REVIEW

Iron and transfusion medicine

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ARTICLE INFO

ABSTRACT

Blood bankers have focused their energy to secure blood transfusion, and only recently have studies been published on the effect of blood donation on iron metabolism. In many facilities, hemoglobin measurement is only performed just before or even during blood donation, but the determination of iron stores is largely ignored. The 2013 paradox of transfusion medicine is due to the fact that blood donation may be harmful and leads to iron deficiency with or without anemia, but for other individuals, it may be a healthy measure preventing type 2 diabetes. The purpose of this review is to discuss iron metabolism in the perspective of blood donation, notably regarding their possible genetic profiles that eventually will discriminate "good" iron absorbers from "bad" iron responders.

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1. Introduction

The importance of iron as well as of iron metabolism has been largely neglected in the transfusion medicine community, even if isolated investigators have made important contributions in this field [1–9]. For most of the blood bankers, the pre-donation value of hemoglobin (Hb) is used as the only hematological criteria allowing the collection of 450 to 500 mL in volunteers. However, all of us clearly know that this amount of blood corresponds to a depletion of about 200 mg of iron, and that repetitive donation may lead to iron deficiency with or without anemia. The problem of iron deficiency without anemia (IDWA) is a difficult one [10–12]. Nevertheless, it should be addressed by physicians involved in blood collection. Inversely, blood donation is an accepted approach to control iron overload, if the patient corresponds to the many criteria that are in place to select blood donors. Therefore, the ultimate development will be the production of "ironomic" tools that will allow us to rapidly identify who are the individuals able to produce enough red blood cells without developing iron deficiency after blood donation, or inversely, who will be protected from iron toxicity by regular blood donation.

2. Iron deficiency with or without anemia

Iron deficiency anemia is a well-known disorder with guidelines clearly establishing assessment, investigation and treatment [13]. It is a major health problem, and iron deficiency anemia ranks number 15 when evaluated in terms of DALYs (disability-adjusted life-years) [14]. IDWA is still a controversial subject particularly regarding its clinical impact and physiological consequences. Iron deficiency affects not only erythropoiesis but also cellular functions involving the immune system, neurotransmitters, DNA synthesis and mitochondrial function [15]. Muscle function, fatigue and effect on attention and cognition are classic features of iron deficiency anemia even though a recent meta-analysis showed a modest effect of iron supplementation on attention and concentration [16]. However most studies included in this meta-analysis were underpowered. In the absence of anemia the association between fatigue and IDWA is still unclear particularly considering the effectiveness of iron supplementation. This question is important considering the high prevalence of iron deficiency without anemia in a French study [17] and in the United States [18]. Several randomized control trials have shown a positive effect of iron supplementation on fatigue [10,12,19]. However, the difficulty of blinding is an important issue because of the effect of iron on stool color. Administering intravenous iron in a placebo controlled randomized clinical trial is probably the best design and Krayenbühl et al. in a subgroup analysis have shown an improvement in fatigue in IDWA women (ferritin below 15 µg/L). However the study with 90 participants was too underpowered to show a statistically significant effect on the whole

group (ferritin below 50 μg/L). Furthermore the question of improving quality of life is still an unsolved issue. A new ongoing multicenter randomized controlled trial with intravenous iron not yet published but presented in a conference showed a positive effect on fatigue and quality of life [20]. All these studies suggest that the improvement of fatigue is independent of hemoglobin, therefore illustrating the impact of iron "to maintain key enzymes in the mitochondrial electron transport system" [15]. Apart from fatigue and cognitive changes, other studies have shown a benefit for endurance [21], athletic performance [22], restless leg syndrome [23], pregnancy [24] and heart failure [25]. All these studies give arguments to a more individualized definition of anemia and iron deficiency. Normal references based on population data do not mean "asymptomatic intervals". For example the Vaucher's study show in women with prolonged fatigue without anemia not only an improvement in fatigue but also a strong improvement in erythropoiesis (hemoglobin and MCV increase and soluble transferrin receptor (sTfR) decrease) with iron supplementation in comparison with placebo. Interestingly in blood donors with IDWA one week after a blood donation, iron supplementation in comparison with placebo had no effect on fatigue and muscular function despite the strong improvement in erythropoiesis [4]. Hence women blood donors are a different population than women with prolonged fatigue. Nevertheless the Waldvogel's study showed that hemoglobin regeneration time was shortened and predonation HB levels were recovered 5 weeks after blood donation while in the placebo group donors were still iron depleted. This consideration is important to increase blood donor return rates. Therefore short-term iron supplementation may be a better approach rather than reducing the frequency of blood donation [26]. More research on donor harm according to iron depletion is clearly needed.

3. Blood donation and iron deficiency

Whole blood donation of 450–500 mL is inevitably associated with iron loss of 200–220 mg, depending on the Hb concentration of the donor [7,27,28], representing 5 to 10% of total body iron. Enteral iron absorption is the only way for the body to replace iron loss. If all the dietary iron (heme- and non-heme iron) could be absorbed by the enterocytes, it would take 15 to 20 days to replace iron loss by blood donation. However, the capacity to increase iron absorption is limited to a maximum of 5 to 7 mg/day depending on serum ferritin concentration [29], which means that at least 40 to 60 days are necessary to refill the depleted iron stores. Only few donors possess sufficient adaptation capacities to deal with the extreme challenges to iron metabolisms by blood donations. Most blood donors do not fully compensate iron loss between consecutive blood donations and as a consequence they develop iron deficiency [30].

However, it is well known, that preselected long term blood donors manage to maintain normal Hb concentration over several years despite regular blood donation [31]. In Zurich, some of us examined multidonation donors for their iron status parameters while undergoing blood donation [32]. Iron parameters such as sTfR and calculated ferritin index (sTfR/log ferritin) remained stable in these preselected donors, despite continuous blood donation; the individual hemoglobin (cHb) remained in the normal range, indicating sufficient iron supply to erythropoiesis. In contrast, serum ferritin was found to be very variable among these donors (variation of ferritin levels according to inflammation was excluded by measuring CRP which was normal in these donors). Obviously, under circumstances of regular blood donation, ferritin did not appear informative for evaluating actual iron stores, an observation also made by Hallberg et al. [33]. The recently discovered iron regulation mechanisms centered on hepcidin [34–36], may now give detailed insights into the physiology of iron metabolisms in blood donors. Consistent with the findings in mice experiments [37–39], Mast et al. have shown that regular blood donation correlates with low serum hepcidin in parallel with low serum ferritin [31]. A sustained decrease of serum hepcidin leads to "high" expression of ferroportin

(Fpn1) at enterocytes and macrophages, allowing better iron absorption in the gut and shifting of iron from the reticuloendothelial store to erythroid precursors [40]. In selected individuals, excessive iron loss by blood donation may be compensated by adequate adjustment of iron metabolisms allowing these individuals to become long term blood donors. In a prospective study of newly recruited blood donors, we confirmed sustained down-regulation of serum hepcidin while on blood donation [41]. However, female donors who revealed already low serum hepcidin at study entry allowing only minor down-regulation of serum hepcidin were much more susceptible to develop significant iron deficiency anemia and thus were deferred from blood donation. Recently, Mast et al. confirmed these observations and postulate the significance of hepcidin response to predict tolerance to ongoing blood donation [42]. However, due to the high variability of hepcidin concentration measured by immunoassays, it might be difficult to use this parameter in individual cases. The use of mass spectrometry should prove to be a useful test in this context [43].

4. Hemoglobin, iron, ferritin and blood donation: how to select blood donors?

The correlation between Ht measurement or Hb concentration determination with total red cell volume is quite poor and only measurements of both plasma and red blood cell volumes are accurate and objective indicators of normality in blood composition [44]. Nevertheless, Hb is the only laboratory value required before blood donation in the vast majority of blood establishments. Mostly, these tests are performed on finger stick samples using portable hemoglobin analyzers, especially on mobile donor drives. Hb values vary between finger stick samples and venous samples. Finger stick samples yield higher Hb values than venous samples [45], which have to be taken into account for developing donor algorithms. Measurement of Hb is not an easy task and noninvasive methods are evaluated [46,47]. Nevertheless, a high rate of blood donors are deferred, notably because of anemia which is most frequently related to iron deficiency [48–50]. Furthermore, a very important factor for developing iron deficiency after blood donation is the frequency of donation. The Council of Europe recommends no more than 4 whole blood donations in female and 6 donations in male donors per year [51]. Some European blood establishments have even lower total numbers of whole blood donations (e.g. in Switzerland 3 donations per year in female and 4 in male donors). With these intervals, the risk of depletion of iron stores should be acceptable in the vast majority of healthy volunteer donors. However, many blood donors still develop iron deficiency or even iron deficient anemia. Considering the shrinking of the donor pool that many blood donation facilities are going to face in the next years, the interest on preventing significant iron deficiency and in particular iron deficiency anemia is increasing. Currently there are many groups investigating laboratory tests and/or prediction models to minimize donor deferral due to low hemoglobin, one of the main reasons leading to a loss of blood donors. At some blood donation centers, larger hematology analyzers and other lab tests such as ferritin or zinc protoporphyrin (ZPP) are available. However the added value of these additional tests to predict iron deficiency or low hemoglobin deferral at the next intended donation is not yet established. Ferritin is used in some blood centers in order to prevent donors from developing iron deficiency without anemia or even overt iron deficient anemia. Ferritin is not a point of care analysis and is rather cost intensive. O'Meara et al. investigated the value of routine ferritin testing and recommended an algorithm at the detection of anemia or iron deficiency without anemia. Donors were offered extending donation interval, change of diet or oral iron supplementation alone or in different combinations, according to donor's needs and wishes. Donors were referred to their GP when medical history was abnormal [3]. With this strategy, they could show that introduction of routine ferritin measurement was improving donor Hb and ferritin when following an

algorithm for donor counseling based on Hb and ferritin, particularly in the group of women of childbearing age.

Stern et al. investigated the value of ferritin, HB and red blood cell indices (MCV and MCHC) to predict low HB deferral at the next visit. This study found that hemoglobin was the best single marker for predicting low HB at the next visit. Ferritin levels were found to be of additional value in blood donors with Hb 5 μg/mL and less above Hb cutoff values [2]. However this finding has not yet been validated prospectively. In a recent study, Kiss et al. showed that red cell indices are of limited value for use as diagnostic tools in blood donors at risk for iron deficiency [52]. Finally, because the presence of pica appeared to be associated with a high probability of iron depletion or deficiency in blood donors, screening questions for pagophagia may prove useful in the ascertainment of iron deficiency in donors and may identify those who would benefit from oral iron [53].

ZPP is another interesting indicator of iron stores: it rises in early iron deficiency, making it a potential useful new marker for early detection of iron deficiency. ZPP can be measured from finger stick samples (point of care testing!) and is a relatively inexpensive test which has been shown in a Dutch cohort of blood donors to be of value in the prediction of low Hb deferral [54]. Before ZPP can be included in donor selection algorithms, more studies are warranted. The Nijmegen group developed a refined prediction model for low hemoglobin donor deferral comprising of Hb level measured at the previous visit, age, seasonality, difference in Hb level between the previous two visits, time since the previous visit, deferral at the previous visit, and total number of whole blood donations in the past 2 years [55]. With this algorithm they predict the ability to prevent donor deferral by inviting only donors who are predicted to be able to donate at the intended donation. An important observation is however that different Hb cut off values for blood donation represent a limit for the application of these refined prediction algorithms in all blood establishments. For instance, the Dutch prediction model could not be validated in Ireland presumably because of different Hb cut off levels [56].

In conclusion measuring hemoglobin at the intended donation is still the single most important lab test in 2013 to predict future low hemoglobin deferral. Additional tests such as ferritin and ZPP are in use and their role still needs to be established. Prediction models using basic values which can be widely used are under way, but still need validation, and they promise to be of great value in the near future to detect earlier blood donors at risk of iron deficiency and iron deficiency anemia.

5. The spectrum of iron overload diseases

Iron-overload diseases are heterogeneous. However, these diseases are typically insidious, causing progressive and irreversible organ injury before clinical symptoms develop. Some iron-overload diseases such as HFE-associated hemochromatosis or beta-thalassemia are relatively common, whereas others are rare. Early diagnosis is important since iron toxicity can be attenuated or prevented.

5.1. Hereditary hemochromatosis

Hereditary hemochromatosis (HH) is a heterogenous disorder at both genetic and phenotypic levels [57], and the genomics of iron overload syndromes is a rapidly growing field of research [58–62]. Since the discovery of the Cys282Tyr mutation of *HFE* in 1996, several types (type 1, types 2A and 2B, type 3, types 4A and 4B) have been described, affecting genes corresponding to HFE, hemojuvelin, hepcidin and ferroportin, respectively. Types 1, 2A, 2B and 3 are autosomal recessive diseases, whereas types 4A and 4B are autosomal dominant disorders. The different clinical presentations as well as the algorithm allowing to evaluate patients presenting with normal transferrin saturation and elevated ferritin have been described elsewhere [57,63]. A spectrum of treatment (from bleeding to liver transplantation [64]) is available. Clinical and

molecular investigations, leading to adapted treatment options are mandatory, because HH may lead to various organ dysfunctions (notably heart failure [65]) or to the development of hepatocarcinoma [66].

5.2. Secondary hemochromatosis, hemosiderosis

Iron overload is observed as secondary to many disorders and can be classified in different groups of diseases. In the first group, the "iron-loading anemias", disorders such thallassemic syndromes, sideroblastic anemia, chronic hemolytic anemia, aplastic anemia, and pyruvate kinase deficiency are observed. In the "chronic liver diseases", several pathologies are encountered: hepatitis C infection, nonalcoholic fatty liver disease (NASH), alcoholic liver disease, or porphyria cutanea tarda. Finally, accumulation of iron may be secondary to red blood cell transfusion, long-term hemodialysis with iron substitution, or to orphan diseases such as acerulopasminemia, African iron overload or neonatal iron overload [67]. In all these diseases, the consequences of iron overload should be carefully determined.

6. Iron and glucose metabolism; from physiopathology to transfusion medicine

Type 2 diabetes (T2D) is a worldwide health burden considering that over 370 million individuals are today affected by the disease. T2D is responsible for a substantial morbidity and increased mortality. Iron homeostasis is closely linked to glucose homeostasis [68–70].

6.1. Iron toxicity and type 2 diabetes

Iron toxicity observed in hereditary hemochromatosis or during transfusional iron overload is associated with high prevalence of secondary diabetes [71]. Conversely, iron deficiency is associated with obesity which is the most common risk factor for developing T2D. How can iron contribute to abnormal glucose homeostasis?

In the experimental model of iron overload that mimics hemochromatosis, mice have a decreased glucose-stimulated insulin secretion and increased insulin sensitivity [72]. Insulin resistance occurs later during the disease in mice and these animals have an increased oxidative stress detected in pancreatic islets resulting to an excess of β-cell apoptosis. In contrast to the experimental mice models of hemochromatosis, both insulin deficiency and insulin resistance are present in human hemochromatosis [73]. However, the β-cell failure observed in humans with hemochromatosis is probably the primary and prerequisite abnormality for developing T2D. This is emphasized by the observation that insulin sensitivity is restored after bloodletting and insulin secretory abilities are only partially improved in patients with hemochromatosis who undergo phlebotomy [73,74].

The pathogenesis of T2D in patients with iron overload (hemochromatosis) compared to diabetic patients with elevated iron levels (inflammatory state and/or elevated iron intake) is probably not similar. As mentioned, in prediabetic individuals with hemochromatosis, insulin sensitivity is initially preserved while β-cell failure is present. In typical prediabetic patients with no hemochromatosis but elevated ferritin levels, insulin resistance is present very early in the course of the disease. This difference may be partially explained by a different response of the adipocytes to the iron load. In mouse models and humans with hemochromatosis, the adipokine "adiponectin" secreted by the adipocytes are elevated [72]. This hormone increases the insulin-sensitivity. Conversely, in diabetes associated with increased iron intake or inflammation, the adiponectin levels are low and may therefore contribute to the insulin resistance state observed in common T2D. During inflammation, ferritin levels increase and a negative relationship is observed between ferritin and adiponectin. In fact, ferritin levels seem to predict adiponectin secretion in a better way than body mass index.

During iron overload, the oxidative stress is increased by the generation of free radicals from iron reacting with hydrogen peroxide and the

trafficking of other micronutrients such as manganese is also altered by iron stores [75–77]. The oxidative stress contributes to β-cell failure and also to hepatic dysfunction and fibrosis. This later alters liver insulin sensitivity and therefore fails to suppress gluconeogenesis in the liver.

6.2. Iron body status and intake as risk factor for developing type 2 diabetes

Several epidemiological studies and meta-analyses have shown that dietary heme iron intake and body stores are associated with an increased risk of T2D [78,79]. The risk of developing T2D is approximately three times greater for an increment of 5 mg/day in dietary heme. Non-heme iron intake as well as supplemental iron seems not to be associated with T2D [78,80–82]. Heme iron is readily absorbed in the body and is therefore more likely to increase iron stores. Ferritin, as biomarker of iron store, has been consistently shown to be an independent risk factor for developing T2D. In a recent systematic review and meta-analysis, Kunutsor et al. have identified nine studies that prospectively evaluated the risk of developing T2D based upon ferritin levels [83]. The effect of elevated ferritin on T2D is about 70% higher in individuals with high ferritin levels compared with those in the bottom quintile. This risk is only slightly attenuated after adjusting for a large range of potential T2D risk factors, including inflammatory markers, HDL-levels and triglyceride levels, smoking, BMI, alcohol consumption and liver enzymes. The critical question underlying these studies is to address whether the association between ferritin (iron store) and the risk of T2D is a causal relationship or a simple association. Since environmental factors contribute to ferritin levels, Mendelian randomization studies have been initiated to answer the question of the direct causal relationship of ferritin levels with diabetes. Two SNPs of the transmembrane protease serin 6 (TMPRSS6) gene have been associated with ferritin levels in population-based studies in GWAS studies. So these genetic variants are positively associated with the levels of ferritin and these SNPs have been also directly associated with T2D, suggesting that the association between ferritin level and diabetes is a causal one [84,85]. However er these studies need to be replicated in a larger consortium of population-based studies where all confounding factors are clearly included in the analysis of the GWAS to perform a Mendelian randomization approach.

6.3. Should iron stores be reduced to treat or to prevent the onset of diabetes?

If the relationship between iron and glucose metabolism is well recognized, data related to the potential beneficial effects of iron depletion are relatively rare in common T2D. In several animal models of T2D, effects of phlebotomy or low iron diet have been studied [72,86]. These iron-depleted animals were protected in part from diabetes and an increase in insulin secretion and sensitivity was demonstrated [72]. In animals, iron-restriction, without inducing anemia, is also associated with increased insulin sensitivity. In humans, this observation has been confirmed in blood donors [87]. In healthy people, frequent blood donation leading to depleted iron stores are associated with reduced incidence of T2D. Insulin sensitivity in these healthy blood donors significantly increased as compared with a control group who had never given blood and matched for several traditional risk factors for T2D. This positive effect on insulin sensitivity is coupled with an anticipated reduction of insulin secretion in frequent blood donors. This implies that iron stores, at least evaluated by the ferritin levels, is not only an independent risk factor for developing diabetes in healthy individuals but also directly associated with insulin resistance. A universal definition of iron overload in healthy persons need therefore to be addressed since lower levels of ferritin may be a better objective of health, at least from a perspective of metabolic homeostasis.

Therapeutic phlebotomy is required in patients with HH. Glucose metabolism has been studied in subjects with newly diagnosed HH [88]. After normalization of ferritin and transferring saturations by venesection for 12 months, subjects with HH improved the glucose tolerance status mainly by increasing insulin sensitivity of peripheral tissues.

In common T2D, Paul Cutler investigated almost 25 years ago, the potential benefits of reducing iron stores in patients with high-ferritin diabetes in the absence of hemochromatosis [89]. Using the iron chelator deferoxamine, diabetic subjects with high ferritin improved drastically fasting glucose, HbA1c, and triglycerides and most of the individuals were free of insulin treatment after iron depletion induced by an iron chelator. These effects were not observed in the control group that included diabetic subjects with normal ferritin levels. Blood-letting was also evaluated in high ferritin T2D patients [90,91]. Three phlebotomies (500 mL) at 2 week intervals had a substantial benefit in these patients compared to subjects that were matched for age, BMI and pharmacological treatment. Reducing iron stores improved HbA1c and insulin sensitivity up to 12 months after the bloodletting. This study was controlled but the small numbers of individuals require confirmation in a larger sample of subjects. Phlebotomy in these individuals improved vascular reactivity which may contribute to the amelioration of insulin action [92].

In patients with metabolic syndrome and clinical evidence of nonalcoholic fatty liver disease (NASH), phlebotomy was shown to decrease blood pressure, fasting glucose, HbA1c and lipid profile 6 weeks after bloodletting [93]. Here again, the results were encouraging but the relative small numbers of individuals included requires the extension of the observation in a larger sample of subjects. A multicenter, randomized and controlled trial was initiated to assess whether the reduction of iron stores by phlebotomy could modify cardiovascular outcomes in patients with peripheral arterial disease [94]. In these symptomatic patients, the all-cause mortality and nonfatal myocardial infarction or stroke were not reduced by the bloodletting.

In summary, epidemiological studies in humans and several animal models have demonstrated a clear association between iron stores and glucose homeostasis as well as diabetes risk. The intervention studies to reduce iron stores are still limited and required confirmation in a larger multicenter randomized trial to fully confirm the potential beneficial effects of reducing iron to treat and/or to prevent the onset of T2D, NASH or metabolic syndrome.

7. The 2013 paradox of transfusion medicine

The transfusion medicine community is apparently faced with two apparently contradictory situations: the consequences of blood donation in the development of iron deficiency with or without anemia and the place of blood donation to treat iron overload and thus, prevent T2D. In some donors, blood donation is "dangerous" whereas in others, it is a beneficial approach and may be a part of the treatment. This paradox certainly will open many ethical discussions: to harm or not to harm, to treat or not to treat; blood donation as being dangerous for the health of the donor or blood donation as a preventive measure or a treatment.

The only possible approach to resolve this paradox will be the development of a global "omic" approach for iron metabolism that will allow us to identify "good (those who will benefit from blood donation)" and "bad (those who will develop iron deficiency with or without anemia)" donors.

In order to achieve this objective, certain basic requirements need to be fulfilled: i) the biochemical principles of iron pathways need to be considered for both, healthy humans and individuals affected by iron overload and deficiency (anemia), respectively, ii) all data need to be examined, since these are pheno- as well as genotypes, which need to be collected in sufficient quality and quantity, and iii) all data need to be analyzed in an adequate manner (statistics and modeling). Today, information about the biochemistry of iron homeostasis and pathological backgrounds, technical platforms for data acquisition and data interpretation tools are in place, and probably more convenient, than ever

before. There is detailed knowledge about the basic biochemical iron-pathways [95–97]. And for the most pronounced pathological situations there are some explanations and some locations identified within these pathways, as exemplified for iron-refractory iron deficiency anemia [98,99]. However, borderline phenotypes still lack recognition, full explanation, or identified causes [100]. It may therefore be of advantage to interpret the presence of iron in the human body without fixed boundaries between health and disease, in a "global" way.

Additional hidden (genetic) predispositions only becoming apparent upon physiological stress, e.g. malnutrition, or blood donation, may be expected. Iron metabolism itself may roughly be segmented into biochemical sub-disciplines and pathological situations may be located therein:

(1) *iron logistics*, that is transport from one place to another, which includes storage and remobilization (Tf, ZIP14), iron preparation for transport by reductase and oxidase (Cybrd1, Cp, Heph) and iron absorption and export (Dmt1, Slc40A1);
(2) *regulation of iron homeostasis* by signal transduction (Smad genes), transcription factors (Usf2), growth factors (Gdf15), cell iron regulators (Irp genes) and the whole world of hepcidin regulation (HFE, Hfe2, Trf2, Hamp);
(3) *sub-pathways* like hypoxia-, heme-, hmj-, and Bmp-pathways (Hif2A, Flvcr1, Neo1, Bmp genes);
(4) *cross-talk to other biochemical, or immunological processes* like Cu-metabolism, Ca-metabolism, or inflammation (Best1, IL1A, IL6, IL6R, Stat3) [62,95,96,101].

Blood donors are tremendously important, and fortunately enough, numerous. Thereby, they fulfill the absolute need for statistical power in health oriented study-projects. First time donors may be seen as statistically representative of the average population, however, a potential bias towards an overrepresentation of individuals unaffected by iron dependent anemia needs to be accounted for. Female donors in childbearing age and repetitive first time donors may be considered as ideal study-subjects for physiological stress of iron depletion, and long term repetitive donors as humans with a nutritionally or genetically reasoned tendency for iron accumulation. Certainly and independent of the above described interpretation, all blood donors are renowned as "healthy" when donating blood. Blood donors will not only be "used" as study subjects, but will benefit as humans from universal findings with respect to iron-metabolism, at the same time.

8. Genomic research

Genomic research is critically dependent upon phenotypic data in general. With respect to genomics of iron metabolism, e.g. "ironomics", this requirement is of even more significance, since physiological phenotypes must be expected as blended results of alternate and compensatory pathways in either directions or unfixed boundaries between health and disease, e.g. iron overload and iron deficiency. Consequently, the best available phenotypic iron measures will be needed to define distinct subgroups of blood donors and to correlate those with genetic findings. Individual donor data sets for ferritin, cHb, transferrin, transferring receptor, sTfr, hepcidin, CRP, and expression of ferroportin may be considered for this purpose. Whereas some of these values will be determined upon first blood donation only, others will be measured repeatedly in appropriate time frames in order to phenotype "physiological" stress resulting from repeated blood donations over time.

Detection of genetic donor polymorphism will focus on SNPs. Focusing in on "tagSNPS", which are representative for haplotypic blocks of genes, allows the identification of genetic variation without genotyping every SNP in a chromosomal region [102,103]. However, dependent on the number of haplotype blocks per gene, which is roughly influenced by its length in base pairs, single SNPs up to several SNPs of potential influence on iron metabolism may be identified for every single gene involved [62,101]. This enlarged candidate gene approach is in contrast to GWAS, which scans the entire genome for common genetic variation. The rationale behind specifically focusing on allelic variation, is that this approach is better suited for detecting genes underlying common and more complex diseases where the risk associated with any given candidate gene is relatively small [104–106]. This approach usually uses the case–control study design.

8.1. A possible protocol

Switching to numbers, a reasonable study protocol for a "global" approach to iron metabolism may involve 20 to 30 genes with an average of 5–10 SNPs per gene as detailed earlier, and may collect pheno- and genotype data of some 12,000–18,000 well selected blood donors considering the cohorts' sex ratio, and percentages of pre-/postmenopausal women, first time donors, and depleting and nondepleting long term donors [62,101]. This means, that with respect to the genetic analysis alone, 1.2 to 5.4 million SNPs would await their detection. Technically, several platforms allow for such projects, of which only matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) will be discussed here. MALDI-TOF MS was initially introduced in proteomics applications, while the full potential for DNA analysis was demonstrated in 1995 [107].

Optimized for the detection of nucleic acids the MALDI-TOF MS (MassARRAY, Sequenom, San Diego, USA) system is currently applied for SNP genotyping (including insertions and deletions), somatic mutation screening, quantitative gene expression and copy number variation analysis, and DNA methylation detection [108–112]. The platform supports multiplexed reactions up to a plex level of 40+ assays (SNPs) per reaction, acquires and interprets data quickly, gives a quantitative output and is highly sensitive [113]. MALDI-TOF MS SNP genotyping is accurate, highly automatable and fast, with a capacity of up to 150,000 SNPs per day [113,114]. Currently, data interpretation seems to be biggest task for the "global" genomic approach of iron metabolism. An oft-cited reason for the lack of success in genetic studies of complex disease, as may be expected in the field of "ironomics", is the existence of interactions between loci. There are numerous difficulties in determining the biological relevance of statistical gene–gene interactions [115]. The search for such interactions may range from simple exhaustive search, over various data-mining/machine learning approaches to Bayesian model selection approaches [115]. Although a starting point, examination of pairwise interactions of gene polymorphisms, e.g. using "BOolean Operation-based Screening and Testing" (BOOST), may not be sufficient [116]. Selected search of three- to five-way interactions conditioned on significant pair-wise results may finally help to unravel the intrinsic of ironomics [117].

9. Perspectives for transfusion medicine

The knowledge of the physiology as well as the pathophysiology of iron metabolism is rapidly changing. The determination of Hb by using $CuSO_4$ (a very old fashioned method, but still in use in many places such as the Service Régional Vaudois de Transfusion Sanguine) is entering medical history. The future is in the present. The classification of blood donors according to a stratification of either iron deficiency or iron overload (and thus of the potential toxicities of iron) is potentially open. Clinical trials associated with GWAS and "omics" approaches will certainly help us to progress and transform donor cares and donor management programs. The future is open!

10. Practice points

Blood donation is always associated with iron depletion. In some individuals, this may lead to iron deficiency with or without anemia. In other individuals, this iron depletion may be beneficial, by decreasing the iron stores which may accumulate according to specific genetic alterations or to other mechanisms such as those present in patients

with metabolic syndrome. Therefore, transfusion medicine is placed in the paradox of harming some donors, or being beneficial, by preventing the development of type 2 diabetes.

11. Research agenda

The development of "ironomics" certainly will help physicians in charge of blood donors by providing tools allowing discriminating "bad" from "good" donors. However, these venues certainly will open ethical debates regarding the definition of a healthy voluntary non-paid donor. Therefore, a combination of research in epidemiology, human sciences as well as in basic sciences will be needed to resolve the new paradoxes of transfusion medicine.

Conflict of interest

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