Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An analysis Based on Ferritin and Transferrin Saturation

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ABSTRACT

Background: Hematopoietic Stem cell Transplant Recipient conditioning regimens include immunosuppressive and myeloablative drugs that cause secondary cell damage and lead to ineffective erythropoiesis, which is one of the mechanisms that triggers iron (Fe) overload (IO). In addition, exogenous Fe from transfused blood components, specifically red blood cells, is what most contributes to excess Fe in the body. Fe toxicity secondary to transfusion is complex to estimate as it is related to the sum of exposure time and exogenous Fe associated with the factors determined based on the environment, genetic metal transport differences, and intrinsic antioxidant defenses.

Keywords: ferritin, iron overload, hematopoietic stem cell transplant, transferrin saturation, transfusion complications.

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Background: Hematopoietic Stem cell Transplant Recipient conditioning regimens include immunosuppressive and myeloablative drugs that cause secondary cell damage and lead to ineffective erythropoiesis, which is one of the mechanisms that triggers iron (Fe) overload (IO). In addition, exogenous Fe from transfused blood components, specifically red blood cells, is what most contributes to excess Fe in the body. Fe toxicity secondary to transfusion is complex to estimate as it is related to the sum of exposure time and exogenous Fe associated with the factors determined based on the environment, genetic metal transport differences, and intrinsic antioxidant defenses.

Objective: This study aimed to assess the impact of IO on HSCT in terms of overall survival (OS), in addition its relationship with red blood cell transfusion support, using serum biomarkers as ferritin and transferrin saturation.

Materials and Methods: A specific laboratorial and clinical data was collected in a sample of 199 bone marrow recipients from two public hospitals at São Paulo, Brazil, in a large period, submitted to allogeneic Hematopoietic stem cell transplantation, between 1996 to 2018.

Results: Data analysis showed that the highest mean ferritin levels in absolute numbers was reached within 100 days after HSCT in 146 (73.4%) patients, followed by a second peak in the ferritin levels in 44 (22.3%) patients 1 year after HSCT, with significantly decreased ferritin levels obtained in subsequent measurements. The sample presented higher TSI 100 days post-transplant, with a mean TSI of 46.93%. This finding correlates with the higher peak ferritin concentration in the same post-transplant period. OS was impacted by the number of red blood cell transfusions; the group with the lowest number of transfusions (<10) achieved greater survival. The study also demonstrated that a mean ferritin level of >1,000 post-HSCT impacted the patient's OS (79% versus 91%; p<0,05). We demonstrated that patients with a TSI of >50% have a lower OS, especially when evaluated after 1 year (80%: TSI > 50% versus 92%: TSI < 50%, p < 0.05). Conclusion: High ferritin and TSI levels cannot be attributed only to transfusion dependence, highlighting that the ferritin level remains high for up to 5 years, even if hemotherapy support is not required. Others factor must be evaluated in further studies to elucidate other mechanisms responsible for iron overload in bone marrow transplantation field.

Keywords: ferritin, iron overload, hematopoietic stem cell transplant, transferrin saturation, transfusion complications.

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I. INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a therapeutic procedure involving the intravenous infusion of hematopoietic stem cells (HSCs) obtained from the blood of a previously selected donor into a properly conditioned recipient with the aim of reconstituting the hematopoietic organ. Recipient conditioning regimens include immunosuppressive and

London Journal of Medical and Health Research

myeloablative drugs that cause secondary cell damage and lead to ineffective erythropoiesis after transplantation, which is one of the mechanisms that triggers iron (Fe) overload (IO).[1,2,3] In addition, the recipient's previous transfusion history is a factor of great importance.

Exogenous Fe from transfused blood components, specifically red blood cells, is what most contributes to excess Fe in the body. The need for transfusion prior to transplantation depends on the underlying disease and varies; however, some diseases require frequent transfusion of blood products, especially those in need of myeloablative chemotherapy, such as acute leukemias and myelodysplastic syndromes.[4] In these cases, the patient undergoes repeated transfusions over a short period, which causes secondary Fe excess and leads to IO immediately before HSCT.

Fe toxicity secondary to transfusion is complex to estimate as it is related to the sum of time ($\Sigma^{time} \times \Delta Time$) of exposure to the reactive iron modulated by environmental and genetic antioxidant factors. This relation is highly nonlinear, making it impossible to predict iron toxicity from iron levels alone. Therefore, ferritin is intracellular but can leak into the plasma when cell membranes are damaged,and can be a useful marker. Thus, serum/plasma ferritin levels are a rough measure of iron loading, but are also sensitive to inflammation and cell membrane leak.

Hemosiderin, which is made up of aggregates of ferritin, is the primary species that is detected by MRI. (reviewed in Coates[5]).

IO diagnosis is suggested in patients with a serum ferritin level of >1,000, evidenced by transferrin saturation index (TSI) values of >50% in men and >45% in women.[6,7] In the context of HSCT, IO is diagnosed by liver biopsy, the gold standard test; however, it is not frequently used in the clinical setting due to its prohibitive risks. IO can also be verified by abdominal magnetic resonance imaging using the T2* protocol, but this test only shows the installed cell damage, estimating the direct quantification of the amount of Fe deposited.

Serum Fe levels, its cellular deposit (ferritin), and the indirect quantification of excess metal in transport (TSI) are used in the early serum IO analysis and in the continuous assessment of Fe metabolism dynamics. These data provide a broad picture of IO prior to HSCT and in periods of greater catabolism, such as after transplantation.

This study aimed to assess the impact of IO on HSCT in terms of overall survival (OS), in addition to assessing its relationship with red blood cell transfusion support.

II. MATERIALS AND METHODS

Patients who underwent allogeneic HSCT from an HLA-identical related, unrelated, and haploidentical donor between 1996 and 2018 in the two institutions, with neoplastic and benign diseases, and who visited the post-transplant outpatient clinic from 2015 to 2019 with no evidence of disease relapse or infectious process were included in the study; of the 426 post-allogeneic transplant patients currently being cared, 199 of them who underwent routine follow-up, were enrolled in this study. These outpatient recipients were aged 18–65 years at the time of transplantation. No restrictions were applied in the conditioning regimen and indications for transplant.

This retrospective historical cohort study had an individualized, observational, and longitudinal epidemiological investigation design. It aimed to establish the relationship between red blood cell transfusions, patient's age, and hematologic disease and its implications on excess Fe after HSCT.

This study was approved by the Research Ethics Committees of Hospital São Paulo and Casa de Saúde Santa Marcelina. All study participants signed an informed consent form in accordance with the Declaration of Helsinki.

The following data were collected from the patients' medical records: time of transplantation, procedure-related complications, underlying disease, previous history of the disease, chemotherapy treatment, and transfusion history. At different times of the transplant, the patients

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

34 Volume 22 | Issue 13 | Compilation 1.0 © 2022 London Journals Press

were invited to visit the outpatient clinics between 2015 and 2019 for an initial diagnosis of IO or follow-up of a previously established diagnosis.

Clinical evaluation and serial laboratory tests such as serum Fe, ferritin, transferrin, and TSI were performed before HSCT and 100 days, 1 year, 3 years, 5 years, and >5 years after HSCT. The ferritin level was measured based on the data provided in the medical records and obtained during routine outpatient visits in patients not hospitalized and not presenting signs of infection or relapse at the time of examination.

The medical records were stored in paper-based documents, microfilms, or electronic systems. The data analyzed were available in the Blood Bank specific system. All clinical and laboratory parameters corresponding to the proposed variables were listed in Excel spreadsheets in the Office XP software.

Descriptive statistics were performed using Excel spreadsheets in the Office XP software along with the inferential analysis performed in the SPSS®. The categorical variables were expressed as absolute and relative frequencies. The distribution of numerical variables, especially the mean and maximum ferritin levels, was evaluated using central tendency measures. The numerical outcome variables were presented in a boxplot for the total sample or divided into categories of interest. Bivariate analyses were carried out as a joint assessment of the numerical and categorical variables using the boxplot. The distribution of the TSI numerical variable in the total sample and the categorical variable was visualized using a boxplot.

Numerical or continuous variables were expressed as central tendency measures of variability, and adherence to normality was evaluated using the Kolmogorov-Smirnov test. Numerical variables were compared in the same individual before HSCT and 100 days after HSCT. A parametric t-test was used to compare the difference between paired samples if the variable had normal distribution at both moments; otherwise, a Wilcoxon test was used, a non-parametric equivalent. The significance level was set at 0.05 for all tests. The OS curves for categories of the

same variable were compared using the Kaplan-Meier test. The survival curves were compared using the log-rank test.

III. RESULTS

The study evaluated 199 patients, of whom 125 (62.8%) were from Casa de Saúde Santa Marcelina and 74 (37.2%) were from UNIFESP; among the total participants, 113 (56.8%) were men (M), while 86 (43.2%) were women (W), with a median age at the time of transplantation of 37 years. The population from the two institutions showed comparable demographic profile. The laboratory data equivalence was also evaluated, demonstrating similar maximum ferritin distribution in the two institutions (**Graph 1**). As for the time to elapsed from HSCT to inclusion in the study, 51.2% of the sample underwent HSCT 1 to 9 years ago (**Figure 1**). The sample included 159 (80%) patients who underwent identical-related allogeneic, 22 (11%) who underwent identical unrelated allogeneic, and 18 (9%) who underwent haploidentical transplants (**Table1).**

Patients with diseases that are indicated for transplant were grouped by etiological similarity and divided into large groups: benign anemia group (aplastic anemia and Fanconi anemia), acute leukemia group, Lymphoproliferative disease, chronic leukemia group, and myelo-dysplastic syndrome group. The sample was also categorized according to disease status at the time of transplant. The data showed a great proportion of patients with acute leukemias, and pre-transplant positive minimal residual disease (MRD+) was observed in 81 (40.7%) patients with this condition. A small part of the sample had lymphoproliferative diseases; however, the proportion of patients in partial remission is higher (**Table 2**).

Post-HSCT was divided into the pre-transplant period, measured from the initiation of chemotherapy treatment and disease-specific clinical support until stem cell infusion, which included HSCT conditioning; immediate post-HSCT period, measured from the first 100 days after HSCT (starting from the time that the stem cells were infused), including the period of

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

immune recovery in which neutrophilic engraftment occurs within 30 days; intermediate post-HSCT period, measured from the first 30–100 days after HSCT; and late post-HSCT period, measured from the first 101 days to 1 year after HSCT, with CD19+ B lymphocyte recovery. A follow-up period of 3 to >5 years after the procedure was called prolonged post-HSCT.[8]

IV. FERRITIN ANALYSIS

The distribution of ferritin level was analyzed in the complete sample before transplantation and 100 days, 1 year, 3 years, 5 years, and more than 5 years after HSCT. The highest mean ferritin value was observed within 100 days after HSCT, with progressively decreased ferritin value 1 year after HSCT. The ferritin level was near normal 5 years after HSCT. (**Table 3**).

Individualized sample analysis showed that the highest mean ferritin levels in absolute numbers was reached within 100 days after HSCT in 146 (73.4%) patients, followed by a second peak in the ferritin levels in 44 (22.3%) patients 1 year after HSCT, with significantly decreased ferritin levels obtained in subsequent measurements.

The ferritin medians of 28 patients with complete Fe metabolism data available in all predetermined periods were evaluated; the highest median value was observed within 100 days after HSCT, it progressively decreased 1 year after HSCT, and was below 1,000 within 3, 5, and >5 years after HSCT (**Table 4**).

4.1 Ferritin analysis associated with red blood cell transfusion

More aggressive diseases such as acute leukemia require more intensive chemotherapy with greater myelotoxic potential and a greater number of transfusions. Two other categories, benign types of anemia (Fanconi Anemia and severe aplastic anemia) and myelodysplastic syndrome, require more blood transfusions, raking second and third in terms of the number of transfusions needed, respectively. On the contrary, lymphoproliferative diseases and chronic leukemias, which require less blood transfusion, rank fourth and fifth (**Graph 2**).

Patients in the quartile with the highest number of transfusions (>20) have a higher pre-transplantation ferritin level; meanwhile, the ferritin levels progressively decrease in patients in the quartile with 20 to 10 transfusions and those in the quartile with less than 10 transfusions **(Graph 2).** Patients in the quartile with 20 transfusions have a higher post-transplant maximum ferritin value; that is, they reach the median ferritin values greater than 2,000 after HSCT. The mean maximum ferritin value subsequently decreased as the number of transfusions in each quartile decreased, with median ferritin levels of 1,500 in the quartile with 20 and 10 red blood cell transfusions and 1,100 in the quartile with less than 10 transfusions (**Graphs 3 and 4).**

4.2 Analysis of the impact of IO

Sample components showed high levels of post-transplant transferrin saturation, especially within 100 days after the transplant, with a median TSI of 43% **(Graph 5)**.

The sample presented higher TSI 100 days post-transplant, with a mean TSI of 46.93%. This finding correlates with the higher peak ferritin concentration in the same post-transplant period **(Table 5).**

OS was also impacted by the number of red blood cell transfusions; the group with the lowest number of transfusions (<10) achieved greater survival. Patients who underwent more than 20 transfusions had lower survival rates **(Graph 6).**

Ferritin-related survival was tested, with a ferritin level of >1,000 impacting survival; the OS decreased within 1 year after transplant, which was maintained for up to 5 years. The ferritin level of <1,000 slightly decreased after 1 and 3 years and remained stable after these periods, with greater survival compared with a ferritin curve of >1,000 (**Graph 7).**

As for OS-related transferrin saturation, greater survival was observed in patients with lower IO levels (TSI < 50%) even before HSCT. The OS was slightly affected within 100 days after the transplant based on the TSI. However, this impact

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

36   Volume 22 | Issue 13 | Compilation 1.0   © 2022 London Journals Press

on survival was much greater when the same parameter was measured 1 year after HSCT (**Graph 8**). This finding shows that TSI is influenced by the transplant, reflecting what was observed with the ferritin level, a parameter peaking at 100 days and 1 year after HSCT. Serum Fe was another marker tested at all study time points and did not impact the survival when evaluated alone; however, it is necessary to calculate the TSI (serum Fe: transferrin × 0.71 = TSI × 100), thus indirectly impacting OS.

OS was impacted by the patients' age and was significant in patients aged below 25 years; the older the recipient, the greater the progressive survival decrease (**Graph 9**).

V. DISCUSSION

The study included 199 patients, of which 125 (62.8%) underwent HSCT at Casa de Saúde Santa Marcelina and 74 (37.2%) at UNIFESP. The population of the two institutions was restricted to the Public Health System (SUS) population and had similar characteristics such as age, sex, and socioeconomic status. The laboratory data collected at both institutions are comparable, with the median maximum ferritin level being similar in both institutions (**Graph 1**). Thus, it was possible to compare the data and treat the participants from both institutions as equivalent.

Yuan et al. evaluated the transfusion support of 1,792 transplant recipients; among them, 1,582 (88.3%) received red blood cell transfusions in the first 30 days after the procedure, with a progressively reduced transfusion need after this period and a consequent interruption in the transfusion support on an average of 60 days post-transplant. The mean number of red blood cell transfusions required within 30 days after HSCT was three units, with no record after 60 days.[9] The results of our study were similar, as none of the participants required red blood cell transfusion within 45 days after HSCT, with increased pre-transplant ferritin levels in patients with more than 20 red blood cell transfusions (**Graph 2**). Therefore, exogenous Fe from transfusions is one of the factors that can contribute to the occurrence of IO in the immediate post-HSCT period.

After complete immune reconstitution, transfusion independence was achieved after reestablishment of erythropoiesis.[10,11] However, within 100 days after HSCT, the ferritin level and TSI values increased, as demonstrated in **Tables 3, 4, and 5,** regardless of the interruption in transfusions. Therefore, high ferritin levels and IO cannot be attributed only to transfusion dependence, highlighting that the ferritin level remains high for up to 5 years, even if hemotherapy support is not required.

IO causes damage as it increases the level of non-transferrin bound Fe (NTBI), which causes oxidative stress, thus increasing the risk of transplant-related toxicity. Excess Fe increases the incidence of early HSCT complications such as acute graft-versus-host disease (GVHD), sinusoidal obstruction syndrome, and infections.

IO leads to a higher incidence of late complications such as chronic GVHD, liver cirrhosis, and cardiomyopathy.[12,13] These complications are directly related to the occurrence of toxicity produced by the use of conditioning regimen and immune-mediated reactions caused by graft-host interaction.

Therefore, it is important to determine the recipient's transplant stage and period of immune reconstitution to examine the dynamics of Fe metabolism at a certain stage. To demonstrate this relationship, data on serum Fe, ferritin, and transferrin levels were collected, and the TSI was calculated before transplant and 100 days, 1 year, 3 years, and 5 years after HSCT.

The discussion on which plasma marker is ideal to estimate IO in the transplanted body is always present and controversial. Ferritin is a widely available and easily evaluated marker; however, as it is an acute phase reactant and a marker of inflammation, ferritin is the most sensitive but not very specific marker for excess Fe. However, ferritin is considered a good screening tool, and a clinically significant IO (intracellular fluid (ICF) >7,000 g/g) is uncommon in patients with a ferritin level of <1,000 ng/ml. A previous meta-analysis found that a ferritin level of >1,000

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

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Volume 22 | Issue 13 | Compilation 1.0

37

ng/ml is a negative prognostic marker for non-relapse-related mortality (risk ratio = 1.7, p = 0.036).[14] For this reason, pre-transplant ferritin level, which demonstrates IO caused by transfusions; mean post-transplant ferritin level, which would be the mean ferritin level from stem cell infusion until late post-transplant; and maximum post-transplant ferritin level, which represents the maximum ferritin value achieved post-transplant, were used as markers.

A prospective analysis conducted by the Kanto Study Group for Cell Therapy divided 190 patients undergoing allogeneic HSCT for acute myeloid leukemia (AML) and MDS into two groups: those with ferritin level of >1,000 and those with a ferritin level of <1,000. Results showed that increased ferritin level decreased the patients' OS when ferritin level >1,000 at 1 year after transplant was compared with ferritin level <1,000 (63% versus 75%, p = 0,017).[15]

This study demonstrated that a mean ferritin level of >1,000 post-HSCT impacted the patient's OS (79% versus 91%; p<0,05) (**Graph 7).** The analysis used here was retrospective in nature and included patients at different transplant timepoints, up to 21 years post-HSCT, thus allowing a long-term analysis of the deleterious effects of IO, unlike Tanaka et al. who only analyzed the IO effects 1 year after HSCT. In addition, the author evaluated all onco-hematologic diseases without proper distinction, while the previous study only evaluated patients with AML and MDS. Another study analyzed the factor (patient's age) that influenced the OS. Patients aged less than 25 years showed higher OS, with older patients presenting a progressively decreased survival (**Graph 9**).

Meyer et al. investigated 290 patients undergoing myeloablative HSCT between 2000 and 2009. They reported a peak in the ferritin level within the first 3 months after HSCT, with the levels gradually returning to normal 5 years after HSCT, which corroborated with our results. According to that study, high ferritin levels are not associated with pre-HSCT transfusion dependence or GVHD. In all analyzed periods, a ferritin level of >1,380 decreased the post-transplant survival (0–6 months, p < 0.001; 6–12 months, p < 0.001; 1–2 years, p = 0.002; 2–5 years, p = 0.002).[10]

In terms of transfusion dependence, a significant difference was found in post-transplant mean maximum ferritin level for each disease. The mean maximum ferritin level was higher in diseases requiring greater transfusion support such as benign anemia, acute leukemia, and MDS.

Meanwhile, diseases mostly requiring outpatient follow-up, such as chronic leukemias and lymphoproliferative diseases, only ranked fourth and fifth in terms of transfusion demand (**Graph 4).** Patients in the quartile with >20 transfusions have a higher post-transplant maximum ferritin value, reaching a median ferritin value of greater than 2,000 in post-HSCT periods. The mean maximum ferritin value subsequently decreased with the reduction in the respective transfusion quartiles, with a median ferritin level of 1,500 in the quartile with 20 and 10 red blood cell transfusions, and a median maximum ferritin level of 1,100 in the quartile with less than 10 transfusions (**Graph 3).** Therefore, these results disagree with that of Meyer et al., as they showed that transfusion is an additional risk factor for post-transplant IO; however, as previously mentioned, the ferritin level remained high for up to 5 years regardless of the interruption in the administration of blood transfusions.

TSI was analyzed before HSCT and 100 days and 1 year after HSCT to assess whether these data reflect IO and follow the mean ferritin data. Then, we verified whether the transferrin saturation values in the sample were high, which resulted in an TSI of 46.93% within 100 days post-transplant and remained high 1-year post-HSCT, in 44% of the patients. This finding validates and corroborates the ferritin data, with the highest mean ferritin concentration in the same post-transplant period (**Table 5**) and excludes the bias of a possible underlying inflammation based on the ferritin values.

As for the prognostic impact of IO on HSCT, Armand et al. reviewed 590 patients undergoing myeloablative HSCT at the Dana-Faber Cancer Institute between 1997 and 2005. When patients with ferritin levels above and below 1,000

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

38   Volume 22 | Issue 13 | Compilation 1.0   © 2022 London Journals Press

pre-HSCT were compared, the 5-year OS rates were 54% and 27%, respectively, while those of patients with single lineage dysplasia (SLD) were 43% and 27%, with decreased OS in the IO group due to non-disease-related mortality. Bazuave et al. corroborated these data in their analysis to some extent; of the 230 post-allogeneic myeloablative HSCT patients, those with a TSI of >69% had a 5-year OS of 5%, while those without IO had a 5-year OS of 52%.[11,14,16] These data corroborate the findings of our study, in which patients with a TSI of >50% (**Graph 8**) have a lower OS, especially when evaluated after 1 year (80%: TSI > 50% versus 92%: TSI < 50%, $p < 0.05$).

The results of this study demonstrated that the type of disease and dependence on red blood cell transfusion resulted in the reduction of patient's survival after HSCT. As illustrated in **Graphs 3 and 6**, the greater the number of red blood cell transfusion (>20 units), the lower the patient's survival. However, transfusion is no longer required in the late post-transplant period (>30 days and <100 days post-HSCT); therefore, the direct influence of transfusion cannot be considered the only factor responsible for this effect, with high pre-HSCT ferritin level being considered another predisposing factor for higher ferritin levels post-HSCT. Therefore, both the mean and maximal ferritin values are valid and should be used as indirect markers of post-transplant IO. This finding indicates that the pre-transplant ferritin levels should be analyzed and measured, and high levels should be controlled early whenever possible. This finding corroborates the results of the studies described above, thus reaffirming that high mean ferritin levels are not a simple consequence of pre-transplant transfusions or of the inflammation caused by use of conditioning regimens in the immediate transplant period.

VI. CONCLUSION

The data presented in this study corroborate the importance of assessing IO in HSCT survivors.

However, few studies estimate the clinical significance of IO post-HSCT using diagnostic parameters directly and indirectly quantifying IO.

A few studies consistently correlate IO to inflammatory markers and plasma Fe levels.

Clinicians caring for HSCT patients in their daily practice need established and validated screening and therapeutic strategies to guide IO management post-HSCT. This series showed that IO is common in HSCT patients, with excessive pre-transplant transfusion being an additional risk factor. IO can occur up to 5 years after HSCT.

Hence, further studies are necessary to identify the causes of IO persistence in HSCT.

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Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

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Volume 22 | Issue 13 | Compilation 1.0   39

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London Journal of Medical and Health Research

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

40 Volume 22 | Issue 13 | Compilation 1.0

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Table 1: Sample characterization

		N	%
Group	Santa Marcelina	125	62.8
	Unifesp	74	37.2
Sex	Men	113	56.8
	Women	86	43.2
AGE of BMT	Mean 37,26 years		
	Median 37 years		
	Standard deviation 12,08		
Disease	Anemias	26	13.1
	Lymphoproliferative	18	9.0
	Acute leucemias	94	47.2
	MDS (myelodysplastic syndrome)	17	8.5
	Chronic leucemias	44	22.1
Pre-HSCT status	Active/refractory disease	70	35.2
	Disease in complete remission	111	55.8
	Disease in partial remission	18	9.0
Transplant Types	Related allogeneic HSCT	159	80
	Identical unrelated allogeneic HSCT	22	11
	Haploidentical allogeneic HSCT	18	9.0
Conditioning Types	Conditioning myeloablative BU / CY / VP / TBI	131	66.8
	Non-myeloablative conditioning FLU / TBI / FLUCYTBI /CYpost	49	23.6
	Reduced intensity conditioning CY / MEL / FLU	19	9.5

*BU: busulfan, CY: cyclophosphamide, VP: etoposide; TBI: total body irradiation, FLU: fludarabine, MEL: melphalan

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

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Table 2: Disease profile and respective status

Disease	Sample N	Disease status
Benign types of anemia Aplastic anemia Fanconi anemia	25(12,5%) 1(0,5%)	24 activity and 1 partial response 1 activity
Lymphoproliferative diseases		
Hodgkin's lymphoma	2 (1%)	2 partial remission
Non-Hodgkin's lymphoma	8 (4%)	7 partial remission 1 activity
Multiple myeloma	8 (4%)	8 partial remission (post autologous 8
Acute leukemias		
Acute lymphoblastic leukemia	32 (16%)	1 activity/remission: 29 MRD+/2MRD-
Acute lymphoblastic leukemia	56 (28.1%)	5 activity/ Remission: 46 MRD+/ 3 MRD-
Ambiguous lineage leukemia	7 (3.5%)	1 activity/ remission 6 MRD+
Dendritic cell leukemia	1 (0.5%)	
Chronic Leukemias		
Chronic myeloid leukemia	38 (19.1%)	26 Activity or refractory/ 12
Chronic lymphocytic leukemia	6 (3.0%)	Partial response 4 activity/ 2partial response
Myelodysplastic syndrome		
RAEB (refractory anemia with excess blasts) 1	1 (0.5%)	1 activity
RAEB 2	13 (6.5%)	13 activity
CMML (chronic myelomonocytic leukemia)	1 (0.5%)	1 activity

Table 3: Ferritin in every patient sample by period

	N	Mean	Median	Standard Deviation	Minimum	maximum	Kolmogorov-Smirnov
Ferritin pre	133	1137.46	780	1139.351	14	7371	<0.001
Ferritin 100 days	139	1847.14	1509	1613.250	29	8000	<0.001
Ferritin 1 year	124	1526.54	960	1506.501	23	8476	<0.001
Ferritin 3 years	95	1006.54	613	949.583	24	5768	<0.001
Ferritin 5 years	91	574.64	377	499.959	17	2350	<0.001
Ferritin >5 years	97	389.79	280	323.594	15	1418	<0.001

Ferritin levels after transplant and 100 days, 1 year, 3 years, 5 years, and >5 years after transplant of all patients at each time point

Table 4: Ferritin levels in patients with complete data in the six periods

	N	Mean	Median	Standard Deviation	Minimum	maximum	Kolmogorov-Smirnov
Ferritin pre	28	1107.70	518.00	1546.441	14	7371	<0.001
Ferritin 100 days	28	1675.16	1348.90	1532.039	29	7558	0.002
Ferritin 1 year	28	1195.00	804.50	1122.756	92	4788	<0.001
Ferritin 3 years	28	994.94	529.00	4894.716	11	3099	<0.001
Ferritin 5 years	28	560.80	413.65	451.099	37	1764	0.060
Ferritin >5 years	28	484.58	313.50	377.192	15	1418	0.001

Median ferritin level before transplant and 100 days, 1 year, 3 years, 5 years, and >5 years after transplant in patients with complete ferritin data on all transplant stages

London Journal of Medical and Health Research

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

42 Volume 22 | Issue 13 | Compilation 1.0 © 2022 London Journals Press

Table 5: Distribution of transferrin saturation index before and 100 days after BMT

TSI in the period mean median	Standard deviation	Minimum	Maximum	CI 95% smaller	CI 95% Greater	Kolmogorov -Smirnov
TSI pre-HSCT 43.91 41.50	28.773	10	97	35.93	52.46	<0,001
TSI 100 days 46.93 46.93 HSCT	22.116	10	102	40.3	53.50	<0.001
TSI greater 40.04 30.50 than 1 year	21.135	12	105	33.7	46.32	<0.001
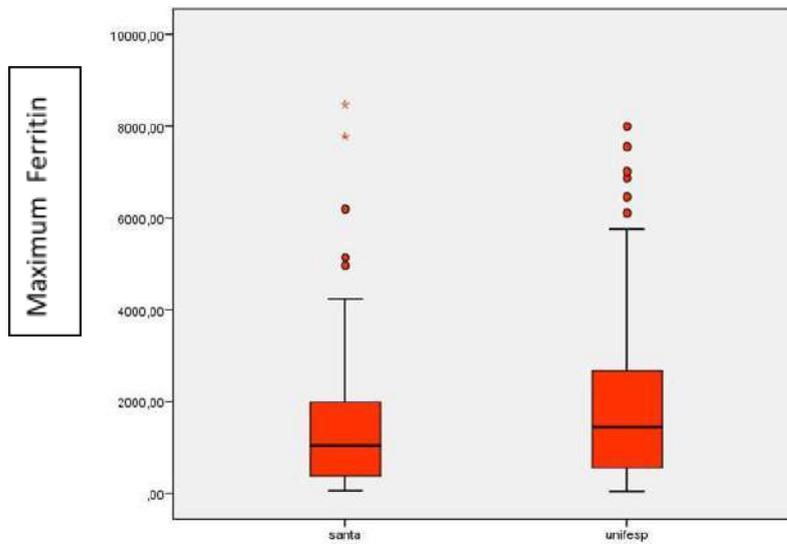
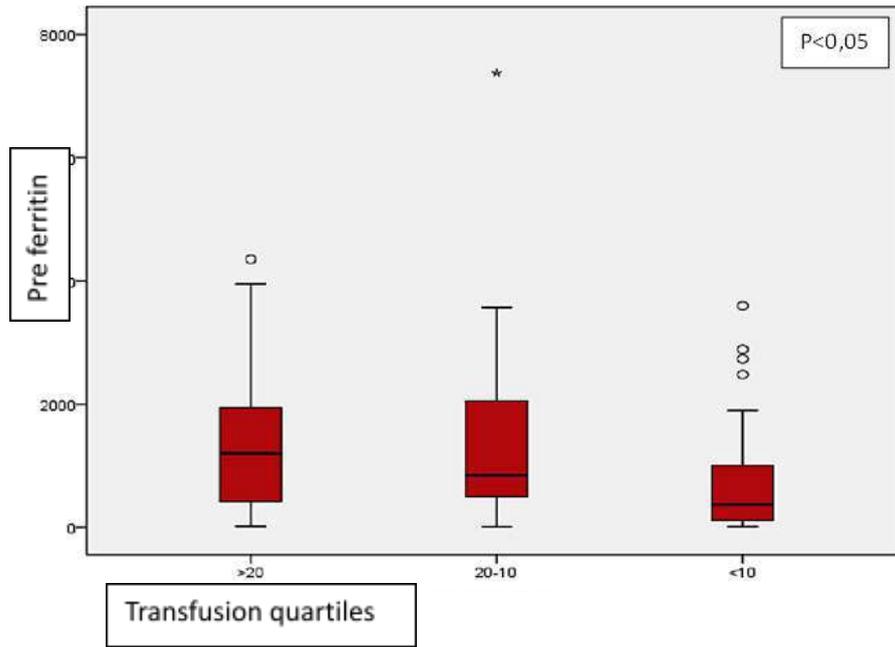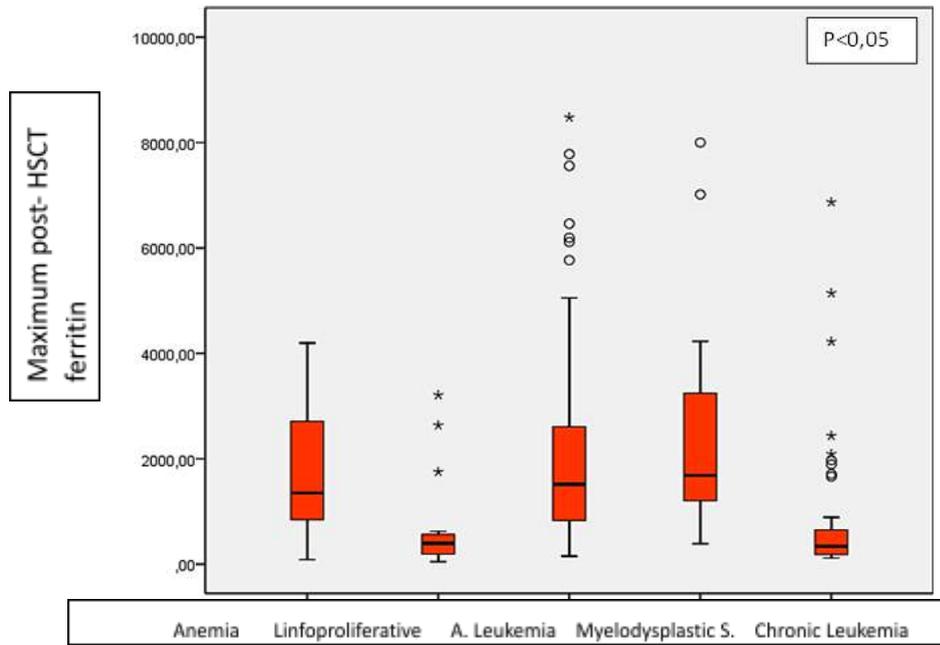*Graph 1:* Maximum ferritin level reported in the institutions
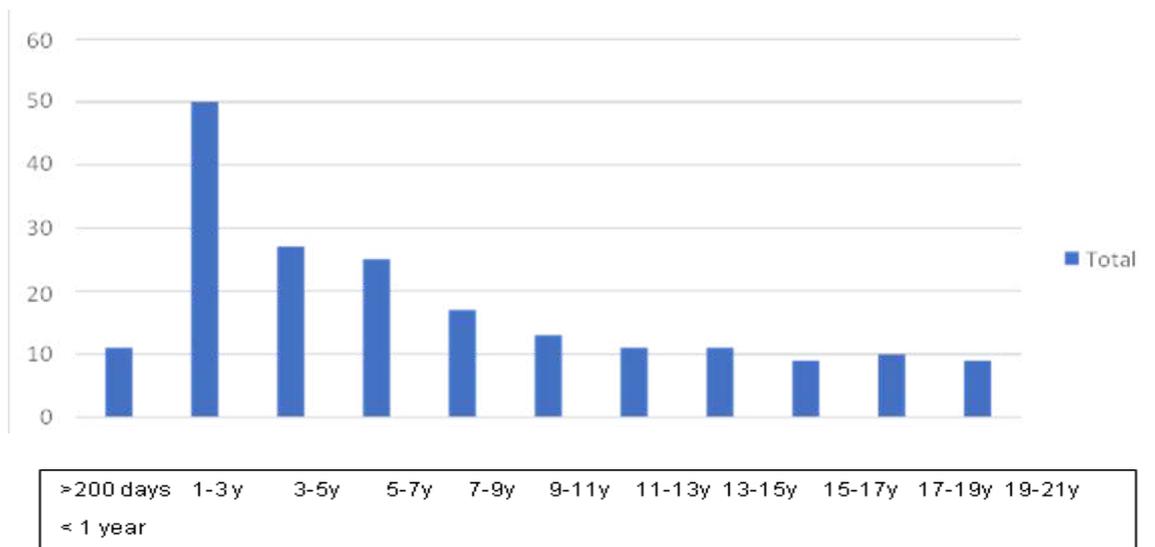
Figure 1: Number of patients by HSCT stage (in years) at the time of inclusion in the study

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

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Volume 22 | Issue 13 | Compilation 1.0

43

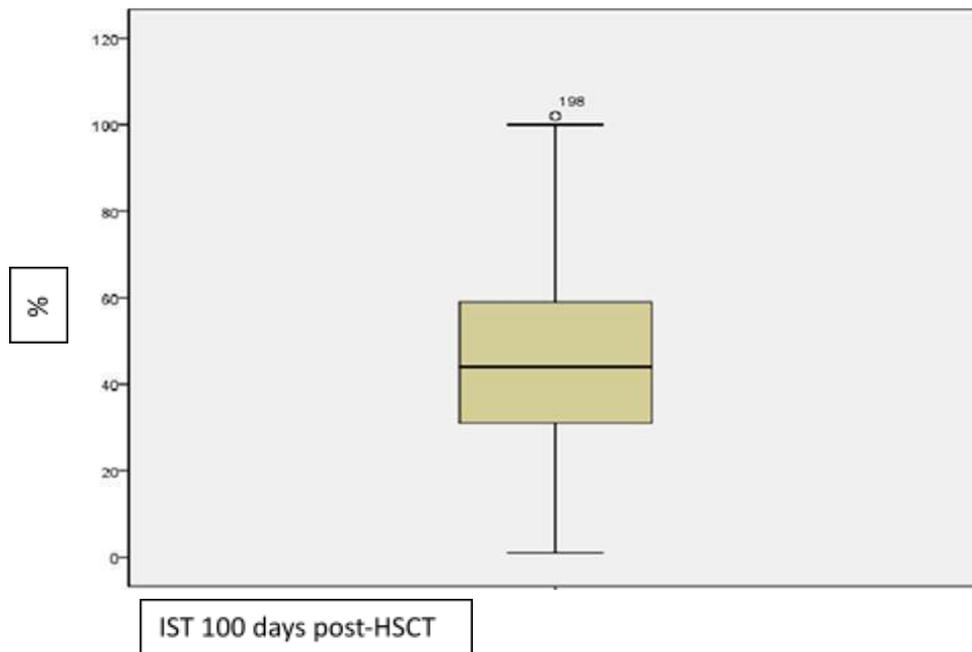London Journal of Medical and Health Research

Graph 2: Pre-HSCT ferritin level. Quartiles by number of packed red blood cell transfusions (>20, 20–10, and <10)
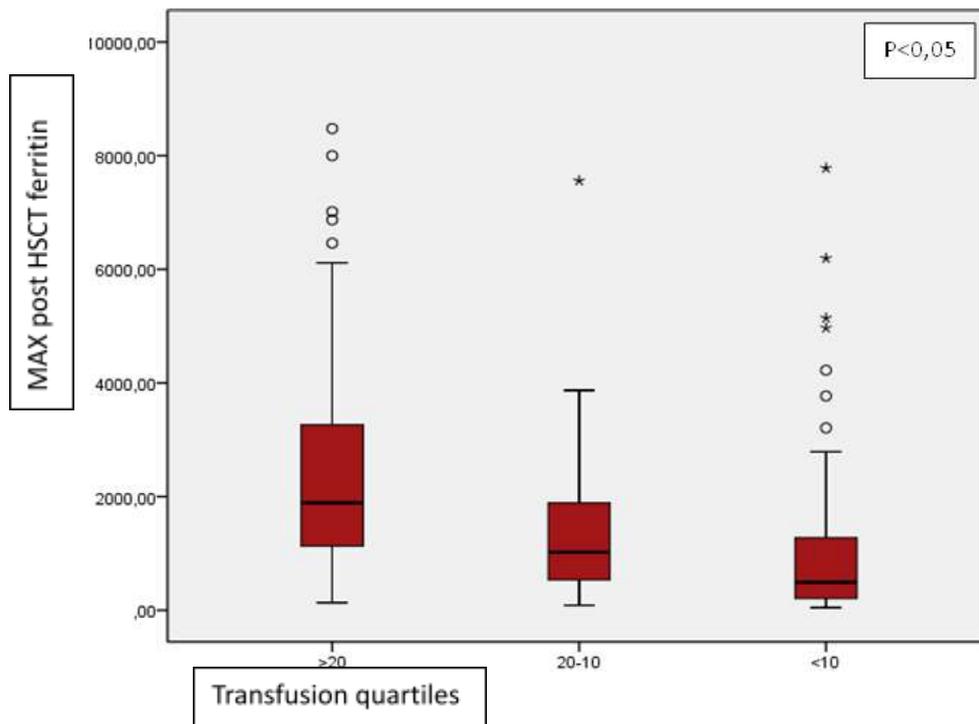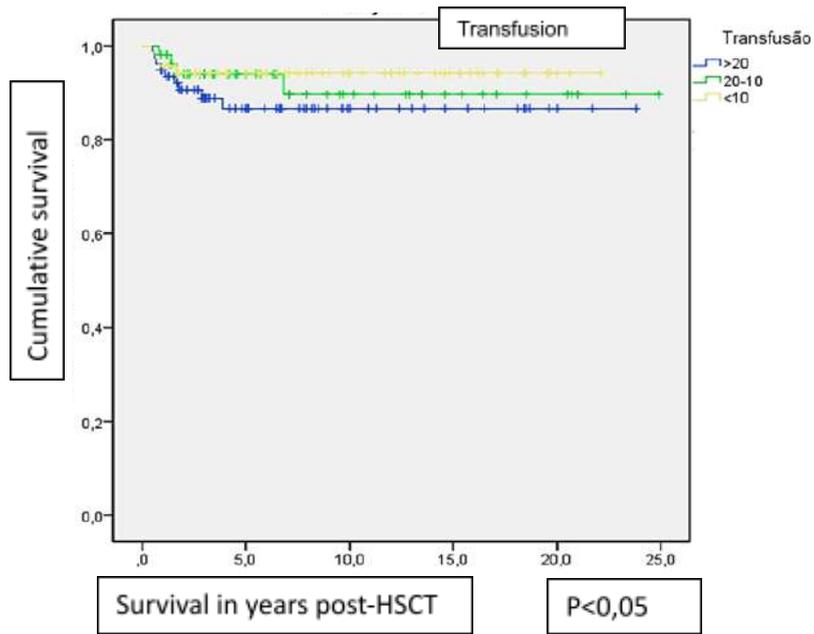

Graph 3: Maximum post-HSCT ferritin level × number of red blood cell transfusions. Quartiles by number of packed red blood cell transfusions (>20, 20–10, and <10)

London Journal of Medical and Health Research

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44   Volume 22 | Issue 13 | Compilation 1.0   © 2022 London Journals Press
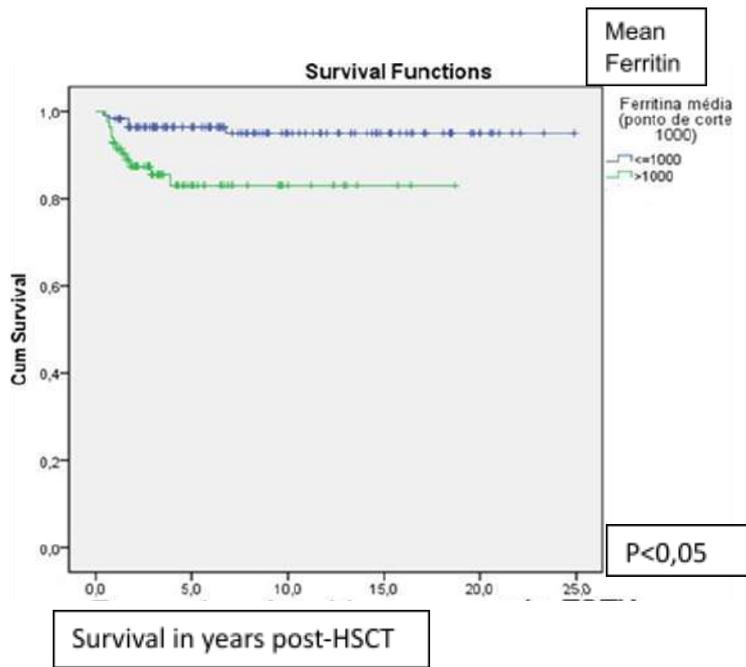
Graph 4: Maximum post-HCST ferritin level versus disease. Diseases: benign types of anemia (AF and AAS), lymphoproliferative disease, acute leukemia, myelodysplastic syndrome, and chronic leukemias
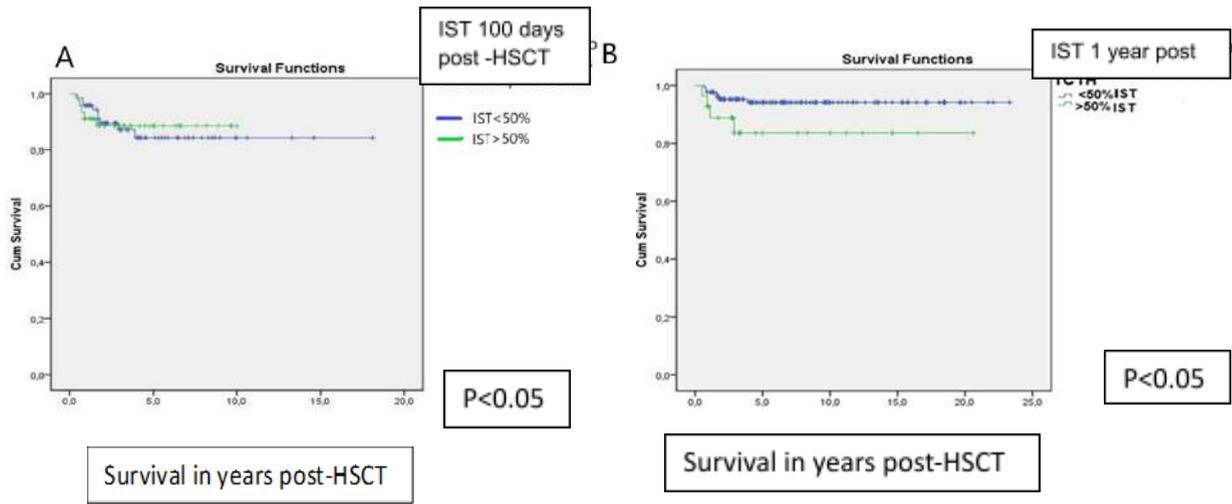

Graph 5: Transferrin saturation index 100 days post-HSCT

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Volume 22 | Issue 13 | Compilation 1.0

45

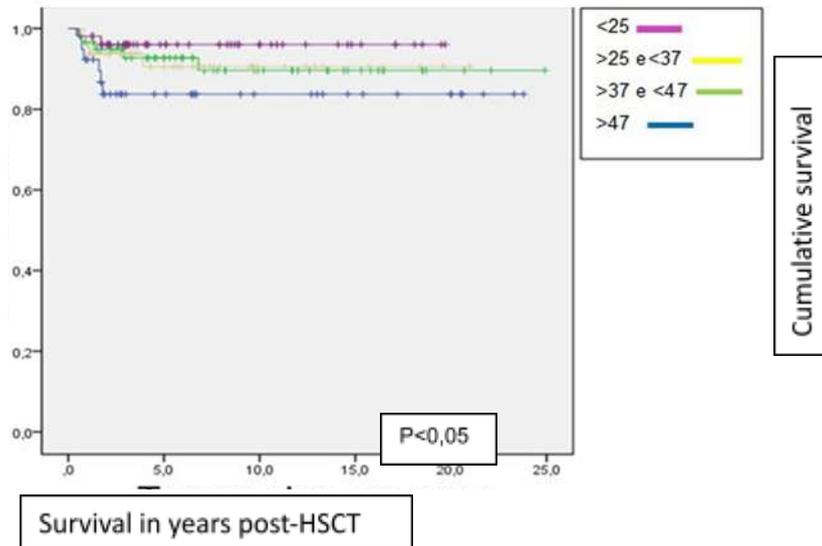London Journal of Medical and Health Research

Graph 6: Survival outcomes according to the number of red blood cell transfusions. Number of transfusions: 0–10, 10–20, and >20

Graph 7: Survival versus mean ferritin level. Survival in years related to ferritin >1,000 and <1,000

Iron Overload in Bone Marrow Transplant Recipients in two Brazilian Centers: An Analysis based on Ferritin and Transferrin Saturation

Graph 8: A. TSI within 100 days after HSCT versus survival. B, TSI 1 year after HSCT versus survival

Graph 9: Survival time in years versus age quartiles at the time of transplant

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Volume 22 | Issue 13 | Compilation 1.0   47

London Journal of Medical and Health Research