RESEARCH ARTICLE

Cardiac iron overload in chronically transfused patients with thalassemia, sickle cell anemia, or myelodysplastic syndrome

Mariane de Montalembert1,2,*, Jean-Antoine Ribeil3,4, Valentine Brousse1,2, Agnès Guerci-Bresler5, Aspasia Stamatoullas6, Jean-Pierre Vannier7, Cécile Dumensnil7, Agnès Lahany8, Mohamed Touati9, Krimo Bouabdallah10, Marina Cavazzana3,4,11,12, Emmanuelle Chauzit13, Amandine Baptiste14, Thibaud Lefebvre2,15,16, Hervé Puy2,15,16, Caroline Elie14, Zoubida Karim2,15, Olivier Ernst17, Christian Rose18

1 Pediatrics Department, Necker Children’s Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France, 2 Laboratory of Excellence GR-Ex, Paris, France, 3 Biotherapy Department, Necker Children’s Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France, 4 Biotherapy Clinical Investigation Center, Groupe Hospitalier Universitaire Ouest, Assistance Publique-Hôpitaux de Paris, INSERM, Paris, France, 5 Hematology Department, Hôpital d’Adultes du Brabois, Vandoeuvre les Nancy, Nancy, France, 6 Centre Henri Becquerel, Rouen, France, 7 Pediatric Oncology and Hematology Unit, Hôpital Charles Nicolle, Rouen, France, 8 Department of Biochemistry, Hôpital Charles Nicolle, Rouen, France, 9 Service d’Hématologie Clinique et de Thérapie Cellulaire, CHU, Limoges, France, 10 Service des Maladies du Sang, Hôpital Haut-Levêque, Pessac, France, 11 Paris Descartes-Sorbonne Paris Cité University, Imagine Institute, Paris, France, 12 INSERM UMR 1163, Laboratory of Human Lymphopoiesis, Paris, France, 13 Département de Pharmacologie clinique et toxicologique, CHU, Bordeaux, France, 14 Paris Descartes Clinical Research Unit, Necker Children’s Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France, 15 INSERM UMR 1149/ERL CNRS 8252, Centre de Recherche sur l’inflammation, Paris, France, 16 French center for Porphyria, Louis Mourier Hospital, Assistance Publique-Hôpitaux de Paris, Colombes, France, 17 Radiology Department, Hospital Huriez, CHRU, Lille, France, 18 Hématologie clinique, Hôpital Saint Vincent de Paul, Université Catholique de Lille, Lille, France

* These authors contributed equally to this work.

Abstract

The risk and clinical significance of cardiac iron overload due to chronic transfusion varies with the underlying disease. Cardiac iron overload shortens the life expectancy of patients with thalassemia, whereas its effect is unclear in those with myelodysplastic syndromes (MDS). In patients with sickle cell anemia (SCA), iron does not seem to deposit quickly in the heart. Our primary objective was to assess through a multicentric study the prevalence of cardiac iron overload, defined as a cardiovascular magnetic resonance T2*<20 ms, in patients with thalassemia, SCA, or MDS. Patient inclusion criteria were an accurate record of erythrocyte concentrates (ECs) received, a transfusion history >8 ECs in the past year, and age older than 6 years. We included from 9 centers 20 patients with thalassemia, SCA, or MDS. Ferritin levels showed a strong association with LIC. Non-transferrin-bound iron was high in the thalassemia and MDS groups but low in the SCA group (P<0.001). Hepcidin...
was low in thalassemia, normal in SCA, and markedly elevated in MDS (P<0.001). Two mechanisms may explain that iron deposition largely spares the heart in SCA: the high level of erythropoiesis recycles the iron and the chronic inflammation retains iron within the macrophages. Thalassemia, in contrast, is characterized by inefficient erythropoiesis, unable to handle free iron. Iron accumulation varies widely in MDS syndromes due to the competing influences of abnormal erythropoiesis, excess iron supply, and inflammation.

Introduction
Cardiac iron overload is the leading cause of death in patients with thalassemia who require chronic transfusion [1]. Cardiac iron overload has also been reported in a minority of patients with myelodysplastic syndromes (MDS) [2–4]. Iron overload has been associated with an increased risk of progression to leukemia and with shorter survival in non-chelated low-risk patients with MDS [4, 5]. The heart does not seem to be an early target for iron accumulation in chronically transfused patients with sickle cell anemia (SCA) [6]. Nevertheless, cardiac iron overload has been reported in a small percentage of chronically transfused young adults with SCA [7]. Hypotheses put forward to explain that the heart is relatively spared in SCA include a later onset of chronic transfusion compared to thalassemia, the use of erythrocytapheresis rather than simple transfusion, chronic inflammation that sequesters iron within reticuloendothelial cells, and efficient erythropoiesis capable of handling the iron from both transfused blood and hemolysis [6–10].

Blood transfusion is increasingly used in patients with MDS or SCA [11], who nevertheless are not routinely screened for iron overload and receive less iron chelation compared to patients with thalassemia [12]. These patients may, therefore, be at risk for iron-related comorbidities.

The primary objective of this study was to assess the prevalence of cardiac iron overload, defined as a cardiovascular magnetic resonance T2⁺ <20 ms, in patients with thalassemia, SCA, or MDS. We also sought to identify factors associated with cardiac iron overload.

Patients and methods
The study was approved by the appropriate ethics committee (Comité de Protection des Personnes d’Ile de France 2) (#21/21/2010) and supported by the AP-HP Department for Clinical Research and Development (DRCID), Paris, France. Written informed consent was approved by the ethics committee; it was obtained from each patient and/or both parents before study inclusion.

Patients
Inclusion criteria were availability of a complete blood transfusion record, history of receiving at least 8 erythrocyte concentrates (ECs) in the past year, age older than 6 years to allow magnetic resonance imaging (MRI) without sedation, and affiliation to an health insurance system. Patients with heart diseases due to causes other than iron overload were not included. The patients were included between March 14, 2012, and February 21, 2014, at nine centers in France.

Data collection
Iron accumulation in the heart and liver was assessed using MRI. Myocardial T2⁺ (ms) and liver iron content (LIC) (mg of iron/g dry weight) were recorded. Within 6 months before or
after MRI, blood was drawn for serum assays of iron, ferritin, non-transferrin-bound iron (NTBI), and hepcidin. NTBI was measured using the FeROSTM eLPI kit and hepcidin by liquid chromatography-tandem mass spectrometry. In patients treated with deferasirox, the drug was assayed in plasma using high performance liquid chromatography coupled with tandem mass spectrometry, as previously described [13].

The following data were collected retrospectively from the medical files of each patient: diagnosis, age at study inclusion, ethnic origin, history of splenectomy, serological status for hepatitis B and C (HCV), age at first transfusion, number of ECs transfused since the diagnosis, number of ECs transfused each year, time since initiation of chronic transfusion, transfusion procedure (simple transfusion, manual exchange transfusion, or erythrocytapheresis), age and ferritin levels at the beginning of chelation, and type of chelation.

Given the scarcity of published data on risk factors for cardiac iron overload in chronically transfused patients, we had no point of reference for estimating the sample size. We estimated that including 50 patients in each diagnostic group would allow us to identify associations linking selected parameters to cardiac iron overload.

Imaging data

All patients underwent prospectively cardiac T2* by the same MRI method according to Anderson et al [14], liver iron content (LIC) according to the MRI method of Gandon et al [15], with 1.5 Tesla Machine and were validated in routine practice, evidencing the comparability of the measurements in the different centers [16].

Statistical tests

Statistical analyses were performed using R software version 2.11.1. Descriptive statistics were computed for each diagnostic group. Continuous variables were described as mean ± SD if normally distributed and as median [min-max] otherwise. Categorical variables were described as n (%).

We used the chi-square test or Fisher’s exact test, as appropriate, to test for associations between categorical variables. To compare distributions of continuous outcomes across groups, ANOVA or the nonparametric Kruskal-Wallis test (when distribution was not normal) was performed. When the results indicated a significant overall difference, the t test or Wilcoxon test was applied to each pair of groups, and corresponding P values were adjusted using the Benjamini-Hochberg method to take the multiple comparisons into account. Spearman’s rho and P value of its corresponding test were calculated to investigate correlations between continuous parameters.

All tests were two-sided. P values ≤0.05 were considered significant.

Results

Descriptive data

One hundred and four patients were included in this study. Eighteen of them were excluded from our analysis (n = 2 did not meet inclusion criteria (total number of ECs received unavailable), n = 16 did not undergo cardiac T2* MRI or its result could not be collected). Thus, 86 patients were analyzed: 20 with thalassemia, 41 with SCA, and 25 with MDS (Table 1). As expected, the patients with MDS were older than were the patients with thalassemia or SCA, and most of them (96%) were of European descent. In the thalassemia group, 70% of patients had a history of splenectomy, a significantly higher proportion than in the other groups (P<0.001). The 12% prevalence of positive HCV serology in the SCA group may be ascribable
to the history of episodic blood transfusions in some SCA patients during their infancy in Africa.

We divided the patients with SCA into two groups depending on whether their transfusion procedure at the time of the study was erythrocytapheresis (n = 11, E-SCA group) or another method (n = 30, including 29 given manual exchange transfusions and 1 simple transfusions, non-E-SCA group). The 25 patients with MDS were distributed as follows according to the WHO classification: refractory anemia with ring sideroblasts (RARS), n = 12; refractory anemia (RA), n = 6; unclassified, n = 2; refractory anemia with excess of blasts (RAEB-1), n = 2; 5q minus syndrome, n = 1; and myelodysplastic/myeloproliferative disease, n = 2. According to the International Prognostic Scoring System (IPSS) [17], they were all low risk: low risk in 15 cases and intermediate 1 in 10 cases.

Table 2 describes the main transfusion and chelation characteristics. Since MDS affects older patients, their age at transfusion initiation was significantly older, and their time on chronic transfusion and total number of ECs received since the diagnosis were significantly lower (\(P<0.01\)). When we compared the number of ECs given per year across groups, we found that the only significant difference was a higher number/year in the E-SCA group compared to the non-E-SCA group (35/y vs 21/y, \(P = 0.03\)).

Chelation was given to 95% of patients in the thalassemia group and 90% of those in the non-E-SCA group, compared to only 72% of patients in the E-SCA group and 72.7% of those in the MDS group. Age at chelation initiation was older in the E-SCA group than in the non-E-SCA group (18 vs. 9 years); the serum ferritin level at chelation initiation was apparently lower in the E-SCA group (1500 ng/mL vs. 2075 ng/mL in the non-E-SCA group), but this variable was missing for nearly half the patients and, therefore, statistical testing was not performed.

Data on chelation were available for 72 of the 86 patients. They are reported in Table 3. Seven patients received no chelation. Of the remaining 65 patients, 55 received deferasirox (14/20 with thalassemia, 25/41 with SCA, and 16/25 with MDS), 9 deferiprone (3/20 with thalassemia and 6/41 with SCA), and 1 deferoxamine. In each diagnostic group, about half the patients given deferasirox had at least one plasma deferasirox assay. Non-adherence to the deferasirox regimen, defined as a plasma level <0.5 μg/mL was found in none of the patients

| Table 1. Main patient characteristics. |
|---------------------------------------|
| N (%) or mean±SD | Thalassemia | SCAa | MDSb | P value |
|------------------|-------------|------|------|---------|
| Number of patients | 20 | 41 | 25 |
| Male gender | 10 (50%) | 21 (51.2%) | 17 (68%) | 0.34 |
| Age (y) at MRIc | 27.5±13.9 | 22.9±13.5 | 69.5±10.4 | <0.001 |

Origin:
- • Africa
- • Europe
- • North Africa
- • Asia
- • Caribbean
- • Other

History of splenectomy
| |
|---|
| 14 (70.0) | 6 (14.6%) | 2 (8%) | <0.001 |

Positive HCVd serology
| |
|---|
| 1 (5.9%) | 5 (13.9%) | 0 (0%) | 0.45 |

aSCA, sickle cell anemia.
bMDS, myelodysplastic syndrome.
cMRI, magnetic resonance imaging.
dHCV, hepatitis C virus.

doi:10.1371/journal.pone.0172147.t001
with MDS, 37.5% of patients with thalassemia, 30% of non-E-SCA patients, and 60% of E-SCA patients.

Table 4 reports the data on blood iron variables, LIC, and $T2^*$.

LIC was significantly elevated in all four groups (median, 10.4-15.2 mg/g dry weight; normal, 0.4-2.2 mg/dry weight), with no significant difference across groups at the time of the study ($P = 0.29$).

Cardiac iron overload defined as $T2^* < 20$ ms was diagnosed in 3 (15%) patients with thalassemia and 4 (16%) patients with MDS. No patient with SCA had $T2^* < 20$ ms, despite non-significantly higher serum ferritin levels ($P = 0.08$) in the two SCA groups. Ferritin levels were strongly associated with LIC both in the overall population and in each group (Fig 1). Serum iron was elevated in the thalassemia and MDS groups but normal in the SCA groups, producing a highly significant difference ($P<0.001$). NTBI was also high in the thalassemia and MDS...

Table 2. Transfusion and chelation data.

|                      | Thalassemia | non-E-SCA$^a$ | E-SCA$^b$ | MDS$^c$ | $P$ value |
|----------------------|-------------|---------------|-----------|---------|-----------|
| N = 20               | N = 30      | N = 11        | N = 25    |         |           |
| Age at transfusion initiation (y) | 8.5 [0-45] | 7 [0-45]     | 16.5 [1-55] | 66 [38-83] | <0.001    |
| Time on chronic transfusion (y) | 10 [1-39]  | 7 [1-22]     | 10.5 [0-25] | 3 [1-10]  | <0.001    |
| Number of ECs$^d$ since diagnosis | 359 [21-1360] | 139 [24-791] | 301 [14-888] | 77 [16-544] | <0.001    |
| Number of ECs$^d$/y | 24 [8-67]  | 21 [4-62]    | 35 [17-58] | 27 [7-65] | 0.09      |

$^a$non-E-SCA, patients with sickle cell anemia given manual exchange transfusions (n = 19) or simple transfusions (n = 1).

$^b$E-SCA, patients with sickle cell anemia managed with erythrocytapheresis.

$^c$MDS, myelodysplastic syndrome.

$^d$EC, erythrocyte concentrate.

doi:10.1371/journal.pone.0172147.t002

Table 3. Chelators used.

|                      | Thalassemia | non-E-SCA$^a$ | E-SCA$^b$ | MDS$^c$ | Total |
|----------------------|-------------|---------------|-----------|---------|-------|
| N = 20               |             |               |           |         |       |
| Intravenous Deferoxamine | 1 (5%)     | 0 (0%)        | 0 (0%)    | 0 (0%)  | 1     |
| Deferiprone          | 3 (15%)     | 5 (16.7%)     | 1 (9.1%)  | 0 (0%)  | 9     |
| Deferasirox         | 14 (70%)    | 19 (63.3%)    | 6 (54%)   | 16 (64%)| 55    |
| No chelation         | 1 (5%)      | 3 (10%)       | 1 (9.1%)  | 2 (8%)  | 7     |
| Unknown              | 1 (5%)      | 3 (10%)       | 3 (27%)   | 7 (28%) | 14    |

$^a$non-E-SCA, patients with sickle cell anemia managed with manual exchange transfusions or simple transfusions.

$^b$E-SCA, patients with sickle cell anemia managed with erythrocytapheresis.

$^c$MDS, myelodysplastic syndrome.

doi:10.1371/journal.pone.0172147.t003

Cardiac iron overload in chronically transfused patients...
groups and considerably lower in the SCA groups (P<0.001). The largest differences across groups were for the serum hepcidin levels, which were low in the patients with thalassemia, normal in those with SCA, and markedly elevated in those with MDS (P<0.001). NTBI was significantly associated with serum iron (rho = 0.46, p<0.001), but with none of the others parameters studied (ferritin, LIC, years of transfusion therapy, hepcidin level). On the whole population, hepcidin level was significantly associated with ferritin (rho = 0.55, p<0.01), LIC (rho = 0.35, p<0.01), and with years of transfusion therapy (rho = -0.31, p<0.01). The association between hepcidin level and ferritin was the only one that remained strong and significant in most of the disease groups (it was non significant for MDS) (Fig 2).

Factors associated with cardiac iron overload

None of the patients with SCA had cardiac iron overload. LIC elevation was associated with cardiac iron overload in the patients with thalassemia (P = 0.04) or MDS (P = 0.03). Surprisingly, cardiac iron overload was not significantly associated with any of the transfusion or chelation parameters, but our study is limited by the short number of patients developing cardiac iron.

Discussion

Over 300 000 children are born each year with major thalassemia or SCA. Cardiac iron overload is the leading cause of death in thalassemia. In the UK, routine MRI T2* monitoring and improved iron chelation have been associated with a 71% decrease in deaths due to thalassemia [18]. The relationship between excess iron and mortality in SCA is more difficult to analyze, because the patients with transfusion-related iron overload are also those who have greater disease severity requiring chronic transfusion [19, 20]. However, although additional long-term
prospective studies are still needed [21], blood transfusion is increasingly used in patients with SCA, particularly for primary and secondary stroke prevention [22]. As a result, iron-related toxicity has been reported in SCA [7, 23]. Clearly, there is a pressing need for evaluating the potential consequences of transfusion iron overload in SCA. Finally, there is increasing evidence that iron chelation benefits patients with MDS [2–5], not only by diminishing the deposition of iron in the liver and heart, but also by alleviating the adverse effects of iron on erythroid precursors [3, 24].

In thalassemia, both the liver and the heart contain excess iron, whereas in SCA the iron deposits selectively in the liver. In a study of 141 patients with SCA who died in adulthood, at a
mean age of 36±11 years, Darbari et al found that 16 (11.4%) had cirrhosis and 10 (7.1%) iron overload; seven of 16 (43.8%) patients with cirrhosis had iron overload compared with 3 of the 125 without cirrhosis (2.4%) (P<0.001) [23]. The heart seems less affected by iron overload in children and young patients [25–27], although iron-related cardiomyopathy has been reported in older patients. Thus, in a series of 201 chronically transfused patients with SCA, 6 had cardiac iron overload, including 1 aged 17 years and 5 aged 22 to 29.6 years [7]. We found no cardiac iron overload in our 41 patients with SCA, whose median age was 22.9±13.5 years, whereas LIC values in the SCA groups were similar to those in the thalassemia and MDS.
groups. These results confirm that, in patients with SCA, iron overload selectively targets the liver, leaving initially the heart relatively spared.

Our finding of severe iron overload with LIC elevation in patients with SCA managed using erythrocytapheresis contrasts with previous reports [10, 28, 29]. Several factors may explain this discrepancy. First, duration of transfusion was longer in our patients than in previous studies. Given the severity of the anemia in the SCA patients included in this study, the centers programmed each automated erythrocytapheresis procedure to increase the hemoglobin level by 1.5 g/dL compared to the pre-procedure value, which can induce substantial accumulation of iron over several years. Second, adherence to chelator therapy seemed low in the E-SCA group: of the 5 patients with plasma deferasirox assays, 3 had low values. Physicians may direct insufficient attention to adherence because of a misconception that erythrocytapheresis prevents totally iron overload. Efforts are needed to raise physician awareness about the risk of iron overload despite erythrocytapheresis.

The 16% prevalence of cardiac iron overload in the MDS group is consistent with earlier data from chronically transfused patients with MDS (18% [30] and 16% [31]). Cardiac iron overload may contribute to heart failure in this older population, in addition to chronic anemia and comorbidities.

Liver iron overload has been reported to antedate cardiac iron overload in patients with thalassemia or MDS [32, 33]. Cardiac iron overload usually develops only after severe hepatic iron overload but not exclusively, since some patients may accumulate iron in heart whereas they have a negative liver iron balance [33]. In spite of the low frequency of cardiac iron overload in our cohort, resulting in a lack of power to detect associations, we found a significant relationship between increased LIC and abnormal T2* in patients with thalassemia (P = 0.04) and MDS (P = 0.03). Some other studies found poor correlation, this discrepancy might be explained by differences in the kinetics of iron uptake and in iron elimination in the heart and the liver [33].

Cardiac iron overload as defined by T2* < 20 ms was absent in SCA but present in some patients with thalassemia or MDS despite similar total numbers of ECs received. The reasons for this finding are unclear. The efficiency of erythropoiesis may play a key role in determining the rate and location of iron deposition. In diseases characterized by inefficient erythropoiesis, whether inherited such as thalassemia or acquired such as MDS, hepcidin production is suppressed and iron absorption increased [34, 35]. Thus, our patients with thalassemia had low hepcidin levels, high serum iron and NTBI, and more organ damages, since iron under NTBI form enters the myocytes through the voltage-dependent L-type calcium channels and induces the development of a cardiomyopathy [36]. Hepcidin levels were normal in SCA patients, whose low NTBI levels resulted probably from massive consummation of iron through effective erythropoiesis, making NTBI less available in the circulation. A recent study compared 5 patients with thalassaemia, 5 with SCA, and 5 with Diamond-Blackfan anemia [37]. The three groups were comparable for age, time on chronic transfusion, and time on chelation. NTBI levels were lowest in the SCA group, suggesting better iron utilization. Further support for this explanation comes from a study of iron overload in 121 chronically transfused children with SCA enrolled in the TWITCH trial [38]. Interestingly, despite a mean time on chronic transfusion of 4.1 ± 2.4 years, mean transferrin saturation was 47.2% ± 23.6%, i.e., lower than the value expected after a comparable transfusion time in patients with thalassemia. We did not collect transferrin saturation data. However, serum iron reliably mirrors transferrin saturation in patients without hypotransferrinemia. In our study, serum iron was normal in the patients with SCA. Conceivably, in SCA, the large amounts of heme-bound iron from transfused blood and hemolysis may be efficiently handled by the liver macrophages, transferred to the bone marrow, and used to produce erythrocytes, thereby preventing the formation of NTBI.
Furthermore, chronic inflammation, which increases hepcidin levels, is a prominent feature of SCA that limits the release of iron release from macrophages [39]. A recent study of adults with SCA showed that the percentage of reticulocytes and levels of erythropoietin, ferritin, and C-reactive protein contributed to variations in hepcidin levels [40]. Hepcidin levels were very high in our patients with MDS. In previously published works, hepcidin levels varied across MDS subtypes [3], due to the conflicting influences of dyserythropoiesis, transfusion-related iron overload, and inflammation [41]. Our patients with MDS were heavily transfused and had high hepcidin levels, without major inflammation (median C-reactive protein, 5.0 mg/L [range, 0-42]), suggesting that iron overload in this population increased the production of hepcidin, albeit not sufficiently to prevent high transfusion-related NTBI release.

In conclusion, in SCA, the liver is the main target for iron accumulation, and the heart is usually initially spared, perhaps because iron is recycled by efficient erythropoiesis and trapped within macrophages due to chronic inflammation. Iron overload can occur in SCA even when erythrocytapheresis is used. In thalassemia, erythropoiesis is inefficient, allowing free iron to deposit within tissues. In MDS, the heterogeneity of the causative diseases and competing influences of abnormal erythropoiesis, iron overload, and inflammation produce widely variable results.

Acknowledgments

We are indebted to Caroline Tourte and Valerie Jolaine (Paris Descartes Clinical Research Unit, Necker Children’s Hospital) for their careful collection and organization of the data in the centers; to Professor Pierre Brissot for fruitful discussions, and to A. Wolfe MD for help in preparing the manuscript.

Author Contributions

Data curation: AB CE.

Formal analysis: MM CE OE CR.

Funding acquisition: MM.

Investigation: MM CE OE CR.

Project administration: MM CR.

Resources: JAR VB AGB AS JPV CD AL MT KB MC TL ZK EC.

Software: CE AB.

Supervision: MM CE ZK OE.

Visualization: MM CE CR.

Writing – original draft: MM CR ZK.

Writing – review & editing: MM CR ZK AB CE HP OE.

References

1. Borgna-Pignatti C, Cappellini MD, De Stephano P, Del Vecchio GC, Forni GL, Gamberini MR, et al. Survival and complications in thalassemia. Ann NY Acad Sci 2005; 1054: 40–47. doi: 10.1196/annals.1345.006 PMID: 16339650

2. Pinto V, Balocco M, Ambaglio I, Derchi G, Malcovati L & Forni GL. Iron overload-related heart failure in a patient with transfusion-dependent myelodysplastic syndrome reversed by intensive combined chelation therapy. Clinical case Reports 2015; 3: 952–954. doi: 10.1002/ccr3.407 PMID: 26576280
3. Shenoy N, Vallumsetla N, Rachmilewitz E, Verma A, Ginzburg Y. Impact of iron overload and potential benefit from iron chelation in low-risk myelodysplastic syndrome. Blood 2014; 124: 873–881. doi: 10.1182/blood-2014-03-663211 PMID: 24923296

4. Rose C, Brechignac S, Vassiliad S, Pascal L, Stamatoullas A, Guerci A, et al. Does iron chelation improve survival in regularly transfused lower risk MDS patients? A multicentre study by the GFM (Groupe francophone des Myéloïdysplasies). Leuk Res 2011; 34: 864–870.

5. Fenaux P, Rose C. Impact of iron overload in myelodysplastic syndromes. Blood Rev 2009; Suppl 1: S15–19.

6. Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, et al. Comparison of organ dysfunction in transfused patients with sickle cell disease or beta thalassemia. Am J Hematol 2005; 80: 70–74. doi: 10.1002/ajh.20402 PMID: 16138345

7. Fang EB, Harmatz PR, Milet M, Ballas S, Casella JF, et al. Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: a report from the multi-centre study of iron overload. Am J Hematol 2007; 82: 255–265. doi: 10.1002/ajh.20809 PMID: 17094096

8. Ballas SK. Iron overload is a determinant of morbidity and mortality in adults with sickle cell disease. Semin Hematol 2001; 38(1 Suppl 1): 30–36.

9. Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, et al. Comparison of organ dysfunction in transfused patients with sickle cell disease or beta thalassemia. Am J Hematol 2005; 80: 70–74. doi: 10.1002/ajh.20402 PMID: 16138345

10. Modell B, Khan M, Darlison M. Survival in beta thalassaemia major in the UK: data from the UK Thalassemia register. Lancet 2000; 355: 2051–2052. doi: 10.1016/S0140-6736(00)02357-6 PMID: 10885361

11. Ballas SK. Iron overload is a determinant of morbidity and mortality in adults with sickle cell disease. Semin Hematol 2001; 38(1 Suppl 1): 30–36.

12. Fung EB, Harmatz PR, Milet M, Ballas SK, De Castro L, Hagar W, et al. Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: a report from the multi-center study of iron overload. Am J Hematol 2007; 82: 255–265. doi: 10.1002/ajh.20809 PMID: 17094096

13. Lucania G, Vitrano A, Filosa A, Maggio A. Chelation treatment in sickle-cell anaemia: much ado about nothing? Br J Haematol 2011; 154: 545–555. doi: 10.1111/j.1365-2141.2011.08769.x PMID: 21707578

14. Adams PJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anaemia and abnormal results on transcranial doppler ultrasonography. N Engl J Med 1998; 339: 5–11. doi: 10.1056/NEJM199807023390102 PMID: 9647873

15. Darbahi DS, Kple-Faget P, Kwagyan J, Rana S, Gordeuk VR, Castro O. Circumstances of death in adult sickle cell disease patients. Am J Hematol 2006; 81: 858–863. doi: 10.1002/ajh.20685 PMID: 16924640

16. Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, et al. Comparison of organ dysfunction in transfused patients with sickle cell disease or beta thalassemia. Am J Hematol 2005; 80: 70–74. doi: 10.1002/ajh.20402 PMID: 16138345

17. Modell B, Khan M, Darlison M. Survival in beta thalassaemia major in the UK: data from the UK Thalassemia register. Lancet 2000; 355: 2051–2052. doi: 10.1016/S0140-6736(00)02357-6 PMID: 10885361

18. Ballas SK. Iron overload is a determinant of morbidity and mortality in adults with sickle cell disease. Semin Hematol 2001; 38(1 Suppl 1): 30–36.

19. Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, et al. Comparison of organ dysfunction in transfused patients with sickle cell disease or beta thalassemia. Am J Hematol 2005; 80: 70–74. doi: 10.1002/ajh.20402 PMID: 16138345

20. Modell B, Khan M, Darlison M. Survival in beta thalassaemia major in the UK: data from the UK Thalassemia register. Lancet 2000; 355: 2051–2052. doi: 10.1016/S0140-6736(00)02357-6 PMID: 10885361

21. Ballas SK. Iron overload is a determinant of morbidity and mortality in adults with sickle cell disease. Semin Hematol 2001; 38(1 Suppl 1): 30–36.

22. Fung EB, Harmatz P, Milet M, Ballas SK, De Castro L, Hagar W, et al. Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: a report from the multi-center study of iron overload. Am J Hematol 2007; 82: 255–265. doi: 10.1002/ajh.20809 PMID: 17094096

23. Lucania G, Vitrano A, Filosa A, Maggio A. Chelation treatment in sickle-cell anaemia: much ado about nothing? Br J Haematol 2011; 154: 545–555. doi: 10.1111/j.1365-2141.2011.08769.x PMID: 21707578

24. Adams PJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anaemia and abnormal results on transcranial doppler ultrasonography. N Engl J Med 1998; 339: 5–11. doi: 10.1056/NEJM199807023390102 PMID: 9647873

25. Darbahi DS, Kple-Faget P, Kwagyan J, Rana S, Gordeuk VR, Castro O. Circumstances of death in adult sickle cell disease patients. Am J Hematol 2006; 81: 858–863. doi: 10.1002/ajh.20685 PMID: 16924640
24. Carmaschella C, Campanella A, de Falco L, Boschetto L, Merlini R, Silvestri L et al. The human counterpart of zebrafish shiraz shows sideroblastic-like microcytic anemia and iron overload. Blood 2007; 110: 1353–1358. doi: 10.1182/blood-2007-02-072520 PMID: 17485548

25. Kaushik N, Eckrich MJ, Parra D, Yang E. Chronically transfused pediatric sickle cell patients are protected from cardiac iron overload. Pediatr Hematol Oncol 2012; 29: 254–260. doi: 10.3109/08880018.2011.630774 PMID: 22309797

26. Wood JC, Tyszka JM, Carson S, Nelson MD, Coates TD. Myocardial iron loading in transfusion-dependent thalassemia and sickle cell disease. Blood 2004; 103: 1934–1936. doi: 10.1182/blood-2003-06-1919 PMID: 14630822

27. Porter J, Garbowski M. Consequences and management of iron overload in sickle cell disease. Hematology Am Soc Hematol Educ Program 2013, 447–456. doi: 10.1182/asheducation-2013.1.447 PMID: 24319218

28. Kim HC, Dugan NP, Silber JJ, Martin MB, Schwartz E, Ohene-Frempong K, et al. Erythrocytapheresis therapy to reduce iron overload in chronically transfused patients with sickle cell disease. Blood 1994; 83: 1136–1142. PMID: 8111053

29. Fasano RM, Leong T, Kaufman M, Sagiv E, Luban NL, Meier ER. Effectiveness of red blood cell exchange, partial manual exchange, and simple transfusion concurrently with iron chelation therapy in reducing iron overload in chronically transfused sickle cell anemia patients. Transfusion 2016; 56: 1707–1715. doi: 10.1111/trf.13558 PMID: 26997031

30. Pascal L, Beyne-Rauppy O, Brechignac S, Marechaux S, Vassilieff D, Ernst O, et al. Cardiac iron overload assessed by T2* magnetic resonance imaging and cardiac function in regularly transfused myelodysplastic syndrome patients. Br J Haematol 2013; 162: 413–427. doi: 10.1111/bjh.12368 PMID: 23656172

31. Roy N, Myerson S, Schuh AH, Bignell P, Patel R, Wainscoat JS, et al. Cardiac iron overload in transfusion-dependent patients with myelodysplastic syndromes. Br J Haematol 2011; 154: 521–524. doi: 10.1111/j.1365-2141.2011.08749.x PMID: 21689086

32. Di Tucci AA, Matta G, Deplano S, Gabbas A, Depau C, Derudas D, et al. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. Haematologica 2008; 93: 1385–1388. doi: 10.3324/haematol.12759 PMID: 18603557

33. Noetzel LJ, Carson SM, Nord AS, Coates TD, Wood JC. Longitudinal analysis of heart and liver iron in thalassemia major. Blood 2008; 112: 2973–2978. doi: 10.1182/blood-2008-04-148767 PMID: 18650452

34. Nemeth E. Hepcidin and beta-thalassemia major. Blood 2013; 122: 3–4. doi: 10.1182/blood-2013-05-502617 PMID: 23828883

35. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anemias. Br J Haematol 2016;

36. Kremastinos DT, Farmakis D. Iron overload in clinical practice. Circulation 2011; 124: 2253–2263. doi: 10.1161/CIRCULATIONAHA.111.050773 PMID: 22083147

37. Porter JB, Walter PB, Neumayer LD, Evans P, Bansal S, Garbowski M, et al. Mechanisms of plasma non-transferrin bound iron generation: insights from comparing Diamond Blackfan anaemia with sickle cell and thalassaemia patients. Br J Haematol 2014; 167: 692–696. doi: 10.1111/bjh.13081 PMID: 25209728

38. Wood JC, Cohen AR, Pressel SL, Aygun B, Imran H, Luchtman-Jones L, et al. Organ iron accumulation in chronically transfused children with sickle cell anemia: baseline results from the TWITCH trial. Br J Haematol 2016; 172: 122–130. doi: 10.1111/bjh.13791 PMID: 26523836

39. Walters PB, Fung EF, Killilea DW, Jiang Q, Hudes M, Madden J, et al. Oxidative stress and inflammation in iron-overloaded patients with β-thalassaemia or sickle cell disease. Br J Haematol 2006; 135: 254–263. doi: 10.1111/j.1365-2446.2006.06277.x PMID: 17010049

40. Karaffin MS, Koch KL, Rakin AB, Nischik D, Rahhal G, Simpson P, et al. Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease. Blood Cells Mol Dis 2015; 55: 304–307. doi: 10.1016/j.bcmd.2015.07.010 PMID: 26460251

41. Santini V, Girelli D, Sanna A, Martinelli N, Duca L, Campostrini N, et al. Hepcidin levels and their determinant in different types of myelodysplastic syndromes. PLoS One 2011; 6(8):e23109, doi: 10.1371/journal.pone.0023109 PMID: 21886780