venous access site • Haematomas

Erythrocytapheresis

A more recent alternative for HH treatment is erythrocytapheresis, a technique that selectively removes RBCs and returns valuable blood components, such as plasma proteins, clotting factors, platelets, etc., to the patient and so is particularly suitable for patients suffering from hypoproteinaemia and/or thrombocytopenia.

With erythrocytapheresis it is possible to remove up to 1000 ml RBCs per single procedure, compared to 200–250 ml RBCs per phlebotomy. Another advantage is the possibility for substitution of the removed RBC volume with saline, albumin or other colloid solutions. This results in fewer haemodynamic changes compared to phlebotomy, making this treatment particularly well suited for patients with cardiac diseases (Rombout-Sestrienkova *et al*, 2014).

Two different approaches are used when applying erythrocytapheresis in daily practice.

In an individualised approach based on sex, body weight, total blood volume (TBV), and actual haematocrit (Hct), erythrocytapheresis allows for a more sophisticated and accurate adjustment for the volume of removed RBCs (Conte *et al*, 1983, 1989; Zoller *et al*, 1988; Kellner & Zoller, 1992; Muncunill *et al*, 2002; Fernandez-Mosteirin *et al*, 2006; Rombout-Sestrienkova *et al*, 2007, 2012, 2016a; Wijermans *et al*, 2009; Poullin & Lefevre, 2011; Rehácek *et al*, 2012; Evers *et al*, 2014). This approach showed that erythrocytapheresis was more efficient than phlebotomy, with a significant reduction in the number treatment procedures (between 50–70%) required in the depletion phase as well as in the maintenance phase (at least 43%). Furthermore erythrocytapheresis produced a significantly reduced treatment duration in the depletion phase and a significant prolongation of inter-treatment interval in the maintenance phase of treatment. One of our studies (Rombout-Sestrienkova *et al*, 2012) also showed that total treatment costs of erythrocytapheresis in the depletion phase are in the same range or even lower than phlebotomy because of a considerable reduction in the number of total treatment procedures, travel costs and costs resulting from work absenteeism. In addition, it was shown that the majority (80%) of patients preferred erythrocytapheresis to phlebotomy (Rombout-Sestrienkova *et al*, 2016a). The decrease in the number of treatment procedures with erythrocytapheresis may also have a positive effect on compliance with lifelong maintenance treatment.

Mariani *et al* (2005) and Kohan *et al* (2000) applied personalised erythrocytapheresis in combination with recombinant human erythropoietin with the aim of increasing the volume of removed RBCs and this method decreased total treatment duration. This approach offered good results in a short time, but cost-effectiveness was not proven.

Another therapeutic approach has been applied by Sundic *et al* (2014) and Stefashyna *et al* (2014). They chose to remove a standard volume of RBCs in each patient (400 and 360 ml respectively), which also led to a reduction in total number of treatments, but without a reduction in the treatment duration. This approach lacks a potential advantage of

erythrocytapheresis because a fixed volume is used instead of personalised removal of RBCs, making it relatively more expensive and therefore less competitive with phlebotomy. Also Stefashyna *et al* (2014) studied a selected population of patients in order to be able to use the removed RBCs for transfusion purposes. However, if patients with HH could be accepted as blood donors, it would be presumably more cost-effective when 2, 3 or even 4 RBCs units could be removed through erythrocytapheresis, depending on the patients' TBV and Hb.

Based on published data and our own experience we currently recommend individually adjusted erythrocytapheresis in the depletion and maintenance phase of treatment.

Treatment regime in the depletion phase. The recommended frequency of erythrocytapheresis procedure is once every 2–3 weeks, depending on Hb recovery. The RBC volume to be removed must be calculated based on individual parameters and is usually between 350–800 ml RBCs. The minimal targeted post procedure Hct must be at least 30%, preferably at 32–34%. In patients without co-morbidities and estimated removed RBC volume ≤500 ml, substitution is not needed. For removal of higher RBC volumes, it is recommended that 30% of the removed RBC volume should be replaced with isotonic saline during the first treatment procedure. Depending on how well the patient tolerates the first procedure, 0–50% of the estimated removed RBC volume can be replaced in all subsequent treatment procedures. Standard replacement of 50–100% of removed volume of RBCs is recommended in patients with cardiac disease.

Treatment regime in the maintenance phase. The frequency of erythrocytapheresis is reduced in the maintenance phase, and varies between 1–3 procedures per year, sometimes only 1 procedure in 1·5–2 years (Rombout-Sestrienkova *et al*, 2016a).

How should treatment be monitored?. Monitoring erythrocytapheresis treatment in the depletion phase is identical to treatment with phlebotomy, although we would recommend more frequent SF measurements, for instance after every 3rd erythrocytapheresis procedure in the early phase of treatment and after every procedure when SF approaches the normal range.

In the maintenance treatment phase the policy is identical to the phlebotomy treatment: SF measurements every 6 months.

Side effects of erythrocytapheresis treatment. The use of apheresis generally leads to a reduction in moderate and severe adverse events compared to whole blood collections (Wiltbank, 2002). This is related to the saline compensation and the longer collection time during apheresis procedures, facilitating trans-capillary refilling of the intravascular compartment (Popovsky, 2004).

The most frequently described but overall still rare adverse reactions during therapeutic apheresis procedures (Table I) include reactions to the citrate used as anticoagulant (e.g. muscle cramps, paraesthesiae, nausea). The frequency of citrate reactions varies between 0·38–7·8% (Lee & Arepally, 2012), depending on the type of apheresis procedure applied. They are relatively rare in erythrocytapheresis because of the restricted volume of infused citrate in the relatively short procedure time (15–45 min for erythrocytapheresis versus 3–5 h for other therapeutic apheresis procedures). Other described side effects, such as dizziness, vasovagal collapse and problems with venous access, are even less frequent and mild of character.

In a RCT comparing erythrocytapheresis with phlebotomy (Rombout-Sestrienkova *et al*, 2012), all adverse events in the erythrocytapheresis group appeared to be mild. In a total of 171 procedures, eight events were reported (4·7%): one case of citrate reaction (0·58%), one vasovagal collapse (0·58%) and six cases of dizziness (3·5%).

Iron chelators

There are three chelating agents currently approved by the US Food and Drug Administration (FDA): deferoxamine, deferiprone and deferasirox.

Deferoxamine has been approved for treatment of secondary iron overload in thalassaemia and is used as a subcutaneous or intravenous infusion. Adverse effects include retinal and auditory neurotoxicity.

Deferiprone is an oral chelator approved for treatment in transfusion-dependent thalassaemia patients when current chelation with deferoxamine is inadequate. Significant side effects of deferiprone include neutropenia and agranulocytosis.

Deferasirox is the latest oral chelator to be approved. Adverse events, such as diarrhoea, headache, nausea, abdominal pain, increased serum creatinine, increased liver enzymes, rash, fatigue and arthralgia, occur in more than 10% of all patients (Phatak *et al*, 2010).

In HH chelation has been used when other treatments were not appropriate or possible, i.e. in severe congestive heart failure, without appropriate access to peripheral veins and in case of severe anaemia (Nagler *et al*, 2011). Treatment with a combination of oral chelators has also been reported in a case of severe juvenile haemochromatosis (Fabio, 2007). Two studies showed that deferasirox, used once daily at 10 mg/kg/day, can reduce iron burden in patients with HH (Phatak *et al*, 2010; Cançado *et al*, 2015). The higher incidence of adverse events with deferasirox (Phatak *et al*, 2010) may have a negative influence for patient compliance and acceptance as a treatment. Currently, the use of chelators is indicated only in selected patients with underlying anaemias or reduced venous access. Newer agents, such as silybin, deferitrin and starch-conjugated deferoxamine, have been evaluated for the use in treatment of chronic iron overload (Borsari *et al*, 2001; Barton, 2007; Harmatz *et al*, 2007).

Future perspectives

Hepcidin deficiency is the cause of iron overload in almost all types of HH (with exception of type 4A). Not surprisingly, hepcidin has become the target for the development of novel therapeutics. New approaches are based on manipulating the mechanism regulating hepcidin production. Currently there are two types of approaches under investigation: first, minihepcidins, which are hepcidin-based agonists, and second, stimulators of hepcidin production acting to promote hepcidin activity through targeting the TMPRSS6 or BMP6 proteins (Fung & Nemeth, 2013).

Supporting treatment options

Proton pump inhibitors (PPIs)

Gastric acid has an important function in the release of non-haem iron, the major form of iron in most diets. Another role lies in the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), after which iron can enter the enterocyte via the divalent metal transporter 1 (DMT1). In vivo studies have shown that the reduction of ferric iron and the formation of ferric chelates are decreased when the pH is above 2·5. PPIs act through inhibition of gastric acid production, increasing the average pH in the stomach to >3·6 over 24 h.

Two studies showed that administration of PPIs to patients with HH inhibits the absorption of non-haem iron (Hutchinson *et al*, 2007) and significantly reduces the number of phlebotomies required to maintain SF below target levels (Van Aerts *et al*, 2016). These studies imply that PPIs could have an additional role in the treatment of selected HH patients. However, the place of PPIs in treatment of HH remains uncertain.

Environmental factors- dietary management

There are no RCTs that have evaluated whether avoidance of certain food components or drinks has a positive effect on the number of treatment interventions needed in HH patients. However, there have been studies about the influence of certain diets on the SF levels.

Fletcher *et al* (2002) and Scotet *et al* (2003) showed a cumulative effect of alcohol with iron overload, increasing oxidative stress and progression of disease. Coexistence of steatosis (Powell *et al*, 2005), as well as hepatitis C infection (Diwakaran *et al*, 2002) has been reported as potentiating liver fibrogenesis.

Therefore it is recommended that patients with HH and liver damage should stop alcohol consumption, and that where there is HH without liver injury, alcohol consumption should be restricted.

Cade *et al* (2005) showed increased absorption of iron caused by vitamin C. However there is no need to discourage people from consuming fresh fruits and the expert opinion is to limit the intake of vitamin C in supplements to 500 mg/day (Nienhuis, 1981; Milward *et al*, 2008).

Kaltwasser *et al* (1998) and, later, Hutchinson *et al* (2010) showed a reduction in both iron resorption and in the rate of iron accumulation by drinking black tea during meals.

Treatment of HH complications

Liver disease

Chronic inflammation due to iron-mediated oxidative stress may lead to fibrosis, cirrhosis and an increased chance of developing HCC. Cirrhosis can ultimately lead to liver failure and death. Liver damage is often worse in people with HH who also have concomitant diseases, such as chronic hepatitis B and or C, and alcoholic or non-alcoholic liver disease. Patients with cirrhosis should be monitored and screened for presence of HCC every 6 months (European Association for the Study of the liver (EASL), 2010). Liver transplantation (LT) is considered an important treatment modality for HH patients with end stage liver disease, such as complicated cirrhosis and HCC. A French study (Bardou-Jacquet *et al*, 2014) demonstrated that LT cured the iron overload phenotype in HH by normalizing hepcidin synthesis, suggesting that HH is a liver disease that can be cured by transplantation. Survival of patients with HH after LT was similar to that of the overall population.

Cardiac disease

HH-induced arrhythmias and congestive heart failure should be treated with a standard regimen including diuretics, angiotensin converting enzyme inhibitors, beta-blockers and aldosterone antagonists (Jesup *et al*, 2009). Treatment with calcium channel blockers and DMT1 blockers has demonstrated a reduction in cardiac iron deposits in thalassaemic mice (Kumfu *et al*, 2012). The use of erythrocytapheresis is, in particular, suitable for this group of patients (Rombout-Sestrienkova *et al*, 2014). Cardiac transplantation is a viable treatment option for patients with severe heart failure but may be avoided by adequate iron removal (Jensen *et al*, 1993; Schofield *et al*, 2000; Rombout-Sestrienkova *et al*, 2014).

Arthralgia, arthritis

Musculoskeletal symptoms should be treated with analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). Colchicine and NSAIDs are usually effective in cases of complicating calcium pyrophosphate deposition disease with acute arthritis (Van Onna *et al*, 2011). There is scope for major benefit from replacement surgery interventions, especially joint arthroplasties. Unfortunately it is unusual for symptoms to be alleviated by iron removal treatment.

Diabetes mellitus

Treatment with oral anti-diabetics or insulin is indicated in accordance with guidelines. Adequate iron removal may improve glucose control, but insulin dependency is not reversible (Niederau *et al*, 1996).

Endocrine diseases

Hypothyroidism and hypogonadism are recognized complications of organ iron deposition and reactive inflammation. Thyroid function tests and testosterone serum levels should be monitored. In case of decreased levels substitution therapy is indicated because reversibility is not to be expected after iron removal.

Treatment of non-*HFE* HH

HH type 2A and 2B, known as juvenile haemochromatosis, are severe forms of non-*HFE* HH. The age of onset is usually below the age of 30 years and is, if not treated adequately, characterized by severe cardiomyopathy and hypogonadism. The basis of treatment is intensive iron removal. Regimes with intensive phlebotomies, sometimes combined with iron chelation, are employed. Our group has successfully applied erythrocytapheresis in the treatment of these patients.

Patients with type 3 and 4B HH are treated the same way as patients with HH type 1.

Unlike patients with type 4B HH, patients with type 4A ferroportin disease, associated with macrophage iron retention, are often asymptomatic and neither require phlebotomy nor tolerate it due to the risk of developing anaemia.

First choice treatment for patients with aceruloplasminaemia is the use of chelators as phlebotomy is not advised due to the risk of developing anaemia. We have however successfully applied erythrocytapheresis in patients who did not tolerate chelators, making personalised erythrocytapheresis a possible alternative.

Discussion

There are still some unsolved issues that are currently under debate regarding the treatment of HH. In particular, the appropriate cut-off point of SF for initiating treatment as well as SF target levels for either depletion or maintenance phase have not been established yet in an evidence-based manner.

Recently Ong *et al* (2015) initiated a RCT to examine whether there is a true benefit for treating patients who are homozygous for p.C282Y mutation with a moderately elevated SF (300–1000 μg/l). The patients are randomized to either erythrocytapheresis or to mock treatment with plasmapheresis. The outcome measures are markers of liver injury, hepatic fibrosis and oxidative stress as well as patient-reported outcome scales, such as modified fatigue impact scale. Until the results of this study become available, we advise following the AASLD guidelines (Bacon *et al*, 2011) despite the apparent illogicality of using values above the upper limit of normal values for starting treatment and SF values between 50–100 μg/l as the target for the maintenance phase.

In the treatment of iron removal one can choose between phlebotomy and erythrocytapheresis. Both procedures have their pros and cons. As is presented in Table I, both of our randomized trials (Rombout-Sestrienkova *et al*, 2012, 2016a) showed higher efficiency of personalised erythrocytapheresis in both the depletion and maintenance phases of HH treatment, especially in patients with higher TBV or body weight and in patients with higher Hb or achievable ΔHct. The advantages of personalised erythrocytapheresis over standard phlebotomy are the ability to remove greater amounts of RBCs in a single procedure and return the plasma components to the patient, thus increasing the efficiency of iron removal with greater intervals between treatments. On the basis of these results the current guidelines of the American Association for Apheresis (ASFA) recommend the use of erythrocytapheresis as a first-line therapy for HH patients (Schwartz *et al*, 2013).

In a recent published small observational study comparing serum iron parameters in males with type 1 HH during the depletion phase using either phlebotomy or erythrocytapheresis (Rombout-Sestrienkova *et al*, 2016b), we showed that erythrocytapheresis might lead to a better recovery of Hb and hepcidin by the start of the next procedure when compared to phlebotomy. However, measurements of serum iron parameters were performed only before treatment procedures (once a week for phlebotomy and once every 2–4 weeks for erythrocytapheresis) and not on a scheduled interval. The outcome of this small study needs to be confirmed in prospectively conducted studies.

On the other hand, it is obvious that phlebotomy is a simple procedure and can be performed in various situations. For erythrocytapheresis, one needs adequate equipment and trained staff. The latter procedure, therefore, is restricted to specially equipped health care centres, where apheresis equipment is available, or alternatively, to blood donation centres. Whole blood or RBCs obtained by either phlebotomy or erythrocytapheresis may be used for transfusion purposes in some countries, but is still not allowed or is under discussion in other countries (Pauwels *et al*, 2013).

In our opinion, the current treatment of HH by personalised erythrocytapheresis seems to be a very efficient and cost-effective approach, which could replace phlebotomy as a first line therapy for the majority of HH patients.

The use of erythrocytapheresis is not recommended for patients with anaemia and is debatable for patients needing low-frequency phlebotomies (1–2 per year).

Furthermore, it would be of great benefit for both patients and physicians if the total number of procedures of phlebotomy or erythrocytapheresis could be predicted when commencing treatment. This will guide patients and physicians in shared decision-making with regard to the most effective treatment modality, and will provide insight in cost-effectiveness

for insurance companies. Currently our group is developing an algorithm to predict the number of procedures required.

Alternative, less invasive therapies have been developed and are under investigation. The use of iron chelators seems to be indicated only in patients without appropriate access to peripheral veins or in case of severe anaemia (Phatak *et al*, 2010; Cançado *et al*, 2015). The (additive) use of PPIs is still under discussion (Van Aerts *et al*, 2016). Long-term follow-up and monitoring of side effects are needed to evaluate these treatments. In the future, hepcidin-targeted therapy could probably provide a more fundamental approach to treatment (Fung & Nemeth, 2013). However, to date, phlebotomy and erythrocytapheresis are still the cornerstones of HH treatment.

In conclusion, phlebotomy is the most widely accepted treatment of HH. However personalised erythrocytapheresis seems a very efficient treatment with a good balance between effectiveness, tolerability and costs, and could be an excellent alternative to phlebotomy.

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Author contributions

Eva Rombout-Sestrienkova: review design; literature review; writing the manuscript. Marian G.J. van Kraaij: critical revision of the manuscript. Ger H Koek: critical revision of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest relevant to the manuscript.

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