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Impact of treatment with iron chelation therapy in patients with lower-risk myelodysplastic syndromes participating in the European MDS registry

by Marlijn Hoeks, Ge Yu, Saskia Langemeijer, Simon Crouch, Louise de Swart, Pierre Fenaux, Argiris Symeonidis, Jaroslav Čermák, Eva Hellström-Lindberg, Guillermo Sanz, Reinhard Stauder, Mette Skov Holm, Moshe Mittelman, Krzysztof Mądry, Luca Malcovati, Aurelia Tatic, Antonio Medina Almeida, Ulrich Germing, Aleksandar Savic, Njetočka Gredelj Šimec, Dominic Culligan, Raphael Itzykson, Agnes Guerci-Bresler, Borhane Slama, Arjan van de Loosdrecht, Corine van Marrewijk, Jackie Droste, Nicole Blijlevens, Marian van Kraaij, David Bowen, Theo de Witte, and Alex Smith
Collaborative Groups: EUMDS Registry Participants)

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Impact of treatment with iron chelation therapy in patients with lower-risk myelodysplastic syndromes participating in the European MDS registry

Authors and Affiliations:
Marlijn Hoeks[1,2], Ge Yu[3], Saskia Langemeijer[4], Simon Crouch[3], Louise de Swart[4], Pierre Fenaux[5], Argiris Symeonidis[6], Jaroslav Čermák[7], Eva Hellström-Lindberg[8], Guillermo Sanz[9], Reinhard Stauder[10], Mette Skov Holm[11], Moshe Mittelman[12], Krzysztof Mądry[13], Luca Malcovati[14], Aurelia Tatic[15], Antonio Medina Almeida[16], Ulrich Germing[17], Aleksandar Savic[18], Njetočka Gredelj Šimec[19], Dominic Culligan[20], Raphael Itzykson[5], Agnes Guerci-Bresler[21], Borhane Slama[22], Arjan van de Loosdrecht[23], Corine van Marrewijk[4], Jackie Droste[4], Nicole Blijlevens[4], Marian van Kraaij[24], David Bowen[25], Theo de Witte[26], and Alex Smith[3], on behalf of the EUMDS Registry Participants

[1]Centre for Clinical Transfusion Research, Sanquin Research, Leiden, The Netherlands; [2]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; [3]Epidemiology and Cancer Statistics Group, Department of Health Sciences, University of York, York, United Kingdom; [4]Department of Hematology, Radboud university medical center, Nijmegen, The Netherlands; [5]Service d'Hématologie, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris and Université Paris 7, Paris, France; [6]Department of Medicine, Division of Hematology, University of Patras Medical School, Patras, Greece; [7]Department of Clinical Hematology, Inst. of Hematology & Blood Transfusion, Praha, Czech Republic; [8]Department of Medicine, Division of Hematology, Karolinska Institutet, Stockholm, Sweden; [9]Department of Haematology, Hospital Universitario y Politécnico La Fe, Valencia, Spain; [10]Department of Internal Medicine V (Haematology and Oncology), Innsbruck Medical University, Innsbruck, Austria; [11]Department of Haematology, Aarhus University Hospital, Aarhus, Denmark; [12]Department of Medicine A, Tel Aviv Sourasky (Ichilov) Medical Center and Sackler Medical Faculty, Tel Aviv University, Tel Aviv, Israel; [13]Department of Haematology, Oncology and Internal Medicine, Warszawa Medical University, Warszawa, Poland; [14]Department of Hematology Oncology, Fondazione Istituto Di Ricovero e Cura a Carettere Scientifico, Policlinico San Matteo, University of Pavia, Pavia, Italy; [15]Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania; [16]Department of Hematology, Hospital da Luz, Lisbon, Portugal; [17]Department of Haematology, Oncology and Clinical Immunology, Universitätsklinik Düsseldorf, Düsseldorf, Germany; [18]Clinic of Hematology - Clinical Center of Vojvodina, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia; [19]Department of Internal Medicine, Division of Hematology, Merkur University Hospital, Zagreb, Croatia; [20]Department of Haematology, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; [21]Service d'Hématologie, Centre Hospitalier Universitaire Brabois Vandoeuvre, Nancy, France; [22]Service d'Hématologie, Centre Hospitalier d'Avignon, Avignon, France; [23]Department of Hematology – Cancer Center Amsterdam VU University Medical Center, Amsterdam, The Netherlands; [24]Unit Transfusion Medicine, Sanquin Blood Bank, Amsterdam, the Netherlands; [25]St. James's Institute of Oncology, Leeds Teaching Hospitals, Leeds, United Kingdom; [26]Department of Tumor Immunology - Nijmegen Center for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands

Correspondence: Theo de Witte. Dept of Tumorimmunology, Radboud Institute for Molecular Life Sciences. P.O.Box 9101, 6500HB Nijmegen, Netherlands. Email: theodewitte@radboudumc.nl
Telephone: +31 (0)651691226

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Authorship

-Contributions: Design: MH, GY, SL, SC, DB, TDW, and AS; provision of patients, assembly of data: GY, SL, LdS, PF, ASY, JC, EHL, GS, RS, MSH, MM, KM, LM, AT, AMA, UG, ASA, NGS, DC, RI, AGB, BS, AvdL, CvM, JD, DB, TdW, and AS; statistical analysis: MH, GY, SL, SC, TDW and AS; manuscript writing: all authors; final approval: all authors.

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Key points

- Secondary iron overload is common in lower-risk MDS patients and is associated with morbidity and mortality
- Iron Chelation Therapy may improve overall survival and hematopoiesis in lower-risk MDS patients

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 independency 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 µg/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 µg/L or a drop of the serum ferritin value below 1000 µg/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 µg/L vs 547 µg/L), and this had increased to 1,221 µg/L prior to start of ICT. 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 non-chelated 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 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).

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.

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.

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 diarrhoea (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 deferasiox-treated patients),

Renal function in non-chelated patients increased similarly over time (Figure 7).

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 trial[29] 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 transplantion.[33]

Toxic iron species are known to catalyze the cellular generation of reactive oxygen species (ROS), which play 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-κB, 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 6-monthly intervals. Subtle changes in patient-related factors in the intervening 6-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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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 (µg/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 (MDSCI)					
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 ringed sideroblasts present

*** LOCF: last observation carried forward (only for cytogenetics and bone marrow blasts)

SD: standard deviation; RBCT: red blood cell transfusion; MDSCI: 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 (µg/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
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 1 Number of registry patients by transfusion and chelation status.

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 µg/L.

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

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

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

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

Legend 5:

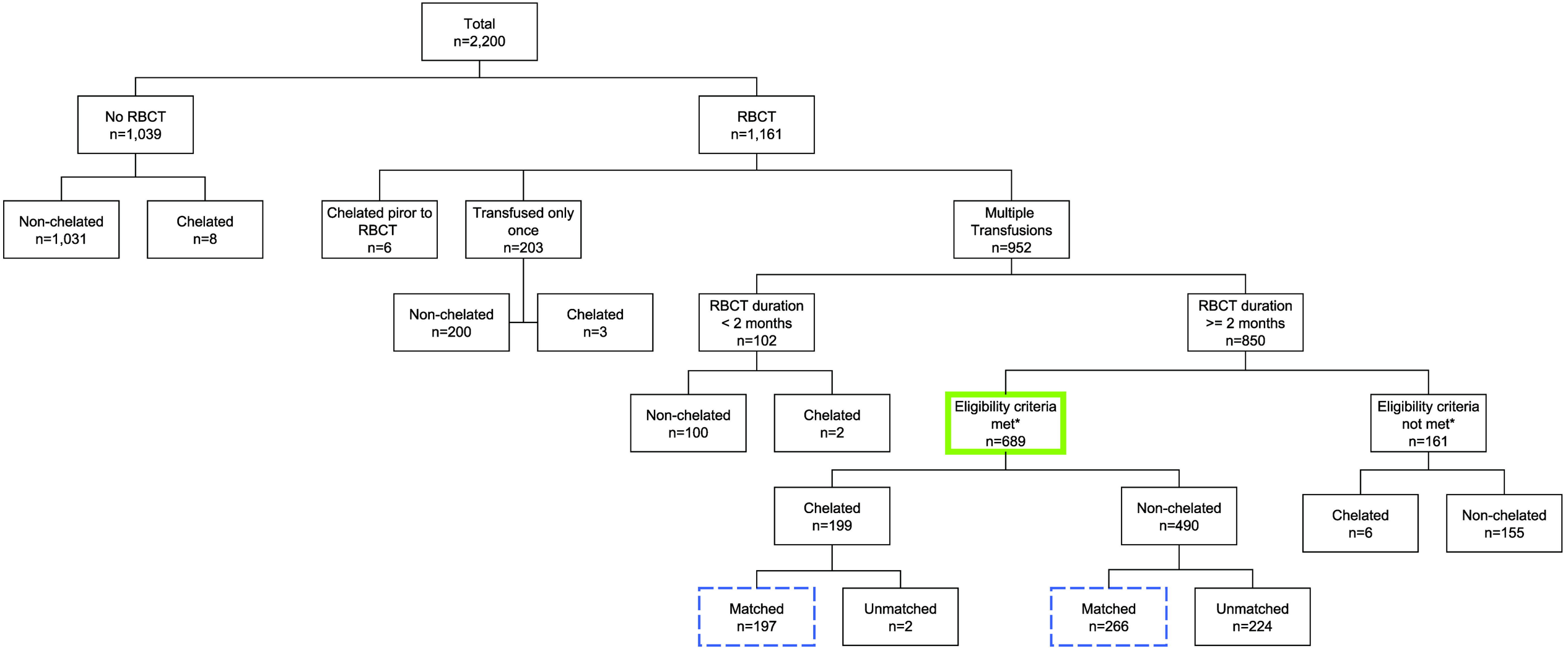
time: eight 6-monthly visits

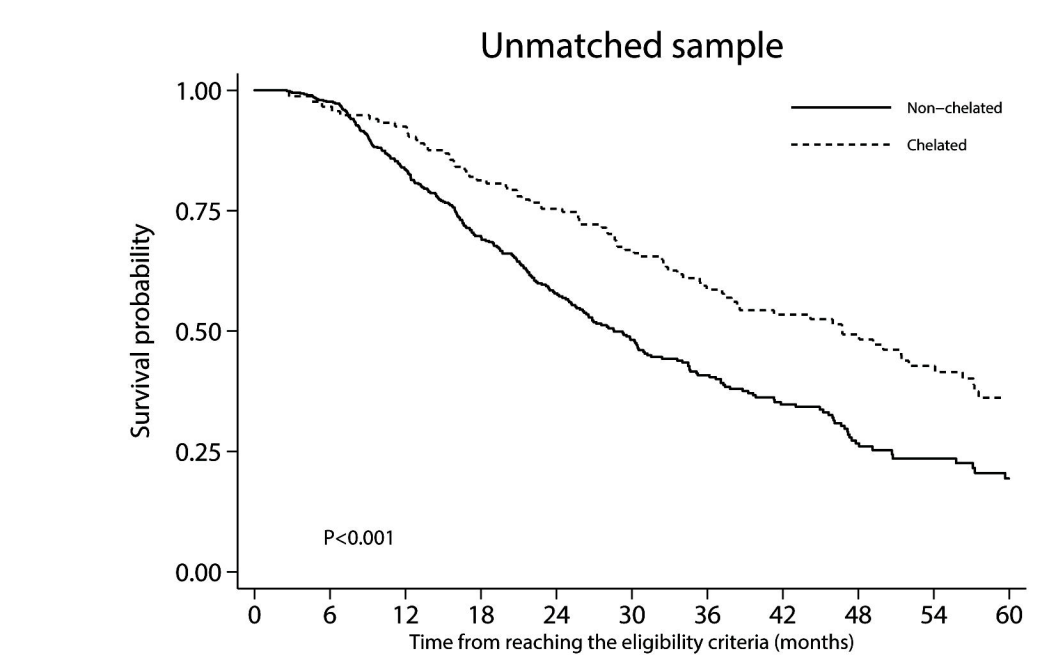
Figure 6 Trajectory analysis in chelated patients with and without response and for non-chelated patients.

Legend figure 6:

6A: Monthly red blood cell transfusion density for chelated patients with and without an erythroid response and for non-chelated patients. 6B: Ferritin levels of patients with and without a ferritin response, defined as a decrease of ≥1000 µg/L or a drop of the serum ferritin value below 1000 µg/L, and for non-chelated patients.

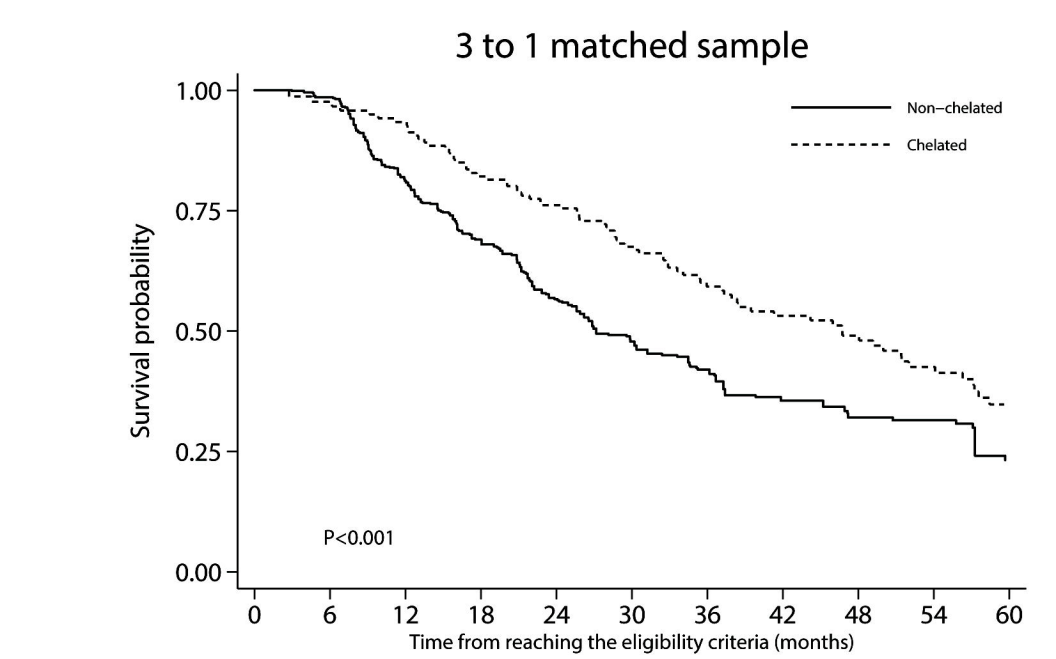
Figure 7 Serum creatinine levels (µmol/L) in chelated and non-chelated patients per visit.


Total
n=2,200
No RBCT
n=1,039
RBCT
n=1,161
Non-chelated
n=1,031
Chelated
n=8
Chelated piror to RBCT
n=6
Transfused only once
n=203
Multiple Transfusions
n=952
Non-chelated
n=200
Chelated
n=3
RBCT duration < 2 months
n=102
RBCT duration >= 2 months
n=850
Non-chelated
n=100
Chelated
n=2
Eligibility criteria met*
n=689
Eligibility criteria not met*
n=161
Chelated
n=199
Non-chelated
n=490
Chelated
n=6
Non-chelated
n=155
Matched
n=197
Unmatched
n=2
Matched
n=266
Unmatched
n=224



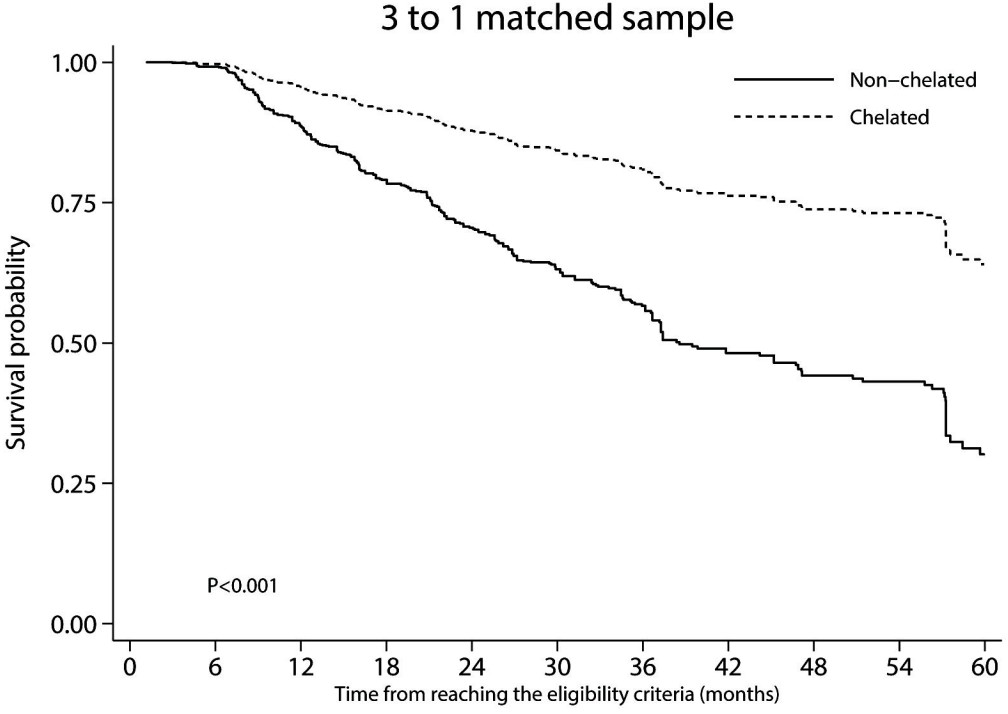
Number at risk

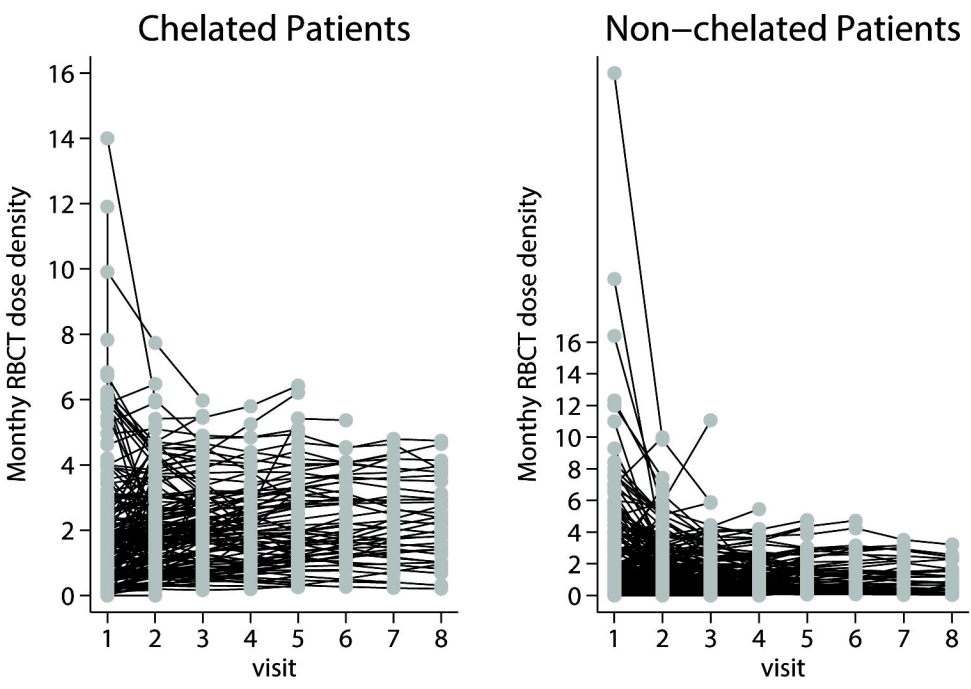
Non−chelated	612	548	389	280	191	138	105	71	42	26	16
Chelated	77	101	128	118	117	101	72	58	47	34	24

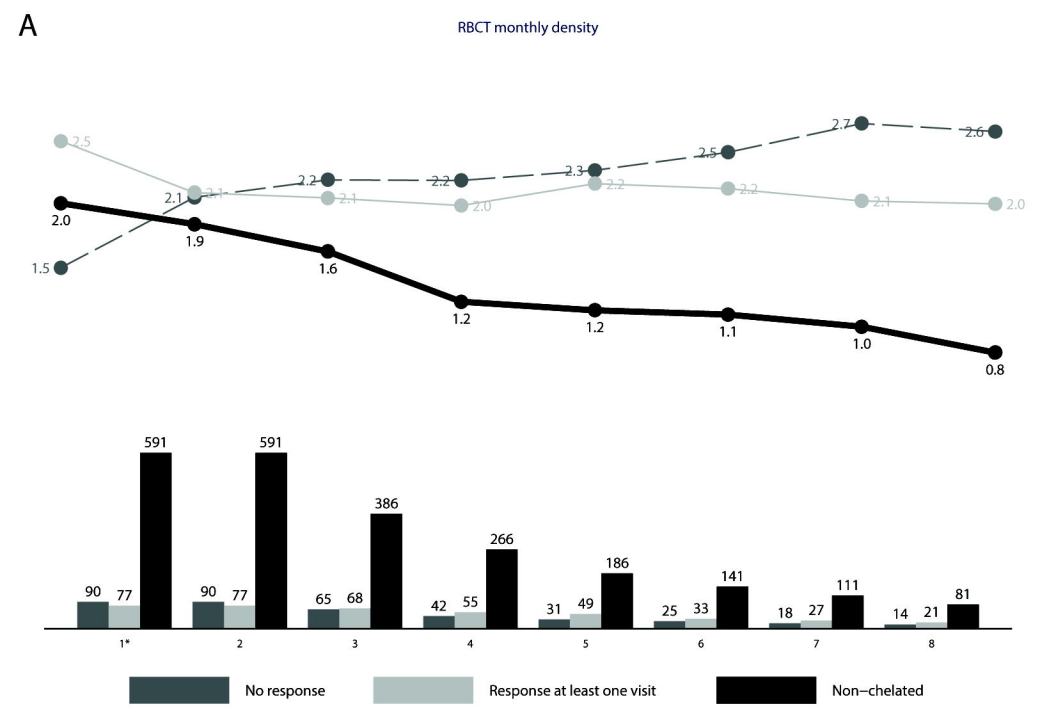


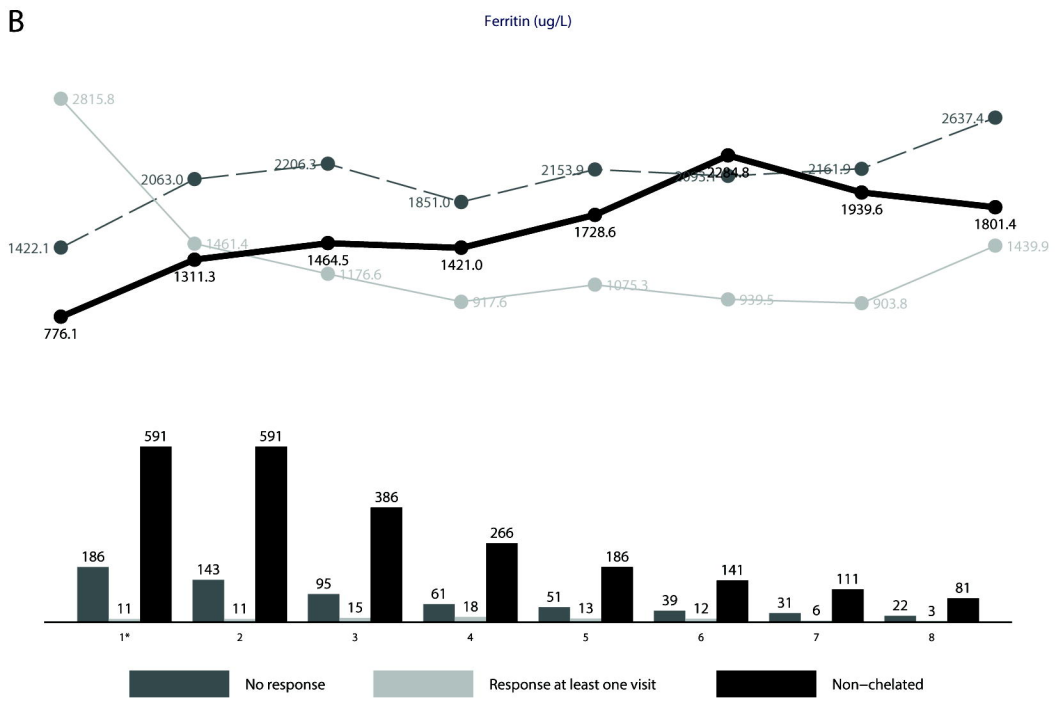
Number at risk

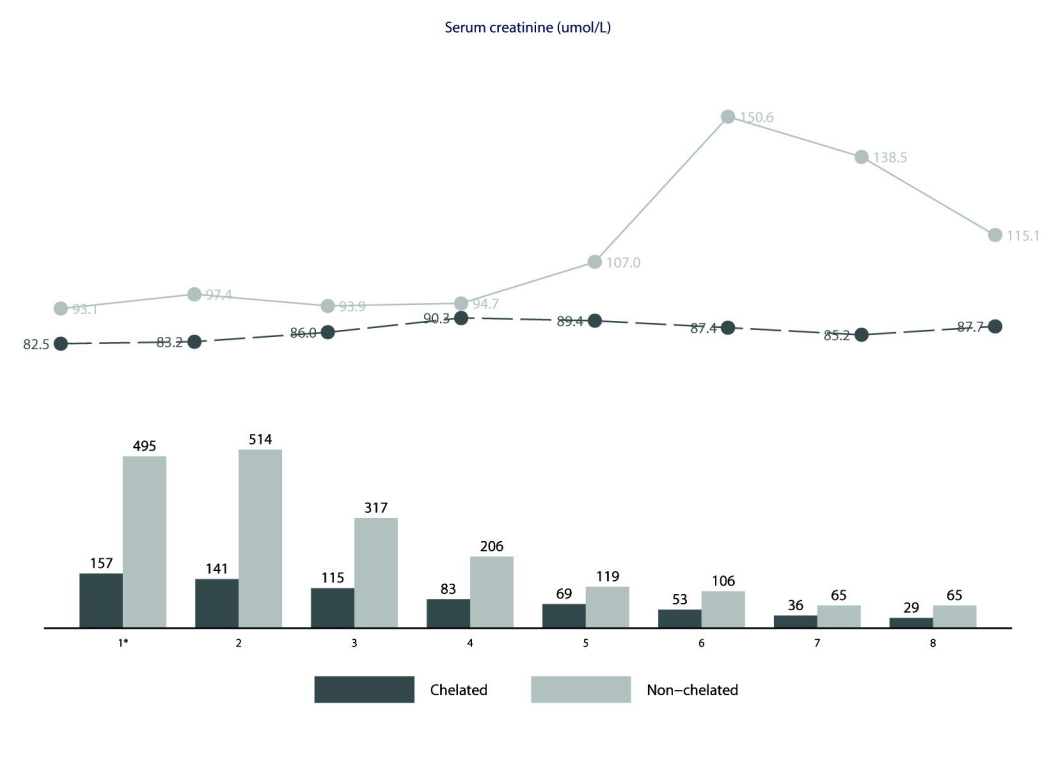
Non−chelated	713	638	434	333	227	172	137	92	69	49	25
Chelated	75	101	128	118	117	101	73	58	47	34	24


3 to 1 matched sample
Non–chelated
Chelated
Survival probability
1.00
0.75
0.50
0.25
0.00
P<0.001
0
6
12
18
24
30
36
42
48
54
60
Time from reaching the eligibility criteria (months)


Chelated Patients
Monthy RBCT dose density
0
2
4
6
8
10
12
14
16
1
2
3
4
5
6
7
8
visit
Non−chelated Patients
Monthy RBCT dose density
0
2
4
6
8
10
12
14
16
1
2
3
4
5
6
7
8
visit


A
RBCT monthly density
2.5
2.1
2.2
2.1
2.2
2.0
2.3
2.2
2.5
2.2
2.7
2.1
2.6
2.0
2.0
1.9
1.5
1.6
1.2
1.2
1.1
1.0
0.8
591
591
386
266
186
141
111
81
90
77
90
77
65
68
42
55
31
49
25
33
18
27
14
21
1*
2
3
4
5
6
7
8
No response
Response at least one visit
Non−chelated


B
Ferritin (ug/L)
2815.8
2063.0
2206.3
2153.9
2161.9
2637.4
1851.0
1939.6
1801.4
1422.1
1461.4
1464.5
1421.0
1728.6
1439.9
1311.3
1176.6
1075.3
939.5
903.8
917.6
776.1
591
591
386
266
186
186
143
95
61
51
39
31
22
141
111
81
11
11
15
18
13
12
6
3
1*
2
3
4
5
6
7
8
No response
Response at least one visit
Non−chelated


Serum creatinine (umol/L)
93.1
97.4
93.9
94.7
107.0
150.6
138.5
115.1
82.5
83.2
86.0
90.3
89.4
87.4
85.2
87.7
495
514
317
206
157
141
115
83
69
119
53
106
36
65
29
65
1*
2
3
4
5
6
7
8
Chelated
Non−chelated

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 tables

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 *N*= 490		Chelated *N*= 199		*P*-Value	*Standardised*	Non-chelated *N*= 490		Chelated *N*= 199		*P*-Value	*Standardised differences***
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%		
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

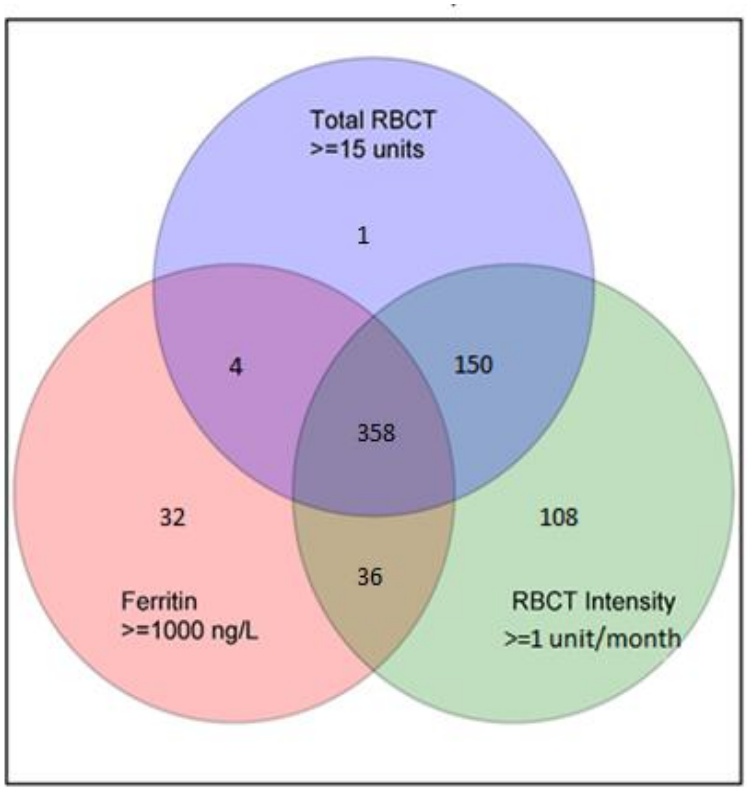
** The standardised difference in percent is the the mean difference as a percentage of the average standard deviation

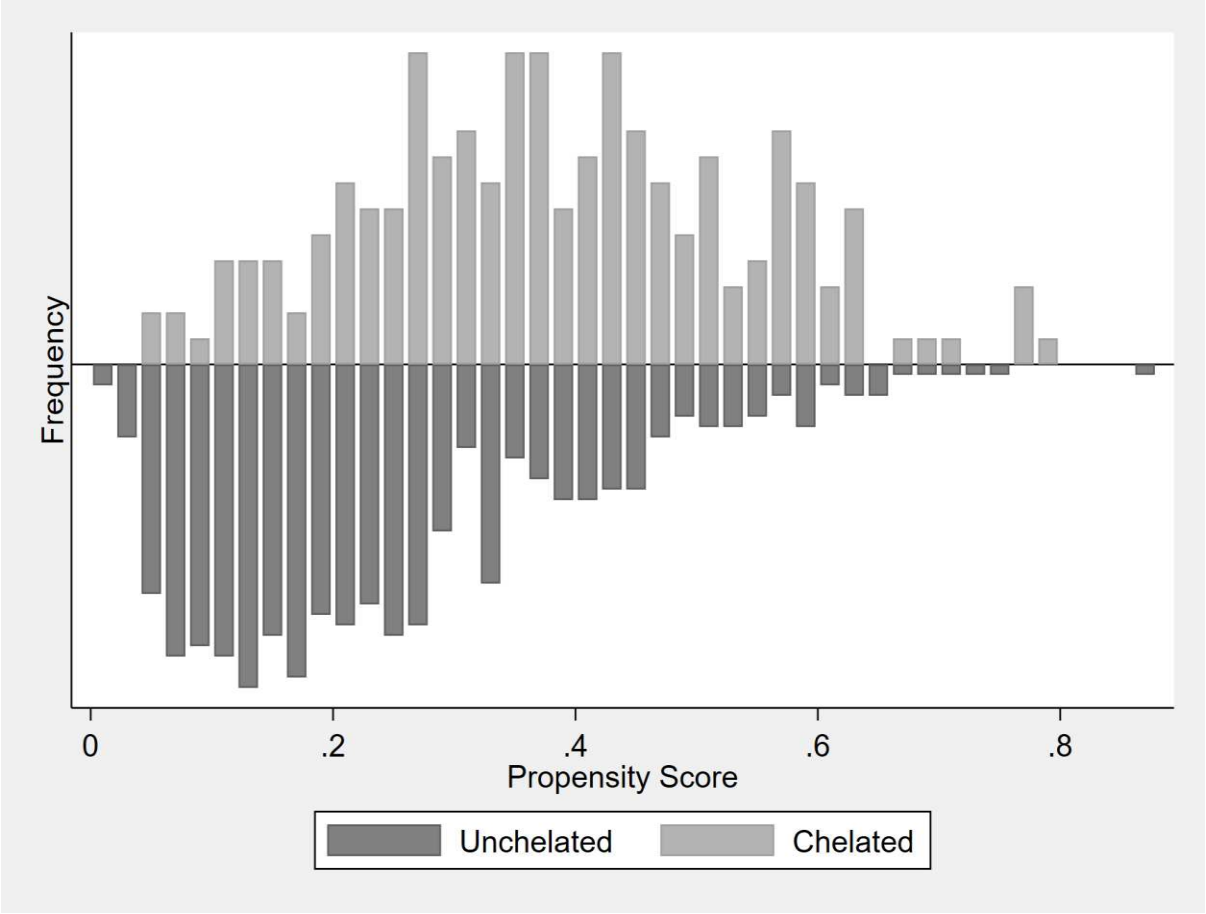
*** Adjusted by age, sex, comorbidity, performance status, RBCT intensity, number of units transfused, IPSS-R, and RS present

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 figures

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.