Red blood cell transfusions in hemato-oncological patients: Don't iron out the consequences

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General introduction

“Niets is zonder bloed geboren.”
(“Nothing is born without blood”- Herwig Hensen, Belgian writer and poet)

Red Blood Cell Transfusions

History

Over time it has been recognized that blood loss was frequently associated with weakness and death, and as a result the old history of blood transfusion is based on the traditional idea of blood being the ‘living-force’ of the body.[1] Drinking blood was thought of as a magical elixir by the ancient Romans and Greeks. Similarly, in the Middle Ages, it was promoted drinking young blood as a means of elderly to regain their youthful vigor.[2]

After discovery of the blood circulation by William Harvey in 1628, the interest in blood transfusions increased. In 1667, the first blood transfusion in humans was reported. Jean-Baptiste Denis, court physician of King Louis XIV of France, transfused 25 centiliter blood of a lamb, a so-called ‘innocent’ animal, into a young boy as so called treatment for insanity. Unfortunately, the result was not that innocent for the young boy because death awaited him. Due to multiple fatal transfusion attempts, the Pope announced a ban on blood transfusion procedures in 1679.[3]

In 1818, the first successful human to human blood transfusion was given by the British gynecologist James Blundell in order to treat post-partum hemorrhage. He wrote: ‘The patient expressed herself very strongly on the benefits resulting from the injection of the blood; her observations are equivalent to this –that she felt as if life were infused into her body.’ Blundell further formulated two basic rules for blood transfusions: 1) humans can only be transfused with human blood; and 2) transfusions are only allowed in case of life-threatening blood loss. Despite these precautions half of the transfused patients died.[3-5]

A major break-through in tackling this high death rate in blood transfusion medicine was the discovery of the AB0 blood groups by the Austrian physician Karl Landsteiner in 1900, explaining earlier failures due to AB0 mismatched transfusions.[6] Together with the discovery of the agglutination technique for compatibility testing in 1907 and the discovery of the Rhesus antigen in 1940, it became possible to safely transfuse blood from one human to another. The development of anticoagulant-preservative solutions made it possible to preserve blood in depots, which was particularly useful during war-time. This was first used in World War I, where transfusions were reported to save lives.[7]

Currently, clinicians have a diversity of blood products at their disposal, which provides the possibility of 'precision' blood transfusion for recipients. While blood transfusions have been shown to be life saving for many patients regarding bleeding after trauma, complicated surgery, and obstetric complications, the precise when and how to transfuse, still requests more elucidation.

This thesis will focus on the management of red blood cell transfusions in one particular group of patients, namely those with hematological malignancies. As by disease and/or treatment hematopoiesis is compromised in these patients, this may lead to severe anemia, and consequently to a high need for red blood cell transfusions.[8,9]

Red blood cell transfusion therapy

Clinical practice

Red blood cell (RBC) transfusion is one of the few treatments that adequately restores tissue oxygenation when oxygen demand exceeds supply in case of anemia. Nevertheless, tissue oxygenation as an indicator for RBC transfusion remains controversial as it is not easily assessed by conventional clinical tests. Few clinical signs like hypotension, oliguria, and impaired consciousness, reliably predict early hypoxemia, and in general, clinicians will not wait for these clinical symptoms to occur before starting RBC transfusion treatment.[3] New tests that are able to indicate failing tissue oxygenation during anemia in clinical patients is of eminent importance to better guide RBC transfusion therapy in general.

Hemoglobin triggers guiding red blood cell transfusion therapy

Currently, clinicians mainly seem to rely on their clinical experience in the decision at what hemoglobin trigger to initiate transfusion.[3] The additional absence of high-grade evidence-based guidelines, therefore effects in a wide variation in RBC transfusion practice throughout the world.[10-13]

Interestingly, often a less restrictive RBC transfusion policy is applied in patients, which likely originates from the still widespread assumption that a high hemoglobin level is beneficial for patients in terms of survival and quality of life. The '10/30-rule' introduced in 1942 by an anesthesiologist, where a RBC transfusion was suggested for surgical patients when their hemoglobin levels dropped below 10 g/dL (~6.3 mmol/L) or their hematocrit below 30%, has contributed to this assumption.[14] This recommendation was later applied to all transfusion settings and resulted in the term 'transfusion trigger': i.e. the critical hemoglobin value in which a RBC transfusion is indicated.[3]

In the late nineties of the last century, after publication of a landmark study on hemoglobin triggers for RBC transfusion in intensive care patients, the interest in RBC transfusion triggers increased rapidly.[15] This study of Hébert et al. compared a restrictive RBC transfusion trigger (7 g/dL ~ 4.4 mmol/L) with a liberal RBC transfusion trigger (10 g/dL ~ 6.3 mmol/L). Although the study showed that the 30-day mortality was similar in both groups, 30-day mortality rates were significantly lower in the restrictive RBC transfusion group as compared with the liberal group in patients who were less acutely ill (8.7 vs. 16.1%, p=0.03) and in patients who were less than 55 years of age (5.7 vs 13.0%, p=0.02).

Afterwards, many RBC transfusion trigger trials in various patient groups have been performed, for example in critically ill children (7 versus 9.5 g/dL), in cardiac surgery (7.5 versus 8.5-9.5 g/dL), and hip surgery patients (8 versus 10 g/dL), and also in the setting of gastro-intestinal bleeding (7 versus 9 g/dL), septic shock (7 versus 9 g/dL), and traumatic brain injury (7 versus 10 g/dL).[16-23] All of these studies reported no disadvantages of a restrictive compared to liberal transfusion strategy, which has led to guidelines recommending restrictive strategies for non-hematological patient groups.[24,25] However, one must note that due to non-blinded study designs, strong selection at inclusion, and non-adherence to protocols, the outcome of these trials have to be interpreted with caution.[26] To date, solid data on restrictive RBC transfusion strategies in hematological patients is still lacking, although hematological patients are one of the most intensively transfused patient groups.[27-41]

Advantages of restrictive RBC transfusion policies may be prevention of transfusion-associated side-effects and reduced costs. However, on an individual level, in some patients a restrictive RBC transfusion strategy may not be the best choice and probably more precision medicine is needed.[24]

In general, RBC transfusions can be avoided in most patients with a hemoglobin level above 7 to 8 g/dL (4.4 to 5.0 mmol/L), however, there is insufficient evidence yet of the safety of restricted transfusion policies in certain clinical subgroups, including acute coronary syndrome, myocardial infarction, neurological injury/traumatic brain injury, acute neurological disorders, stroke, thrombocytopenia, cancer, hematological malignancies, and bone marrow failure.[24] For the latter subgroups, studies are ongoing.[25,42]

Side-effect of transfusions

While quality of life may be improved by transfusion of RBCs,[43] additional beneficial effects are unclear. Evidence and quantification of such a benefit is of special

importance, since transfusions may also include negative effects on outcome through risk of transmission of infectious diseases, allo-immunization, hemolytic reactions, and other transfusion reactions. Moreover, transfusions are associated with immuno-modulation and secondary iron overload.[3,44] Worldwide scandals with transmission of human immunodeficiency virus (hiv) and hepatitis C via transfused blood products in the late 80's and 90's, gained attention on the negative effects of transfusion. Figure 1 depicts the incidence of transfusion-associated side-effects. The incidence of a chronic, but serious, complication of RBC transfusions, like secondary iron overload, still has to be established. Estimations in regularly transfused patient groups vary from 25 to 100%.[45-48] With this, iron overload is probably much more common compared to most other transfusion-associated side-effects.

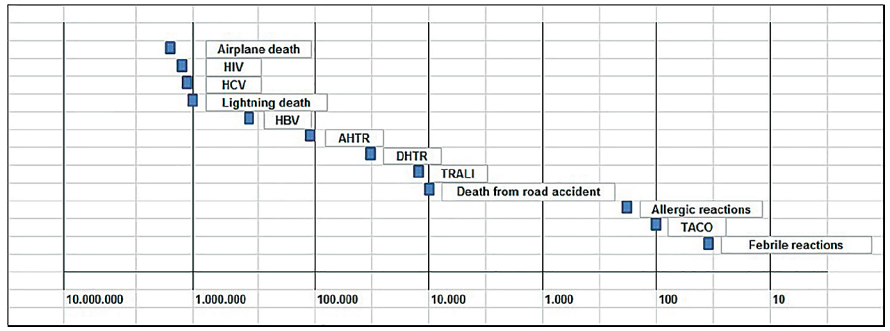

Figure 1 Adverse effects of RBC transfusion as compared with other unrelated risks.

HIV: Human Immunodeficiency Virus; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AHTR: Acute Hemolytic Transfusion Reaction; DHTR: Delayed Hemolytic Transfusion Reaction; TRALI: Transfusion-Related Acute Lung Injury; TACO: Transfusion Associated Cardiac Overload. Adapted from Carson et al.[50]

Red blood cell transfusion therapy in hematology patients

To date, RBC transfusion therapy still is the cornerstone of supportive care in hematology patients. Almost 20% of all RBC transfusions in Europe are given to support treatment and/or disease-related anemia in patients with hematological diseases.[8,9]

Despite the substantial usage of RBC transfusion in hematological patients, there is a paucity of good quality data on RBC transfusion strategies in this patient group. This may result in a large variation of RBC transfusion strategies throughout countries, centers, and even individual physicians.

Fortunately, attention for this knowledge gap is rapidly increasing over the past few years. Patient blood management programs currently focus on various medical patient groups, including hemato-oncological patients.[25,49]

Iron and transfusion

Physiology

Iron is an essential element for the human body, mainly because of its ability to accept and donate electrons by switching between ferrous (Fe2+) and ferric (Fe3+) ions.[44] The energy production by this redox reaction plays a major role in many metabolic pathways as oxygen transport in the hemoglobin molecule, DNA synthesis, and the cytochrome P-450 enzymes involved in degradation of potential toxic substances.[44]

The human body contains 2.5 to 4.0 grams of iron.[44] Since, our body has no active iron secretion system and an excess of iron can be extremely toxic, the quantity of iron in the body is tightly regulated, primarily by the rate of iron absorption from the gut.[51] About 1-2 mg of iron is lost daily through shedding of duodenal enterocytes and skin cells.[44,52] Additionally, 1 mg of iron is lost daily from menstruation in women.[44] Only 1-2 mg iron per day needs to be absorbed in order to maintain iron homeostasis.[52]

In the circulation, iron is bound to transferrin, the major iron transport protein. Iron then enters the intracellular pool, mainly in red blood cells as a component of hemoglobin and as ferritin in hepatocytes and macrophages, as part of the reticuloendothelial system.[53] Iron recycled from damaged or senescent red blood cells, remains stored in macrophages or is released back into the circulation bound to transferrin for production of new red blood cells in the bone marrow, or for storage in hepatocytes.[54] Figure 2 demonstrates an overview of the iron metabolism.

Hepcidin, a small peptide hormone, produced by the liver, is discovered as the key regulator of iron metabolism.[55] Hepcidin blocks the iron absorption in the duodenal enterocytes and release of iron stored in macrophages by degradation and internalization of the cellular iron transporter ferroportin.[52,54,55] The hepcidin-ferroportin interaction is central to iron metabolism in humans, as regulatory molecules mainly act by modulating this interaction. Hepcidin production is regulated by iron stores through the bone morphogenetic protein (BMP) signalling pathway.[56] It was only recently that a new hormone involved in hepcidin regulation

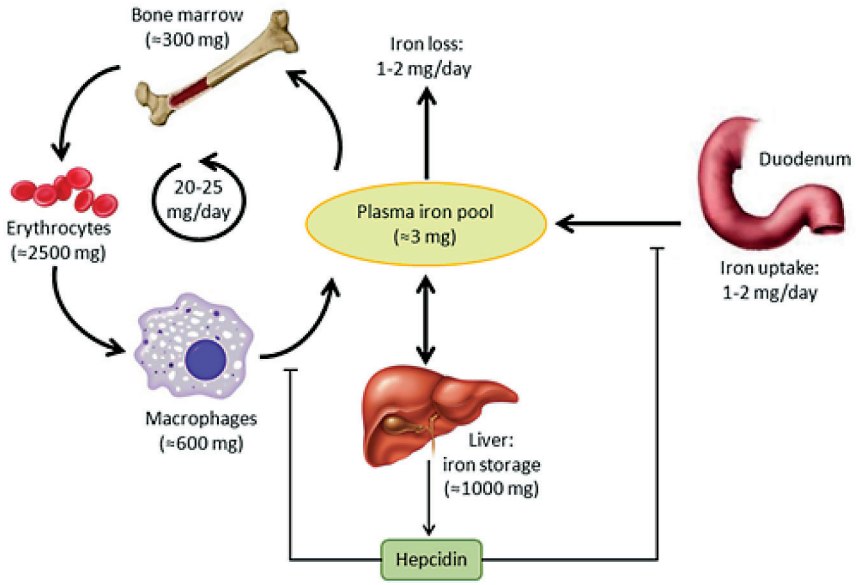

Figure 2 Short overview of iron metabolism, previously published in TvB 2015, Hoeks et al, published with the courtesy of N. Sonneveld.

was identified: erythroferrone.[57] Erythroferrone is produced by erythroblasts in response to erythropoietin and mediates hepcidin suppression during stress erythropoiesis.[57] Hepcidin production is increased in case of iron overload and decreased in iron deficiency, anemia, and hypoxia.[55]

Pathophysiology

Iron overload occurs when the binding capacity of transferrin for iron is exceeded, resulting in non-transferrin bound iron (NTBI). NTBI and its redox active component labile plasma iron (LPI) are small molecules which are readily absorbed by body tissues where it leads to increased levels of storage iron and labile cellular iron. The liver, endocrine system, and myocardium are the most susceptible to toxic iron accumulation.[58] When the amount of the labile cellular iron exceeds the capacity of the cell to produce new ferritin molecules, reactive oxygen species (ROS) are being formed.[58,59]

ROS are associated with lipid peroxidation and organelle damage, resulting in cell death and fibrosis mediated by transforming growth factor-1.[60] ROS are also known to damage DNA, which could lead to genomic instability, mutagenesis, and cell death or neoplasia.[58,61] Iron overload and subsequent oxidative stress may contribute to genomic instability of the pre-leukemic clone and may result in clonal evolution of a myelodysplastic syndrome (MDS) towards acute myeloid leukemia.[62]

Iron overload can be either acute or chronic. Acute iron overload results from intake of iron salts or from an overdose of iron-containing supplements. Chronic iron overload is more frequent and could result from long-term intake of iron-containing supplements, chronic liver disease, several hereditary disorders of iron metabolism like hereditary hemochromatosis, ineffective erythropoiesis as for example seen in thalassemia, and from frequently administered RBC transfusions.[58] One RBC unit approximately contains 200 mg of iron, which is about 100 times the quantity of daily absorbed iron.[44]

Secondary iron overload due to multiple RBC transfusions is a potential threat to frequently transfused patients as it may cause significant organ damage e.g. to liver, heart, and endocrine organs.[63,64] Adverse effects of iron overload, specific for patients with hematological malignancies, include: inferior survival after hematopoietic stem cell transplantation (HSCT), increased risk of bacterial and fungal infections, and impaired hematopoiesis.[65-70] Moreover, patients with hematological malignancies may be more prone to develop iron toxicity-related cardiac disease than other patients. Cardiac remodelling, may be additionally induced by long-standing anemia and cardiomyopathy by various chemotherapeutical agents.[71] Cardiac failure is indeed the most common non-leukemic cause of death (51%) among MDS patients, and fatal cardiac failure is significantly more frequent in transfusion-dependent patients.[72]

Secondary iron overload already occurs in patients who received about 20 RBC transfusions, while after 30 RBC transfusions, the positive predictive value for significant hepatic iron overload reaches 96%.[69,73]

Despite increasing evidence for iron toxicity, monitoring and management of secondary iron overload in patients receiving multiple RBC transfusion, such as patients with hematological malignancies, is still not common practice.[74] This may be due to the fact that only in hemoglobinopathies treatment of transfusion-associated iron overload has shown to be beneficial in limiting organ damage and even mortality. Clinicians on the other hand, may perceive secondary iron overload in hemato-oncological patients of minor importance as a contributable

factor for overall survival and may experience treatment by iron chelation therapy (ICT) too much of a burden. Finally, imprecision of serum markers for monitoring iron overload, the invasiveness (biopsy) or unavailability (MRI) of accurate diagnostics might also play a role.[54] All of these factors contribute to a lack of studies on secondary iron overload and low enrollment of patients in studies investigating this side-effect of RBC transfusion. Furthermore, uniform guidelines on monitoring and treatment of iron overload are absent.[54,73]

Diagnosis of secondary iron overload

Detection of secondary iron overload is challenging since early symptoms, like fatigue and abdominal discomfort, are nonspecific. This may delay its diagnosis until organ damage and dysfunction are clinically apparent.[44,58]

The most frequently used parameter to detect iron overload in clinical practice is serum ferritin. Generally, serum ferritin is indicative for iron stored in macrophages, which is proportional to the total body iron.[58] However, it may lack clinical significance as iron toxicity usually occurs at the time that transferrin capacity is exceeded and NTBI and consequently LPI are produced. This may occur after chronic RBC transfusion therapy, but recently it has been recognized that in lower-risk MDS patients LPI production is already frequently seen early after patients becoming transfusion-dependent and before serum ferritin levels are elevated.[75] LPI is suggested to be a predictive factor for inferior survival in lower-risk MDS patients.[75]

A drawback of serum ferritin is its lack of specificity for detecting iron overload because of its property of being an acute phase protein. Specificity can be improved by serial measurements and concurrent measurement of C-reactive protein.

Another parameter for detecting secondary iron overload is transferrin saturation. It is especially useful to detect the location of iron overload. Elevated values of transferrin saturation indicate parenchymal iron overload, whereas a transferrin saturation values within the reference range could indicate reticuloendothelial iron overload.[54] This distinction between location of iron overload is not merely academic, but has considerable clinical consequences. Reticuloendothelial iron loading is relatively safe as the iron is contained inside, for example, macrophages. Iron loading in parenchymal cells is, however, extremely toxic resulting in organ damage.[44,58]

Assessment of the liver iron concentration by liver biopsy is still the golden standard in the detection of iron overload.[76] However, tissue biopsies of liver or even myocardium are not likely to be performed in hematological patients due to its risk of complications.[54] Currently, the use of the T2* magnetic resonance imaging (MRI) is increasing for non-invasive assessment of liver and myocardial iron concentration. T2* MRI values of less than 20 milliseconds are associated with high liver iron content and a significant decrease in left ventricular function in thalassemia patients.[77] Additionally, a correlation between the total RBC transfusion burden and increased liver T2* values was demonstrated in patients with MDS.[78]

Treatment of secondary iron overload

Phlebotomy is a safe and cost-effective treatment for secondary iron overload in many conditions like, for example, hereditary hemochromatosis.[79] Due to anemia, the use of phlebotomies is often of limited value in patients with hematological malignancies. Therefore, iron chelation therapy can be considered in case of secondary iron overload in anemic patients. Three iron chelating agents are available: desferoxamine (Desferal®), deferiprone (Ferriprox®), and deferasirox (Exjade®) of which the first is administered subcutaneously or intravenously and the latter two are administered orally.[80] Iron chelation therapy is known to improve outcome in multi-transfused hemoglobinopathy patients,[81-83] but whether it actually decreases morbidity and mortality in patients hematological malignancies needs to be further investigated as most studies were executed in small or highly selected patient groups or suffered from methodological problems.[84-87]

Thesis outline

This thesis focuses on the variability of RBC transfusion management and the screening and management of transfusion-associated iron overload in patients with hematological malignancies.

Since evidence-based guidelines for RBC transfusion support in patients with hematological malignancies are currently lacking, we expect a large variation in clinical practice. Therefore, in **chapter 2**, the Dutch RBC transfusion practice among hematologists is evaluated by means of a survey. Assessing the actual RBC transfusion practice and management of secondary iron overload of patients with hematological malignancies, could be the starting point for further research and eventually improvement of current RBC transfusion guidelines for these patients.

Likewise, since abundant literature on the use of restrictive or liberal RBC transfusion strategies in patients with hematological malignancies is lacking, **chapter 3** provides a summary and meta-analysis of all available literature on this important topic.

Chapter 4 quantifies the relation between the cumulative administered RBC transfusions with bone marrow iron scores as an indicator of secondary overload and whether bone marrow iron scores obtained from routinely performed bone marrow aspirate samples could be clinically applicable to assess bone marrow iron overload.
The knowledge of temporal changes in iron parameters in transfused lower-risk myelodysplastic syndrome patients is scarce. Similarly, the impact of toxic iron species as NTBI and LPI on survival in lower-risk MDS patients remains unclear. **Chapter 5** describes the results of a sub study within a large European dataset: the EUMDS registry, in order to gain -by means of repeated monitoring of iron parameters- more insight in the pathophysiology of secondary iron overload and the impact of toxic iron species on survival in lower-risk MDS patients.

As mentioned in the introduction, secondary iron overload results in morbidity and mortality in intensively transfused patients. Iron chelation therapy evidently improves outcomes in hemoglobinopathy patients. However, whether iron depletion by the use of iron chelation therapy also improves outcome in patients with hematological malignancies has still to be elucidated. In **chapter 6** the effect of iron chelation therapy on clinical outcome in patients with lower-risk myelodyplastic syndromes is evaluated with two different statistical models in the EUMDS registry.

Lastly, **chapter 7** discusses the important topics of this thesis and provides perspectives for future research within the field.

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