

**PUBLICATION OF THE SUPERIOR HEALTH COUNCIL No. 8672****Acceptance of *HFE* haemochromatosis gene mutation carriers as blood donors**

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**1. INTRODUCTION AND ISSUE**

For quite a while now, a controversy has raged amongst patients and carriers of the haemochromatosis mutation, general practitioners and blood establishments over whether or not haemochromatosis patients should be allowed to give blood.

At the moment, most blood establishments reject haemochromatosis patients as donors of whole blood and blood components. In Belgium, this approach is based on two provisions of the Act of 5 July 1994 regarding blood and blood products of human origin (modified by the Royal Decree of 1 February 2005) according to which blood and blood products for transfusion purposes shall only be collected from volunteer donors who shall receive no compensation in return. Donations from haemochromatosis patients (probably) fail to meet the disinterested and voluntary donation requirement, as they are carried out for therapeutic purposes. It follows that there is a risk that the donor will withhold information that is liable to stand in the way of their giving blood. The exclusion criteria for potential donors mentioned in the appendix of the Act referred to above also include metabolic disorders. Haemochromatosis can be taken to be such a disorder.

In a previous advisory report, the Superior Health Council (SHC, 2004) emphasised the paramount importance of the altruistic nature of blood donations, mainly in order to ensure the safety of the blood components. At the time, a request to depart from this principle in the case of haemochromatosis patients was declined.

However, critics to this approach have emphasised that excluding haemochromatotics results in the loss of a group of regular, loyal and very willing donors. It is important to note that a traditional donor population includes a certain number of individuals who are carriers of a mutation related to haemochromatosis. Indeed, most new donors are recruited among young adults at an age at which there is not yet any clinical manifestation of potential case of haemochromatosis.

Moreover, there has never been enough concern over the quality of the blood from haemochromatotic donors to carry out genetic or biological screenings aimed at identifying blood donors with undiagnosed preclinical haemochromatosis.

The greater vigilance and improved performance of the diagnostic tools used in the context of hereditary haemochromatosis entail a risk of losing ever more donors as a result of this diagnosis (Conry-Cantilena, 2001). This raises the question whether it is still justified to exclude all haemochromatotics from donating blood, given the considerable efforts that are continuously required to maintain the blood supply at an appropriate level.

In some countries (Norway, Sweden, South Africa, Canada, Australia), the blood from haemochromatosis patients is used for transfusion purposes without there having been any side effects reported in the recipients (Jeffrey & Adams, 1999). Several other countries have recently adopted a more flexible and/or more fine-tuned approach to blood donations from haemochromatotics (FDA, 2001; UKBTS, 2005; NZBS, 2006; Nicholson, 2009; Danic & Bigey, 2009). Yet taking such a step requires certain precautions, an overview of which is provided in APPENDIX 1.

These developments call for a revision of the previous SHC advisory report. For haemochromatosis patients to be allowed to give blood, the components obtained from these donors must be shown to be just as safe and effective as those that are currently used for transfusion purposes. Apart from this, what are the conditions on which haemochromatosis patients could safely be allowed to give blood? As already mentioned above, it is to be feared that implementing the usual eligibility criteria in selectively accepting donors with haemochromatosis will pose safety risks. Indeed, these potential donors could be tempted to withhold or play down information on potential impediments to donating blood.

This report aims at issuing up-to-date advice based on scientific arguments regarding the exclusion/eligibility of haemochromatotics as donors.

We will provide an answer to the following questions regarding the use for transfusion purposes of blood components from venesections carried out on haemochromatosis patients.

- Are there any risks involved in giving blood for the haemochromatotics themselves?
- Are there any risks involved in haemochromatotics giving blood for the safety of the blood components?
- If there are no risks involved in haemochromatotics giving blood, or if the risk is acceptable, is it at all desirable that these individuals should give their blood?
- Should haemochromatotics meet certain specific requirements in order to be authorised to give blood?
- Are there any particular organisational requirements to abide by in the event of haemochromatotics being allowed to give blood?

We also verify whether the advisory report is acceptable from an ethical point of view.

In Belgium, 39,088 therapeutic venesections were carried out in 2009 (NIHDI, 2009): 45 % in a department of internal medicine, 22 % in gastroenterology and 15 % in departments of clinical biology. 10 % are carried out by the general practitioner.

In order to update the advisory report, the issue was submitted to the working group "Blood and blood products", which includes the necessary experts.

## 2. ADVICE

For individuals who have been diagnosed as carriers of an *HFE* gene mutation but do not require therapeutic venesections, there are no additional requirements or restrictions as regards their acceptance as blood donors. This especially concerns heterozygous carriers of a mutation of the *HFE* gene, but also homozygous carriers or people with a compound mutation without any iron build-up (variable penetrance of the disease). The situation of these individuals only needs to be considered insofar as they look upon their anomaly as a (non-altruistic) reason to give their blood.

The blood of all those with hereditary haemochromatosis and with (a history of) iron overload (*i.e.* including those undergoing maintenance treatment) cannot be used for transfusion purposes for the following reasons:

- It is difficult to guarantee that the blood donation is voluntary and altruistic;
- If there is any organ damage, the counter-indication for blood donation is definitive;
- During iron-depletion therapy,
  - o it is possible that multiple blood donations are carried out during the same serological window for viral infections;
  - o there is a higher risk of bacterial contamination;
  - o there is a risk of iron toxicity;
  - o there is a contradiction with the healthy-donor principle;
- Even in the maintenance phase of treatment, the following issues have been described:
  - o a higher risk of bacterial contamination;
  - o a risk of iron toxicity;
  - o disruptions in the homeostasis of other metal ions;
- To date, the blood drawn from haemochromatosis patients has not been shown to entail a greater risk of viral contamination;
- The transfusional efficacy of blood components from haemochromatosis patients has not been determined yet;
- In light of the interactions that were found to occur with macromolecules, the influence of by no means insignificant amounts of biometals and/or heavy metals in the plasma of haemochromatosis patients is liable to compromise the robustness of certain processes pertaining to the biological validation of the blood donation. The blood establishments should implement appropriate operational modalities to safeguard the quality of these processes;
- Research is only just beginning into the impact of disruptions in physiological processes other than iron homeostasis;
- Allowing haemochromatosis patients to give blood will result in a considerable number of practical problems and operational requirements, which form a potential source of error.

All these findings have led the SHC to take the view that<sup>1</sup> additional research is required as regards the susceptibility to infections and appropriateness of blood taken from haemochromatosis patients with no complications, even if the serum ferritin has returned to normal levels, before allowing their blood to be used for blood transfusion purposes.

### 3. FURTHER DETAILS AND ARGUMENTATION

#### Abbreviations used:

ALT = alanine aminotransferase; anti-HBc = antibody to hepatitis B core antigen; CMV = cytomegalovirus; DNA = deoxyribonucleic acid; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HEV = hepatitis E virus; HGV = hepatitis G virus; *HFE* = type 1 haemochromatosis gene; HH = hereditary haemochromatosis; HIV = human immunodeficiency virus; HTLV = human T-lymphotropic virus; LPI = labile plasma iron; NAT = nucleic acid testing; NTBI = non-transferrin-bound iron; PCR = polymerase chain reaction; TTVI = transfusion transmissible viral infections.

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<sup>1</sup> One member of the working group has taken a minority position as regards part of the conclusion (see section 3.3).

### 3.1. Methodology

This advisory report is a revision of the previous advisory report of the SHC on haemochromatosis and blood donations (SHC, 2004). The arguments on which the previous advisory report was based were re-examined in the light of the most recent data from the scientific literature, the practical experience of the blood establishments and the opinion of the experts. The findings were tested against the position of foreign blood establishments and legislators.

The literature was reviewed on the basis of reference lists from relevant articles recently published in the main clinical transfusion journals, including articles available on-line prior to publication. More data were obtained by searching through the PubMed and Embase databases using the search terms "hemochromatosis", "haemochromatosis", "HFE" and "iron overload".

### 3.2. Further details

This document and the advice pertaining to it only concern the traditional form of hereditary haemochromatosis (type 1). This genetic disorder is caused by mutations of the *HFE* gene.

#### Advisory report of 2004

The SHC advisory report no. 8059 on haemochromatosis and blood donations (SHC, 2004) was formulated as follows: "*The 1994 Act explicitly confirms the principle that blood donations shall be voluntary and that there shall be no financial compensation in return. All blood donations shall be totally disinterested. The altruistic intentions of donors who give their blood on a voluntary basis with no financial compensation are of paramount importance to keep the blood supply in our country safe. Haemochromatosis patients, who require their blood to be drawn for therapeutic purposes, therefore do not qualify as volunteer donors, thus failing to meet the set requirements. No exemption can therefore be granted, for this or for any other reasons.*"

Apart from this general refusal based on the principle that all blood donations should be voluntary and altruistic, the following concrete arguments were brought against allowing such donations:

- during the initial phase of treatment, blood is drawn up to twice a week. A haemochromatosis patient carrying a recent viral infection could therefore give several units of blood during the serological window, thus contaminating several recipients;
- blood with a high iron content, such as that drawn during the initial phase of treatment, is not suitable for patients with a chronic need for transfusion (risk of secondary iron overload);
- the exclusion criteria for blood donation, such as hepatitis, liver disorder, cirrhosis, diabetes and hepatocellular carcinoma, are more frequently found in haemochromatosis patients;
- haemochromatosis patients display a higher risk of bacteraemia caused by siderophilic bacteria such as *Yersinia enterocolitica* and *Vibrio vulnificus*;
- there is one study that has shown a higher prevalence of hepatitis B markers in haemochromatosis patients than in the control group.

Moreover, the view was taken that there was no compelling reason to change the selection criteria and extend the donor pool to haemochromatosis patients for the sake of the blood supply.

The proposal of allowing the blood establishments to carry out therapeutic venesections free of charge for the patient was turned down because this would have resulted in blood being frequently drawn from at-risk individuals and subsequently needing to be destroyed.

### 3.2.1. Hereditary haemochromatosis: genetic polymorphism and connection with clinical symptoms

Hereditary haemochromatosis (HH) is one of the most common congenital disorders among individuals of northern European descent (Fix & Kowdley, 2007). This disorder is caused by a deficient expression of the iron regulatory hormone hepcidin, which causes the intestine to absorb abnormally high amounts of iron (Andrews, 2012). Because the body has no active mechanisms for iron excretion, the excess iron builds up in the organs and tissues and may reach toxic levels, with adverse effects on the organs.

The liver, endocrine organs, joints and the heart are particularly liable to be affected, the main late-onset complications being liver cirrhosis, diabetes mellitus and cardiomyopathy. This is a slow process, which means that haemochromatosis may remain clinically unnoticed for a very long time, with its symptoms usually observed after the age of 50. Among the first to appear are rheumatological symptoms (Carroll et al., 2011; Guggenbuhl et al., 2011).

Hereditary haemochromatosis is an autosomal recessive condition. The most common form – also known as type 1 haemochromatosis (Van Steenberghe, 2008) – is caused by a mutation of the *HFE* gene. At the clinical level, the most important mutation is found at position 282 of the *HFE* protein (p.Cys282Tyr or C282Y). A second common mutation in haemochromatosis (p.His63Asp or H63D) can, in combination with C282Y (compound heterozygosity), result in iron levels that are abnormal to different extents. This too may sometimes lead to iron overload (Jackson et al., 2001).

Some 0.4 % of the northern and western European population are homozygous for C282Y, whilst some 2 % are carriers of the compound genotype H63D/C282Y (Hanson et al., 2001; EASL, 2010). These percentages are higher in some countries or regions (Ireland, Scandinavia, Denmark, Brittany) in which there is a high prevalence (0.5 – 1 %) of this mutation, but are lower elsewhere. Thus, in our neighbouring countries the Netherlands, Germany and France (except for Brittany), an average 0.1 % only of the population is homozygous and the mutation is even less frequent in southern Europe. The *HFE* genotypes are practically non-existent in people who are not of European descent. The H63D polymorphism shows less geographic variation, with 2 % of the European population carriers of the homozygous genotype (EASL, 2010).

The prevalence of the disorder among the most affected demographic groups is estimated at 5 in 1,000 (Edwards et al., 1988; Tan et al., 1999). On average, heterozygous carriers of a mutation of the *HFE* gene show slightly higher haemoglobin levels than those without this mutation and are less prone to iron deficiency. However, they never show any iron overload (Allen et al., 2008). For heterozygous carriers, there are therefore no particular conditions or restrictions as regards their ability to give blood. Indeed, the hepcidin-mediated feedback loop is functional enough in all heterozygous carriers of these mutations and prevents excessive iron uptake in the intestine. As a result, only C282Y homozygotes and carriers of the compound genotype can develop clinical haemochromatosis (Clark et al., 2010). 80 to 85 % of patients with a clinically significant iron overload are C282Y homozygotes (Van Vlierberghe et al., 2000; Hanson et al., 2001; EASL, 2010), whereas some 5 % of haemochromatosis patients are H63D/C282Y compound heterozygotes. Established iron overload with no C282Y homozygosity or H63D/C282Y compound genotype can be a potential sign of other types of haemochromatosis (which may or may not be hereditary).

Though C282Y homozygotes often show elevated iron levels (elevated biochemical penetrance), only a small portion of them will actually develop an iron overload disease (low clinical penetrance) (Whitlock et al., 2006; Bacon & Britton, 2008; Clark et al., 2010; EASL, 2010). The clinical penetrance reaches some 28 % in men and 1 % in women (Allen et al., 2008).

Treatment is indicated when the serum ferritin levels are found to rise. It initially involves removing around 450 mL of blood once or twice a week (induction phase) until the serum ferritin levels have dropped to 20 – 50 µg/L (Adams & Barton, 2007). This intense iron-depletion phase of treatment takes between 6 – 24 months, or longer if the iron overload is severe (Poullin & Lefèvre, 2011). This is usually followed by lifelong maintenance therapy, which involves venesections two to four times a year in order to keep the ferritin levels below 50 or 100 µg/L (Bolan et al., 2001; HAS, 2005; Swinkels et al., 2009; EASL, 2010). There is significant variation in the precise frequency with which venesections are carried out during the maintenance stage. They are performed more often on men – in exceptional cases up to 8 times a year – than on women. Iron depletion by means of deferoxamine or oral iron chelating agents can be an alternative to venesection in certain specific situations (Swinkels et al., 2009; Camaschella & Hoffbrand, 2011).

The number of carriers of *HFE* gene mutations who never show any iron build-up may be estimated at 1 million, which amounts to 10 % of the Belgian population (Van Vlierberghe et al., 1999). To date, neither the precise prevalence of HH among the Belgian population (EASL, 2010), nor the regional differences based on the density of population groups that are not of northern or western European ancestry have ever been determined, yet it is to be expected that the prevalence of C282Y/C282Y homozygotes is 0.1 %.

On the basis of the prevalences mentioned above, it can be calculated that, among the Belgian population (see Table 1), around 217,000 individuals are H63D homozygotes, around 48,500 H63D/C282Y compound heterozygotes and some 10,900 C282Y homozygotes.

**Table 1.** Demographic data for Belgium (INS, 2010) and assessment for the number of homozygote or compound heterozygote carriers for H63D or C282Y and the number of haemochromatosis patients.

Gender	Population					
	Total	with H63D homozygosity	with H63D/C282Y* compound heterozygosity	with C282Y homozygosity	with H63D/C282Y and symptomatic iron overload	with C282Y/C282Y and symptomatic iron overload
Men	5,312,221	106,244	23,746	5,312	24	1,487
Women	5,527,684	110,554	24,708	5,527	2 <sup>Δ</sup>	55

\* Calculated on the basis of the prevalences of H63D and C282Y, and taking into account a population in Hardy-Weinberg equilibrium.

<sup>Δ</sup> Among the 49 patients with compound heterozygosity monitored by D. Walkden (*pers. comm.*), only 4 women (8.2 %) required therapeutic venesection.

Based on the clinical penetrance of 28 %, it is believed that 1,487 male patients suffer from symptomatic iron overload linked to C282Y homozygosity of the *HFE* gene, whereas 55 women (1 %) are thought to show iron overload. The clinical penetrance is extremely low among H63D/C282Y compound heterozygotes (about 0.1 % according to Allen et al., 2008). Thus, in Belgium, a little over 1,500 HH patients are currently undergoing therapeutic venesections.

Recent observations (Allen et al., 2010) support the prevalence of associated symptoms in cases where serum ferritin levels remain both normal and mildly elevated ( $< 1,000 \mu\text{g/L}$ ) at an age at which the disease is expected to have developed. For concentrations  $< 1,000 \mu\text{g/L}$ , the prevalence of associated symptoms is not higher in C282Y homozygotes for the HFE gene than in individuals who are not carriers of the mutations. In this study, 36 % of men and 5 % of women with C282Y homozygosity have elevated ferritin levels. As a result, it can be assessed that, in the near future, 425 Belgian men and 221 Belgian women may require treatment aimed at bringing down the iron levels not only in the liver but also in extra-hepatic organs in order to reduce the risks of haemochromatosis to a minimum.

This revised version of the previous advisory report takes the view that carriers of *HFE* mutations with no pathological iron overload only require further examination insofar as they mistakenly look upon their anomaly as a (non-altruistic) incentive to give their blood: if these individuals feel that their health will benefit from their giving blood, the anamnesis may not be very reliable. It is therefore important for general practitioners and the doctors carrying out the collections to provide correct information.

Individuals with hereditary haemochromatosis who may require treatment are C282Y homozygotes or H63D/C282Y compound heterozygotes with serum ferritin concentrations  $> 1,000 \mu\text{g/L}$ .

As regards those who have been diagnosed as carriers of an *HFE* gene mutation without requiring therapeutic venesections, there are no particular requirements as regards their acceptance as blood donors.

### 3.2.2. Reliability of haemochromatosis patients as donors

Haemochromatosis patients reap personal benefit from their blood donations. In fact, these donations are part of the treatment for their disease. In an American study, 46 % of haemochromatotic donors declared that this was the main incentive for their giving blood. If the venesection cannot be carried out as a blood donation at a blood establishment, this will have to be done at a hospital, which is a less attractive solution, both financially and socially. The influence of the financial advantage is apparent from e.g. the observation that the proportion of American HH patients having their venesections performed at a blood establishment is significantly higher among patients with no medical insurance (McDonnell et al., 1999).

In a survey conducted among haemochromatosis patients, 0.5 % of the respondents admitted to having given their blood as part of their maintenance therapy without mentioning their disease (McDonnell et al., 1999). The purely altruistic drive to give blood – which is an important factor in terms of the safety of the blood from volunteer, disinterested donors (MacPherson, 1998) – can, as a result, be supplanted by less noble intentions in which the personal interest of the donor overrides concerns regarding the safety of the recipient.

The possibility of having therapeutic venesections performed for free can constitute a reason for HH patients to fail to disclose their disease and refrain from mentioning any risk factors that could stand in the way of their giving blood during the anamnesis (SHC, 2004). This risk remains if the policy is to accept HH patients according to the usual selection criteria for blood donors. The practical experience in support of this theoretical risk is mainly anecdotal. Also, published data on this subject are scarce. A single report involving 130 donors with free access to venesection regardless of any potential unsuitability for allogeneic blood donation reports that 4 (3 %) had also mentioned risky behaviour they had previously failed to disclose when the acceptance for venesections depended on the selection requirements for blood donors (Leitman et al., 2003).

In a large anonymous written enquiry among blood donors, Sanchez et al. (2001) found that, on the one hand, unreported risk factors for TTVI (transfusion transmissible viral infections) did not appear more frequently among HH patients than among donors with no health problems (2 % and 3.1 %, respectively). Yet it is striking that the HH donors had often failed to disclose their disease in order to be able to give blood!

Moreover, there is another financial advantage that HH patients benefit from by being allowed to give blood. Indeed, in principle, blood donations are taken from healthy donors. Patients who are afraid they will be unable to take out a life insurance (Delatycki et al., 2002) or another type of insurance (CHS, 2012), to take out a loan or to find employment after having been diagnosed with a disease may fail to reveal any counter-indications to giving blood in order to gain donor status.

The reliability of haemochromatosis patients as blood donors may be compromised by the possibility of having therapeutic venesections performed free of charge to the patient. This can constitute an incentive to fail to disclose risk factors that could form an impediment to giving blood.

### 3.2.3. Ethical issues regarding blood donations from haemochromatosis patients

Haemochromatosis patients are driven to refrain from disclosing their diagnosis by fear of discrimination or stigmatisation regarding their ability to take out insurance, find employment or adopt, as well as their options in terms of education and family relations (Barash, 2000). Moreover, HH patients feel that excluding them as donors is tantamount to denying them an opportunity of helping others in an altruistic fashion. This is all the more difficult to accept for HH patients who gave blood prior to the diagnosis being made. The fact that their blood is "wasted" can be an additional source of irritation and may be reason enough for them not to disclose their disease (Brissot et al., 2011).

Even though the health benefits involved mean that blood donations by haemochromatosis patients are not entirely altruistic (SHC, 2004) and that these donations may therefore be looked upon as less acceptable compared to donations that are purely disinterested (SANQUIN, 2005), Pennings (2005) takes the view that they can nonetheless be considered acceptable from an ethical and social point of view.

This author argues that the altruistic nature of blood donations by HH patients can be safeguarded by doing away with all their advantages:

1. the financial incentive: e.g. by making all therapeutic venesections free of charge to the patient (Grindon, 1993);
2. the health benefit: by separating (non-altruistic) venesections from (altruistic) blood donations.

Once the venesection has been performed, the patient may freely decide whether or not their blood may be used for transfusion purposes, which makes them volunteer altruistic donors. According to the same author (Pennings, 2005), blood donations from haemochromatosis patients are not felt to be demotivating by other volunteer donors.

Another ethical issue concerns the unequal access to healthcare and/or inequalities in the care provided to patients. In fact, if the acceptance of haemochromatosis patients as blood donors differs from one jurisdiction to another, this may result in the emergence of a "medical tourism" phenomenon (CHS, 2012b). It is therefore advisable that the different blood establishments in this country take the same position as regards haemochromatosis patients.

The altruistic nature of blood donations from haemochromatosis patients can be preserved by separating therapeutic venesections from blood donations, as well as by doing away with the financial incentives. Harmonising the access to treatment and the care provided to the patients should also be taken into consideration.

### 3.2.4. Are there any risks involved in giving blood for the haemochromatotics themselves?

The exclusion criteria mentioned in the appendix to the Act of 5 July 1994 regarding blood and blood products of human origin (modified by the Royal Decree of 1 February 2005) that apply to haemochromatosis – metabolic disorders, chronic conditions – aim at protecting the donors. However, we raise the question whether there is any real risk for donors with HH but no complications or apparent organ damage.

The very nature of this condition means that patients with HH face a lesser risk of developing iron deficiency than ordinary donors (Barton et al., 2001; Røsvik et al., 2010). As a result of the greater iron uptake, the iron stores are systematically compensated for to reach pre-donation levels after normal-frequency donations.

Yet a recent study (Mast et al., 2011) has shown that the protective effect of *HFE* mutations against the appearance of anaemia induced by frequent blood donations mainly concerns the higher initial values for haemoglobin and the iron status. In the event of repeated blood donations, the measured haemoglobin and iron values evolve in a parallel way in those with and without *HFE* mutations. In contrast to what may be expected if haemochromatosis were to provide overall protection against blood-donation induced iron deficiency, *HFE* mutations did not turn out to be more frequent in the population of long-term and frequent donors than they were in new donors. Along the same lines, Adams & Barton (2010) report cases in which HH patients showed symptomatic iron deficiency and anaemia after having given blood for transfusion purposes, without having revealed their diagnosis and without having had their serum ferritin checked by their general practitioners.

On the other hand, Adams & Barton (2010) have also come across HH patients with uncontrolled iron accumulation who self-medicated by giving their blood without mentioning their diagnosis – e.g. out of fear of rejection. Indeed, each time blood is removed, this results in iron loss which in turn reinforces the underlying weak hepcidin state, thus perpetuating the excessive intestinal uptake of iron (Piperno et al., 2007; van Dijk et al., 2008).

Venesections in HH patients who do not meet the safety criteria for blood donors – as a result of organ damage or other medical issues that may or may not have been caused by iron overload – constitute a risk by definition and require facilities in which the necessary care can be provided in the event of any problems occurring.

There is no reason to assume that haemochromatosis patients who meet the usual selection criteria for blood donors are exposed to any greater risk when giving blood than normal donors. However, it is necessary to monitor the manner in which their iron overload evolves both clinically and biologically. In order to avoid the risk of a patient being no longer properly monitored, an integrated care plan should be implemented.

### 3.2.5. Are there any risks involved in haemochromatotics giving blood for the microbiological safety of the collected blood components?

The iron overload that comes with haemochromatosis has varying implications for the susceptibility to infections.

Indeed, type 1 haemochromatosis involves iron overload primarily in the parenchymal cells, but also iron loss in the reticuloendothelial system (Cairo et al., 1997; Knutson & Wessling-Resnick, 2003; Jacolot et al., 2010). This iron deficiency in the macrophages turns out to hinder the growth of many intracellular organisms (Paradkar et al., 2008). These organisms include bacteria that are known to be transfusion-transmissible: *Coxiella burnetii*, *Chlamydia pneumoniae*, *Yersinia enterocolitica*, *Yersinia pestis* (Moalem et al., 2004).

Conversely, the presence of iron overload compromises the ability of phagocytes to kill micro-organisms and many pathogenic micro-organisms have in turn developed iron accumulation strategies, thus increasing their virulence (Bullen et al., 1999). The uptake of iron from plasma glycoproteins with a high affinity for iron has been observed for many protozoa, fungi, bacteria and viruses (Khan et al., 2007; Johnson & Wessling-Resnick, 2012).

The association of haemochromatosis with certain infections has been well described. Thus, Khan et al. (2007) made a list of 16 pathogenic micro-organisms that cause viral, bacterial or fungal infections, half of which have a fatal outcome. In fact, there are continuing reports on new associations with other micro-organisms (Vetter et al., 2010; CDC, 2011; Church et al., 2011; Galan et al., 2011; Greaves et al., 2012; Siegel et al., 2012).

### 3.2.5.1. Viral infections

The data pertaining to viral infections are contradictory. A French study (Deugnier et al., 1991) found that there was significantly higher prevalence of hepatitis B markers (mainly anti-HBc) among 272 haemochromatosis patients than among the general population. This is in line with previous studies that had suggested that there is a link between iron overload in the hepatocytes and a susceptibility to HBV infection (Lustbader et al., 1983; Senba et al., 1985; Zhou et al., 1987). This cannot be accounted for either by previous stays in the hospital with serological testing, sexual behaviour, drug addiction or stays in an endemic country (except for anti-HBc). Another French study (Jouanolle et al., 1991) found that the prevalence of hepatitis C markers (anti-HCV) was 6 times higher among 137 haemochromatosis patients than among a matching control population. However, in all of these cases, this can be accounted for by a previous history of orthopaedic surgery with blood transfusion.

On the other hand, an important observational study involving 52,650 American donors has found that there was no greater seroprevalence of transfusion-transmissible infections (HBsAg, anti-HBc, HCV, HTLV, syphilis) nor a greater prevalence of disturbed ALT values among 97 haemochromatotic donors than among 50,079 donors without haemochromatosis (Sanchez et al., 2001). However, their comparison of the prevalences is distorted, as blood donors are usually younger than people with haemochromatosis and the proportion of males is significantly higher among the latter. Also, the authors are unable to do away with the selection bias observed during the analysis of the questionnaires.

Leitman et al. (2003) did not find any seroconversion for transfusion-transmissible infections in 130 haemochromatotics – 76 % of whom were C282Y homozygotes – who had given blood 1,402 times over a period of 27 months. However, given the very low baseline frequency of seroconversion, this observation is not conclusive.

A clear risk factor for TTVI during the intense iron-depletion stage is the high collection frequency, viz. up to twice a week. A haemochromatosis patient may therefore give several units of blood during the serological window of a recently acquired viral infection and thus contaminate not one but several recipients (SHC, 2004). Greaves et al. (2012) suggest that there is a direct link between iron overload and the HIV viral load in HIV infected individuals. Thus, the CMV and HIV viruses have been shown to down-regulate the expression of the HFE protein in order to access the intracellular iron stores (Drakesmith et al., 2003; Vahdati-Ben Arieh et al., 2003).

On the other hand, the fact that HH donors are regular donors can contribute to the safety of their blood donations (AABB, 2002).

It should be noted that the research carried out among blood donors did not include the other viruses recorded by Khan et al. (2007) and more recently by Vetter et al. (2010) and Siegel et al. (2012), viz. CMV, parvovirus B19, HEV, HGV, HPV. Most reported clinical cases were observed in patients at an advanced stage of the disorder.

Greater certainty concerning the safety of blood from HH donors for transfusion purposes can only be obtained by conducting prospective studies, which, given the very low incidence of TTVI, will have to be very extensive and therefore difficult to carry out.

### 3.2.5.2. Bacterial infections

Haemochromatosis with iron overload is concomitant with a greater susceptibility to certain bacterial infections, partly due to the increased virulence of siderophilic bacteria (SHC, 2004), and partly due to the weaker immune system (Moura et al., 1998; Walker & Walker, 2000; Ashrafian, 2003). The iron-regulatory hormone hepcidin plays a key role in this context (Ashrafian, 2003; Ganz, 2011).

There are several case reports on haemochromatosis patients with septicaemia caused by siderophilic bacteria such as *Escherichia coli* (Christopher, 1985; Corke et al., 1995), *Vibrio cholerae* non-O1 (Fernández et al., 2000), *Vibrio vulnificus* (Blake et al., 1979; Murphy, 1987; Klontz et al., 1988; Tefany et al., 1990; Gerhard et al., 2001; Barton & Acton, 2009) and *Yersinia enterocolitica* (Abbott et al., 1986; Cauchie et al., 1987; Nouel et al., 1991; Crosbie et al., 2005). A clear association has also been found to exist between haemochromatosis and the development of liver abscesses due to a *Yersinia enterocolitica* infection: 64 % of the 45 reported cases were registered among HH patients (Bergmann et al., 2001). Torp-Pedersen et al. (2012) have described a case of *Vibrio cholerae* non-O1 infection in a haemochromatosis patient following contact with water from the Baltic Sea during the summer, and which caused an intracerebral abscess. All these reports confirm the severity of these infections in patients with iron overload. Yet, there are no reports on the frequency with which these bacterial infections occur in haemochromatosis patients.

Following the greater susceptibility of HH patients to *V. vulnificus* infections, they are advised against eating raw shellfish (Bacon et al., 2011). However, there are no reports of transfusion incidents caused by *V. vulnificus*.

Conversely, *Yersinia enterocolitica* is known to be a transfusion-transmissible pathogen. Transfusing contaminated red blood cell concentrates is, among other things, liable to cause fatal post-transfusion sepsis (CDC, 1997).

A recent and detailed review of the literature on 55 published cases of transfusion-borne *Y. enterocolitica* infection (Guinet et al., 2011) does not make it possible to confirm or rule out a connection with haemochromatosis in the donors. In actual fact, the authors did not find any explicit mention of the iron status of the donors or recipients when examining the case descriptions (F. Guinet, *pers. comm.*). There was only a single case in which it was explicitly mentioned that the donor had no family history of haemochromatosis. An additional case has just been published by Boyer et al. (2012).

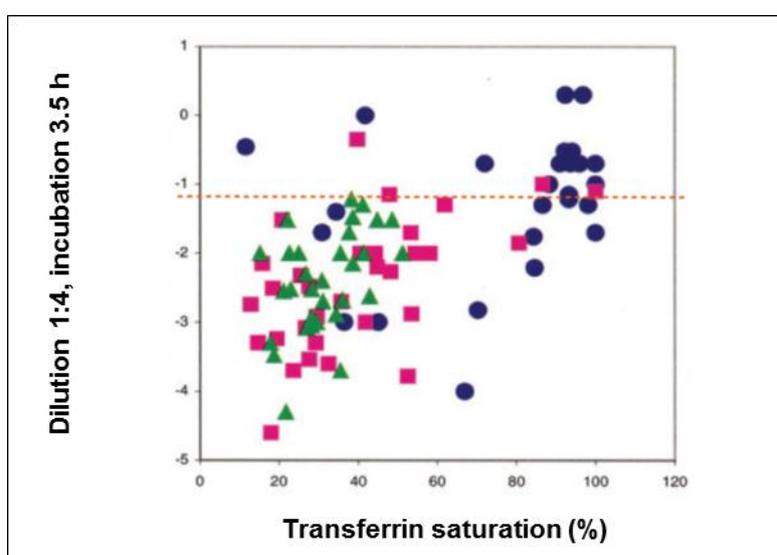
Several comparative studies have also been devoted to the relationship between haemochromatosis and susceptibility to bacterial infections. *Vibrio vulnificus* does not survive in the blood of healthy donors, but thrives in blood from HH patients with iron overload (Bullen et al., 1991). Jolivet-Gougeon et al. (2007) did not find a higher seroprevalence of *Yersinia* infections in 236 HH patients than in 306 healthy donors.

The same authors (Jolivet-Gougeon et al., 2008) found that the antibacterial activity against *Salmonella enterica* Typhimurium LT2 was weaker in the serum of patients with iron overload. They further consider this antibacterial activity to be related to the transferrin saturation levels (see Figure 1).

In 14 of the 26 HH patients in the iron-depletion induction phase (53.8 %), the antibacterial activity was weaker compared to that of healthy controls. Among 32 patients in the maintenance phase of treatment<sup>2</sup>, four (12.5 %; 95% CI<sup>3</sup>: 4 – 29 %) still show antibacterial activity that is as weak as that in patients in the iron-depletion induction phase of treatment.

**Figure 1.** Relationship between the degree of serum antibacterial activity against intracellular bacteria and transferrin saturation level in haemochromatosis patients according to Jolivet-Gougeon et al. (2008).

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- haemochromatosis patients in the iron-depletion induction phase of treatment.
- haemochromatosis patients in the maintenance phase of treatment
- ▲ healthy individuals

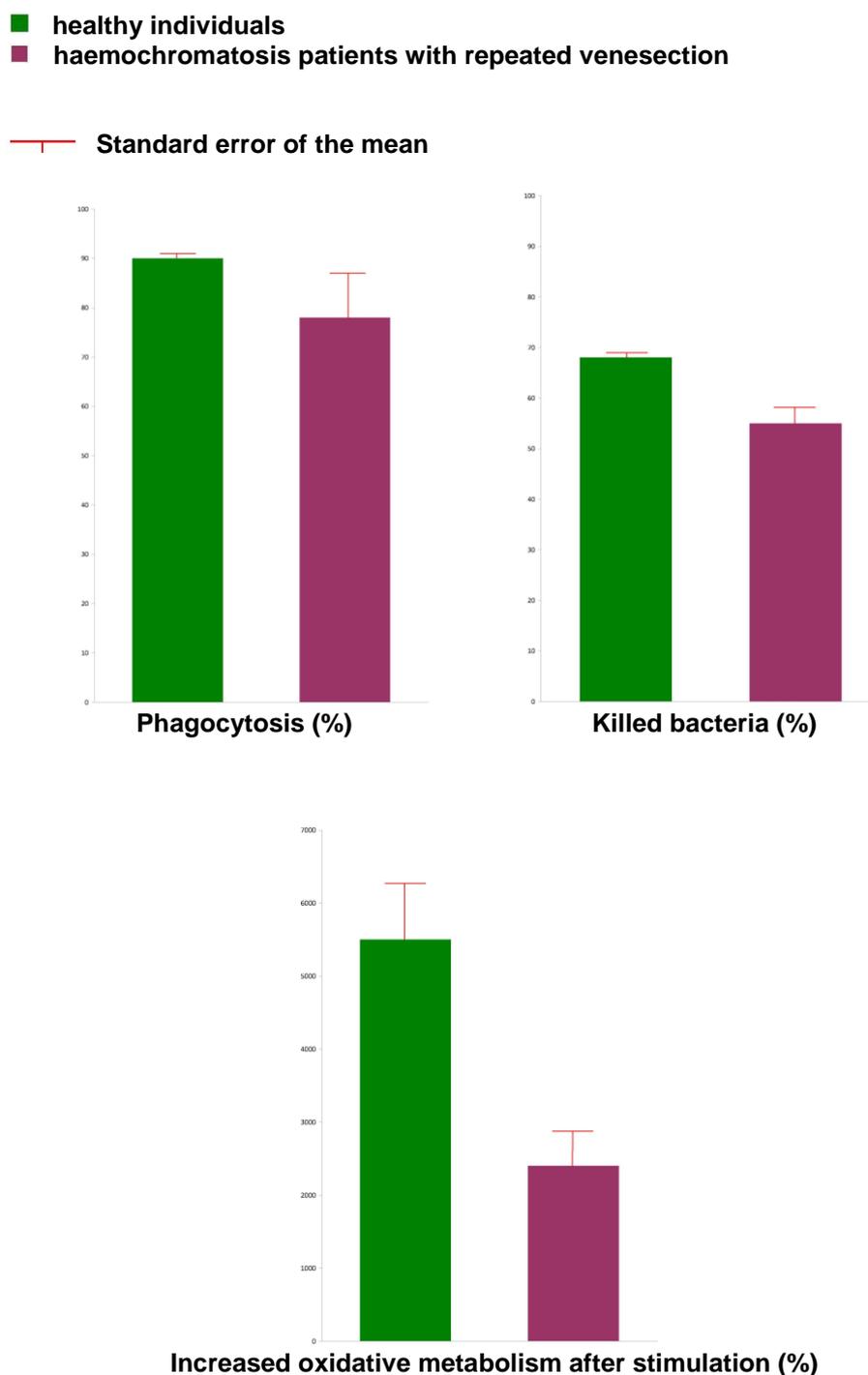
For the purpose of this *in vitro* study, the authors excluded patients with complications such as diabetes mellitus or cardiomyopathy. Few patients were older than 55; the eldest was aged 61.

Previously, Patruta et al. (1998) had found that the antibacterial activity against *Escherichia coli* was inhibited in haemochromatosis patients in their comparative trial on the ability of polymorphonuclear leukocytes to destroy extracellular bacteria (see Figure 2). These authors had observed *in vitro* that the destruction of bacteria inside the phagocytes declined by a significant 13 %, as well as a significantly strong inhibition (56 %) of the oxidative metabolism of these cells, and an 11 % lower, non-significant phagocytic activity.

<sup>2</sup> With serum ferritin levels  $21.29 \mu\text{g/L} \pm 11.85 \mu\text{g/L}$ . Beamish et al. (1974), Van Der Weyden et al. (1983), Edwards et al. (1989), Patruta et al. (1998) and Bolan et al. (2001) show that such patients usually show a higher transferrin saturation than healthy individuals.

<sup>3</sup> According to the modified Wald method (Agresti & Coull, 1998). With 3 points not visible on the graph, the upper value of the confidence interval can vary between 27 and 36 %.

**Figure 2.** Assessment of the ability of polymorphonuclear leukocytes to kill extracellular bacteria in haemochromatosis patients (based on Patruta et al., 1998).



It is important to note that the inhibition of antibacterial activity was measured in HH patients upon entering the maintenance phase of treatment.<sup>4</sup> Most of these patients were older than 55; the eldest was aged 85. Venesection was last performed at least 4 weeks earlier.

<sup>4</sup> With serum ferritin levels of  $200.4 \mu\text{g/L} \pm 101.6 \mu\text{g/L}$  and transferrin saturation levels of  $47.6 \% \pm 10.2 \%$ .

It should be noted that the research carried out among blood donors did not include the other<sup>5</sup> bacteria listed by Khan et al. (2007), Church et al. (2011), Galan et al. (2011) and Torp-Pedersen et al. (2012): viz. *Gemella haemolysans*, *Listeria monocytogenes*, *Plesiomonas shigelloides*, *Vibrio cholerae non-O1*, *Negativicoccus succinicivorans*. Ritger et al. (2011) and Frank et al. (2011) also reported on a case that shows that patients with hereditary haemochromatosis are more susceptible to certain infections, even attenuated ones. In this particular case, this concerned a fatal infection with an attenuated *Yersinia pestis* strain. Pletschette et al. (1992) and Weinberg (1999) warn against an increased susceptibility to *Capnocytophaga canimorsus* infections in individuals with iron overload who have sustained a dog bite. Yet there is no specific case report on haemochromatosis patients.

Conry-Cantilena (2001) suggest that it is possible that *Yersinia* infections in people with iron overload rule out any blood donations as a result of their severity.

### 3.2.5.3. Fungal infections

Increased susceptibility to fungal infections in people with iron overload (Nevitt, 2011) is of concern for transplant patients (Maertens et al., 1999; Alexander et al., 2006). In this context, iron overload is not relevant to the issue of transfusion-transmissible infections. In contrast, two fatal clinical cases of mucormycosis have been described that did not involve a transplantation setting (Khan et al., 2007).

However, pathogenic fungi require between  $10^{-7}$  à  $10^{-6}$  M iron to grow, which means that serum and other biological fluids and tissues – which contain  $< 10^{-15}$  and as low as  $10^{-24}$  M iron – should be fungistatic for all species (Symeonidis & Marangos, 2012). This suggests that the blood collected from HH patients does not involve a greater risk of transmitting a potential fungal infection than blood collected from the usual donors.

The studies mentioned above indicate that blood collected from HH patients undergoing depletion-phase as well as maintenance-phase treatment may entail a higher risk of bacterial contamination.

There may also be an increased risk of viral or bacterial contamination that is linked to the higher donation rates during the iron-depletion induction phase of treatment.

Conversely, these studies have not been able to show to date that the blood drawn from haemochromatosis patients entails a greater potential risk of viral contamination.

The blood collected from HH patients should not involve a greater risk of transmitting a potential fungal infection than blood collected from ordinary donors.

### 3.2.6. Are there any risks involved in haemochromatotics giving blood for the quality of the collected blood components?

Hereditary haemochromatosis is not transfusion-borne. However, the use of blood collected from patients undergoing therapeutic venesections for transfusion purposes is subject to debate. The apparent wastage of precious blood units is weighed against the possibility that these components could contain unknown and perhaps undesirable elements.

It is also important to take into account the mechanisms through which iron overload induces cellular dysfunction, especially as regards the red blood cell and platelet function, as well as interactions with plasma proteins.

<sup>5</sup> Nairz et al. (2009) have shown that the underlying causes of the *HFE* gene mutation in haemochromatosis induce macrophage iron depletion, whilst the latter have an improved ability to counter intracellular pathogens like *Salmonella* or mycobacteria. Research is also being conducted on how macrophages counter extracellular bacteria (G. Weiss, *pers. comm.*).

Iron and other biometals such as copper, zinc, manganese, cobalt or nickel are examples of metals that are of crucial importance for the organism to function properly. Many of the properties that make it possible for biometals to carry out essential biochemical activities and structural purposes in a vast number of proteins, including enzymes, and other cellular constituents, are also a potential source of toxicity.

### **3.2.6.1. Iron overload and risk of cancer**

With a few exceptions, almost all cells use iron as a fundamental cofactor in their biochemical activity, such as oxygen transport, energy metabolism, DNA synthesis and free-radical detoxification. However, under aerobic conditions, iron catalyses the propagation of ROS (reactive oxygen species) and the generation of highly reactive radicals. As iron readily passes from the reduced ( $\text{Fe}^{2+}$ ) to the oxidized ( $\text{Fe}^{3+}$ ) form and vice-versa, disruption of the cellular redox equilibrium requires only catalytic amounts of the metal (Wang & Pantopoulos, 2011). The "oxidative stress" this leads to plays a part in the appearance of cellular macromolecule damage and tissue injury, ultimately leading to signs of clinical disease. The DNA damage leads to genomic instability as a result of mutations, hypermethylation or shorter telomere length (Prá et al., 2012).

Haemochromatosis patients suffering from liver cirrhosis are at an increased risk of hepatocellular carcinoma (Elmberg et al., 2003). A recent meta-analysis conducted by Ellervik et al. (2012) has also shown that there is a connection between high transferrin saturation and an increased risk of cancer. In a prospective population study by the same authors, this is, among other things, revealed by the existence of a positive correlation between the C282Y/C282Y genotype and the appearance of cancer in men.

The precise mechanism that makes haemochromatosis patients more prone to cancer, mainly liver cancer, is not known. The accumulation of iron and the "oxidative stress" it generates cannot be ruled out as a possible cause. Gannon et al. (2011) also consider the possible influence of antigen presentation by the *HFE* protein or the response to the accumulation of misfolded proteins in the cells.

As some consider it highly unlikely that the increased risk of cancer – or possibly of a precancerous situation – in the donor means that the potential recipient too is exposed to an increased risk (Edgren et al., 2007; Yang et al., 2010), this observation can be extrapolated to HH donors.

The risk of cancer being transmitted through blood components collected from HH patients can only be determined with greater certainty by conducting research on this particular subgroup.

### **3.2.6.2. Iron overload in the recipient**

Mammals do not excrete iron in any specific way and its uptake from food is therefore narrowly controlled. Thus, transfusing 10 to 15 red blood cell concentrates from normal donors, each of which contains around 200 to 250 mg of iron<sup>6</sup>, causes the transferrin to reach saturation. Multiple transfusions can induce secondary haemochromatosis (post-transfusional haemosiderosis), leading to iron overload in which the serum ferritin levels are found to reach at least 1,000  $\mu\text{g/L}$ , with or without organ damage (AFSAPPS, 2011; CBO, 2011). Moreover, iron overload is linked to the intensity of the treatment required in paediatric oncology (Gurram et al., 2012; Ruccione et al., 2012). It has also been connected to the prognosis of patients with a chronic HCV infection who do not respond to treatment (Franchini et al., 2008).

<sup>6</sup> Calculated on the basis of the haemoglobin concentration in the blood by taking 0.34 % as the iron saturation value.

65 % of the iron contained in the human body is bound to the haemoglobin molecules in the red blood cells and the erythroid cells, some 8 % is bound to myoglobin molecules and some 30 % of the iron in the organism is stored as ferritin or haemosiderin in the spleen, bone marrow and liver. The iron in the bloodstream is usually bound to transferrin, plasma heme or serum ferritin. The serum iron is the level of free circulating iron in the blood plasma; normal levels can reach 1.8 mg/L.

Haemochromatosis patients:

- usually show normal erythrocyte production and destruction rates, as well as normal biochemical haemoglobin analyses;
- display more red blood cells, with the latter containing haemoglobin levels that are elevated by about 1 g per 100 mL, especially at the beginning of the iron-depletion induction phase of treatment (Barton et al., 2000; Bolan et al., 2001);
- have a mean globular volume, mean corpuscular haemoglobin as well as mean corpuscular haemoglobin concentration that remain well above normal after the induction phase of treatment (Barton et al., 2000);
- display red blood cells with ferritin levels that are 15 to 60 times more elevated than those in healthy individuals (Cazzola et al., 1983; Novembrino et al., 2005);
- present an intracellular ferritin concentration that is 1,000 times higher in the lymphocytes than in the red blood cells, yet to our knowledge, no studies have been published on an increase in lymphocyte ferritin in haemochromatosis patients. In actual fact, the lymphocyte count has been reported to decline (Macedo et al., 2012);
- undergoing the iron-depletion induction phase of treatment as well as certain asymptomatic individuals with HH may display serum ferritin levels that lie well above the 1,000 µg/L threshold. In the maintenance phase of treatment, therapeutic venesections are carried out when the serum ferritin is around 100 – 150 µg/L, *i.e.* below the levels measured in healthy individuals;
- may display a transferrin saturation<sup>7</sup> that reaches 100 %, whilst it is around 35 % in healthy individuals. After the iron-depletion induction phase, the mean transferrin saturation is lower but rapidly returns to elevated levels (Beamish et al., 1974; Edwards et al., 1989; Bolan et al., 2001);
- have serum iron levels that are over twice those found in healthy individuals. After the iron-depletion induction phase, the mean values are lower but are likely to go back up during the maintenance phase of treatment, with the iron being constantly eliminated by the macrophages in HH patients.

The red blood cell concentrates from HH donors present a low risk of iron overload in the recipient, especially as the likelihood of the same patient receiving several blood units from a HH donor is minimal:

- with red blood cell concentrates only containing a low amount of plasma, there will only be an insignificant increase in the amount of iron administered during blood transfusion;
- the lymphocytes are ill-equipped to detoxify the iron burden through stimulation of ferritin synthesis (Walker & Walker, 2000);
- each red blood cell ferritin molecule is able to store up to 4,500 iron atoms (saturation at 56 %). Based on the 3,200 µg/L increase observed by Novembrino et al. (2005), the iron level in concentrates from haemochromatosis patients is around 0.9 mg higher than that in concentrates from healthy donors;
- based on a 5 g increase of haemoglobin per concentrate, the iron level is around 17 mg higher (*i.e.* around 7 %) than that in concentrates from healthy donors. However, after transfusion, this amount will be significantly diluted.

<sup>7</sup> These patients show moderate hypo-transferrinaemia.

Plasma, and, to a lesser extent, platelet concentrates obtained by pooling buffy coats from HH donors also entail a low risk of iron overload in the recipient:

- with serum ferritin only containing a low amount of iron (saturation at 12.4 %), elevated serum ferritin corresponds to minimal amounts of circulating iron (Brissot et al., 2012);
- each transferrin molecule is able to store up to 2 iron atoms (saturation at 0.15 %). Based on the difference in saturation (*i.e.* 100 % versus 35 %) and dosage (*i.e.* 2 g/L versus 3 g/L), the iron level in concentrates from haemochromatosis patients is around 0.75 mg higher than that in concentrates from healthy donors;
- the excess serum iron can reach around 1 mg (Nielsen et al., 1995) compared to concentrates from healthy donors. Yet the excess iron will be significantly diluted following the transfusion of plasma and platelet concentrates.

Elevated amounts of toxic forms of iron have been shown to appear in pathological states of iron overload (Batey et al., 1980). Comparative studies have been conducted on the relationship between the haemochromatosis treatment phases and the presence of toxic forms of iron (Aruoma et al., 1988; Loréal et al., 2000; Le Lan et al., 2005). In patients in the depletion-induction phase of treatment, there is a significant rise in the blood levels of iron as non-transferrin-bound iron (NTBI) and labile plasma iron (LPI): up to 5 µmol/L (Brissot et al., 2012). However, during the maintenance phase of treatment, the LPI drops to similar values to those obtained in healthy controls, whilst the serum NTBI concentration is often still found to be twice as high. Prus & Fibach (2011) have shown that red blood cells readily absorb the NTBI that builds up as labile iron and generates reactive oxygen species. This can affect the properties of the red blood cells *in vivo* (Ghoti et al., 2007).

Chitambar and Wereley (2001) have conducted an *in vitro* study on the iron uptake in a B-lymphoid cell line from a C282Y homozygous haemochromatosis patient. The non-transferrin-bound iron uptake was significantly higher, as well as cell susceptibility to iron-induced oxidative stress.

### **3.2.6.3. Disruption of metal ion homeostasis**

A poor regulation of the iron uptake also affects the intestinal absorption and storage of other biometals or heavy metals in the organs. Sargent et al. (1979), Barton et al. (1998) and Onalaja & Claudio (2000) have drawn up a list of several analyses on the homeostasis of chromium, zinc, manganese, cobalt, nickel and lead in HH patients.

Other studies report a build-up of cadmium (Åkesson et al., 2000) or lead (Wright et al., 2004; Hopkins et al., 2008) in the blood of these patients or confirm the lower blood concentration of manganese (Henn et al., 2011). Becket & Ball (2012) show that *HFE* mutations or an iron overload do not seem to have a significant impact on the blood concentration of selenium, copper or zinc.

Most of these studies do not draw a distinction between patients in the iron-depletion induction phase of treatment and those in the maintenance phase. They also do not take into account the influence of the duration of the venesection treatment on the blood content of these metals (Åkesson et al., 2000).

The uptake of cadmium rises in haemochromatotics in the maintenance phase of treatment. This may be due to DMT1 stimulation, a mucosal divalent cation transporter molecule (Åkesson et al., 2000). The authors mentioned above found that the cadmium blood content – which also reflects the body load of cadmium – is proportional to the duration of venesection treatment. A similar finding was made for the lead concentrations in HH patients' blood (Åkesson et al., 2000). Other authors have also reported increased lead concentrations in the blood and organs of haemochromatosis patients (Barton et al., 1994; Hopkins et al., 2008).

The importance of these findings in terms of the safety of blood from haemochromatotic donors intended for transfusion purposes is difficult to assess. Redox-inactive metals, such as cadmium and lead, exhibit toxic effects due to their binding to sulfhydryl groups of proteins as well as to glutathione exhaustion (Jomova & Valko, 2011).

A population study has shown that even low environmental or dietary exposure to cadmium can result in bone demineralisation, leaving them more fragile as a result (Staessen et al., 1999; Åkesson et al., 2000; Thomas et al., 2011). Increased exposure to cadmium also has several other adverse health effects (Nawrot et al., 2010).

An important fraction of the circulating blood lead and cadmium is transported by the red blood cells (Foulkes, 2000). Apart from that, the metals in the blood of HH patients will be transferred through plasma transfusion, and, to a lesser extent, transfusion of platelets obtained through buffy coat pooling.

On the other hand, the amount of additional cadmium and/or lead that reaches a patient following the transfusion of red blood cell concentrates from HH donors is extremely low. Still, the cytotoxic potential of these metals has to be considered to be more serious in the event of exposure to several metals and/or in the case of young recipients (children and newborns).

#### **3.2.6.4. Characteristics of blood components from individuals with hereditary haemochromatosis**

Blood from haemochromatosis patients collected during the depletion phase of treatment is believed to contain more young red blood cells as a result of the higher venesection rates. This could even make them more suitable for transfusion. However, this theoretical advantage has never been confirmed (Tan et al., 1999). On the other hand, it has also never been shown that a higher reticulocyte count results in a loss of quality of the blood during storage, or in shorter post-transfusion survival (AABB, 2002). Still, there has been no systematic analysis yet of the erythroid parameters in haemochromatosis (Barton et al., 2000; Li & Ginzburg, 2010).

A Dutch observational study (Luten et al., 2008) has compared the red blood cell concentrates of HH patients with iron overload with those of standard regular donors. No significant difference could be found for the tested parameters during a 50-day storage period. The red blood cell concentrates from haemochromatosis patients met with the *in vitro* transfusion criteria. However, to our knowledge, no study has been published to date to confirm the functional equivalence *in vivo* (cf. de Korte & Verhoeven, 2004; Barshtein et al., 2011; Hess, 2012) of such red blood cells for transfusion purposes.

Iron overload mediated "oxidative stress" plays a part in the appearance of damage to the cellular macromolecules, including polysaccharide depolymerisation and DNA oxidation (Broedbaek et al., 2009; Weinberg, 2010). Ghashghaeinia et al. (2012) have obtained data that show that "oxidative stress" does indeed affect the susceptibility of all circulating red blood cells.

According to Broedbaek et al. (2009), haemochromatosis patients undergo severe "oxidative stress", but the markers of nucleic acid oxidation themselves return to the control values after the iron-depletion phase of treatment.

To our knowledge, no study has been published that shows there is no potential irreversible damage to the nucleic acids of the red blood cell progenitors collected from HH patients. However, Akoev et al. (1992) and Akoev et al. (1997) have pointed to changes in the surface molecules of red blood cells collected from these patients. Moreover, Prus & Fibach (2011) have shown that mature red blood cells as well as erythroid cells at all stages of maturation readily absorb the NTBI that builds up as labile iron and generates reactive oxygen species.

In addition, these red blood cells have to withstand irradiation (Knight et al., 1992), as red blood cell concentrates are currently irradiated before being administered to certain recipients – foetuses, newborns, some cancer or transplant patients, as well as in the event of transfusions between relatives (SHC, 2010). It follows that the transfusion efficacy of red blood cells from haemochromatosis patients needs to be examined.

It is noteworthy that, apart from "oxidative stress", a biochemical interaction has just been found to occur in a blood component collected from haemochromatosis patients as a result of direct binding of the iron to enzymes. In actual fact, a recent study has shown that an elevated serum iron content in these patients may prevent platelet aggregation (Lynch & Soslau, 2012). The efficacy of platelets transfused into the circulation of different recipients therefore requires verification.

NTBI iron and/or other metals may affect the proteins in the blood collected from HH donors. It has been shown that intravenous iron infusion during haemodialysis treatment results in *in vivo* oxidation of the plasma proteins, mainly fibrinogen and albumin (Oettl & Stauber, 2007).

To our knowledge, no functional study has been published in support of the functional equivalence *in vivo* of single-donor plasma collected from haemochromatosis patients for transfusion purposes. In addition, Pankow et al. (2008) have reported that the average fibrinogen levels were significantly lower in C282Y homozygotes compared to healthy subjects.

To date, it has not been established whether the increased risk of cancer in haemochromatosis patients constitutes a hazard for the safety of certain recipients.

Red blood cell concentrates from donors with haemochromatosis do not entail any specific risk of iron overload in the recipient, but there has been a risk of iron toxicity identified.

The potential excess cadmium and/or lead in the blood of the patient will be transfused through red blood cell concentrates in particular. Excess amounts of other metals will chiefly be transfused through plasma and, to a lesser extent, through platelet concentrates obtained through buffy coat pooling.

There is no evidence to date as regards the transfusion efficacy of blood components from haemochromatosis patients.

### 3.2.7. Safety of the process and biological validation of the donation

Though there do not appear to be any definite data on this subject, we need to be aware of the fact that most of the specific operational requirements (see 3.2.8; see Appendix 1) involved in accepting haemochromatotics as blood donors may result in an increased risk of errors occurring in the various processes performed at the transfusion centre, from the moment the blood is collected to that when it is actually distributed (Tan et al., 1999).

Heme iron is a well-known inhibitor of nucleic acid amplification reactions used during genomic screening in a transfusion setting (Neumaier et al., 1998). With the biometals interacting with the macromolecules, the fact that the former are found in significant amounts in the blood of HH patients means that they are liable to compromise the robustness of screening tests based on nucleic acid amplification (NAT). When this biometal overload goes unnoticed, quantitative PCR inhibition can severely affect the precision and sensitivity of the results obtained (Bar et al., 2012).

In Belgium, certain viral inactivation or pathogen reduction methods that depend on a chemical reaction with nucleic acids are implemented for plasma or platelets (SHC, 2007; SHC, 2011). To our knowledge, no study has been published to date in support of the efficacy of these techniques in the specific case of blood collected from haemochromatosis patients.

The bications in the serum are liable to affect antigen conformation or interfere with the formation of antigen-antibody complexes (Tate & Ward, 2004). However, the antibody detection techniques usually remain unaffected by sample haemolysis.

In light of the interactions that were found to occur with macromolecules, the influence of by no means insignificant amounts of biometals and/or heavy metals in the plasma obtained from haemochromatosis patients is liable to compromise the robustness of certain processes pertaining to the biological validation of the donated blood.

The blood establishments should have appropriate operational modalities to safeguard the quality of these processes.

### 3.2.8. Preliminary conditions and operational requirements in the event of haemochromatosis patients being allowed to give blood

Allowing haemochromatosis patients to give blood will have a number of effects on the daily operations in the blood establishments and curative facilities, as well as on the legislation on blood donations (see Appendix 1).

1. In order to safeguard the altruistic and voluntary nature of blood donations, it is of paramount importance to eliminate the therapeutic and potential financial benefits linked to removing blood and using it as a blood donation:

- therapeutic venesections should be carried out in a blood establishment *prior to* collecting the pre-donation anamnesis;
- obtaining donor status cannot give these patients/donors an advantage over non-donor patients in the eye of insurance companies;
- therapeutic venesections performed in a blood establishment should abide by the same billing policy as regards the financial burden to HH patients/donors, even if they are not suitable for allogeneic donation.

2. Blood collections carried out in a setting in which the blood establishment does not strictly abide by the legal selection criteria pose specific problems. This issue needs to be solved by drawing up uniform procedures to be implemented by the Belgian blood establishments:

- in order to preserve the safety of the donor, there should be appropriate medical supervision to monitor the specifics of the disease and the observations made should be recorded in the medical file;
- in order to prevent the quality of donor selection from being compromised, ordinary collections should be separated from collections made for therapeutic venesection purposes;
- all necessary precautions should be taken to prevent crossovers between the path taken by the ordinary blood donations and that of the blood removed for therapeutic purposes;
- it is necessary to guarantee that blood that is unsuitable for transfusion purposes<sup>8</sup> will follow a path that is completely separate, from the moment it is collected to that when it is destroyed.

<sup>8</sup> For example, upon the patient's return from a country in which malaria is endemic (blood donations postponed for a 4-month period).

3. There should be provisions made for donors with haemochromatosis who are permanently deferred from giving blood. A specific case in this respect are HH donors who have reached the age limit for giving blood.

This can be approached in either of two ways:

- the blood establishments continue to perform the venesections: this can result in a long-lasting, unnecessary and possibly even a high-risk burden for the blood establishment;
- the patients are referred back to the therapeutic network: this may not leave the patient/donor at a financial disadvantage so as to avoid the risk of their withholding important information (cf. above).

4. There should be good-quality cooperation and clear agreements should be made with the treating physicians of donors with haemochromatosis: they are not only in charge of prescribing the venesections as well as determining their frequency, but also of the clinical and biological follow-up. The role of the treating physician in planning and following up on the maintenance treatment of haemochromatosis based on blood donations should be discussed with the donor.

5. It is important that the medical profession be provided with suitable information in order to ensure that haemochromatosis patients (including asymptomatic carriers of the C282Y mutation with no iron overload) be given correct counselling on the appropriateness/possibility of giving blood (Sanchez et al., 2001).

6. Providing appropriate information to the public should allow haemochromatosis patients to know whether or not they are eligible for blood donation (Levstik & Adams, 1998).

7. In order to be able to collect all donations from patients requiring over 4 venesections a year, the Act of 5 July 1994 needs to be modified by extending the variance from the 4-donations-a-year upper limit on blood donations to donors with haemochromatosis as well as by abolishing the yearly limit of 32 mL per kg body weight for these donors.

There are a considerable number of operational requirements as well as regulatory problems involved in allowing haemochromatosis patients to give blood.

From an ethical point of view, it is of paramount importance that the blood establishments perform the venesections *prior* to collecting the anamnesis for the blood donation.

It is also necessary to remove all potential financial advantages linked to collecting blood through venesection/blood donation.

The operational requirements also make it necessary to separate blood obtained through therapeutic venesections from ordinary donations.

### 3.2.9. Discussion

Though there are a number of publications available on blood donations from people with hereditary haemochromatosis, there is not enough evidence in the literature on the safety or risks involved in using this blood to support conclusions and recommendations with a strong evidence base (Conry-Cantilena & Klein, 2000; Whitlock et al., 2005; Swinkels et al., 2009).

This finding is far from surprising, as the main tool for patient management – the evidence provided by randomised controlled trials – cannot apply to donor selection. Moreover, there is still a scarcity of clinical or epidemiological trials available, given the limited geographical distribution of the C282Y mutation. Another reason is that few countries have departed from the principle that only healthy individuals may give blood. In addition, many molecular repercussions that are inherent to *HFE* gene mutations have only been discovered fairly recently (Rohrlich et al., 2005; Åkesson et al., 2006; Andrews, 2008; Jolivet-Gougeon et al., 2008; Ganz, 2011; Ellervik et al., 2012; Lynch & Soslau, 2012).

Still, the SHC has been able to glean certain elements that make it possible to issue a new advisory report on blood donations from haemochromatosis patients.

When assessing the eligibility of a given individual as a donor, the point of departure must always be that there may never be any increased risk involved either for the safety of the donors themselves, or that of the recipients of their blood components. The general principle according to which only healthy individuals may give blood is a corollary to this principle.

In this revision of the SHC advisory report (SHC, 2004), a distinction is drawn between carriers of the *HFE* haemochromatosis gene mutation for which a genetic diagnosis has been made but who do not require therapeutic venesections, and individuals with clinical haemochromatosis who require programmed venesections to remain healthy.

The SHC finds that the affected individuals who require regular venesections are either C282Y homozygotes or H63D/C282Y compound heterozygotes with high concentrations of serum ferritin and complaints caused by the build-up of iron (see Table 1). As for all other carriers of *HFE* gene mutations, the genetic diagnosis does not usually call for programmed therapeutic venesections (Swinkels et al., 2006).

This evaluation has made it possible to reveal that these carriers do not display any pathological iron overload. Compared to healthy donors, homozygous carriers who do not yet present any significant iron overload, may be protected against infections caused by many intracellular pathogens due to the iron deficiency in the macrophages (see 3.2.5). As result, the SHC takes the view that all these asymptomatic carriers<sup>9</sup> may continue to give blood under the usual conditions for donor eligibility.

A recurring argument in the discussions on blood donations from patients with hereditary haemochromatosis is the non-altruistic drive to give blood, which can cause them to provide an unreliable anamnesis at the time of donor selection, making their donations less safe. The literature confirms that a significant incentive for patients to donate their blood instead of undergoing conventional therapeutic venesection is the fact that blood donations are free of charge (McDonnell et al., 1999; Sanchez et al., 2001). Yet there is but limited evidence that donor selection safety may be compromised.

On the one hand, it is a known fact that even so-called volunteer donors do not always give blood for altruistic reasons. Blood donations may also confer other (putative or real) benefits, such as free blood tests, a day off work, positive health effects, etc., which may also play a part.

The issue of the non-altruistic incentives to give blood and the potential problems they cause for the safety of the blood donations need to be addressed by removing all the financial advantages of donating blood instead of undergoing purely therapeutic venesections.

<sup>9</sup> including the 217,000 individuals with H63D homozygosity, some 48,500 H63D/C282Y compound heterozygotes, and around 9,300 C282Y homozygotes with no symptoms of iron overload (see Table 1).

In practice, this can be done by having therapeutic venesections performed in blood establishments, in the hospital, or at the patient's home and applying the same billing policy as regards the financial burden to the HH patient/donor. In addition, the fact that some patients achieve donor status may not give them an advantage over non-donor patients in the eye of insurance companies.

This would make it possible to address the ethical objections against the potentially non-altruistic incentive for people with HH to give blood. From an ethical point of view, it is also advisable to separate the non-altruistic component (the venesection) from the altruistic component (the blood donation) (Pennings, 2005). Discussing the details of these ethical issues went beyond the scope of this advisory report.

The analysis of the literature first of all focussed on the safety issues mentioned above. In this advisory report, an additional distinction is drawn between haemochromatosis patients in the intense iron-depletion induction phase of treatment and those in the maintenance phase.

In this respect, we can draw the following conclusions:

- There is no reason to state that haemochromatosis patients who meet the usual blood donor selection criteria are exposed to any greater risk when giving blood than ordinary donors. What is important is that there be clinical and biological monitoring of their iron build-up and the way it evolves.

Performing venesections on haemochromatosis patients who do not meet the safety criteria for blood donors entails a risk per se and requires facilities in which they can receive the necessary care in the event of any problems occurring.

- The blood collected from haemochromatosis patients should not involve a greater risk of transmitting a potential fungal infection than blood collected from ordinary donors.

Throughout the intensive phase of treatment, iron is mobilised from tissue deposits. Due to the frequency with which venesections are performed, several collections can occur during the same serological window for viral infections. One *in vitro* trial has revealed that blood collected from these patients can entail an increased risk of bacterial contamination (Jolivet-Gougeon et al., 2008). This risk is even greater as a result of the higher collection rates. The risk of excess cadmium being transferred is proportional to the number of times venesection is performed. During this iron-mobilisation phase, the tissues and cells of these patients continue to undergo severe "oxidative stress" and the redox-reactive iron level rises considerably.

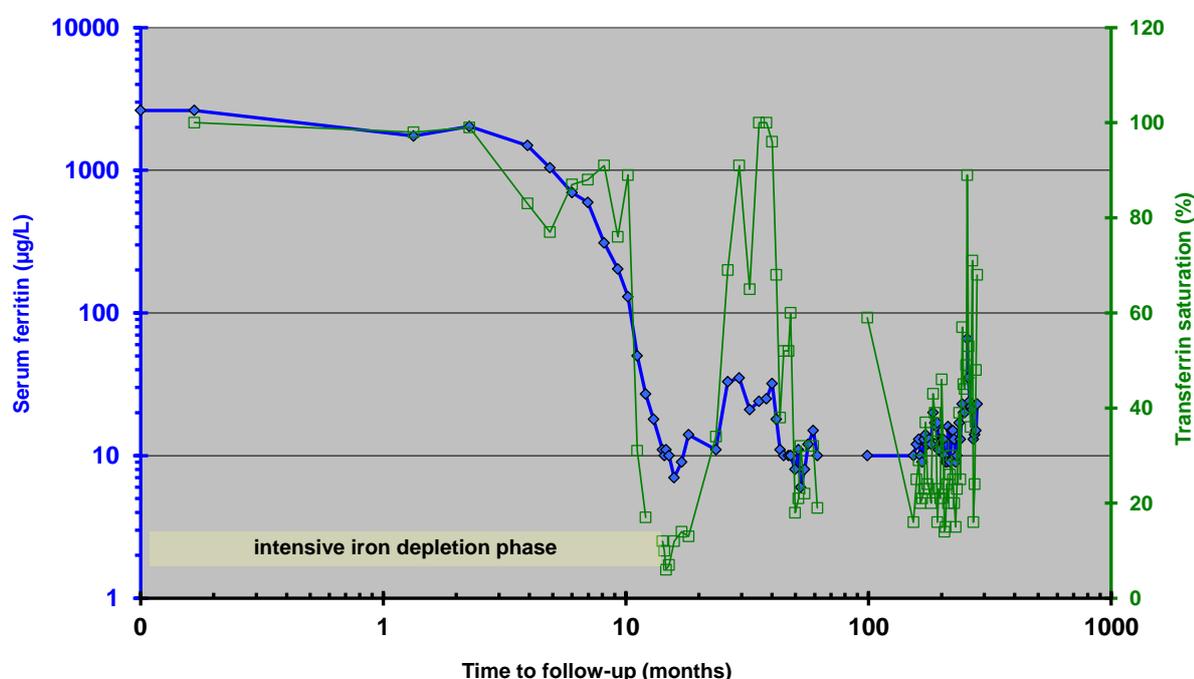
In the light of these observations, blood donations from these patients go against the healthy-donor principle. As a result, HH patients in the induction phase of treatment clearly cannot be accepted as blood donors. In view of the increased risk of bacterial contamination, Jolivet-Gougeon et al. (2008) have recently drawn the same conclusion, as well as De Buck et al. (2012) on the basis of a systematic review of the literature.

There are plenty of data according to which the availability of iron can and does in fact play a crucial role in many different clinical infections (Patruta & Hörl, 1999; Weinberg, 1999; Bullen et al., 2006; Nairz & Weiss, 2006; Vento et al., 2006).

- Due to the cytotoxic potential of iron and/or other metals, the SHC advises, as a precautionary measure, against carrying out transfusions with fresh frozen plasma and buffy-coat platelet concentrates prepared from blood collected from haemochromatosis patients.

- During the maintenance phase of treatment, occasional venesection is performed to maintain patient health; there is no longer any sustained mobilisation of iron from the tissue deposits. It is important to note that transferrin saturation is usually higher in patients in the maintenance phase of treatment than in healthy individuals and that this saturation can reach levels that are as high as those of patients who are starting the intense iron-depletion phase of treatment (see Figure 3). Moreover, there is still an increased level of redox-reactive iron in the blood and disruptions in the homeostasis of other metal ions have been described. The research has not yet established that blood taken from patients in the maintenance phase of treatment entails a higher potential risk of viral contamination, but an increased risk of bacterial contamination has been identified (Patruta et al., 1998; Jolivet-Gougeon et al., 2008; see Figures 1 and 2). Thus, Jolivet-Gougeon et al. (2008) show that, as the transferrin saturation rises, the antibacterial activity declines proportionally (Figure 1). The risk of excess-cadmium transfer is proportional to the number of times venesection is performed; its absorption increases during the maintenance phase of treatment (Åkesson et al., 2000).

**Figure 3.** Evolution of serum ferritin and transferrin saturation in haemochromatosis patients (with kind authorisation from P. Nielsen and R. Fischer).



The red blood cell concentrates from haemochromatosis patients in the maintenance phase of treatment intrinsically meet with the *in vitro* quality criteria for transfusion. Still, given the fact that venesection is less effective against e.g. joint damage, diabetes and other parameters (Norris et al., 2010; Richette et al., 2010), the impact of severe "oxidative stress" on the erythroid cells needs to be determined.

The transfusion efficacy of blood components from haemochromatosis patients has not yet been established.

- The meta-analysis carried out by Ellervik et al. (2012) shows that elevated transferrin saturation levels are associated with an increased risk of cancer in both women and men. Support for these authors' conclusion is provided by the results of a randomised controlled trial on the effect of iron reduction (Zacharski et al., 2008) as well as by a cohort of regular blood donors (Edgren et al., 2008), which suggest that this risk is lower when the iron is reduced.

It is not possible to entirely rule out that transfusing blood from haemochromatosis patients entails an increased risk of cancer in recipients who already have an underlying iron overload (for example, thalassemia patients and those with myelodysplastic syndrome without chelator-induced removal of the iron build-up, all types of haemochromatosis patients, ...).

- To this day, studies on haemochromatosis linked to *HFE* protein mutations mainly focus on the terminal outcome of this condition – iron overload. Yet the protein encoded by the *HFE* gene is similar to proteins of the class 1 major histocompatibility complex and the mutant C282Y protein also seems to play a part in immune defects (Arosa et al., 1997; de Almeida & de Sousa, 2008). The SHC points out that, apart from disruptions in the iron homeostasis, research on the impact of any deregulations in other physiological processes is only just beginning (Rohrlich et al., 2005; Adams et al., 2008; Gray et al., 2009; Norris et al., 2010; Richette et al., 2010; Martins et al., 2011; Mitchell et al., 2011; Ravasi et al., 2012).

All these findings have led the SHC to take the view that additional research is required as regards the susceptibility to infections and the appropriateness of blood taken from haemochromatosis patients with no complications, even those whose serum ferritin levels have returned to normal, before allowing their blood to be used for blood transfusion purposes.

Regardless of the ethical and safety issues linked to blood donations from HH patients, the additional yield of usable blood components is also a recurring argument in this debate (see Appendix 2). It is difficult to provide an accurate assessment for this in this advisory report. Based on American assessments, European data on the prevalence and clinical penetrance of HH homozygosity as well as the current therapeutic venesection figures for Belgium, it is to be expected that allowing HH patients to give blood during the maintenance phase of treatment would result in a maximum yearly increase of 0.4 % in the number of usable blood donations for the Belgian transfusion blood production.

Allowing haemochromatosis patients to give blood will lead to a considerable number of practical problems and operational requirements, the burden and feasibility of which will have to be weighed against the additional yield in usable blood donations, which is believed to be relatively low. The treating physician shall only prescribe venesection-donations for patients who are entitled to have their blood removed in a blood establishment on the basis of the donor selection criteria. The ultimate decision on whether or not the patient should be allowed to donate blood for transfusion purposes is taken exclusively by the blood establishment physician.

### 3.3. Conclusions

This assessment has made it possible to show that only a minority of carriers of a HFE hereditary haemochromatosis gene mutation display pathological iron overload. The SHC takes the view that **asymptomatic** carriers may continue to give blood according to the usual conditions for donor eligibility. This especially concerns heterozygous carriers of a mutation of the *HFE* gene, but also homozygous carriers or people with a compound mutation without any iron build-up. Moreover, accepting haemochromatotics as regular blood donors currently depends on the identification of C282Y homozygotes among young adults, *i.e.* before the appearance of considerable iron-induced organ damage.

The SHC takes the view that the precautionary principle can be invoked when considering implementing a new procedure with unknown potential adverse effects, or when such effects are observed without being able to establish a definite causal link with a suspected factor. Transfusing blood components collected from haemochromatosis **patients** who have undergone regular venesection as a means to preserve their health is worth considering. Indeed, such a practice raises obvious ethical considerations. Also, the epidemiological findings that pertain to it are but partial and debatable. Not only are the relevant epidemiological or clinical investigations scarce, partial or simply non-existent, but failing to put these donations to use will not cause any problems for the Belgian blood component supply. The SHC therefore takes the view that the precautionary principle should be applied with respect to the question submitted.

There is no specific risk involved in haemochromatotics giving blood for the safety of the donors themselves, though there may be for the recipient of these blood components.

Donor acceptance does not apply to therapeutic venesections for the following reasons:

- it is possible that blood is removed on several occasions during the same window period for viral infections in the intense iron-depletion phase of treatment;
- there is a higher risk of bacterial contamination;
- there is a risk of iron toxicity;
- this is not in keeping with the healthy-donor principle.

The SHC emphasises that it is of paramount importance to guarantee that the **anamnesis is reliable** and that the blood is donated for **altruistic reasons** before allowing the use of blood components taken from haemochromatosis patients for transfusion purposes. Furthermore, accepting haemochromatosis patients as blood donors will result in a considerable number of practical problems and operational requirements.

Minority position: One member of the working group, J. Coene, does not agree with the conclusion regarding the use of red blood cells taken from hereditary haemochromatosis patients in the maintenance phase of treatment for transfusion purposes. He bases his view on a different assessment of the available data and also has a different interpretation of the precautionary principle. However, J. Coene does agree with the other recommendations in this advisory report.

Keywords	MeSH terms*	Sleutelwoorden	Mots clés	Schlüsselwörter
haemochromatosis; hemochromatosis	hemochromatosis	hemochromatose	hémochromatose	Hämochromatose
iron overload	iron overload	ijzeroverbelasting	surcharge en fer	Eisenüberladung
blood donation	blood donation	bloedgeven	don de sang	Blutspende

\* MeSH (*Medical Subject Headings*): controlled vocabulary thesaurus for indexing articles for PubMed.

#### 4. REFERENCES

- AABB. Blood donors and hemochromatosis: questions and answers. Association Bulletin 2002; #02-7.
- Abbott M, Galloway A, Cunningham JL. Haemochromatosis presenting with a double Yersinia infection. *J Infect* 1986;13:143-5.
- Adams PC, Barton JC. Haemochromatosis. *The Lancet* 2007;370:1855-60.
- Adams PC, Barton JC. How I treat hemochromatosis. *Blood* 2010;116:317-25.
- Adams PC, Kertesz AE, Valberg LS. Rate of iron reaccumulation following iron depletion in hereditary hemochromatosis. Implications for venesection therapy. *J Clin Gastroenterol* 1993;16:207-10.
- Adams PC, Passmore L, Chakrabarti S, Reboussin DM, Acton RT, Barton JC, et al. Liver diseases in the hemochromatosis and iron overload screening study. *Clin Gastroenterol Hepatol* 2006;4:918-23.
- Adams PC, McAlister V, Chakrabarti S, Levstik M, Marotta P. Is serum hepcidin causative in hemochromatosis? Novel analysis from a liver transplant with hemochromatosis. *Can J Gastroenterol* 2008;22:851-3.
- Adams PC, Speechley M, Barton JC, McLaren CE, McLaren GD, Eckfeldt JH. Probability of C282Y homozygosity decreases as liver transaminase activities increase in participants with hyperferritinemia in the hemochromatosis and iron overload screening study. *Hepatology* 2012;55:1722-6.
- AFSSAPS. Agence française de sécurité sanitaire des produits de santé. Fiche Technique Hémosidérose. Saint-Denis: AFSSAPS; 2011. [accessed 2012 March 28]. Available from: <http://www.anism.sante.fr/content/download/34141/447026/version/1/file/FicheTechniqueHemosidrose.pdf>
- Agresti A, Coull BA. Approximate is better than "Exact" for interval estimation of binomial proportions. *The American Statistician* 1998;52:119-26.
- Åkesson A, Stål P, Vahter M. Phlebotomy increases cadmium uptake in hemochromatosis. *Environ Health Perspect* 2000;108:289-91.
- Akoev VR, Shcherbinina SP, Shnyrov VL. [Microcalorimetric assay of erythrocyte ghosts in hereditary hemochromatosis]. *Gematol Transfuziol* 1992;37:34-6.
- Akoev VR, Shcherbinina SP, Matveev AV, Tarakhovskii IuS, Deev AA, Shnyrov VL. [Study of structural transitions in erythrocyte membranes during hereditary hemochromatosis]. *Biull Eksp Biol Med* 1997;123:279-84.
- Alexander J, Limaye AP, Ko CW, Bronner MP, Kowdley KV. Association of hepatic iron overload with invasive fungal infection in liver transplant recipients. *Liver Transpl* 2006;12:1799-804.
- Allen K. Hereditary haemochromatosis: Diagnosis and management. *Austr Fam Physician* 2010;39:938-41.
- Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008;358:221-30.
- Allen KJ, Bertalli NA, Osborne NJ, Constantine CC, Delatycki MB, Nisselle AE, et al. HFE Cys282Tyr homozygotes with serum ferritin concentrations below 1000 µg/L are at low risk of hemochromatosis. *Hepatology* 2010;52:925-33.
- Andrews N. Forging a field: the golden age of iron biology. *Blood* 2008;112:219-30.
- Andrews N. Closing the iron gate. *NEJM* 2012;366:376-7.
- Arosa FA, Oliveira L, Porto G, da Silva BM, Kruijer W, Veltman J, et al. Anomalies of the CD8+ T cell pool in haemochromatosis: HLA-A3-linked expansions of CD8+CD28- T cells. *Clin Exp Immunol* 1997;107:548-54.
- Aruoma OI, Bomford A, Polson R, Halliwell B. Non-transferrin-bound-iron in plasma from hemochromatosis patients: effect of phlebotomy therapy. *Blood* 1988;72:1416-9.
- Ashrafian H. Hepcidin: the missing link between hemochromatosis and infections. *Infect Immun* 2003;71:6693-700.
- Bacon BR, Britton RS. Clinical penetrance of hereditary hemochromatosis. *NEJM* 2008;358:291-2.

- Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS. Diagnosis and management of hemochromatosis: 2011 practice guidelines by the American association for the study of liver diseases. *Hepatology* 2011;54:328-43.
- Bar T, Kubista M, Tichopad A. Validation of kinetics similarity in qPCR. *Nucleic Acids Res* 2012;40:1395-406.
- Barash CI. Genetic discrimination and screening for hemochromatosis: then and now. *Genet Test* 2000;4:213-8.
- Barton JC, Bottomley SS. Iron deficiency due to excessive therapeutic phlebotomy in hemochromatosis. *Am J Hematol* 2000;65:223-6.
- Barton JC, Acton RT. Hemochromatosis and *Vibrio vulnificus* wound infections. *J Clin Gastroenterol* 2009;43:890-3.
- Barton JC, Patton MA, Edwards CQ, Griffen LM, Kushner JP, Meeks RG, et al. Blood lead concentrations in hereditary hemochromatosis. *J Lab Clin Med* 1994;124:193-8.
- Barton JC, McDonnell SM, Adams PC, Brissot P, Powell LW, Edwards CQ, et al. Management of hemochromatosis. *Ann Intern Med* 1998;129:932-9.
- Barton JC, Grindon AJ, Barton NH, Bertoli LF. Hemochromatosis probands as blood donors. *Transfusion* 1999;39:578-85.
- Barton JC, Bertoli LF, Rothenberg BE. Peripheral blood erythrocyte parameters in hemochromatosis: evidence for increased erythrocyte hemoglobin content. *Lab Clin Med* 2000;135:96-104.
- Barton JC, Preston BL, McDonnell SM, Rothenberg BE. Severity of iron overload in haemochromatosis: effect of volunteer blood donation before diagnosis. *Transfusion* 2001;41:123-9.
- Barton JC, Edwards CQ, Phatak PD, Britton RS, Bacon BR. *Handbook of Iron Overload Disorders*. Cambridge (UK): Cambridge University Press; 2010.
- Barton JC, Barton JC, Acton RT, So J, Chan S, Adams PC. Increased risk of death from iron overload among 422 treated probands with HFE hemochromatosis and serum levels of ferritin greater than 1000 µg/L at diagnosis. *Clin Gastroenterol Hepatol* 2012;10:412-6.
- Barshtein G, Manny N, Yedgar S. Circulatory risk in the transfusion of red blood cells with impaired flow properties induced by storage. *Transfus Med Rev* 2011;25:24-35.
- Batey RG, Lai Chung Fong P, Shamir S, Sherlock S. A non-transferrin-bound serum iron in idiopathic hemochromatosis. *Dig Dis Sci* 1980;25:340-6.
- Beamish MR, Walker R, Miller F, Worwood M, Jacobs A, Williams R, et al. Transferrin Iron, Chelatable Iron and Ferritin in Idiopathic Haemochromatosis. *Br J Haematol* 1974;27:219-28.
- Beckett JM, Ball MJ. Effect of hereditary haemochromatosis genotypes and iron overload on other trace elements. *Eur J Nutr* 2012;51: *in press*.
- Bergmann TK, Vinding K, Hey H. Multiple hepatic abscesses due to *Yersinia enterocolitica* infection secondary to primary haemochromatosis. *Scand J Gastroenterol* 2001;36:891-5.
- Blacklock HA, Dewse M, Bolard C, Hudson P, Barnhill D, Jackson S, et al. Blood donation by healthy individuals with haemochromatosis. *NZ Med J* 2000;113:77-8.
- Blake PA, Merson MH, Weaver RE, Hollis DG, Heublein PC. Disease caused by a marine *Vibrio*. Clinical characteristics and epidemiology. *NEJM* 1979;300:1-5.
- Bolan CD, Conry-Cantilena C, Mason G, Rouault TA, Leitman SF. MCV as a guide to phlebotomy therapy for hemochromatosis. *Transfusion* 2001;41:819-27.
- Boyer F, David C, Lemarié C, Lefort C, Coat C, Poirier-Caruso D, et al. Sepsis à *Yersinia* lors d'une transfusion: de la pratique à la théorie. *Transfus Clin Biol* 2012;19:298.
- Brissot P, Ball S, Rofail D, Cannon H, Jin VW. Hereditary hemochromatosis: patient experiences of the disease and phlebotomy treatment. *Transfusion* 2011;51:1331-8.
- Brissot P, Ropert M, Le Lan C, Loréal O. Non-transferrin bound iron: A key role in iron overload and iron toxicity. *Biochim Biophys Acta* 2012;1820:403-10.
- Brittenham GM, Klein HG, Kushner JP, Ajioka RS. Preserving the national blood supply. *Hematology Am Soc Hematol Educ Program* 2001:422-32.
- Broedbaek K, Poulsen HE, Weimann A, Kom GD, Schwedhelm E, Nielsen P, et al. Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. *Free Radic Biol Med* 2009;47:1230-3.

- Bryant J, Cooper K, Picot J, Clegg A, Roderick P, Rosenberg W, et al. A systematic review of the clinical validity and clinical utility of DNA testing for hereditary haemochromatosis type 1 in at-risk populations. *J Med Genet* 2008;45:513-8.
- Bullen JJ, Spalding PB, Ward CG, Gutteridge JM. Hemochromatosis, iron and septicemia caused by *Vibrio vulnificus*. *Arch Intern Med* 1991;151:1606-9.
- Bullen JJ, Griffiths E, Edmiston CE. *Iron and Infection: Molecular, Physiological, and Clinical Aspects*. 2<sup>nd</sup> edition. Chichester; New York: John Wiley & Sons; 1999.
- Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Natural resistance, iron and infection: a challenge for clinical medicine. *J Med Microbiol* 2006;55:251-8.
- Byrne SL, Krishnamurthy D, Wessling-Resnick M. Pharmacology of Iron Transport. *Annu Rev Pharmacol Toxicol* 2012;52: *in press*.
- Cairo G, Recalcati S, Montosi G, Castrusini E, Conte D, Pietrangelo A. Inappropriately high iron regulatory protein activity in monocytes of patients with genetic hemochromatosis. *Blood* 1997;89:2546-53.
- Camaschella C, Hoffbrand AV. Iron Overload. In: Hoffbrand AV, Catovsky D, Tuddenham EG, Green AR, eds. *Postgraduate haematology*. 6<sup>th</sup> edition. Oxford (UK): Wiley-Blackwell; 2010. p. 47-60.
- Carroll GJ, Breidahl WH, Bulsara MK, Olynyk JK. Hereditary hemochromatosis is characterized by a clinically definable arthropathy that correlates with iron load. *Arthritis Rheum* 2011;63:286-94.
- Cauchie P, Vincken W, Peeters O, Charels K. Hemochromatosis and *Yersinia enterocolitica* septicemia. *Dig Dis Sci* 1987;32:1438.
- Cazzola M, Dezza L, Bergamaschi G, Barosi G, Bellotti V, Caldera D, et al. Biologic and clinical significance of red cell ferritin. *Blood* 1983;62:1078-87.
- CBO. Centraal BegeleidingsOrgaan. Richtlijn Bloedtransfusie. Utrecht: CBO; 2011.
- CBS. Canadian Blood Services. New Partnership with the Canadian Hemochromatosis Society. Ottawa: CBS; 2001. [accessed 2011 December 5]. Available from: [http://www.blood.ca/CentreApps/Internet/UW\\_V502\\_MainEngine.nsf/resources/2001/\\$file/2001-05-25-Partnership-with-hemochromatosis-society\\_eng.pdf](http://www.blood.ca/CentreApps/Internet/UW_V502_MainEngine.nsf/resources/2001/$file/2001-05-25-Partnership-with-hemochromatosis-society_eng.pdf)
- CDC. Centers for Disease Control and Prevention. Red blood cell transfusions contaminated with *Yersinia enterocolitica* - United States, 1991-1996, and initiation of a national study to detect bacteria-associated transfusion reactions. *MMWR* 1997;46:553-5.
- CDC. Centers for Disease Control and Prevention. Fatal laboratory-acquired infection with an attenuated *Yersinia pestis* Strain--Chicago, Illinois, 2009. *MMWR* 2011;60:201-5.
- Chitambar CR, Wereley JP. Iron transport in a lymphoid cell line with the hemochromatosis C282Y mutation. *Blood* 2001;97:2734-40.
- Christopher GW. *Escherichia coli* bacteremia, meningitis, and hemochromatosis. *Arch Intern Med* 1985;145:1908.
- CHS. Canadian Haemochromatosis Society. Iron Filings Printemps 2012. Insurance issues. Richmond: CHS; 2012. [accessed 2012 August 10]. Available from: [http://www.toomuchiron.ca/newsletter/2012\\_spring\\_en.pdf](http://www.toomuchiron.ca/newsletter/2012_spring_en.pdf)
- CHS. Canadian Haemochromatosis Society. Iron Filings Printemps 2012. Heading to the United States? Richmond: CHS; 2012b. [accessed 2012 August 10]. Available from: [http://www.toomuchiron.ca/newsletter/2012\\_spring\\_en.pdf](http://www.toomuchiron.ca/newsletter/2012_spring_en.pdf)
- Church DL, Simmon KE, Sporina J, Lloyd T, Gregson DB. Identification by 16S rRNA gene sequencing of *Negativicoccus succinicivorans* recovered from the blood of a patient with hemochromatosis and pancreatitis. *J Clin Microbiol* 2011;49:3082-4.
- Clark P, Britton LJ, Powell LW. The diagnosis and management of hereditary haemochromatosis. *Clin Biochem Rev* 2010;31:3-8.
- Conry-Cantilena C. Phlebotomy, blood donation, and hereditary hemochromatosis. *Transfus Med Rev* 2001;15:136-43.
- Conry-Cantilena C, Klein HG. Hemochromatosis patients as blood and tissue donors. In: Barton JC, Edwards CQ, eds. *Hemochromatosis: Genetics, pathophysiology, diagnosis and treatment*. Cambridge (UK): Cambridge University Press; 2000. p. 564-73.

- Corke PJ, McLean AS, Stewart D, Adams S. Overwhelming gram negative septic shock in haemochromatosis. *Anaesth Intensive Care* 1995;23:346-9.
- Courtois F, Danic B. Hémochromatose génétique et don de sang. Synthèse d'un groupe de travail multidisciplinaire (janvier 2001). *Rev Med Interne* 2001;22:1165-7.
- Crosbie J, Varma J, Mansfield J. *Yersinia enterocolitica* infection in a patient with hemochromatosis masquerading as proximal colon cancer with liver metastases: report of a case. *Dis Colon Rectum* 2005;48:390-2.
- Cruickshank MK, Ninness J, Curtis A, Barr RM, Flanagan PR, Ghent CN, et al. Usefulness of erythrocyte ferritin analysis in hereditary hemochromatosis. *CMAJ* 1987;136:1259-64.
- Danic B, Bigey F. Les contre-indications au don de sang. Impact de l'arrêté du 12 janvier 2009. *Transfus Clin Biol* 2009;16:209-13.
- de Almeida SF, de Sousa M. The unfolded protein response in hereditary haemochromatosis. *J Cell Mol Med* 2008;12:421-34.
- De Buck E, Pauwels NS, Dieltjens T, Compennolle V, Vandekerckhove P. Is blood of uncomplicated hemochromatosis patients safe and effective for blood transfusion? A systematic review. *J Hepatol* 2012;57:1126-34.
- de Korte D, Verhoeven AJ. Quality determinants of erythrocyte destined for transfusion. *Cell Mol Biol* 2004;50:187-95.
- Delatycki M, Allen K, Williamson R. Insurance agreement to facilitate genetic testing. *Lancet* 2002;359:1433.
- Del Castillo A, Gordo S, Aldámiz T, De la Calle F, Álvarez-Sala LA. Infectious diseases in diagnosed patients of genetic hemochromatosis. *Am J Hematol* 2009;84:E366.
- Deugnier Y, Battistelli D, Jouanolle H, Guyader D, Gueguen M, Loréal O, et al. Hepatitis B virus infection markers in genetic haemochromatosis. A study of 272 patients. *J Hepatol* 1991;13:286-90.
- Drakesmith H, Chen N, Ledermann H, Screatton G, Townsend A, Xu XN. HIV-1 Nef down-regulates the hemochromatosis protein HFE, manipulating cellular iron homeostasis. *PNAS* 2005;102:11017-22.
- EASL. European Association For The Study Of The Liver. Clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010;53:3-22.
- Edgren G, Hjalgrim H, Reilly M, Tran TN, Rostgaard K, Shanwell A, et al. Risk of cancer after blood transfusion from donors with subclinical cancer: a retrospective cohort study. *Lancet* 2007;369:1724-30.
- Edgren G, Reilly M, Hjalgrim H, Tran TN, Rostgaard K, Adami J, et al. Donation frequency, iron loss, and risk of cancer among blood donors. *J Natl Cancer Inst* 2008;100:572-9.
- Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Eng J Med* 1988;318:1355-62.
- Edwards CQ, Griffen LM, Kaplan J, Kushner JP. Twenty-four hour variation of transferrin saturation in treated and untreated haemochromatosis homozygotes. *J Intern Med* 1989;226:373-9.
- EFS. Etablissement Français du Sang. Rapport d'activité 2010. Paris: EFS; 2011. [accessed 2011 December 2]. Available from: <http://www.donusang.net/rewrite/heading/1/758/l-efs.htm?idRubrique=758>
- Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Risk of cancer by transferrin saturation levels and haemochromatosis genotype: population-based study and meta-analysis. *J Intern Med* 2012;271:51-63.
- ElMBERG M, Hultcrantz R, EkboM A, Brandt L, Olsson S, Olsson R, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology* 2003;125:1733-41.
- EUROSTAT. Eurostat - European Commission Statistics Database. Population on 1 January by age and sex. Brussels: EUROSTAT; 2010. [accessed 2011 October 26]. Available from: [http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search\\_database](http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database)

- FAMHP. Federal Agency for Medicines and Health Products. Total de l'activité des Etablissements de transfusion de Belgique. Statistiques 2006-2010/Overzicht van de activiteiten van de bloedinstellingen. Statistieken 2006-2010. Brussels: FAMHP; 2011. [accessed 2011 October 31]. Available from: [http://www.fagg-afmps.be/fr/binaries/Sang%202006%20-%202010\\_tcm291-73802.pdf](http://www.fagg-afmps.be/fr/binaries/Sang%202006%20-%202010_tcm291-73802.pdf)
- Fernández JM, Serrano M, De Arriba JJ, Sánchez MV, Escribano E, Ferreras P. Bacteremic cellulitis caused by non-01, non-0139 *Vibrio cholerae*: report of a case in a patient with hemochromatosis. *Diagn Microbiol Infect Dis* 2000;37:77-80.
- Fix OK, Kowdley KV. Hereditary hemochromatosis. *Minerva Medica* 2007;99:605-17.
- Foulkes EC. Transport of toxic heavy metals across cell membranes. *Proc Soc Exp Biol Med* 2000;223:234-40.
- Franchini M, Targher G, Capra F, Montagnana M, Lippi G. The effect of iron depletion on chronic hepatitis C virus infection. *Hepato Int* 2008;2:335-40.
- Frank KM, Schneewind O, Shieh WJ. Investigation of a researcher's death due to septicemic plague. *NEJM* 2011;364:2563-4.
- Galan SR, Kann PH, Gress TM, Michl P. Spontan-bakterielle Peritonitis durch *Listeria monocytogenes* nach Genuss von kontaminiertem Käse bei einer Patientin mit Hämochromatose. *Z Gastroenterol* 2011;49:832-5.
- Gannon PO, Medelci S, Le Page C, Beaulieu M, Provencher DM, Mes-Masson AM, et al. Impact of hemochromatosis gene (HFE) mutations on epithelial ovarian cancer risk and prognosis. *Int J Cancer* 2011;128:2326-34.
- Ganz T. Hepcidin and iron regulation, 10 years later. *Blood* 2011;117:4425-33.
- Gerhard GS, Levin KA, Price Goldstein J, Wojnar MM, Chorney MJ, Belchis DA. *Vibrio vulnificus* septicemia in a patient with the hemochromatosis HFE C28Y mutation. *Arch Pathol Lab Med* 2001;125:1107-9.
- Ghashghaieina M, Cluitmans JC, Akel A, Dreischer P, Toulany M, Köberle M, et al. The impact of erythrocyte age on eryptosis. *Br J Haematol* 2012;157:606-14.
- Ghoti H, Amer J, Winder A, Rachmilewitz E, Fibach E. Oxidative stress in red blood cells, platelets and polymorphonuclear leukocytes from patients with myelodysplastic syndrome. *Eur J Haematol* 2007;79:463-7.
- Gray SG, Crowe J, Lawless MW. Hemochromatosis: as a conformational disorder. *Int J Biochem Cell Biol* 2009;41:2094-7.
- Greaves DE, Griffiths WJ, Lever AM. Does venesection reduce HIV viral load in patients with hereditary haemochromatosis? *Antivir Ther* 2012;17:*in press*.
- Grindon AJ. Blood donation by patients with hemochromatosis [Reply]. *JAMA* 1993;270:2929.
- Guggenbuhl P, Brissot P, Loréal O. Haemochromatosis: The bone and the joint. *Best Pract Res Clin Rheumatol* 2011;25:649-64.
- Guinet F, Carniel E, Leclercq A. Transfusion-transmitted *Yersinia enterocolitica* sepsis. *Clin Inf Dis* 2011;53:583-91.
- Gurram MK, Newman W, Kobrinsky N. Prevalence of iron overload in pediatric oncology patients after blood transfusion. *Clin Adv Hematol Oncol* 2012;10:363-5.
- Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. *Am J Epidemiol* 2001;154:193-206.
- HAS. Haute Autorité de Santé. Prise en charge de l'hémochromatose liée au gène HFE (hémochromatose de type 1). Saint-Denis La Plaine: HAS; 2005. [accessed 2012 August 7]. Available from: [http://www.has-sante.fr/portail/jcms/c\\_450900/hemochromatose-recommandations](http://www.has-sante.fr/portail/jcms/c_450900/hemochromatose-recommandations)
- Henn BC, Kim J, Wessling-Resnick M, Téllez-Rojo MM, Jayawardene I, Ettinger AS, et al. Associations of iron metabolism genes with blood manganese levels: a population-based study with validation data from animal models. *Environ Health* 2011;10:97.
- Héroult B, Assari S, Colombat P, Binet C, Courtois F, Roubinet F. Les structures de soins dans les établissements de transfusion sanguine, pourquoi faire? *Transfus Clin Biol* 2007;14:127-31.
- Hess JR. Scientific problems in the regulation of red blood cell products. *Transfusion* 2012;52:1827-35.

- HHS. US Department of Health and Human Services. Food and Drug Administration. Guidance for industry: variances for blood collection from individuals with hereditary hemochromatosis. Rockville (MD): Center for Biologics Evaluation and Research; 2001. [accessed 2011 October 19]. Available from: <http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm080393.pdf>
- Hopkins MR, Ettinger AS, Hernández-Avila M, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, et al. Variants in iron metabolism genes predict higher blood lead levels in young children. *Environ Health Perspect* 2008;116:1261-6.
- INS. Institut national de statistique. Population de nationalité belge et étrangère par sexe. Bruxelles: INS; 2010. [accessed 2011 December 19]. Available from: [http://statbel.fgov.be/fr/binaries/3111414\\_fr\\_tcm326-55755.xls](http://statbel.fgov.be/fr/binaries/3111414_fr_tcm326-55755.xls)
- Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, et al. HFE mutations, iron deficiency and overload in 10,500 blood donors. *Br J Haematol* 2001;114:474-84.
- Jacolot S, Yang Y, Paitry P, Férec C, Mura C. Iron metabolism in macrophages from HFE hemochromatosis patients. *Mol Genet Metab* 2010;101:258-67.
- Janssen MCH, Swinkels DW. Hereditary haemochromatosis. *Best Pract Res Clin Gastroenterol* 2009;23:171-83.
- Jeffrey G, Adams PC. Blood from patients with hereditary hemochromatosis - a wasted resource? *Transfusion* 1999;39:549-50.
- Johnson EE, Wessling-Resnick M. Iron metabolism and the innate immune response to infection. *Microbes Infect* 2012;14:207-16.
- Jolivet-Gougeon A, Ingels A, Danic B, Aussant-Bertel F, Férec C, Loreal O, et al. No increased seroprevalence of anti-Yersinia antibodies in patients with type 1 (C282Y/C282Y) hemochromatosis. *Scand J Gastroenterol* 2007;42:1388-9.
- Jolivet-Gougeon A, Loreal O, Ingels A, Danic B, Ropert M, Bardou-Jacquet E, et al. Serum transferrin saturation increase is associated with decrease of antibacterial activity of serum in patients with HFE-related genetic hemochromatosis. *Am J Gastroenterol* 2008;103:2502-8.
- Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011;283:65-87.
- Jouanolle H, Boucher E, Ruffault A, Andre P, Gubguen M, Guyader D, et al. Prevalence of hepatitis C virus antibody in homozygous genetic hemochromatosis: a study of 137 patients [poster abstract]. Third International Conference on Haemochromatosis. Düsseldorf, 25-26 July 1991.
- Khan FA, Fisher MA, Khakoo RA. Association of hemochromatosis with infectious diseases: expanding spectrum. *Int J Inf Dis* 2007;11:482-7.
- Klontz KC, Lieb S, Schreiber M, Janowski H, Baldy L, Gunn RA. Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiologic features in Florida cases, 1981-1987. *Ann Intern Med* 1988;109:318-23.
- Knight JA, Searles DA, Blaylock RC. The effect of metal chelators on lipid peroxidation in irradiated erythrocytes. *Ann Clin Lab Sci* 1992;22:417-22.
- Knutson M, Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. *Crit Rev Biochem Mol Biol* 2003;38:61-88.
- Leitman SF, Browning JN, Yau YY, Mason G, Klein HG, Conry-Cantilena C, et al. Hemochromatosis subjects as allogeneic blood donors: a prospective study. *Transfusion* 2003;43:1538-44.
- Le Lan C, Loréal O, Cohen T, Ropert M, Glickstein H, Lainé F, et al. Redox active plasma iron in C282Y/C282Y hemochromatosis. *Blood* 2005;105:4527-31.
- Levstik M, Adams PC. Eligibility and exclusion of hemochromatosis patients as voluntary blood donors. *Can J Gastroenterol* 1998;12:61-3.
- Li H, Ginzburg YZ. Crosstalk between iron metabolism and erythropoiesis. *Adv Hematol* 2010;2010:605435.
- Loréal O, Gosriwatana I, Guyader D, Porter J, Brissot P, Hider RC. Determination of non-transferrin-bound iron in genetic hemochromatosis using a new HPLC-based method. *J Hepatol* 2000;32:727-33.

- Lustbader ED, Hann HWL, Blumberg B. Serum ferritin as a predictor of host response to hepatitis B virus infection. *Science* 1983;22:423-5.
- Luten M, Roerdinkholder-Stoelwinder B, Rombout-Sestrienkova E, de Grip WJ, Bos HJ, Bosman GJ. Red cell concentrates of hemochromatosis patients comply with the storage guidelines for transfusion purposes. *Transfusion* 2008;48:436-41.
- Lynch S, Soslaw G. Iron levels found in hemochromatosis patients inhibit  $\gamma$ -thrombin induced platelet aggregation. *Platelets* 2012;23:611-6.
- Macedo MF, Porto G, Costa M, Vieira CP, Rocha B, Cruz E. Low numbers of CD8+ T lymphocytes in hereditary haemochromatosis are explained by a decrease of the most mature CD8+ effector memory T cells. *Clin Exp Immunol* 2010;159:363-71.
- MacPherson J. On volunteer donors and the pursuit of a safe blood supply. *ABC Newsletter* 1998;38:15-8.
- Maertens J, Demuyneck H, Verbeken EK, Zachee P, Verhoef GE, Vandenberghe P, et al. Mucormycosis in allogeneic bone marrow transplant recipients: Report of five cases and review of the role of iron overload in the pathogenesis. *Bone Marrow Transplant* 1999;24:307-12.
- Martins R, Silva B, Proença D, Faustino P. Differential HFE gene expression is regulated by alternative splicing in human tissues. *PLoS One* 2011;6:e17542.
- Mast AE, Lee T-H, Schlumpf KS, Wright DJ, Johnson B, Carrick DM, et al. The impact of HFE mutations on haemoglobin and iron status in individuals experiencing repeated iron loss through blood donation. *Br J Haematol* 2012;156:388-401.
- McDonnell SM, Grindon AJ, Preston BL, Barton JC, Edwards CQ, Adams PC. A survey of phlebotomy among persons with hemochromatosis. *Transfusion* 1999;39:651-6.
- Mitchell RM, Lee SY, Simmons Z, Connor JR. HFE polymorphisms affect cellular glutamate regulation. *Neurobiol Aging* 2011;32:1114-23.
- Moalem S, Weinberg ED, Percy ME. Hemochromatosis and the enigma of misplaced iron: implications for infectious disease and survival. *BioMetals* 2004;17:135-9.
- Moura E, Verheul AF, Marx JJ. A functional defect in hereditary haemochromatosis monocytes and monocyte-derived macrophages. *Eur J Clin Invest* 1998;28:164-73.
- Murphy PA. Personal Communication in OMIM® Online Mendelian Inheritance in Man. MIM Number: 235200. Baltimore, MD: Johns Hopkins University; 1987. [accessed 2012 June 18]. Available from: <http://omim.org/>
- Nairz M, Weiss G. Molecular and clinical aspects of iron homeostasis: from anemia to hemochromatosis. *Wien Klin Wochenschr* 2006;118:442-62.
- Nairz M, Theurl I, Schroll A, Theurl M, Fritsche G, Lindner E, et al. Absence of functional Hfe protects mice from invasive *Salmonella enterica* serovar Typhimurium infection via induction of lipocalin-2. *Blood* 2009;114:3642-51.
- Nawrot TA, Staessen JA, Roels HA, Munters E, Cuypers A, Richart T, et al. Cadmium exposure in the population: from health risks to strategies of prevention. *BioMetals* 2010;23:769-82.
- Neumaier M, Braun A, Wagener C. Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics. *Clin Chem* 1998;44:12-26.
- Nevitt T. War-Fe-re: iron at the core of fungal virulence and host immunity. *BioMetals* 2011;24:547-58.
- Newman B. Hemochromatosis blood donor programs: marginal for the red blood cell supply but potentially good for patient care. *Transfusion* 2004;44:1535-7; author reply 1537-8.
- Nicholson A. Hereditary Haemochromatosis - Diagnosis & Management from a GP perspective. ICGP Quality in Practice Committee; 2009. [accessed 2012 June 18]. Available from: [http://www.icgp.ie/library\\_catalogue/index.cfm/id/47329/event/catalogue.item.view.html](http://www.icgp.ie/library_catalogue/index.cfm/id/47329/event/catalogue.item.view.html)
- Nielsen P, Fischer R, Engelhardt R, Dresow B, Gabbe EE. Umfassende, nichtinvasive Diagnostik von Eisenstoffwechselstörungen. *Hamb Ärztebl* 1995;49:347-52.
- NIHDI. National Institute of Health and Disability Insurance. Therapeutic phlebotomy [A. De Swaef, *pers. comm.* 7 October 2011]. Brussels: NIHDI; 2011.
- Norris S, White M, Mankan AK, Lawless MW. Highly sensitivity adhesion molecules detection in hereditary haemochromatosis patients reveals altered expression. *Int J Immunogenet* 2010;37:125-33.

- Nouel O, Voisin PM, Vaucel J, Dartois-Hoguin M, Le Bris M. Association d'une septicémie à *Yersinia enterocolitica*, d'une hémochromatose idiopathique et d'un traitement par déferoxamine: un cas. *Presse Med* 1991;20:1494-6.
- Novembrino C, Porcella A, Conte D, de Vecchi AF, Buccianti G, Lonati S, et al. Erythrocyte ferritin concentration: analytical performance of the immunoenzymatic IMx-Ferritin (Abbott) assay. *Clin Chem Lab Med* 2005;43:449-53.
- NZBS. New Zealand Blood Service. Haemochromatosis information. 111/02602. Epsom: NZBS; 2006.
- Oetl K, Stauber RE. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. *Br J Pharmacol* 2007;151:580-90.
- Onalaja AO, Claudio L. Genetic susceptibility to lead poisoning. *Environ Health Perspect* 2000;108(Suppl 1):23-8.
- Pankow JS, Boerwinkle E, Adams PC, Guallar E, Leidencker-Foster C, Rogowski J, et al. HFE C282Y homozygotes have reduced low-density lipoprotein cholesterol: the Atherosclerosis Risk in Communities (ARIC) Study. *Transl Res* 2008;152:3-10.
- Paradkar PN, De Domenico I, Durchfort N, et al. Iron depletion limits intracellular bacterial growth in macrophages. *Blood* 2008;112:866-74.
- Park SK, Hu H, Wright RO, Schwartz J, Cheng Y, Sparrow D, et al. Iron metabolism genes, low-level lead exposure, and QT interval. *Environ Health Perspect* 2009;117:80-5.
- Patruta SI, Hörl WH. Iron and infection. *Kidney Int Suppl* 1999;69:S125-30.
- Patruta SI, Edlinger R, Sunder-Plassmann G, Hörl WH. Neutrophil impairment associated with iron therapy in hemodialysis patients with functional iron deficiency. *J Am Soc Nephrol* 1998;9:655-63.
- Pennings G. Demanding pure motives for donation: the moral acceptability of blood donations by haemochromatosis patients. *J Med Ethics* 2005;31:69-72.
- Piperno A, Girelli D, Nemeth E, Trombini P, Bozzini C, Poggiali E, et al. Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. *Blood* 2007;110:4096-100.
- Pletschette M, Köhl J, Kuipers J, Schmidt RE. Opportunistic *Capnocytophaga canimorsus* infection. *Lancet* 1992;1;339:308.
- Poullin P, Lefèvre PA. Intérêt des érythrophérèses à la phase initiale du traitement des hémochromatoses génétiques de type 1: expérience à propos de 30 cas. *Transfus Clin Biol* 2011;18:553-8.
- Power TE, Adams PC. Hemochromatosis patients as voluntary blood donors. *Can J Gastroenterol* 2004;18:393-6.
- Prá D, Rech Francke SI, Henriques JA, Fenech M. Iron and genome stability: an update. *Mutat Res* 2012;733:92-9.
- Prus E, Fibach E. Uptake of non-transferrin iron by erythroid cells. *Anemia* 2011;2011:945289.
- Ravasi G, Pelucchi S, Trombini P, Mariani R, Tomosugi N, Modignani GL, et al. Hepcidin expression in iron overload diseases is variably modulated by circulating factors. *PLoS One* 2012;7:e36425.
- Richette P, Eymard C, Deberg M, Vidaud D, de Kerguenec C, Valla D, et al. Increase in type II collagen turnover after iron depletion in patients with hereditary haemochromatosis. *Rheumatology* 2010;49:760-6.
- Ritger K, Black S, Weaver K, Jones J, Gerber S, Conover C, et al. Fatal laboratory-acquired infection with an attenuated *Yersinia pestis* Strain--Chicago, Illinois, 2009. *MMWR* 2011;60:201-5.
- Rochette J, Le Gac G, Lassoued K, C. Férec C, Robson KJH. Factors influencing disease phenotype and penetrance in HFE haemochromatosis. *Human Genetics* 2010;128:233-48.
- Rohrlich PS, Fazilleau N, Ginhoux F, Firat H, Michel F, Cochet M, et al. Direct recognition by alphabeta cytolytic T cells of Hfe, a MHC class Ib molecule without antigen-presenting function. *PNAS* 2005;102:12855-60.
- Røsvik AS, Ulvik RJ, Wentzel-Larsen T, Hervig T. Blood donors with hereditary hemochromatosis. *Transfusion* 2010;50:1787-93.
- Ruccione KS, Mudambi K, Sposto R, Fridey J, Ghazarossian S, Freyer DR. Association of projected transfusional iron burden with treatment intensity in childhood cancer survivors. *Pediatr Blood Cancer* 2012;59:697-702.

- Sanchez AM, Schreiber GB, Bethel J, McCurdy PR, Glynn SA, Williams AE, et al. Prevalence, donation practices, and risk assessment of blood donors with hemochromatosis. *JAMA* 2001;286:1475-81.
- SANQUIN. Stichting Sanquin Bloedvoorziening. Primaire hemochromatose en bloedafname. Amsterdam: SANQUIN; 2005 [accessed 2011 October 19]. Available from [http://www.sanquin.nl/Sanquin-nl/sqn\\_donorstart\\_nl.nsf/All/Folders-Voor-Donoren.html?opendocument&highlight=hemochromatose](http://www.sanquin.nl/Sanquin-nl/sqn_donorstart_nl.nsf/All/Folders-Voor-Donoren.html?opendocument&highlight=hemochromatose)
- SANQUIN. Stichting Sanquin Bloedvoorziening. IJzerstapeling en therapeutische bloedafname. Amsterdam: SANQUIN; 2011 [accessed 2012 July 9]. Available from [http://www.sanquin.nl/repository/documenten/nl/product-dienst/272540/Brochure\\_IJzerstapeling.pdf](http://www.sanquin.nl/repository/documenten/nl/product-dienst/272540/Brochure_IJzerstapeling.pdf)
- Sargent T, Lim TH, Jenson RL. Reduced chromium retention in patients with hemochromatosis, a possible basis of hemochromatotic diabetes. *Metabolism* 1979;28:70-9.
- Schaub R, Dauresa J-P, Seifer D, Lapierre C, Michel H. Enquête nationale sur les saignées dans l'hémochromatose génétique (HG). *La Revue de médecine interne* 2012;33(Suppl 1):S79.
- Senba M, Nakamura T, Itakura H. Statistical analysis of relationship between iron accumulation and hepatitis B surface antigen. *Am J Clin Pathol* 1985;84:340-2.
- SHC. Superior Health Council. Avis relatif à une demande concernant l'hémochromatose/Advies van de HGR betreffende een aanvraag in verband met hemochromatose. Advisory report no. 8059. Brussels: SHC; 2004.
- SHC. Superior Health Council. La viro-inactivation du plasma frais congelé/Virusinactivatie van vers bevroren plasma. Advisory report no. 8339. Brussels: SHC; 2007.
- SHC. Superior Health Council. Bonnes pratiques de transfusion à l'usage des hôpitaux/Goede transfusiepraktijken in ziekenhuizen. Advisory report no. 8381. Brussels: SHC; 2010.
- SHC. Superior Health Council. L'efficacité clinique de la réduction des pathogènes en vue d'une implémentation pour les concentrés plaquettaires/De klinische doeltreffendheid van pathogeenreductie met het oog op implementatie voor bloedplaatjesconcentraten. Advisory report no. 8700. Brussels: SHC; 2011.
- Siegel EM, Patel N, Lu B, Lee JH, Nyitray AG, Huang X, et al. Circulating biomarkers of iron storage and clearance of incident human papillomavirus infection. *Cancer Epidemiol Biomarkers Prev* 2012;21:859-65.
- Staessen JA, Roels HA, Emelianov D, Kuznetsova T, Thijs L, Vangronsveld J, et al. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. *Lancet* 1999;353:1140-4.
- STATBEL. Direction générale Statistique et Information économique. Mouvement de la population 1988-2007. Population - Chiffres population 1990-2010. Bruxelles: STATBEL; 2010. [accessed 2012 January 26]. Available from: <http://statbel.fgov.be/fr/statistiques/chiffres/population/mouvement/>
- Stuart BJ & Viera AJ. Polycythemia vera. *Am Fam Physician* 2004;69:2139-44.
- Swinkels DW, Janssen MCH, Bergmans J, Marx JJM. Hereditary hemochromatosis: genetic complexity and new diagnostic approaches. *Clin Chem* 2006;52:950-68.
- Swinkels DW, Jorna ATM, van Bokhoven MA, Castel A, van Deursen CTBM, Giltay JC, et al. Samenvatting van de richtlijn "Diagnostiek en behandeling van hereditaire hemochromatose". *Ned Tijdschr Hematol* 2009;6:143-9.
- Symeonidis A, Marangos M. Iron and Microbial Growth. In: *Insight and Control of Infectious Disease in Global Scenario*. Roy PK, ed. InTech; 2012. [accessed 2012 May 29]. Available from: <http://www.intechopen.com/books/insight-and-control-of-infectious-disease-in-global-scenario/>
- Tan L, Khan MK, Hawk JC 3rd, for the Council on Scientific Affairs, American Medical Association. Use of blood therapeutically drawn from hemochromatosis patients. *Transfusion* 1999;39:1018-26.
- Tate J, Ward G. Interferences in immunoassay. *Clin Biochem Rev* 2004;25:105-20.
- Tefany FJ, Lee S, Shumack S. Oysters, iron overload and *Vibrio vulnificus* septicaemia. *Australas J Dermatol* 1990;31:27-31.

- Thomas LD, Michaëlsson K, Julin B, Wolk A, Åkesson A. Dietary cadmium exposure and fracture incidence among men: A population-based prospective cohort study. *J Bone Miner Res* 2011;26:1601-8.
- Torp-Pedersen T, Nielsen XC, Olsen KE, Barfod TS. [Intracerebral abscess after infection with non-toxicogenic *Vibrio cholerae*.][Article in Danish] *Ugeskr Laeger* 2012;174:498-9.
- UKBTS. United Kingdom Blood Transfusion Services. Guidelines for the Blood Transfusion Services in the United Kingdom. 7<sup>th</sup> edition. London: TSO; 2005.
- Vahdati-Ben Arie S, Laham N, Schechter C, Yewdell JW, Coligan JE, Ehrlich R. A single viral protein HCMV US2 affects antigen presentation and intracellular iron homeostasis by degradation of classical HLA class I and HFE molecules. *Blood* 2003;101:2858-64.
- Van Der Weyden MB, Fong H, Salem HH, Batey RG, Dudley FJ. Erythrocyte ferritin content in idiopathic haemochromatosis and alcoholic liver disease with iron overload. *Br Med J* 1983;286:752-4.
- van Dijk BA, Laarakkers CM, Klaver SM, Jacobs EM, van Tits LJ, Janssen MC, et al. Serum hepcidin levels are innately low in HFE-related haemochromatosis but differ between C282Y-homozygotes with elevated and normal ferritin levels. *Br J Haematol* 2008;142:979-85.
- Van Steenberghe W. Hereditaire hemochromatose: snel evoluerende inzichten in een lang gekende ziekte. *Tijdschr Geneesk* 2008;64:551-60.
- Van Vlierberghe H, Messiaen L, Hautekeete M, Elewaut A, De Paepe A. Erfelijke hemochromatose. Van keltten en ijzeren tijdperk tot genetische diagnose. *Tijdschr Geneesk* 1999;55:143-7.
- Van Vlierberghe H, Messiaen L, Hautekeete M, De Paepe A, Elewaut A. Prevalence of the Cys282Tyr and His63Asp mutation in Flemish patients with hereditary hemochromatosis. *Acta Gastroenterol Belg* 2000;63:250-3.
- Vento S, Cainelli F, Cesario F. Infections and thalassaemia. *Lancet Infect Dis* 2006;6:226-33.
- Vetter S, Hartmann D, Jakobs R, Riemann JF. Seltene akute Hepatitis bei einer Hämochromatosepatientin: eine Zoonose? *Med Klin* 2010;105:305-9.
- Walkden D, Badami K, McGonigal G. Phlebotomy patterns in haemochromatosis patients and their contribution to the blood supply. *N Z Med J* 2012;125:29-34.
- Walker EM, Walker SM. Effects of iron overload on the immune system. *Ann Clin Lab Sci* 2000;30:354-65.
- Wang J, Pantopoulos K. Regulation of cellular iron metabolism. *Biochem J* 2011;434:365-81.
- Weinberg ED. Iron loading and disease surveillance. *Emerg Inf Dis* 1999;5:346-52.
- Weinberg ED. The hazards of iron loading. *Metallomics* 2010;2:732-40.
- Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2006;145:209-23.
- Wright RO, Silverman EK, Schwartz J, Tsaih SW, Senter J, Sparrow D, et al. Association between hemochromatosis genotype and lead exposure among elderly men: the normative aging study. *Environ Health Perspect* 2004;112:746-50.
- Yang H, Lee J, Seed CR, Keller AJ. Can blood transfusion transmit cancer? A literature review. *Transfus Med Rev* 2010;24:235-43.
- Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. *J Natl Cancer Inst* 2008;100:996-1002.
- Zhou XD, De Tolla L, Custer RP, Lonton WT. Iron, ferritin, hepatitis B surface and core antigens in the livers of Chinese patients with hepatocellular carcinoma. *Cancer* 1987;59:1430-7.

## 5. APPENDIXES

### **APPENDIX 1. Different modalities for the collection and use of blood from haemochromatosis patients in other countries**

Blood from individuals with HH but no symptoms is considered intrinsically safe for transfusion. Countries that allow the use of blood from haemochromatosis patients usually follow the same line of policy for venesection-donations, regardless of whether or not the blood is actually used.

#### **Canada** (Levstik & Adams, 1998; Adams & Barton, 2010)

- HH patients in the induction phase of treatment are not accepted as blood donors;
- the blood of patients in the maintenance phase of treatment who are "in good health" and who meet the usual safety requirements may be used for transfusion;
- the legal provisions regarding the donation intervals (56-day minimum) apply to haemochromatosis patients, who therefore may give blood up to 6 times a year;
- the Canadian Blood Services do not perform any purely therapeutic venesections.

#### **United States** (Brittenham et al., 2001; HHS, 2001)

- initial requirements:
  - o blood components from haemochromatosis patients should be labelled as such;
  - o when less than 8 weeks have passed since the previous donation, a medical examination is required on the day of the donation.

These two regulations were found to be an impediment to the use of blood from individuals with HH for allogeneic transfusion purposes in spite of the blood itself being considered safe;

- the blood establishments can obtain a variance to the requirements mentioned above provided the following conditions are met:
  - o the blood of haemochromatosis patients must be suitable according to the usual criteria for blood intended for allogeneic transfusion purposes;
  - o blood banks that accept the blood of haemochromatosis patients for transfusion purposes have to provide the venesections free of charge for all haemochromatosis patients, even if the donors are ineligible as allogeneic blood donors;
  - o blood collections may be carried out more frequently than every eight weeks for donors who have presented a physician's prescription for therapeutic venesection;
- the *AABB Standards for Blood Banks and Transfusion Services* allow the use of blood from therapeutic venesections for allogeneic transfusion purposes (AABB, 2002);
- it is up to the blood establishments themselves to decide whether or not they make use of this possibility. Many US blood banks have declined obtaining these variances and prefer to collect blood units from volunteer donors only (Adams & Barton, 2010);
- most of the patients who receive treatment in the blood establishments are required to have their serum ferritin levels measured and other assessments made in their physician's surgery (Adams & Barton, 2010).

**The Netherlands (SANQUIN, 2005; SANQUIN, 2011)**

- Sanquin accepts HH patients who present a medical prescription for therapeutic venesection;
- the blood collected is not used for transfusion but is intended for scientific purposes instead;
- the venesections cannot be performed in the blood bank if there are any medical reasons against this, such as an infectious disease or heart problems.

**United Kingdom (UKBTS, 2005)**

- HH patients in the induction phase of treatment are not accepted as blood donors, but those who require regular venesection for the maintenance of their health can be;
- before accepting these patients, the collecting physician must ensure that the following criteria are met:
  - o the altruistic-donations-principle must be guaranteed;
  - o the usual donor selection criteria must be met, except for donation frequency; if it is clinically appropriate for individuals to donate more frequently than the minimum donation interval, specific permission must be obtained from the designated medical officer;
  - o the patient is under the continuing care of a physician who is able to offer therapeutic venesection if they are not eligible for blood donation.

**New Zealand (NZBS, 2006)**

- NZBS accepts HH patients as donors both during the intense iron-depletion phase of treatment as well as during maintenance therapy, provided they initially meet the donor acceptance criteria and their liver function tests are normal;
- only HH patients undergoing maintenance therapy can donate blood at mobile collection sites;
- donor acceptance requires an initial medical examination and referral by the treating physician; the latter remains in charge of the treatment follow-up;
- in the event of a risk for the recipient resulting in temporary deferral: blood collections are continued by NZBS;
- in the event of permanent deferral: subsequent venesections are performed by the healthcare services.

**Ireland (Nicholson, 2009)**

- since June 2007, the Irish Blood Transfusion Service has run a dedicated clinic one day per week in Dublin for haemochromatosis patients who also meet the donor eligibility requirements; similar clinics have been established since in 4 other centres;
- these patients must be referred by a physician;
- ferritin levels are measured and the venesection is performed; the clinic must keep the medical record to ensure that the haemochromatosis will be properly monitored by the hospital or the prescribing physician; the collected blood can then be offered for transfusion purposes;
- if patients need to be temporarily deferred from donating blood, they can continue to have venesections performed, but the blood cannot be used for transfusion purposes until the deferral period is over; this service had to be temporarily suspended.

**France** (Danic & Bigey, 2009)

- hereditary haemochromatosis with no phenotypic expression was already no longer considered a counter-indication to blood donation (Courtois & Danic, 2001);
- therapeutic venesections are carried out in a health centre (Centre de Soins) in cooperation with a medical team that monitors the condition (Hérault et al., 2007);
- since April 2009, hereditary haemochromatosis is no longer considered a counter-indication to blood donation;
- venesection- blood donations need to be performed in a blood establishment that also has a health centre (which means that it can never be performed at a mobile collection venue) and in cooperation with the medical team in charge of the follow-up;
- the health centre physician receives the informed consent of the patient regarding the use of their venesection blood as a donation. This physician then informs the collecting physician. Prior to carrying out the "venesection-donation", the latter conducts the medical interview aimed at identifying any counter-indications for the donor and the recipient;
- key role of the health centre physician in charge of the haemochromatosis follow-up: no blood donations are allowed from patients with iron overload induced complications; if there is a clear medical counter-indication that may or may not be related to the haemochromatosis, the "venesection-donation" is not offered to the patient;
- health centre physicians who both carry out the collections and provide care can perform both the medical follow-up of the condition and assess the patient's eligibility for blood donation;
- the usual deferral criteria apply, except for the interval between donations and the number of blood donations per year;
- in the event of a temporary or permanent deferral from blood donations, the patient returns to the therapeutic network and the venesections can be performed at the health centre if there is no direct counter-indication.

## **APPENDIX 2. What is the significance of accepting HH patients as blood donors for the blood supply?**

There are wide discrepancies in the figures from the literature regarding the number of additional donations for transfusion that may be obtained by accepting haemochromatosis patients as donors.

The effect on the blood supply depends on three factors: the prevalence of the disease among the population, the number of venesections performed on HH patients and the number of donations that qualify for transfusion on the basis of donor selection and product suitability.

### ***1. Donor selection, product suitability and assessment for the blood supply***

In a US retrospective study, 67.3 % of the 211 haemochromatosis index cases<sup>10</sup> were considered eligible for blood donation at the moment of diagnosis (Barton et al., 1999). This suitability was significantly lower than that among volunteer donors (93.5 %). This difference is partly due to the disorder itself and to the readiness to give blood, and partly to the risk of transfusion-borne transmission of viral infections.

On average, the eventual suitability of the products taken from HH patients is 66.3 % lower than that of products taken from volunteer donors (93.6 %), but is relatively higher during the maintenance phase of treatment (87.6 %) than during the depletion phase (64.6 %). Due to the greater collection frequency, the number of usable donations obtained during the depletion phase of treatment is, in absolute figures, much greater than the yield of a one-year maintenance therapy (1,029 vs. 106). The number of usable donations obtained during the maintenance phase of treatment only represents about one tenth of the total number of usable donations from HH patients. The main reasons for rejecting these products are believed to be low haemoglobin and haematocrit levels in the donors, as well as elevated ALT values.

The lack of agreement in terms of composition as well as the different selection criteria applied to haemochromatosis index cases and the donor population in general make it difficult to draw a direct comparison.

By combining the donor eligibility and product suitability percentages, the proportion of usable blood components from venesections performed on HH patients in the maintenance phase of treatment amounts to 59 %. Based on this percentage and on a 0.4 to 0.5 % prevalence of homozygosity in Americans of Caucasian descent, the AABB has estimated the number of potential additional donations in the USA to be between 300,000 and 3 million (AABB, 2002). The significant lack of precision regarding this assessment is due to the lack of certainty regarding the prevalence of the disorder and the number of blood donations per year during maintenance therapy.

In an alternative analysis based on the number of asymptomatic HH patients that also takes into account the number of donations during the depletion phase of treatment, Conry-Cantilena & Klein (2000) calculated that the number of additional blood units would reach 53,000, which amounts to 0.4 % of the total annual production from voluntary donations. This boils down to an 0.18 to 0.80 % increase of the blood supply established by Newman (2004) in a study conducted at 16 US blood transfusion centres after implementing the FDA variance (HHS, 2001) that allows blood to be taken from HH patients for allogeneic transfusion purposes.

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<sup>10</sup> Index case: initial member of a family who – based on iron overload – is diagnosed as suffering from haemochromatosis.

Also in North America, Leitman et al. (2003) carried out a prospective trial during which 130 HH patients were granted free access to venesections in a university blood transfusion centre, both for iron-depletion as well as for maintenance therapy. 76 % of the donors appeared to be eligible for allogeneic blood donation. An assessment was made after 27 months: as it turned out, the HH patients had provided 14 % of the red blood cell concentrate supply at the research centre. Another point that needs emphasising is the donor loyalty exhibited by the HH patients. Such a significant contribution from HH patients is only possible within a hospital transfusion-department setting. It requires extensive screening and active recruitment – of the HH patients and their relatives –, careful communication and rigorous follow-up, it also involves administrative costs and additional laboratory testing (Newman, 2004). In Canada, experience has shown that, without properly raising the awareness of the blood establishments and HH patients, there is little to be gained in terms of additional blood donations by accepting these patients as donors (Levstik & Adams, 1998; Tan et al., 1999).

Finally, we need to take into account the fact that many haemochromatotics are part of the donor population anyway, even in countries in which the diagnosis of haemochromatosis is an exclusion criterion. These donors will not really provide any additional blood donations if the legislation were to become less stringent regarding haemochromatosis and blood donations (AABB, 2002). Two US trials have shown that 23.2 % and 37 % respectively of newly diagnosed HH patients were volunteer blood donors (Barton et al., 1999; McDonnell et al., 1999) and Sanchez et al. (2001) estimate that the prevalence of unidentified HH donors in 8 US transfusion centres – from which most of the known haemochromatosis patients are excluded from blood donation – reaches 0.8 %.

Genetic population screening would make it possible to identify more people with HH but, taking into account the limited clinical penetrance of the anomaly, it is not advisable to do so (Newman, 2004; Whitlock et al., 2006; Bryant et al., 2008; Swinkels et al., 2009; Camaschella & Hoffbrand, 2010; EASL, 2010; Bacon et al., 2011).

As regards other countries than the USA, there are only few articles available on the eligibility of donors with HH and their donations. A New Zealand prospective trial (Blacklock et al., 2000) has revealed that 56 % of 53 haemochromatosis patients – both in the depletion and maintenance phase of treatment – are eligible for allogeneic blood donation if the local donor selection criteria are applied. On average, they provided 13 donations a year, but no mention was made of the ultimate suitability of these donations. The Etablissement Français du Sang (EFS) performs some 70,000 therapeutic venesections each year. These venesections qualify as blood for transfusion purposes if these patients have a good general state of health and if there are no counter-indications for blood donation. According to the EFS, 20 to 30 % of the therapeutic venesections could therefore be used for transfusion purposes (EFS, 2011).

## **2. Estimates for the Belgian blood supply**

It is possible to provide an approximation of the potential number of blood donations from haemochromatosis patients on the basis of the prevalence of C282Y homozygosity and the clinical penetrance of hereditary haemochromatosis (cf. Table 2).

Given the fact that the clinical penetrance of iron overload is so low in women (see section 3.2.1) and that, on average, the iron overload appears 10 years later in women than in men, the contribution of men and women should be assessed separately.

It is also necessary to take into account the age limits. In general, the iron overload is only diagnosed after the age of 50 in men. In Belgium, blood donations are authorised up to the age of 70. Finally, we assume that only HH patients in the maintenance phase of treatment are allowed to give blood for the purpose of this assessment.

**Table 2.** Assessment of the number of haemochromatosis patients eligible for blood donation.

Gender	Population			
	Having reached the age for HH expression*	Having reached the age for HH expression*, younger than 71	With symptomatic iron overload linked to <i>HFE</i> <sup>Δ</sup>	With symptomatic iron overload, younger than 71
<b>Men</b>	1,822,925	1,305,812 <b>(71,6 %)</b>	1,487	<b>1,065</b>
<b>Women</b>	1,396,747	614,966 <b>(44,0 %)</b>	55	<b>24</b>

\* from the age of 50 in men, from the age of 60 in women.

<sup>Δ</sup> cf. Table 1.

Given the distribution by age among the Belgian population (EUROSTAT, 2010), we can state that among the total male and female population with a potential risk of iron overload (over the age of 50 for men, and 60 for women, respectively), 71.6 % of men are eligible for blood donation, compared to 44 % of women. However, if we only take into consideration the clinically observable case of symptomatic iron overload, this would amount in practice to 1,065 potential male donors, whereas for women, this would concern no more than 24 individuals.

It is not exactly known how many of these potential donors are in the iron-depletion induction phase of treatment, but it can be assessed to be 10 %, given the fact that this intense phase of treatment takes around 24 months on average in the age group at which HH patients would be allowed to give blood (see above). Some 90 % of the 1,089 potential donors are therefore in the maintenance phase of iron-depletion and do in fact qualify for blood donations.

By taking as a point of departure a rate of 3 blood donations a year, 59 % of which are suitable for transfusion (see APPENDIX 2, 1.), and assuming that all HH patients offer to give blood, the contribution of these 926 blood donors with HH undergoing maintenance therapy<sup>11</sup> would reach 1,735 usable red cell concentrates per year, which amounts to some 0.33 % of the yearly deliveries of red cell concentrates (519,530 units, cf. FAMHP, 2011).

Needless to say that the value of this calculation depends on the accuracy of the annual number of donations taken into consideration, of the proportion of venesections performed during the depletion phase of treatment and during maintenance therapy as well as of the average age at diagnosis.

The known number of therapeutic venesections carried out in Belgium makes it possible to make a more direct assessment of the number of potential donations from HH donors. 39,088 therapeutic venesections were carried out in 2009 (NIHDI, 2009): if, among these venesections, 1 out of 11 was performed as part of the maintenance therapy for haemochromatosis (Barton et al., 1999), this would yield 3,553 additional donations per year, which would amount to a 0.65 % increase (549,266 donations, cf. FAMHP, 2011).

<sup>11</sup> Based on this line of reasoning, as well as 2 venesections a year (Walkden et al., 2012), some 20 usable concentrates could be collected from compound heterozygotes each year (see Table 1).

This assessment does not take into account the fact that therapeutic venesections are also prescribed for a whole series of patients with conditions other than *HFE*-related haemochromatosis (Stuart & Viera, 2004; Barton et al., 2010). This concerns e.g. patients with primary polycythaemia, non-*HFE* haemochromatosis, ferroportin related haemochromatosis, secondary iron overload, porphyria cutanea tarda, chronic hepatitis C<sup>12</sup>, etc.

Moreover, the ultimate gain in terms of usable blood components is lower as a result of the fact that individuals with HH stop donating blood more frequently, especially as a result of liver conditions and endocrine problems (e.g. diabetes), which cannot be improved through iron depletion (Clarke et al., 2010).

Accepting haemochromatosis patients undergoing maintenance therapy as blood donors results in a fairly limited additional yield of usable blood donations, viz. around 0.4 % of the annual blood supply in Belgium, provided that all patients offer to give blood at each venesection.

### **3. Shortages in the Belgian blood supply**

Each venesection will result in lower iron levels, which in turn will worsen the underlying low-hepcidin state, thus perpetuating the excessive iron absorption by the intestine (see section 3.2.4). It follows that it is important to understand that each haemochromatosis patient is required to abide by the time intervals between venesections (Barton & Bottomley, 2000; Bolan et al., 2001) and should therefore refrain from offering to give blood on other occasions, including in response to calls to donate blood in times of temporary shortage.

Since September 2009, an inventory has been made of the blood supplies available in the transfusion centres and blood establishments for delivery to the hospitals every week. An overview is provided to the transfusion centres and blood establishments. In addition, these supplies have also been published on the FAMHP website for over a year now. Since then, the total supply of blood components has never dropped below the critical threshold (which amounts to the supply for half a week). It follows that the Belgian blood supply is not affected by any structural shortage.

In the light of the research conducted by Adams et al. (1993) and Allen et al. (2010), it is to be expected that the numerous cases of moderate haemochromatosis will be managed in a less aggressive manner. This approach aims at normalising the iron-indices rather than really achieving iron-depletion.

Some experts now advise normalising the serum ferritin at < 300 µg/L for men and menopausal women, and at < 200 µg/L for non-menopausal women (Janssen & Swinkels, 2009; Allen, 2010). A follow-up aimed at monitoring the ferritin and preventing it from reaching high levels, *i.e.* > 1,000 µg/L, should be initiated every 3 – 5 years, which significantly reduces the number of venesections.

It should also be noted that some 15 % of the index cases with serum ferritin levels over 1,000 µg/L at the moment of diagnosis, die prematurely as a result of this iron overload, in spite of their having received treatment (Barton et al., 2012).

<sup>12</sup> Carriers of H63D *HFE* mutations usually only have a moderate iron overload (Allen et al., 2008), but a few authors have reported a strong clinical association of hepatitis C with this mutation (Adams et al., 2006; Del Castillo et al., 2009; Adams et al., 2012). As regards these patients, the therapeutic venesections are not prescribed for *HFE*-related haemochromatosis.

A national survey conducted on venesections performed for genetic haemochromatosis in France (Schaub et al., 2012) has revealed that 39 % of patients wish to have their venesections performed in the hospital in order to see the physician, whereas 28 % prefer to have them performed at home by a nurse.

Finally, it is entirely possible for efficient and safe medication to turn out to be active enough to make venesections obsolete (Andrews, 2008; Byrne et al., 2012).

## 6. COMPOSITION OF THE WORKING GROUP

All experts joined the working group *in a private capacity*. The names of the members and experts of the Superior Health Council are indicated with an asterisk\*.

The following experts were involved in drawing up this advisory report:

BENOIT Yves	paediatric haemato-oncology	UGent
BRUSSELMANS Koen	blood and blood products; molecular and cell biology	ISP
COENE José	transfusion	Dienst voor het Bloed, Rode Kruis – Vlaanderen
DENEYS Véronique*	immunology-haematology; transfusion	Service du Sang, Croix Rouge de Belgique; UCL
DE PAEP Rudi*	intensive care	UZA
GERARD Christiane	immunology-haematology; transfusion	CHU Sart-Tilman; ULg
LATINNE Dominique*	haematological biology	UCL
LAMBERMONT Micheline*	transfusion	ULB; Service du Sang, Croix Rouge de Belgique
LOIX Sébastien	anaesthesiology; intensive care	Hôpital Jolimont La Louvière
MUYLLE Ludo*	blood, tissues and cells	UA; UZA; FAMHP
SELLESLAG Dominik	internal medicine, haematology	AZBrugge
SZABO Bertrand	transfusion	Clinique Reine Astrid Malmédy
THOMAS Isabelle*	virology	ISP
VAN DER LINDEN Philippe	anaesthesiology	CHU Brugman
ZACHEE Pierre	haematology	ZNA

This working group was chaired by Véronique DENEYS, the scientific secretary was Roland HÜBNER.

## About the Superior Health Council (SHC)

The Superior Health Council is a federal body that is part of the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC takes no decisions on the policies to follow, nor does it implement them. It does, however, aim at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, members of scientific institutions), 200 of whom are appointed experts of the Council. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a referring committee) and 4) the final endorsement of the advisory reports by the Board (ultimate decision-making body). This coherent set of procedures aims at allowing the SHC to issue advisory reports based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

The advisory reports drawn up by the working groups are submitted to the Board. Once they have been endorsed, they are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website ([www.css-hgr.be](http://www.css-hgr.be)), except as regards confidential advisory reports. Some of them are also communicated to the press and to target groups among healthcare professionals.

The SHC is also an active partner in developing the EuSANH network (European Science Advisory Network for Health), which aims at drawing up advisory reports at the European level.

In order to receive notification about the activities and publications of the SHC, you can send a mail to [info.hgr-css@health.belgium.be](mailto:info.hgr-css@health.belgium.be) .