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

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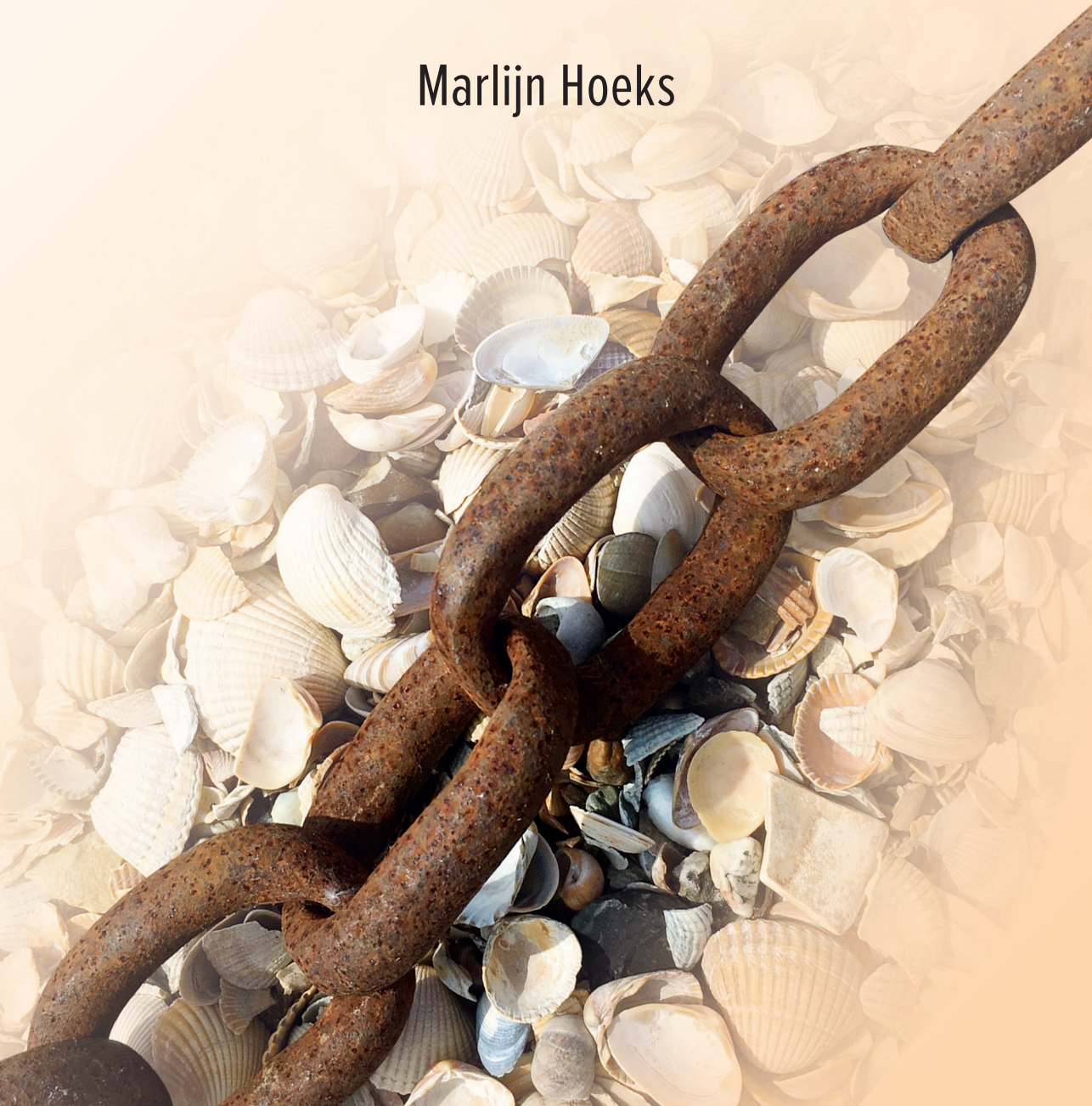
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RED BLOOD CELL TRANSFUSIONS IN HEMATO-ONCOLOGICAL PATIENTS

DON'T IRON OUT THE CONSEQUENCES

Marlijn Hoeks



RED BLOOD CELL TRANSFUSIONS IN HEMATO-ONCOLOGICAL PATIENTS

DON'T IRON OUT THE CONSEQUENCES

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1

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 ABO blood groups by the Austrian physician Karl Landsteiner in 1900, explaining earlier failures due to ABO 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.

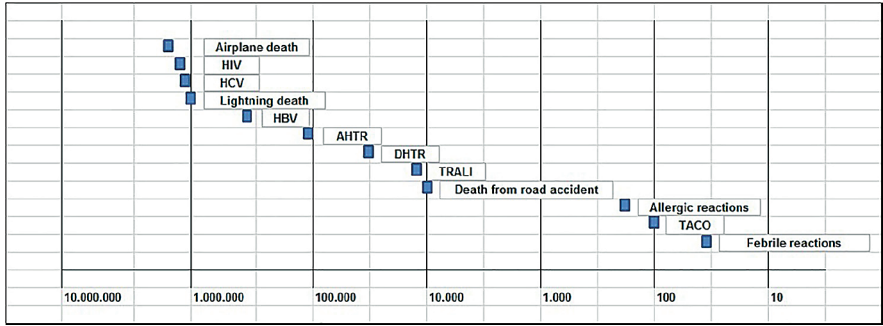


Figure 1 Adverse effects of RBC transfusion as compared with other unrelated risks.

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

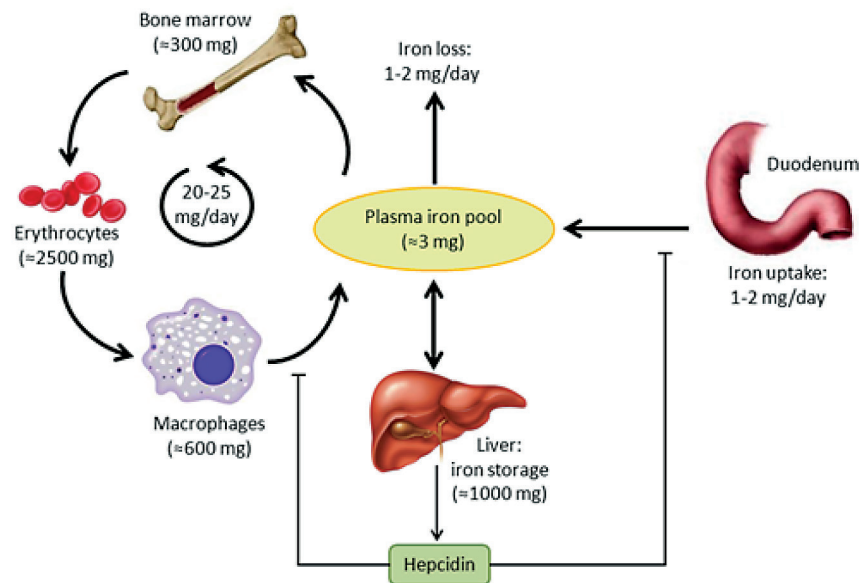


Figure 2 Short overview of iron metabolism, previously published in TvB 2015, Hoeks et al, published with the courtesy of N. Sonneveld.

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 chemotherapeutic 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 myelodysplastic 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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2

RED BLOOD CELL TRANSFUSION SUPPORT AND MANAGEMENT OF SECONDARY IRON OVERLOAD IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES IN THE NETHERLANDS: A SURVEY

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Abstract

Background and objectives: Evidence-based guidelines on optimal triggers for red blood cell (RBC) transfusion in patients with hematological malignancies exist, but the evidence is weak. Secondary iron overload is an often overlooked chronic complication of RBC transfusions and also here guidelines are either lacking or lack international consensus. Our aim was to evaluate the triggers for RBC transfusion support and management of secondary iron overload among hematologists in the Netherlands.

Materials and methods: For this cross-sectional study, all hematologists and hematologists in training in the Netherlands were sent a web-based, 25-question survey including three clinical scenarios. The survey distribution took place between November 19, 2015 and January 26, 2016.

Results: Seventy-seven responses were received (24%), well distributed among community and university hospitals. A wide variation in hemoglobin triggers existed: 5.6-9.5 g/dL (median: 8.0 g/dL). Personalization of this trigger was mostly based on (estimated) cardiopulmonary compensation capacity of patients. About 65% of respondents reported two RBC units per transfusion episode (range 1-3). For monitoring secondary iron overload, serum ferritin was most frequently measured (97%), while a value of 1000-1500 µg/L was the most common cut-off to initiate treatment (39%). For 81% of respondents, phlebotomies were the first choice of treatment, although often the hemoglobin level was considered a limiting factor.

Conclusion: Our results confirm large reported variation in daily practice among hematologists in the Netherlands regarding RBC transfusion support and management of secondary iron overload. Future studies providing better evidence are needed to improve guidelines specific for patients with hematological malignancies.

Introduction

Almost twenty percent of all red blood cell (RBC) transfusions in Europe are given to patients with hematological malignancies to compensate their disease and treatment-related anemia [1,2]. The beneficial effect of these RBC transfusions on e.g. quality of life, bleeding and other clinical outcomes remains however, difficult to quantify [3-5]. While studies on restrictive RBC transfusion strategies show no disadvantages in other patient groups, limited data is available on such strategies in patients with hematological malignancies [5-7]. Hemoglobin triggers and number of RBC units given per transfusion episode may therefore vary in daily practice.

Furthermore, apart from acute transfusion reactions, secondary (transfusion-related) iron overload, a more chronic complication, occurs in many patients with hematological malignancies who undergo hematopoietic stem cell transplantation or are treated for myelodysplastic syndrome (MDS) [8,9]. Secondary iron overload often 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% [10,11].

Although the negative impact of iron overload on mortality and morbidity is well established in various patient groups, for instance thalassemia patients [12-14], this remains to be elucidated in patients with hematological malignancies. Recent data suggest inferior overall survival for transfusion-dependent MDS patients as a consequence of secondary iron overload [15,16]. Moreover, patients with hematological malignancies may be more prone to develop iron toxicity-related cardiac disease. Cardiac remodeling, namely, is additionally induced by long-standing anemia and cardiomyopathy by various chemotherapeutical agents [17]. Indeed, cardiac failure is the most common non-leukemic cause of death (51%) among patients with MDS, and fatal cardiac failure is significantly more frequent in transfusion-dependent MDS patients [18].

Furthermore, data on the effect of iron chelation therapy in patients with hematological malignancies are scarce as most studies were executed in small or highly selected patient groups or suffer from serious methodological problems like confounding by indication [16, 19-21].

In summary, for optimal RBC transfusion strategies for patients with hematological malignancies as well as monitoring and treatment of secondary iron overload, high-grade evidence is unavailable. As a result, we assumed a wide variation in the daily practice of RBC support and management of iron overload in the Netherlands.

The aim of the current study was to evaluate RBC transfusion support and management of secondary iron overload in patients with hematological malignancies among hematologists and hematology trainees in the Netherlands by means of an online survey.

Materials and methods

In this cross-sectional study, all hematologists and hematologist trainees in the Netherlands were asked to complete a structured, 25-question online survey. The questions related to clinical factors influencing initiation of RBC transfusion, numbers of RBC units given per transfusion episode, and clinical factors influencing detection and management of secondary iron overload regarding adult patients with hematological malignancies. Additionally, three clinical scenarios were presented in order to test consistency of replies with the previous responses to similar questions. For questions with a quantitative nature, a 5-point Likert scale was used (never, sometimes, regularly, often, always) [22]. The complete survey is provided in the supplementary material. Incomplete surveys were included in the analysis.

This web-based survey was approved by the members of the working party “non-oncological hematological diseases” of the Dutch Hematology Association and was distributed by email. The survey distribution included one reminder and took place between November 19, 2015 and January 26, 2016.

In order to guard anonymity, participants were not asked for identifying information such as age and gender. No incentives were provided.

Descriptive statistical methods (frequencies and histograms) were used for the analyses using IBM SPSS statistics, version 23 (Armonk, NY: IBM Corp.).

Results

Study cohort

In total, of 325 sent surveys, 77 responses were received (24%). The respondents represented all eight university hospitals and 29 community hospitals (39%) across the Netherlands. Ninety-nine percent of the respondents attended relevant hematology conferences, either European, American or Dutch in the preceding three years; whereas only 20% attended specific conferences on transfusion medicine in the preceding three years. According to respondents, 14 out of 37 institutions

(38%) employed an in-house consultant in transfusion medicine and 18% of the respondents reported being a transfusion medicine consultant.

RBC transfusion triggers

Sixty-six percent of the responders, representing 27 centers, reported existing institutional RBC transfusion guidelines specific for patients with hematological malignancies.

Table 1 shows the reported hemoglobin triggers for hemodynamically stable, hospitalized patients and outpatients without severe co-morbidities. The triggers ranged from 5.6 to 9.5 g/dL (median 8.0 g/dL). For hemodynamically stable hospitalized patients, the most frequently reported hemoglobin trigger was 8.0 g/dL (42%) and for outpatients 7.2 g/dL (34%).

Table 1			
Hemoglobin trigger g/dL	(mmol/L)	Hospitalized patients number (%)	Outpatients number (%)
< 5.6	(<3.5)	1 (1)	0 (0)
6.4	(4.0)	1 (1)	6 (8)
7.2	(4.5)	17 (22)	26 (34)
8.0	(5.0)	32 (42)	24 (31)
8.8	(5.5)	16 (21)	20 (26)
9.6	(6.0)	10 (13)	1 (1)
Total		77 (100)	77 (100)
Reported hemoglobin triggers for hemodynamically stable hospitalized patients and outpatients without severe comorbidities			

The number of RBC units given per transfusion episode for hospitalized patients and outpatients is depicted in figure 1. Most commonly, two RBC units were given per transfusion episode (range 1-3). Additionally, 16% of all respondents considered weight and/or total blood volume when ordering a specific number of RBC units.

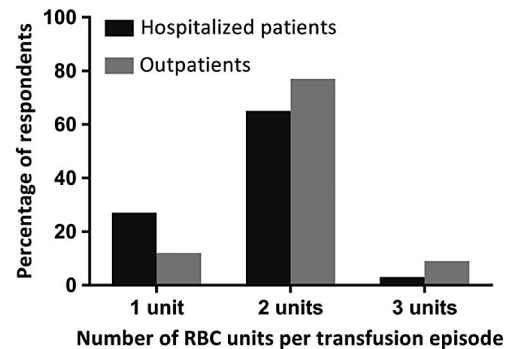


Figure 1 Number of RBC units per transfusion episode.

RBC: Red Blood Cell.

Figure 2 shows a subset of clinical factors possibly influencing the hemoglobin trigger for RBC transfusion. A higher hemoglobin level was maintained after recent cardiac ischemia or cardiac failure (New York Heart Association (NYHA) grade II-IV). In this respect, also higher age, dyspnea, signs of hypoxia, bleeding with or without accompanied thrombocytopenia, and quality of life were often considered by the respondents.

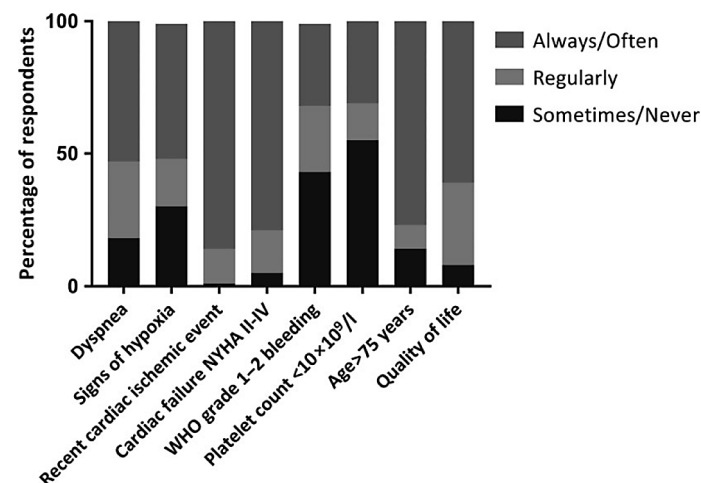


Figure 2 Clinical factors influencing RBC transfusion initiation.

NYHA: New York Heart Association; WHO: World Health Organization.

Particularly in the outpatient setting, various logistic reasons caused non-adherence to a common trigger. Difficulties to organize an outpatient transfusion appointment, the travel time for the patient to the hospital and the time between appointments, played a role in 64%, 71%, and 75% of the respondents, respectively. RBC transfusions in the neutropenic phase during intensive chemotherapy or hematopoietic stem cell transplantation will be guided and dependent on measurements of the complete blood count. The frequency of measurement of these blood counts, varied considerably from once or twice a week in 7% of respondents, three to four times a week in 58%, to five to seven times a week in 35%. Besides hemoglobin level, 17% of the respondents performed additional tests to determine whether RBC transfusion was required. Of these respondents, 20% considered the patient's vital signs, 20% serum ferritin levels, whereas 60% considered the hemoglobin increment after previous RBC transfusions to guide further transfusion.

Monitoring of secondary iron overload

Twenty-six percent of the respondents reported to comply with a local guideline regarding detection and treatment of iron overload and 61% reported to comply to the national guideline [23]. Three respondents reported to comply with the national as well as a local guideline.

Factors that led respondents to initiate monitoring of secondary iron overload were: the total RBC transfusion burden (77%, [20-29 RBC units, 58%]), a transfusion intensity of ≥ 2 RBC units per month (52%), and a hematopoietic stem cell transplantation in the medical history (46%).

Figure 3 shows the iron parameters used by the respondents to monitor iron overload. Serum ferritin (97%) was the most frequently measured iron parameter, either alone (55%) or in combination with C-reactive protein (43%). Additional diagnostic tests that were reported included: transferrin saturation, serum iron, total iron-binding capacity, and bone marrow iron staining. Forty-two percent of all respondents never performed the currently available, most specific tests for iron overload: a biopsy of liver or myocardial tissue or a T2* MRI of heart and/or liver, while 58% considered one of these additional tests.

For the 58% of respondents who performed a MRI or biopsy, the following indications were mentioned (multiple answers were possible): an elevated serum ferritin level (44%, with most reported cut-off values of 1000-1500 $\mu\text{g/L}$ [38%] and 1500-2000 $\mu\text{g/L}$ [24%] respectively), increased liver enzymes (46%), decreased liver function (e.g. low serum albumin levels, disturbed production of coagulation

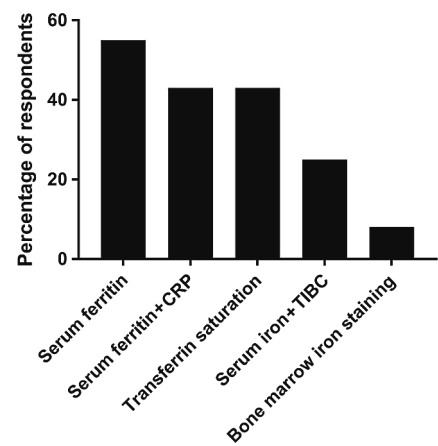


Figure 3 Iron parameters used in the detection of iron overload.

CRP: C-reactive protein; TIBC: total iron binding capacity.

factors [26%]), occurrence of cardiac failure (27%), or endocrinopathy (14%). Unexplained fatigue, arthromyalgia, bronze skin, transfusion intensity, total RBC transfusion burden, and cardiac arrhythmia in the absence of inflammation were less common reasons for performing additional diagnostic tests for secondary iron overload.

Management of iron overload

Figure 4 shows determining factors for treatment of secondary iron overload. For most of the respondents, the serum ferritin level was the reason to start treatment (86%). A serum ferritin level of 1000-1500 µg/L (39%), 1500-2000 µg/L (21%), and 2000-2500 µg/L (24%) were the most commonly reported cut-off values. For 75% of respondents, a total RBC transfusion burden of 20-29 units was reason to initiate iron overload treatment.

For 81% of all respondents, phlebotomies were the first choice for treating iron overload when hemoglobin levels were sufficiently high. Conversely, iron chelation therapy was the first choice in 20% of respondents. In all respondents (multiple answers possible), deferasirox was the most commonly prescribed iron chelation agent (91%), followed by deferiprone (9%), and deferoxamine (5%). Nine percent of all respondents never prescribed iron chelation therapy. The hemoglobin level was the most important clinical factor influencing the choice of treatment (phlebotomy versus iron chelation; 87%). Factors influencing this treatment choice are summarized in figure 5.

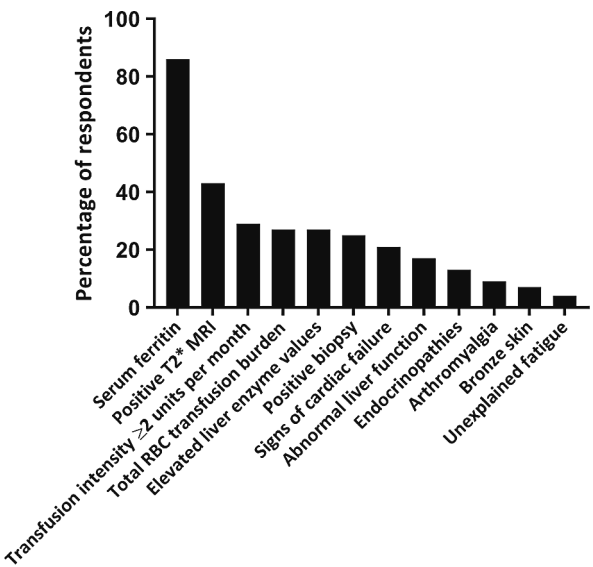


Figure 4 Clinical and laboratory factors for initiating management of secondary iron overload.

MRI: Magnetic Resonance Imaging; RBC: Red Blood Cell.

Reasons to refrain from starting iron chelation therapy in spite of a high serum ferritin level were (multiple answers possible): comorbidity limiting prognosis (75%), limited life expectancy of less than one year (70%) or less than three years (41%), age ≥85 years (49%), renal dysfunction (50%), possible side effects (26%), drug-drug interactions (25%), reduced quality of life (20%), expected lack of treatment compliance (16%), and costs (3%).

The most frequently reported reasons for cessation of iron chelation therapy were: a dismal prognosis (46%), side effects/intolerance (38%), and comorbidity (13%), but also reaching a certain low serum ferritin level (71%, [500 µg/L 93%]) and transfusion-independency (26%) were reasons to stop. On the contrary, twenty-five percent of the respondents state that –once started– they usually do not stop iron chelation therapy.

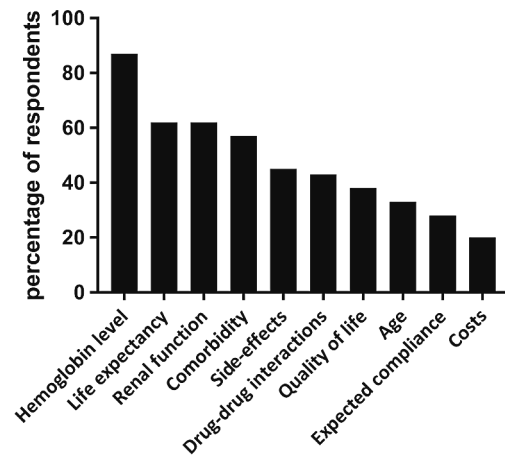


Figure 5 Clinical factors influencing treatment choice in management of iron overload

Discussion

This survey among hematologists and hematologist trainees in the Netherlands shows large reported variation in daily practice, not only regarding RBC transfusion support but also for the monitoring and management of secondary (transfusion-associated) iron overload in patients with hematological malignancies.

First, hemoglobin triggers for RBC transfusion differed among the respondents. For hemodynamically stable, hospitalized patients, a hemoglobin level of 8.0 g/dL (5 mmol/L) was the most commonly reported trigger. For outpatients this was 7.2 g/dL (4.5 mmol/L). However, the range varied considerably from 5.6 g/dL up to 9.6 g/dL. Patient-specific adaptation of these triggers by the respondents was mostly based on the (estimated) cardiopulmonary compensation capacity and age. These triggers may be derived from the RBC transfusion triggers for normovolemic anemia patients with an acute bleeding according to the Dutch transfusion guideline, the so-called '4-5-6 rule' in which a RBC transfusion is initiated at a hemoglobin trigger of 6.4 g/dL (4 mmol/L) in young and otherwise healthy patients. This trigger is adjusted to 8.0 g/dL or 9.6 g/dL (5 mmol/L or 6 mmol/L) dependent on comorbidities and other clinical factors [7]. However, the observed variation of hemoglobin triggers, reported in our survey, is much wider.

Second, when initiating a RBC transfusion, the number of RBC units given per transfusion episode also differed widely among the respondents. Interestingly, in a more concrete clinical setting simulated by *clinical scenario 1 and 2* (supplementary textbox 1 and 2), respondents initiate RBC transfusion at a higher hemoglobin trigger than the responses in the survey suggest. Moreover, respondents tended to transfuse more RBC units per transfusion in the scenario setting than was reported in the survey.

These differences in RBC transfusion practice, either hemoglobin triggers or number of RBC units per transfusion episode, likely originate from a lack of high-grade evidence-based guidelines for this specific group of patients. Interestingly, often a less restrictive transfusion policy is applied in actual patients. This is probably due to the widespread believe that a high hemoglobin level is beneficial for patients according to the '10/30-rule' introduced in 1942, in which a RBC transfusion was suggested in surgical patients when the hemoglobin levels drops below 10 g/dL or the hematocrit below 30% [24]. The variation in hemoglobin triggers in our study are also in line with a recently published survey regarding RBC transfusion practice in leukemia patients in the United States [25]. Although for the present study, the outpatient hemoglobin levels were slightly lower when compared with the inpatient hemoglobin triggers, which is in contrast to the US study. The travel distance to hospitals for outpatients in the Netherlands is probably much shorter, which facilitates a more restrictive transfusion approach in the outpatient setting. Nevertheless, both our study as well as the US study suggest at least the risk for over-transfusion in these patients, while a recent meta-analysis performed by our group suggests no differences in mortality rates and safety outcomes when applying more restrictive RBC transfusion strategies compared with more liberal strategies in patients with hematological malignancies [26].

Currently, a number of studies on RBC transfusion triggers and iron overload monitoring and treatment in patients with hematological malignancies are being carried out. For example, the preliminary results from the randomized, controlled TRIST study, comparing a restrictive (7 g/dL) and a liberal (9 g/dL) hemoglobin trigger, suggest no differences in quality of life and other clinical outcomes in patients with hematological malignancies undergoing hematopoietic stem cell transplantation [27]. Many more studies on RBC transfusion strategies in patients with hematological malignancies (REDDS ISRCTN26088319; EnhanceRBC NCT02099669; 1versus2CGR NCT02461264; REAL study ISRCTN 96390716) and studies on efficacy of iron chelation therapy (TELESTO NCT00940602; EUMDS registry NCT00600860) are ongoing. With the results of these studies, we will gain better insight into transfusion triggers and iron overload monitoring and

treatment in one of the most frequently transfused patient groups, namely patients with hematological malignancies.

Besides, reduced RBC use may lead to a decrease in transfusion-associated complications like secondary iron overload. One unit of RBC contains 200-250 mg of iron and thus over 100 times the physiological uptake of 1-2 mg per day by the gut. Frequent RBC transfusions in these already vulnerable patients may therefore lead to iron-mediated toxic effects.

In our study, the most important reasons for the respondents to initiate monitoring of secondary iron overload were: a total RBC transfusion burden of 20-29 units and a transfusion intensity of ≥ 2 RBC units per month. The total RBC transfusion burden of 20-29 units is similar to the Dutch guideline for patients with myelodysplastic syndromes [23], clearly basing iron overload monitoring on a high net iron dose received as RBC transfusions. Additionally, although not an ideal marker for monitoring iron overload, serum ferritin is the most frequently used biomarker. The combination of serum ferritin levels with a normal level of C-reactive protein, will improve the sensitivity to demonstrate secondary iron overload, since it rules out a high serum ferritin level due to an acute phase reaction. Transferrin saturation, another biomarker for iron body stores, could be most useful for defining the site of iron overload. High values, $\geq 70\%$ for women and $\geq 80\%$ for men, namely suggest parenchymal (toxic) iron loading, whereas normal values can indicate reticulo-endothelial iron loading [28].

Additionally intriguing is the fact that the most sensitive and specific tests for secondary iron overload such as T2*MRI or a tissue biopsy of heart and/or liver, are not considered by 42% of the respondents [28]. Possibly, T2* MRI is not widely available, but understandably, tissue biopsies of liver and heart, are less likely to be performed due to its risk of complications in these vulnerable patients.

For most hematologists, phlebotomies were regarded as the first choice iron-lowering therapy (81%). Again, the survey results seem to be discordant with the responses provided in *clinical scenario 3* (supplementary textbox 3), in which much less respondents (54%) preferred phlebotomies over iron chelation therapy (46%). This may be explained by the hemoglobin level in the scenario, but also the lack of consensus to guide therapy, is a likely explanation. Iron chelation therapy, of course, has some clear advantages in daily practice. It can be administered orally, whereas phlebotomies are more invasive and require more time and effort from the hospital personnel. However, side effects of iron chelation therapy as well as its costs should also be considered.

Limited life expectancy and limited prognosis due to comorbidity were the most common reasons for our respondents to refrain from iron chelation therapy in case of iron overload in this specific patient group. This was also seen in an European survey among physicians treating transfusion-dependent MDS patients [29]. A serum ferritin level of ≤ 500 $\mu\text{g/L}$ was a general threshold for stopping iron chelation therapy (93% of respondents), whereas clinical and laboratory factors such as normalizing liver or cardiac function were not. Next to limited drug efficacy, a dismal prognosis and side effects/intolerance were the most common reasons to stop iron chelation therapy.

Strengths and limitations:

This is the first large survey among hematologists and hematologist trainees in the Netherlands in which important questions regarding RBC transfusion support and diagnosis and management of secondary iron overload in patients with hematological malignancies are addressed.

The limitation inherent to most surveys is the moderate response rate. However, we consider our survey's response rate quite reasonable with a representation of all eight university hospitals, which treat the majority of patients with hematological malignancies, and 39% of all community hospitals. Second, not all hematologists in the Netherlands treat patients with hematological malignancies. However, we were not able to differentiate this from the available email addresses, so the actual response rate is somewhat underestimated.

Thirdly, the results of the survey for practicing hematologists and hematologists in training were not separated. It is possible that some of the variation found in this study, could be due to this fact.

Furthermore, no actual data on clinical practice were collected. So in theory, the responses reported in the survey and the actual clinical practice could differ. Since all responses were anonymized and we tested the responses in the survey with the results of the clinical scenarios, this is not expected to be a major problem.

Finally, as in all surveys, response bias cannot be excluded. The relatively high amount of transfusion medicine consultants (18%) indicates that such a selection indeed exists in our survey; still this would rather result in an underestimation of the existing variability in daily practice.

In conclusion, the results of this survey indicate large reported variation in RBC transfusion support and assessment and management of transfusion-associated iron overload in Dutch patients with hematological malignancies. Proper evidence-based guidelines on these subjects may reduce this variability.

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Supplementary material

S1 Survey

Dear colleague,

In the context of a PhD research project, we would like to ask you to complete a survey. The aim of this survey is to evaluate the daily practice of red blood cell transfusion support and management of secondary iron overload in the Netherlands.

The survey is approved by the members of the working party 'non-oncological hematological diseases of the Dutch Hematology Association.'

We will ask you in which center you are employed in order to look at differences between centers, these data will be anonymized.

The survey will take 10-15 minutes to complete. You can start the survey by clicking on the link below:

<https://response.questback.com/stichtingsanquinbloedvoorzieni/dagelijkse-praktijkerytrocytentransfusie>

Thank you for your cooperation!

Used abbreviations

ASA: American Society of Anesthesiology; NYHA: New York Heart Association; WHO: World Health Organization; RBC: Red Blood Cell; AST: Alanine transaminase; GGT: Gamma-glutamyl transpeptidase; T2* MRI: transversal relaxation magnetic resonance imaging.

Remarks

The original survey is in Dutch, this is a translated version.
Mmol/L were converted to g/dL for the manuscript.

1. In which treatment center are you currently employed?
2. Which of the following congresses did you attend in the preceding three years?
Multiple answers possible.
 - ☐ European Hematology Association congress
 - ☐ American Society Hematology congress
 - ☐ International Society of Thrombosis and Hemostasis congress
 - ☐ International Society of Blood Transfusion congress
 - ☐ Dutch Hematology congress
 - ☐ European Bone Marrow Transplantation congress
 - ☐ Other: American Association of Blood Banks congress, World Federation of Hemophilia congress, European Association of Hemophilia and Allied Disorders congress
 - ☐ None of the above
3. Does your center employ an in-house consultant in transfusion medicine?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
4. Are you a transfusion medicine consultant yourself?
 - ☐ Yes
 - ☐ No
5. Does your center have institutional guidelines on RBC transfusion support specific for hemato-oncological patients?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
6. Do you comply with a guideline for the management of secondary iron overload?
 - ☐ Yes; a local guideline
 - ☐ Yes; a national guideline
 - ☐ Yes; an international guideline
 - ☐ No

- ☐ I don't know
- ☐ If yes, please specify which guideline you comply with.

- 7a. At which hemoglobin level do you usually initiate a red blood cell transfusion in a hemodynamically stable, 45-year-old male patient without significant comorbidities (ASA class I-II) during intensive chemotherapy or hematopoietic stem cell transplantation?
 - ☐ < 3.5 mmol/L
 - ☐ 3.5 to 3.9 mmol/L
 - ☐ 4.0 to 4.4 mmol/L
 - ☐ 4.5 to 4.9 mmol/L
 - ☐ 5.0 to 5.4 mmol/L
 - ☐ 5.5 to 5.9 mmol/L
 - ☐ 6.0 to 6.4 mmol/L
- 7b. At which hemoglobin level do you usually initiate a red blood cell transfusion in a hemodynamically stable, 45-year-old male patient without significant comorbidities (ASA class I-II) with chronic anemia (e.g. myelodysplastic syndrome) in the outpatient clinic.
 - ☐ < 3.5 mmol/L
 - ☐ 3.5 to 3.9 mmol/L
 - ☐ 4.0 to 4.4 mmol/L
 - ☐ 4.5 to 4.9 mmol/L
 - ☐ 5.0 to 5.4 mmol/L
 - ☐ 5.5 to 5.9 mmol/L
 - ☐ 6.0 to 6.4 mmol/L
8. What factors, next to hemoglobin level and diagnosis/treatment, influence your decision to initiate a red blood cell transfusion?
Response categories: never, sometimes, regularly, often, always
 - ☐ Dyspnea
 - ☐ Signs of hypoxia (oxygen saturation < 90%; PaO₂ < 7.8 kPa or < 60 mmHg)
 - ☐ Recent ischemic cardiac event (in the previous 3 months)
 - ☐ Tachycardia (>100 beats/minute)
 - ☐ Cardiac arrhythmia
 - ☐ Cardiac failure (NYHA grade II-IV)
 - ☐ WHO grade 1-2 bleeding
 - ☐ WHO grade 3-4 bleeding
 - ☐ Platelet count of <10x10⁹/L without bleeding
 - ☐ Platelet count of <10x10⁹/L with WHO grade 1-2 bleeding

- ☐ Age ≤40 years
☐ Age ≥75 years
☐ Age ≥85 years
☐ Quality of life
☐ Patients' request
☐ Hospitalized patient versus outpatient
☐ Logistical reasons (e.g. inability to organize an outpatient transfusion appointment)
☐ Patients travel distance to hospital
☐ Time to next appointment
9. How many times a week is a complete blood count routinely performed in your center in the neutropenic phase during intensive chemotherapy or hematopoietic stem cell transplantation?
- ☐ Daily
☐ 5 to 6 times a week
☐ 3 to 4 times a week
☐ 1 to 2 times a week
☐ Not applicable
☐ Other, namely:
10. Do you perform additional tests, next to the hemoglobin level, to guide initiation of a red blood cell transfusion?
- ☐ Yes, namely:
☐ No
11. How many red blood cell units per transfusion episode do you routinely order for a hemodynamically stable, 45-year-old male patient without significant comorbidities (ASA class I-II) during intensive chemotherapy or hematopoietic stem cell transplantation?
- ☐ 1 unit
☐ 2 units
☐ 3 units
☐ Other, namely:
12. How many red blood cell units per transfusion episode do you routinely order for a hemodynamically stable, 45-year-old male patient without significant comorbidities (ASA class I-II) with chronic anemia (e.g. myelodysplastic syndrome) in the outpatient setting.
- ☐ 1 unit
☐ 2 units
☐ 3 units
☐ Other, namely:
13. Do you consider a patient's weight and/or blood volume to guide red blood cell transfusion support?
- ☐ Yes
☐ No
☐ I don't know
14. In which patient groups do you perform screening for secondary iron overload, assuming this has consequences for the treatment?
- Multiple answers possible.*
- ☐ All patients who were previously transfused with RBCs
☐ Patients receiving ≥2 RBC per month
☐ Total RBC transfusion burden of 10-19 RBC units
☐ Total RBC transfusion burden of 20-29 RBC units
☐ Total RBC transfusion burden of 30-39 RBC units
☐ Total RBC transfusion burden of 40-49 RBC units
☐ Total RBC transfusion burden of ≥50 RBC units
☐ Patients who underwent intensive (clinical) chemotherapy
☐ Patients who underwent autologous or allogeneic hematopoietic stem cell transplantation
☐ None of the above
☐ Other, namely:
15. What laboratory test(s) would you order when screening for secondary iron overload?
- Multiple answers possible.*
- ☐ Serum ferritin
☐ Serum ferritin in combination with C-reactive protein
☐ Serum iron in combination with total iron binding capacity
☐ Transferrin saturation
☐ Bone marrow iron staining
☐ Other, namely:

16a. What clinical factors influence your decision to perform a MRI T2* and/or biopsy of liver and/or heart?

Multiple answers possible.

- ☐ I have never/rarely performed an MRI and/or biopsy in this respect
- ☐ Certain serum ferritin level
- ☐ Certain total RBC transfusion burden
- ☐ Disturbed liver enzymes (AST, GGT)
- ☐ Disturbed liver function (e.g. ↓ serum albumin levels, ↓ clotting factor production)
- ☐ Signs of cardiac failure
- ☐ Cardiac arrhythmia in absence of inflammation
- ☐ Occurrence of endocrinopathy (e.g. diabetes mellitus type 2, hypothyroidism)
- ☐ Occurrence of unexplained fatigue
- ☐ Occurrence of unexplained arthromyalgia
- ☐ Occurrence of an abnormal brown/bronze skin
- ☐ RBC transfusion intensity ≥ 2 units/month
- ☐ Other, namely:

When 'certain serum ferritin level' was indicated in question 16a.

16b. What serum ferritin level would lead you to perform a MRI T2* and/or biopsy of liver and/or heart?

- ☐ 500-1000 $\mu\text{g/L}$
- ☐ 1000-1500 $\mu\text{g/L}$
- ☐ 1500-2000 $\mu\text{g/L}$
- ☐ 2000-2500 $\mu\text{g/L}$
- ☐ 2500-3000 $\mu\text{g/L}$
- ☐ >3000 $\mu\text{g/L}$
- ☐ other, namely:

When 'certain total RBC transfusion burden' was indicated in question 16a.

16c. Which total RBC transfusion burden would lead you to perform a MRI T2* and/or biopsy of liver and/or heart?

- ☐ 10-19 RBC units
- ☐ 20-29 RBC units
- ☐ 30-39 RBC units
- ☐ 40-49 RBC units
- ☐ ≥ 50 RBC units
- ☐ Other, namely:

17a. What factors would lead you to initiate treatment of secondary iron overload?

Multiple answers possible.

- ☐ Certain serum ferritin level
- ☐ Certain total RBC transfusion burden
- ☐ Transfusion intensity ≥ 2 RBC units/month
- ☐ Disturbed liver enzymes (AST, GGT)
- ☐ Disturbed liver function (e.g. low serum albumin levels, clotting factor production)
- ☐ Signs of cardiac failure
- ☐ Cardiac arrhythmia in absence of inflammation
- ☐ Signs of iron overload on a T2* MRI
- ☐ Signs of iron overload in a liver and/or myocardial biopsy
- ☐ Occurrence of endocrinopathy (e.g. diabetes mellitus type 2, hypothyroidism)
- ☐ Occurrence of unexplained fatigue
- ☐ Occurrence of unexplained arthromyalgia
- ☐ Occurrence of an abnormal brown/bronze skin
- ☐ Other, namely:

When 'certain serum ferritin level was indicated in question 17a.

17b. What serum ferritin level would lead you to initiate treatment for secondary iron overload?

- ☐ 500-1000 $\mu\text{g/L}$
- ☐ 1000-1500 $\mu\text{g/L}$
- ☐ 1500-2000 $\mu\text{g/L}$
- ☐ 2000-2500 $\mu\text{g/L}$
- ☐ 2500-3000 $\mu\text{g/L}$
- ☐ >3000 $\mu\text{g/L}$
- ☐ other, namely:

When 'certain total RBC transfusion burden' was indicated in question 17a.

17c. Which total RBC transfusion burden would lead you to initiate treatment for secondary iron overload?

- ☐ 10-19 RBC units
- ☐ 20-29 RBC units
- ☐ 30-39 RBC units
- ☐ 40-49 RBC units
- ☐ ≥ 50 RBC units
- ☐ Other, namely:

18a. Can you indicate your preferences regarding treatment of secondary iron overload: phlebotomies when the hemoglobin level is considered sufficiently high?

- ☐ First choice
- ☐ Second choice
- ☐ Not an option

18b. Can you indicate your preferences regarding treatment of secondary iron overload: iron chelation therapy?

- ☐ First choice
- ☐ Second choice
- ☐ Not an option

19. Which clinical factors influence your treatment choice for secondary iron overload?

Multiple answers possible.

- ☐ Hemoglobin level
- ☐ Comorbidity
- ☐ Drug interactions with comedication
- ☐ Renal dysfunction
- ☐ Costs
- ☐ Possible adverse effects
- ☐ Life expectancy
- ☐ Age
- ☐ Expected therapy compliance
- ☐ Quality of life
- ☐ Other, namely:

20. What iron chelating agent(s) do you prescribe to your patients with secondary iron overload?

Multiple answers possible.

- ☐ I never/rarely prescribe iron chelation therapy
- ☐ Deferoxamine (Desferal™)
- ☐ Deferasirox (Exjade™)
- ☐ Deferiprone (Ferriprox™)
- ☐ Other, namely:

21. Which clinical factors would guide your decision to not treat a patient with iron chelation therapy despite a high serum ferritin level?

Multiple answers possible.

- ☐ Limited life expectancy ≤ 1 year
- ☐ Limited life expectancy ≤ 3 years
- ☐ Limited life expectancy ≤ 5 years
- ☐ Age ≥ 75 years
- ☐ Age ≥ 85 years
- ☐ Comorbidity limiting prognosis
- ☐ Renal dysfunction
- ☐ Drug interaction with comedication
- ☐ Possible side effects of iron chelation therapy
- ☐ Low quality of life
- ☐ Expected low therapy compliance
- ☐ Costs of iron chelation therapy
- ☐ Other, namely:

22a. Which clinical factors would guide your decision to stop iron chelation therapy?

Multiple answers possible.

- ☐ I usually do not stop iron chelation therapy once it is started
- ☐ Having reached a certain serum ferritin level
- ☐ Normalization of liver values
- ☐ Normalization of cardiac function
- ☐ Normalization of cardiac rhythm
- ☐ Normalization of T2*MRI liver and/or heart
- ☐ Normalization of liver and/or myocardial biopsy
- ☐ Disappearance of endocrinopathy (e.g. diabetes mellitus type 2, hypothyroidism)
- ☐ Disappearance of arthromyalgia
- ☐ Limited life expectancy
- ☐ Having reached transfusion-independency
- ☐ Other, namely:

22b. What serum ferritin level would lead you to stop iron chelation therapy?

- ☐ $< 500 \mu\text{g/L}$
- ☐ $< 1000 \mu\text{g/L}$
- ☐ $< 1500 \mu\text{g/L}$
- ☐ $< 2000 \mu\text{g/L}$
- ☐ $< 2500 \mu\text{g/L}$
- ☐ Other, namely:

Clinical scenario 1

A 23-year-old patient with acute lymphoid leukemia is currently admitted to the hospital for remission-induction chemotherapy. Her hemoglobin level is 4.4 mmol/L. She has no specific complaints and all vital signs are normal. It is likely her hemoglobin level will decrease in the next few days.

23a. Would you initiate a RBC transfusion at this moment?

- ☐ Yes
- ☐ No
- ☐ I don't know

23b. If yes: How many RBC units would you order at this moment?

- ☐ 1 RBC unit
- ☐ 2 RBC units
- ☐ 3 RBC units
- ☐ 4 RBC units
- ☐ Other, namely:

23c. If no: Do you schedule a RBC transfusion for the next day?

- ☐ Yes
- ☐ No
- ☐ I don't know

23d. If yes: How many RBC units would you order for the next day?

- ☐ 1 RBC unit
- ☐ 2 RBC units
- ☐ 3 RBC units
- ☐ 4 RBC units
- ☐ Other, namely:

Clinical scenario 2

A 74-year-old patient with a myelodysplastic syndrome with an excess of blasts (MDS RAEB-1) is known to have a moderate chronic obstructive pulmonary disease (Gold class 2) and peripheral vascular disease. He is treated with demethylating therapy since he is not a candidate for intensive chemotherapy. For treating disease-related anemia, he received 30 RBC units in total. He visits the outpatient clinic to receive the fourth cycle of demethylating therapy. His hemoglobin level is 5.6 mmol/L, he has no specific health complaints and his vital signs are normal.

24a. Would you initiate a RBC transfusion at this moment?

- ☐ Yes
- ☐ No
- ☐ I don't know

24b. Would you initiate a RBC transfusion when his hemoglobin level would be 4.5 mmol/L?

- ☐ Yes
- ☐ No
- ☐ I don't know

24c. Are you going to monitor this patient for secondary iron overload?

- ☐ Yes
- ☐ No
- ☐ I don't know

24d. Would you treat this patient for secondary iron overload if there are signs of secondary iron overload?

- ☐ Yes
- ☐ No
- ☐ I don't know

Clinical scenario 3

A 58-year-old patient recently underwent an allogeneic stem cell transplantation due to a myelodysplastic syndrome with an excess of blasts (MDS RAEB 2). His total RBC transfusion burden is 50 units. His hemoglobin level is currently 6.8 mmol/L; his serum ferritin level is 3500 µg/L. There are no signs of active infection, inflammation or cardiac failure. All liver enzyme values are within normal ranges.

25a. Do you consider treatment for secondary iron overload at this moment?

- ☐ Yes
- ☐ Only if a T2* MRI or biopsy of liver and/or heart indicates iron overload
- ☐ No
- ☐ I don't know

25b. If you chose 'only if a T2* MRI or biopsy of liver and/or heart indicates iron overload': What treatment would you prefer?

- ☐ phlebotomies
- ☐ Iron chelation therapy
- ☐ Other, namely:

Supplementary textbox 1

Clinical scenario 1

A 23-year-old patient with acute lymphoid leukemia is currently admitted to the hospital for remission-induction chemotherapy. Her hemoglobin level is 7.1 g/dL. She has no specific complaints and all vital signs are normal. It is likely her hemoglobin level will decrease in the next few days.

Thirty-five percent of the respondents would, at this point, give this patient a RBC transfusion. Two RBC units would be ordered by 74% of these respondents, followed by one RBC unit in 15% and three RBC units in 11% of respondents. Of the 65% who would not immediately give a RBC transfusion, 14% would order a RBC transfusion for the next day. In case the patients' hemoglobin level would be 6.5 g/dL, 94% of the respondents would give a RBC transfusion right away. Sixty-two percent would order 2 RBC units for this transfusion episode, 22% one unit, 15% three units and 1% four units for a single transfusion episode.

Supplementary textbox 2

Clinical scenario 2

A 74-year-old patient with a myelodysplastic syndrome with an excess of blasts (MDS RAEB-1) is known to have a moderate chronic obstructive pulmonary disease (Gold class 2) and peripheral vascular disease. He is treated with demethylating therapy since he is not a candidate for intensive chemotherapy. For treating disease-related anemia, he received 30 RBC units in total. He visits the outpatient clinic to receive the fourth cycle of demethylating therapy. His hemoglobin level is 8.9 g/dL, he has no specific health complaints and his vital signs are normal.

Ninety-two percent of the respondents would not initiate a RBC transfusion at this point, while 97% of all respondents would if his hemoglobin level were 7.3 g/dL. Screening for secondary iron overload was considered by 59% of the respondents; 49% would treat in case of secondary overload (according to local guidelines), whereas 26% would only treat in case of iron overload specific complaints. Surprisingly, 20% of the respondents who performed screening, would eventually not treat this patients for secondary iron overload (5% of respondents did not know yet whether to treat him or not).

Supplementary textbox 3

Clinical scenario 3

A 58-year-old patient recently underwent an allogeneic stem cell transplantation for a myelodysplastic syndrome with an excess of blasts (MDS RAEB 2). His total RBC transfusion burden is 50 units. His hemoglobin level is currently 10.8 g/dL; his serum ferritin level is 3500 µg/L. There are no signs of active infection, inflammation or cardiac failure. All liver enzyme values are within normal ranges.

At this point, 88% of the respondents consider iron lowering therapy, of whom 17% would first perform an additional T2*MRI and/or a liver or heart biopsy to determine the presence of iron overload. From the 88% of respondents considering iron lowering therapy, 54% would choose for phlebotomies and 46% for iron chelation therapy.



3

IMPACT OF RED BLOOD CELL TRANSFUSION STRATEGIES IN HAEMATO-ONCOLOGICAL PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Summary

Haemato-oncological patients receive many red blood cell (RBC) transfusions, however evidence-based guidelines are lacking. Our aim is to quantify the effect of restrictive and liberal RBC transfusion strategies on clinical outcomes and blood use in haemato-oncological patients. A literature search, last updated on August 11th 2016, was performed in PubMed, EMBASE, Web of science, Cochrane, CINAHL and Academic Search Premier without restrictions on language and year of publication. Randomized controlled trials and observational studies, which compared different RBC transfusion strategies in haemato-oncological patients were eligible for inclusion. Risk of bias assessment according to the Cochrane collaboration's tool and Newcastle-Ottawa scale was performed. After removing duplicates, 1,142 publications were identified. Eventually, fifteen studies were included, reporting on 2,636 patients. The pooled relative risk for mortality was 0.68 (95% CI 0.46 to 1.01) in favour of the restrictive strategy. The mean RBC use was reduced with 1.40 units (95% CI 0.70 to 2.09) per transfused patient per therapy cycle in the restrictive strategy group. There were no differences in safety outcomes. All currently available evidence suggests that restrictive strategies do not have a negative impact regarding clinical outcomes in haemato-oncological patients, while it reduces RBC blood use and associated costs.

Introduction

Twenty to thirty percent of all red blood cell transfusions (RBC) in Europe are given to patients with haematological diseases, mostly acute myeloid leukaemia (AML), myelodysplastic syndromes (MDS), lymphoma, and haemoglobinopathies (Tinegate *et al*, 2016; Bruun *et al*, 2016). In general, RBC transfusions are given to optimize the quality of life during disease- or treatment-mediated anaemia and to improve platelet dependent haemostasis (Valeri *et al*, 1998; Valeri *et al* 2011; Ho 1998). The beneficial effect of RBC transfusion on disease outcomes, however, remains difficult to quantify. This is important, as transfusions may include negative effects on outcome through risk of transmission of infectious diseases and transfusion reactions, and their association with immuno-modulation and iron overload (Klein 1999; Shander *et al*, 2009). Current blood transfusion guidelines still lack high-grade evidence for optimal RBC transfusion strategies in haemato-oncological patients (Carson *et al*, 2012; BCSH guideline 2001; Haas *et al*, 2011). This likely explains why RBC transfusion strategies vary widely amongst haematologists and between centres.

Over the past twenty years many studies have reported no disadvantages of restrictive compared to liberal RBC transfusion strategies, which has led to guidelines recommending restrictive strategies for non-haemato-oncological patient groups (Hebert *et al*, 1999; Villanueva *et al*, 2009; Carson *et al*, 2011; Hajjar *et al*, 2010; Holst *et al*, 2014; Holst *et al*, 2015; Lacroix *et al*, 2007; Robertson *et al*, 2014). However, to this day, solid data on restrictive RBC transfusion strategies in haemato-oncological patients is lacking. Although various RBC transfusion strategies, such as lowering haemoglobin triggers and single-unit versus double-unit transfusion have been studied, the individual sample sizes of these studies are too small to draw definite conclusions. We therefore conducted a systematic review including a meta-analysis to quantify the effect of restrictive and liberal RBC transfusion strategies on clinical outcomes and blood use in haemato-oncological patients.

Methods

Literature search

For this systematic review with meta-analysis, a literature search was performed in PubMed (1946 to 2016), EMBASE (1947 to 2016), Web of science (1900 to 2016), Cochrane library (1992 to 2016, issues: trials and methods studies), CINAHL (1937 to 2016), and Academic Search Premier (1975 to 2016) with assistance of a qualified

librarian. No restrictions were made on language, year of publication or patients’ age. Both randomized controlled trials and observational studies were eligible study designs for inclusion in this review. We have searched for all possible relevant clinical outcomes and use of blood products in haemato-oncological patients. The complete eligibility criteria are listed in table I. Titles and abstracts of all publications identified by the search were independently evaluated for eligibility by two reviewers. Publications deemed irrelevant by both researchers were excluded. In case of disagreement, a third independent reviewer’s opinion determined in- or exclusion for further study. All possible eligible studies were included for full-text review. Furthermore, we searched the reference lists of eligible studies to identify additional relevant publications and contacted researchers who registered a RCT in the clinicaltrials.gov and ISRCTN registry for possible unpublished data. The search strategy is provided in the appendix. The search was updated on August 11th 2016.

Table I Eligibility criteria	
Eligibility Criteria of Included Studies	
Population	-Haemato-oncological patients
Intervention	-Restrictive versus liberal haemoglobin trigger -Double- versus single-unit strategy -Haemoglobin content-based strategy -Other predefined RBC transfusion strategies
Clinical outcomes	-All-cause mortality -Cardiac events (myocardial infarction, cardiac dysfunction, cardiac arrhythmias) -Thromboembolic events (deep venous thrombosis, pulmonary embolism, other) -Incidence and severity of bleeding -Treatment response -Quality of life
Blood use	-Reduction in RBC use -Reduction in PLT use -Costs on blood products
Exclusion criteria	-Reviews -Case series -Animal studies

Risk of bias assessment

Risk of bias was assessed at study level by using the Cochrane collaboration’s tool for bias assessment for RCTs (Higgins *et al*, 2011). Each item of the Cochrane collaboration’s tool was rated as “high”, “low”, or “unclear”. RCTs with a high or unclear risk of bias on the following domains: sequence generation, allocation concealment, incomplete outcome data, selective outcomes reporting, and other sources of bias, were excluded from pooling by meta-analysis. A lack of blinding, which is almost impossible in transfusion studies, was allowed.

For observational studies, stars were awarded to the items of the Newcastle-Ottawa quality assessment scale at study level (Wells *et al*, 2000). The domains considered in this scale are: 1) selection (e.g. representativeness of the exposed cohort and selection of non-exposed cohort); 2) comparability of the cohorts on the basis of design or analysis (e.g. whether results were adjusted for possible confounders); 3) outcome (assessment of outcome measures and adequacy of follow-up). A total of nine stars could be achieved. Studies with less than five stars were classified as a ‘high risk of bias’. Additionally, studies were considered as having an ‘unclear risk of bias’ when they scored zero stars at the comparability domain. The studies with a high of bias were excluded from pooling for meta-analysis.

Data extraction and missing data

The data extraction was performed by a first reviewer and checked by a second using a prespecified data extraction form. When the same data were published more than once, the study with the most complete data was included. Where necessary mean and standard deviations were calculated from medians, ranges and quartiles with the assumption of an underlying normal distribution (Wan *et al*, 2014). When we identified data to be missing or unclear in published literature, authors were contacted. When combined data on benign and malignant haematological diseases were reported, we extracted the data of the malignant haematological diseases separately when possible. When this was not possible, we only included studies if little admixture existed.

Definition of RBC transfusion strategies

A low haemoglobin transfusion trigger, a single-unit transfusion policy, and a haemoglobin-content based strategy (in which the required haemoglobin amount for the patient and haemoglobin level of the RBC unit were matched by a computer system which reduces the number of RBC units) were considered ‘restrictive’ strategies as reported by the authors. A high haemoglobin transfusion trigger and a double-unit policy were considered ‘liberal’ strategies as reported by the authors.

Statistical analyses

Statistical analyses were all performed in STATA 14 (College station, TX:StataCorpLP). Statistical heterogeneity was assessed by I² tests. Percentages of 25%, 50% and 75% correspond to a low, moderate and high level of heterogeneity (Higgins *et al*, 2003). In case meta-analysis was feasible due to the availability of all needed data, pooled relative risks for dichotomous outcomes and weighted mean differences for continuous outcomes and corresponding 95% confidence intervals (CI) were calculated using the random effects model of DerSimonian and Laird (DerSimonian & Laird, 1986). A random effects model was chosen as most conservative analysis method and because of the unavoidable differences in patient groups and interventions. Furthermore, in case of little or no heterogeneity this model produces similar results as a fixed effects model. Forest plots for pooled data are presented. Reduction in blood use is reported as units per therapy cycle, meaning the number of units given within a whole course of chemotherapy, e.g. remission-induction or consolidation course.

Sensitivity analyses

The Newcastle-Ottawa quality assessment scale is quite strict about assessing comparability, exclusion strategies based on this scale may not always be justified (Hernán *et al*, 2002). Therefore, to enable a more complete overview, we performed a prespecified sensitivity analysis, including the observational studies with an unclear risk of bias due to a ‘zero’ score at comparability. We did not perform sensitivity analyses regarding patients’ age because we assume that only the size but not the direction of the effects will change between different age categories.

Results

Search results

After removing duplicates 1,142 publications were identified by the search of which 1,060 did not meet the inclusion criteria and four lacked an abstract. Seventy-eight publications, four RCTs and 74 observational studies, were evaluated for full-text review (figure 1). No additional relevant publications were identified when searching the reference lists of eligible studies. From a total of fifteen remaining publications, four RCTs, one non-randomized intervention trial and ten observational cohort studies, reporting on 2,636 patients, were included. These selected publications differed between disease type, study design and type of RBC transfusion strategy. The search strategy is listed in the appendix. Two out of three authors of included studies responded to our request for additional information. For one study supplementary data could thus be included in our meta-analysis (Webert *et al*,

2008); information on a registered but withdrawn RCT did not lead to includable results (Jansen *et al*, 2005).

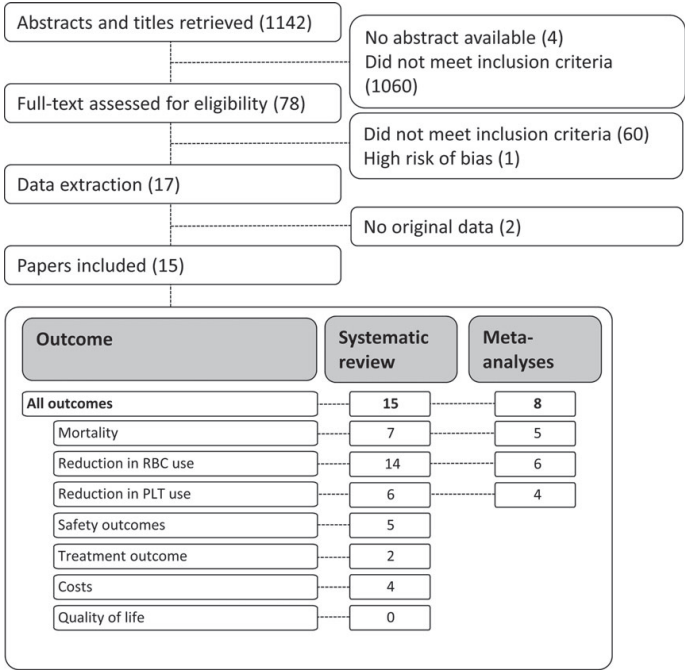


Figure 1 Flow chart

Risk of bias assessment

Tables II and III provide information on the bias risk assessment. Two RCTs were classified as ‘low risk of bias’ (DeZern *et al*, 2016; Webert *et al*, 2008). A lack of blinding in the RCT of DeZern *et al* was allowed as blinding in transfusion studies is almost impossible. Five ‘low risk of bias’ observational studies were included (Berger *et al*, 2012; Hoeg *et al*, 2013; Jansen *et al*, 2004; Lightdale *et al*, 2012; Paananen *et al*, 2009) and one was excluded because of a high risk of bias (Bishop *et al*, 2005). The data of six studies with a low score on the comparability domain were accounted for in the sensitivity analysis in case their data was suitable for pooling by meta-analysis.

Table II Cochrane Collaboration's tool for assessing risk of bias

Reference

	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Atila 2011	?	?	?	+	?	+
DeZern 2016	+	+	-	+	+	+
Robitaille 2013	+	+	?	+	+	-
Webert 2008	+	+	+	+	+	+

+ Low risk of bias; - High risk of bias; ? Unclear risk of bias

Table III Newcastle Ottawa bias assessment scale

Reference	Selection	Comparability	Outcome	Total
Allameddine 2015	****	-	**	*****
Arslan 2004	***	-	***	*****
Bercovitz 2011	***	-	**	*****
Berger 2012	****	**	**	*****
Bishop 2005	**	-	**	****
Butler 2015	****	-	***	*****
Hoeg 2013	****	*	**	*****
Jansen 2004	****	**	**	*****
Lightdale 2009	****	**	**	*****
Mear 2014	****	-	**	*****
Paananen 2009	***	**	***	*****
Patil 2013	****	-	**	*****

Study characteristics

Table IV depicts the characteristics per study. All studies were published between 2004 and 2016. Although various haematological malignancies were included, fourteen out of the fifteen studies reported results mainly or partly on patients with acute leukaemia receiving either intensive chemotherapy or hematopoietic stem cell transplantation (HSCT). Eight of the fifteen studies reported on restrictive (70 g/L to 97 g/L) versus liberal (80 g/L to 120 g/L) haemoglobin triggers, three on single- versus double-unit RBC transfusion strategy, one study on both of the former mentioned strategies, and three on computer-based strategies. The large overlap between both strategies was mainly due to one study with a very broad definition of the restrictive strategy (73 up to 97 g/L in presence of anaemia-related symptoms) (Hoeg *et al*, 2013). We performed a post-hoc sensitivity analysis excluding this particular study to assure the validity of the results concerning restrictive strategies. When this study was excluded the range of restrictive haemoglobin triggers of the remaining studies was 70 g/L to 88 g/L. Four studies reported results regarding paediatric patients (Bercovitz *et al*, 2011; Lightdale *et al*, 2012; Paananen *et al*, 2009; Robitaille *et al*, 2013); the remaining studies reported on adult patients.

Outcomes

Mortality. Five studies, of which four observational studies and one RCT, were suitable for meta-analysis on mortality (Berger *et al*, 2012; DeZern *et al*, 2016; Hoeg *et al*, 2013; Jansen *et al*, 2004; Lightdale *et al*, 2012). The results of the meta-analysis are stratified for study design and presented in figure 2. The relative risk for mortality was 0.68 (95% CI 0.46 to 1.01; 571 patients) in favour of the restrictive RBC transfusion strategies. Hence, restrictive transfused patients had a 32% lower chance of dying as compared to the liberal transfused patients. Heterogeneity between the studies was low, I^2 0.0% ($p=0.99$). Similar results were obtained after sensitivity analysis excluding the study with a broad definition of a restrictive transfusion strategy (Hoeg *et al*, 2013), resulting in a RR of 0.67 (0.41 to 1.09). This was also the case after sensitivity analysis including observational studies with an unclear risk of bias according to the Newcastle Ottawa quality assessment scale (Patil *et al*, 2013) with a RR of 0.71 (0.48 to 1.04). The forest plot stratified for 45-60 and 90-100 days mortality is presented in the appendix.

Reduction of RBC transfusions. Fourteen studies investigated RBC use. Six of these studies, of which two RCTs and four observational studies, reported data which could be pooled by meta-analysis (Berger *et al*, 2012; DeZern *et al*, 2016; Jansen *et al*, 2004; Lightdale *et al*, 2012; Paananen *et al*, 2009; Webert *et al*, 2008). For three of these studies the means and SDs of the data were estimated

Table IV Study characteristics

Reference	Study design	Patients R/L	Age (yr) R/L	n R/L	Treatment R/L	RBC strategy R/L	Hb difference (g/L) R/L	Included in meta-analysis and/or systematic review	Outcome R/L
Allameddine 2015	Prospective cohort study, retrospective control group	All haemato-oncological inpatients	NM	764 (382/382)	NM	Single/ double-unit	NM	SR. Did not contain data suitable for pooling by MA	RBC use: R: 18% reduction of total units/year
Arslan 2004	Non-randomized Intervention study	Acute leukaemia (n=38), lymphoma (n=11), MM (n=2)	Median: 38	51 (51/51)	NM	Hb-content based/ unit-based. Computer-based reduction of initial ordered RBC units with , patients as their own controls	NA	SR. Excluded from MA (low score on comparability in NOS)	RBC use: R: 31% reduction
Atilla 2011	RCT	Acute leukaemia (n=44), lympho-proliferative disorders (n=17), plasma cell disorders (n=11), bone marrow defects (n=15), sickle cell (n=1), solid tumour (n=1)	Median: 46	89. 178 transfusion episodes (92/86)	NM	Hb-content based/unit-based. Computer-based reduction of ordered RBC units	NM	SR. Excluded from the MA (unclear risk of bias)	RBC use: R: 21% reduction of total RBC units in the study period
Bercovitz 2011	Retrospective cohort study comparing 2 centres	Paediatric patients 1-21 years with first HSCT	NM	457** (283/174)	Autologous HSCT (25/ 21); allogeneic HSCT (258/ 153)	Hb trigger: 80 g/L/ 100 g/L	Mean Hb study period: 96 /110	SR. Excluded from MA (low score on comparability in NOS)	Mean RBC use: 4.34 (SD 2.35)/ 4.93 (SD 3.01)
Berger 2012	Retrospective cohort study	Inpatient leukaemia (AML n=102, ALL n=17), and HSCT patients (lymphoma n=12, others 8). Disease stage in therapy cycles: good: R 37, L 28; intermediate R 35, L 33 ; poor: R 66, L 73	Median 49	139 (62/ 77)	Induction CT (69/ 67); consolidation (20/15); reinduction: (10/ 6); allogeneic HSCT: (38/43); autologous HSCT: (1/3)	Single/ double-unit	NM	MA+SR	60 day mortality: 6.5%/7.8% 100 day mortality: 9.7%/14.3% Median RBC use: 6 (Q1-Q3 3-10)/8 (Q1-Q3 4-13) Median PLT use: 5 (Q1-Q3 3-9)/5 (Q1-Q3 2-9)
Butler 2015	Retrospective cohort study	Intensive CT or HSCT for patients with leukaemia, MDS, lymphoma or MM	Mean: 51.5/52.7	97 (69/28)	Allogeneic HSCT: (21/ 10); autologous HSCT: (29/ 10); intensive CT: (19/8)	Introduction of clinical decision support system. Hb trigger: 80 g/L /physician-driven RBC request	NM	SR. Excluded from MA (low score on comparability in NOS)	Mean RBC use: 4.8 (SD 4.9)/5.9 (SD 5.4) Mean PLT use: 5.5 (SD 5.4)/7.4 (SD 6.8)

Reference	Study design	Patients R/L	Age (yr) R/L	n R/L	Treatment R/L	RBC strategy R/L	Hb differ- ence (g/L) R/L	Included in meta- analysis and/or systematic review	Outcome R/L
Dezern 2016	RCT	Acute leukaemia patients(AML 50/ 23; ALL 7/7; APL 2/0)	Median: 56/63	89 (30/ 59)	Intensive CT	Hb trigger: 70 g/L + single-unit/ 80 g/L + single-unit	Mean Hb: 77/86	MA+SR	60 day mortality: 5%/10% Mean RBC use: 8.2 (SD 4.2)/11.3 (SD 5.4). Median PLT use in episodes: 9 (IQR 5.5-12.5)/9 (IQR 7-12). Therapy response: CR 42%/57%; MRD by FCM 19%/7%; MRD by molecular features 7%/7%; PR + transfusion dependence 8%/7%; treatment failure 24%/20%
Hoeg 2013	Retrospective cohort study	AML patients	Mean age: 68.7/ 65.4	150 (65 '09+'10)/ 53 '06+'07)	Intensive CT	Only RBC transfusion in presence of anaemia-related symptoms when Hb is between 73 g/L and 97 g/L / standard Hb trigger of 97 g/L	NM	MA+SR	90 days mortality: 20%/27% RBC use: 1.84/1.70 units per 10 patient-days PLT use: 1.41/ 0.91 units per 10 patient-days
Jansen 2004	Retrospective cohort study	Acute myeloid leukaemia. Risk classification: good (7/6); intermediate (19/ 28); poor (11/10); unknown (1/2)	Mean age: 43.2/ 42.5	84 (38/46)	Intensive CT	Hb trigger: 72-88 g/L, dependent on age and symptoms/ 96 g/L. Comparing 2 locations of same academic hospital	Mean Hb: 80/93	MA+SR	30-day mortality: 3%/2% Mean RBC use: 9.6 (SD 3.9, sem 0.6)/ 10.8 (SD 2.9, sem 0.4) units/patient/first 31 days of CT Mean PLT use: 7.5 (SD 3.8, sem 0.6)/ 8.5 (SD 4.9, sem 0.7) units/patient/first 31 days of CT Therapy response: CR 58%/59%; PR 18%/24%; NR 18%/15%
Lightdale 2012	Retrospective cohort study	First HSCT in children with hematologic malignancies (n=62, 28/34); lymphoma and solid tumour (n=50, 28/22); non-malignant hematology (n=18, 12/6); neuroblastoma (n=11, 7/4)	Median age: 6/ 6	141 (75/66)	Autologous HSCT: (26/22); allogeneic HSCT: (49/ 44)	Hb trigger: 70 g/L / 90 g/L	Median Hb level: 68/ 88	MA+SR	100 day mortality: 17.3%/25.8% Median RBC use: 3 (IQR 2.5)/ 4 (IQR 3.8)
Mear 2014	Prospective cohort study with historical control group	AML or allogeneic SCT	NM	269 (75/194)	Remission induction CT	Single/ double- unit	NM	SR. Excluded from MA (low score on comparability in NOS)	Mean RBC use: 7.92 (SD 5.39)/ 9.27 (SD 7.42)
Paananen 2009	Retrospective cohort study	Children with treatment for ALL	Median age: 4.2/5.1	40 (20/20)	Intensive CT	Hb trigger: 80 g/L / 90-100 g/L, comparing two centres	NM	MA+SR	Median RBC use: 2.5 (Q1-Q3 1-7, range 0-12)/2.5 (Q1-Q3 1.5-5.5, range 0-18) units/induction course Median PLT use: 0 (Q1-Q3 0-1.5, range 0-11)/0 (Q1-Q3 0-1, range 0-8) units/induction course

Table IV Continued									
Reference	Study design	Patients R/L	Age (yr) R/L	n R/L	Treatment R/L	RBC strategy R/L	Hb difference (g/L) R/L	Included in meta-analysis and/or systematic review	Outcome R/L
Patil 2013	Retrospective cohort study	MM patients	NM	200 (100/100)	Autologous HSCT	Hb trigger: 70 g/L + single-unit / non-specified more liberal Hb trigger + double-unit	NM	SR. Excluded from MA (low score on comparability in NOS)	100 day mortality: 3%/2% 1 year mortality: 11%/8%
Robitaille 2013	RCT	Children with AML, MDS or benign disease	Mean age: 9.3/ 14,0	6 (3/ 3)	Myeloablative BMT	Hb trigger: 70 g/L / 120 g/L	R 96/127	SR. Excluded from the MA (unclear risk of bias)	100 day mortality: 0%/0% Mean RBC use: 1.33 (SD 0.47)/ 8.67 (SD 4.50)
Webert 2008	RCT	Acute leukaemia or other hematologic malignancy	Mean age: 50.8/ 45.3	60 (29/31)	Remission induction CT (acute leukaemia) or myeloablative allogeneic SCT (haematologic malignancy)	Hb trigger: 80 g/L / 120 g/L	NM, estimated from graph: 93/122	MA+SR	Mean RBC use*:2.5 (SD 2.27)/3.25 (SD1.67) units/ patient/treatment cycle Mean PLT use*: 4.25 (SD 3.33)/4.30 (SD 3.17) units/ patient/treatment cycle
R: restrictive; L: liberal; RBC: red blood cell; Hb: haemoglobin; NM: not mentioned; SR: systematic review; MA: meta-analysis; NOS: Newcastle Ottawa assessment Scale; RCT: randomized controlled trial; (H)SCT: (hematopoietic) stem cell transplantation; SD: standard deviation; AML: acute myeloid leukaemia; ALL: acute lymphoid leukaemia; CT: chemotherapy; MDS: myelodysplastic syndrome; PLT: platelet; MM: multiple myeloma; APL: acute promyelocytic leukaemia; IQR: inter quartile range; CR:						complete remission; MRD: minimal residual disease; FCM: flow cytometry; PR: partial remission; NR: no response; BMT: bone marrow transplantation; sem: standard error of the mean. *calculated from original data provided by the authors **data on ASCT for solid tumours are excluded from our analysis			

from medians (Berger *et al*, 2012; Lightdale *et al*, 2012; Paananen *et al*, 2009). The observed reduction in blood use for restrictive strategies was a weighted mean difference of 1.40 RBC units (95% CI 0.70 to 2.09; 684 patients, figure 3). This is best interpreted as a reduction of 1.40 RBC units on average for each therapy cycle of a restrictive transfused patient compared to patients receiving transfusions more liberally. Heterogeneity between the studies was moderate ($p=0.14$, I^2 39.4%). The sensitivity analysis including observational studies with an unclear risk of bias according to the Newcastle Ottawa quality assessment scale (Bercovitz *et al*, 2011; Butler *et al*, 2015; Mear *et al*, 2014) showed similar results: WMD 1.16 (95% CI 0.66 to 1.67). The results of the studies that could not be included in the meta-analyses are presented in table IV (Allameddine *et al*, 2015; Arslan *et al*, 2004; Atilla *et al*, 2011; Hoeg *et al*, 2013; Robitaille *et al*, 2013).

Modulation of platelet transfusions. Four studies, one RCT and three observational studies, were suitable to be included into the meta-analysis to assess whether the RBC transfusion strategy could have influenced the amount of PLT transfusions (Berger *et al*, 2012; Jansen *et al*, 2004; Paananen *et al*, 2009; Webert *et al*, 2008). For two of these studies the means and SD were estimated from medians (Berger *et al*, 2012; Paananen *et al*, 2009). The weighted mean difference of platelet use between restrictive and liberal strategies was 0.16 (95% CI -0.52 to 0.83; 454 patients, figure 4). The small point estimate indicates little to no difference. However, the wide 95% confidence interval indicates limited statistical precision to support this conclusion. Heterogeneity between these studies was low ($p=0.57$, I^2 0.0%). The sensitivity analysis, including the study with an unclear risk of bias according to the Newcastle-Ottawa quality assessment scale (Butler *et al*, 2015), did not influence the results: WMD 0.06 (-0.60 to 0.72).

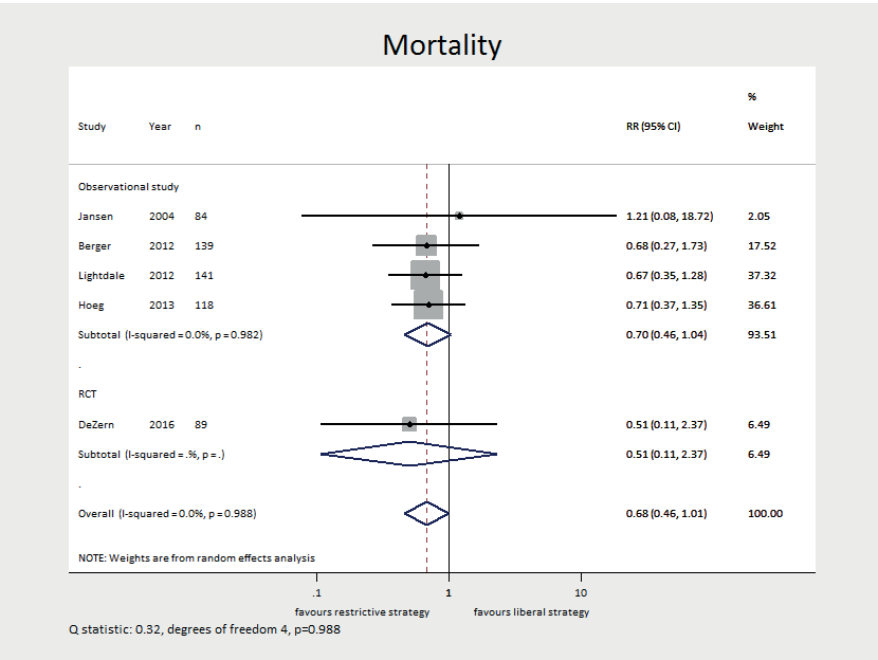


Figure 2 Forest plot mortality

Safety. Only five of the fifteen selected studies have reported on safety outcomes: cardiac events, thromboembolic events and bleeding, respectively (Berger *et al*, 2012; DeZern *et al*, 2016; Jansen *et al*, 2004; Robitaille *et al*, 2013; Webert *et al*, 2008).

One study reported data on cardiac events in AML patients in the first thirty-one days after start of combination chemotherapy, but no differences were found in incidence of cardiac arrhythmias or cardiac dysfunction between the restrictive and the liberal strategy (Jansen *et al*, 2004).

Another RCT (Robitaille *et al*, 2013) comparing a haemoglobin trigger of 70 g/L to a 120 g/L trigger to assess the effect on the length of neutropenia in children undergoing myeloablative haematopoietic SCT for AML, MDS or immune deficiency. After inclusion of six patients the study was terminated by the data safety monitoring board because of the occurrence of severe cases of veno-occlusive disease (VOD) in all of the patients assigned to the liberal RBC transfusion group. No data on thromboembolic events were reported in any of the other studies.

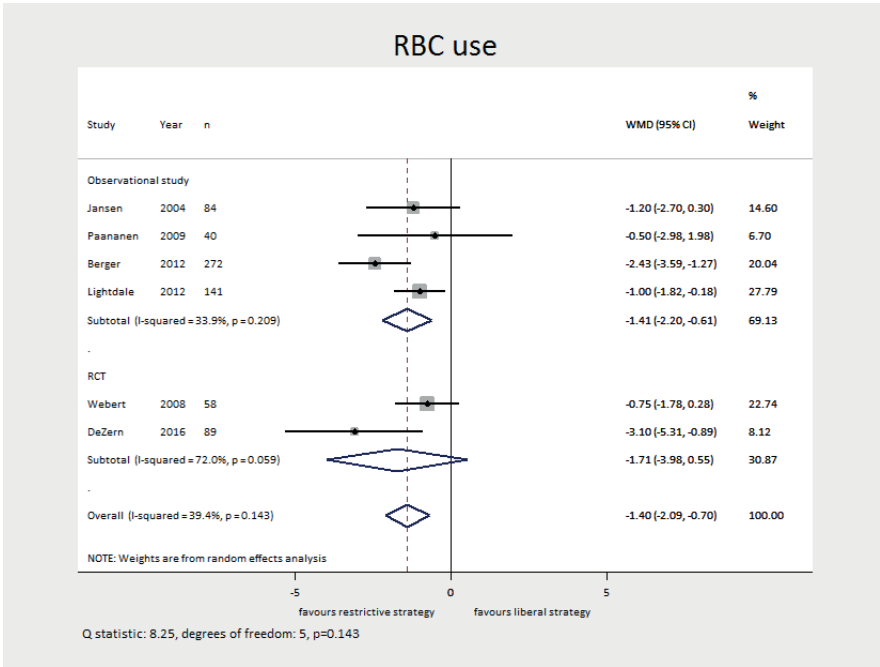


Figure 3 Forest plot RBC use

Incidence of bleeding was reported by four studies (Berger *et al*, 2012; DeZern *et al*, 2016; Jansen *et al*, 2004; Webert *et al*, 2008). No differences in the occurrence of any bleeding or clinically significant bleeding were found by a study in sixty patients between the liberal and restrictive RBC transfusion group (120 g/L versus 80 g/L), RR 1.05 (95% CI 0.87 to 1.26) and RR 0.94 (95% CI 0.69 to 1.27) (Webert *et al*, 2008). Also three other studies reported no differences in the incidence of severe bleeding comparing either a double- and single-unit RBC strategy (Berger *et al*, 2012) or lowering of the haemoglobin trigger (DeZern *et al*, 2016; Jansen *et al*, 2004) (see table IV).

Treatment response. Only two studies (DeZern *et al*, 2016; Jansen *et al*, 2004) reported on treatment response of 173 acute leukaemia patients. No differences were observed comparing restrictive with liberal RBC transfusion strategies (data depicted in table IV).

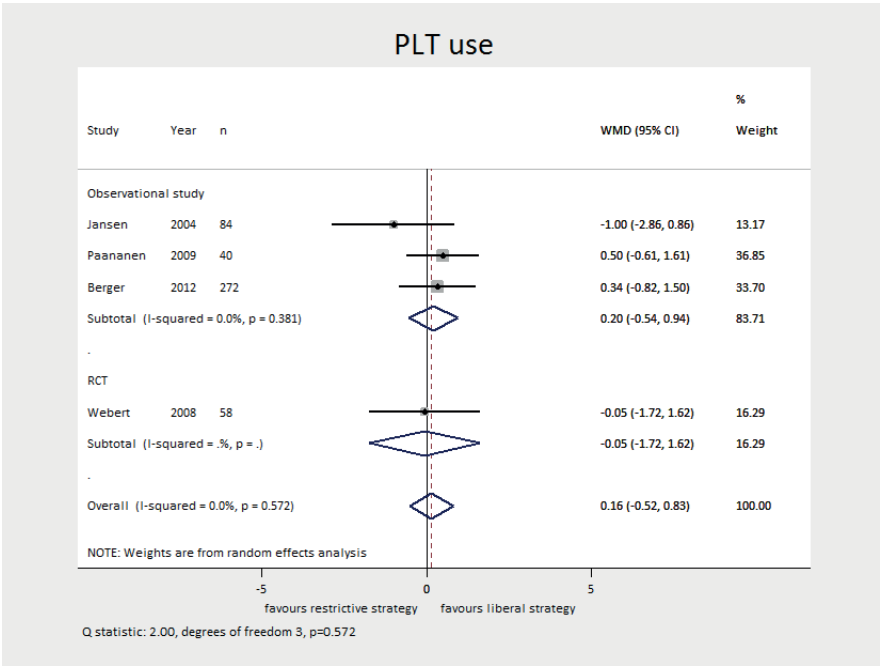


Figure 4 Forest plot PLT use

Costs. Four studies reported on the reduction of costs due to reduction of RBC transfusions (Berger *et al*, 2012; Lightdale *et al*, 2012; Paananen *et al*, 2009; Allameddine *et al*, 2015) (see table V). All studies show a considerable reduction of costs either per patient or per department.

Quality of life. None of the studies reported on quality of life.

Table V Costs		
Reference	RBC transfusion strategy	Reduction of costs due to reduction in RBC use
Allameddine 2015	Single- versus double-unit	€40,000.- per year per haematology hospital ward
Berger 2012	Single- versus double-unit	€2,534.- per patient per therapy cycle**
Lightdale 2012	Hb trigger: 70 g/L versus 90 g/L	€1,278.- per HSCT patient**
Paananen 2009	Hb trigger: 80 g/L versus 90-100 g/L	€335.- per patients per whole ALL treatment*

RBC: red blood cell; Hb: haemoglobin; ALL: acute lymphoid leukaemia; HSCT: haematopoietic stem cell transplantation. *paediatric RBC units (which are smaller and cheaper) were more often used in the restrictive group compared to the liberal group.**US dollars were converted to Euros

Discussion

The results of our meta-analysis suggest that a restrictive RBC transfusion strategy has no negative impact on all-cause mortality in haemato-oncological patients. Patients in the restrictive transfused group tended to have even lower mortality rates. The confidence interval, however, is wide; therefore more patients are needed to confirm or refute such conclusions.

Looking at mortality as an outcome, several specific possibilities of bias have to be addressed. Changes over time such as chemotherapy regimens or infection prophylaxis, which were often not stated in the included studies, may have influenced the result of the meta-analysis on mortality as an outcome. Moreover, intensive chemotherapy and the related transfusion support are mostly applied to patients that have an acceptable performance score and no severe comorbidities, therefore the results cannot be generalized to all patients. General confounding by indication, however, is unlikely since the patients in either the restrictive or liberal transfusion strategy groups in the included studies were comparable with regard to nature and/or severity of the haematological malignancy.

As expected, restrictive RBC transfusion strategies lead to a reduction in the use of RBC units. As a consequence, lower use of blood products will diminish the possible risks of adverse events like transmission of infectious diseases, transfusion

reactions, immunomodulation, and secondary iron overload. Therefore, restrictive RBC strategies will not only reduce costs for the blood products itself, but also costs for nursing and blood bank staff activities, pre-administration testing, transport, haemovigilance and the management of adverse events. The real difference in costs is, however, difficult to quantify because data on managing possible adverse events due to low haemoglobin levels as a result of a restrictive strategy are unknown.

On the other hand restrictive RBC transfusion strategies could lead to lower haematocrit levels as compared with liberal RBC transfusion strategies. While low haematocrit levels have been associated with a higher risk of bleeding (Valeri *et al*, 1998; Valeri *et al* 2011; Ho 1998) a compensatory increased need for platelet products could therefore be presumed. However, in the studies included in our systematic review, no differences were found in either incidence of bleeding events or in the use of platelet products in the more restrictive versus more liberal RBC transfusion strategies. Again, these studies lack power to draw definite conclusions.

Moreover, more restrictive transfusion strategies and thus lower haemoglobin levels could lead to decreased oxygen supply to the coronary arteries and thus theoretically to more cardiac events. Only one study with a relatively small number of patients addressed this variable as a safety outcome, but did not find a change in incidence of cardiac arrhythmia and cardiac dysfunction. Also none of the other studies reported on the incidence of myocardial events. Since reports on comorbidity at baseline are lacking in the included studies, no firm conclusions can be drawn whether more restrictive RBC transfusion strategies are also safe for haemato-oncological patients with cardiac or pulmonary comorbidities. In patients with sepsis on the intensive care unit, who are partly comparable to intensively treated haemato-oncological patients, no differences in relative risk of fatal and non-fatal myocardial infarction in restrictive versus liberal RBC transfusion strategies were reported (Holst *et al*, 2014; Holst *et al*, 2015).

Finally, lower haemoglobin levels by restrictive strategies might comprise quality of life (QoL). No studies, however, reported on differences in quality of life with regard to RBC transfusion strategies. That is a shortcoming because QoL is a common reason to initiate RBC transfusion. Only one RCT is performed addressing QoL in MDS patients comparing different haemoglobin triggers (72 g/L versus 96 g/L) (Jansen *et al*, 2005). Unfortunately, this study suffered from a too low accrual to allow definite conclusions on the effect of QoL.

In another study a strikingly increased incidence of VOD in children undergoing myeloablative bone marrow transplantation (BMT) in the liberal transfusion arm led to a preliminary termination of the study. No other studies have ever reported on haemoglobin levels as a risk factor for VOD and several other confounders might have influenced the occurrence of VOD in this myeloablative BMT setting (Robitaille *et al*, 2013).

Strengths and limitations

The strength of our analysis is that we have conducted a large literature review including both randomized controlled and observational studies. Additionally, no limitations were made on language and publication date and authors were contacted to supply missing data and to explain unclarities in their studies. Because data from RCTs on this subject is scarce, the combination with 'low risk of bias' observational studies has given us the opportunity to retrieve all relevant up-to-date data on this subject.

The limitation inherent in the present form of analyses is the heterogeneity in patients and study outcomes. Also in our study, not all data could be pooled and even although studies that included both non-malignant diseases or patients with solid tumours were excluded, some admixture still exists.

Another limitation is that not every study reported on the difference in haemoglobin levels per treatment arm. Therefore, non-compliance of haematologists to a specific RBC strategy in RCTs might have led to smaller differences in post-transfusion haemoglobin levels in both arms. Theoretically, this would only result in an underestimation of the outcome effect.

Third, despite excluding one observational study in the sensitivity analysis which had a broad definition of a restrictive strategy (up to 97 g/L) (Hoeg *et al*, 2013), an overlap in restrictive versus liberal triggers remains and could have led to smaller differences between groups. Again, this would in our opinion lead to further underestimation of the outcome effect.

Fourth, the effect of different RBC strategies on clinical outcomes in patients with pre-existing cardiac or pulmonary comorbidity could not be assessed since comorbidity at baseline was not reported in the included studies.

The final limitation involves that, for meta-analyses of continuous outcomes, mean and standard deviations are required. So, when only median and (interquartile) ranges were reported, we estimated the mean and SD by a formula which assumes

a normal data distribution to circumvent this problem (Wan *et al*, 2014). Although the best possible estimation from the available data, this, however, need not to be true in all cases.

Future research and conclusion

Fortunately, more insight in QoL and adverse events by either restrictive or liberal red blood cell transfusions in haemato-oncological patients will become more evident in the near future. Both the REDDS study in the UK (ISRCTN26088319) and the EnhanceRBC study in Canada (NCT02099669) are investigating restrictive (85-100 g/L) versus liberal (110-125 g/L and 110-120 g/L, respectively) RBC transfusion strategies in MDS patients including QoL. The EnhanceRBC study will also assess the incidence of adverse events of RBC transfusions and transfusion requirements. The Canadian TRIST study, will also investigate QoL among other clinical outcomes in patients undergoing either autologous or allogeneic HSCT allocated to a restrictive or liberal haemoglobin trigger (70 versus 90 g/L) (Tay *et al*, 2011; NCT01237639).

There are more on-going studies on RBC transfusion in haemato-oncological patients. Chantepie *et al* (2015) in France are investigating the effect of a single-versus double-unit RBC transfusion strategy on clinical outcomes in patients undergoing remission induction therapy, and autologous or allogeneic HSCT (NCT02461264). The 'REAL study' from Stanworth and colleagues, soon to be opened in the UK and Sweden, will assess the feasibility of conducting a larger RCT on the effect of different haemoglobin triggers (70 versus 90 g/L) on haemato-oncological patients, including outcomes as overall survival and QoL (personal communication).

Hence, in the near future our understanding of the effects of different RBC transfusion strategies on haemato-oncological patients will increase considerably and enable proper evidence and guideline-based use of RBCs in this patient group. Our systematic review with meta-analysis, although with inherent limitations, is the first to summarize the currently available data on RBC transfusion strategies in haemato-oncological patients as one of the most intensely transfused patient groups.

Supplementary appendix

Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.14641>

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4

BONE MARROW IRON SCORE AS AN INDICATOR FOR SECONDARY IRON OVERLOAD IN ACUTE MYELOID LEUKEMIA PATIENTS

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Abstract

Objectives: Secondary iron overload due to red blood cell transfusions (RBCT) is associated with increased morbidity and mortality. However, attention for secondary iron overload and its side effects in patients with hematological malignancies may need improvement. The aim of this study was to determine the number of transfused RBCT needed to reach a maximum bone marrow iron score (BMIS).

Methods: BMIS was independently assessed by two researchers on consecutive bone marrow samples of 35 acute myeloid leukemia (AML) patients. The slides were blinded to both researchers to prevent bias. A Kaplan-Meier survival analysis was performed for estimation of the proportion of patients reaching a maximum BMIS.

Results: In total, 141 bone marrow specimens from 35 patients were included. The median number of RBCT to reach a maximum was 20 units (range 6-42, IQR 15-26), after a mean of 1.64 chemotherapy courses (SD 0.99).

Conclusions: In conclusion, the cumulative RBCT number is associated with BMIS. Due to the considerable variation in number of RBCT to reach a maximum BMIS, BMIS instead of only considering the cumulative RBCT number, may be a valuable indicator of secondary iron overload in AML patients. BMIS could guide iron-lowering therapy and/or transfusion strategies in an early stage.

Introduction

Secondary iron overload due to multiple red blood cell transfusions (RBCT) is a potential threat to frequently transfused patients as it may cause significant organ damage e.g. to liver, heart, and endocrine organs.¹ Adverse effects of iron overload, specific for patients with hematological malignancies, include: worse survival after hematopoietic stem cell transplantation (HSCT), increased risk of bacterial and fungal infections, and impaired hematopoiesis.²⁻⁶

Despite increasing evidence for iron toxicity, monitoring and management of secondary iron overload in patients with hematological malignancies is still not common practice.⁷ This may be partly due to the imprecision of serum markers for monitoring iron overload, the invasiveness of accurate diagnostics, and the possible drawbacks of iron chelation therapy (ICT).^{8,9} A further contributing factor could be the lack of uniform guidelines on monitoring and treatment of iron overload.^{9,10}

Acute myeloid leukemia (AML) patients receive many RBCT due to disease-related as well as therapy-related anemia. Bone marrow aspirates are routinely performed for assessing disease status and therapy response. Estimation of bone marrow iron contents using Perl's iron staining is an easy to apply technique and has been demonstrated to strongly predict body iron contents.^{11,12} As such, we hypothesize that bone marrow iron score (BMIS) is associated with the cumulative RBCT number and could be an indicator of secondary iron overload in AML patients. The aim of this study was to quantify the number of RBCT needed to reach a maximum bone marrow iron score (BMIS).

Materials and methods

Patients with either AML 'de novo' or a myelodysplastic syndrome with refractory anemia with excess blasts (MDS-RAEB), treated according to current intensive AML treatment regimens, were identified from the records of the Leiden University Medical Centre (2007-2016), the Netherlands. The study protocol has been approved by the medical ethical committee of the Leiden University Medical Center and is in accordance with the current version of the Helsinki declaration. Inclusion criteria were: ≥ 20 RBCT, a complete transfusion history, and availability of bone marrow aspirate samples. The 20 RBCT threshold was chosen since several guidelines suggest iron overload development after only 20 RBCTs.^{6,10} Moreover, we do not expect to find a completely different rate of iron loading in

patients who ultimately receive more or less than 20 RBCTs, so this will not likely lead to selection bias. Likewise, we stained consecutive bone marrow aspirate samples, therefore a maximum BMIS before this threshold could also be observed. When the quality of bone marrow specimens was poor due to technical issues, patients were excluded from the study.

Consecutive bone marrow samples of the individual patients, from diagnosis until the end of study, were stained with a standardized Perl's staining.¹¹ BMIS were independently assessed by two trained researchers according to a pre-specified protocol (supplementary Table 1 and 2). The maximum BMIS is 11 corresponding with a very strongly positive BMIS. In order to prevent observer bias, both researchers were blinded for all clinical information. A weighted Cohen's kappa was calculated to assess the inter-rater reliability. A time-to-event analysis with RBCT as the time component was performed to assess the median number of RBCT needed to reach the maximum BMIS. Patients not reaching a maximum BMIS at the end of follow-up were censored. We further compared age and gender of patients who reached a maximum BMIS after a small number of RBCT with patients who reached a BMIS after a higher number of RBCT (p10 versus p90 and the lowest versus highest quartile).

Results

Evaluating 110 consecutive AML patients (2007-2016), 39 received less than 20 RBC units, 10 had an incomplete transfusion history, and from 13 patients insufficient bone marrow specimens were available due to missing baseline specimens. From the remaining 48 patients, 35 patients were selected on the basis of the quality and optimal timing of bone marrow specimens relative to received RBCT. In total, 141 bone marrow specimens (median: 4 per patient [range 2-8]) from 35 patients were included (Table 1). Ninety-one percent of patients had AML 'de novo', whereas 9% had MDS-RAEB. The mean age was 57.8 years (SD 14.0), 51% were males. Twenty-nine patients (83%) underwent HSCT. The cumulative number of received red blood cell transfusions at each bone marrow assessment is provided in supplementary table 3. The inter-rater agreement for assessing BMIS was found to be 'excellent' ($\kappa = 0.89$; SE: 0.06). Six patients were censored from the survival analysis for not reaching a maximum BMIS at the end of follow-up.

Table 1 Patient characteristics

	Mean ($\pm$ SD)	N	%
Gender			
Female		17	49
Male		18	51
Age (years)	57.8 ($\pm$ 14.0)		
Diagnosis			
AML 'de novo'		32	91
MDS-RAEB		3	9
Number of RBCTs received	45.8 ($\pm$ 18.7)		
20 – 40		16	46
> 40		19	54
HSCT			
yes		29	83
no		6	17
HSCT type			
Autologous		1	3
Allogeneic		28	97
-non myeloablative conditioning		20	71
-myeloablative conditioning		7	25
-reduced intensity conditioning		1	4

SD: standard deviation; n = amount of individuals; % = percentage of total cohort (n=35); MDS-RAEB: myelodysplastic syndrome, refractory anemia with excess blasts; RBCT: red blood cell transfusion; HSCT: hematopoietic stem cell transplantation.

The median number of RBCT to reach a maximum BMIS was 20 units (range 6-42, IQR 14-26), after a mean of 1.64 chemotherapy courses (SD 0.99) (Figure 1). The median number of RBCT to reach the maximum BMIS was not statistically different for males (18.0) and females (20.0 [p=0.32]). It required 78.5 days (median) to reach a maximum BMIS (IQR 54-152). Supplementary Figure 1 shows the spaghetti plot in which the slopes of individual patients are displayed. There were no statistically significant differences in age and gender between the patients who reached a maximum BMIS after a small or high number of RBCT (p10 versus p90 and lowest versus highest quartile).

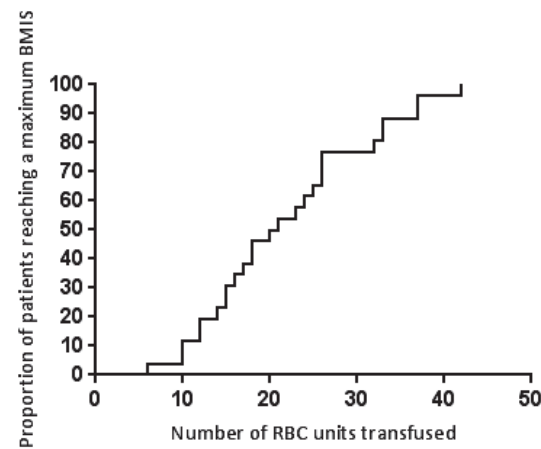


Figure 1 Kaplan Meier curve for proportion of patients reaching a maximum BMIS.

Curve of proportion of patients reaching a maximum bone marrow iron score (corresponds to score 11: very strongly positive bone marrow iron score, see supplementary table 2) according to the number of RBC units transfused.

Discussion

Our study demonstrates that BMIS on routinely collected bone marrow aspirates is easy to perform and has a high inter-rater reliability. Therefore, BMIS can provide the clinician a quick, easy, and cheap indication of the degree of secondary iron overload in AML patients.

The number of received RBCT of 20 units is in line with other studies indicating that secondary iron overload could occur after transfusion of only 20 RBC units.^{6,10} The number of RBCT for reaching a maximum BMIS varied considerably across patients. Cell lysis, due to chemotherapy, radiation or leukemia itself, are additional risk factors for developing iron overload. So determining BMIS, instead of only considering the cumulative RBCT transfusions, is of extra value.

Intriguingly, some patients reach a maximum BMIS after only six RBCT, while others need up to 42 RBCT. Gender and age did not explain this difference in this patient group. Other factors like genetic factors in iron metabolism and transfusion intensity may play a role.^{12,13}

A previous study, evaluating BMIS on bone marrow biopsies of ten patients with hematological malignancies who underwent HSCT, showed a strong correlation between BMIS ($r=0.8$, $p=0.006$) and biochemical bone marrow and liver iron contents ($r=0.82$, $p=0.004$).¹⁴ Another, more recent, study in 125 patients who underwent HSCT for acute leukemia or lymphoma, suggested that increased BMIS prior to allogeneic HSCT was likewise strongly correlated with elevated serum ferritin levels and poorer survival ($p<0.001$).¹⁵ However, in clinical practice, bone marrow aspirates are more frequently performed than bone marrow biopsies due to improved diagnostic methods like immuno-phenotyping and molecular diagnostics, and it is also less invasive for the patients. So, performing BMIS on bone marrow aspirates as we did in our study, is probably more applicable to the current clinical practice.

Currently, tissue biopsy and T2* MRI are regarded as the most specific and sensitive diagnostic tests for detecting iron overload. Yet, these tests are not often performed because of invasiveness (biopsy), unavailability, or expense (T2* MRI). Instead serum ferritin has been used as a surrogate marker for iron overload, however, its property of being an acute phase protein decreases its specificity to detect iron overload in the presence of concurrent infection and/or inflammation, which is often the case in AML patients. Furthermore, no convincing evidence exists regarding the use of serum ferritin to monitor secondary iron overload in AML patients.^{9,10} BMIS could therefore be an attractive alternative test to demonstrate presence of secondary iron overload in AML patients in an early stage.

Treatment of secondary iron overload in AML patients remains, however, challenging. Hemoglobin levels are often not sufficiently high to perform phlebotomies, while drug interactions or adverse events could hamper the use of iron chelation therapy (ICT).⁸ ICT in AML patients has to be the subject of further study and should at least show a clear benefit on clinical outcomes like survival with an acceptable toxicity profile. Alternatively, more restrictive RBCT strategies may help prevent secondary iron overload. Such restrictive strategies do not seem to have a direct negative impact on clinical outcomes in patients with hematological malignancies.^{16,17} BMIS could guide iron-lowering therapy and/or transfusion strategies in AML patients already in an early stage.

Limitations

Since this is a retrospective study, we were dependent on the number of available bone marrow specimens. A maximum BMIS could have occurred earlier in time than observed in our study. So the median number to reach a maximum BMIS could even be smaller in clinical practice.

Due to paucity of other measures of clinically relevant iron overload, we were unable to correlate these biochemical markers like serum ferritin or T2* MRI to BMIS. However, as mentioned above, the clinical relevance of the currently used measures of iron overload needs confirmation. Rather, BMIS is expected to be more specific for iron overload than serum ferritin and is easier, cheaper, and more accessible to perform than T2* MRI. Moreover, assessment of bone marrow iron scores is a direct measure of iron at tissue level, while T2*MRI is an indirect measure, which so far has been validated for liver and cardiac iron content, but not for bone marrow iron content. Future studies should address the relation between T2* MRIs and bone marrow iron scores. A strong association between the two would in our opinion further increase the value of measurement of iron in bone marrow samples, especially since this method is much more convenient and cost efficient than T2* MRI.

In conclusion, the cumulative RBCT number is associated with BMIS. A maximum BMIS was found after a median of 20 RBC units, however, variation exists. We therefore expect added value of the use of BMIS in clinical practice. BMIS could serve as an indicator for secondary iron overload in AML patients and may guide treatment. Further studies to relate BMIS to the currently regarded most specific tests and clinical outcomes are warranted.

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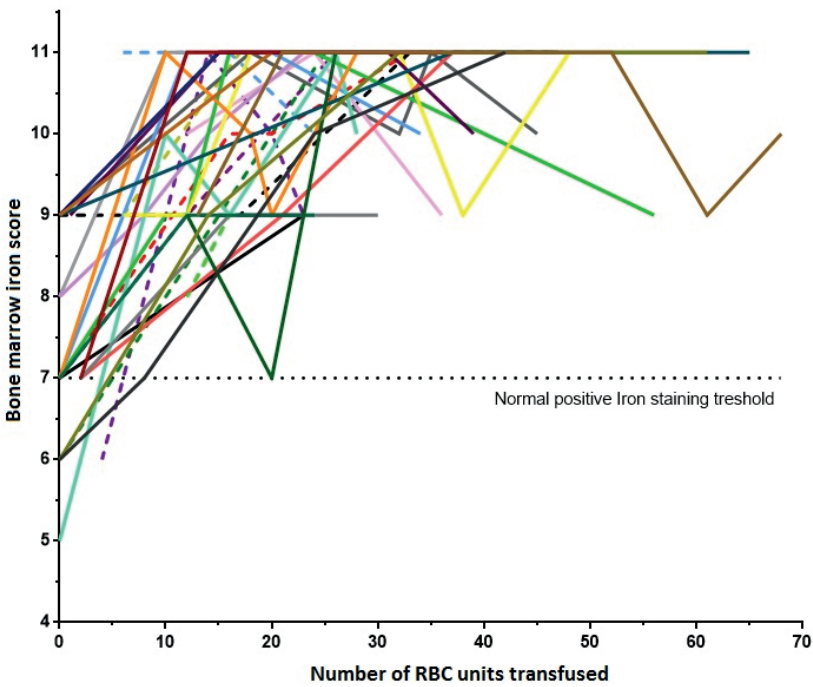
Supplementary files

Supplementary Table 1	Perls' iron staining protocol
- Fix bone marrow specimens with cooled methanol for 5 minutes at room temperature. - Mix Potassiumhexacyanoferate(II)-Trihydrate 5% and 0.2N Hydrochloric acid in a 1:1 ratio 30 minutes prior to usage. - Remove methanol and apply the staining solution on the bone marrow specimens and incubate for 30 minutes at room temperature. - Rinse the bone marrow specimens with tap water. - Perform counterstaining with Nuclear Fast Red 0.1% for 15 minutes at room temperature in closed petri dishes in a flow cabinet. - Rinse bone marrow specimens with tap water and air-dry at room temperature.	

Supplementary Table 2 Scoring system Perls' staining	
Overall score	Definition and magnification
Negative	No detectable iron (100x)
Trace	No detectable iron (16x), iron detectable (100x)
Weakly positive	Little iron detectable (16x), iron detectable (40x)
Normal positive	Iron detectable (16x)
Strongly positive	Iron clearly detectable (16x)
Very strongly positive = maximum BMIS	Iron clearly detectable in all particles, parts without staining are hardly present (16x)
Perl's staining scoring system. The categories: negative, trace, weakly positive, normal positive, strongly positive and very strongly positive corresponds to a bone marrow iron score of 1, 3, 5, 7, 9, and 11 , respectively. Scores in between categories were rated in between, for example, a strongly to very strongly scores corresponds to a bone marrow iron score of 10.	

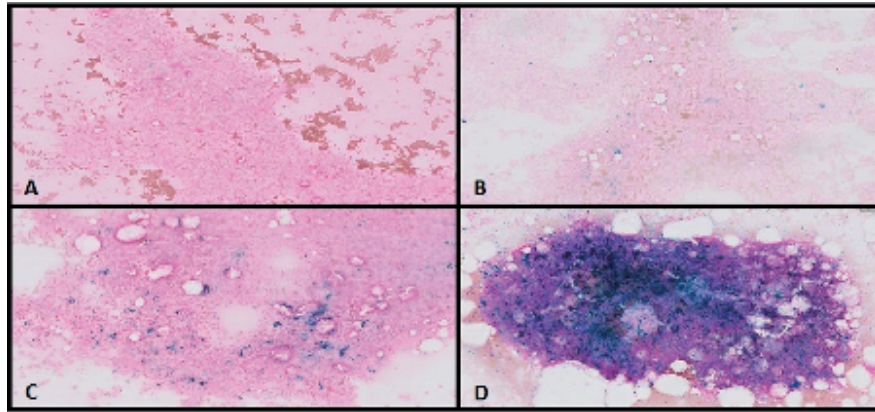
Supplementary table 3								
Patient	RBCT at BM 1	RBCT at BM 2	RBCT at BM 3	RBCT at BM 4	RBCT at BM 5	RBCT at BM 6	RBCT at BM 7	RBCT at BM 8
1	0	12	16	48				
2	1	15	17	31	39			
3	2	17	21	23				
4	2	16	30					
5	0	12	14	22	26			
6	2	21	23	37	41	56		
7	0	10	18	20	28			
8	0	6	12	18	32	38	48	
9	0	10	12	16	24	34	56	
10	0	10	16	26	28			
11	0	12	18	20	21	34		
12	0	10	16	41	45			
13	0	8	14	23	25	35		
14	0	12	24	36				
15	0	18	32	35	38	40		
16	0	10	16	26	34			
17	0	17	33	46	67	69		
18	0	16	20	33				
19	0	4	12	24	28			
20	6	17	23	25				
21	0	12	16	18	31			
22	0	25	29	47				
23	6	16	24					
24	4	14	20	23				
25	0	8	26					
26	0	16	23					
27	0	2	8	12	20	22	24	42
28	1	3	13	19	21	23	27	40*
29	2	12	24	36				
30	0	20	22					
31	0	14	32	38	61			
32	12	20	22	26				
33	0	12	24					
34	0	37	49	65				
35	0	15	21	23	29	41	47	

Cumulative number of red blood cell transfusions received at each bone marrow biopsy for each individual patient. Patient 28 had >8 bone marrow biopsies performed: the cumulative number of RBCT at bone marrow biopsy 9, 10, 11, and 12 were 42, 52, 61, and 68, respectively. RBCT=Red Blood Cell Transfusion, BM=Bone Marrow biopsy.



Supplementary Figure 1 Spaghetti plot demonstrating the trajectories of bone marrow iron scores over time in relation to the received number of red blood cell transfusions for individual acute myeloid leukemia patients.

Bone marrow iron scores: 5=weakly positive; 6=weakly to normal positive; 7=normal positive; 8=normal to strongly positive; 9=strongly positive; 10=strongly to very strongly positive; 11= very strongly positive/ maximum bone marrow iron score. RBC= Red Blood Cell.



Supplementary Figure 2 Examples bone marrow iron staining categories.

Panel A Weakly positive (bone marrow iron score 5); panel B normal positive (bone marrow iron score 7); panel C strongly positive (bone marrow iron score 9); panel D very strongly positive/maximum bone marrow iron score (bone marrow iron score 11).

TOXIC IRON SPECIES AND OXIDATIVE STRESS IN LOWER-RISK MYELOYDYSPLASTIC SYNDROME PATIENTS: NATURAL COURSE AND EFFECTS ON OUTCOME

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Abstract

Background: Lower-risk MDS (LR-MDS) patients are prone to iron toxicity due to red blood cell transfusions (RBCT) and/or ineffective erythropoiesis. Non-transferrin bound iron (NTBI) and labile plasma iron (LPI) are toxic iron species that may mediate cellular damage via oxidative stress. The pathophysiology of iron metabolism and its relation with RBCT in LR-MDS patients is not completely understood. The aim of the present study is to describe the natural course of iron and oxidative stress parameters and its effect on outcome in LR-MDS patients.

Methods: Iron and oxidative stress parameters were analyzed from consecutive samples of LR-MDS patients included in the EUMDS registry. Descriptive statistics were used with stratification for transfusion-dependency and ring sideroblast status. Linear quantile mixed models were used for assessing the change of parameters over time and time-dependent cox models for survival analysis.

Results: 1,040 samples of 256 LR-MDS patients were analyzed. The transfusion-dependent group with ring sideroblasts had the highest levels and steepest increase of serum ferritin, transferrin saturation, LPI, and NTBI. Hepcidin levels were high in transfusion-dependent patients, however, strikingly lower in transfusion-dependent patients with ring sideroblasts, reflecting ineffective erythropoiesis. Malondialdehyde levels, as a marker of oxidative stress, increased over time with the steepest increase in transfusion-dependent patients. Elevated LPI levels were associated with inferior overall survival with an adjusted hazard ratio of 2.7 (95% CI 1.5-5.0).

Conclusion: Iron toxicity is associated with inferior survival in LR-MDS patients. RBCT and iron chelation strategies may be optimized in order to improve supportive care in LR-MDS patients.

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of acquired clonal hematopoietic stem cell disorders, characterized by abnormal differentiation and maturation of hematopoietic cells, bone marrow failure, and genetic instability with an enhanced risk of progression to acute myeloid leukemia.¹ Currently, red blood cell transfusions (RBCT) remain the cornerstone of supportive care in these patients, despite other treatment options such as erythroid stimulating and immune-modulating agents.² Transfusion dependency in lower-risk MDS (LR-MDS) patients has been described in literature as having a negative impact on patient outcome, mainly due to its association with severe bone marrow failure.³ However, iron toxicity due to frequently administered RBCT or as a result of ineffective erythropoiesis and subsequent suppression of hepcidin production, may be an additional negative prognostic factor in itself.⁴⁻⁷ Patients with ring sideroblasts (MDS-RS) are of particular interest because of their pronounced ineffective erythropoiesis.⁸⁻¹² In recent years, much progress has been made in unraveling the iron metabolism with the discovery of key regulators of this process like hepcidin and erythroferrone,^{13, 14} however, the pathophysiology of iron metabolism in MDS is not completely understood.

Increasing evidence indicates that iron toxicity can arise much earlier after start of transfusion-dependency than previously believed.^{4, 7, 15} Oxidative stress occurs when reactive oxygen species (ROS) production exceeds antioxidant enzyme systems, and is believed to play a role in cellular damage.¹⁶⁻¹⁸ Malondialdehyde (MDA) resulting from lipid peroxidation of polyunsaturated fatty acids, is a biomarker of oxidative stress.^{18, 17} In vitro data suggest a constitutional defective ROS balance in MDS, acute myeloid leukemia, and other malignancies, indicating a possible role of oxidative stress in the etiology of MDS.^{16, 19} Exceedingly high ROS levels are associated with iron toxicity and may promote stem cell exhaustion and subsequent apoptosis.²⁰

Several reports link oxidative stress to iron toxicity, disease development and progression towards acute myeloid leukemia in MDS patients. However, less is known about the prognostic impact of ROS in MDS patients.^{7, 21}

Accordingly, in vitro and in vivo studies on iron chelation or antioxidant therapies²²⁻²⁴ demonstrated reduced levels of intra- and extracellular free iron species and a reduction of oxidative stress parameters.²⁵ EUMDS registry studies also showed that overall survival was superior for chelated patients even after correction for all relevant confounding variables, when compared with non- chelated patients.²⁶

Summarizing, the pathophysiology of iron metabolism and its relation with cumulative RBCT in LR-MDS patients is not completely understood. Improved insights in levels and roles of key players of iron metabolism and oxidative stress during treatment with transfusions in the various MDS subtypes may provide leads for novel diagnostic and iron reducing treatment strategies.

The aim of the present research is twofold: 1) to describe iron and oxidative stress parameters over time in patients with LR-MDS and 2) to assess the effect of iron and oxidative stress parameters on overall and progression-free survival.

Methods

Study design and participants

The EUMDS registry prospectively collects observational data on LR-MDS patients from 146 centers in 16 countries in Europe and Israel as of December 2007. Patients with newly diagnosed MDS, according to the World Health Organization 2001 classification, were included, restricted to patients with a low or intermediate-1 score according to the international prognostic scoring system (IPSS).²⁷ IPSS was the prognostic indicator at the start of the registry, in accordance with the currently used prognostic score, the revised IPSS (IPSS-R) was reconstructed afterwards. The ethics committees of all participating centers approved the protocol and all patients provided written informed consent.

Clinical data were collected at baseline and at each 6-monthly outpatient routine follow-up visit via a bespoke web-based database on: comorbidity, red blood cell transfusions, concomitant treatment, quality of life, peripheral blood counts, bone marrow pathology and cytogenetics, and MDS progression to higher risk MDS or acute myeloid leukemia (AML). Serum samples were collected prospectively at registration and with six-monthly intervals from 259 patients included in six countries participating in this study. Conventional iron parameters such as serum ferritin levels, serum iron, transferrin saturation were measured in all patients. For this sub study we analyzed hepcidin, growth differentiation factor 15 (GDF15), soluble transferrin receptor (sTfR), non-transferrin bound iron (NTBI), LPI, and MDA. Subjects were prospectively followed until death, loss to follow-up, or withdrawal of informed consent.

Biochemical assays

All iron parameters were measured centrally at the department of Laboratory Medicine of the Radboudumc, Nijmegen, The Netherlands, which is a worldwide

reference laboratory with respect to diagnostics and analysis of iron disorders. Serum samples were collected just prior to transfusion in transfusion-dependent patients and stored at -80 degrees Celcius before shipping on dry ice to the central lab. Serum ferritin, iron, and transferrin saturation (TSAT) were measured with routine assays. Elevated TSAT levels were defined as a TSAT >80% as formation of toxic iron species is known to occur from this TSAT level.⁴ Analysis of serum NTBI (measured in May 2014, September 2015, March 2016, and June 2018) was based on the chelation-ultrafiltration-detection approach. NTBI is mobilized in the serum by weak iron-mobilizing chelators. The chelated NTBI is separated from transferrin-bound iron by ultrafiltration and detected by colorimetry.²⁸ The lower limit of detection (LLOD) of the assay is 0.47 $\mu\text{mol/L}$. The reference range as assessed in 33 healthy volunteers is <0.47 to 1.98 $\mu\text{mol/L}$.

The LPI analysis (measured in May 2014, September 2015, March 2016, and June 2018) was based on the measurement of the redox-active and rapidly chelatable fraction of NTBI. The assay measures iron catalyzed radical generation in the presence of a low ascorbate concentration. Fluorogenic redox sensitive probe dihydrorhodamine-123 was used to measure radical generation; iron catalyzed radical generation was calculated by subtracting the radical generation in the presence of 50 $\mu\text{mol/L}$ of the iron chelator deferiprone.²⁹ The LLOD of the assay is 0.24 $\mu\text{mol/L}$. The reference range as assessed in 33 healthy volunteers is <0.24 to 1.00 $\mu\text{mol/L}$. In order to evaluate the upper limit of the reference range of our assay, we measured LPI in 50 individuals (≥ 60 years) included in the Lifelines project. Lifelines is a large multi-disciplinary prospective population-based cohort study which examines, in a unique three-generation design, the health and health-related behaviors of persons living in the north of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical, and psychological factors which contribute to the health and disease of the general population, with special focus on multi-morbidity and complex genetics.³⁰ LPI levels were analyzed in an age-matched population with and without anemia in order to obtain age-matched reference values for LPI.

The hepcidin-25 assay (performed in February 2001, March 2016, and September 2015) is based on a combination of weak cation exchange chromatography and time-of-flight mass spectrometry, using a hepcidin analogue as internal standard.³¹ Median reference values in a Dutch reference population were 4.5 nmol/L for men, 2.0 nmol/L for premenopausal women, and 4.9 nmol/L for postmenopausal women. (www.hepcidinanalysis.com, accessed on May 1st 2018).

GDF15 levels (measured in 2012) were analyzed by using a DuoSet (R&D Systems, Minneapolis, MN) enzyme-linked immunosorbent assay for human GDF15 following the manufacturer's protocol. Serum sTfR was measured immunonephelometrically by using polystyrene particles coated with a monoclonal antibody specific to human sTfR on a BN II System (Dade Behring Marburg GmbH, Marburg, Germany).

Malondialdehyde (MDA) was measured in duplicate by spectrofluorometry.³² The LLOD of the assay is 0.12 $\mu\text{mol/L}$. The reference range of serum MDA as measured in an adult Dutch population is 0.16 to 0.64 $\mu\text{mol/L}$. Reference values in an elderly population are lacking. Therefore, MDA was measured in an age-matched reference population (≥ 60 years) with and without anemia in the Lifelines cohort ($n=319$). The LLOD of this assay was 0.22 $\mu\text{mol/L}$.

Statistical analysis

The Spearman rank test was used to evaluate the association between the iron parameters. We stratified the results by transfusion-dependency per visit and the presence of ring sideroblasts. When evaluating the temporal changes in iron parameters, we excluded patients from the timepoint they received iron chelation therapy. A linear quantile mixed model, a linear mixed model comparing predefined quantiles, in our case medians, instead of means, was used. The betas (median change per visit) and corresponding 95% confidence intervals (CI) were reported.

In a sensitivity analysis we stratified the patients according to expected classical iron overload, according to one of the predefined criteria (RBCT intensity of ≥ 1 RBC unit/month during a six-month period between visits or serum ferritin level ≥ 1000 $\mu\text{g/L}$).²⁶

Overall survival was defined as the time from MDS diagnosis to death or, in the case of progression-free survival, to date of progression or death; patients still alive at the end of follow-up were censored. Time-dependent Kaplan Meier curves and cox proportional hazards models were used to evaluate the effect of toxic iron parameters on overall and progression-free survival. Covariates included in the cox model for survival were: age at diagnosis and IPSS-R at baseline.

Missing data were presumed to be missing completely at random, because of the fact that in a random number of patient samples did not contain enough serum to perform all iron and oxidative stress parameters.

All analyses were undertaken in Stata 15 (StataCorp, College Station, TX).

Results

Patients

In total, 259 consecutive patients from the six participating countries, were included in this sub study. Over five six-monthly visits, 1,040 samples were collected (visit 1: 256; visit 2: 251; visit 3: 222; visit 4: 176; visit 5: 135). The median age at diagnosis was 74 years (range 37 to 95); 66% were males. Patients were diagnosed with the following WHO 2001 MDS subtypes: RCMD 44.5%, RARS 21.9%, RA 17.6%, RAEB-1 6.3%, RCMD-RS 3.9%, 5q-syndrome 3.9%, and MDS-U 2.0% (Table 1). The IPSS-R risk groups were: very low/low 76.2%, intermediate 9%, high/very high 1.6%, and unknown 13.3%, mainly on account of cytogenetics not being performed. The majority of the included patients were, at diagnosis, transfusion-independent without ring sideroblasts (non-RS, TI) (55.9%), 18.8% were transfusion-independent with ring sideroblasts (RS, TI), 18.4% were transfusion-dependent without ring sideroblasts (non-RS, TD), and 7% were transfusion-dependent patients with ring sideroblasts (RS, TD). Table 1 further describes the patient characteristics, including Karnofsky performance status, comorbidity index, the use of iron chelation, erythroid stimulating agents, and hypomethylating agents, overall survival, and causes of death. The median follow-up time was 6.6 years (95% CI 5.9 to 7.0).

Reference values as assessed in the Lifelines cohort

The LPI value was <0.24 $\mu\text{mol/L}$ in all 50 age-matched elderly individuals included in the Lifelines cohort, irrespective of gender, age, and presence of anemia. MDA reference values were measured in 319 age-matched individuals included in the Lifelines cohort of which 166 individuals had anemia and 153 individuals had normal haemoglobin values. The MDA levels of anemic individuals were: median 0.78 $\mu\text{mol/L}$, mean 0.92 $\mu\text{mol/L}$, range 0.22 to 4.50; for non-anemic individuals MDA levels were: median 0.91 $\mu\text{mol/L}$, mean 0.99 $\mu\text{mol/L}$, range 0.22 to 3.10. Mean MDA levels tend to increase with age, till the age of 85 with a mean MDA level at 65 years of 0.88 $\mu\text{mol/L}$ that increased to 1.06 $\mu\text{mol/L}$ at the age of 85. The MDA reference range for the non-anemic group (-2 and $+2$ SD) was 0.22 to 2.33 $\mu\text{mol/L}$.

Correlation of iron parameters

LPI is positively correlated with transferrin saturation (TSAT) ($r=0.15$, $p<0.001$, figure 1, panel A). The LPI values increase exponential at TSAT values above 80%. This effect is most pronounced in the transfusion-dependent groups, but also observed in the RS, TI group. NTBI is similarly correlated with TSAT ($r=0.56$, $p<0.001$, figure 1, panel B), however a more gradual increase of NTBI is observed at increasing TSAT levels as opposed to LPI.

Table 1 Baseline characteristics		
		N (%)
Total		256 (100.0)
Sex:	Males	169 (66.0)
	Females	87 (34.0)
Age:	35-44	2 (0.8)
	45-54	7 (2.7)
	55-64	51 (19.9)
	65-74	78 (30.5)
	75+	118 (46.1)
	Mean (sd)	72.1 (9.5)
	Median (min – max)	74.0 (37.0 - 95.0)
MDS Diagnosis:	RCMD	114 (44.5)
	RARS	56 (21.9)
	RA	45 (17.6)
	RAEB-1	16 (6.3)
	RCMD-RS	10 (3.9)
	5q-Syndrome	10 (3.9)
	MDS-U	5 (2.0)
Group	non-RS,TI	143 (55.9)
	non-RS,TD	47 (18.4)
	RS,TI	48 (18.8)
	RS,TD	18 (7.0)
IPSS-R category	very low/low	195 (76.2)
	intermediate	23 (9.0)
	high/very high	4 (1.6)
	Not known	34 (13.3)
IPSS category	Low risk	144 (56.3)
	Intermed-1	75 (29.3)
	Intermed-2	1 (0.4)
	Not known	36 (14.1)
Karnofsky performance status	Able to work and normal activity	193 (75.4)
	Unable to work	48 (18.8)
	Unable to care for self	1 (0.4)
	Not known	14 (5.5)

Table 1 Continued		
		N (%)
Comorbidity index	Low risk	158 (61.7)
	Intermediate risk	79 (30.9)
	High risk	19 (7.4)
EQ-5D index score	Mean (sd)	0.77 (0.24)
	Median (p10 – p90)	0.80 (0.52 - 1.00)
ESA	No	159 (62.1)
	Yes	97 (37.9)
Iron chelation	No	241 (94.1)
	Yes	15 (5.9)
	<i>desferoxamine</i>	5 (2.0)
	<i>deferiprone/deferasirox</i>	11 (4.3)
Hypomethylating agents	No	245 (95.7)
	Yes	11 (4.3)
Overall survival	Median (95% CI)	4.8 (3.9 – not reached)
Cause of death	MDS unrelated	15 (34.1)
	MDS related	24 (54.5)
	Unknown	5 (11.4)
Follow up time (censored last EUMDS visit)	Median (95% CI)	6.6 (5.9 - 7.0)

sd: standard deviation; MDS: myelodysplastic syndrome; RCMD: refractory cytopenia with multilineage dysplasia; RARS: refractory anemia with ring sideroblasts; RA: refractory anemia; RAEB: refractory anemia with excess blasts; RCMD-RS: refractory cytopenia with multilineage dysplasia with ring sideroblasts; MDS-U: myelodysplastic syndrome unspecified; RS: ring sideroblasts; TI: transfusion-independent; TD: transfusion-dependent; IPSS(-R): (revised) international prognostic scoring system; EQ5D: EuroQoL five dimension scale; ESA: erythroid stimulating agents.

NTBI and LPI are positively correlated ($r=0.19$, $p<0.001$). The correlation was the strongest in the RS, TD group ($r=0.53$, $p<0.001$), but also observed in the non-RS, TD group ($r=0.22$, $p<0.001$).

MDA was not correlated with serum ferritin, LPI, and TSAT ($r=-0.03$, $p=0.481$; $r=0.05$, $p=0.281$; $r=0.06$, $p=0.204$, respectively), and weakly correlated with NTBI ($r=0.09$, $p=0.069$). MDA was negatively correlated with the hemoglobin level

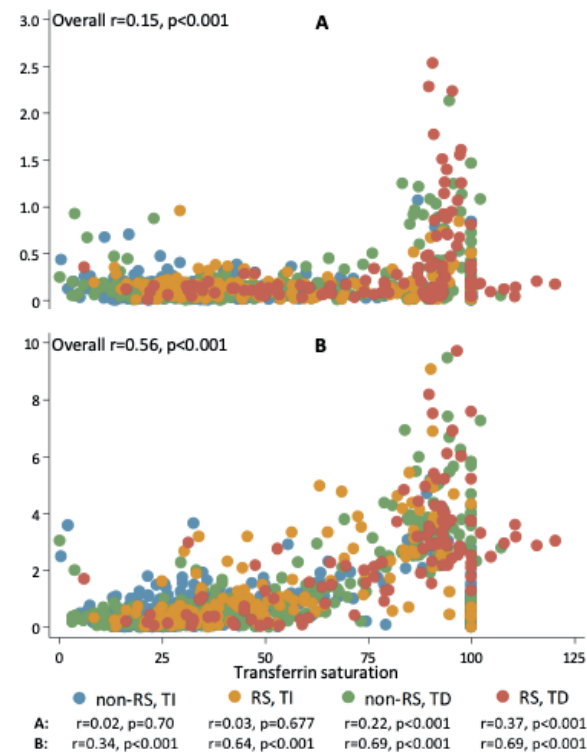


Figure 1 Scatter plots of labile plasma iron and transferrin saturation (A) and non-transferrin bound iron and transferrin saturation (B).

RS: ring sideroblasts; transfusion-independent; TD: transfusion-dependent.

($r=-0.1$, $p=0.033$). This effect was most prominent in the non-RS, TI and RS, TD groups. MDA was not significantly correlated with transfusion density ($r=0.05$, $p=0.254$).

Temporal changes in iron parameters by transfusion-dependency and presence of ring sideroblasts

Serum ferritin levels were elevated in all subgroups with a mean value of 858 $\mu\text{g/L}$ at visit 5. The highest serum ferritin levels were observed in the RS, TD group (mean at visit 5: 2092 $\mu\text{g/L}$), followed by the non-RS, TD group (mean at visit 5: 1399 $\mu\text{g/L}$, table 2). Serum ferritin increases significantly per visit in the RS, TD group (beta 454.46 $\mu\text{g/L}$, 95% CI 334.65 to 574.27, $p<0.001$, table S1), but not in the other groups.

All subgroups, except for the non-RS, TI, have elevated TSAT values. TSAT is most markedly increased in the RS, TD group with a mean TSAT of 70% at visit 1 and 88% at visit 5 (table 2). In both transfusion-dependent groups the increase in median per visit was 2.47% (95% CI 0.46 to 4.47) for the non-RS, TD group and 3.66% (95% CI 0.91 to 6.4) for the RS, TD group (table S1).

LPI was only elevated in the RS, TD group with a mean value of 0.59 $\mu\text{mol/L}$ at visit 5. Per visit LPI increased with 0.01 $\mu\text{mol/L}$ (95%CI 0 to 0.02, $p=0.01$) for all subgroups. NTBI, on the other hand, is elevated in all subgroups, with again the highest values in the RS, TD group (mean 3.09 $\mu\text{mol/L}$ at visit 5). The non-RS, TD group and the RS, TI group had similarly increased mean NTBI values at visit 5 (1.85 and 1.82 $\mu\text{mol/L}$, respectively) (table 2). The increase in median NTBI level was 0.27 $\mu\text{mol/L}$ (95% CI 0.15 to 0.39) per visit in the non-RS, TD group (table S1).

Hepcidin levels were the highest for the non-RS, TD group (16.0 nmol/L at visit 5). Interestingly, hepcidin levels were lower in the RS, TD group, probably reflecting residual ineffective erythropoiesis (table 2). Median hepcidin levels increase over time with 0.57 nmol/L (95% CI 0.34 to 0.79) per visit for all subgroups ($p<0.001$).

GDF-15 values, a protein produced by erythroid precursors, involved in the communication between bone marrow and liver in case of increased erythropoiesis by inhibiting hepcidin synthesis, were increased in all subgroups (table 2). The RS subgroups have higher GDF-15 levels compared to the non-RS groups, reflecting the increased erythropoiesis in both RS groups. For RS, TI versus non-RS, TI: 3885 versus 2468 pg/ml at visit 5 and for RS, TD versus non-RS, TD: 5460 versus 3620 pg/ml at visit 5 (table 2). The increase in median GDF-15 levels per visit for all subgroups is 229.08 pg/ml (95% CI 133.23 to 423.93) (table S1).

The mean sTfR levels were within the reference range in all subgroups except for the RS-TI group in which showed increased levels, reflecting an increased erythropoiesis (table 2). Interestingly, a decrease of sTfR over time was observed in the RS, TD group (-0.12 mg/l per visit, 95% CI -0.2 to -0.04), whereas the sTfR levels increased over time in the RS-TI group (0.11 mg/l per visit, 95% CI 0.07 to 0.15) and remained stable in the non-RS TD and non-RS TI groups (table S1).

MDA levels were within the reference range (0.22 to 2.33 $\mu\text{mol/L}$) in the non-RS, TI group and above the upper limit of the reference range in all other subgroups with the highest levels observed in the RS, TD group (mean 8.9 $\mu\text{mol/L}$ at visit 5) (table 2). Median MDA levels did not change significantly over time in all subgroups (table S1). Because the number of MDS patients with MDA levels above the upper limit of the

Table 2 Iron sub study parameters for the first five visits										
	Visit									
	1		2		3		4		5	
Total patients	N	Mean (sd)	N	Mean (sd)	N	Mean (sd)	N	Mean (sd)	N	Mean (sd)
	256		251		222		176		135	
Ferritin										
Non-RS, TI	252	488 (588)	213	605 (747)	183	702 (1057)	150	822 (1093)	115	858 (1275)
Non-RS, TD	140	280 (318)	99	274 (308)	86	251 (256)	60	262 (371)	51	263 (223)
RS, TI	46	935 (863)	55	1145 (1070)	53	1297 (1510)	47	1083 (1128)	32	1399 (1519)
RS, TD	48	505 (567)	35	356 (283)	26	343 (244)	21	493 (416)	16	441 (314)
	18	919 (559)	24	1096 (650)	18	1627 (1151)	22	2104 (1505)	16	2092 (1918)
TSAT										
Non-RS, TI	254	50 (29)	217	50 (29)	191	49 (31)	158	52 (31)	120	54 (31)
Non-RS, TD	143	44 (29)	100	43 (28)	91	43 (29)	66	46 (31)	52	41 (28)
RS, TI	46	57 (28)	57	54 (30)	55	55 (33)	48	49 (31)	34	56 (32)
RS, TD	48	53 (26)	36	52 (27)	26	41 (20)	22	56 (29)	17	57 (27)
	17	70 (29)	24	71 (26)	19	73 (33)	22	76 (23)	17	88 (15)
LPI										
Non-RS, TI	255	0.17 (0.25)	216	0.18 (0.45)	187	0.17 (0.22)	152	0.17 (0.23)	116	0.22 (0.35)
Non-RS, TD	143	0.14 (0.12)	100	0.11 (0.12)	88	0.11 (0.06)	62	0.11 (0.11)	52	0.12 (0.08)
RS, TI	46	0.19 (0.33)	57	0.30 (0.77)	54	0.21 (0.31)	48	0.18 (0.17)	32	0.25 (0.33)
RS, TD	48	0.16 (0.18)	35	0.15 (0.15)	26	0.15 (0.11)	21	0.16 (0.16)	16	0.11 (0.07)
	18	0.41 (0.61)	24	0.27 (0.50)	19	0.36 (0.37)	21	0.36 (0.47)	16	0.59 (0.69)
NTBI										
Non-RS, TI	254	1.03 (1.28)	216	1.15 (1.41)	188	1.18 (1.53)	152	1.18 (1.46)	116	1.50 (1.78)
Non-RS, TD	141	0.64 (0.69)	100	0.72 (0.73)	88	0.58 (0.52)	62	0.53 (0.60)	52	0.69 (0.76)
RS, TI	47	1.44 (1.62)	56	1.50 (1.84)	55	1.49 (1.53)	48	1.28 (1.55)	32	1.85 (2.06)
RS, TD	48	1.20 (1.33)	36	1.15 (1.30)	26	1.44 (1.97)	21	1.67 (1.86)	16	1.82 (1.86)
	18	2.51 (2.09)	24	2.15 (1.89)	19	2.69 (2.44)	21	2.36 (1.68)	16	3.09 (2.15)
hepcidin										
Non-RS, TI	246	8.6 (12.0)	206	9.4 (11.4)	188	10.2 (13.4)	154	10.2 (12.1)	116	8.9 (11.1)
Non-RS, TD	140	6.2 (6.2)	98	6.4 (6.7)	89	5.6 (4.8)	62	6.5 (6.8)	52	5.2 (5.0)
RS, TI	44	20.4 (21.7)	53	18.8 (16.6)	55	21.0 (19.3)	48	15.7 (14.2)	32	16.0 (13.5)
RS, TD	44	5.0 (5.8)	32	3.9 (3.5)	26	3.7 (2.8)	22	3.8 (3.7)	16	3.9 (3.5)
	18	7.8 (5.2)	23	8.4 (6.6)	18	9.3 (10.0)	22	15.0 (16.9)	16	11.7 (17.0)
GDF-15										
Non-RS, TI	99	3051 (3034)	92	3309 (2892)	76	3372 (2993)	76	4216 (4058)	62	3516 (2935)
Non-RS, TD	53	2428 (1846)	40	2318 (1726)	32	2382 (2053)	24	2756 (2842)	25	2468 (2008)
RS, TI	13	2467 (1365)	20	3488 (2149)	20	3644 (2525)	26	4176 (2412)	18	3620 (2283)
RS, TD	26	4578 (4917)	19	3113 (2678)	16	4083 (3714)	13	4129 (4449)	8	3885 (2860)
	7	3187 (1418)	13	6369 (4706)	8	5226 (4546)	13	7082 (6508)	11	5460 (4613)
sTfR										
Non-RS, TI	99	1.5 (0.9)	92	1.7 (1.1)	77	1.7 (1.2)	76	2.0 (2.7)	61	1.6 (0.9)
Non-RS, TD	53	1.5 (0.9)	40	1.7 (1.2)	32	1.8 (1.5)	24	1.6 (0.8)	25	1.5 (0.7)
RS, TI	13	1.3 (0.8)	20	1.4 (1.2)	21	1.2 (0.9)	26	2.4 (4.5)	17	1.5 (1.2)
RS, TD	26	1.8 (0.8)	19	1.9 (0.8)	16	2.0 (0.7)	13	2.3 (0.8)	8	2.0 (0.7)
	7	1.3 (1.1)	13	1.9 (1.2)	8	1.6 (0.9)	13	1.6 (1.2)	11	1.6 (1.2)
MDA										
Non-RS, TI	121	3.0 (11.3)	101	2.0 (3.8)	74	1.4 (1.8)	68	2.1 (3.4)	48	3.9 (7.1)
Non-RS, TD	45	2.2 (3.7)	31	1.8 (2.4)	22	2.1 (2.7)	14	1.5 (1.6)	13	1.4 (0.8)
RS, TI	31	1.5 (1.8)	32	1.3 (0.9)	25	0.9 (0.5)	23	1.6 (3.2)	12	2.6 (2.3)
RS, TD	34	6.2 (20.6)	22	2.0 (3.4)	16	1.0 (0.5)	18	3.0 (4.7)	12	3.5 (7.9)
	11	1.2 (0.9)	16	3.6 (8.0)	11	1.8 (2.2)	13	2.6 (2.8)	11	8.9 (11.1)

sd: standard deviation; RS: ring sideroblasts; TI: transfusion-independent; TD: transfusion-dependent; TSAT: transferrin saturation; LPI: labile plasma iron; NTBI: non-transferrin bound iron; GDF-15: growth differentiation factor 15; sTfR: soluble transferrin receptor; MDA: malondialdehyde.

sd: standard deviation; RS: ring sideroblasts; TI: transfusion-independent; TD: transfusion-dependent; TSAT: transferrin saturation; LPI: labile plasma iron; NTBI: non-transferrin bound iron; GDF-15: growth differentiation factor 15; sTfR: soluble transferrin receptor; MDA: malondialdehyde.

reference range was limited, we describe differences between the MDS subgroups and between MDA levels below and above the median MDA level (1 $\mu\text{mol/L}$) at visit 1 separately in table 3.1 and 3.2 respectively. MDA levels were more frequently above 1 $\mu\text{mol/L}$ in patients with MDS subtype: RA, RCMD-RS, and 5q- syndrome. The MDA levels at diagnosis were markedly higher in the RCMD-RS group compared to all other MDS subtypes (Table 3.1). Not surprisingly, in the group with MDA levels above the median, the transfusion density was markedly higher as compared with the group with a MDA level below the median. Comorbidity index was lower in the group with MDA levels above the median as compared with the patients with a MDA level below the median, whereas quality of life was similar for both groups (Table 3.2). In order to provide more information on the temporal changes of MDA over time we analyzed the differences in MDA levels at baseline and at the highest MDA level after baseline, grouped by transfusion status and presence of ring sideroblasts (Table 3.3). Patients who were transfusion independent and became transfusion dependent were categorized separately. Overall MDA levels increase over time ($p<0.0001$). The steepest increase was observed in the patients who became transfusion dependent after baseline in both RS and non-RS groups, with the highest median MDA levels over time in the RS TD group.

Table 3.1 MDA by MDS diagnosis at first visit			
	N	Mean (sd)	Median (min - max)
Total	121	3.0 (11.3)	1.0 (0.0 - 120.0)
RA	17	3.8 (5.5)	1.3 (0.0 - 18.0)
RARS	38	2.5 (4.3)	1.1 (0.5 - 23.0)
RCMD	51	1.2 (1.0)	0.9 (0.0 - 4.7)
RCMD-RS	7	18.3 (44.8)	1.3 (0.6 - 120.0)
RAEB-1	4	1.0 (0.5)	0.9 (0.6 - 1.7)
5q-Syndrome	4	4.1 (4.6)	2.2 (1.1 - 10.9)

Table 3.2 Baseline characteristics comparing patients with MDA levels below or above the median*		
	MDA below median (<=1 $\mu\text{mol/L}$)	MDA above median (>1 $\mu\text{mol/L}$)
Number of patients	61	60
Mean age (sd), years	74.2 (8.6)	70.0 (11.1)
		p=0.02
Sex N (%), males	45 (73.8)	31 (51.7)
		p=0.012
MDS diagnosis:		
RA	4 (6.6)	13 (21.7)
RARS	19 (31.1)	19 (31.7)
RCMD	32 (52.5)	19 (31.7)
RCMD-RS	3 (4.9)	4 (6.7)
RAEB-1	3 (4.9)	1 (1.7)
5q-Syndrome	0 (0.0)	4 (6.7)
		p=0.021
Group:		
non-RS, TI	22 (36.1)	23 (38.3)
Non-RS, TD	17 (27.9)	14 (23.3)
RS, TI	17 (27.9)	17 (28.3)
RS, TD	5 (8.2)	6 (10.0)
		p=0.94
Transfusion density:		
Mean (sd)	0.54 (1.28)	1.14 (4.25)
Median (min-max)	0.00 (0.00 - 8.30)	0.00 (0.00 - 30.44)
0	45 (73.8)	41 (68.3)
>0 - <0.75	1 (1.6)	3 (5.0)
≥0.75 - ≤1.75	8 (13.1)	10 (16.7)
>1.75	7 (11.5)	6 (10.0)
		p=0.688
IPSSR category:		
very low/low	45 (73.8)	47 (78.3)
Intermediate	8 (13.1)	2 (3.3)
high/very high	3 (4.9)	1 (1.7)
Unknown	5 (8.2)	10 (16.7)
		p=0.098

Table 3.2 Continued

	MDA below median ($\leq 1\mu\text{mol/L}$)	MDA above median ($> 1\mu\text{mol/L}$)
IPSS category:		
Low risk	7 (11.5)	11 (18.3)
Intermed-1	31 (50.8)	31 (51.7)
Intermed-2	22 (36.1)	18 (30.0)
Unknown	1 (1.6)	0 (0.0)
		p=0.516
Karnofsky performance status:		
Able to work and normal activity	3 (4.9)	2 (3.3)
Unable to work	0 (0.0)	0 (0.0)
Unable to care for self	20 (32.8)	9 (15.0)
Unknown	38 (62.3)	49 (81.7)
		p=0.060
Comorbidity index:		
Low risk	34 (55.7)	37 (61.7)
Intermediate risk	22 (36.1)	20 (33.3)
High risk	5 (8.2)	3 (5.0)
		p=0.700
EQ-5D index score (N):	51	31
Mean (sd)	0.73 (0.29)	0.80 (0.18)
Median (p10-p90)	0.80 (0.33 - 1.00)	0.77 (0.62 - 1.00)
		p=0.28
EQ-5D VAS score (N):	58	56
Mean (sd)	68.88 (20.92)	74.84 (16.83)
Median (p10-p90)	75.00 (30.00 - 90.00)	75.00 (50.00 - 100.00)
		p=0.0972
ESA use during observation period (%):	21 (34.4)	26 (43.3)
		p=0.315
Iron chelation use during observation period (%):	5 (8.2)	5 (8.3)
		p=0.978
Hypomethylating agent use during observation period (%):	2 (1.9)	0 (0.0)
		p=0.644
Overall survival Median (95% CI):	4.0 (3.1 - 7.1)	7.1 (5.1 - .)

Table 3.2 Continued

	MDA below median ($\leq 1\mu\text{mol/L}$)	MDA above median ($> 1\mu\text{mol/L}$)
Cause of death:	35 (100.0)	27 (100.0)
MDS unrelated	10 (28.6)	7 (25.9)
MDS related	17 (48.6)	13 (48.1)
Unknown	8 (22.9)	7 (25.9)
		p=0.952
*at first visit		
MDA: malondialdehyde; RA: refractory anemia; RARS: refractory anemia with ring sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; RCMD-RS: : refractory cytopenia with multilineage dysplasia with ring sideroblasts; RAEB: refractory anemia with excess blasts; RS: ring sideroblasts; TI: transfusion-independent; TD: transfusion-dependent; sd: standard deviation; IPSS(-R): (revised) international prognostic scoring system; EQ5D: EuroQoL five dimension scale; ESA: erythroid stimulating agent; CI: confidence interval.		

Table 3.3 MDA at baseline and highest available measurement after baseline by ring sideroblast and transfusion status

		MDA at baseline		Highest MDA level		P ¹
		N	Mean (sd)	N	Mean (sd)	
Total	132	2.8 (10.7)	1.0 (0.0 - 120.0)	132	3.2 (5.7)	<0.0001
non-rs, ti	32	1.9 (3.2)	1.0 (0.0 - 18.0)	32	2.2 (3.0)	0.0741
non-rs, ti-td	17	1.8 (2.5)	0.9 (0.5 - 10.9)	17	4.1 (7.2)	0.0351
non-rs, td	31	1.6 (1.8)	1.0 (0.3 - 9.9)	31	1.4 (1.1)	0.5112
rs, ti	29	6.8 (22.3)	1.0 (0.5 - 120.0)	29	3.5 (6.0)	0.0727
rs, ti-td	10	2.0 (1.4)	1.7 (0.6 - 4.3)	10	10.7 (12.0)	0.0284
rs, td	13	1.4 (1.4)	1.1 (0.1 - 4.9)	13	2.1 (2.3)	0.0041

¹ Wilcoxon matched-pairs signed-ranks test

Temporal changes in iron parameters in patients with expected classical iron overload

Median serum ferritin, TSAT, NTBI, LPI, and hepcidin levels were notably higher in the iron overload group when compared with the non-iron overload group (table S2), especially in the group with ring sideroblasts in combination with iron overload (data not shown). Interestingly, MDS patients with ring sideroblasts and iron overload have lower hepcidin levels than patients without ring sideroblasts but with iron overload, reflecting ineffective erythropoiesis. Median TSAT, NTBI, ferritin, and hepcidin levels also show a significant increase over time (table S2).

Median TSAT, LPI, NTBI, ferritin, and hepcidin levels increase when the transfusion density dose increases with the highest values for the transfusion density category of >1.75 units/month (table S3). TSAT increased significantly over time in the mid and high dose density groups, LPI levels remained stable over the groups in the three dose density groups, NTBI increased significantly over time in the non-transfused and in the mid and high dose density groups, whereas ferritin levels showed an overall increase with statistical significance in the high dose density group. Furthermore, hepcidin levels increased over time in the high density group. Median MDA levels were stable over time, however, it must be noted that the MDA group by dose density is small.

Overall survival

Figure 2 demonstrates a Kaplan-Meier curve for overall survival, stratified by LPI above or below the lower limit of detection (LLOD) and transfusion status as time-varying variables. The patients who are transfusion-dependent with elevated LPI levels have inferior overall survival compared to the other subgroups. The patients who are transfusion-independent with a LPI level below the LLOD have the best overall survival. The corresponding Cox-model shows an adjusted hazard ratio (HR) for overall survival, corrected for age at diagnosis and IPSS-R category, of 2.7 (95% CI 1.5 to 5.0) for the elevated LPI group (table 4). With the transfusion-independent group with a LPI value <LLOD as a reference, the HR for overall survival in the transfusion-independent group with elevated LPI was 4.5 (95% CI 1.4 to 13.9), for the transfusion-dependent group without elevated LPI: 3.9 (95% CI 1.5 to 10.4), and for the transfusion-dependent group with elevated LPI: 6.7 (95% CI 2.5 to 17.6). Interestingly, having an elevated LPI level influenced the HR for survival more than expected on basis of the transfusion status. High LPI levels in transfusion-independent patients were mostly observed in patients with ring sideroblasts.

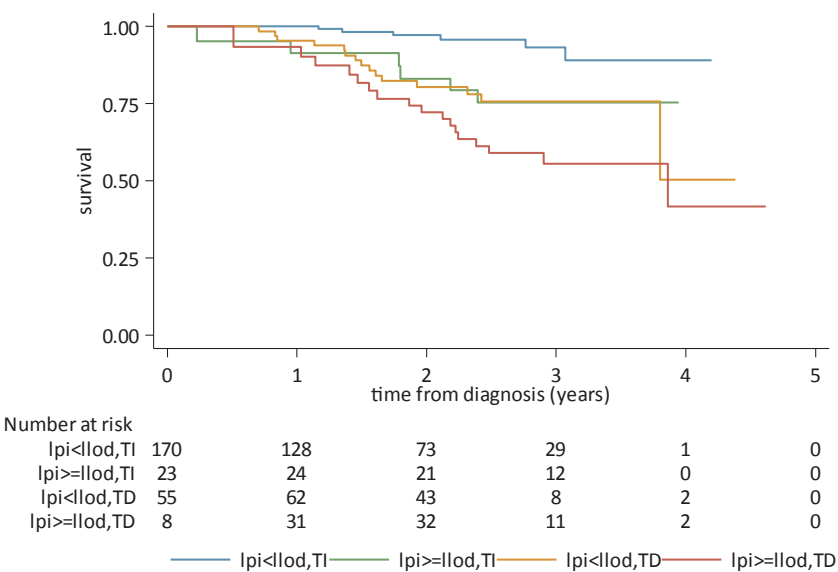


Figure 2 Kaplan Meier curve overall survival stratified by labile plasma iron above or below the lower limit of detection and transfusion status as time-dependent variables.

LPI: labile plasma iron; LLOD: lower limit of detection; TI: transfusion-independent; TD: transfusion-dependent.

The adjusted HR for overall survival for elevated NTBI was 1.6 (95% CI 0.8 to 3.1, p=0.17) (table 5). Transfusion-independent patients with normal NTBI levels have superior overall survival when compared with the other subgroups, who have significantly increased HRs for overall survival (table 5).

Elevated TSAT (>80%) alone did not influence overall survival with an adjusted HR of 1.4 (95% CI 0.7 to 2.5, p=0.33) (table S4). Transfusion-dependency influenced the survival negatively. Similarly, when we analysed TSAT as a continuous variable, elevated TSAT alone did not influence overall survival independent of transfusion-dependency (p=0.195). However, when repeated the analysis (TSAT <80 and ≥80 and continuously) in the whole EUMDS registry, TSAT alone did influence OS with an adjusted HR of 2.1 (95% CI 1.6 to 2.7, p<0.001) and 1.009 (95% CI 1.004 to 1.014, p<0.001) respectively. Transfusion-dependent patients with a TSAT ≥80% had the worst overall survival with an adjusted HR of 4.2 (95% CI 2.9 to 5.9, p<0.001).

Table 4 Cox model of overall survival by labile plasma iron level (above or below the lower limit of detection) and transfusion status as time-varying variables

	Unadjusted		Adjusted ¹	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
Labile plasma iron < LLOD	1	-	1	-
Labile plasma iron ≥ LLOD	2.8 (1.5 - 5.1)	0.001	2.7 (1.5 - 5.0)	0.001
Labile plasma iron < LLOD, TI	1	-	1	-
Labile plasma iron ≥ LLOD, TI	4.1 (1.3 - 12.9)	0.01	4.5 (1.4 - 13.9)	0.01
Labile plasma iron < LLOD, TD	4.9 (1.9 - 12.7)	0.001	3.9 (1.5 - 10.4)	0.006
Labile plasma iron ≥ LLOD, TD	8.3 (3.2 - 21.4)	<0.001	6.7 (2.5 - 17.6)	<0.001

¹adjusted for age at diagnosis and IPSS-R category
IPSS-R: revised international prognostic scoring system; CI: confidence interval; LLOD: lower limit of detection; TI: transfusion-independent; TD: transfusion-dependent

Table 5 Cox model of overall survival by non-transferrin bound iron and transfusion status as time-varying variables

	Unadjusted		Adjusted ¹	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
Non-transferrin bound iron normal ²	1	-	1	-
Non-transferrin bound iron elevated ²	2.0 (1.1 - 3.6)	0.03	1.6 (0.8 - 3.1)	0.17
Non-transferrin bound iron normal, TI	1	-	1	-
Non-transferrin bound iron elevated, TI	4.6 (1.5 - 14.5)	0.01	4.5 (1.4 - 14.4)	0.01
Non-transferrin bound iron normal, TD	6.1 (2.6 - 14.6)	<0.001	5.2 (2.1 - 12.6)	<0.001
Non-transferrin bound iron elevated, TD	5.3 (2.1 - 13.3)	<0.001	3.7 (1.4 - 9.9)	0.01

¹adjusted for age at diagnosis and IPSS-R category; ²above or below upper limit of reference range.
CI: confidence interval; TI: transfusion-independent; TD: transfusion-dependent

Elevated MDA levels alone (above the median of 1.0 μmol/L) were not associated with inferior overall survival in this patient population, adjusted HR 0.9 (95% CI 0.4 to 2.4, p=0.86) (table S5).

Progression-free survival

In line with the effect of LPI on overall survival, progression-free survival is significantly inferior in transfusion-dependent patients with LPI levels above the LLOD (HR 9.2, 95% CI 3.8 to 22.5, p<0.001) (table S6).

Discussion

The results of this study suggest that patients with myelodysplastic syndrome (MDS) who are transfusion dependent and have a MDS subtype with ring sideroblasts (RS) have the highest levels for markers that reflect iron toxicity compared to transfusion-independent patients and patients without ring sideroblasts. Likewise, hepcidin levels were the highest in the transfusion-dependent, non-RS group, but importantly, hepcidin levels were lower in the transfusion-dependent patients with ring sideroblasts. Despite the excess of iron administered through red blood cell transfusions, hepcidin levels are lower than expected, thereby increasing the dietary uptake of iron from the gut and release from the reticuloendothelial system. Transfusion dependency is a known risk factor for iron toxicity, however, ineffective erythropoiesis in RS subgroups evidently leads to additional iron toxicity possibly leading to increased morbidity and even mortality. Therefore, transfusion-dependent MDS patients with ring sideroblasts should be closely monitored for signs of iron toxicity and treated accordingly.

Labile plasma iron (LPI) and non-transferrin bound iron (NTBI) are positively correlated with transferrin saturation (TSAT) with the highest levels occurring at TSAT levels above 80%. LPI and NTBI are currently not routinely measured in clinical practice, however, high TSAT levels may indicate the presence, but not the height of LPI and NTBI (>LLOD) in a relatively simple and inexpensive way.

Our data suggest that LPI levels above the LLOD are associated with inferior overall and progression-free survival, irrespective of transfusion status. This underlines the hypothesis that iron toxicity occurs much earlier than previously assumed. This highlights the importance of rational red blood cell transfusion strategies in lower-risk MDS patients. Several trials addressing red blood cell transfusion strategies in this patient group are ongoing. The optimum between quality of life and physical activity on the one hand and iron toxicity on the other

hand, is a topic for future research. Another open question is whether iron chelation therapy should be initiated earlier in the treatment course of transfusion-dependent MDS patients. Whether early initiation of iron chelation therapy influences clinical outcome in MDS patients is the subject of ongoing clinical trials. Another topic for further research is the use of hepcidin agonists. In RS patients hepcidin agonists could reduce iron uptake from the gut and release of iron from the reticuloendothelial system in order to prevent iron toxicity.

Iron toxicity is associated with oxidative stress, which occurs when reactive oxygen species (ROS) production exceeds antioxidant enzyme systems. ROS are believed to play a role in cellular damage.^{7, 16} Malondialdehyde (MDA), resulting from lipid peroxidation, is a biomarker of oxidative stress, which is associated with mutagenesis and cell death.¹⁶⁻¹⁸ Overall MDA levels increased significantly over time in our patient group. The steepest increase was observed in the patients who became transfusion-dependent after baseline in both RS and non-RS groups, with the highest median MDA levels over time in the RS TD group. Median overall survival was inferior in the patients with an elevated MDA, however, this was mostly attributed to the patient's transfusion status. High MDA levels in transfusion-dependent patients could lead to organ damage by iron toxicity as well as mutagenesis and clonal instability possibly resulting in a higher risk for progression to higher risk MDS or AML.

Strengths and limitations

The strength of this study is that data and blood samples from low-risk MDS patients through Europe were prospectively collected. The samples were tested for iron and oxidative stress parameters in one central reference laboratory with a dedicated iron expertise center. Another strong point is the assessment of reference ranges for LPI and MDA for our assays in an elderly reference population, as those data were not available from literature.

A limitation of this study is that the measurement of MDA levels may be subject to analytical and pre-analytical disturbances.³³ We analyzed MDA with the same badges of reagents in controlled laboratory circumstances in order to prevent analytical disturbances. Pre-analytical disturbances due to problems with sample handling, processing, and storage of the blood samples collected at multiple sites cannot be excluded. Measurement of oxidative stress is thereby challenging. Better techniques and stringent sample processing to measure oxidative stress may increase knowledge on the role of oxidative stress on MDS in the future.

In conclusion, our study demonstrates that iron toxicity, as defined by the presence of LPI, is associated with inferior overall and progression-free survival in lower-risk MDS patients. The cumulative dose of red blood cell transfusions and additionally important, the presence of ineffective erythropoiesis, contribute to iron toxicity and needs to be prevented as it is associated with increased morbidity and mortality. More restrictive red blood cell transfusion strategies and effective iron chelation therapy could prevent or reverse these unwanted effects.

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Supplemental methods

Analysis of malondialdehyde

Malondialdehyde (MDA) a biomarker of lipid peroxidation, indicative of oxidative stress, was measured in lithium heparin anti-coagulated plasma, or standard solution was added to 1mL reagents consisting of 10 mmol/L 2-thiobarbituric acid in phosphate buffer (0.1 mol/L, pH3). The solution was vortexed and incubated for one hour at 96°C. Thereafter, samples were placed on ice for 5minutes after which 2 mL of butanol was added. The mixture was shaken twice for one minute to extract TBA-MDA adduct and then centrifuged at 1500x g for 5 minutes at 4°C. For fluorometric measurement of the supernatant a Shimadzu RFF500 spectro-fluorometer was used. The results were quantified by comparison with the standard curve, obtained with 1,1,3,3-tetramethoxy-propane, ranging from 0-10 µmol/L. The CV for this assay was <10% for both intra- and interassay variations. (Conti 1991).

Table S1 Iron and oxidative stress parameters for the first five visits
(median, p10 and p90) coefficients from linear quantile regression

	Visit						Visit				Beta (95% CI)	p
	1		2		3		4		5			
	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)		
Total patients	256		251		222		176		135			
Ferritin (µg/L)	252	305 (58 - 1078)	213	357 (69 - 1427)	183	293 (61 - 1794)	150	349 (69 - 2322)	115	374 (61 - 2560)	122.03 (99.1 - 144.96)	<0.001
Non-RS, TI	140	188 (47 - 577)	99	202 (40 - 541)	86	177 (36 - 584)	60	181 (45 - 471)	51	206 (51 - 603)	-2.83 (-494.73 - 489.08)	0.99
Non-RS, TD	46	673 (106 - 1982)	55	883 (109 - 2432)	53	809 (97 - 2554)	47	673 (120 - 2372)	32	858 (180 - 3169)	328.88 (-992.37 - 1650.13)	0.63
RS, TI	48	326 (123 - 1108)	35	271 (91 - 751)	26	275 (107 - 796)	21	388 (175 - 999)	16	359 (191 - 818)	-8.27 (-95.09 - 78.55)	0.85
RS, TD	18	889 (108 - 1382)	24	1116 (230 - 1856)	18	1439 (518 - 2622)	22	2081 (223 - 3560)	16	1178 (474 - 4657)	454.46 (334.65 - 574.27)	<0.001
TSAT (%)	254	39 (19 - 100)	217	41 (19 - 97)	191	37 (16 - 100)	158	40 (19 - 100)	120	42 (21 - 100)	1.5 (0.26 - 2.74)	0.02
Non-RS, TI	143	34 (18 - 100)	100	33 (18 - 100)	91	33 (17 - 100)	66	34 (17 - 100)	52	30 (17 - 100)	0.28 (-1.12 - 1.68)	0.7
Non-RS, TD	46	51 (26 - 100)	57	54 (16 - 96)	55	42 (14 - 100)	48	37 (16 - 100)	34	53 (20 - 100)	2.47 (0.46 - 4.47)	0.02
RS, TI	48	44 (24 - 95)	36	46 (24 - 95)	26	37 (22 - 73)	22	56 (22 - 92)	17	51 (26 - 93)	1.31 (-0.24 - 2.87)	0.1
RS, TD	17	84 (31 - 94)	24	78 (31 - 94)	19	90 (16 - 102)	22	81 (45 - 100)	17	93 (59 - 100)	3.66 (0.91 - 6.4)	0.01
LPI (µMol/L)	255	0.12 (0.03 - 0.31)	216	0.11 (0.03 - 0.27)	187	0.12 (0.03 - 0.34)	152	0.12 (0.02 - 0.30)	116	0.12 (0.03 - 0.67)	0.01 (0 - 0.02)	0.01
Non-RS, TI	143	0.12 (0.02 - 0.20)	100	0.08 (0.02 - 0.19)	88	0.11 (0.03 - 0.19)	62	0.09 (0.02 - 0.18)	52	0.11 (0.04 - 0.20)	0 (-0.01 - 0.01)	0.84
Non-RS, TD	46	0.09 (0.02 - 0.34)	57	0.12 (0.04 - 0.76)	54	0.16 (0.04 - 0.36)	48	0.15 (0.02 - 0.44)	32	0.13 (0.03 - 0.88)	0.02 (-0.02 - 0.06)	0.33
RS, TI	48	0.12 (0.02 - 0.33)	35	0.13 (0.02 - 0.25)	26	0.13 (0.03 - 0.34)	21	0.11 (0.03 - 0.30)	16	0.10 (0.01 - 0.20)	0 (-0.01 - 0.01)	0.81
RS, TD	18	0.17 (0.08 - 1.77)	24	0.13 (0.06 - 0.39)	19	0.18 (0.04 - 1.14)	21	0.16 (0.07 - 1.25)	16	0.26 (0.01 - 1.60)	0.07 (-0.02 - 0.16)	0.11

Table S1 Continued

	Visit						Visit				Beta (95% CI)	p
	1		2		3		4		5			
	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)		
NTBI (μMol/L)	254	0.53 (0.10 - 2.92)	216	0.57 (0.13 - 2.94)	188	0.56 (0.12 - 3.33)	152	0.53 (0.10 - 3.24)	116	0.66 (0.15 - 4.17)	0.08 (0.03 - 0.12)	0
Non-RS, TI	141	0.44 (0.10 - 1.25)	100	0.51 (0.15 - 1.50)	88	0.45 (0.12 - 1.13)	62	0.40 (0.06 - 0.92)	52	0.49 (0.06 - 1.43)	-0.01 (-0.03 - 0.02)	0.56
Non-RS, TD	47	0.79 (0.10 - 3.82)	56	0.67 (0.10 - 3.85)	55	0.73 (0.18 - 3.75)	48	0.57 (0.08 - 4.25)	32	0.93 (0.19 - 5.47)	0.27 (0.15 - 0.39)	<0.001
RS, TI	48	0.66 (0.11 - 3.85)	36	0.45 (0.07 - 3.33)	26	0.58 (0.07 - 3.52)	21	0.90 (0.14 - 4.15)	16	1.00 (0.26 - 4.92)	0.09 (-0.03 - 0.2)	0.14
RS, TD	18	2.46 (0.33 - 5.39)	24	1.92 (0.25 - 4.25)	19	3.20 (0.06 - 5.20)	21	2.52 (0.26 - 4.93)	16	2.85 (0.42 - 6.90)	0.22 (0.01 - 0.42)	0.04
Hepcidin (nmol/L)	246	4.7 (0.8 - 21.7)	206	5.2 (0.7 - 24.2)	188	5.6 (0.5 - 24.5)	154	5.7 (1.3 - 25.8)	116	4.7 (0.8 - 22.4)	0.57 (0.34 - 0.79)	<0.001
Non-RS, TI	140	4.2 (0.8 - 14.3)	98	4.3 (0.5 - 14.8)	89	4.4 (0.5 - 13.8)	62	4.0 (1.6 - 13.1)	52	3.4 (0.8 - 10.8)	-0.02 (-0.38 - 0.33)	0.89
Non-RS, TD	44	13.2 (1.1 - 51.0)	53	18.1 (1.2 - 39.1)	55	17.7 (0.4 - 41.8)	48	12.2 (0.4 - 37.9)	32	12.6 (1.3 - 32.3)	0.86 (-0.62 - 2.35)	0.26
RS, TI	44	3.4 (0.8 - 9.2)	32	2.8 (0.5 - 8.2)	26	3.1 (0.5 - 7.5)	22	2.5 (1.1 - 8.9)	16	3.1 (0.7 - 10.6)	-0.04 (-0.36 - 0.29)	0.82
RS, TD	18	7.8 (1.5 - 15.9)	23	7.2 (2.1 - 19.5)	18	8.9 (1.5 - 19.5)	22	10.7 (1.3 - 37.6)	16	4.2 (1.0 - 41.5)	1.67 (0.52 - 2.82)	0
GDF-15 (ng/L)	99	2189 (938 - 5956)	92	2491 (1000 - 7290)	76	2470 (1016 - 7982)	76	2932 (887 - 8058)	62	2556 (1045 - 7488)	229.08 (133.23 - 324.93)	<0.001
Non-RS, TI	53	1831 (731 - 4658)	40	1735 (721 - 4596)	32	1568 (615 - 5684)	24	1520 (574 - 7615)	25	1667 (633 - 5736)	118.87 (-68.36 - 306.09)	0.21
Non-RS, TD	13	1856 (1204 - 4828)	20	2971 (1067 - 6895)	20	2583 (1844 - 7166)	26	3494 (1609 - 8058)	18	3064 (1398 - 8037)	523.54 (254.19 - 792.89)	<0.001
RS, TI	26	2774 (996 - 11083)	19	2402 (1067 - 7354)	16	2694 (1223 - 10303)	13	3099 (1474 - 5435)	8	2780 (1331 - 9554)	225.25 (-35.96 - 486.46)	0.09
RS, TD	7	2883 (1869 - 5370)	13	5035 (2571 - 11253)	8	3538 (830 - 15167)	13	4517 (869 - 20000)	11	5166 (1053 - 7933)	363.24 (-45.59 - 772.07)	0.08
sTfR (mg/L)	99	1.3 (0.7 - 2.8)	92	1.3 (0.7 - 3.1)	77	1.4 (0.7 - 3.0)	76	1.4 (0.7 - 3.4)	61	1.3 (0.8 - 2.7)	0.03 (-0.03 - 0.09)	0.38
Non-RS, TI	53	1.2 (0.8 - 2.7)	40	1.3 (0.8 - 2.9)	32	1.4 (0.9 - 3.0)	24	1.4 (0.9 - 2.8)	25	1.2 (0.9 - 2.7)	0.02 (-0.02 - 0.05)	0.32
Non-RS, TD	13	1.1 (0.6 - 2.5)	20	1.0 (0.5 - 2.8)	21	1.1 (0.4 - 1.8)	26	1.2 (0.4 - 3.6)	17	1.2 (0.6 - 2.2)	0 (-0.69 - 0.69)	1
RS, TI	26	1.6 (0.9 - 3.3)	19	1.8 (1.0 - 3.1)	16	2.0 (1.1 - 2.8)	13	2.2 (1.3 - 3.5)	8	2.2 (1.0 - 2.8)	0.11 (0.07 - 0.15)	<0.001
RS, TD	7	0.9 (0.4 - 3.1)	13	1.9 (0.6 - 3.4)	8	1.4 (0.6 - 3.1)	13	1.4 (0.5 - 3.2)	11	1.4 (0.4 - 3.6)	-0.12 (-0.2 - -0.04)	0.01
MDA (μMol/L)	121	1.0 (0.5 - 4.1)	101	1.1 (0.5 - 3.9)	74	0.9 (0.4 - 2.0)	68	1.0 (0.5 - 6.7)	48	1.2 (0.7 - 11.9)	0 (-47.8 - 47.8)	1
Non-RS, TI	45	1.1 (0.5 - 3.7)	31	0.9 (0.4 - 3.9)	22	1.1 (0.4 - 4.6)	14	0.9 (0.5 - 2.4)	13	1.1 (0.6 - 2.6)	-0.03 (-0.31 - 0.25)	0.84
Non-RS, TD	31	1.0 (0.4 - 2.8)	32	1.1 (0.7 - 1.6)	25	0.8 (0.1 - 1.7)	23	0.8 (0.3 - 1.9)	12	1.6 (0.8 - 6.4)	-0.17 (-0.41 - 0.07)	0.17
RS, TI	34	1.1 (0.6 - 10.2)	22	0.9 (0.4 - 4.3)	16	0.9 (0.4 - 1.4)	18	0.9 (0.5 - 12.8)	12	1.1 (0.5 - 3.8)	-0.88 (-14.4 - 12.65)	0.9
RS, TD	11	1.1 (0.6 - 1.4)	16	1.1 (0.4 - 5.6)	11	0.9 (0.5 - 4.1)	13	1.4 (0.5 - 7.6)	11	1.9 (0.7 - 28.5)	0.5 (-1.03 - 2.03)	0.52
Beta: coefficient indicating the median value change per visit; CI: confidence interval; RS: ring sideroblasts; TI: transfusion-independent; TD: transfusion-dependent; TSAT: transferrin saturation; LPI: labile plasma iron; NTBI: non-transferrin bound iron; GDF-15: growth differentiation factor 15; STfR: soluble transferrin receptor; MDA: malondialdehyde.												

Table S2 Iron parameters by classical iron overload status, linear quantile mixed model

	Visit						Visit				Beta (95% CI)	p
	1		2		3		4		5			
	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)		
Total patients	256		251		222		176		135			
TSAT (%)	254	39 (19 - 100)	217	41 (19 - 97)	191	37 (16 - 100)	158	40 (19 - 100)	120	42 (21 - 100)	1.50	0.018
non-IO*	204	36 (19 - 100)	177	36 (18 - 100)	158	34 (16 - 100)	120	35 (18 - 100)	97	35 (19 - 100)	0.31	0.978
IO	50	64 (26 - 100)	40	78 (36 - 97)	33	90 (30 - 100)	38	87 (35 - 100)	23	91 (40 - 100)	3.48	0.003
LPI (μMol/L)	255	0.12 (0.03 - 0.31)	216	0.11 (0.03 - 0.27)	187	0.12 (0.03 - 0.34)	152	0.12 (0.02 - 0.30)	116	0.12 (0.03 - 0.67)	0.012	0.013
non-IO	204	0.12 (0.03 - 0.26)	176	0.10 (0.02 - 0.20)	154	0.12 (0.03 - 0.24)	115	0.11 (0.02 - 0.19)	93	0.10 (0.03 - 0.20)	0.00004	0.988
IO	51	0.11 (0.03 - 0.38)	40	0.14 (0.04 - 0.95)	33	0.18 (0.04 - 0.87)	37	0.17 (0.05 - 0.91)	23	0.32 (0.06 - 1.13)	0.024	0.551
NTBI (μMol/L)	254	0.53 (0.10 - 2.92)	216	0.57 (0.13 - 2.94)	188	0.56 (0.12 - 3.33)	152	0.53 (0.10 - 3.24)	116	0.66 (0.15 - 4.17)	0.078	0.001
non-IO	203	0.50 (0.10 - 1.94)	176	0.46 (0.13 - 2.08)	155	0.47 (0.10 - 1.86)	115	0.39 (0.06 - 1.67)	93	0.53 (0.09 - 2.78)	0.010	0.503
IO	51	1.00 (0.10 - 4.37)	40	2.27 (0.27 - 4.00)	33	2.94 (0.51 - 4.03)	37	2.70 (0.53 - 5.20)	23	2.84 (0.86 - 7.25)	0.31	<0.001
Ferritin (μg/L)	252	305 (58 - 1078)	213	357 (69 - 1427)	183	293 (61 - 1794)	150	349 (69 - 2322)	115	374 (61 - 2560)	122.0	<0.001
non-IO	202	250 (54 - 680)	173	250 (55 - 748)	150	247 (56 - 821)	112	240 (51 - 655)	92	298 (56 - 727)	13.5	<0.001
IO	50	1086 (280 - 2674)	40	1471 (1109 - 3008)	33	1861 (1135 - 5126)	38	1961 (1164 - 4223)	23	2560 (1280 - 5081)	482.9	<0.001
Hepcidin (nmol/L)	246	4.7 (0.8 - 21.7)	206	5.2 (0.7 - 24.2)	188	5.6 (0.5 - 24.5)	154	5.7 (1.3 - 25.8)	116	4.7 (0.8 - 22.4)	0.57	<0.001
non-IO	197	4.2 (0.8 - 14.3)	169	4.4 (0.5 - 16.9)	155	4.9 (0.5 - 17.3)	116	4.5 (1.3 - 13.1)	93	3.6 (0.8 - 13.7)	0.13	0.382
IO	49	10.0 (1.4 - 51.0)	37	19.7 (2.3 - 50.0)	33	18.6 (4.8 - 46.2)	38	15.1 (1.2 - 46.3)	23	14.6 (0.7 - 48.5)	2.12	<0.001
MDA (μMol/L)	121	1.0 (0.5 - 4.1)	101	1.1 (0.5 - 3.9)	74	0.9 (0.4 - 2.0)	68	1.0 (0.5 - 6.7)	48	1.2 (0.7 - 11.9)	-0.003	1.00
non-IO	91	1.1 (0.5 - 4.1)	74	1.0 (0.4 - 4.1)	52	0.9 (0.4 - 1.9)	47	1.0 (0.5 - 9.0)	38	1.2 (0.6 - 15.7)	0.32	0.024
IO	30	1.0 (0.4 - 3.5)	27	1.1 (0.7 - 1.9)	22	0.9 (0.6 - 2.0)	21	1.1 (0.6 - 3.1)	10	1.4 (0.8 - 2.8)	0.028	0.807

*Iron overload (IO): RBCT intensity of ≥ 1 RBC units/month during a six month-period between visits or serum ferritin level ≥ 1000 μg/L. sd: standard deviation; Beta: coefficient indicating the median value change per visit; CI: confidence interval; TSAT: transferrin saturation; LPI: labile plasma iron; NTBI: non-transferrin bound iron; MDA: malondialdehyde; RBC: red blood cell; RBCT: red blood cell transfusions.

Table S3 Iron parameters by transfusion density category, quantile mixed model

	Visit										Beta (95% CI)	P
	1		2		3		4		5			
	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)		
Total patients	256		251		222		176		135			
TSAT (%)	254	39 (19 - 100)	217	41 (19 - 97)	191	37 (16 - 100)	158	40 (19 - 100)	120	42 (21 - 100)	1.5 (0.26 - 2.74)	0.018
None	199	36 (19 - 100)	138	34 (18 - 100)	120	33 (17 - 100)	93	35 (19 - 100)	71	35 (19 - 100)	1.06 (-0.91 - 3.03)	0.292
>0 - <0.75 units/month	10	54 (28 - 98)	19	41 (16 - 100)	23	37 (14 - 100)	22	36 (18 - 74)	19	39 (19 - 100)	-1.85 (-5.29 - 1.59)	0.291
≥0.75 - ≤1.75 units/month	22	51 (28 - 88)	24	60 (38 - 100)	19	85 (9 - 100)	20	66 (22 - 100)	17	90 (32 - 100)	4.71 (0.4 - 9.01)	0.032
>1.75 units/month	23	69 (23 - 100)	36	64 (26 - 94)	29	87 (18 - 100)	23	80 (26 - 100)	13	93 (68 - 100)	5.16 (0.93 - 9.38)	0.017
LPI (μMol/L)	255	0.12 (0.03 - 0.31)	216	0.11 (0.03 - 0.27)	187	0.12 (0.03 - 0.34)	152	0.12 (0.02 - 0.30)	116	0.12 (0.03 - 0.67)	0.01 (0 - 0.02)	0.013
None	199	0.12 (0.02 - 0.28)	137	0.09 (0.02 - 0.20)	117	0.11 (0.03 - 0.19)	88	0.10 (0.02 - 0.19)	69	0.11 (0.03 - 0.20)	0.01 (0 - 0.02)	0.007
>0 - <0.75 units/month	10	0.17 (0.09 - 1.24)	19	0.14 (0.05 - 0.27)	22	0.16 (0.04 - 0.18)	22	0.13 (0.05 - 0.19)	19	0.09 (0.03 - 0.21)	-0.01 (-0.03 - 0.01)	0.2
≥0.75 - ≤1.75 units/month	22	0.09 (0.04 - 0.18)	24	0.14 (0.04 - 0.39)	19	0.18 (0.05 - 1.14)	19	0.14 (0.01 - 0.53)	15	0.15 (0.03 - 1.39)	0.02 (-0.12 - 0.15)	0.815
>1.75 units/month	24	0.11 (0.04 - 0.70)	36	0.11 (0.04 - 0.76)	29	0.16 (0.04 - 0.87)	23	0.19 (0.05 - 0.68)	13	0.32 (0.01 - 0.94)	0.05 (-0.05 - 0.15)	0.345
NTBI (μMol/L)	254	0.53 (0.10 - 2.92)	216	0.57 (0.13 - 2.94)	188	0.56 (0.12 - 3.33)	152	0.53 (0.10 - 3.24)	116	0.66 (0.15 - 4.17)	0.08 (0.03 - 0.12)	0.001
None	197	0.50 (0.10 - 2.08)	138	0.51 (0.11 - 2.08)	117	0.47 (0.12 - 1.61)	88	0.42 (0.07 - 2.66)	69	0.51 (0.07 - 2.78)	0.05 (0.01 - 0.09)	0.012
>0 - <0.75 units/month	11	0.97 (0.33 - 3.40)	18	0.38 (0.08 - 1.40)	23	0.67 (0.07 - 1.58)	22	0.52 (0.14 - 1.67)	19	0.61 (0.09 - 2.27)	0.07 (-0.14 - 0.28)	0.533
≥0.75 - ≤1.75 units/month	22	0.80 (0.23 - 3.36)	24	1.26 (0.24 - 4.28)	19	2.81 (0.06 - 5.20)	19	1.53 (0.05 - 5.21)	15	2.58 (0.42 - 6.00)	0.29 (-0.01 - 0.59)	0.055
>1.75 units/month	24	1.26 (0.10 - 3.73)	36	1.30 (0.12 - 4.03)	29	2.64 (0.18 - 4.42)	23	2.48 (0.16 - 4.68)	13	2.84 (1.35 - 7.40)	0.35 (0.07 - 0.63)	0.014
Ferritin (μg/L)	252	305 (58 - 1078)	213	357 (69 - 1427)	183	293 (61 - 1794)	150	349 (69 - 2322)	115	374 (61 - 2560)	122.03 (99.1 - 144.96)	<0.0001
None	196	253 (54 - 732)	136	230 (55 - 576)	115	223 (49 - 673)	86	213 (47 - 650)	68	250 (51 - 625)	37.87 (3.87 - 71.88)	0.029
>0 - <0.75 units/month	11	708 (92 - 1570)	17	394 (13 - 1146)	23	550 (97 - 905)	21	650 (198 - 1303)	19	407 (61 - 1242)	-3.86 (-63.88 - 56.16)	0.9
≥0.75 - ≤1.75 units/month	22	579 (87 - 1382)	24	865 (433 - 1856)	19	1428 (65 - 2554)	20	1092 (198 - 2785)	15	1014 (474 - 3209)	532.95 (-14716 - 1213.06)	0.125
>1.75 units/month	23	1003 (288 - 2682)	36	1330 (513 - 3044)	26	1727 (370 - 5136)	23	2231 (426 - 4948)	13	2755 (1280 - 5081)	851.36 (642.11 - 1060.62)	<0.0001

Table S3 Iron parameters by transfusion density category, quantile mixed model

	Visit										Beta (95% CI)	P
	1		2		3		4		5			
	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)	N	Median (p10-p90)		
Hepcidin (nmol/L)	246	4.7 (0.8 - 21.7)	206	5.2 (0.7 - 24.2)	188	5.6 (0.5 - 24.5)	154	5.7 (1.3 - 25.8)	116	4.7 (0.8 - 22.4)	0.57 (0.34 - 0.79)	<0.0001
None	192	4.1 (0.8 - 14.3)	132	4.0 (0.5 - 13.6)	118	4.2 (0.5 - 12.8)	89	3.8 (1.3 - 12.1)	69	3.4 (0.7 - 11.8)	0.09 (-0.11 - 0.29)	0.363
>0 - <0.75 units/month	10	9.7 (2.1 - 35.6)	18	5.7 (0.3 - 14.1)	23	9.2 (1.0 - 19.6)	22	11.1 (3.0 - 17.6)	19	8.1 (1.0 - 26.5)	-0.18 (-1.39 - 1.03)	0.771
≥0.75 - ≤1.75 units/month	20	7.0 (0.5 - 33.5)	22	8.8 (0.7 - 26.8)	19	9.5 (0.3 - 46.2)	20	12.9 (2.0 - 25.9)	15	13.5 (3.2 - 28.4)	0.03 (-1.33 - 1.39)	0.969
>1.75 units/month	24	13.3 (1.5 - 67.9)	34	19.6 (3.0 - 44.5)	28	19.1 (3.1 - 58.3)	23	18.3 (0.3 - 46.3)	13	10.5 (0.7 - 48.5)	2.82 (0.11 - 5.52)	0.041
MDA (μMol/L)	121	1.0 (0.5 - 4.1)	101	1.1 (0.5 - 3.9)	74	0.9 (0.4 - 2.0)	68	1.0 (0.5 - 6.7)	48	1.2 (0.7 - 11.9)	0 (-47.8 - 47.8)	1
None	86	1.0 (0.5 - 4.2)	53	0.9 (0.4 - 4.1)	38	1.0 (0.4 - 4.2)	34	0.9 (0.5 - 6.7)	25	1.1 (0.5 - 2.7)	-0.38 (-11.95 - 11.19)	0.949
>0 - <0.75 units/month	4	1.2 (0.4 - 1.2)	8	1.3 (0.6 - 5.6)	7	0.6 (0.0 - 1.2)	11	0.7 (0.1 - 1.5)	8	2.6 (0.7 - 29.7)	1.14 (-2.29 - 4.57)	0.514
≥0.75 - ≤1.75 units/month	18	1.3 (0.7 - 4.1)	14	1.2 (0.7 - 1.6)	12	1.1 (0.4 - 2.0)	11	1.0 (0.6 - 4.1)	10	1.8 (0.7 - 9.2)	-0.06 (-0.83 - 0.71)	0.871
>1.75 units/month	13	1.0 (0.4 - 1.9)	26	1.1 (0.6 - 2.9)	17	0.8 (0.6 - 1.9)	12	1.2 (0.4 - 3.1)	5	1.8 (0.9 - 15.7)	-0.08 (-0.23 - 0.07)	0.271

TSAT: transferrin saturation; LPI: labile plasma iron; NTBI: non-transferrin bound iron; MDA: malondialdehyde.

Table S4 Cox model of overall survival by transferrin saturation level (above or below 80%) and transfusion status as time-varying variables

	Unadjusted		Adjusted ¹	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
Transferrin saturation (%) < 80	1	-	1	-
Transferrin saturation (%) ≥ 80	1.4 (0.8 - 2.5)	0.30	1.4 (0.7 - 2.5)	0.33
Transferrin saturation < 80, TI	1	-	1	-
Transferrin saturation ≥ 80, TI	2.0 (0.6 - 6.5)	0.22	2.0 (0.6 - 6.3)	0.24
Transferrin saturation < 80, TD	5.2 (2.2 - 12.4)	<0.001	4.0 (1.6 - 9.8)	0.003
Transferrin saturation ≥ 80, TD	4.5 (1.8 - 11.5)	0.001	3.7 (1.4 - 9.7)	0.01

¹adjusted for age at diagnosis and IPSS-R category
CI: confidence interval; TI: transfusion-independent; TD: transfusion-dependent.

Table S5 Cox model of overall survival by malondialdehyde level and transfusion status as time-varying variables.

	Unadjusted		Adjusted ¹	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	P
Malondialdehyde normal ²	1	-	1	-
Malondialdehyde elevated ²	0.7 (0.3 - 1.8)	0.46	0.9 (0.4 - 2.4)	0.86
Malondialdehyde normal ² , TI ³	1	-	1	-
Malondialdehyde elevated ² , TI ³	0.4 (0.1 - 1.5)	0.19	0.6 (0.2 - 2.2)	0.48
Malondialdehyde normal ² , TD ⁴	2.7 (1.1 - 6.7)	0.03	1.9 (0.7 - 4.8)	0.19
Malondialdehyde elevated ² , TD ⁴	2.0 (1.0 - 4.0)	0.05	1.9 (0.9 - 3.7)	0.09

¹adjusted for age at diagnosis and IPSS-R category; ²below/above median MDA level (1μmol/L) .
CI: confidence interval; TI: transfusion-independent; TD: transfusion-dependent.

Table S6 Cox model of progression-free survival by labile plasma iron and transfusion status as time-varying variables.

	Unadjusted		Adjusted ¹	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
Labile plasma iron < LLOD	1	-	1	-
Labile plasma iron ≥ LLOD	3.3 (1.9 - 5.9)	<0.001	3.2 (1.8 - 5.7)	<0.001
Labile plasma iron < LLOD, TI	1	-	1	-
Labile plasma iron ≥ LLOD, TI	4.4 (1.6 - 12.7)	0.005	4.7 (1.6 - 13.4)	0.004
Labile plasma iron < LLOD, TD	5.0 (2.1 - 12.0)	<0.001	4.6 (1.9 - 11.4)	0.001
Labile plasma iron ≥ LLOD, TD	10.6 (4.4 - 25.3)	<0.001	9.2 (3.8 - 22.5)	<0.001

¹adjusted for age at diagnosis and IPSS-R category; ²below the lowest level of detection
CI: confidence interval; LLOD: lowest limit of detection; TI: transfusion-independent; TD: transfusion-dependent.

IMPACT OF TREATMENT WITH IRON CHELATION THERAPY IN PATIENTS WITH LOWER-RISK MYELODYS- PLASTIC SYNDROMES PARTICIPATING IN THE EUROPEAN MDS REGISTRY

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Abstract

Iron overload due to red blood cell transfusions is associated with morbidity and mortality in lower-risk myelodysplastic syndrome patients. Many studies suggested improved survival after iron chelation therapy, but valid data are limited. The aim of this study was to assess the effect of iron chelation on overall survival and hematological improvement in lower-risk myelodysplastic syndrome patients in the European MDS registry. We compared chelated patients with a contemporary, non-chelated control group within the European MDS registry, that met the eligibility criteria for starting iron chelation. A Cox proportional hazards model was used to assess overall survival, treating receipt of chelation as a time-varying variable. Additionally, chelated and non-chelated patients were compared using a propensity-score matched model. Of 2200 patients, 224 received iron chelation. The hazard ratio and 95% confidence interval for overall survival for chelated patients, adjusted for age, sex, comorbidity, performance status, cumulative red blood cell transfusions, IPSS-R, and presence of ringed sideroblasts was 0.50 (0.34-0.74). The propensity-score analysis, matched for age, sex, country, red blood cell transfusion intensity, ferritin level, comorbidity, performance status, and IPSS-R and additionally corrected for cumulative red blood cell transfusions and presence of ringed sideroblasts, demonstrated a significantly improved overall survival for chelated patients with a hazard ratio of 0.42 (0.27-0.63) compared to non-chelated patients. Up to 39% of chelated patients reached an erythroid response. In conclusion, our results suggest that iron chelation may improve overall survival and hematopoiesis in transfused lower-risk myelodysplastic syndrome patients.

This trial was registered at www.clinicaltrials.gov as #NCT00600860.

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematopoietic stem cell disorders characterized by abnormal differentiation and maturation of hematopoietic cells, bone marrow failure and genetic instability, with an enhanced risk of progressing to acute myeloid leukemia.¹ Iron overload, as a consequence of frequently administered red blood cell transfusions (RBCT) and/or ineffective erythropoiesis, is a common finding in MDS. The effects of toxic iron species in other iron loading diseases, such as primary hemochromatosis, thalassemia and sickle cell anemia are well known, but the consequences in MDS are less clear.²⁻⁴ With an expected median survival of 2.4 to 11.8 years in lower-risk MDS (LR-MDS) patients,⁵ these patients are prone to long-term accumulation of iron due to RBCT as well as direct iron toxicity due to the formation of reactive oxygen species (ROS).⁶

Several studies have reported beneficial effects of iron chelation therapy (ICT) on overall survival (OS) and other clinical outcomes in MDS patients with iron overload.⁷⁻¹⁰ However, valid data on the effect of ICT are limited as most studies are executed in small or highly selected patient groups or suffer from serious methodological problems such as confounding by indication. Performing a randomized, controlled trial for this research question is cumbersome due to a widespread belief of patients in the beneficial effects of ICT and additionally, the personal opinion on ICT of many treating physicians, which may negatively affect enrollment. Likewise, patients included in a randomized, controlled trial do not generally reflect the actual LR-MDS patient group, which are usually elderly patients with multiple comorbidities.

In addition to the possible beneficial effects of iron chelation therapy on overall survival, increasing evidence indicates hematological improvement in patients during treatment with iron chelators.¹¹⁻¹⁶ Next to improvement in hemoglobin, platelet, and neutrophil levels, transfusion independence is achieved in a minority of chelated patients.^{11,12,14} The underlying mechanisms are still unclear.¹⁷

The aim of this study was to evaluate the effect of iron chelation therapy on OS, hematological improvement, and ferritin levels in lower-risk MDS patients in the EUMDS Registry.

Methods

The EUMDS registry prospectively collects observational data on LR-MDS patients from 142 centers in 16 countries in Europe and Israel. Patients were included within 100 days of MDS diagnosis according to the World Health Organization 2001 classification, restricted to patients with a low or intermediate-1 score according to the international prognostic scoring system (IPSS).¹⁸ IPSS was the current prognostic indicator at the start of the registry, in accordance with the currently used prognostic score, the revised IPSS (IPSS-R) was reconstructed afterwards. The ethics committees of all participating centers approved the protocol and all patients provided written informed consent. Data were collected at baseline and at each 6-monthly outpatient routine follow-up visit. Data were collected on: comorbidity, transfusion history, use of iron chelators (agent, time frame; no drug doses or schedules were collected), peripheral blood values, conventional iron parameters (e.g. serum ferritin), bone marrow pathology, and progression to higher-risk MDS or acute myeloid leukemia. Subjects were prospectively followed until death, loss to follow-up, or withdrawal of informed consent.

In Europe, three iron chelators are available for treatment of secondary iron overload, but availability varies between countries. We analyzed all patients, chelated or non-chelated, who are eligible for receiving ICT based on at least one criterion for starting ICT (cumulative ≥ 15 RBC units, RBCT intensity of ≥ 1 unit/month during a six-month period, or serum ferritin level > 1000 $\mu\text{g/L}$), thereby preventing immortal time bias. As chelated and non-chelated patients may differ in characteristics that affect outcome, two different approaches were performed in order to control for potential bias: 1) Analysis of all eligible chelated and non-chelated patients using receipt of ICT as a time-varying covariate; adjusting for covariates related to both ICT receipt and OS: sex, age, comorbidity, performance status, RBCT intensity, number of units transfused, IPSS-R, and presence of ringed sideroblasts; 2) Propensity score (PS), i.e. conditional probability for being treated with ICT on basis of patient characteristics, matching of the same group. Variables included in the PS were: age, sex, country, RBCT intensity, ferritin level, MDS comorbidity index, performance status, and IPSS-R. A 3-to-1 nearest neighbor matching method with replacement and caliper (0.2) was applied.¹⁹ Additionally, we used a robust sandwich estimator to correct for intra-individual correlation of multiply used controls. Further details on the PS matching are provided in the supplementary methods.²⁰⁻²² OS was defined as the time from eligibility for ICT to death; subjects still alive were censored at the last follow-up date. Cox proportional hazards regression models and Kaplan-Meier survival curves were applied and hazard ratios (HRs) with 95% confidence intervals (95% CIs) were reported.²³

Erythroid responses were defined as a reduction in RBCT density (number of RBCT over time, see supplementary methods for definition and details) or as transfusion independency at least once as the transfusion density was reduced to zero, platelet responses were assessed according to the modified international working group (IWG) criteria.²⁴ Ferritin responses were defined as a decrease of ≥ 1000 $\mu\text{g/L}$ or a drop of the serum ferritin value below 1000 $\mu\text{g/L}$.

All analyses were undertaken in Stata 15 (StataCorp, College Station, TX).

Results

Patient population

Data were extracted from the EUMDS registry on July 5, 2017, 2,200 patients, diagnosed between December 3, 2007 and April 25, 2017, had been registered, of which 1,161 patients received at least one RBCT and 224 patients received iron chelation therapy (ICT) (figure 1). A small proportion of patients had received ICT without being transfused or prior to starting RBCT, these subjects generally had a high ferritin level and were excluded from subsequent analyses. Of the 1,161 transfused patients, 850 patients had been transfused for a duration of ≥ 2 months. Out of these 850 patients, 689 met the eligibility criteria. Supplementary figure 1 summarizes the number of patients who reached each criterion. At the time of analysis, 236 patients were deceased (154 non-chelated, 82 chelated) and nine patients progressed to high-risk MDS or AML (4 non-chelated, 5 chelated).

Comparing outcome of chelated versus non-chelated patients using ICT as a time-dependent variable

Table 1 shows the characteristics of the 689 patients who met one of the eligibility criteria at the visit prior to meeting one of the eligibility criteria; the date of this visit is when the patients enter this analysis. The mean age of the 199 chelated patients was 70 years and these patients were younger than the non-chelated patients whose mean age was 76 years. Median time from date of diagnosis to date of meeting the eligibility criteria was seven months in the non-chelated and eight months in the chelated subjects. The median follow-up period from study entry for chelated and non-chelated patients was 39.4 months (range 4.1 – 106.6) and 27.1 months (range 2.5 – 105.6), respectively. Non-chelated subjects had a higher number of cumulative units transfused than chelated subjects (4 vs 2 units) at time of inclusion and, on average, chelated patients had 13 units transfused prior to commencing ICT. The latter had a higher median ferritin level recorded at baseline (675 $\mu\text{g/L}$ vs 547 $\mu\text{g/L}$), and this had increased to 1,221 $\mu\text{g/L}$ prior to start of ICT.

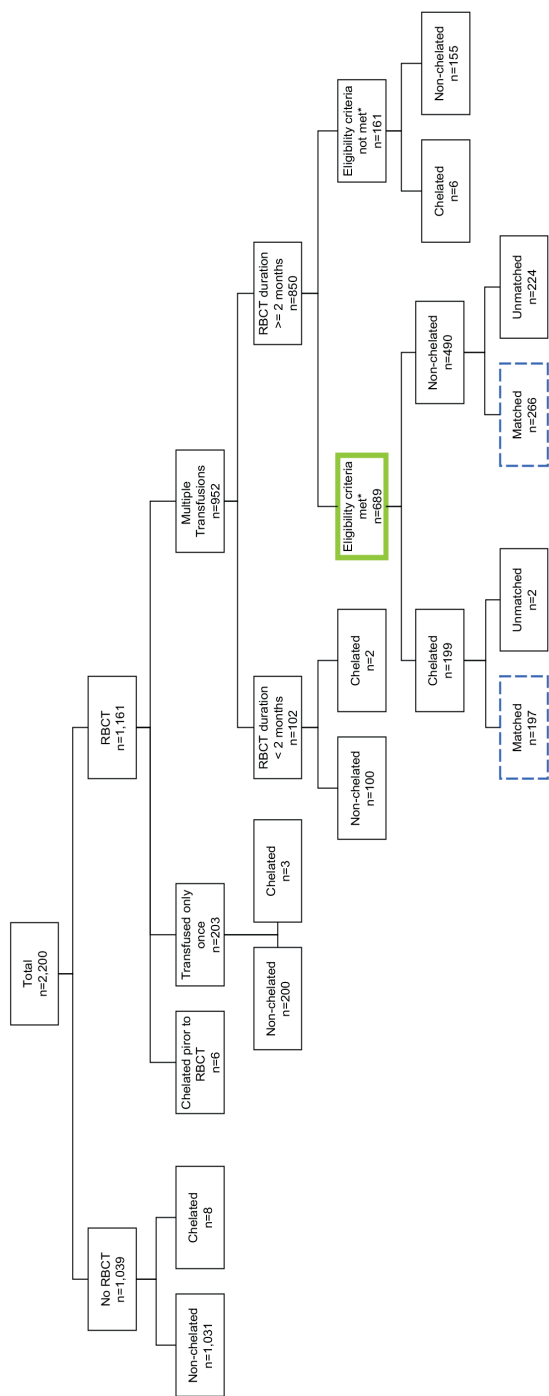


Figure 1 Number of registry patients by transfusion and chelation status.

RBCT = Red Blood Cell Transfusion
*Cumulative RBCT units ≥ 15 or RBCT intensity of ≥ 1 RBC unit/month or Serum Ferritin >1000 $\mu\text{g/L}$.

Whilst non-chelated and chelated subjects had similar IPPS-R scores, chelated patients had fewer co-morbidities as measured by the MDS-CI score and a better performance status as measured by Karnofsky performance status. Overall survival was estimated using receipt of ICT as a time-dependent variable – hence the number of patients reported in the risk table in Figure 2 reflects the time when a subject commences ICT. The hazard ratio for overall survival in the univariate analysis was 0.57 (95% CI: 0.45-0.73) (Figure 2, Table 1). This benefit increased when adjusted for the factors in Table 1 and the following variables: sex, RBCT intensity, and the presence of ringed sideroblasts (HR: 0.50, 95% CI: 0.34-0.74). No statistically significant interactions were detected by using a sophisticated prediction-type model. When we restricted the analysis to patients who were treated with deferasirox (the largest group), thereby excluding possible differences between patients using different chelators, the crude HR for OS was 0.53 (95% CI: 0.40-0.69) and the adjusted HR for OS was 0.38 (95% CI: 0.24-0.60). Out of the 199 chelated patients, 150 received deferasirox as the initial chelator, 36 deferoxamine, and 13 deferiprone, and differences were seen in the baseline characteristics by type of chelator with deferasirox treated patients being younger and fitter. Twenty-two patients switched from one chelator to another, or were treated with all three chelators consecutively (Supplementary Table 1), but usually the treatment period of the second chelator was shorter than the treatment period of the first chelator. The median time on chelation for all 199 patients was 13 months (range 3-41 months) and patients who were initially treated with deferoxamine had inferior overall survival compared to deferasirox treated patients (Table 1: adjusted HR: 2.46, 95% CI: 1.12-5.41). The overall survival of deferoxamine-treated patients was similar to non-chelated patients (adjusted HR: 0.98, 95% CI: 0.52-1.86).

Matching of chelated and unchelated patients by propensity scores

The variables used in the propensity score matching are described in supplementary table 2 for all eligible patients by chelation status; initially excluding any missing variables and then after multiple imputation (MI). Along with factors already shown in table 1, there was a difference by country as to whether a patient was treated with ICT; patients in the UK were less likely to be treated.

The overlap of propensity scores of both groups (chelated and non-chelated), which is essential for PS matching, was good for the majority of the patients (Supplementary figure 2). The matched MI dataset included 197/199 chelated cases and identified 591 non-chelated controls. There were no differences by sex, RBCT intensity, cumulative RBCT units, serum ferritin levels, comorbidity, performance status, IPSS-R, presence of ringed sideroblasts, quality of life (QoL), and country between both groups (Table 2). Figure 3 shows the unadjusted survival plot by ICT status with

Table 1 Baseline characteristics of non-chelated and chelated patients at the visit prior to reaching the eligibility criteria and estimates of overall survival.					
	Non-chelated	Chelated	Deferasirox	Deferoxamine	Deferiprone
Total	490	199	150	36	13
No. of countries with chelated patients	17 / 17	17 / 17	14 / 17	9 / 17	6 / 17
Mean age at eligible (SD)	76 (10)	70 (9)	70 (9)	72 (9)	70 (10)
Time from diagnosis (months)					
Inclusion, median (p10-p90)	7 (0 - 35)	8 (0 - 32)	9 (0 - 36)	6 (0 - 30)	7 (0 - 33)
Inclusion, mean (SD)	14 (16)	13 (15)	14 (16)	9 (11)	12 (13)
Chelation median (p10-p90)	NA	17 (4 - 46)	17 (4 - 47)	13 (2 - 39)	22 (5 - 51)
Chelation mean (SD)	NA	21 (17)	21 (18)	17 (13)	26 (18)
Number of units transfused					
Median (range)	4.0 (1.0 - 33.0)	2.0 (0.0 - 28.0)	2.5 (0.0 - 28.0)	3.0 (0.0 - 18.0)	2.0 (0.0 - 8.0)
Median at start of chelation (range)	NA	13.0 (2.0 - 91.0)	12.0 (2.0 - 75.0)	10.5 (2.0 - 75.0)	24.5 (2.0 - 91.0)
Ferritin (ug/L)					
Median (p10-p90)	547.0 (116.0 - 1384.0)	675.0 (256.0 - 1573.0)	683.0 (264.0 - 1600.0)	682.0 (256.0 - 1920.0)	525.0 (190.5 - 918.1)
Median at start of chelation (p10-p90)	NA	1221.0 (475.8 - 3000.0)	1210.0 (449.3 - 2832.0)	1173.0 (335.0 - 3000.0)	2202.0 (475.8 - 4900.0)
Comorbidity (MDS/CI)					
Low risk	308 63.2%	150 75.8%	118 79.2%	23 63.9%	9 69.2%
Intermediate risk	149 30.6%	43 21.7%	28 18.8%	11 30.6%	4 30.8%
High risk	30 6.2%	5 2.5%	3 2.0%	2 5.6%	0 0.0%
Performance status					
Unable to care for self	8 2.0%	1 0.6%	1 0.8%	0 0.0%	0 0.0%
Unable to work	132 32.3%	36 20.2%	21 15.9%	12 34.3%	3 27.3%
Able to work and normal activity	269 65.8%	141 79.2%	110 83.3%	23 65.7%	8 72.7%
Prognostic indicator (IPSS-R)					
Reaching criteria (LOCF**)					
Very low	48 13.4%	22 13.5%	18 14.6%	2 6.9%	2 18.2%
Low	199 55.6%	95 58.3%	66 53.7%	21 72.4%	8 72.7%
Intermediate	111 31.0%	46 28.2%	39 31.7%	6 20.7%	1 9.1%
High	38 10.6%	9 5.5%	6 4.9%	3 10.3%	0 0.0%
Very high	3 0.8%	1 0.6%	1 0.8%	0 0.0%	0 0.0%
Duration of treatment with chelation (months)					
Median (p10-p90)	NA	13 (3 - 41)	14 (3 - 41)	9 (1 - 34)	13 (2 - 30)
Ever received ESAs					
No	312 63.7%	115 57.8%	85 56.7%	22 61.1%	8 61.5%
Yes	178 36.3%	84 42.2%	65 43.3%	14 38.9%	5 38.5%
Ever received hypomethylating					
No	460 93.9%	184 92.5%	136 90.7%	36 100.0%	12 92.3%
Yes	30 6.1%	15 7.5%	14 9.3%	0 0.0%	1 7.7%
Ever received lenalidomide					
No	467 95.3%	179 89.9%	136 90.7%	33 91.7%	10 76.9%
Yes	23 4.7%	20 10.1%	14 9.3%	3 8.3%	3 23.1%
Overall Survival (OS)*					
Unadjusted	1	0.57 (0.45 - 0.73)	1	1.99 (1.18 - 3.35)	0.42 (0.10 - 1.71)
Adjusted**	1	0.50 (0.34 - 0.74)	1	2.46 (1.12 - 5.41)	0.30 (0.02 - 3.58)

* HRs and 95% CI were estimated using receipt of chelation as a time-varying covariate
 ** adjusted by age at eligibility criteria, sex, comorbidity, performance status, number of units transfused, IPSS-R, and ring-sideroblast present
 *** LOCF: last observation carried forward (only for cytogenetics and bone marrow blasts)
 SD: standard deviation; RBCT: red blood cell transfusion; MDS/CI: myelodysplastic syndrome specific comorbidity index; IPSS-R: revised international prognostic scoring system

Table 2 Baseline characteristics for all matched subjects included in the propensity analyses

Covariates		Matched* data with imputations**					
		Non-chelated		Chelated		P-Value	Standardized differences***
		N = 591		N = 197			
Age (years)		71 (11)		70 (9)		0.364	-0.077
Sex						0.797	-0.021
	Female	210	35.5%	72	36.5%		
	Male	381	64.5%	125	63.5%		
RBCT Intensity (per month)		0.7 (1.0)		0.6 (1.0)		0.484	-0.058
Cumulative RBCT units		4.5 (4.9)		4.3 (4.7)		0.570	-0.047
Ferritin level (ug/L, median, p25-p75)		730.6 (494.6-977.3)		683.6 (504-915.5)		0.328	-0.086
Comorbidity (MDSCI)						0.965	-0.004
	Low risk	440	74.5%	150	76.1%		
	Intermediate risk	145	24.5%	42	21.3%		
	High risk	6	1.0%	5	2.5%		
Performance status						0.279	0.090
	Unable to care for self	4	0.7%	1	0.5%		
	Unable to work	135	22.8%	38	19.3%		
	Able to work and normal activity	452	76.5%	158	80.2%		
Prognostic indicator (IPSS-R)						0.914	0.009
	Very low	83	14.0%	22	11.2%		
	Low	337	57.0%	120	60.9%		
	Intermediate	134	22.7%	45	22.8%		
	High	34	5.8%	9	4.6%		
	Very high	3	0.5%	1	0.5%		
Ring-sideroblast present						0.445	0.062
	Yes	419	70.9%	134	68.0%		
	No	172	29.1%	63	32.0%		
Platelet level (10^9/L, median, p25-p75)		162.5 (99.2-294)		224.0 (121-324)		0.086	0.148
Haemoglobin level (g/dL, median, p25-p75)		8.8 (8.2-9.8)		8.4 (7.7-9.5)		0.021	-0.194

Table 2 Continued

Covariates	Matched* data with imputations**				
	Non-chelated		Chelated	P-Value	Standardized differences***
	N = 591		N = 197		
Quality of Life (EQ-5D)					
	Index (mean, SD)	0.7 (0.2)	0.7 (0.2)	0.186	0.125
	VAS (mean, SD)	64.8 (21.0)	68.1 (19.9)	0.083	0.165
Country				0.140	-0.122
	Austria	25 4.2%	10 5.1%		
	Croatia	9 1.5%	1 0.5%		
	Czech Republic	58 9.8%	25 12.7%		
	Denmark	15 2.5%	8 4.1%		
	France	113 19.1%	40 20.3%		
	Germany	23 3.9%	8 4.1%		
	Greece	80 13.5%	23 11.7%		
	Israel	11 1.9%	5 2.5%		
	Italy	11 1.9%	5 2.5%		
	Netherlands	17 2.9%	7 3.6%		
	Poland	22 3.7%	8 4.1%		
	Portugal	2 0.3%	1 0.5%		
	Romania	34 5.8%	11 5.6%		
	Republic of Serbia	8 1.4%	2 1.0%		
	Spain	11 1.9%	5 2.5%		
	Sweden	97 16.4%	20 10.2%		
	UK	55 9.3%	18 9.1%		
Overall Survival (OS)					
	Unadjusted	1.0	0.70		
			(0.51 – 0.95)		
	Adjusted****	1.0	0.42		
			(0.27 – 0.63)		

Note: Continuous variables are reported as mean (standard deviation), while categorical variables are reported as number (percent). * Matched by age, gender, country, RBCT intensity, ferritin level, comorbidity, performance status, and IPSS-R at eligibility. ** Multiple imputations in RBCT intensity, ferritin level, comorbidity, performance status, and IPSS-R at eligibility for non-chelated patients. *** The standardized difference in percent is the mean difference as a percentage of the average standard deviation. **** Adjusted by age, sex, comorbidity, performance status, RBCT intensity, number of units transfused, IPSS-R, and RS present. RBCT: red blood cell transfusion; MDSCI: myelodysplastic syndrome specific comorbidity index; IPSS-R: revised international prognostic scoring system; EQ-5D: European Quality of Life - 5 dimensions

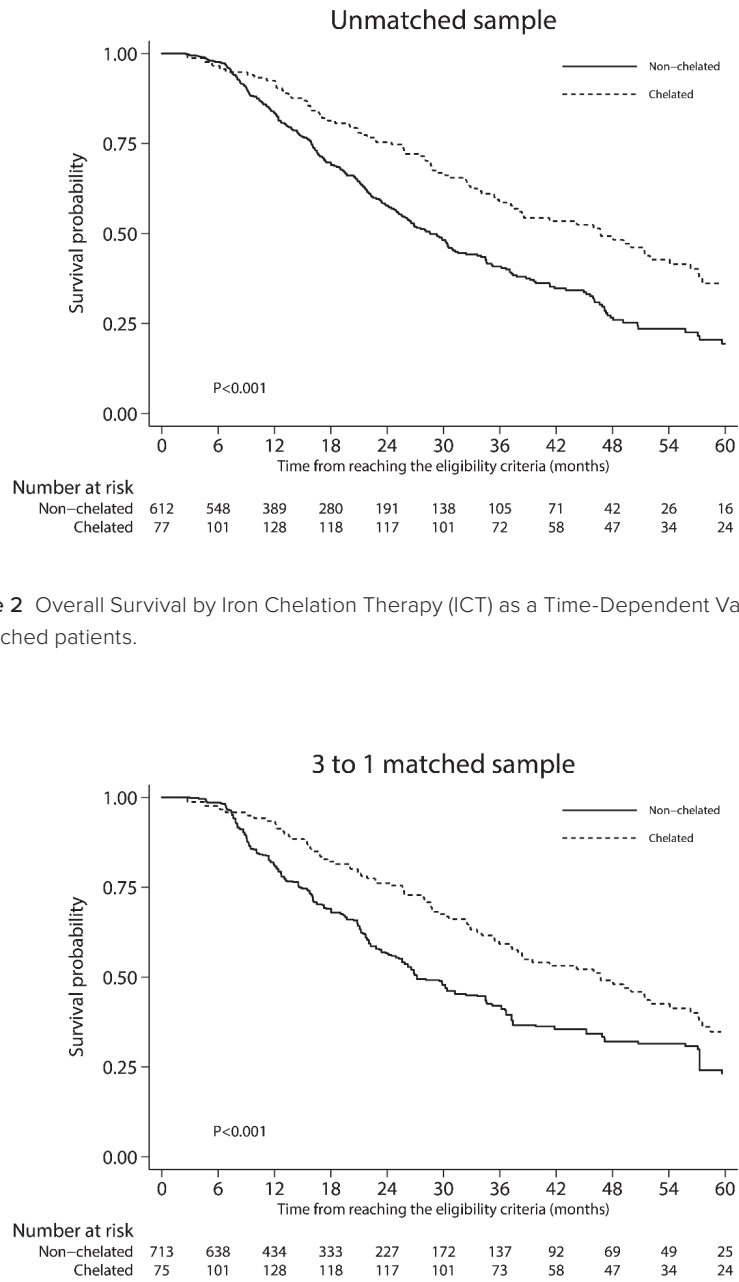


Figure 2 Overall Survival by Iron Chelation Therapy (ICT) as a Time-Dependent Variable in unmatched patients.

receipt of ICT as a time-dependent variable for the matched patients. A multivariate Cox proportional hazard model was used to adjust for potential confounders (age, sex, comorbidity, performance status, monthly RBCT intensity, number of RBC units transfused, IPSS-R, and presence of ringed sideroblasts). The estimated crude and adjusted hazard ratios were 0.70 (95% CI: 0.51-0.95) and 0.42 (0.27-0.63), respectively (Table 2) and the adjusted survival curve is shown in Figure 4. When we again restricted the analysis to the deferasirox treated patients, the crude HR for OS was 0.63 (95% CI: 0.45-0.88) and the adjusted HR was 0.34 (95% CI: 0.22-0.53).

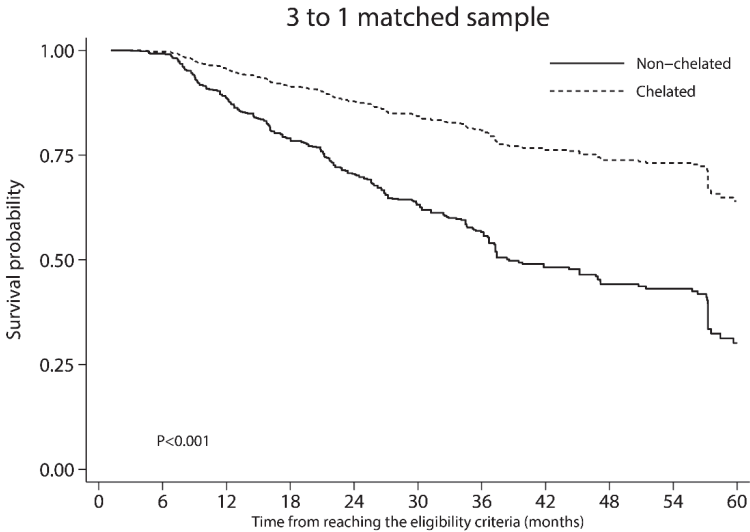


Figure 4 Adjusted Overall Survival by Iron Chelation Therapy (ICT) as a Time-Dependent Variable in matched patients.

The distribution of ESA and lenalidomide-treated patients among chelated and non-chelated patients at time of eligibility were similar in the unmatched and matched sample. A sensitivity analysis excluding the treatment of ESA and lenalidomide showed similar results.

Figure 3 Overall Survival by Iron Chelation Therapy (ICT) as a Time-Dependent Variable in matched patients.

Impact of iron chelation therapy on hematopoiesis and ferritin levels

Figure 5 shows the changes in transfusion density over eight visits in chelated and non-chelated patients. Forty-eight (62.3%) of the 77 responding patients were treated with ESA and 16 (20.8%) were treated lenalidomide during chelation therapy. Compared to visit 1, 61 of the 197 chelated patients (31.0%) had a reduction in transfusion density, i.e. an absolute decrease, during at least one visit interval, 2 patients (1.0%) maintained the same density throughout, and 134 (68.0%) never had a reduction in transfusion density. For those patients who showed a reduction, the average value in the monthly rate was -1.63 units per month (SD: 2.12, median: -0.96) compared to visit 1. Figure 6A shows the monthly red blood cell transfusion density for chelated patients with and without an erythroid response and non-chelated patients. In terms of becoming transfusion independent, 35 (17.8%) of the 197 treated patients had at least one visit interval during, approximately six months, which they had not received any further transfusions and 19 (9.6%) of the 197 patients were transfusion independent during more than one visit interval after starting chelation therapy. In total, 54 patients (27.4%) became (temporarily) transfusion independent.

In total, 77 chelated patients had an erythroid response: 61 patients had a reduction in transfusion density, and 16 patients who did not have a reduction in transfusion density became transfusion independent during at least one visit interval. We observed hematological responses with all chelating agents.

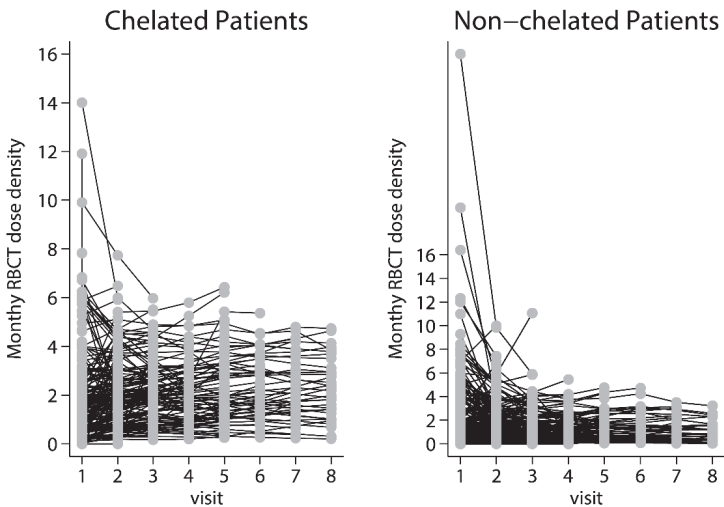


Figure 5 Changes in transfusion density over time in chelated and non-chelated patients.

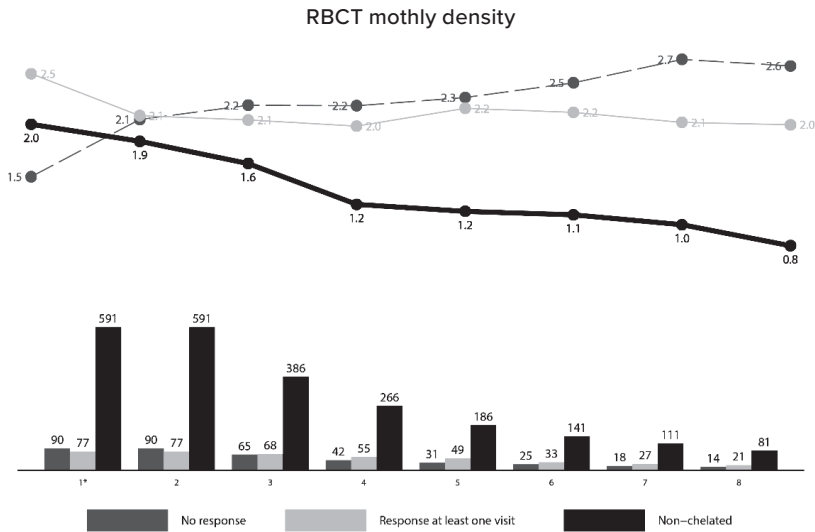


Figure 6A Trajectory analysis of monthly red blood cell transfusion density in chelated patients with and without an erythroid response and for non-chelated patients.

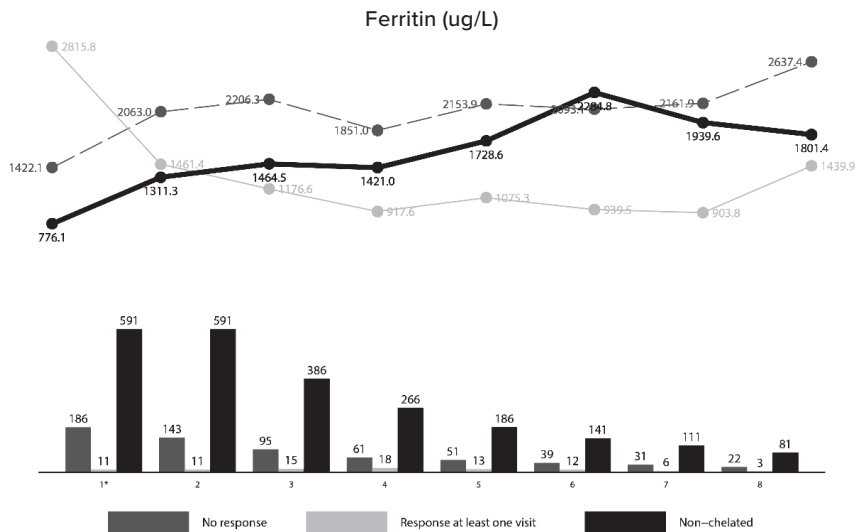


Figure 6B Trajectory analysis ferritin levels in chelated patients with and without a ferritin response and for non-chelated patients.

A subgroup of chelated patients had a platelet response (22.9%) at least temporary over time. Median platelet counts were in the normal range in both the chelated and non-chelated group.

Figure 6B demonstrates ferritin levels of chelated patients with and without a ferritin response and non-chelated patients. Fifteen (51.7%) of the 29 responding patients were treated with ESA and 5 (17.2%) were treated with lenalidomide. A subgroup of patients had a ferritin response (5.6-23.5%) over time. Responding patients showed ongoing mean serum ferritin levels around 1000 µg/L, whereas non-responding chelated patients had mean ferritin values around 2100 µ/L.

Follow-up chelated patients

On average, chelated patients did not start therapy until 17 months after diagnosis (Table 1). Of the 199 chelated patients, at the time of the analysis, follow-up was ongoing for 148 patients, for seven patients their disease had progressed to higher risk MDS/AML, 29 patients had died, and 4 have missing values of treatment dates (those four patients are still ongoing), 9 patients had withdrawn from the study (four of these because of disease progression and five after starting intensive treatment like an allogeneic stem cell transplantation), and 6 were lost to follow-up. Most patients (101 of the 148 ongoing patients) were receiving chelation at the time of the last report. Twenty of the 199 chelated patients switched from deferasirox to another chelating agent.

Reasons for cessation of iron chelation therapy

Information on reasons of cessation of ICT was not routinely recorded in the study, however, information about the deferasirox treated patients was available for 7 patients: fatigue and diarrhea (1 patient), physician's choice (1 patient), economic reasons (1 patient), renal failure (1 patient), no effect (1 patient), dyspepsia (1 patient) and lower limb cramps and dosage change (1 patient).

Renal function

Non-chelated patients had slightly higher median creatinine values compared to chelated patients at time of eligibility (non-chelated: median 86 µmol/L [p10-p90: 61-135]; chelated: median 79 µmol/L [p10-p90: 59-107]). Forty-four chelated patients had higher serum creatinine levels at the first visit after discontinuing chelation compared with creatinine levels at time of eligibility ($p=0.02$ for all chelating agents and $p=0.03$ for deferasirox-treated patients), (Figure 7).

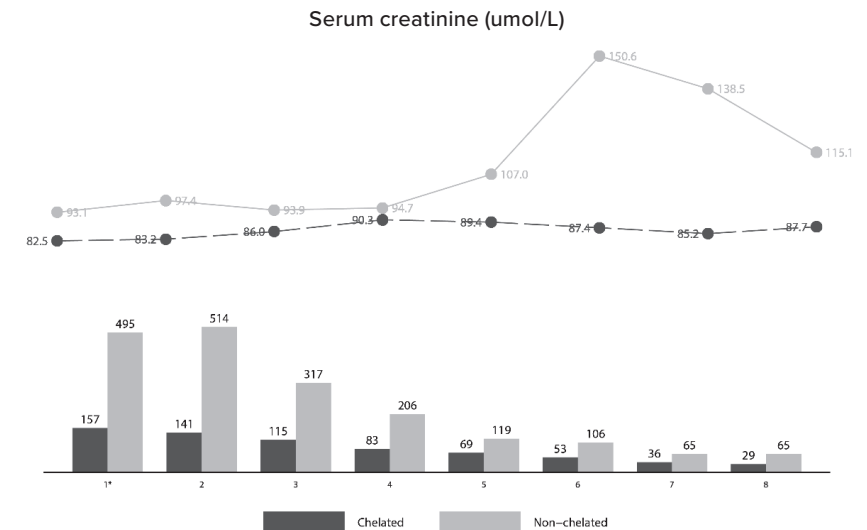


Figure 7 Serum creatinine levels (umol/L) in chelated and non-chelated patients per visit.

Discussion

The results of this study indicate that iron chelation therapy (ICT) may improve OS in transfusion dependent lower-risk MDS patients (LR-MDS). Our results are in line with several previously reported studies.^{7-10,12,25-28} Some of these studies attempted to correct for confounding factors, but still suffered from confounding by indication. This generally results in an overestimation of the beneficial effect of ICT on OS in LR-MDS patients. Currently, one randomized controlled trial has been reported on this subject. The randomized, placebo-controlled, TELESTO trial²⁹ evaluated the event-free survival (EFS) (a composite outcome, including non-fatal events related to cardiac and liver function, and transformation to acute myeloid leukemia or death) and safety of deferasirox versus placebo in low and intermediate-1-risk MDS patients. This study demonstrated an EFS risk reduction of 36.4% in the deferasirox arm ($p=0.015$). However, the median overall survival in the deferasirox-treated arm was not different (HR 0.83, 95% CI 0.54-1.28, $P=0.200$) when compared with placebo, but more than 50% of the placebo-treated patients switched to ICT after study treatment discontinuation (the placebo drug). The results of the TELESTO study are in line with our results, however, the included patients may not represent 'real-life' elderly MDS patients with multiple comorbidities,

as reflected by the mean age of 61 years old of the patients included in TELESTO study compared to the mean age of 70 years in the EUMDS Registry study. Furthermore, low accrual rates and the cross-over to ICT after cessation of the placebo, affected the statistical power of the TELESTO study.

Meanwhile, well-designed prospective observational data, reflecting, ‘real-life’ data, contribute to the better understanding of the effect of ICT on OS in LR-MDS patients. Recently, a study from the Canadian MDS registry demonstrated a superior OS for 83 chelated patients compared to non-chelated patients (5.2 vs. 2.1 years, $p < 0.001$).³⁰ The patients in this study were selected at the onset of transfusion-dependency. Chelated patients became transfusion-dependent at a much longer interval from diagnosis than non-chelated patients (median 18 versus 6 months) and OS was calculated from the time of becoming transfusion-dependent. Even after matching, some incomparability between the two groups remained in factors like concurrent treatment, presence of ringed sideroblasts, and ferritin levels. Therefore, confounding cannot be excluded in this study. Nevertheless, their conclusions are in accordance with our findings, supporting the probable beneficial effect of iron chelation therapy on OS in LR-MDS patients.

The mechanisms by which iron chelation therapy influences OS after a relatively short exposure to iron chelation therapy (median duration of 13 months) is not completely understood. A recently published study of the EUMDS Registry, as well as the follow-up data of this study, demonstrated detectable labile plasma iron (LPI) levels to be associated with inferior overall survival in LR-MDS patients.^{31,32} The risk of dying prematurely in patients with detectable LPI levels occurred too early in this study to explain this risk by classical iron overload due to organ toxicity (e.g. liver and heart) after long-term transfusions, but this indicates a direct toxic effect associated with elevated LPI levels.³¹

Likewise, increasing evidence supports that increased LPI levels may be a general predictor of an increased non-relapse mortality during and after hematopoietic stem cell transplantation.³³

Toxic iron species are known to catalyze the cellular generation of reactive oxygen species (ROS), which plays a key role in cellular damage.^{34,35} ROS damage (mitochondrial) DNA, with potential consequent genomic instability, mutagenesis and cell death. ROS are associated with leukemic transformation of the MDS clone.⁶ Moreover, iron chelation therapy is associated with a decrease of LPI and ROS.^{6,16} Overall, the present study indicates that iron chelation therapy may partly counteract the unfavorable consequences of secondary iron overload.

In up to 31.0% of chelated patients a reduction in transfusion density was observed during at least one visit interval. Likewise, 27.4% of the responding patients became, at least temporarily, transfusion independent. Platelet responses were less frequently observed. However, platelet count in this context was less relevant because the platelet counts in both groups were within the normal range, and will not likely lead to severe bleeding complications. Contemporary treatment with ESA and/or lenalidomide may have enhanced these responses.

Several previous studies recorded hematological responses to ICT.¹¹⁻¹⁶ While the percentage of patients with hematological responses in these studies are in line with the present study, none of the former studies included a control group in their analyses. One of the factors playing a role is the relatively short period of ICT (median 13 months) in this study. The duration of ICT may improve by the introduction of a better tolerated formulation of deferasirox.³⁶ Usually, ICT is prescribed relatively late after detection of signs of iron overload. Earlier initiation of ICT may prevent or decrease the occurrence of transfusional iron toxicity on hematopoiesis. Moreover, we recorded data only at six-monthly intervals. Short duration hematological responses in between visits may be missed by this approach. But on the other hand, short-lasting responses may not be clinically relevant.

Preclinical studies have shown a beneficial effect of ICT on hematopoiesis.^{35,37} Inhibition of the transcription factor NF- κ B, involved in many cellular processes, and modulation of mammalian target of rapamycin (M-TOR) signaling, a major regulator of cell death and proliferation, have been proposed to play a role.¹⁷ Future studies should address this issue appropriately.

In the trajectory analyses, ferritin responses occurred in up to 23.5% of the chelated patients. Serum ferritin levels have been reported frequently as a prognostic marker in LR-MDS patients, but serum ferritin is an imprecise surrogate marker for secondary iron overload and toxicity.^{38,39} This is reflected by the observation that a relatively small proportion of chelated patients have a considerable decrease in serum ferritin levels, while these patients show a significant survival benefit. Serum ferritin levels are influenced by the stage of MDS and by concurrent infection and inflammation, which is common in LR-MDS patients.³⁸ Additionally, no convincing evidence exists regarding its use for monitoring secondary IO in MDS patients.^{38,40} Currently, tissue biopsy and MRI T2* are regarded as most specific and sensitive diagnostic tests for detecting IO.³⁸ However, the clinical utility of these assays remains unclear in MDS and invasiveness (biopsy), unavailability, and expense (MRI T2*) hampers their general

use in clinical practice. LPI, as discussed above, is associated with inferior survival in LR-MDS patients.^{31,32} Future studies are warranted to evaluate the effect of ICT on LPI levels as a measure of iron toxicity. Also measures of oxidative stress, including malondialdehyde, a long-lasting lipid peroxidation product, formed as a consequence of oxidative stress from iron overload, are possible future markers for detecting and monitoring of the biological consequences of secondary IO in LR-MDS patients, should they be proven to correlate with clinical outcomes.^{41,42}

Analysis of renal function demonstrated that iron chelation therapy is associated with an increase of creatinine levels. In some patients this will be clinically relevant and/or a reason to stop or lower the dose of iron chelation therapy. In other patients an increase in creatinine levels will not affect cessation of iron chelation therapy.

Strengths and limitations

This large cohort, with prospectively collected ‘real-life’ data from diagnosis, provides an unique opportunity to study the effect of iron chelation therapy in a large number of lower-risk MDS patients in daily practice. An important strength is that the results are widely generalizable to this, mostly elderly, patient population with multiple comorbidities, who are typically excluded from clinical trials. The variation in iron chelation practice across the different countries, due to variable interpretation of the poor quality outcome data for ICT in MDS, made it possible to compare the effects of ICT on OS to a non-chelated control group. In Europe, unlike in the United States, socio-economic status does not influence the prescription of ICT (either deferoxamine or deferasirox) because the costs are covered by the health insurance systems.

Since conventional statistical modeling is limited by the number of covariates to be added to a model, propensity-score matched analysis is able to incorporate more confounding factors in the model, including country-specific effects. Confounding by indication, a common problem in observational studies, is maximally reduced by using the propensity-score matched method and therefore a major strength of this study. To our knowledge, we are the first to apply this method in order to adequately deal with confounding in this setting.

Limitations of our study include the moderate sample size of the deferoxamine and deferiprone groups. Additionally, differences in dosing schemes and therapy compliance could not be considered in our analysis. This prevented us from drawing definitive conclusions of the effect of the separate iron chelators on OS. Data were collected at the scheduled six-monthly intervals. Subtle changes in patient-related factors in the intervening six-month period may have been missed.

Not all patients can be matched by the propensity score approach. This might introduce selection bias. However, the same magnitude and direction of the results were seen in the analysis of the unmatched samples. Therefore, in this case, propensity-score matching will probably not have led to significant selection bias. Finally, despite using a large control group, eligible for using iron chelation therapy, and a propensity-score matched analysis corrected for many known and measured confounders, we cannot exclude residual confounding. Considering the size of the effect, it is unlikely that residual confounding would explain the difference found between chelated and non-chelated patients.

In summary, the results of this study suggest that iron chelation therapy may improve OS and hematopoiesis in transfused LR-MDS patients.

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Supplementary material

Supplementary Method Section

General

In the EUMDS registry, clinical information was collected via a bespoke web-based database on: concomitant diseases, transfusion history, use of iron chelators (chelating agent, start date and end date; no drug doses or schedules were collected), peripheral blood values, conventional iron parameters (serum ferritin, transferrin saturation), concomitant treatments (lenalidomide, erythroid stimulating agents [ESA], and hypomethylating therapy), and bone marrow pathology.

As information is recorded at 6-monthly time-points and the patients may have reached the criteria for using iron chelation therapy between visits, the visit prior to reaching the criteria was selected.

Propensity score matched method

The main purpose of PSM was to balance the distribution of observed covariates at the time of meeting the eligibility criteria in both the chelated and non-chelated groups, so there should be no systematic differences in the distribution and overlap of covariates between the two groups after matching.²⁰ The causal effect of ICT on outcome was estimated in two stages. In the first stage, the propensity score (PS) or the conditional probability of receiving ICT among eligible subjects were estimated using multivariate logistic regression using the characteristics below, identified a priori to be involved in the decision to treat a patient with ICT; A PS graph was used to check visually if the common support condition was satisfied, i.e. if there was sufficient overlap.²¹ To examine the balance in this study, we computed standardized differences that were defined as the difference between chelated and non-chelated means of each factor, divided by the pooled standard deviation. Absolute values of standardized differences <0.1 indicated sufficient balance.²⁰ A p-value of 0.01 or lower was considered to be statistically significant.

Missing data in PS estimations could result in biased estimates, and it may also shrink the pool of potential matches. The following methods were used to impute missing values: 1) last observation carried forward (LOCF) approach: For many patients bone marrow assessments were not repeated after initial diagnosis, accordingly karyotype and bone marrow blast count, required for the calculation of the IPSS-R at each visit, may be missing. A LOCF approach for only these two components of the IPSS-R was applied; 2) Multiple imputation (MI) approach: For missing values of RBCT intensity, serum ferritin level, MDS comorbidity index,

Karnofsky performance status, and IPSS-R, a MI approach was applied to create 20 multiple complete data sets consisting of all non-chelated patients and all visits since the last visit prior to meeting the eligibility criteria.²² The imputation model also included age, sex, and cumulative RBCT units.

Transfusion dose density

We used the beginning of the time interval in which the first transfusion started after diagnosis as the starting point of time to calculate the cumulative number of transfusion units received and time interval by the end of each subsequent visit. Transfusion dose density was calculated by dividing the cumulative number of units by the time since the starting time point and standardised to monthly value.

Supplementary Table 1 Description of iron chelator use				
	Unmatched	Sample	Matched	Sample
	N	%	N	%
No iron chelation	490	71.12	591	75.00
Deferasirox only	135	19.59	134	17.01
Deferoxamine only	30	4.35	29	3.68
Deferiprone only	12	1.74	12	1.52
Deferasirox and deferoxamine	13	1.89	13	1.65
Deferasirox and deferiprone	4	0.58	4	0.51
Deferoxamine and deferiprone	2	0.29	2	0.25
All of the three	3	0.44	3	0.38
Total	689	100	461	100

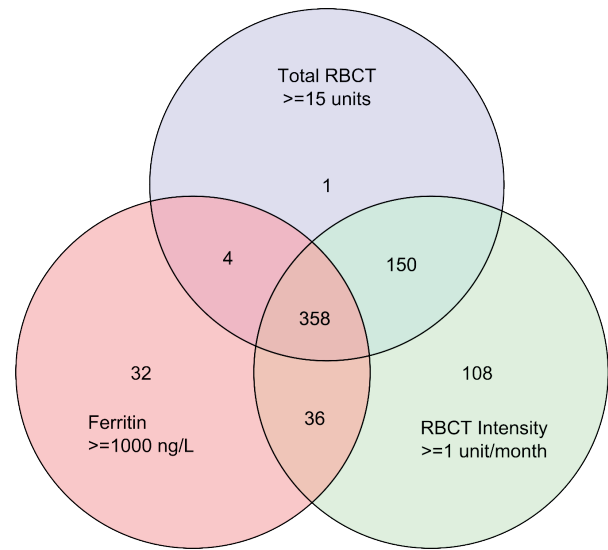
Supplementary table 2 Baseline characteristics for all unmatched transfused non-chelated and chelated patients with missing values and imputed values.

Covariates		Unmatched data with missing values					Unmatched data with imputations*						
		Non-chelated		Chelated		P-Value	Standardised differences**	Non-chelated		Chelated		P-Value	Standardised differences**
		N= 490		N= 199				N= 490		N= 199			
Age (years)		76 (10)		70 (9)		0,000	-0,554	76 (10)		70 (9)		0,000	-0,554
Sex						0,405	0,070					0,405	0,070
	Female	194 39,6%		72 36,2%				194 39,6%		72 36,2%			
	Male	296 60,4%		127 63,8%				296 60,4%		127 63,8%			
RBCT Intensity (per month)		0,5 (0,8)		0,6 (1,0)		0,038	0,169	0,5 (0,8)		0,6 (1,0)		0,046	0,161
Ferritin level (ug/L, median, p25-p75)		547,0 (251.2-878.8)		675,0 (434.9-992)		0,046	0,204	693,5 (382-884)		685,8 (504-921)		0,152	0,124
Comorbidity (MDSCI)						0,001	-0,291					0,001	-0,296
	Low risk	308	63,2%	150	75,8%			309	63,1%	151	75,9%		
	Intermediate risk	149	30,6%	43	21,7%			151	30,8%	43	21,6%		
	High risk	30	6,2%	5	2,5%			30	6,1%	5	2,5%		
Performance status						0,001	0,313					0,001	0,290
	Unable to care for self	8	2,0%	1	0,6%			8	1,6%	1	0,5%		
	Unable to work	132	32,3%	36	20,2%			151	30,8%	39	19,6%		
	Able to work and normal activity	269	65,8%	141	79,2%			331	67,6%	159	79,9%		
Prognostic indicator (IPSS-R)						0,138	-0,137					0,106	-0,139
	Very low	48	12,0%	22	12,7%			49	10,0%	22	11,1%		
	Low	199	49,9%	95	54,9%			276	56,3%	121	61,1%		
	Intermediate	111	27,8%	46	26,6%			124	25,3%	45	22,7%		
	High	38	9,5%	9	5,2%			38	7,8%	9	4,5%		
	Very high	3	0,8%	1	0,6%			3	0,6%	1	0,5%		
Country						0,001	-0,283					0,001	-0,283
	Austria	22	4,5%	10	5,0%			22	4,5%	10	5,0%		
	Croatia	3	0,6%	1	0,5%			3	0,6%	1	0,5%		
	Czech Republic	39	8,0%	25	12,6%			39	8,0%	25	12,6%		
	Denmark	24	4,9%	8	4,0%			24	4,9%	8	4,0%		
	France	88	18,0%	40	20,1%			88	18,0%	40	20,1%		
	Germany	7	1,4%	8	4,0%			7	1,4%	8	4,0%		
	Greece	34	6,9%	23	11,6%			34	6,9%	23	11,6%		
	Israel	20	4,1%	5	2,5%			20	4,1%	5	2,5%		
	Italy	19	3,9%	5	2,5%			19	3,9%	5	2,5%		
	Netherlands	10	2,0%	8	4,0%			10	2,0%	8	4,0%		

Supplementary table 2 Baseline characteristics for all unmatched transfused non-chelated and chelated patients with missing values and imputed values.

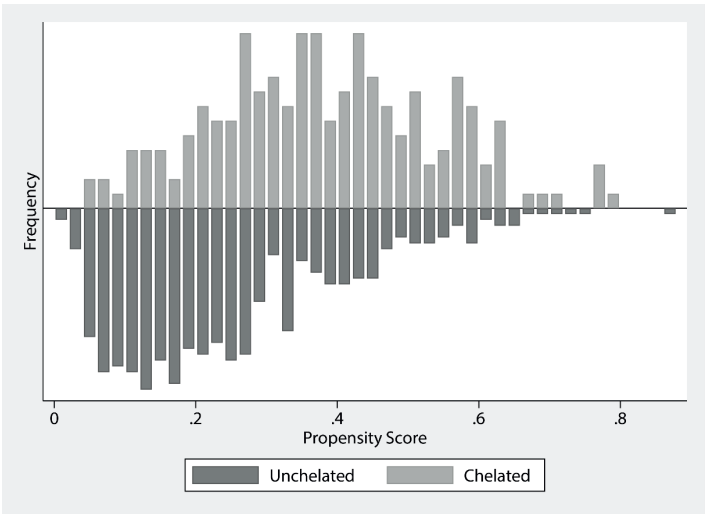
Covariates	Unmatched data with missing values				Unmatched data with imputations*			
	Non-chelated		Chelated	P-Value	Non-chelated		Chelated	P-Value
	N= 490		N= 199		N= 490		N= 199	
				Standardised differences**				Standardised differences**
Poland	15	3,1%	9	4,5%	15	3,1%	9	4,5%
Portugal	15	3,1%	1	0,5%	15	3,1%	1	0,5%
Romania	11	2,2%	11	5,5%	11	2,2%	11	5,5%
Republic of Serbia	7	1,4%	2	1,0%	7	1,4%	2	1,0%
Spain	32	6,5%	5	2,5%	32	6,5%	5	2,5%
Sweden	34	6,9%	20	10,1%	34	6,9%	20	10,1%
UK	110	22,4%	18	9,0%	110	22,4%	18	9,0%

Note: Continuous variables are reported as mean (standard deviation), while categorical variables are reported as number(percent). * Multiple imputations in RBCT intensity, ferritin level, comorbidity, performance status, and IPSS-R at eligibility criteria for unchelated patients. ** The standardised difference in percent is the the mean difference as a percentage of the average standard deviation. *** Adjusted by age, sex, comorbidity, performance status, RBCT intensity, number of units transfused, IPSS-R, and RS present. RBCT: red blood cell transfusion; MDSCI: myelodysplastic syndrome specific comorbidity index; IPSS-R: revised international prognostic scoring system; EQ-5D: European Quality of Life - 5 dimensions



Supplementary figure 1 Proportion of subjects meeting the eligibility criteria (n=689).

RBCT = Red Blood Cell Transfusion



Supplementary figure 2 Overlap of propensity scores for the chelated and non-chelated groups.

7

SUMMARY AND FUTURE PERSPECTIVES



Summary and future perspectives

Red blood cell transfusion therapy remains the cornerstone of supportive care in the treatment of patients with hematological malignancies. This thesis focuses on red blood cell transfusion strategies and monitoring and management of secondary iron overload in patients with hematological malignancies.

Red blood cell transfusion strategies

Since evidence-based guidelines for red blood cell transfusion support in patients with hematological malignancies are currently lacking, a large variation in clinical practice was expected.¹⁻³ In chapter 2, we evaluated, by means of a survey, the current Dutch red blood cell transfusion practice among hematologists. For this cross-sectional study, all hematologists and hematologists in training, currently working in the Netherlands, were asked to complete a web-based 25-question survey encompassing three clinical scenarios. The survey was distributed between November 2015 and January 2016. Seventy-seven responses, well distributed among community and university hospitals, were sent back (24%). Often, one hematologist filled in the survey as a representative for his colleagues in his/her department.

As was expected, a wide variation existed in hemoglobin triggers with a median of 8.0 g/dL (range between 5.6 to 9.5 g/dL). Personalization of the hemoglobin triggers was mostly based on the (estimated) cardiopulmonary compensation capacity of the patients. The majority of the respondents (65%) reported to transfuse two red blood cell units per transfusion episode (range 1-3 units).

The rationale for the used hemoglobin triggers reported in this study might be based on either the in the Netherlands broadly used '4-5-6 rule', which was developed for normovolemic anemia patients with acute bleeding, or according to the '10/30' rule. While the 4-5-6 rule personalizes the Hb transfusion trigger on age, the severity of the ongoing bleeding, and cardiovascular compensation capacity, the 10/30 rule recommends a red blood cell transfusion in surgical patients when the hemoglobin levels drop below 10 g/dL or the hematocrit level below 30%.^{2,4} Currently, these recommendations concerning red blood cell transfusion are regarded outdated and certainly not meant for non-bleeding hemato-oncological patients. Because of this it is likely that at least a part of the hemato-oncological patients may have received unnecessary red blood cell transfusions. Studies providing good quality evidence are needed to improve the rationale of red blood cell transfusion support of patients with hematological malignancies but will also provide guidelines that clinicians are willing to adhere to.

After the landmark trial of Hébert in 1999, in which a restrictive (7 g/dL) versus a liberal (9 g/dL) red blood cell transfusion strategy was compared in critically ill patients, many studies have focused on restrictive versus liberal red blood cell transfusion strategies in various patient groups and reported no disadvantages of a restrictive red blood cell transfusion strategy.⁵⁻¹² However, no robust evidence exists for a restrictive red blood cell transfusion strategy in patients with hematological malignancies, mostly due to limited sample size of studies including these patients. Therefore, we conducted a systematic review on the relevant studies in this respect, and performed a meta-analysis to evaluate the effect of restrictive and liberal red blood cell transfusion strategies in hemato-oncological patients on clinical outcomes and blood use. **Chapter 3** describes the results of this systematic review and meta-analysis. Our extensive literature search, last updated on August 2016, was performed in PubMed, EMBASE (Excerpta Medica Database), Web of Science, Cochrane, CINAHL (Cumulative Index to Nursing and Allied Health Literature), and Academic Search Premier without restrictions on language and year of publication. Both randomized controlled trials and observational studies were included. Risk of bias assessment was performed according to the Cochrane collaboration's tool for randomized controlled trials and the Newcastle-Ottawa scale for observational trials. After removing duplicates, 1142 publications were identified. Eventually, 15 studies, reporting on 2636 patients, were included.¹³⁻²⁷ The main result of the study showed that the pooled relative risk for mortality was 0.68 (95% CI 0.46 to 1.01) in favor of the restrictive strategy when compared to the liberal strategy. Additionally, no differences in safety outcomes were observed. The mean reduction of red blood cell use with the restrictive strategy was 1.4 units (95% CI 0.70 to 2.09) per transfused patient per therapy cycle as compared to the liberal strategy group. There are some drawbacks with regard to this study. Not all studies reported on the post-transfusion hemoglobin levels. Therefore, non-adherence of hematologists to the study protocols delineating an identical transfusion strategy in both arms might have led to smaller differences in the post-transfusion hemoglobin levels. This may have resulted in smaller effects on outcome. In order to guide red blood cell transfusion therapy in hemato-oncological patients, more information on quality of life and physical performance after transfusion is needed as well. Notwithstanding these limitations, we conclude that the current available evidence suggests that restrictive red blood cell transfusion strategies at least has no negative impact on clinical outcomes in hemato-oncological patients. In addition, while it reduces red blood cell use, it may also diminishes adverse events and certain treatment costs.

Monitoring and management of secondary iron overload

Despite increasing evidence for iron toxicity, monitoring and management of secondary iron overload in patients receiving multiple red blood cell transfusion, such as patients with hematological malignancies, is still not common practice.²⁸ While in hemoglobinopathies, treatment of transfusion-associated iron overload has shown to be beneficial in limiting organ damage and even mortality,^{29,30} clinicians treating hemato-oncological patients may perceive secondary iron overload of minor importance as a contributable factor for overall survival and iron chelation therapy itself 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 impair uniform diagnostics and treatment protocols for this form of iron overload.³¹

Finally, all of these factors contribute to a lack of studies and of enrollment of hemato-oncological patients in studies on transfusion-associated iron overload. Because of this, evidence-based guidelines for clinicians are also lacking.^{31,32}

In chapter 2, we evaluated the daily practice regarding monitoring and management of secondary iron overload, among hematologists in the Netherlands by means of a survey. The main factors that led respondents to initiate monitoring of iron overload were: a total red blood cell transfusion burden of 20-29 units or a transfusion intensity of ≥ 2 red blood cell units per month. Serum ferritin was the most frequently measured iron parameter (97% of the respondents). The T2* Magnetic Resonance imaging (MRI), a reliable way to quantify liver and cardiac iron content, was less often mentioned (58% of the respondents). High serum ferritin levels (84%) and a high red blood cell transfusion burden: >20 units were regarded the most important reasons to start iron lowering therapy. With sufficiently high hemoglobin levels, phlebotomies were preferred by 81% of the respondents. When iron chelation therapy was used, deferasirox is the most frequently used iron chelating agent. The main reasons to refrain from the initiation of iron chelation therapy despite apparent iron overload were: comorbidity with a limited life expectancy and high age (≥ 85 years). The results of this survey suggest that there is quite a variation in daily practice among hematologists regarding red blood cell transfusion support and monitoring and management of secondary iron overload. The development of evidence-based guidelines is of great importance in improving supportive care in hemato-oncological patients.

Tissue biopsy of liver and/or myocardium is still considered the conventional golden standard for the diagnosis of iron overload. Disadvantages of such tissue biopsies, however, are besides invasiveness and risk of bleeding, that they are

also prone to sampling error.³³ Therefore T2* MRI is gaining ground, although this technique is not widely available and costly. Bone marrow examinations may be considered as an alternative for examining tissue iron overload. These are routinely performed for the diagnosis and follow-up of hematological malignancies and iron staining of bone marrow specimens is a simple and inexpensive way to assess body iron content.^{34,35} When an iron staining on a bone marrow specimen is performed, the amount of iron, i.e. bone marrow iron score, both in macrophages, erythroblasts, as well as free iron, can be estimated by a grading system.³⁴ High bone marrow iron scores indicate bone marrow tissue iron overload.

In chapter 4, we quantified the relation between the cumulative administered red blood cell transfusions and bone marrow iron scores as an indicator of secondary iron overload and whether bone marrow iron scores obtained from routinely performed bone marrow aspirate samples could be clinically applicable to assess body iron overload.

From acute myeloid leukemia patients, the bone marrow iron scores of consecutive bone marrow samples were independently assessed by two trained researchers. The slides were blinded to the researchers in order to prevent bias. In total, 141 bone marrow specimens of 35 patients were included. The median number of red blood cell transfusions to reach a maximum bone marrow iron score was 20 units (range 6-42, interquartile range 15-26) after a mean of 0.99 intensive chemotherapy courses. In conclusion, the cumulative red blood cell transfusion number is associated with bone marrow iron scores. Due to the considerable variation in number of red blood cell transfusion to reach a maximum bone marrow iron score (n=6-42), assessing bone marrow iron scores instead of only considering the cumulative red blood cell transfusions, may be a valuable indicator of secondary iron overload in acute myeloid leukemia patients. In the majority of our study population, no subsequent serum ferritin levels were available. The relation between serum ferritin levels and bone marrow iron scores may be subject for future research. **In chapter 4**, we therefore conclude that bone marrow iron scores could guide iron-lowering therapy and/or transfusion strategies in an early stage.

Myelodysplastic syndrome (MDS) patients have, similar to acute myeloid leukemia patients, an even more increased risk of the development of iron overload. Not only the frequently administered red blood cell transfusions may cause iron toxicity, but a contributing factor may be the ineffective erythropoiesis, mostly seen in MDS patients with ringed sideroblasts.³⁶ The current knowledge of iron homeostasis in transfused lower-risk myelodysplastic syndrome patients is scarce. Similarly, the impact of toxic iron species as non-transferrin bound iron (NTBI) and

labile plasma iron (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, which aimed 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. In total, 259 consecutive patients from six participating countries, were included in the study. The median age was 74 years (range 37-95), 66% were males. The patients were divided into 4 subgroups. The majority of the included patients were transfusion-independent without ringed sideroblasts (non-RS, TI) (55.9%), 18.8% were transfusion-independent with ringed sideroblasts (RS, TI), 18.4% were transfusion-dependent without ringed sideroblasts (non-RS, TD), and 7% were transfusion-dependent patients with ringed sideroblasts (RS, TD). The median follow-up time was 6.6 years (95% CI 5.9 to 7.0). Serum ferritin levels were elevated in all subgroups with a mean value of 858 µg/L at the fifth six-monthly visit. The highest serum ferritin values were observed in the RS, TD group with a mean at visit 5 of 2092 µg/L. All subgroups, except for the non-RS, TI, had elevated transferrin saturation (TSAT) values. TSAT was most markedly increased in the RS, TD group with a mean TSAT of 70% at visit 1 and 88% at visit 5. LPI was only elevated in the RS, TD group with a mean value of 0.59 at visit 5. NTBI, on the other hand, is elevated in all subgroups, with again the highest values in the RS, TD group. Malondialdehyde (MDA) levels, a marker of oxidative stress, were normal in the non-RS, TI group and elevated in all other subgroups with the highest levels observed in the RS, TD group. Median MDA levels did not change significantly over time in all subgroups. Elevated LPI and NTBI levels were associated with inferior overall survival with an adjusted hazard ratio of 2.7 (95% CI 1.5 to 5.0) and 1.6 (95% CI 0.8 to 3.1), respectively. Similar results were observed for progression-free survival, for all subgroups. In conclusion, a clear association between iron toxicity and inferior overall and progression-free survival is suggested by these data.

As mentioned in the introduction, secondary iron overload may result in morbidity and mortality in intensively transfused patients. Iron chelation therapy evidently improves outcomes in hemoglobinopathy patients.^{29,30} However, whether iron depletion by the use of iron chelation therapy also improves outcome in patients with hematological malignancies has still to be elucidated. Randomized controlled trials on this topic are troublesome, so well executed observational studies are of imminent importance. Therefore, **in chapter 6** the effect of iron chelation therapy on overall survival and hematological improvement in patients with lower-risk myelodysplastic syndromes was evaluated with two different statistical models in the EUMDS registry. In order to prevent immortal time bias -an unequal comparison

of two groups not considering the fact that a treated individual must be alive at time of initiation of treatment in contrast to the control group, which leads to an overestimation of survival in the treated group-, we compared chelated patients with a non-chelated control group, which met the eligibility criteria for starting iron chelation therapy. The hazard ratio for overall survival for chelated patients, adjusted for relevant confounding factors, was 0.50 (95%CI 0.34-0.74). The propensity-score matched analysis demonstrated a significantly improved overall survival for chelated patients with a hazard ratio of 0.42 (95%CI 0.27-0.63) compared to non-chelated patients. Up to 39% of the chelated patients reached, at least temporary, an erythroid response. The results of this study suggest that iron chelation therapy may improve overall survival and hematopoiesis in transfused lower-risk myelodysplastic syndrome patients.

Future perspectives

From our studies some major questions remain:

How restrictive should we transfuse red blood cells in hemato-oncological patients?

Presently, a number of studies concerning red blood cell transfusion strategies in patients with hematological malignancies are being carried out.

Data on red blood cell transfusion strategies in patients who undergo hematopoietic stem cell transplantation are scarce as noticed in our systematic review and meta-analysis in **chapter 3**. The 'TRIST'- study will answer this question. This non-inferiority randomized controlled trial evaluates the effect of a restrictive (target 7-9 g/dL) compared to a liberal red blood cell transfusion strategy (target 9-11 g/dL) in hemato-oncological patients who underwent a stem cell transplantation with as outcomes quality of life, transplant-related mortality, and transfusion requirements. Preliminary data report on 300 enrolled patients undergoing hematopoietic stem cell transplantation (150 allogeneic and 150 autologous). Comparing the liberal and restrictive groups, statistical non-inferiority was observed for quality of life and other associated clinical outcomes. The median number of transfused red blood cell units was lower in the restrictive group as compared with the liberal group, although not statistically significant: 2 (range 2-6) versus 4 (range 2-6), respectively.³⁷

Another interesting study, in this respect, is the REDDS-1 study (ISCTN26088319). The REDDS-1 study is a randomized controlled pilot study that assesses red blood cell transfusion triggers and quality of life in transfusion-dependent MDS patients. The primary outcome is the evaluation of protocol adherence when implementing a restrictive (target 8.5 to 10 g/dL) and a liberal (target 11 to 12.5 g/dL) red blood cell transfusion strategy. Preliminary results of this pilot study suggest that performing a larger study is logistically feasible in assessing the relation of red blood cell transfusion triggers and quality of life in this specific patient group. Large differences were observed in the amount of transfused red blood cell units between the two arms: median number of red blood cell units transfused per patient 6 units (inter quartile range [IQR] 4-7) for the restrictive group and 11 (IQR 8-14) for the liberal group. Post hoc exploratory analyses favored the liberal arm for patient reported outcomes such as quality of life. There were no significant safety concerns in both arms.³⁸

Other relevant ongoing trials are:

- the RBC-Enhance trial (NCT02099669), which evaluates a restrictive (target 8.5-10 g/dL) and a liberal (11-12 g/dL) red blood cell transfusion strategy in MDS patients on quality of life, adverse events, and transfusion requirements.
- the SMD-transfu study (NCT03643042), not yet recruiting, will investigate the effect of a restrictive (target 8-10 g/dL) and a liberal (target 10-12 g/dL) red blood cell transfusion strategy on quality of life. Also activity as estimated by 'time to up and go'-test and costs will be evaluated.
- the 1versus2CGR study (NCT02461264), a randomized multicenter trial, which evaluates a single and double unit transfusion strategy in hematological patients receiving chemotherapy for acute leukemia or hematopoietic stem cells on clinical outcome and transfusion requirements.
- the REAL study (ISRCTN 96390716), a randomized controlled pilot study, which investigates the effect of a restrictive red blood cell transfusion strategy (≤ 7 g/dL) and a liberal strategy (≤ 9 g/dL) on clinical outcomes and transfusion requirements in acute myeloid leukemia patients.
- Our group currently performs an observational study, 'the OPTIMAL study' on the clinical red blood cell transfusion practice in acute myeloid leukemia patients in the Netherlands. Similar to our survey, described in **chapter 2**, we expect a large variation in transfusion triggers and red blood cell units given in one transfusion episode. This study will guide a trial with different transfusion strategies in the future in acute myeloid leukemia patients.

Hence, in the near future our understanding of the effects of red blood cell transfusion strategies in patients with hematological malignancies will increase considerably. New ways to evaluate quality of life and physical activity, such as wearables which register pulse rate, and physical activity, will be investigated in this context. Such good quality data will eventually enable the development of evidence-based guidelines on how restrictive we should transfuse each particular patient. Only this will lead to adherence of clinicians and eventually improve patient care. In this context, also costs should be considered.

How should we -apart from the restrictive use of RBC transfusions- eventually monitor and manage secondary iron overload?

Ideally, iron toxicity in individual patients should be concisely monitored. Measurement of pre- and post-transfusion markers of iron toxicity such as labile plasma iron and non-transferrin bound iron, or even markers of oxidative stress would enable individualized treatment of secondary iron overload. Evidence that early initiation of iron chelation therapy is beneficial on clinical outcomes as overall and progression-free survival and safe in our hemato-oncological patient group, is essential.

The definitive results of the TELESTO study will be published soon. This randomized, placebo-controlled trial evaluated event-free survival -a composite outcome including non-fatal events related to cardiac and liver function, transformation to acute myeloid leukemia, and death- and safety of treatment with deferasirox or placebo in low and intermediate-1-risk MDS patients. Two hundred-twenty-five patients were included (deferasirox n=149, placebo n=76), with a median age of 61.0 years old. The event-free survival was reduced by 36.4% in the deferasirox-treated patients ($p=0.015$) compared to the placebo group. However, overall survival was not statistically different between the two groups. The TELESTO study is the only randomized controlled trial evaluating the effect of deferasirox on event-free and overall survival. There are, however, several limitations to this study. First of all, due to enrollment problems, the total number of included patients was reduced from over 600 to 225 patients with an adjustment of the randomization factor from 1:1 to 2:1, which affects the statistical power of the study. Secondly, the outcome, event-free survival, may not be a relevant clinical outcome. Overall survival, in this respect, is more clinically relevant as differences in for example liver function are of less importance compared to overall and progression-free survival. Finally, the median age of the included patients in the TELESTO study is 61.0 years, whereas the median age in the normal MDS population is above 70 years of age. Therefore, the results of the TELESTO study are less generalizable to the mostly elderly MDS patient with multiple comorbidities.³⁹

The crucial question whether early start of low dose iron chelation therapy is beneficial in preventing iron toxicity in chronic transfusion-dependent patients, like MDS patients, is still not answered. Several studies address this important topic: NCT03387475 evaluates transfusion-independency after 12 months use of low dose deferasirox after erythroid stimulating agents in refractory or relapsing MDS patients; another study, NCT03920657, a phase II study, will evaluate the effect of low dose deferasirox on hepatic iron content and iron parameters.

Another pressing question is whether iron chelation therapy in patients who underwent an allogeneic stem cell transplantation is efficacious and safe. Study NCT03659084 will investigate this question in acute myeloid leukemia patients with relapse-free survival, with occurrence of graft versus host disease, infections, and toxicity as endpoints. Two other studies, NCT01159067 and NCT00602446, will evaluate the safety, tolerability, and efficacy of low dose deferasirox in patients who underwent an allogeneic stem cell transplantation.

Regarding all these studies, the next coming years, important information on the treatment of iron overload/iron toxicity in patients with hematological malignancies will be gained. This may lead to an improvement in the treatment in one of the most overlooked transfusion-associated side-effect, namely iron overload.

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8

NEDERLANDSE SAMENVATTING



Nederlandse samenvatting

In Europa wordt 20-30% van alle rodebloedcelproducten toegediend aan patiënten met een hematologische ziekte zoals leukemie, myelodysplastisch syndroom (MDS, aanmaakstoornis in het beenmerg) en thalassemie (aangeboren afwijking van de rode bloedcel). Deze ziekten zelf, maar ook de behandeling ervan verstoren de normale aanmaak van bloed in het beenmerg. Rodebloedceltransfusies dienen bij deze patiënten om potentieel ernstige gevolgen van diepe bloedarmoede tegen te gaan en een redelijke kwaliteit van leven te handhaven. Rodebloedceltransfusies kunnen echter acute bijwerkingen geven zoals koortsreacties, bloedafbraak door vorming van antistoffen tegen eiwitten op de rodebloedcelmembraan van de donor, risico op het overdragen van infectieziekten van donor op de patiënt en volume-overbelasting. Een chronische, maar niet minder ernstige, bijwerking van rodebloedceltransfusies is de stapeling van ijzer dat in grote hoeveelheden in de rode bloedcellen van de donor aanwezig is. IJzer is een essentieel element dat een belangrijke rol speelt in diverse fysiologische processen, waaronder het zuurstoftransport door de rode bloedcellen, energieproductie in lichaamscellen en inactivatie van vrije zuurstofradicalen (toxische zuurstofmoleculen welke kunnen leiden tot orgaanschade). Een overmaat aan ijzer is echter extreem toxisch. Het geeft schade aan onder andere hart- en levercellen en is geassocieerd met een verminderde overleving. Het menselijk lichaam heeft geen mogelijkheid een overmaat aan ijzer actief uit te scheiden, daarom wordt de ijzeropname in het lichaam strikt gereguleerd door het leverhormoon hepcidin. Bij bloedarmoede wordt er minder hepcidin aangemaakt, waardoor de 'ijzerpoorten' in de darm open gaan staan om meer ijzer te kunnen opnemen. Erythroferrone is een recent ontdekt hormoon dat door voorlopers van rode bloedcellen wordt geproduceerd en de werking van hepcidin remt. Dit proces wordt in gang gezet bij bloedarmoede onder invloed van EPO en zorgt er via verlaging van hepcidin voor dat er meer ijzer beschikbaar komt voor de aanmaak van rode bloedcellen. Een rodebloedceltransfusie bevat 200-250 mg aan ijzer terwijl het dagelijks verbruik en opname via de darm slechts 1-2 mg bedraagt. Met één rodebloedceltransfusie krijg je dus het 100-voudige van je gebruikelijke dagelijkse ijzeropname binnen. Het herhaald toedienen van rodebloedceltransfusies leidt dan ook tot de vorming van toxische ijzerwaarden welke aanleiding kunnen geven tot eerder genoemde bijwerkingen als hartfalen, leverfalen en een verminderde overleving. Bij sommige hematologische ziekten zoals thalassemie en het myelodysplastisch syndroom (met name vormen met aanwezigheid van bepaalde rode voorlopercellen, de zogenaamde ringsideroblasten) speelt nog een additioneel mechanisme een rol. Doordat er een verworven of aangeboren afwijking is in de rode bloedcellen, worden deze abnormale rode bloedcellen,

aangemaakt in het beenmerg, versneld weer afgebroken. Hierbij komt opnieuw ijzer vrij wat schadelijk kan zijn. Daarnaast is er bloedarmoede waardoor er via verlaging van hepcidin meer ijzer uit de darm wordt opgenomen. Hierdoor ontstaat een vicieuze cirkel met bloedarmoede en ijzerstapeling tot gevolg. Samengevat, bij hematologische ziekten met bloedarmoede en zeker die waarbij herhaald toedienen van rodebloedceltransfusies nodig is, worden toxische ijzerwaarden gevonden welke aanleiding kunnen geven tot eerder genoemde bijwerkingen als hartfalen, leverfalen en een verminderde overleving.

Dit proefschrift richt zich op strategieën voor rodebloedceltransfusies en de klinische effecten en behandeling van secundaire ijzerstapeling bij patiënten met hemato-oncologische ziekten.

Hoofdstuk 1 geeft een algemene inleiding van dit proefschrift. Het beschrijft de historie van het ontstaan van de transfusiegeneskunde tot aan de huidige klinische praktijk. Voor- en nadelen van een restrictief (beperkt) versus een liberaal (minder beperkt) transfusiebeleid voor rode bloedcellen worden besproken. Daarnaast wordt de fysiologie van de ijzerhomeostase in meer detail beschreven waarin onder andere de werking van recent ontdekte belangrijke hormonen als hepcidin en erythroferrone wordt uitgelegd. Tevens wordt de diagnose en behandeling van ijzerstapeling in de huidige praktijk uiteengezet.

In hoofdstuk 2 worden de resultaten beschreven van een vragenlijstonderzoek onder hematologen en hematologen in opleiding in Nederland. Hierin werd hun beleid en mening bevraagd omtrent rodebloedceltransfusies en de diagnose en behandeling van secundaire ijzerstapeling bij patiënten met een hemato-oncologische maligniteit. Naast 25 standaardvragen werden er klinische scenario's voorgelegd aan de respondenten. Aangezien goed onderbouwde richtlijnen vooralsnog ontbreken, werd een grote variatie in de klinische praktijk verwacht. De hemoglobine waarde waarbij een rodebloedceltransfusie werd gegeven varieerde inderdaad van 3,5 mmol/L tot 6,0 mmol/L; de meerderheid van de respondenten transfundeert echter bij een hemoglobine waarde van 5,0 mmol/L. Per transfusie-episode werden over het algemeen twee rodebloedcelproducten toegediend; dit varieerde echter ook van één tot drie eenheden per keer. De rationale voor de gebruikte transfusiedrempels is mogelijk gebaseerd op de '4-5-6 regel', welke is ontwikkeld om het transfusiebeleid te sturen bij normovolemische patiënten met acuut bloedverlies, ofwel op de '10/30 regel', ontwikkeld in de jaren '40. Waar de '4-5-6 regel' de transfusiedrempel personaliseert op basis van leeftijd, ernst van bloedverlies en de cardiopulmonale reservecapaciteit, adviseert de '10/30 regel' bij chirurgische patiënten de hemoglobine waarde

boven de 10 g/dL (~6,3 mmol/L) of een hematocrietwaarde boven de 30% te houden. Deze 'regels' worden tegenwoordig als gedateerd beschouwd en zijn zeker niet bedoeld voor het transfusiebeleid bij hemato-oncologische patiënten en krijgt hierdoor in ieder geval een deel van deze patiënten onnodige rodebloedceltransfusies. Dit is onwenselijk gezien het mogelijk optreden van bijwerkingen waaronder chronische ijzerstapeling. Nieuwe, goed uitgevoerde studies zijn dus nodig om het transfusiebeleid bij hemato-oncologische patiënten beter te sturen en te uniformeren.

Een manier om het risico op ijzerstapeling te reduceren is het restrictief toedienen van rodebloedceltransfusies. In vele patiëntgroepen is gebleken dat het restrictief toedienen van rodebloedceltransfusies niet slechter of zelfs beter is dan een liberaal transfusiebeleid wanneer men kijkt naar klinische uitkomsten als overleving. Echter in de hemato-oncologische patiëntengroep is dit effect van restrictiever transfunderen op overleving, maar ook op behoud van kwaliteit van leven nog niet goed bekend. Daarom hebben wij, na een uitgebreide literatuurstudie, een systematische review en een meta-analyse verricht naar het effect van een restrictief versus een liberaal rodebloedtransfusiestrategie op klinische uitkomsten en bloedverbruik bij hemato-oncologische patiënten. **Hoofdstuk 3**, beschrijft de resultaten van deze studie. De uitkomsten van patiënten die behandeld waren met een restrictief transfusiebeleid waren niet slechter, en soms beter, als men kijkt naar totale overleving ten opzichte van de patiënten behandeld met een liberaler transfusiebeleid. Daarnaast werden er geen verschillen geobserveerd wat betreft de veiligheid. Niet verrassend was dat in de restrictief getransfundeerde groep, het totale bloedverbruik minder was ten opzichte van de liberaal getransfundeerde groep. Gegevens over verschillen in de kwaliteit van leven en fysieke inspanningsmogelijkheden tussen de restrictief en liberaal getransfundeerde groep bleken nog te ontbreken en zijn onderwerp zijn van lopende studies. Ondanks dat concluderen we dat het huidige beschikbare bewijs suggereert dat een restrictief rodebloedceltransfusiebeleid geen negatieve invloed heeft op klinische uitkomsten bij hemato-oncologische patiënten, terwijl het bloedverbruik, bijwerkingen en bepaalde behandelkosten potentieel verminderen.

De gouden standaard voor het vaststellen van de diagnose van ijzerstapeling is nog altijd een weefselbiopt van lever en/of hart. Dit is echter een invasief onderzoek met risico op bloedingen en sampling error. Daarom wordt steeds vaker gekozen voor een T2* MRI om ijzerstapeling in hart en/of lever te diagnosticeren. Echter, deze techniek is duur en niet overal beschikbaar. Beenmergonderzoek kan worden beschouwd als een goed alternatief om de weefselijzerstapeling te schatten. Het beenmerg wordt al routinematig onderzocht bij patiënten met hemato-oncologi-

sche ziekten, ter diagnose en tijdens de follow-up. IJzerkleuring van beenmerg-aspiraten is een simpele en goedkope manier om de ijzervoorraad in het lichaam te schatten. De beenmergijzerscore kan worden geschat aan de mate van ijzer-aankleuring van macrofagen en erytroblasten en het vrij aanwezige ijzer door middel van een gevalideerd gradatiesysteem. Hoge beenmergijzerscores zijn indicatief voor ijzerstapeling in het beenmergweefsel. **In hoofdstuk 4** evalueerden we de relatie tussen cumulatief toegediende rodebloedceltransfusies en beenmergijzerscores als een indicator van secundaire ijzerstapeling in het beenmerg bij patiënten met acute myeloïde leukemie. Het cumulatief toegediende aantal rodebloedceltransfusies was inderdaad geassocieerd met een maximale beenmergijzerscore, echter werd een grote variatie geobserveerd in het aantal transfusies dat nodig was om een maximale beenmergijzerscore te behalen. Daarom kan het waardevol zijn de beenmergijzerscore te bepalen in plaats van alleen het cumulatief aantal rodebloedceltransfusies in acht te nemen. We concluderen dat beenmergijzerscores ijzerverlagende therapie en/of transfusiestrategieën kan sturen in een vroeg stadium zonder dat patiënten hiervoor extra worden belast.

In vergelijking met acute myeloïde leukemie, hebben patiënten met een myelodysplastisch syndroom (MDS) een nog groter risico op het ontwikkelen van ijzerstapeling. Niet alleen door frequent toegediende rodebloedceltransfusies, maar ook door de eerdergenoemde extra, maar door de ziekte ineffectieve, aanmaak van rode bloedcellen. Ondanks dat de ijzerfysiologie in de afgelopen jaren steeds beter is ontrafeld, is er nog veel te onderzoeken op het gebied van de ijzerhomeostase in laag-risico MDS patiënten. Daarnaast zijn er weinig gegevens over de impact van toxische ijzerwaarden als niet aan transferrine gebonden ijzer (NTBI) en labiel plasma ijzer (LPI) op de overleving in deze patiëntengroep. **Hoofdstuk 5** beschrijft de resultaten van een substudie binnen een grote Europese dataregistratiestudie: de EUMDS registratie waarbij klinische - en laboratoriumdata worden verzameld van laag-risico MDS patiënten in 16 Europese landen en Israël. Het doel was meer inzicht te verschaffen in de pathofysiologie en de impact van secundaire ijzerstapeling bij deze patiëntengroep. Hierbij werd onderscheid gemaakt in transfusie-afhankelijke en transfusie-onafhankelijke patiënten en de aan- dan wel afwezigheid van ringsideroblasten. In de transfusie-afhankelijke groep met ringsideroblasten werden de hoogste waarden geobserveerd van serum ferritine, transferrinesaturatie, NTBI, LPI en malondialdehyde als een marker van oxidatieve stress. Verhoogde LPI en NTBI waarden zijn geassocieerd met een verminderde totale en progressie-vrije overleving.

Het verlagen van ijzer door middel van ijzerchelatietherapie verbetert de uitkomsten bij patiënten met een hemoglobinoopathie als thalassemie en sikkelcelziekte.

Of ijzerchelatietherapie de uitkomsten van MDS patiënten verbetert is onduidelijk. Daarom evalueerden wij **in hoofdstuk 6** het effect van ijzerchelatietherapie op de totale overleving en verbetering van de bloedaanmaak in patiënten met laag-risico MDS binnen de EUMDS-registratiestudie. Na uitgebreide correctie voor versturende factoren werd een overlevingsvoordeel gezien in de patiënten die behandeld werden met ijzerchelatie ten opzichte van de controlegroep. Bij een deel van de patiënten behandeld met ijzerchelatietherapie werd een verbetering van het hemoglobinegehalte gezien of zelfs tijdelijke transfusie-onafhankelijkheid. De resultaten van deze studie suggereren dat ijzerchelatie de totale overleving en de bloedaanmaak verbetert in laag-risico MDS patiënten.

De bovenstaande studies zijn opgezet met als doel het transfusiebeleid en de diagnose en behandeling van secundaire ijzerstapeling bij hemato-oncologische ziekten te verbeteren. In dit proefschrift hebben we laten zien dat er in de huidige Nederlandse praktijk veel variatie is in transfusiebeleid en behandeling van ijzerstapeling onder hematologen. Dit vraagt in ieder geval om een meer evidence-based aanpak. Er zijn geen aanwijzingen gevonden dat een restrictief rodebloedceltransfusiebeleid een negatieve invloed heeft op de klinische uitkomsten van hemato-oncologische patiënten, terwijl het mogelijk bijwerkingen kan voorkomen. Het verrichten van een ijzerkleuring op beenmerg kan inzicht geven in de mate van ijzerstapeling en kan de behandeling sturen zonder dat de patiënt wordt blootgesteld aan extra onderzoeken. Met name transfusie-afhankelijke MDS patiënten met aanwezigheid van ringsideroblasten in het beenmerg hebben een hoog risico op ijzerstapeling. De aanwezigheid van toxische ijzerparameters is geassocieerd met een verminderde overleving bij laag-risico MDS patiënten. De behandeling van ijzerstapeling met ijzerchelatietherapie resulteert in een betere overleving en verbetering van de bloedaanmaak en moet derhalve overwogen worden in de behandeling van deze patiëntengroep.

Lopende en toekomstige studies zullen moeten bepalen welk geïndividualiseerd rodebloedceltransfusiebeleid bij hemato-oncologische patiënten in zowel de klinische als poliklinische setting het beste is. Naast de hemoglobinewaarde zijn hierbij kwaliteit van leven en verbetering van de fysieke activiteit van belang. Hiernaast zal de timing en dosis van inzetten van ijzerverlagende therapie worden onderzocht, waarbij het vroeg starten van (een lagere doses) ijzerchelatietherapie mogelijk nog extra kan bijdragen tot verdere verbetering van de overleving, leidend tot een betere bloedaanmaak en preventie van ijzerstapeling-gemedieerde orgaanschade. Naar verwachting zullen met die resultaten goed onderbouwde, geüniformeerde richtlijnen kunnen worden opgesteld voor de ondersteuning van onze hemato-oncologische patiënten.

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**CURRICULUM VITAE
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Curriculum Vitae

Marlijn Hoeks was born on the 20th of July, 1984, in Best, The Netherlands. She finished her secondary school (Gymnasium) in 2002 at the Rythovius College in Eersel, The Netherlands. In 2002, she initiated her medical training at the Radboud University in Nijmegen, The Netherlands. She obtained her medical degree in July 2008. In December 2008, she started her clinical training in internal medicine at the Radboudumc in Nijmegen (Prof. Dr. J. de Graaf). In 2011, she continued her training at the TweeSteden Ziekenhuis in Tilburg (Dr. T.K.A. Wierema) for one year, after which she returned to the Radboudumc. From April 2013, she started her differentiation into hematology. In 2015, she discontinued her traineeship for doing a PhD project at the Center of Clinical Transfusion Research, Sanquin Leiden (Prof. Dr. J.J. Zwaginga and Prof. Dr. N.M.A. Blijlevens) and the department of clinical epidemiology of the Leiden University Medical Center. She combined her PhD project with the training in clinical epidemiology. Her thesis is focused on red blood cell transfusions and secondary iron overload in hemato-oncological patients. The results from her thesis project are described and discussed in this thesis. In January 2019, she finished her traineeship and continued working in the Radboudumc as an internist-hematologist with a special focus on benign hematology and transfusion medicine.

Marlijn is married to John and together with their son Daan and daughter Eef they live in Eersel.

List of publications

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- Theo de Witte, Luca Malcovati, Pierre Fenaux, David Bowen, Argiris Symeonidis, Moshe Mittelman, Reinhard Stauder, Guillermo Sanz, Jaroslav Čermák, Saskia Langemeijer, Eva Hellström-Lindberg, Ulrich Germing, Mette Skov Holm, Krzysztof Mądry, Aurelia Tatic, António Medina Almeida, Aleksandar Savic, Inga Mandac Rogulj, Raphael Itzykson, **Marlijn Hoeks**, Hege Gravdahl Garelius, Dominic Culligan, Ioannis Kotsianidis, Lionel Ades, Arjan A. van de Loosdrecht, Corine van Marrewijk, Ge Yu, Simon Crouch, Alex Smith, on behalf of the EUMDS Registry Participants. Novel dynamic outcome indicators and clinical endpoints in MDS developed during the first 10 years of the EUMDS Registry: the MDS-RIGHT project perspective. Submitted.

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List of abbreviations

AHTR	acute hemolytic transfusion reaction
AML	acute myeloid leukemia
BM	bone marrow
BMIS	bone marrow iron score
BMP	bone morphogenetic protein
BMT	bone marrow transplantation
CI	confidence interval
DHTR	delayed hemolytic transfusion reaction
DNA	deoxyribonucleic acid
EFS	event-free survival
EQ-5D	euro quality of life 5 dimension scale
ESA	erythropoiesis stimulating agents
EUMDS	european mds registry
GDF-15	growth differentiation factor 15
Hb	hemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HR	hazard ratio
HSCT	hematopoietic stem cell transplantation
ICT	iron chelation therapy
IO	iron overload
IPSS(-R)	(revised) international prognostic scoring system
IQR	interquartile range
IWG	international working group
LLOD	lower limit of detection
LOCF	last observation carried forward
LPI	labile plasma iron
LR-MDS	lower-risk myelodysplastic syndrome
MDA	malondialdehyde
MDS	myelodysplastic syndrome
MDSCI	myelodysplastic syndrome comorbidity index
MDS-RAEB	myelodysplastic syndrome- refractory anemia with excess of blasts
MDS-U	myelodysplastic syndrome unclassifiable
MI	multiple imputation
MRI	magnetic resonance imaging
NTBI	non-transferrin bound iron
NYHA	New York heart association
OS	overall survival
PFS	progression-free survival
PLT	platelet

PS	propensity score
QoL	quality of life
RA	refractory anemia
RARS	refractory anemia with ring sideroblasts
RBC	red blood cell
RBCT	red blood cell transfusion
RCMD	refractory cytopenia with multilineage dysplasia
RCT	randomized controlled trial
ROS	reactive oxygen species
RR	relative risk
RS	ring sideroblasts
SD	standard deviation
sTfR	soluble transferrin receptor
TACO	transfusion associated cardiac overload
TD	transfusion-dependent
TI	transfusion-independent
TRALI	transfusion-related acute lung injury
TSAT	transferrin saturation
VOD	veno-occlusive disease
WHO	world health organization
WMD	weighted mean difference
5q-	deletion of the long arm of chromosome 5

