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► **To cite this version:**

Norbert Gattermann, Eliezer A. Rachmilewitz. Iron overload in MDS–pathophysiology, diagnosis, and complications. *Annals of Hematology*, 2010, 90 (1), pp.1-10. <10.1007/s00277-010-1091-1>. <hal-00585166>

HAL Id: hal-00585166

<https://hal.science/hal-00585166v1>

Submitted on 12 Apr 2011

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Iron overload in MDS – pathophysiology, diagnosis and complications

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Running Title: Iron overload in MDS

Keywords: iron overload, myelodysplastic syndromes, oxidative stress, non-transferrin-bound iron, labile plasma iron, iron chelation

Word count: 4393

Number of Figures: 6

Number of Tables: 1

Abstract

Many patients with myelodysplastic syndromes (MDS) become dependent on blood transfusions and develop transfusional iron overload, which is exacerbated by increased absorption of dietary iron in response to ineffective erythropoiesis. However, it is uncertain whether there is an association between iron accumulation, clinical complications and decreased likelihood of survival in MDS patients. Here we discuss our current understanding of the effects of transfusion dependency and iron overload in MDS, indicate our knowledge gaps, and suggest that particular emphasis should be placed on further characterizing the role of redox-active forms of labile iron, which may be as important as the total iron burden.

Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal bone marrow disorders characterized by ineffective hematopoiesis and peripheral blood cytopenias. Genomic instability of the MDS clone often leads to disease progression, which results in overt leukemia in 0–32% of patients depending on MDS subtype [1]. Eighty percent of patients have a hemoglobin level of less than 10 g/dl at diagnosis, and most of these will become transfusion dependent [2]; the development of iron overload (IO) is unavoidable in these patients. Moreover, iron accumulation in MDS patients is an ongoing process as ineffective erythropoiesis provides a signal that stimulates intestinal iron absorption. The molecular nature of that signal remained elusive for decades, but there is now evidence that ineffective erythropoiesis is associated with increased secretion of growth differentiation factor 15 and/or TWSG1 [3,4] by maturing erythroblasts, leading to suppressed hepcidin production in the liver [5]. Since hepcidin downregulates iron absorption in the duodenum, lack of hepcidin causes unrestrained intestinal iron uptake. Although this mechanism contributes to iron overload in MDS, it is not the main cause, and rarely leads to serum ferritin (SF) levels above 1000 ng/ml at diagnosis [6]. The main cause of iron overload in MDS is chronic transfusion therapy.

Most of our knowledge regarding the detrimental effects of transfusional iron overload stems from the experience in β -thalassemia major. The question is whether a similar association between iron accumulation, clinical complications and decreased likelihood of survival is also true of MDS patients. Here we discuss current understanding of the effects of transfusion dependency and iron overload in MDS, indicate the gaps in our knowledge, and suggest that particular emphasis should be placed on further characterizing the role of redox-active forms of labile plasma iron (LPI) and of labile cellular iron (LCI).

Pathophysiology of iron overload

Intestinal iron absorption is controlled by hepcidin, a peptide hormone that generally adapts duodenal iron absorption to the demands of erythropoiesis and regulates the release of iron from macrophages [7,8]. Iron is stored in ferritin, an

intracellular iron storage protein primarily found in the liver and in the macrophage system. During infection and other inflammatory states, hepcidin production is increased, leading to a decrease in both intestinal iron absorption and macrophage iron release. The resulting decrease in transferrin saturation helps to deprive circulating microorganisms of iron, but also restricts iron availability for erythropoiesis, thereby causing anemia of chronic inflammation.

Circulating iron is bound to transferrin and when the binding capacity of transferrin is exceeded, non-transferrin-bound iron (NTBI) species appear in the plasma [9]. The portion of NTBI with the weakest binding to plasma biomolecules is labile plasma iron (LPI), which is redox-active and able to permeate organs such as the heart and liver, where it contributes to the generation of reactive oxygen species (ROS) [10]. While hydrogen peroxide and superoxide are comparatively non-toxic and can actually function as physiological signaling molecules, their reaction with unliganded or incompletely liganded iron ions creates more damaging oxygen radicals, in particular the extremely reactive hydroxyl radical (OH•) [11]. While LPI is detected almost exclusively in pathological conditions, labile cellular iron (LCI) is a normal component that is regulated to serve the cellular iron requirements and to prevent an excess of redox-reactive iron that may trigger cellular damage. High LCI levels catalyze increased ROS generation through the Haber-Weiss and Fenton reactions, which may eventually overwhelm the cell antioxidant capacity and deplete cellular antioxidants like reduced glutathione (GSH), resulting in tissue oxidative damage and organ dysfunction (Fig 1).

Decreased life expectancy in transfusion-dependent MDS: identifying the culprit

Transfusion dependency is strongly associated with decreased survival in MDS patients (Fig. 2), as shown by Malcovati *et al* who demonstrated a dose-dependent impact of transfusion requirements on overall and leukemia-free survival [12]. These observations were recently corroborated in a large, retrospective, multicenter analysis from Spain [13]. However, the association between transfusion dependency and decreased life expectancy in patients with MDS might have several causes. Patients may develop clinical complications

resulting from iron overload due to inadequate iron chelation therapy; however, transfusion dependency could merely indicate a more severe bone marrow disease with complications independent of iron overload.

As the severity of bone marrow disease is at least partly determined by karyotype anomalies, one would expect transfusion dependency to lose its prognostic value when the karyotype is taken into account. However, several studies have shown that this is not the case. Transfusion dependency is a risk factor that is independent of cytogenetic risk groups in MDS [12], suggesting that the prognostic influence of transfusion dependency is not solely based on the severity of the underlying bone marrow disease but also on an additional component, which is most likely to be the effects of iron overload. A recent multivariate analysis [13] showed that the prognostic impact of IO was independent of the World Health Organization (WHO) classification-based scoring system (WPSS) which already incorporates transfusion dependency [14]. Therefore, even after transfusion requirement had been taken into account, IO with SF levels above 1000 ng/ml remained an independent prognostic factor, both for overall and leukemia-free survival.

This issue is portrayed in Figure 3 which points out that transfusion dependency clearly has a negative effect on overall survival because it is a consequence of severe bone marrow disease, reflecting the complications of chronic anemia, infections and bleeding. In addition, transfusion dependency causes iron overload, thereby creating a new medical problem which has its own negative impact on survival. This impact is not restricted to patients with low-risk MDS, but was also documented in refractory anemia with excess blasts (RAEB)-1 and even in RAEB-2 [13].

Chee *et al* concluded that neither SF nor number of red blood cell (RBC) transfusions affect overall survival in patients with refractory anemia with ring sideroblasts (RARS) [15]. However, since 72% of the RARS patients had received only a mean of 23 units of packed red cells, and since diTucci had demonstrated that at least 100 units are required before cardiac T2* becomes abnormal [16], the absence of more evidence of cardiac failure in the study by Chee *et al.* is not surprising. Moreover, because autopsies were not performed and almost half of the patients who died had no cause of death available, the results of that study have to be interpreted with caution [17].

Increased risk of leukemic transformation in MDS patients with iron overload: is there a link?

Both the Italian and the Spanish groups reported that iron overload is related to the risk of leukemic transformation [13,18]. Although this could be due to severe bone marrow disease, the Spanish study showed that the predictive value of IO was independent of the WPSS. Why should iron overload affect the frequency of leukemic transformation? It has been shown that endogenous production of ROS may be elevated in myeloid malignancies as a consequence of the underlying genetic changes [19]. Increased ROS can drive a cycle of genomic instability leading to DNA double-strand breaks and error-prone repair, promoting the acquisition of further genomic changes [20]. Increased LCI, generated by dysregulated cellular iron metabolism or increased LPI, may further exacerbate oxidative stress and thus aggravate the genomic instability of the pre-leukemic clone, thereby promoting clonal evolution towards acute leukemia [21].

On the other hand, a recent matched-pair analysis from the Düsseldorf MDS Registry, including 93 patients with various types of MDS undergoing long-term chelation therapy and 93 matched patients receiving supportive care only, did not show a significant difference in the frequency of acute myeloid leukemia transformation between the groups (10% vs. 12% [2 years after diagnosis] and 19% vs. 18% [5 years after diagnosis], respectively) [22]. These results do not indicate that the risk of leukemic transformation is decreased by iron chelation. However, achieving such an effect may require constant elimination of LPI by effective chelation, in order to suppress oxidative stress. LPI levels were not measured in this retrospective analysis but should be included in prospective studies.

Oxidative stress in MDS

The concept of oxidative stress in MDS (Fig. 4) is supported by laboratory investigations. Oxidized pyrimidine nucleotides were identified in the progenitor-enriched bone marrow CD34+ compartment from MDS patients but not in CD34- MDS cells or CD34+ cells from normal subjects [23]. MDS CD34+ blood cells also had oxidized pyrimidine nucleotides compared with MDS CD34- cells. The

authors suggested that intracellular ROS production plays a role in the pathogenesis of ineffective hemopoiesis and, consequently, that there is a rationale for therapeutic use of antioxidants in MDS. When four antioxidant enzymes were studied in normal, MDS and acute myeloid leukemia bone marrow cells [24], their expression was most frequently increased in MDS/acute myeloid leukemia granulocytes, but less so in CD34+ cells. The authors concluded that the CD34+ compartment in MDS is under oxidative stress, with maturing cells being selected according to their augmented antioxidant defense. Another study showed that patients with low-risk MDS also had increased oxidative DNA damage in more differentiated CD34- bone marrow cells [25]. The authors suggested that oxidative damage contributes to genomic instability and disease progression in low-risk MDS. However, besides increased oxidative stress, impaired DNA repair capacity may also contribute to irreversible oxidative DNA damage [26]. Using flow cytometry techniques, evidence of oxidative stress was found in RBC, platelets and granulocytes from patients with MDS. ROS were higher while reduced GSH was lower in RBC and platelets compared with normal cells. In neutrophils, no difference was found in ROS, while the GSH levels were lower. A correlation ($r = 0.6$) was found between serum ferritin levels of the patients and the ROS in their RBC and platelets [27].

ROS-induced damage may be an important trigger of the increased apoptotic activity that occurs in hematopoietic precursors of MDS patients. The anti-oxidant *N*-acetylcysteine significantly reduced apoptosis in an *in-vitro* assay of the clonogenic potential of MDS progenitors, suggesting a useful role for ameliorating oxidative stress in MDS patients [28]. Oxidative stress in MDS may partly be related to labile, redox-active iron. Elevated levels of NTBI have been observed in the plasma of patients with MDS, even in patients not receiving transfusions [29]. Furthermore, LCI was found to be increased in MDS erythroid cells [30]. Significant changes in intra- and extracellular free iron species and oxidative stress parameters were demonstrated during treatment of iron-loaded MDS patients with the oral iron chelator deferasirox. In RBC, there was a significant decrease in LCI, mean levels of ROS, and membrane lipid peroxidation, with a concomitant increase in GSH during deferasirox therapy [30].

Iron-related oxidative stress may be aggravated by mitochondrial abnormalities in MDS, which may lead to further mitochondrial impairment [31]. Mitochondrial DNA (mtDNA) is particularly susceptible to oxidative damage. Gao *et al* recently demonstrated that after 3–5 days of exposure to high iron, rat cardiac myocytes exhibited damage to mtDNA reflected by diminished amounts of near full-length 15.9-kb polymerase chain reaction product with no change in the amounts of a 16.1-kb product from a nuclear gene [32]. With the loss of intact mtDNA, cellular respiration declined and mRNAs for three electron transport chain subunits and 16S mRNA encoded by mtDNA were decreased, whereas no decrements were found in four subunits encoded by nuclear DNA. The authors concluded that long-term damage to cells and organs in iron-overload disorders involves interactions between iron and mitochondrial ROS resulting in cumulative damage to mtDNA, impaired synthesis of respiratory chain subunits and respiratory dysfunction.

Finally, ROS may also be involved in mediating the suppression of hepcidin in MDS patients with iron overload [33] because they can repress the hepcidin gene by preventing C/EBPalpha and STAT-3 binding to the hepcidin promoter. Therefore, it was not surprising that serum hepcidin levels increased after amelioration of oxidative stress parameters by deferasirox treatment [34].

Clinical consequences of iron overload in MDS

As most patients with MDS are elderly, comorbidities may render them more susceptible to iron toxicity; it is therefore important to evaluate whether iron-related complications can diminish their life expectancy.

Hepatic complications

Transfusion-dependent MDS patients usually develop hepatic IO as documented by magnetic resonance imaging (MRI) (Table I). Although MDS patients generally do not live long enough to develop end-stage liver cirrhosis, a recent Japanese study suggests that hepatic damage contributes to non-leukemic death [35]. Patients dying from cardiac or hepatic failure had received more than twice the number of RBC units than those who died from other causes. Thirty-seven of 38

MDS patients who died from cardiac or hepatic failure had a SF level of ≥ 1000 ng/ml.

Diabetes

A retrospective, age-matched analysis in a large US Medicare database showed that diabetes occurred significantly more frequently in patients with MDS than in the overall Medicare population (40% vs 33.1%; $p < 0.01$) [36] and patients with MDS requiring RBC transfusions had a greater prevalence than those who were independent of transfusions (44.4% vs. 37.1%; $p = 0.1$). Iron overload, attributable to transfusion therapy and/or ineffective erythropoiesis may have contributed to pancreatic endocrine insufficiency. In contrast to cardiac failure, it is difficult to invoke concomitant anemia as a confounding factor in the development of diabetes.

Cardiac failure

Cardiac problems seem to be the most frequent and serious complication of IO in MDS [37,38]. In the above-mentioned US Medicare population, significantly more MDS patients (73.2%) suffered cardiac-related events during 3-year follow-up, which exceeded the Medicare population (54.5%; $p < 0.01$). Moreover, a cardiac event occurred significantly more often in transfused than in non-transfused patients (82.4% vs. 67.1%; $p < 0.001$). Nevertheless, it is difficult to assess the impact of myocardial IO because old age, cardiac comorbidities, and the effects of chronic anemia also contribute to cardiac abnormalities in patients with MDS [39].

The frequency and magnitude of cardiac iron overload in MDS is inconsistent. The available studies employing MRI are given in Table 1, which shows that only a minority of MDS patients have cardiac iron deposition detectable by MRI [40,16,41,42]. Almost 40 years ago, Buja and Roberts performed a post-mortem study of cardiac iron deposits in 131 transfused adult patients, excluding thalassemia and sickle cell anemia. Among those who had received more than 75 units of blood, the majority showed cardiac iron deposits (Fig. 5). Grossly visible cardiac iron deposits were always associated with cardiac dysfunction and usually chronic heart failure [43].

A cardiac MRI study conducted in MDS reported that all patients with evidence of cardiac IO ($T2^* < 20$ ms) died of heart failure within a few months [16]. The authors concluded that myocardial iron deposition in MDS should be prevented rather than treated, because iron-mediated mechanisms may cause cardiac tissue damage before cardiac iron levels are detectable by MRI.

There are data from animal studies suggesting that cardiac function may be more vulnerable to iron overload than liver function. Wood and colleagues [44] correlated $T2^*$ data drawn from studies of cardiac iron in a gerbil model with studies of left ventricular ejection fraction (LVEF) in patients with thalassemia major. This analysis indicated that myocardial damage sufficient to impair LVEF occurs at tissue iron concentrations > 2 mg/g dry weight of tissue (Fig. 6). By comparison, hepatic iron concentrations > 22 mg/g dry weight are associated with the development of fibrosis and cirrhosis [45]. Therefore, cardiac dysfunction may occur at lower tissue iron concentrations than liver dysfunction [44].

How to diagnose iron overload in MDS

Iron overload in MDS is generally diagnosed by measuring serum ferritin levels, as this methodology is widely available and inexpensive. This approach is associated with a number of limitations as measurements become imprecise at high SF levels and ferritin is an acute-phase reactant that is increased during infection, inflammation and malignancy. Therefore, concomitant testing of C-reactive protein is recommended. Nevertheless, serial SF assessment, performed in the same testing laboratory, can provide a reliable assessment of increasing or decreasing total body iron burden. In MDS patients undergoing myeloablative hematopoietic stem cell transplantation, it has recently been shown that elevated SF levels were strongly correlated with the number of RBC units transfused, i.e. the magnitude of iron loading, and that a single pre-transplantation measurement of SF level was sufficient for prognostic modelling [46].

Transfusion history, though, is an important indicator of iron overload in patients with MDS, and the number of transfusions a patient receives will help to guide decisions about initiating chelation therapy. Establishing methods of monitoring

individual patient transfusion burden will facilitate this process, and guidelines from the MDS Foundation suggest this may include encouraging the use of a personal RBC transfusion diary, obtaining blood bank transfusion tracking data and requesting automatic alerts to physicians after a patient receives 20 units of blood [17]. On average, patients with MDS reach a SF level of 1000 ng/ml after receiving 21 RBC units [12]. It is important to be aware that, while SF levels correlate well with hepatic IO in thalassemia, an association with cardiac iron overload does not appear to exist and therefore SF levels cannot predict impending cardiac iron overload [47].

If iron overload is suspected based on elevated serum ferritin, a transferrin saturation >55% in men or >50% in woman supports the diagnosis; transferrin saturations above 70%–80% are associated with the appearance of LPI [48]. The diagnosis of iron overload can be confirmed by measuring liver iron concentration (LIC) by T2*MRI, superconducting quantum interference device (SQUID) or liver biopsy, although the latter is rarely performed in MDS patients as there is an increased risk of bleeding due to thrombocytopenia and/or platelet dysfunction. Similar to SF levels, LIC does not appear to reflect cardiac iron loading, since a long latent period relative to hepatic iron loading predates the development of myocardial iron loading in transfusion-dependent MDS patients. The data obtained in thalassemia major suggests that there is a threshold of LIC and SF above which cardiac iron starts to accumulate. The SF threshold appears to be around 1500 ng/ml [49].

Since cardiac iron measurement by T2*MRI requires considerable expertise, it is often replaced by assessment of cardiac function using echocardiography. While this method is useful to diagnose ventricular dysfunction, it is also prone to interobserver variability. In the context of secondary hemochromatosis, echocardiography is a crude method because a decrease in LVEF is a late event in the development of cardiac IO. The emphasis should be on preventing rather than diagnosing iron-related organ dysfunction. Accordingly, measurements of NTBI and/or LPI at diagnosis and during follow-up may be helpful in assessing the duration and intensity of exposure to redox-active plasma iron, which in turn may correlate with the risk of clinical complications. As yet, only a few

laboratories have established the methodology for measuring NTBI/LPI, but it is hoped that accurate and reproducible methods will become more widely available in the near future.

Is there a survival benefit of iron chelation in MDS?

If iron overload causes clinical complications that decrease the likelihood of survival in MDS, iron chelation therapy should achieve the opposite. This idea was first supported by a small retrospective series from Canada [50], then corroborated by a larger study from France [51], and recently underscored by a matched-pair analysis from the Düsseldorf MDS Registry [22]. These studies consistently show that patients with lower-risk MDS who receive iron chelation therapy fare significantly better than those who remain untreated. However, one must be aware of a problem that is common to all these retrospective studies. As the decision to chelate was not randomized, it is impossible to exclude that patients may have been more likely to receive iron chelation if, based on unmeasurable factors, their physicians considered them to have a good prognosis. This potential bias can only be avoided by a prospective randomized placebo-controlled trial. Such a trial was recently started and is projected to recruit 630 MDS patients at 126 centers worldwide.

Guidelines for the management of iron overload in MDS

Guidelines for the management of iron overload in MDS are currently based on a low level of evidence and usually make inferences from the more established data obtained in thalassemia major. Nevertheless, a number of consensus statements and practice guidelines have been developed by various groups to outline best practice [17,52-59]. These guidelines vary somewhat in their recommendations for initiating iron chelation therapy and strategies for the ongoing management of IO [60]. Overall, they favor starting treatment if more than 20 blood transfusions have been given and SF levels exceed 1000–2000 ng/ml, with the goal to maintain SF levels below 1000 ng/ml. There is general agreement that the MDS patients most likely to benefit from chelation therapy are

those who have a good survival expectancy according to the WHO classification system [61] and the IPSS [62]. Most of the previous guidelines recommend deferoxamine (DFO) for iron chelation, while those published more recently also recommend the use of deferasirox [60].

Efficacy of chelation therapy in MDS

Data on the efficacy of chelation therapy in MDS are limited to a relatively small number of studies. One of the earliest studies followed 11 patients for up to 60 months of treatment with DFO and observed an improvement in hematopoietic output [63]. A greater than 50% reduction in transfusion requirement was noted in seven patients (64%), while five (45.5%) became completely transfusion independent. Neutrophil and platelet counts improved in 78% and 64% of the patients, respectively. SF levels decreased clearly in nine patients (82%), while LIC assessed by MRI decreased in all the patients although the decrease was minor in five patients. The best hematopoietic responses were observed in patients with the greatest efficacy based on change in LIC. However, the demanding continuous parenteral DFO treatment may lead to difficulties with compliance, particularly in elderly patients with MDS.

The first commercially available alternative to DFO was deferiprone, an oral chelator given three times a day. A pilot study evaluating oral deferiprone in three MDS patients did not observe decreased SF levels in any patient and was stopped prematurely due to the concerns over the risk of agranulocytosis [37]. A larger study in 18 patients with MDS reported a 25% median decrease in SF (from 3113 ng/ml at baseline) during 1 year of treatment [64]. Agranulocytosis developed in one patient and recurred on rechallenge, thus necessitating treatment withdrawal.

More recently, once-daily oral deferasirox has become commercially available and has demonstrated efficacy in decreasing iron burden in patients with MDS [65-68]. Doses of 10–30 mg/kg/day maintain or decrease SF and LIC in a dose- and iron intake-dependent manner and have also been shown to produce sustained reductions in LPI levels [65,67,68]. The 1-year EPIC (Evaluation of

Patients' Iron Chelation with Exjade) deferasirox clinical trial enrolled the largest cohort of MDS patients (n=341) evaluated for iron chelation therapy [68]. Overall median SF decreased significantly at 1 year (-253 ng/ml; $P = 0.002$) and decreases occurred irrespective whether patients had previously received chelation therapy or were chelation naïve; changes in SF were reflective of dose adjustments and ongoing iron intake. The discontinuation rate in MDS patients was 48.7%.

Greenberg et al. [69] recently reported the results of a prospective study evaluating the effects of deferasirox on liver iron concentration (LIC), LPI and pharmacokinetics (PK) along with SF values in MDS patients with IPSS Low- and Intermediate-1 risk profile and evidence of iron overload. Twenty-four heavily transfused MDS patients were enrolled in a planned 52 weeks of therapy. PK studies showed dose-proportional total drug exposure. The data demonstrated that deferasirox was well tolerated and effectively reduced LIC, LPI and SF in the iron-overloaded patients with MDS who completed 24 and 52 weeks of therapy despite ongoing receipt of RBC transfusions.

Similar to observations with DFO, deferasirox has been shown to improve hemoglobin levels and reduce transfusion requirements in a small population (n=5) of patients with MDS and primary myelofibrosis [70,71].

It should be noted that deferasirox treatment is less well tolerated in the elderly MDS population when compared with young thalassemia patients. Diarrhea is the most common side effect, which can usually be managed by dose reduction but sometimes necessitates discontinuation of the drug. Anecdotal reports suggest that diarrhea is diminished when deferasirox is taken in the evening. A significant rise in serum creatinine occurs in approximately 25% of MDS patients, but progressive increases can be avoided with appropriate dose reductions. The observed safety profile of deferasirox in MDS may be related to pre-existing comorbidities, concomitant medication use and the advanced age of the MDS patient population.

Discussion

The discussion about the importance of iron overload in MDS underscores the need for a prospective trial investigating the potential survival benefit of iron chelation therapy. We suggest that until results of such a trial become available, one should not ignore the potential impact of unliganded or inadequately liganded 'free iron' that may cause cell and tissue damage. Chelation experience in thalassemia major suggests that substantial clinical benefit can be derived from detoxification of redox-active iron species, as supported by an experimental model of heart cells [72,73] and the beneficial effects of deferasirox on arterial function [74]. Moreover, Piga *et al* recently demonstrated a link between NTBI and heart disease in thalassemic patients with transfusional iron overload [48], as NTBI levels were significantly higher in patients with heart disease than those without. All patients with heart disease had transferrin saturation above 70% and measurable NTBI levels. Conversely, no patients without NTBI and/or with transferrin saturation of less than 70% had preclinical or clinical evidence of heart disease. The authors support the concept of a threshold level of excess iron (indicated by a transferrin saturation of 70–80%) beyond which the normal regulatory mechanisms protecting against iron toxicity are overwhelmed.

We hope that in the near future treatment decision and monitoring will be supported by LPI and LCI measurements. Measuring the toxic forms of iron in plasma and cells at diagnosis and follow-up, preferentially in parallel with oxidative stress parameters, and correlating the results with clinical complications of IO, with and without iron chelation therapy, will enable a more comprehensive and meaningful assessment of iron overload. Measuring LPI may also help to assess patient compliance.

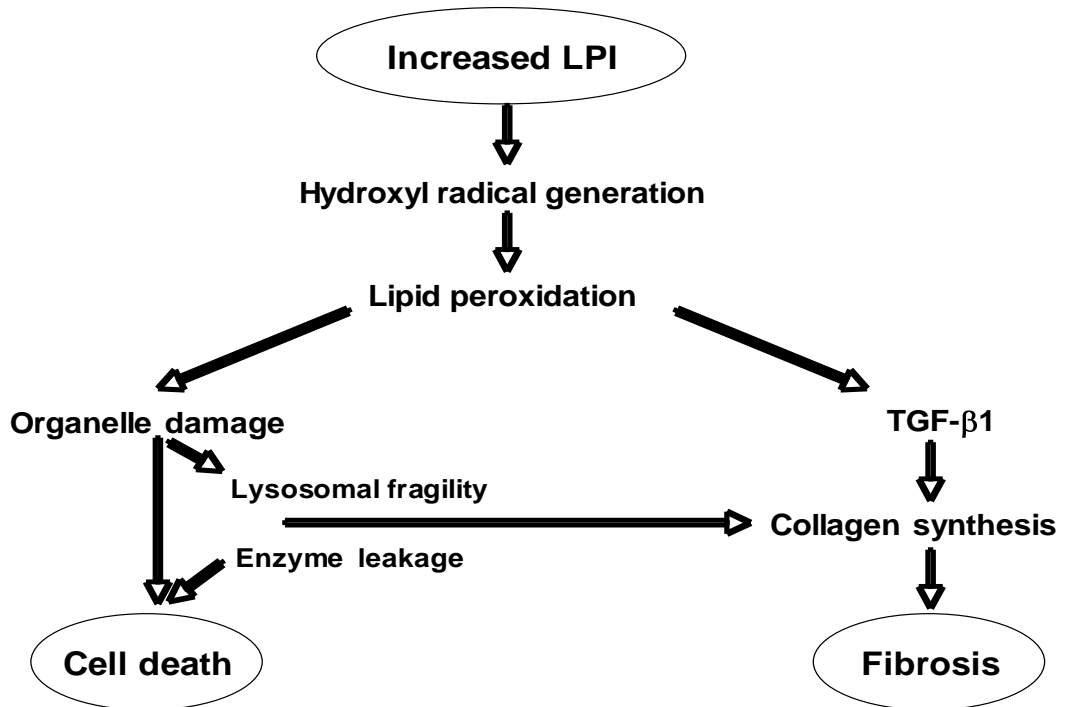
We feel that, currently, initiating chelation therapy should be tailored to the individual patient, based on the type of MDS and its prognosis, patient's age and comorbidities, transfusion history, measurements of iron burden, and the intensity of the current transfusion regimen. Though it may be difficult to recruit patients, a well-designed prospective trial is required to corroborate the current practice guidelines supporting the use of chelation therapy in transfusion-dependent patients with lower-risk MDS.

Table I. MRI detection of cardiac and liver iron overload in MDS

Normal ranges: cardiac T2* > 20 milliseconds (ms), hepatic T2* > 6 ms.

	Number of patients with MDS with cardiac T2* <20 ms	Average no. of packed RBC units transfused	Serum ferritin (mean; ng/ml)	Average hepatic T2* (ms)
(Glanville <i>et al</i> , 2006) [41]	3/7	208	5865	1.4
(Chacko <i>et al</i> , 2007) [40]	1/11	116	4400	1.5
(Konen <i>et al</i> , 2007) [42]	1/10	50	4250	2-3
(Di Tucci <i>et al</i> , 2008) [16]	3/22	125	2300	1.5

Fig 1. Pathophysiology of iron overload. Adapted from: [75]



LPI, labile plasma iron; TGF, transforming growth factor

Fig 2. Impact of transfusion dependency on survival in MDS [12]

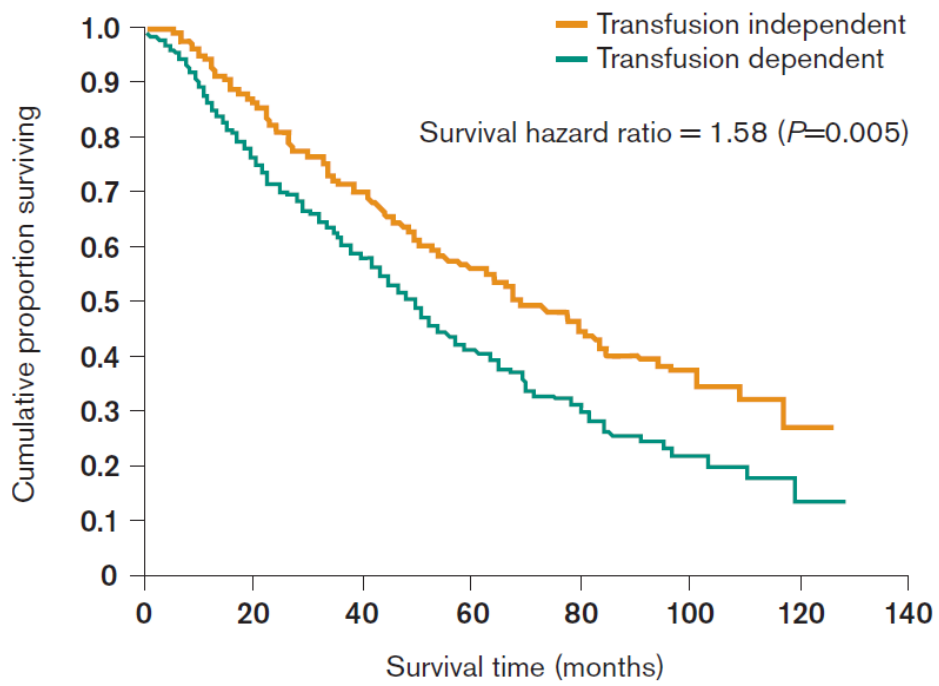


Fig. 3 Independent impact of transfusion dependency and iron overload on survival in patients with MDS.

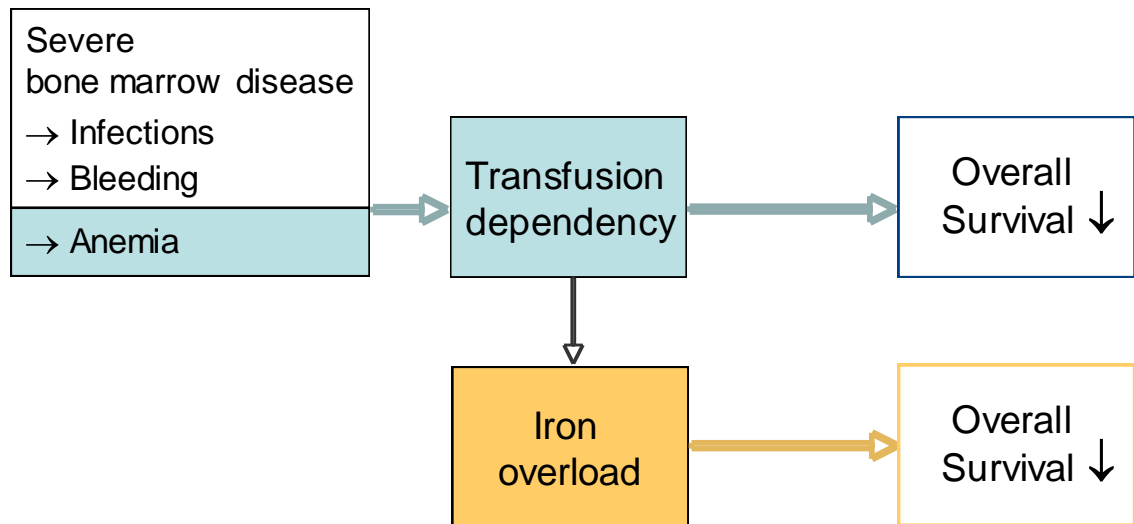
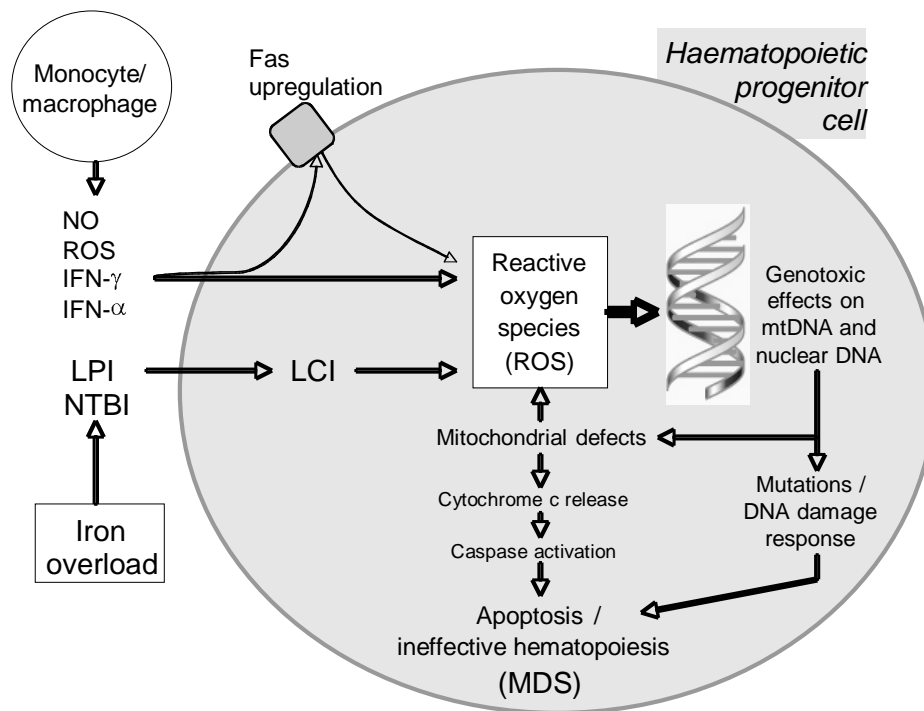


Fig 4. The role of oxidative stress in MDS. Adapted from: [76]



IFN, interferon; LCI, labile cell iron; LPI, labile plasma iron; MDS, myelodysplastic syndromes; NO, nitric oxide; NTBI, non-transferrin-bound iron; ROS, reactive oxygen species.

Fig 5. Relationship between transfusions and cardiac iron in MDS. Based on data from [43]

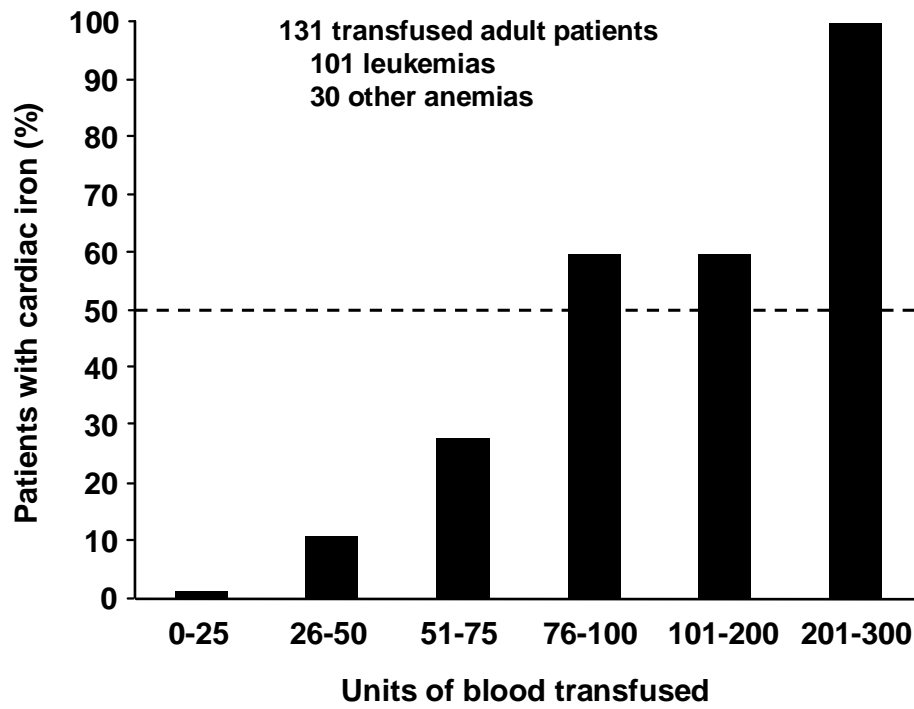
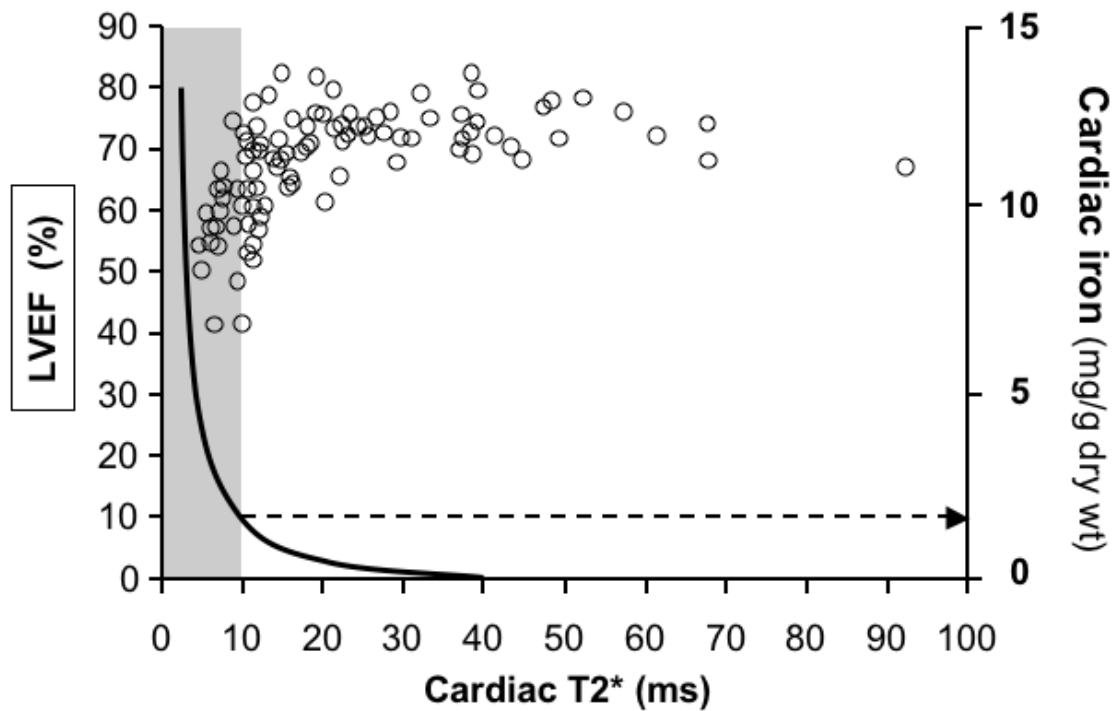


Fig 6. Relationship between cardiac T2*, cardiac function, and cardiac iron.

Black circles show the relationship between cardiac T2* and cardiac function as assessed by LVEF in patients with iron overload. The grey shaded area indicates the 'danger zone' for cardiac iron overload, where patients are at risk of deteriorating LVEF. The black curve represents T2* measurements and cardiac tissue iron concentrations obtained from animal studies. The dotted line indicates that entry into the 'danger zone' corresponds to a cardiac iron concentration of about 2 mg/g dry wt. Adapted from [44].



LVEF, left ventricular ejection fraction

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