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Dear transplant colleagues

In 2019 we celebrated the 40th anniversary of the first bone marrow transplant (Tmo) in our country, with the pioneering spirit of Professor Ricardo Pasquini, Eurípides Ferreira and his team, a fact that was undoubtedly a milestone and the driving force for us to arrive where we are. Today, we are 84 Tmo-enabled centers in Brazil and we have seen the great success of these teams, demonstrating a process of maturation of our transplant recipients.

Our company was founded in 1996 by a group of specialists and within this same premise. Today we are prominent in the worldwide transplanting community, having entered into several partnerships with international entities, such as ASCT, LABMT, CIBMTR, FACT, among others.

We have a research group at GEDECO (Grupo de Estudo Doença Enxerto Contra o hospedeiro e complicações tardias) ,coordinated by our dear Dr. Mary Flowers and Dr Afonso Celso Vigorito. This started small as a group of studies on graft disease and because of its quality and empathy, it has now become the gateway to cooperative studies on various topics in our society. SBTMO also maintains a Pediatrics Group, a flow cytometry group, a multidisciplinary group and one of data managers. Every two years, a consensus of indications and complications of transplants is performed, which serves as a guide for the guidance of specialists and public policies.

Faced with this scenario, in a natural way, arose the need to have a journal that could disseminate the work of this scientific community, doctors and multidisciplinary professionals, thus strengthening our interaction with transplantation professionals from various countries.

It is with this spirit of joy and hope that we launched the first volume of JBMCT, Journal of Bone Marrow Transplantation and Cellular, which will certainly be a periodical to publicize the work of all those who believe that science , research and caring for patients, is the best way to improve our walking.

Fernando Barroso Duarte　　　　Nelson Hamerschlak

Summary

ORIGINAL ARTICLES

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ANALYSIS OF EXPANSION MESENCHYMAL STROMAL CELLS IN PATIENTS WITH LOW RISK MYELODYSPLASTIC SYNDROME

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ABSTRACT

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis, cytopenias and dysplasia and one or more lineages. The stratification of MDS is made based on the percentage of bone marrow blasts, number of cytopenias and karyotype at diagnosis. Somatic mutations in the p53 tumor suppressor gene are found in approximately 50% of all human tumors, making it the most commonly mutated gene. The expression of p53 protein and the study of mutations is especially needed in the prognosis of MDS. In this context, the study aims to evaluate the expansion of mesenchymal stromal cells (MSCs) and the expression of p53 protein in patients with SMD, low risk, according to the International Prognostic System (IPSS), in order to demonstrate the importance of these evaluations also diagnostics. This is a cross-sectional analytical study with review 3 adult patients of both sexes, the diagnosis of low-risk MDS receiving outpatient treatment at the University Hospital Walter Cantídio (HUWC). MSCs were characterized by immunophenotyping and screening of mutation of the p53 gene by Real Time PCR System (Applied Biosystems). For data analysis, the statistical software was used GraphPadPrism 5.0. Statistical differences between groups were checked by Student t or Mann-Whitney's test significance level was $p < 0.05$ for all analyzes. The results showed a smaller expansion of MSCs in the bone marrow of patients with MDS compared with a control group. A survey of mutation of the p53 gene was negative in all patients. The results demonstrate an impairment in the growth of MSCs in patients with MDS, collaborating with the hypothesis that medullary microenvironment in MDS may be compromised contributing greater understanding of disease mechanisms. However studies with larger sample should be conducted in order to establish the best results.

Key words: MDS; hematopoietic cells; mesenchymal cells; TP53 mutation.

INTRODUCTION

Mesenchymal stromal cells (MSCs) are a group of clonogenic, cells present in the bone marrow stroma, with potential to differentiate into various cell lineages. They propitiate the production and differentiation of hematopoietic stem cells in the bone microenvironment. In the bone marrow match 0.01% to 0.0001% [1,2]. MSCs are multipotent expressing positivity for CD73, CD90 and CD105 markers, and lack of expression of CD14, CD34, CD45, CD19, HLA-DR, CD3, CD11b, CD8, CD4, CD16 and CD56 in 95% of the cells in cultures. MSCs can be isolated from bone marrow by various methods, expandable pontencial maintaining their pluripotency and growth, with a doubling time which varies with the donor [3,4].

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis, cytopenias and dysplasia and one or more lineages. The stratification of MDS is made based on the percentage of bone marrow blasts, number of cytopenias and karyotype at diagnosis. Somatic mutations in the p53 tumor suppressor gene are found in approximately 50% of all human tumors, making it the most commonly mutated gene. The expression of p53 protein and the study of mutations is especially needed in the prognosis of MDS [4].

Several in vitro studies show that the bone marrow of patients with MDS has a high rate of cell proliferation and cell death (apoptosis). The paradox in a hypercellular marrow peripheral cytopenias in MDS can be attributed to several mechanisms, such as changes in its own hematopoietic cells, changes in the expression of molecules involved in apoptosis (Fas, Bcl-2, caspase), abnormalities in the cell cycle as well as presence of changes in the stroma [4,5] component.

The SMD has a high rate of ineffective hematopoiesis, manifested by anemia, neutropenia and / or thrombocytopenia. Besides the fact that the impairment also appears to occur in the bone marrow microenvironment, and MSCs. The ineffective hematopoiesis, is characterized by increased apoptosis, present in approximately 75% of patients with MDS [6,7,8].

In this context, this study aims to evaluate the expansion of MSCs in cultures of patients with low-risk MDS and compare with those of healthy donors. Moreover, determining the expression of p53 gene in patients with MDS MSCs.

CASUÍTICA AND METHODS

Casuistry

This is a cross section of 3 adult patients, two females and one male, the diagnosis of low-risk MDS in a clinical service specializing in Fortaleza - Ceará. Risk stratification was performed by the International Prognostic Scoring System Revised (IPSS-R). Patient samples were obtained from bone marrow, during the period January to December 2013. Clinical data related to age, sex, blood count, bone marrow biopsy and bone were collected for analysis of medical records. The inclusion criteria in this study were samples at diagnosis, free of any type of treatment and availability of suitable cells for analysis.

All samples were obtained only after patients or guardians agree to participate and sign the "Statement of Consent", approved by the Federal University of Ceará Research Ethics Committee of the University Hospital Walter Cantídio (HUWC).

The control group (n=4) of MSCs was obtained from the Cell Culture Laboratory and Molecular Analysis of Hematopoietic Cells, Center for Experimental Research / Hospital de Clinicas de Porto Alegre.

Isolation, cultivation and expansion of MSCs

The procedure for isolation, cultivation and expansion of MSCs was performed at the Laboratory of the Bank Umbilical Cord Blood Center of Ceará-Hemoce. The criteria adopted for the characterization of MSCs were those of the International Society for Cellular Therapy (ISCT) [9].

MSCs were isolated from bone marrow samples from patients with MDS (3 samples) and control subjects (6 samples) in culture medium poor in high concentrations of glucose and amino acids and proteins (fetal bovine serum). After counting the cells of the bone marrow aspirate about 1x 〖〗 10 ^ 6 cells / ml were subjected to culture in bottles of 25 cm ^ 2 in α-MEM medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with antibiotics and with 15% fetal bovine serum (fetal bovine Serum Standard - ^ TM HyClone, Logan, UT, USA). Cells were cultured in a humidified 37 ° C incubator with 5% CO 〖〗 _2. After 3 to 5 days, it was able to remove nonadherent cells and new culture medium added. Every 2 or 3 days, the medium was changed and the cell culture was maintained until reaching a confluence of 70-90%.

When they reach this confluence, MSCs were subjected to treatment with 1 ml of trypsin-EDTA 1x (0.05% Tripsin 0.53 mM EDTA, Gibco 〖〗 ^ TM Carlsbad, CA, USA) for 2-4 minutes at 37 ° C. After inactivation of trypsin, cell suspension was washed, resuspended in culture medium and plated at a density of 5x 10 〖〗 ^ 4 cells / cm ^ 2. Upon reaching the 3rd passage, the cells were subjected to the analyzes provided.

immunophenotyping

In flow cytometry, the cell suspension passes through a channel system which generates a laminar flow cell. A light beam hits these cells suffering deviation according to the physical characteristics of the same: cell size, granularity, internal complexity of the cell.

The monoclonal antibodies used is conjugated with three different fluorochromes: phycoerythrin (PE phycoeritrin the English), fluorescein isothiocyanate (FITC, fluorescein isothiocyanate English), PerCP (peridinin chlorophyll English). Positive and negative controls were included for proper calibration of the device, analyze the results and define the positivity of the sample.

The labeling of cells occurred after culturing MSCs reach the third pass, they were trypsinized, centrifuged, and the supernatant was discarded, leaving approximately 1.5 mL of media then held for cell counting. To perform labeling cells with monoclonal antibodies it takes a minimum of 5x 〖〗 10 ^ 5 cells per tube, so after counting was performed in adjusting the final volume of cell suspension to that amount of cells were in a volume of 100 ul in which were added 5µl of a fluorochrome-labeled antibody (FITC, PE or PerCP). After addition of the antibody sample was incubated in the dark for 15 minutes, then washed with 1x PBS, centrifuged and supernatant discarded, the cell pellet was added 100 ul of 1x PBS. Once the cell suspension has been marked by the technique described, proceeded to the acquisition of fluorescence intensity in the cytometer.

Immunophenotyping of cells was performed using monoclonal antibodies which recognize antigens on the cell surface membrane. For the identification of these cells was assembled a panel containing the following markers CD105 PE (Serothec, Oxford, England), CD73 PE, CD45 FITC, CD14 PE, CD34 FITC, CD90 PE, CD13 PE (Becton Dickinson, San Jose, CA, USA), CD140B PE, CD146 PE and CD31 FITC.

The sequencing of the TP53 gene

Mutational analysis of the TP53 gene was performed in the Laboratory of Molecular Biology of the Transplant Center Bone Marrow (CEMO) Cancer Institute (INCA) in Rio de Janeiro, by direct sequencing. Exons 3 - 9 gene were amplified by PCR from DNA extracted from MSCs. The PCR primers and conditions for amplification of genomic DNA followed established by the International Agency for Research on Cancer (p53.iarc.fr/ProtocolsAndTools.aspx). All PCR products were confirmed by 1.5% agarose gel, purified using the Wizard SV Gel kits and PCR Clean-Up (both Promega) and sequenced by an automatic sequencer 16 capillaries (ABI PRISM ® 3100 Genetic Analyzer, Applied Biosystems). The sequence data files were analyzed using Mutation Surveyor (SoftGenetics) software. All variants were found compared with databases: Cosmic, dbSNP, and 1000 genomes UniProtKB

Statistical Analysis

Results were expressed as mean ± standard error of the mean. For data analysis, the statistical software was used GraphPadPrism 5.0. Statistical differences between groups were checked by Student t or Mann-Whitney tests. The level of significance was set at $p< 0.05$ for all analyzes.

RESULTS

A total of three patients with low-risk MDS were analyzed for the expansion of mesenchymal cells and compared with a control group consisting of individuals considered healthy. Of the three patients studied one being female 74 years old, diagnosed with SMD hypocellular variant hypocellular marrow and 0.8% blasts; bone marrow biopsy with 20% diseritropoese and dismegacariocitopoese and Normal reticulin; Karyotype 46, XX; immunohistochemistry for p53 and negative for CD34 positive megakaryocytes; IPSS intermediate 1 with good clinical outcome. Patient with 58 year old female with pancytopenia; hypocellular marrow with 4% blasts; with hypercellular bone marrow biopsy, 50% of diseritropoese and dismegacariopoese; karyotype 46XX. The male patient of 78 years; CRDM; IPSS intermediate 1; karyotype 46, XY, normocellular marrow with moderate and mild diseritropoese and disgranolopoese dismegacariopose and presence of 0.9% blasts; hypercellular bone marrow biopsy with diseritropoese, disgranulopoese and dismegacariopoese and reticulin grade 1; immunohistochemistry for p53 positive focal nuclear pattern.

TABLE 1 - Clinical characteristics of patients with myelodysplastic syndrome diagnosis (n = 3)

VARIABLES	PATIENT 1	PATIENT 2	PATIENT 3
Age (years)	78	54	78
Gender	Female	Male	Female
Cytogenetics, n (%)	Karyotype Normal	Karyotype Normal	karyotype Normal
IPSS	Intermediate 1	Intermediate 1	Intermediate 1
IPSS- R	Low	Low	Low
hematological Prâmetros			
RBC /1012/L	3.72	3.22	3.81
Hemoglobin, g/dL	10.8	10.6	12.1
Hematocrit, %.	32.7	31.0	34.7
leukocytes /L	3.700	2.924	3273
Platelet/L	116.000	42090	49530

In Figure 1 we can see confirmation of the origin of MSCs through the characteristic profile by immunophenotyping.

In relation to research the expansion of mesenchymal cells in patients compared to the control group we observed a significant decrease in the group of MDS patients compared to the control group. The analysis of mutations in the p53 gene was negative in patients with MDS MSCs.

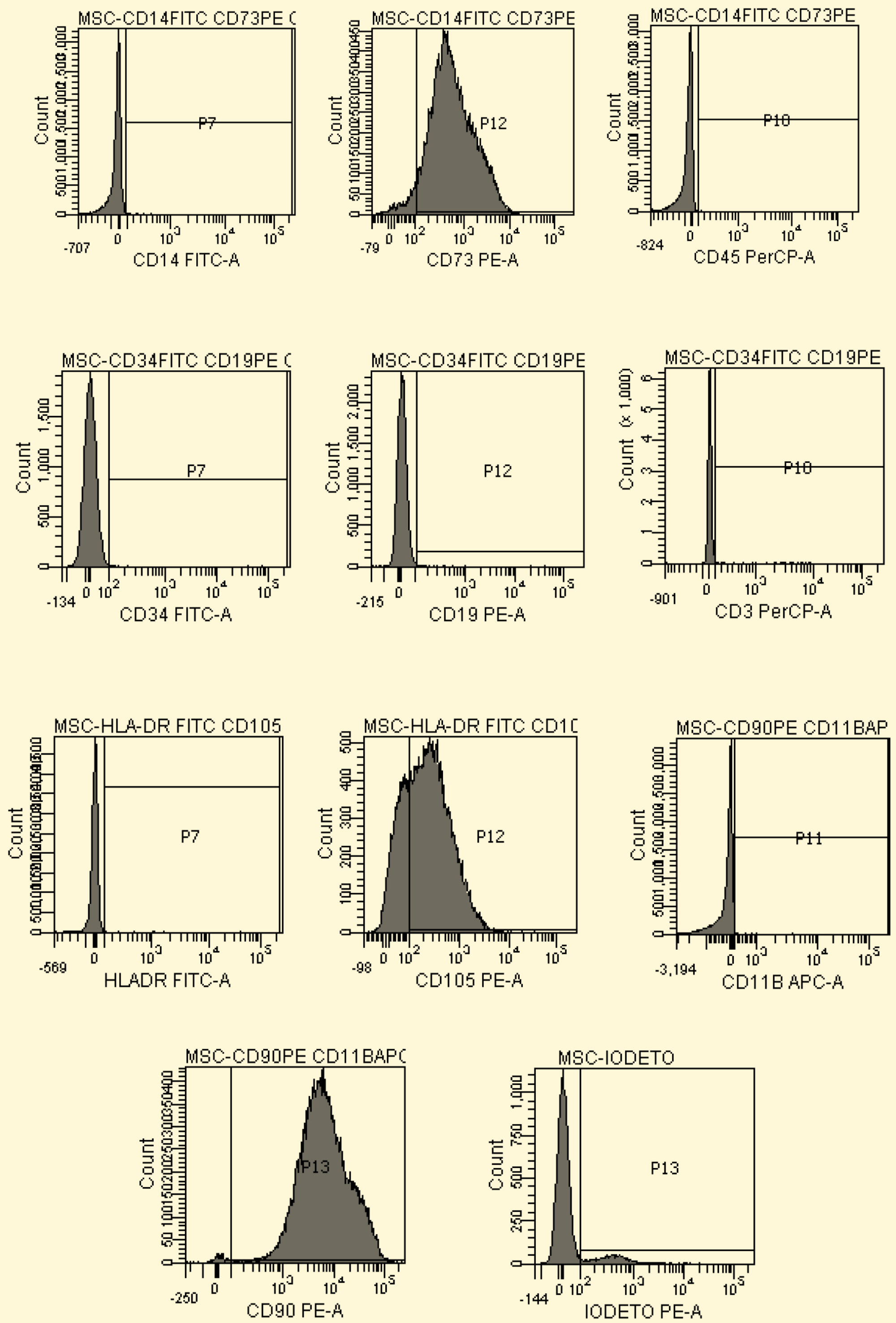

FIGURE 1 - Phenotypic analysis of MSCs in patients with low-risk MDS (n = 3). Feasibility: 89.7% (10.3% dead cells)

TABLE 2 - Analysis of expansion of MSCs in patients with low-risk myelodysplastic syndrome and apparently healthy individuals

MONONUCLEAR CELLS RECOVERED FROM THE BAG AND FILTER		p0	p1	p2	p3
CONTROL	15.700.000	1.099.000	48.081.250	9.676.351.563	84.635.821.667
CONTROL	150.000.000	12.400.000	640.666.667	125.730.833.333	541.480.788.889
CONTROL	28.000.000	6.981.333	1.087.924.444	381.226.857.407	2.328.025.342.568
CONTROL	194.000.000	19.788.000	1.261.485.000	147.698.868.750	746.371.616.750
CONTROL	7.400.000	740.000	46.250.000	12.738.020.833	
CONTROL	304.500.000	23.548.000	1.138.153.333	314.414.858.333	
PATIENT	1.075.000	2.200.000	6.306.667	40.867.200	250.652.160
PATIENT	4.060.000	4.300.000	32.480.000	329.130.667	3.774.031.644

TABLE 3 - Characterization of mutation of TP53 in MSCs in patients with low-risk MDS (n = 3)

PATIENT	MUTATION IN TP53
1	Absent
2	Absent
3	absent

DISCUSSION

In culture, MSCs are a population of cells with the morphological appearance of fibroblasts, adherent to plastic. The half-life is limited, with an average doubling time of 33 hours and a maximum overlap of about 40. Expands As the number lost their multipotential capacity and undergo apoptosis. The cell cycle studies in cultured human MSC show that while a small fraction of these cells proliferating (approximately 10% of cells are in S + G2 + M phase) are most cells in the G0/G1 phase, comprising a minority of resting cells [10,11].

Some aspects regarding the interactions between the neoplastic clone and the bone microenvironment has been rumored as one of the mechanisms of the pathophysiology of MDS. However, studies on the subject are scarce and therefore requiring research characterizing the bone marrow stromal cells in healthy individuals and in patients with malignant hematological diseases [12].

The development of MDS is a complex process, for which we propose a model with successive steps. In this model, an abnormal clone could interact with hematopoietic marrow microenvironment providing the altered neoplastic growth with normal shifting [13] hematopoiesis.

Studies evaluating the functionality and molecular phenotyping aspect of MSCs in patients with MDS have been documented. However the results are conflicting. In this study the degree of purity of MSCs was 89.7% of the cells present in the sample, we can affirm that the data obtained are in effect for these cells. We found that the pattern of growth of MSCs in patients with low-risk MDS was different from healthy subjects. There was a significant reduction in the MSCs expanssão of MDS patients compared to healthy bone marrow. The growth pattern of MSCs is controversial because some studies have described altered expansion [11,14], while others have observed a similar growth of normal bone marrow [15] standard. The discrepancies may result attributed to the large variation in the growth of MSC in MDS subtypes or methodological used, among others.

Regarding the immunohistochemical study of MSCs found that there was no difference in the pattern of patients with low-risk MDS, relative to healthy individuals. These results corroborate with the literature, which state that most studies agree that MSCs from MDS patients are identical to normal [2,15] markers. Studies, but has shown that the expression of CD90, CD104 and lower CD105é MSCs in MDS patients [4,10,11]. Finding attributed to alteration of the marrow stroma and hematopoietic cells.

Regarding the analysis of mutation of p53 gene mutation was not observed in MCSs in patients with MDS. Additional studies are needed to elucidate the mechanisms involved in the regulation of MCSs in MDS, so that we can establish the prognostic value of MCSs, the pathophysiology in this disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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MYELODYSPLASTIC SYNDROME / SECONDARY ACUTE MYELOID LEUKEMIA: ROLE OF THE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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ABSTRACT

Secondary Acute Myeloid Leukemia (s-AML) refers to the development of leukemia after cytotoxic therapy, immunosuppressive therapy, radiation or an antecedent hematological disorder, such as Myelodysplastic Syndrome (MDS). A s-AML corresponds to 10% to 30% of AML cases and is defined by the presence of at least 20% of blast cells, representing a category of disease with a poor prognosis. Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only option with curative potential for patients with s-AML, but recurrence after HSCT emerges as a frequent cause of treatment failure and course with high mortality. We report the case of a patient with s-AML after MDS, who underwent HSCT due to refractoriness to other treatments, recovering the bone marrow with dysplasia, being classified as AREB1.

Key words: Secondary Myeloid Leukemia; Myelodysplastic Syndrome;Autologous Hematopoietic Stem Cell Transplantation; Relapse; Diagnosis

INTRODUCTION

Myelodysplastic Syndromes (MDS) are an hematological disease characterized by peripheral cytopenias and displaced changes in the bone marrow which present progress of approximately one third of patients to acute myeloid leukemia (AML). The distinction between AML and MDS consists mainly on cytomorphological analyzes, since MDS has variable hematopoiesis and myeloblast count is less than 20%, while s-AMLs' myeloblasts are ≥ 20% [1, 2] HEUNG *et al.*, 2019]) .

The s-AML is different from the AML de novo, due to previous exposure to chemotherapy and / or radiotherapy treatments, secondary to diseases such as MDS, Chronic Myelomonocytic Leukemia (CMML), Chronic Myeloid Leukemia (CML), and other variables. The s-AML has a less effective response to induction therapy, with a higher recovery rate and a worse prognosis [4,5], which causes factors, such as: presence of comorbidities, drug resistance, justified cytogenetic and molecular changes or worse prognosis of AML compared to AML de novo.

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only potentially curative option for patients with s-AML secondary to MDS, being indicated in primary induction failure or relapse refractory to chemotherapy [4,7]. However, for patients not eligible for HSCT, the treatment of choice is with

Hypomethylating Agents (HMA), such as low-dose cytarabine or supportive care.[7,8].

The clinical management of these patients is a major challenge. Thus, the aim of this study was to report the case of a patient diagnosed with Acute Myeloid Leukemia secondary to Myelodysplastic Syndrome, treated with chemotherapy and submitted to Allo-HSCT, with relapse before six months and in the reassessment presented bone marrow with dysplastic morphological changes, being classified as AREB1 MDS , according to the WHO classification.

Clinical Case

Patient, male, 38 years old, came to our service in July 2018, asymptomatic, with a history of papulo-erythematous lesion on the first finger of his right hand and with laboratory tests that showed anemia (Hb: 8.2g / dL) with anisocytosis , leukopenia (1500 / mm^3) and neutropenia (225 / mm^3). The initial treatment was with vitamins B1 (thiamine nitrate), B6 (pyridoxine hydrochloride), B12 (cyanocobalamin) and folic acid. Upon returning in September of the same year, he maintained anemia (Hb: 8.1 g / dL) and neutropenia (404.8 / mm^3) .The myelogram was performed and showed hypercellularity, with dysplasias in about 60% of the cells in the three hematological lines and 23% of blasts (Figure 1, immunophenotyping showed positive markers for CD13, CD33, CD34, CD45 and CD117, BCR-ABL and FLT3 were negative and the karyotype without structural changes (46 XY).At the time, he was diagnosed with Acute Myeloid Leukemia with FAB maturation, LMA M2, secondary to MDS. Treatment was started with chemotherapy following the 3 + 7 protocol with cytarabine and idarubicin and the MEC protocol (mitoxantrone, etoposide and cytarabine). The patient was refractory to treatment, being indicated for the realization of the HSCT.

The allogeneic related bone marrow transplant was performed in April 2019 with the reduced intensity conditioning regimen (RIC) with BUFLU (Busulfan and Fludarabine). The patient evolved with acute Graft Versus Host Disease (GVHD) in the skin, grade IV and in the fourth month after HSCT, still undergoing immunosuppressive therapy, pancytopenia with anemia was observed (Hb: 10.8 g / dL), leukopenia (1189 / mm^3) and thrombocytopenia (35,580 / mm^3). The myelogram showed dyserythropoiesis and dysmegakaryopoiesis > 20% and the presence of 6% of explosions (Figure 2). Bone marrow biopsy showed hypocellularity, with hypoplasia and dysplasias ≥ 10% of the erythroid, granulocytic and megakaryocytic series and absence of fibrosis. An immunophenotyping with 7.7% of immature cells and HPN clones in less than 40%. The patient was then reassessed and confirmed the diagnosis of MDS AREB1, stratified according to the score (IPSS-R) as high risk, and the use of Azacitidine was started, at a dose of 75 mg / m^2 for 5 days. Currently, the patient is stable, with (Hb: 12.4g / dL), leukocytes (3100 / mm^3) and platelets (180,000 / mm^3).

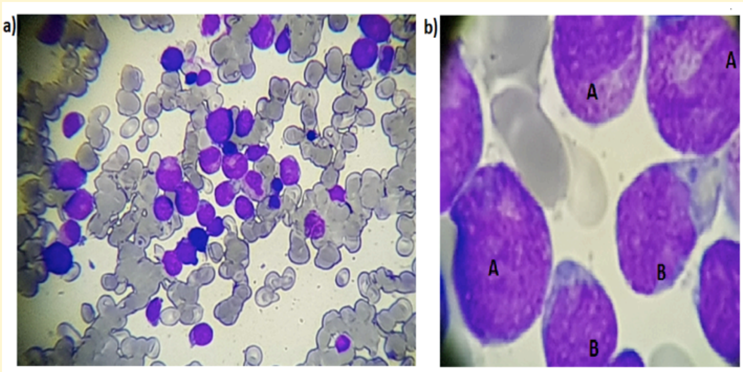

FIGURE 1- *a) Hypercellular bone marrow for the age, with dysplasias in the three hematological lines: erythroid (6%), granulocytic (20%) and megakaryocytic (30%) with dysplasias in about 6% of the cells, presence of 23% of blasts suggestive of AML. b) Myelogram at diagnosis, hypercellular medullary aspirate with the presence of 52% normal promyelocytes (A) and 9% myeloblasts (B) with regular nuclei, some with nucleoli present and Auer rod.*

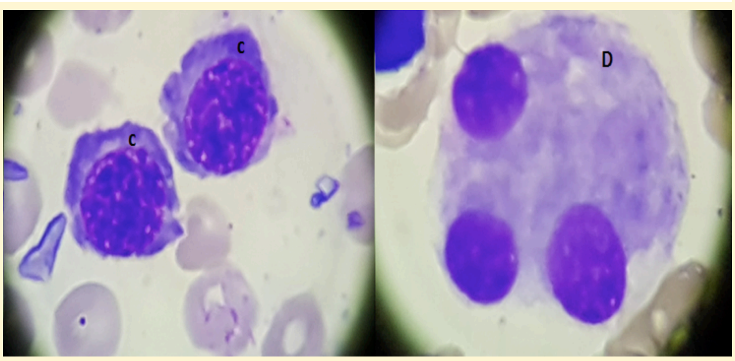

FIGURE 2 - *Myelogram after Allo-TCTH. C: Dysplastic erythroblasts with maturative asynchrony. D: dysplastic megacarioblasts.*

Discussion

MDS affects individuals of all age groups, being more prevalent in the elderly, on average of 65 to 70 years old, being characterized by the association of dysplastic hematopoiesis and peripheral cytopenias. Usually the patient is diagnosed with anemia, accompanied by thrombocytopenia; often, in the first stage, they are asymptomatic. It is noteworthy that MDS can progress to acute myeloid leukemia (AML), in about 20 to 30% of cases, 1 to 2 years after diagnosis, being more common in patients with high-risk MDS (MALCOVATI et al ., 2006; YE *et al.*, 2019) [1, 9].

Therefore, s-AML with MDS are associated with worse prognosis, when related to de novo or primary AML, since studies demonstrate low rates of remission to conventional treatments and HSCT (BARRET *et al.*, 2010 SENG SAYADETH *et al.*, 2018; NOMDEDEU *et al.*, 2017). [8, 10]

The patient was asymptomatic, at the first consultation, with laboratory of anemia, neutropenia and thrombocytopenia which after three months evolved to AML-M2. The patient was refractory to conventional chemotherapy treatment. When performing the HSCT, he evolved with GVHD grade IV on skin. Four months after HSCT, the patient was reassessed with a laboratory compatible with MDS AREB1, stratified according to the score (IPSS-R) as high risk, and the use of Azacitidine was started. Currently, the patient is stable with mild anemia and leukopenia and without transfusion dependence.

The incidence of relapse was 37%, in two years, in patients with s-AML with SMD after HSCT, with overall survival (OS) exceeding 45% of the cases with RIC or MAC conditioning regimen, in which GVDH is one of the post-transplant complications in 39% of patients (SENGSAYADETH *et al.*, 2018) [10].

Many factors can be attributed to justify the failure of the HSCT in this case. The relapse of the primary disease can occur after the HSCT, if the initial conditioning regimen is insufficient as it does not establish an effect of the graft against the neoplastic condition. It is also noteworthy that it can occur after the period of effective catching, if the immune system weakens or becomes tolerant to residual disease, or if the disease suffers immune escape through the clonal selection of immune-resistant parents. In addition, it must be known that, occasionally, the disease may recur, in the donor cells, as an event de novo, masked as a relapse (BARRET *et al.*, 2010).

Hypomethylating agents (azacytidine, decitabine) alone or in combination with donor lymphocyte infusion (DLI) appear to be among the most promising therapeutic options for the treatment of post-transplant relapse due to the direct antileukemic efficacy and immunomodulatory capacity of this therapy. Other treatment options for these cases are intensive chemotherapy or second HSCT, something for patients who do not achieve complete remission or long-term remission (Granfeldt et al., 2015). [11] In this case, the patient is being treated with hypomethylation and with prospects of performing a second HSCT.

Therefore, this clinical case demonstrates a rare event, with challenges related to treatment since there is no protocol to be followed for the relapse to primary disease.

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EVALUATION OF PLATELETS TRANSFUSION IN PATIENTS UNDERGOING HIGH DOSE CHEMOTHERAPY FOR BONE MARROW TRANSPLANTATION

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ABSTRACT

Introduction: Microvascular endothelial damage is a well-recognized complication of bone marrow transplantation (BMT) and the mechanisms of this disorder are still poorly understood. The objective of this scenario is to evaluate the relationship between inflammatory markers and other factors that influence platelet consumption and platelet transfusion yield, as well as the presence of embolic and / or vascular thrombotic events in patients submitted to high-dose chemotherapy conditioning for Bone marrow transplant.

Material and Methods: Prospective analysis of patients, including 25 patients who underwent autologous and allogenic BMT. The patients were evaluated in relation to previous radiotherapy, CD34 + cell count, period of neutropenia, body mass index (BMI), ferritin, reactive C protein (RCP), relating these factors to the number of platelet transfusions, platelet refractoriness and vascular events such as sinusoidal obstruction syndrome (SOS) and bone marrow grafting syndrome.

Results: Only BMI> 25 Kg / m2 of the studied variables presented a statistically significant value (p = 0.003) in relation to the lower need for transfusion of platelet concentrate. For platelet refractoriness and / or vascular events none of the variables was statistically significant. The conditions found in the 3 cases of platelet refractoriness and in the 2 cases of vascular events have characteristics like those described in the literature.

Conclusion: Although the cause is unclear, we agree with data reported in the literature that patients with high BMI have a lower need for transfusion of platelets. Small sampling limits our comparisons and significant statistical inference; however, we cannot rule out the relevance of a descriptive analysis of the results, especially if we consider that each patient should be evaluated in an individualized way in clinical practice

Key words: BMT, endothelial lesion, platelet refractoriness, platelets transfusion

INTRODUCTION

Recent studies have shown that endothelial cells are much more than just vessel lining, these cells can control vascular smooth muscle tone by nitric oxide (NO), conserve different concentrations of procoagulants depending on the functional requirements and play an immunological role through interaction with circulating leukocytes.[1]

When endothelial function is disturbed, for instance, in cases of inflammatory conditions the endothelial surface rapidly converts from a non-thrombotic state to a procoagulant state, this change is due to desregulation of anticoagulant factors as well as activation of prothrombotic mediators. [2,3]

Some authors have demonstrated the interaction of several mechanisms in the association among obesity, metabolic syndrome, endothelial injury and platelet activation. Adipose tissue secretes proinflammatory cytokines such as: Interleukin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-a), affecting both endothelial function and glucose metabolism. [4,5,6]

During hematopoietic stem cells transplant, endothelial cells can be activated and damaged by chemotherapy contained in the conditioning regimen, cytokines produced by injured cells, bacterial endotoxins translocated through the injured gastrointestinal tract, and by the complex process of graft versus host reaction. [7]

Microvascular endothelial dysfunction is a process recognized as a complication of bone marrow transplantation (BMT) and the mechanisms related to this disorder are still poorly understood. Transplant associated endothelial disorder is correlated to a group of complications such as, thrombotic microangiopathy, sinusoidal obstruction syndrome (SOS) and graft-versus-host disease (GVHD). [7]

Thrombocytopenia is frequently seen in the BMT scenario and it often requires platelets transfusions. In adult recipients of autologous hematopoietic stem cell transplantation (HSCT) randomized trials have demonstrated that they receiving platelet transfusion at the first sign of bleeding is better than prophylactically, principal for a prespecified subgroup of patients who undergoes autologous stem cell transplantation. [8,9]

The role of clinical knowledge related to variants linked to platelet recovery is important and assessment of risk factors associated with prolonged recovery include; use of radiation and its toxic effects on the bone marrow, a high mononuclear cell count in the receptor, fever and the presence of SOS. The variables related to the shortest time of thrombocytopenia are CD34 + counts and the early recovery of neutrophil counts. [7,10,11] Diagnosis of vascular complications in patients undergoing BMT is challenging, since there are so few markers of endothelial lesion available in clinical practice.[7]

In this context, the objectives of this article are to evaluate the relationship between inflammatory markers, available in clinical practices in our country, like serum ferritin and C-reactive protein (CRP) and other circumstances that influence platelet consumption and platelet transfusion increments, as well as the presence of thromboembolic and/or vascular events in patients submitted to high-dose chemotherapy-based regimes as conditioning for BMT.

METHOD:

A prospective analysis of patients was performed between March 2016 and October 2017 at the Bone Marrow Transplantation Service of the University Hospital of the Federal University of Juiz de Fora (HU-UFJF), where both autologous and allogeneic bone marrow transplantation were studied, being excluded those who did not present the necessary data to reach the evaluation of the objectives proposed or who did not sign the free and informed consent. This study was approved by the Human Research Ethics Committee of the HU-UFJF (CEP HU-UFJF), with its opinion nº. 1,466,443 and CAAE: 52091415.0.0000.5133.

Patients:

Patients who would be submitted to autologous and allogenic BMT of both sexes and any age were included in the study. The diagnosis of Bone Marrow Aplasia was an exclusion criterion since their characteristics being quite heterogeneous in relation to the rest of the studied patients, especially when observed the dependence of transfusion support in the pre BMT period. Patients were evaluated in relation to previous radiotherapy, CD34+ cell count, period of neutropenia, body mass index (BMI), ferritin, C-reactive protein (CRP), relating these factors to the number of platelet transfusions, platelet refractoriness and events such as SOS and Engraftment Syndrome following hematopoietic stem cell transplantation.

Sample collection:

To evaluate the inflammatory situation prior to infusion of high dose chemotherapy we collected: the CRP and ferritin at hospitalization, as well as considered the weight at the beginning of conditioning regimen to calculate the BMI. Five milliliters (ml) of whole blood were collected from each participant in an anticoagulated tube with ethylenediamine tetra acetic acid (EDTA) during the service collection routine. Quantification of CD34 + cells was performed on a double platform, cytometry was performed on the Fluorescence Activated Cell Analyzer, FACSCalibur, Becton Dickinson (BD) flow cytometer and cytometry analysis was performed on the Cell Quest analysis software according to the ISHAGE protocol (International Society of Hemotherapy and Graft Engineering).

Platelet increment:

For the calculation of platelet refractoriness, the CCI formula (correct count increment) was used, and those patients who presented post-transfusion 24-hour platelet yield (ICC-24 - collected between 18 and 24 hours post-transfusion) were considered, refractory less than 4500 platelets per ml in at least two transfusions, preferably consecutive, with compatible ABO platelets.[12]

$CCI = IP \times SC \times 10^{11} / n$

at where:

IP = increase in platelet count (x 10^9/L) (post-transfusion count - pre-transfusion count)

SC = body surface (m^2)

n = number of transfused platelets (x 10^{11}/L)

Serum ferritin was considered elevated when greater than 300 ng/mL, BMI altered when greater than 25 kg/m^2, CRP when greater than 2 mg/mL. For the diagnosis of SOS we used the modified Seattle Criteria: Presence before day 20 after BMT of two or more of the following: Bilirubin ≥ 2 mg /dl, Hepatomegaly, right upper quadrant pain, Ascites or unexplained weight gain of >2% baseline and the Engraftment Syndrome based on the Maiolino criteria, characterized by cutaneous rash, aseptic fever and pulmonary infiltrates or diarrhea 24 hours before or at the moment of grafting. [13]

Patients received irradiated platelets when the counts were less than 10,000 to 20,000 mm^3 platelets. One unit of platelet concentrate per 10 kg of patient weight was transfused per transfusion episode when random platelets were used, and single platelets donor apheresis collections were considered equivalent to 6 units of random platelets.

Data analysis:

After the assessment of platelets transfusion need in conjunction with the presence of thromboembolic events and platelet refractoriness, it was compared based on the values found in the relation with factors that could be related to a greater transfusion dependence and consequent increased life risk to the patients submitted to HSCT. The factors analyzed were radiotherapy, type of transplant (Autologous / Allogenic), preconditioning CD34 + cells, febrile neutropenia, days of neutropenia, BMI, use of two or more antibiotics, ferritin and RCP. The medians of platelets transfusions per transfusional episodes are considered as the most correct method to obtain an estimate of the consumption of Platelet Concentrate (PC), since a normal distribution between the groups was not found.

The analyzes were performed in the Statistical Package program for Social Science (SPSS) version 17.0. For the statistically significant values, the value of $p < 0.05$ was considered for the rejection of the null hypothesis

RESULTS

A total of 25 individuals with a median age of 38.8 years (14 to 61 years), 13 (52%) males and 12 (48%) females, 3 patients were excluded because they presented a diagnosis of bone marrow aplasia.

The characteristics of the evaluated patients are shown in Table 1. Of the evaluated variables, only BMI presented a statistically significant relationship ($p = 0.003$) with the number of transfused platelets concentrates, as an altered BMI (>25 Kg/m2) an indicative of lower platelets transfusions. For platelet refractoriness and/or vascular events none of the variables was statistically significant.

There was no difference between autologous and allogeneic BMT patients according to the number of transfused platelets concentrates (p=0,063), platelet refractoriness (p=0.13) and vascular events (p=0.13). Although there is a lower transfusion consumption of platelets in patients with high BMI (Table 2), the median of platelet concentrates per transfusion episodes of patients with normal BMI and those with high BMI was not statistically significant (High BMI x Normal BMI: 10.5 x 13 units of platelets, $p = 0.137$). (Graph 1)

Patients with platelet refractoriness are described in table 3. Vascular complications were present in 2 patients, one with SOS and another with Engraftment Syndrome, described in Table 4, where attention is drawn to the ferritin level of patient 1 and the number of CD34 + cells infused to the patient 2.

Discussion:

The results of this prospective cohort show a limitation of a study sample size. However, the understanding of the impact related to platelets transfusion events, refractoriness and certain pathologies with vascular characteristics are important.

Although the data postulate the lack of detection or inexistence of a significant relationship between inflammatory markers, platelet transfusion increment, as well as the presence of thromboembolic and /or vascular events, they are in agreement with preexisting data reported in the literature, where patients with high BMI have lower need of platelets transfusion.[14]

Although the cause is unclear, it can be inferred an association with the pro-inflammatory state, which is caused largely by IL-6 present in the circulation produced by adipose tissue. IL-6 acts strongly on the proliferation of megakaryocyte progenitors and synergistically with thrombopoietin in the stimulation of megakaryopoiesis.[15] It is also observed a procoagulant state in obese and metabolic syndrome, which are characterized by high Tissue Factor levels, von Willebrand Factor, Factor VIII, Fibrinogen and platelet aggregation secondary to dyslipidemia and endothelial dysfunction present in subjects with high body weight.[5,16]

Patients undergoing BMT require serial platelet transfusions secondary to an intense and persistent thrombocytopenia, this situation is even more serious when the patient develops refractoriness to platelets transfusion. The frequency of patients with platelet refractoriness observed in the study (12%) was similar to those reported in the literature. Sherrill et al reported 13% of patients with platelet refractoriness otherwise others studies reported approximately half, ranging from 24% to 34%.[17]

Although the bleeding risk of patients receiving an allogenic transplantation was greater than those receiving an autologous transplantation[8], there was no impact in statistical analysis, between these two groups. This fact may occurred because there is a limitation of the small sample size could be explained by the reason that BMT is not a frequent procedure and performed in a single institution. The patient's characteristics at the refractory group demonstrates that exposure to a higher frequency of transfusion can lead to a platelet transfusion refractoriness, as in patient 3, who had a metallic heart valve and required full anticoagulation during the period of thrombocytopenia for this reason he was maintained with serial platelet transfusions in order to keep a platelet count around 50,000 mm^3.Other factors related to a worse post-transfusion platelet increament and platelet refractory, present in the study, which coincide with the literature were SOS, fever and the presence of splenomegaly. The increased spleen is documented as a factor of platelet refractoriness and lower interval between platelets transfusions.[18, 19]

In SOS, there is evidence that both thrombocytopenia and platelet refractoriness, probably related to disordered endothelial activation, are early markers of its presence. The low platelet increment of these patients may be related to endothelial lesion resulting from the chemotherapy program submitted to the patient with an increase in platelet adhesion to the damage endothelium, resulting in a leakage of platelets from the circulation.[17,20,21] Iron overloads is also associated with sinusoidal obstruction syndrome and ferritin levels greater than 1000 ng / dL in the pre-transplant period are an independent risk factor for this disease.[22] The results of our research do not corroborate the evidence related to high ferritin levels, as an independent risk at the pre-transplantation evaluation for vascular events, even though when we analyze the single SOS event, attention is drawn to the ferritin level of the patient in question, disproportionate to the sample. Although the single event is not significant in relation to the sample size, it presents a pattern like those described in the literature. [23, 24]

Engraftment Syndrome, the second vascular event diagnosed during the study period, presents a risk of pulmonary complications like transfusion-related lung injury. This syndrome often starts with fever and hypoxia at the time of leukocyte recovery and presents a possible and well-known correlation with the high number of infused CD34+ cells,[13, 25, 26] studies have shown that for a successful grafting the number of CD34+ cells is an important factor, with a dose of 3.5-5 x 10^6 cells/kg/weight being the optimal value. [11] The infusion of CD34+ > or = 5 x 10^6/kg, although it is related to a lower need for hemotherapy support, it raises the risk for Engraftment Syndrome,[13, 27] according to the only patient who evolved with this condition and received 7.52 x 10^6 / kg.

Onco-hematological patients classically presents clinical conditions and are submitted to therapies that interfere in the response to platelet transfusion. The conditions found in the 3 patients with platelet refractoriness and about the 2 patients with vascular events, they present features described in the literature, reinforcing the importance of the presence of these factors as a cause of refractoriness and vascular/endothelial involvement in patients submitted to BMT. Endothelial markers studies may help in the early identification of patients at risk of developing vascular complications, such as venocclusive disease and Engraftment Syndrome, enabling the beneficial introduction of curative and prophylactic therapies.

Conclusion:

It is possible that larger samples demonstrate other factors that influence the number of platelets transfusion events and the platelet transfusion increment of patient undergoing high doses of chemotherapy protocols and BMT. A small sampling limits comparisons and significant statistical inference, however, we cannot rule out the relevance of a descriptive analysis of the results, especially considering that each patient should be evaluated in an individualized way in clinical practice.

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Table 1 - Demographic, clinical and laboratory characteristics

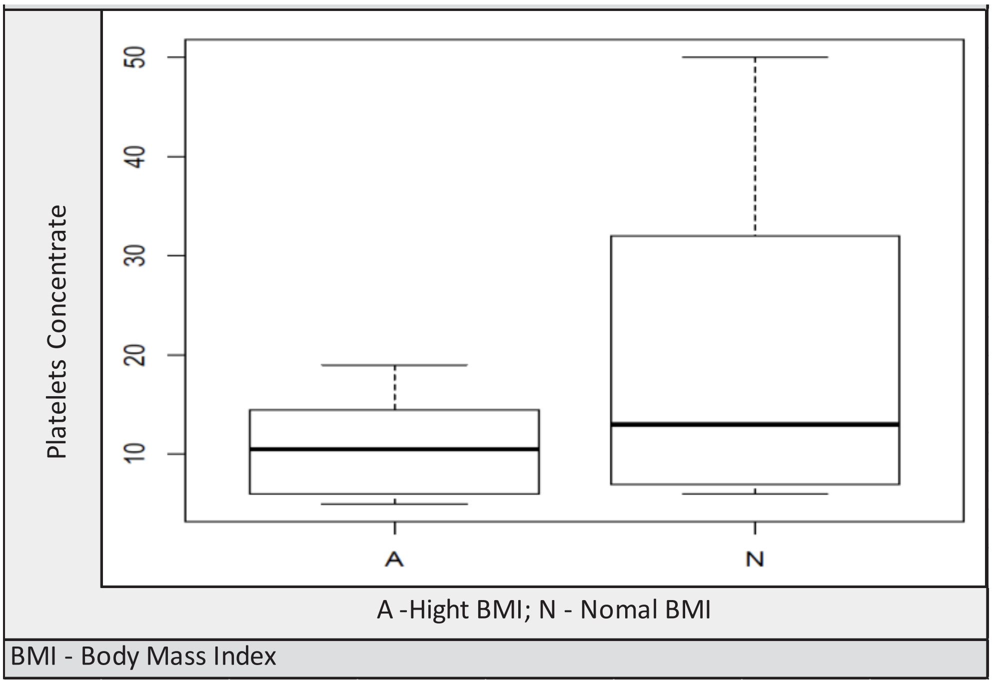
AGE	AVERAGE	39 14- 61	%
Sex	Male	13	52
	Female	12	48
Diagnostics	MM	10	40
	HL	4	36
	NHL	4	16
	AML	1	4
	CML	1	4
Radiotherapy	Yes	7	28
	No	18	72
Type of transplant	Autologous	19	76
	Allogenic	6	24
Weight (kg)	Average	76,2 Kg	
High BMI	Yes	16	64
	No	9	36
Vascular Events	Yes	2	8
	No	23	92
High Ferritin	Yes	9	36
	No	16	64
High RCP	Yes	19	76
	No	6	24
Platelet transfusion	Yes	19	76
	No	6	24
Unsatisfactory 24h ICC	Yes	3	12
	No	22	88

MM - Multiple Myeloma; LH - Hodgkin's Lymphoma; LNH - Non-Hodgkin's lymphoma; AML - Acute Myeloid Leukemia; LMC - Chronic Myeloid Leukemia; BMI - Body Mass Index; RCP - Reactive C Protein; CCI - Corrected Increment Count
Source: Prepared by the Author

Table 2 - Characteristics of patients with increased BMI

CHARACTERISTICS	BMI (KG/M2)				
	< 18,4	18,5 a 24,9	25 a 29,999	30 a 34,9	35 a 39,9
Age	38	35,8 ± 9,9	37,7 ± 10,1	38 ± 10,9	51,33 ± 15,1
Weight (kg)	46	71,3 ± 5,6	73,28 ± 12,1	90,3 ± 17,9	88,5 ± 8,4
Duration of Neutropenia	13	10,71 ± 2,1	8,9 ± 1,8	9,25 ± 0,9	7,33 ± 0,6
Days with Fever	1	5 ± 3,2	1,4 ± 1,5	4,5 ± 2,5	2,66 ± 2,1
Ferritin (ng/mL)	139	639 ± 743	301 ± 306,7	619 ± 925,3	333 ± 83,8
RCP (mg/l)	32	11,9 ± 9,8	15,14 ± 23,6	29 ± 40,3	7,5 ± 4,8
Patients with vascular events (%)	0	4	4	0	0
Patients with more than 2 events of Platelet					
Transfusion (%)	4	24	12	8	0
Patients with unsatisfactory CCI 24h (%)	0	8	4	0	0

Data given in Median ± Standard Deviation
BMI - Body Mass Index RCP - Reactive C Protein; CCI - Correct Count Increment
Source: Prepared by the Author



Graph 1 - Median of Platelets Concentrate per transfusion event

Table 3 - Patients with platelet refractoriness

CHARACTERISTICS	PATIENT 1	PATIENT 2	PATIENT 3
Age	50	33	61
Sex	Female	Male	Male
Diagnosis			
	LNH	LH	MM
Transplant	Allogenic	Allogenic	Autologous
BMI (Kg/m2)	22,3	21,6	29,1
Fever	Yes	Yes	No
Ferritin (ng/ml)	2000	1341	79
RCP (mg/l)	4	1	16
SOS	Yes	0	0
Splenomegaly	No	Yes	No
Transfusion reaction	No	No	No
Bleeding	No	No	No

LH - Hodgkin's Lymphoma; LNH - Non-Hodgkin's Lymphoma; MM - Multiple Myeloma; BMI - Body Mass Index; RCP - C Reactive Protein; SOS - Sinusoidal Obstruction Syndrome
Source: Prepared by the Author

Table 4 - Patients with vascular events

CHARACTERISTICS	VASCULAR EVENT	
	Patient1	Patient 2
	SOS	Engraftment Syndrome
Age	50	25
Sex	Female	Female
Diagnosis	LNH	LNH
Previous radiotherapy	Yes	No
Transplant	Allogenic	Autologous
Conditioning	MEL + FLU	LEAM
CD 34+ cells	3,57	7,52
Days of neutropenia	13	9
BMI (kg / m 2)	22,3	30,8
Days of fever	5	4
Ferritin(ng/mL)	2000	34
RCP (mg/l)	4	8
Platelets Transfusion Events	7	3
Unsatisfactory ICC 24 h	Yes	No

SOS - Sinusoidal Obstruction Syndrome; LNH - Non-Hodgkin's lymphoma; MEL - Melphalan; FLU - Fludarabine; LEAM - Lomostine, Etoposide, Cytarabine, Melphalan; BMI - Body Mass Index; RCP - Reactive C Protein; CCI - Corrected Increment Count.
Source: Prepared by the Author

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WHAT IS THE ROLE OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) IN THE SCENARIO OF NEW DRUGS FOR MULTIPLE MYELOMA (MM)

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Patients with multiple myeloma (MM) in clinical conditions to be referred to autologous hematopoietic stem cell transplantation (AHSCT) generally start therapy with an induction chemotherapy followed by high-dose alkylating and AHSCT (1). The ideal regimen and the number of pre-AHSCT induction is still a controversial subject, however, opting for at least three to four cycles of chemotherapy including a drug with immunomodulatory action, a proteasome inhibitor, with a corticosteroid, are advised as the first line before AHSCT [3].

It was defined that triple therapies are preferred as induction before transplantation [2,4], and with a better understanding of the pathophysiology of MM new therapies with agents that overcome the responses of established therapies, such as pomalidomide and the new proteasome inhibitors (carfilzomib and ixazomib), has emerged [5].

The current scenario of treatment of MM patients who are candidates for AHSCT includes new agents with many studies, such as the one that assesses the use of daratumumab (Dara) in association with bortezomib, lenalidomide and dexamethasone (Dara-VRd) in the induction and consolidation after TACTH [6]. This study has demonstrated the safety and efficacy of this association, as well as in the CASSIOPEIA clinical trial, which evidenced the benefit of the association of Daratumumab, with the classic VTD (bortezomib, thalidomide and dexamethasone) scheme, increasing the depth of the therapeutic response after TACTH [7].

First-line AHSCT has been questioned, several studies assessed the role of AHSCT in this scenario comparing to its use in first relapse [8,9,10,11]. The EMN02/HO95 study, patients were randomly to receive four cycles of bortezomib, melphalan and prednisone (VMP) or AHSCT after high-dose melphalan, 1197 patients were eligible for the randomization, of whom 702 were assigned to AHSCT and 495 to VMP. The median progression-free survival (PFS) was significantly improved with AHSCT compared with VMP [10].

The IFM trial used induction therapy based on VRd with initial or delayed consolidation with AHSCT. A total of 700 patients radomized for VRd 8 cycles, with lenalidomide maintenance and AHSCT only in relapse, and VRd 3 cycles with AHSCT in the first line, with consolidation of 2 VRd cycles and lenalidomide maintenance. An increase in PFS survival was observed, in addition to deeper responses, with the transplant done early, but with no difference in overall survival (OS). However, 79% of patients who had disease progression in the non-AHSCT arm were submitted to a rescue AHSCT, which may justify the similarities in the OS [11].

In the IFM/DFCI 2009 trial, patients with negative minimal residual disease (MRD) pre-maintenance showed an improvement in PFS (> 80% in 3 years) compared to patients with positive MRD [12]. The impact of negative MRD on OS can also be seen with this scheme, being more frequent in those undergoing first-line AHSCT than in patients who received only 8 cycles of VRd [11]. These findings confirm that the absence of minimal residual disease is an important treatment target for myeloma [13,14] and suggest that the use of high-dose chemotherapy and transplantation after induction therapy with VRd may help to this goal.

The use of other proteasome inhibitors such as carfilzomib has also been tested in a randomized study comparing: carfilzomib, lenalidomide and dexamethasone (KRd) followed by AHSCT plus consolidation with KRd (KRd-AHSCT-KRd) versus KRd 12 Cycles versus carfilzomib, cyclophosphamide and dexamethasone (KCd). The rates of MRD negativity, sCR, ≥CR, ≥VGPR were significantly higher with KRd-AHSCT-KRd and KRd12 vs KCd. No differences were observed in MRD and in the best overall response (sCR, ≥CR, ≥VGPR) between KRd-ASCT-KRd and KRd12, requiring longer follow-up to assess survival [15].

Several other alternatives to avoid AHSCT in the first line have been proposed using different strategies such as MRD and cytogenetic risk stratification, despite these attempts, most studies have shown an increase in PFS and a consequent improvement in response with the consolidation with TACTH despite the scheme used and the consolidation [16].

Although most intense therapies have been suggested with the association of 4 drugs from different classes [6, 7], and some studies try to remove AHSCT in an initial moment [8,9,10,11], none has been able to demonstrate its "uselessness". Thus, in a phase when the therapy with four drugs starts to appear as "gold standard" in the treatment of the newly diagnosed patient, the IMWG recommendation remains up to date regarding the use of ASCTH in the first line and today the main objective is to achieve a sustained MRD negative in order to "cure" these patients.

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MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA IN THE CONTEXT OF HEMATOPOETIC STEM CELL TRANSPLANTATION

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ABSTRACT

Minimal or measurable residual disease (MRD) is considered the most important independent prognostic factor in acute lymphoblastic leukemia (ALL). MRD status after clinical remission has been used to establish the risk of relapse and therapeutic stratification, identifying patients who can benefit from therapeutic intensification, including allogeneic stem cell transplantation (alloSCT). The pre alloSCT MRD also identifies patients eligible for transplant and those with low or high risk of relapse after transplantation, according to the level of MRD detected. However, MRD status post-alloSCT has been shown to be a more powerful predictor of relapse than pre-transplant MRD. In addition, it is important to take into account that there are some factors to be considered to better interpret MRD information, these include: the method used for MRD assessment and its sensitivity and specificity, which may vary according to each specific time point of evaluation; the treatment regimen used; and the identification of genetic lesions that combined with MRD information can further improve the management of patients with ALL.

Key Words: Minimal residual Disease (MRD), Acute Lymphoblastic Leukemia (ALL), allogeneic Stem Cell Transplantation (allo-SCT)

INTRODUCTION

Minimal or measurable residual disease is, by definition, a sub-microscopic disease that can be detected by sensitive methods that more accurately monitor disease kinetics during and after the treatment of hematological malignancies. MRD quantification can assess the response to treatment of individual patients by the magnitude of the disease burden reduction and establish the risk of disease relapse. In acute lymphoblastic leukemia, MDR is considered the most important predictable relapse factor and it has been widely used by most cooperative ALL treatment groups to guide treatment decisions.1-11 The MRD level can identify patients who need treatment intensification, including with allogeneic SCT (alloSCT). Here, the impact of the status of peri and post-transplant MRD in patients with ALL will be discussed, as well as some aspects that must to be taken into account for a better interpretation of the results of MRD by clinical hematologists.

PROGNOSTIC SIGNIFICANCE OF MRD

MRD assessment at the end of induction therapy is useful to identify patients with a low risk of relapse. Any persisting MRD level at the end of consolidation therapy is associated with a high risk of relapse and indicates the need for therapy intensification.[9,10,12,13]

The MRD persistence at ≥10– 3 before SCT in children with high-risk relapsed ALL reflects a disease which is highly resistant to conventional intensive chemotherapy, and which requires prospective investigation of new treatment strategies with innovative targeted or immunotherapeutic approaches.[14]

Many studies have demonstrated the prognostic impact of MRD pre- and post-alloSCT. A study by the BFM group included children and adolescents with relapsed ALL, eligible to receive alloSCT in ≥ second remission (CR2). The methods used for MRD detection included both multiparametric flow cytometry (MFC) and quantitative real time PCR (RTqPCR). The MRD cutoff value of less than 10-4 leukemic cells turned out to be a feasible discriminator between patients at high (≥ 10-4 leukemic cells) or low risk (< 10-4 leukemic cells) for subsequent relapse. In other words, patients who underwent transplantation with an MRD < 10-4 leukemic cells, had a higher event free survival (EFS) and lower cumulative incidence of relapse (CIR) than those who underwent alloSCT with an MRD load of ≥ 10-4 leukemic cells. [15]

However, a meta-analysis study showed that although positive MRD (MRD+) before alloSCT was a significant negative predictive factor of relapse-free survival (RFS), EFS and overall survival (OS), a MRD+ result prior to transplantation was not associated with a higher rate of non-relapse mortality. [16]

Based on MRD status, patients stratified as high-risk of relapse have been shown to benefit from alloSCT, but the maintenance of MRD positivity after transplantation correlates with a poor outcome. [13, 17-20] Retrospective studies with pediatric and adult ALL patients have shown that patients with undetectable MRD by MFC or by RTqPCR before myeloablative alloSCT had a better outcome than patients with any level of MRD+. Patients with MRD+ after transplantation had significantly worse outcomes than patients with undetectable MRD after transplantation. [10, 3] and 10-4 were the minimum discriminatory MRD detection limits used in these studies. [13,21] Similar results were observed using a more sensitive method for MRD detection such as next generation sequencing (NGS), in which any post-SCT NGS-MRD positivity resulted in an increased risk of relapse whereas the absence of detectable NGS-MRD pre-SCT defined good-risk patients. 22 In addition, patients who converted from MRD+ to MRD-negative after transplant had been in remission for at least two years after alloSCT.13 The kinetics of MRD by MFC in pediatric patients with ALL in the peri-haploidentical SCT was also important in predicting the risk of relapse. [23]

A multicenter study in pediatric ALL patients compared the prognostic value of pre-alloSCT and post-alloSCT MRD kinetics. Definitions of MRD status were: undetectable MRD was considered as MRD negative; detectable MRD< 10-4 (RTqPCR) or < 0.01% (MFC) was MRD low positive; MRD ≥10-4 to <10-3 (RTqPCR) or ≥0.01 to <0.1% (MFC) was MRD high positive; and MRD ≥10-3 (RTqPCR)) or ≥0.1% (MFC) was MRD very high positive. They demonstrated that patients with detectable MRD pre-SCT and MRD post-SCT had a significantly lower EFS and higher CIR, especially those with higher MRD levels. But there was no effect on outcomes when MRD pre-SCT was detected at the lowest levels (<10-4) in patients who achieved post-SCT undetectable MRD. However, after transplantation even low levels of MRD were always highly predictive of relapse (p = 0,001). Furthermore, any detectable MRD level on days +180 and +365 was highly predictive of relapse and poor survival. Conversely, patients who were MRD negative on day +365 had long-term survival. In conclusion, the risk of relapse was more strongly influenced by MRD post-SCT than by MRD pre-SCT. [19]

On the other hand, MRD monitoring is much less frequently used after alloSCT because chimerism monitoring provides an alternative for early relapse detection. However, there is evidence that Ig/TCR-based MRD has higher sensitivity and specificity compared with chimerism analysis. [24]

Time points for MRD assessment in ALL patients eligible for allo-SCT

MRD levels at different time points have different prognostic values for relapse. The most suitable time points for MRD assessment are not consensual, however, the following studies can guide clinical strategies in patients with ALL.

Pre allo-SCT MRD assessment

One study has demonstrated the kinetics of MRD reduction in high-risk relapsed ALL patients before alloSCT are heterogeneous. The study noted that patients achieved a rapid or slow reduction in MRD during the treatment period from induction therapy to directly before transplantation. Some patients who initially had a deeper therapeutic response experienced an increased in the MRD level during this period. Therefore, the study concluded that MRD assessments should be done early before consolidation therapy and before each chemotherapy cycle, including immediately before alloSCT. [14] In another prospective study in children with relapsed ALL treated according to the BFM study protocols, MRD was measured by RTpPCR, at a median of 13 days before alloSCT to assess the prognostic significance of MRD before transplantation. [15]

MRD was assessed by RTqPCR (BCR-ABL1 transcript with 10-5 sensitivity) within 30 days before allo-SCT, in patients who received chemotherapy combined with tyrosine-kinase inhibitors (TKIs) before transplantation. Undetectable MRD was one of the factors most influential in RFS for adult patients with Philadelphia+ (Ph1+) ALL transplanted in first clinical remission (CR1) (p = 0.0004). [25]

These studies indicate that MRD assessment should be done very close to alloSCT.

Post allo-SCT MRD assessment

The accuracy of MRD measurements (by MFC and RT-pPCR) in predicting relapse was investigated at days +30, +60, +90, and +180 post-SCT. From day +60 onwards, the discriminatory power of MRD detection was greater to predict the probability of relapse.18 On the other hand, especially at earlier times after transplantation (day + 30), the detection of NGS-MRD after SCT, was better in the prediction of relapse than MFC-MRD (p <0.0001). [22]

The evaluation of factors which may impact the outcome in pediatric patients with ALL undergoing alloSCT, such as MRD+ pre SCT, the status of remission (CR2, CR3), non-TBI conditioning regimen, absence of aGVHD by day+190 post-transplant, can define groups with a high risk of relapse who can benefit from the more frequent MRD assessment and early therapeutic intervention. [19]

EVALUATING AN ALL MRD RESULT

Some important information must be considered and added to MRD results to refine outcome prediction in ALL patients, such as the leukemia biology, the sensitivity of the method used for MRD detection, the time points of assessment and the treatment regimen used.

Methods of MRD detection

Knowledge of the characteristics and limitations of each method is essential for the correct interpretation of MRD results. The sensitivity and specificity of methods for measuring MRD are different and vary during ALL treatment. This means that MRD detection is influenced by the method used at each given evaluation time point.

Molecular methods include the use of RTqPCR to detect leukemia-specific or patient-specific molecular markers, such as fusion gene transcripts and immunoglobulin / T cell receptor (Ig / TCR) gene rearrangements. Multiparametric flow cytometry is based on the detection of "different from normal" immunophenotypes. These methods reach limits of 10-4 to 10-5 for MRD detection, which means 1 leukemic cell in 10.000 to 100.000 normal cells. Recently, new high-throughput technologies to quantify MRD have been introduced: NGS for Ig/TCR rearrangements.22,26 and next generation flow (NGFlow) based on immunophenotyping.27 These reach limits of detection of 10-6 to 10-7 (1: 1.000.000 to 1: 10.000.000 normal cells) and 10-5 to10-6 respectively.

It must be emphasized that, although these methods reach high sensitivity, a negative result of MRD does not necessarily mean eradication of the disease, rather, that the disease burden may be below the detection limit of the method used. [28]

Molecular methods of MRD detection

Both Ig/TCR RTqPCR and Ig/TCR NGS require a diagnostic sample as a reference to identify the leukemia-specific index rearrangements that are monitored throughout therapy. False negative MRD results may occur using RTpPCR/ IgTCR rearrangements due to clonal evolution in immature leukemic blasts, which can lead to the loss of the leukemia-specific Ig / TCR sequence. On the other hand, false positive MRD results can be a consequence of massive bone marrow regeneration after treatment which can cause nonspecific annealing of the primer. [28]

Although well standardized, assessment of MRD by RTq PCR/ IgTCR rearrangements is time consuming and labor intensive, requiring technical expertise. [29]

The NGS of the Ig/TCR gene rearrangements can overcome some of the limitations of RTqPCR and can increase sensitivity, provided that sufficient numbers of cells are analyzed.

NGS does not require the construction of patient-specific oligonucleotides, because the same multiplex PCR assay can be used to identify and follow-up the index sequence. [28,30] NGS also offers the advantage of being able to track minor subclones, responsible for driving relapse, which may not be identified by other methods. A disadvantage of NGS-based MRD detection is the need for large amounts of cells / DNA that can limit its usefulness. This often represents a serious limitation in the aplastic samples during treatment. [28,30]

NGS is still not well standardized and clinically validated, although there is evidence that NGS is more sensitive to identifying clinically significant MRD than other methods. [20,22,26,29] For example, NGS-

MRD post-alloSCT has been shown to be more predictive of relapse and survival than MFC-MRD, suggesting a role for this technique in defining patients who would be eligible for post-transplant interventions. [22]

Both molecular methods are expensive and are not widely available in Brazil.

RTqPCR is also used to detect MRD in patients Ph1+ ALL, detecting the BCR-ABL1 fusion gene with a detection threshold in the range10-4 to 10-5. One disadvantage of this method is that these PCR assays in which the p190 transcript is present are not fully standardized like p210 for Chronic Myeloid Leukemia, which makes it difficult to interpret the results.30 RTq PCR Ig / TCR rearrangements may be more specific than BCR-ABL1 for MDR monitoring in patients with Ph1+ ALL.30,31 MRD measured by MFC and/or RTpPCR produced largely equivalent results in a threshold of 0.01%, which is the limit used to define MRD positivity in Ph1+ALL patients. Both methods have been proven to provide a more accurate quantification of residual leukemic cells than BCR-ABL1 transcripts. [31]

However, there are conflicting data: studies comparing BCR-ABL1 MRD and Ig/TCR MRD demonstrated significant differences in detection, in favor of BCR-ABL1 fusion transcript.28 It seems that Ig/TCR and BCR-ABL1 MRD may provide distinct insights into MRD kinetics of different leukemic subpopulations in response to therapy. [28,30-32]

Despite the potential disadvantages, PCR for BCR-ABL1 is the recommended method for MRD assessment of Ph1+ ALL in the North American consensus, because it is superior to conventional MFC in predicting outcomes in this ALL subtype. [30]

MRD by multiparametric flow cytometry

MFC is based on the identification of leukemia-associated immunophenotypes (LAIPs) and the differences in blast cell immunophenotypes in relation to the maturation patterns of their normal counterparts. MFC is faster compared to molecular methods, which makes it useful for immediate therapeutic decisions. Indeed, MFC is less labor intensive and more widely available than PCR methods. MFC has high applicability (LAIPs can be identified in more than 90% of patients with ALL) and do not require information about the diagnostic immunophenotype. [28,30]

Sensitivity of conventional MFC-MRD detection is about 1 log lower than that for the molecular methods (10-4), [12,33] although the concordance between the paired RTpPCR and MFC-MRD results has been demonstrated in children and in adults. [19,31,34]

MFC performance can be influenced by the similarities between leukemic lymphoblasts and regenerative lymphoid precursors. [35] In addition, phenotypic shifts that occur in residual leukemic cells, as well as in normal regenerative cells during therapy, can lead to false-negative or false-positive results. [35]

The conventional MFC-MRD has limited interlaboratory standardization, which makes the interpretation of results susceptible to the experience of each flow cytometry analyst. As a result, this heterogeneity of approaches to MRD detection generates the differences in sensitivity and specificity of tests among laboratories. The development of NGFlow, a fully standardized MFC technology, can decrease the subjectivity of the interpretation of MRD assays, reaching high sensitivity of the test (10-6) which is directly related to the number of cells (> 5 million) analyzed. [27,29] Like other methods, NGFlow MRD requires training and knowledge. [29] Therefore, MFC-MRD requires significant technical expertise [28-30]

Anti-CD19 therapies influence the detectability of residual leukemic cells, due to the partial or total loss of the main markers for the detection of MRD of B cell precursor (BCP)- ALL. [36] Different approaches are necessary for the detection of MRD in the context of anti-CD19 immunotherapies. [36] However, there is no consensus on the best MFC strategies for this purpose.

In summary, conventional MFC and RTpPCR can achieve sensitivity levels similar to those of NGS, up to a detection limit of 10-4 for MRD assessment, but NGS can achieve a higher degree of sensitivity and specificity than both. [20,28,30] However, NGFlow has been shown to achieve similar sensitivity to RTpPCR in the MRD of BCP-ALL.27 To date, there have been no comparative studies on the sensitivity between NGS and NGFlow.

Genetic factors and MRD response

Genetic abnormalities associated with some subtypes of ALL are significantly associated with MRD status during treatment. [37] Adult patients with Philadelphia-like ALL, KMT2A -MLL gene rearrangement, and early T-cell precursor ALL (ETP-ALL) appear to have relatively poor outcomes regardless of MRD status (at a sensitivity level of 10-4). These disease subtypes are also more likely to have persistent MRD, despite intensive therapy.30 Patients with high risk cytogenetics are generally associated with poor outcomes, even achieving a good response with undetectable MRD at any moment during treatment. In a cohort of 3113 patients treated on UKALL2003, the distributions of MRD results at the end of induction therapy were different in groups of patients with different genetic subtypes (p <0.001). [38] Patients with good-risk cytogenetics (ETV6-RUNX1, high hyperdiploidy) demonstrated faster clearance of leukemic cells (MRD by PCR Ig/TCR rearrangement with a limit of detection of 1x10-5), while patients with high-risk cytogenetics (iAMP21, KMT2A rearrangement, haploid/ hypodiploid) and T-cell acute lymphoblastic leukemia responded more slowly. [38] Intermediate-risk patients who had genetic heterogeneity and variable MRD kinetic: TCF3-PBX1 or t(1;19) exhibited a fast disease clearance, but these patients needed more intensive therapy to avoid relapses. [38,39] Other BCP- ALL with normal or abnormal cytogenetics, and also alterations of copy number, such as ABL-class fusions, JAK-STAT abnormalities, IKZF1 deletion, IKZF plus usually have slower disease clearance with prolonged persistence of MRD. [38] Although the risk of relapse is directly proportional to the level of MRD in each genetic risk group, the absolute risk of relapse associated with a specific level of MRD varies according to the genetic subtype. The integration of genetic information and MRD results can improve risk algorithms for treatment decisions. [38-41]

Hypodiploidy: in a retrospective cohort, the Children's Oncology Group observed that alloSCT has no impact on the outcome of children and young adults with hypodiploid BCP-ALL in CR1. Patients with MRD < 0.01% by MFC at end of induction therapy had 5-year EFS of 66.3% ± 7.9% with alloSCT (n = 39) and 60.3 ± 9.2% without (n = 35; p = 0.77). Five-year OS was 79.5% ± 6.7% with SCT and 66.7% ± 8.8% without (p= 0.39). Furthermore, CIR did not differ significantly between chemotherapy and SCT groups (p=0.22) [42].

***KMT2A (MLL)* rearrangements:** these occur more frequently in BCP- ALL, but also in a small fraction of T-ALL (5-10% of T-ALL patients), mainly in pediatric patients (80% infants), in different proportions and types of molecular lesions. [39,43] The presence of these molecular signatures associated with MRD status determines a high proportion of refractory diseases, despite intensive therapies. [30,38,39]

***BCR–ABL*–like or Philadelphia like ALL:** is a subgroup of BCP -ALL which has a gene expression profile similar to that of BCR–ABL1–positive ALL, with a high frequency of IKZF1 alterations, but lacking the BCR–ABL1 fusion protein. This subtype comprises 10% of the cases of BCP-ALL in children and 25% of the cases of ALL in adolescents and young adults. [39] The spectrum of genetic alterations is diverse, including rearrangements involving tyrosine kinase genes such as ABL and PDGFR, which respond to TKI. [44,45] Other rearrangements target JAK and EPOR, which are sensitive to JAK inhibitors in preclinical studies. [46] In addition, rearrangements involving the cytokine receptor gene CRLF2, which were identified in 50% of patients with BCR-ABL1 like ALL, are often associated with JAK mutations and also potentially sensitive to JAK inhibition. [47,48] In most studies, CRLF2 rearrangements are associated with a poor prognosis, particularly in cases with concomitant IKZF1 alterations. [48] However, risk-oriented therapy, including intensive chemotherapy with or without alloSCT based on the level of MRD during induction therapy, can eliminate the poor prognosis of this group of patients. [33]

***IKZF1* deletions:** these also occur in a subset of patients with poor-response, high-risk ALL without any known chromosomal rearrangement IKZF1 plus is characterized by IKZF1 deletions co-occurring with other copy number alterations. [39] IKZF1 plus had no prognostic impact in patients with undetectable MRD after induction therapy, but in patients with persistent positive MRD, they faced a 10-fold higher relapse rate in stratified analyses by MRD levels, describing a very poor and MRD-dependent prognostic profile in BCP-ALL [49]

***CRLF2* rearrangements:** these are also observed in 50% of ALL patients with Down syndrome, responsible for the inferior outcome due to the increased risk of relapse. In addition, these patients also have high rate of treatment–related mortality. [39,48]

ALL with intrachromosomal amplification of chromosome 21 (iAMP 21): this is considered an ALL subtype of high-risk cytogenetics and requires an intensive treatment modality. [38,50,51] Intensification of chemotherapy has ended the poor prognosis once associated with this ALL subtype. [50] The BFM group considered that MRD alone identifies high-risk patients with iAMP21. [52]

Philadelphia chromosome (BCR-ABL1)- Ph1+ ALL: this occurs in about 3% of children with ALL and has been considered associated with poor outcome, despite intensive chemotherapy regimens and alloSCT. The introduction of TKI has markedly improved outcomes, avoiding alloSCT in MRD negative patients, but relapse remains the main cause of treatment failure. [31,53] MRD kinetics in children with Ph1+ ALL who reached MRD ≤ 10-4 leukemic cells at the end of induction therapy, evaluated by

RTqPCR (Ig/TCR and BCR-ABL1 fusion transcript with sensitivity of 10-4) [53] or by MFC and Ig/TCR rearrangements31 suggest that early MRD negativity was related to lower risk of relapse and that they could achieve high survival rates without alloSCT. Persistence of MRD in children with Ph1+ ALL at later time points of therapy was associated with a higher incidence of disease relapse. [53] Similar results were observed in adult Ph1+ ALL patients. [32] The incidence of Ph1+ALL is 20-30% of adult patients with ALL. Achieving a deeper molecular response (RTqPCR for BCR-ABL1 transcripts with a limit of detection of 10-4 to 10-5) with intensive chemotherapy plus one TKI has been associated with superior outcomes, despite not undergoing alloSCT in first remission. [54] MRD has also been shown to predict outcomes in patients with Ph1+ ALL in a variety of situations, such as in patients undergoing regimens based on non-intensive induction therapy, including TKI plus corticosteroids, as well as TKI plus chemotherapy, whether or not followed by consolidation with allo-SCT, according to age, molecular response, clinical eligibility and donor availability. [55,61] .In these series, the complete molecular remission achieved until 1 or 2 cycles of the induction therapy is associated to higher disease free survival (DFS) and lower CIR. [55,61] On the other hand, based on MRD kinetics by the evaluation of BCR-ABL1 transcript, patients who underwent alloSCT in CR1, after chemotherapy plus dasatinib, showed a significant difference in DFS ($p = 0.0018$) and CIR ($p = 0.0015$) between early stable molecular responders (after 2 cycles of treatment) and poor molecular responders. However, there was no difference between early stable molecular responders and late molecular responders. [62]

The role of alloSCT is controversial with the resulting improvements seen by incorporating TKIs into first-line regimens for Ph1+ ALL. Although the therapy intensification with alloSCT still represents a good curative option, the introduction of novel approaches with ITK and immunotherapeutic agents is likely to improve the outcome of these patients further, and might mean that SCT can be avoided in a proportion of cases. [54,61] Nevertheless in any situation, MRD plays a role in guiding the best treatment choices.

Although several studies have shown the impact of molecular lesions on the ALL prognosis based on retrospective studies, it is difficult to incorporate this information into the MRD data to refine the prognosis in the face of therapeutic intensification. [30] Controlled studies can associate this information and establish treatment algorithms to improve the management of patients with ALL.

T Immunophenotype ALL and MRD response

T-ALL shows a slower blast clearance compared with BCP-ALL in the context of identical therapy, proving that they are biologically different diseases. The AIEOP-BFM 2000 protocol evaluated the impact of MRD by PCR in 464 T-ALL. This study showed that patients with MRD $< 0.01\%$ at the end of induction therapy has the most favorable prognosis, however, patients who became MRD negative by the end of consolidation also had a favorable outcome. [7] In contrast, patients who continued to show a high MRD level ($\geq 0.1\%$) at the end of consolidation phase had a high relapse risk. [7]

MRD is also prognostic in early T-cell precursor ETP-ALL, a more aggressive subset of T-ALL, which accounts for 15% of all T-cell ALL in children and 35% in adult T-cell disease. It is also associated with high MRD levels post-induction therapy and also inferior long-term outcomes. [63,64] The low frequency of this type of leukemia makes it difficult to guide treatment, although there is a consensus on more intensive treatment for this group of patients. [63] Therapy intensification, mainly based on high MRD status, resulted in a comparable outcome for ETP-ALL and non-ET-ALL patients. [65]

Adult T-ALL treatment groups demonstrated that patients who did not achieve molecular remission (MRD > 10-4) after induction therapy have a lower survival rate than patients with MRD-negative (< 10-4). [64]

The GMALL group has reported a beneficial effect of alloSCT in patients with early and mature T immunophenotype, who had 10 years of OS of 25% without allo-SCT vs 59% for those who underwent allo-SCT. [66]

Genetic lesions in T–ALL are diverse and complex, but their prognostic impact is not well defined and they are not widely used for risk stratification.39 Mutation of the NOTCH1/FBXW7 was found in at least 60% of adult patients with T-ALL, which has been described as a good-risk group with significantly higher OS and lower CIR rates in patients without PTEN or NK-RAS mutations. However, this result was not reproducible among the treatment groups and there are limitations in the use of these data for treatment decisions. [64]

SAMPLES FOR MRD ASSESSMENT

Bone marrow (BM) samples are preferable used for BCP-ALL instead of peripheral blood (PB) for ALL MRD, regardless of the method used, because the frequencies of BCP-ALL cells in paired PB and BM samples are significantly higher in BM than in PB, ranging from 1 to 3 logs. On the other hand, a strong correlation can be observed between the frequencies of T-ALL cells in PB and BM, but the differences can occur up to 1 log, in favor of BM samples. [29]

MRD ASSESSMENT REPORT

To allow a correct interpretation of the MRD results, the MRD report must provide clear information about the MRD result and the MRD technique used, including the limits of detection and quantification achieved by the specific assay used, which are parameters of the sensitivity of the method. [67,68]

CONCLUSION

Relapse remains the main cause of treatment failure in patients with ALL who have undergone allogeneic SCT. Currently, MRD is the most important prognostic parameter that can guide clinical decisions in this scenario. However, it is essential to have criteria to incorporate MRD results into clinical management Evaluation of each information discussed below and how the treatment used can impact the therapeutic response are crucial. Thus, a more accurate choice of a better treatment option for each ALL-patient can be made.

CONFLICTS OF INTEREST

Author declare no conflicts of interest

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INCIDENCE OF MUCOSITIS IN PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AT A SINGLE CENTER

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ABSTRACT

Goal: The aim of this study was to describe the incidence of oral mucositis (OM) in patients undergoing autologous hematopoietic stem cell transplantation (auto-HSCT), relating it to the main clinical factors. Methodology: Descriptive analysis based on a randomized clinical study was conducted with patients undergoing HSCT at the University Hospital of Federal University of Juiz de Fora between January 2018 and June 2019. The World Health Organization oral toxicity scale was used to assess the degree of oral mucositis and adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) 4.0 version. Results: Thirty-eight patients were evaluated. The incidence of OM and severe oral mucositis (SOM) was 57.9% and 21.0%, respectively. The mean duration of OM was 7.2 ± 2.6 days and the lomustine, etoposide, cytarabine and cyclophosphamide protocol (LEAC) presented the longest mean time 8.1 ± 3.1 days (p-value 0.02). The number of viable CD34+ cells and the onset day of neutropenia were predictors of SOM. Conclusion: The incidence of OM in patients undergoing HSCT was lower than reported in the literature, being more severe in patients who received less CD34+ cells and in patients with early onset of neutropenia.

Keywords: hematopoietic stem cell transplantation; mucositis; risk factors

INTRODUCTION

Mucositis is the most frequent consequence of antineoplastic drugs toxicity during Hematopoietic Stem Cell Transplantation (HSCT), resulting in changes in patients 'oral microbiota and a significant impact on their quality of life [1, 2, 3, 4, 5]. Different levels mucositis grade and its incidence were described by Bashir *et al.* (2019) [6] in patients with multiple myeloma underwent auto-HSCT who had the conditioning regimen with melphalan alone replaced by busulfan plus melphalan.

Inflammatory lesions in the gastrointestinal mucosa characterize mucositis and its pathophysiology involves a complex process of molecular and cellular events that include five phases: initiation, primary damage response, amplification, ulceration and healing [7, 8].

The occurrence of fever and infection is related to mucosal barrier injuries. Different studies often show the fever as a consequence of neutropenia, however, lesions on mucosal barrier also leads to infections. Considering the infections after the chemotherapy protocol for HSCT lesions of the mucous barrier are more important than neutropenia, and should therefore be carefully evaluated [9, 10]. Mucositis affects the patient´s nutritional status and is related to parenteral nutrition recommendation, the use of opioids, as well as the increase in hospitalization time and costs [11, 12]. Patients undergoing HSCT who developed a high degree of mucositis according to oral mucositis assessment scale (OMAS) resulted in a 45% increase in hospital costs [11].

Nutrition has an important role on health maintenance and either mucositis and malnutrition (in many cases related to mucositis) compromise the nutritional status of patients. The prevalence of malnutrition is over 75% among children and adolescents with cancer [13].

In 2014, a systematic review was published to update the Clinical Practice Guidelines of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC / ISOO). The recommended intervention therapies with level I or II evidence consisted of: cryotherapy, recombinant human keratinocyte growth factor-1 (KGF-1/ palifermin), low intensity laser therapy (wavelength at 650 nm, power of 40 mW, and energy dose of 2 J / cm2), mouthwash with benzidamine [14].

Therefore, the aim of this study was to determine the incidence and clinical impact of mucositis in patients undergoing auto-HSCT, relating them to the main clinical factors.

PATIENTS AND METHODS

A descriptive analysis based on a randomized clinical study was carried out with patients submitted to HSCT at the University Hospital of Federal University of Juiz de Fora (HU-UFJF) between January 2018 and June 2019. All participants signed a free and informed consent. This study was previously approved by the Human Research Ethics Committee of the HU-UFJF and the ethical principles were in accordance with Declaration of Helsinki on human subject research.

This study included all patients admitted to the HSCT Unit of HU-UFJF from January 2018 to June 2019 for the auto-HSCT who had not yet started the conditioning phase. Following the protocol used at the HSCT Unit, all the patients were submitted to laser therapy to prevent mucositis. In summary, the protocol consists of prophylactic low-level scanning therapy with 1J/cm2 (600-690 nm) from the first day of conditioning until hospital discharge and, in case of lesions, direct application to the area with 2J/cm2 (790-830 nm).

The conditioning protocol used for patients diagnosed with multiple myeloma was melphalan (Mel) 200 mg/m2 and Mel 140 mg/m2 for those age >65 years. For patients with Hodgkin lymphoma or non-Hodgkin lymphoma, the protocol was CBV (cyclophosphamide 6 mg/m2, carmustine 300 mg/m2, and etoposide 1200 mg/m2) or LEAC (lomustine 300 mg/m2, etoposide 1000 mg/m2, cytarabine 4000 mg/m2, and cyclophosphamide 5400mg/m2 and LEC (lomustine 200 mg/m2, etoposide 1000 mg/m2, cyclophosphamide 6000 mg/m2).

Mucositis was evaluated according to the oral toxicity scale of World Health Organization (WHO) and is described in table 115. The evaluation period of the patients was from the first day of conditioning chemotherapy until the day of the end of neutropenia. Each patient was categorized according to the highest level reached during this period. Oral mucositis grade equal or higher to 3 was classified as SOM.

This study included the relationship between the number of stem cells, characterized by the expression of CD34, received by the patient in the auto-HSCT with the incidence of OM.

The National Cancer Institute criteria version 4.0 was used for grading of adverse events (AEs) during the study. The AEs evaluated were nausea, emesis, dysphagia, dyspepsia, diarrhea, and xerostomia.

The collected data were analyzed using R Commander program. Categorical data was described using frequencies and percentages and associations with OM were verified by the C2 test or Fisher's exact test. The collected data were analyzed using R Commander program. Categorical data was described using frequencies and percentages and associations with OM were verified by the C2 test or Fisher's exact test. Quantitative data were presented using means, medians, SDs, ranges, and univariate analysis, performed with the t test or Mann–Whitney test. The statistical tests were two-sided at a significance level of 5%.

RESULTS

Thirty-eight patients submitted to the auto-HSCT were evaluated in the period and 57.9% of them were male. The average age was 53 years, ranging from 18 to 70 years. The characteristics of the patients included in this study are shown in Table 2.

The number of days in neutropenia varied between 6 and 15 days with an average of 9.3 ± 2.0. The neutropenia was started between D-2 to D+6 with an average of 3.0 ± 2.2 days, whereas the end varied between D+9 ± D+13 and an average of 11.2 ± 1.0.

More than half of the patients had some degree of OM (57.9%;n = 22) and 36.4% of them had SOM (Figure 1).

Regarding the duration of OM was observed an average of 7.2 ± 2.6 days (D+ 3 - D+ 14.0). The beginning of OM signs occurred on average at 4.4 ± 2.5 days, varying between D-2 and D+8, and day D+5 the symptoms appeared in most of patients. The end of OM occurred in an average of 10.6 ± 1.1 days (D+8 – D+13), with a median of 11.0 days.

Comparing the mean days of OM in patients submitted to different chemotherapy conditioning protocols the following results were determined: MEL (3.2 days ± 3.5), CBV (2.0 ± 4.0), LEAC (8.1 ± 3 , 1), LEC (5.7 ± 5.5) p-value 0.020 (Figure 2).

The average length of stay in the hospital without OM was evaluated and no statistically significant difference was found (p = 0.203) among the chemotherapy protocol groups (Figure 3).

Based on multivariate analysis, the incidence of SOM (21.0%) was related to the number of CD34+ cells/kg infused as well as the day of the beginning of neutropenia, as shown in Table 3. Other variables evaluated were gender, age, diagnosis, chemotherapy conditioning protocol, neutropenia duration and body mass index prior to treatment and none of these had influence on the incidence of SOM.

DISCUSSION

The incidence of OM in patients undergoing auto-HSCT with different conditioning protocols assessed during a 17–month period between 2018 and 2019 is describe in this article.

The use of laser therapy is recommended for prevention and treatment of OM and several parameters must be considered as wavelength (nm), power (mW), amount (J/cm2)and rate (mW/cm2) of energy supplied to the tissues and time of application(s)16. The laser protocol applied in this study is in accordance with the MASCC/ISOO Clinical Practical Guidelines for The Management of Mucositis Secondary to Cancer Therapy [17].

The neutropenia duration was approximately 9 days, similar to the previously work performed by our group (2017) [18], in which a nutritional supplementation was applied to patients undergoing HSCT and shows overall mean duration of neutropenia of 9.87 days varying 6.80 days.

In this work was observed a lower incidence of OM in comparison to studies previously reported in the scientific literature. The occurrence of OM was identified in 60.7% of patients submitted to HSCT 18. [20] reported that treatment-related mucositis affects over 75% of patients undergoing HSCT. Chaudhry *et al.* (2016) [7] systematically reviewed the incidence and severity of OM in patients undergoing allogeneic HSCT and found that 73,2% of patients (total of patients equal to 395 in 8 myeloablative regimen studies) exhibited OM of any degree. A total of 9.5% of the patients experienced OM grade 1 and 79,7% of the patients showed OM between grades 2 and 4.

The begging of OM symptoms usually starts at the end of the conditioning regimen or 4 days later according to the literature [21, 22]. Many studies show that OM average duration varies from 5 to 9 days (maximum of 12 days) in patients undergoing allogeneic HSCT23-26. Patients supplemented with whey protein concentrate during a study to prevent OM presented mean duration of mucositis of 8.4 ± 3.50 days (minimum of 3 and maximum of 16) in the group with a lower dose of supplementation and 7.0 ± 3.4 days (minimum of 4 and a maximum of 17) in the group with a higher dose18.

The conditioning chemotherapy had higher correlation to the incidence and grade of OM compared to patients age. The incidence of SOM was higher in patients submitted to administration of busulfan plus cyclophosphamide as a conditioning regimen when compared to other protocols [19].

Comparative analysis for incidence of OM among researches depends on the chemotherapy applied protocols. Studies show that the conditioning protocol has an impact on the evolution of OM 6, 19. However, in this study, the incidence of mucositis was not correlated to the chemotherapy applied protocols applied. Thus, it was possible to compare the incidence of mucositis among all patients. Although, we observed that the duration of mucositis was longer in patients undergoing the LEAC protocol.

Fleming *et al*. (2014) [27] found no correlation between the amount of stem cells received by patients and the incidence of mucositis in patients submitted to auto-HSCT. However, in the present study, we found that the amount of stem cells infused was inversely proportional to the incidence of SOM. Therefore, we conclude that the number of stem cells infused into the patient in the auto-HSCT as well as the day of onset of neutropenia are predictors of the incidence of severe mucositis.

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Table 1 - World Health Organization Oral Mucositis Classification

SCALE	0	1	2	3	4
Oral toxicity scale (WHO)	No alterations	Pain, sensibility and erythema	Erythema and ulcers, able to swallow solid foods	Ulcers (liquid diet only)	Ulcer, extensive mucositis (unable to feed)

Source: World Health Organization Oral15

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CHALLENGES AND STRATEGIES USED TO INCREASE THE REPORT OF BRAZILIAN HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) DATA TO THE CENTER FOR INTERNATIONAL BLOOD AND MARROW TRANSPLANT RESEARCH (CIBMTR)

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ABSTRACT

To increase the report of Brazilian hematopoietic stem cell transplantation (HSCT) data to the Center for International Blood and Marrow Transplant Research (CIBMTR), the Data Managers Working Group (GTGD) of the Brazilian Society of Bone Marrow Transplants (SBTMO), and the Sao Paulo State Bone Marrow Association (AMEO) developed several strategies since 2016: training data managers (GDs) in national and international HSCT meetings, the development of a free online teaching course (EAD) in Portuguese on Transplant Essential Data (TED), online and presential training course for new data managers offered by AMEO, the approval by the National Committee of Ethics in Research (CONEP) of a national multicenter protocol to formalize sharing data of Brazilian transplants with the CIBMTR, and the first multicenter evaluation our HSCT results using the CIBMTR Data Back to Center. The contract between SBTMO and CIBMTR was signed in 2019 and GTGD of the SBTMO was officially created. These actions resulted in an increase from 24 to 41 transplant centers registered at the CIBMTR from 2016 to 2019. The process of increasing adherence and continuity of HSCT reports to the CIBMTR is complex and requires commitment of all professionals involved HSCT. The success of this process depends on education of the GD and the involvement of all HSCT directors.

Keywords: Database. Hematopoietic Stem Cell Transplantation. Information system.

INTRODUCTION

Hematopoietic stem cell transplants (HSCT) are used to treat many onco-hematological and benign diseases1. For many patients, it is the only treatment option that offers potential for curing their disease,2 as well as offering quality of life. According to the estimate of the Brazilian Society of Bone Marrow Transplantation (SBTMO) and the records of the Brazilian Association of Organ Transplantation (ABTO), about 37,000,000 procedures were performed in the country from 1979 to 20193. The Brazilian Transplant Registry (RBT), ran by the ABTO, provides some quantitative indicators and survival data. In 2019, 3,805 transplants were reported: 1,428 allogeneic and 2,377 autologous4. However, in Brazil, there is not any specific and consolidated HSCT registry. Many Brazilian centers do not have electronic information system and/or medical records that meet their needs, and those who do have such tools, often do not have their data organized in a standardized and integrated way, what may make difficult or impossible to analyze many indicators, such as outcomes and transplant-related complications, multicenter studies, and benchmarking.

However, there are registries developed and made available globally, such as the Center for International Blood and Marrow Transplant Research (CIBMTR), a North American platform created in 2004, merging the International Bone Transplant Transplant Registry (IBMTR) and the National Marrow Donor Program (NMDP)5. The CIBMTR offers an online platform, where centers performing HSCT and/or cell therapy worldwide can insert their data and retrieve relevant data for multicenter studies or for the center, including self-evaluation and benchmarking. Therefore, the objective of this manuscript is to describe the challenges, strategies and results obtained since 2016 with the GTGD, AMEO and SBTMO collaboration to expand and improve the inclusion of Brazilian transplant centers to the CIBMTR.

METHODOLOGY

Brazil's relationship with the CIBMTR begun with the affiliation of the Hospital de Clínicas – Universidade Federal do Paraná (HC-UFPR) to the former IBMTR, in the 1980s, before NMDP and IBMTR formed the CIBMTR. After that, other Brazilian centers joined the CIBMTR, but Brazilian data entry varied over time (Figure 1).

In 2016, a partnership between HC-UFPR, Hospital Amaral Carvalho (HAC) and Hospital Israelita Albert Einstein (HIAE) originated the data managers' working group (GTGD). Subsequently, in 2018, the Bone Marrow Association of the State of São Paulo (AMEO) developed an online training course for new data managers (GD) working at centers authorized to perform Unrelated Donor Transplants. This program, funded by the Brazilian Government (National Program to Support Oncological Attention - Pronon), included a scholarship to the Data Managers and a notebook for programs at public transplant centers. Tools developed by two transplant centers using Access and REDCap to capture all CIBMTR data fields and enable later filling of the online CIBMTR forms were shared to all interested institutions. The development of instruments and strategies to improve adherence to reporting to the CIBMTR has been gradually implemented and important changes are foreseen in the area of HSCT. The GTGD is consolidated and the mission, vision and values of the group were established (Figure 2).

RESULTS

Recognizing the importance of the CIBMTR, several initiatives were developed to train Brazilian professionals with support from the CIBMTR: consecutive visits to the CIBMTR were performed, the first in October 1996 by the GD from the HC-UFPR, then, in February 2009, GD from UNICAMP, in March 2016, GDs from HAC and HIAE, and in 2019, GDs from Biosana's and Ameo. The 2016's visit resulted in a partnership between SBTMO and CIBMTR that offered the first Brazilian GD meeting at the annual meeting of the SBTMO, with approximately 15 participants. This GD meeting was repeated annually with support from the CIBMTR and the number of participants gradually increased, reaching 52 participants from 29 centers in 2019.

Since first Meeting of GDs in 2016, a voluntary work of the GDs from HC-UFPR, HAC and HIAE started. Also, in 2016 the GDs created the first Brazilian online training for filling in the CIBMTR forms. This EAD tool was made available free of charge to all HSCT centers in Brazil; 65,535 people accessed the tutorial, and 573 completed the pre-TED training (Form 2400) and 202 completed the post-TED training (Form 2450). The result of this training was presented at the BMT Tandem Meeting in 2017 and received the award for best work in the GDs category. [6]

In 2017, the HIAE Research Ethics Committee (CEP) approved a multicenter trial submitting data to the CIBMTR, entitled "Multicenter Registry of Autologous and Allogeneic Hematopoietic Stem Cell Transplants (HCT) for malignant and non-malignant diseases performed in Brazil and reported at the Center for International Blood and Transplant Research (CIBMTR)". With this approval, it was possible to make some analyses, with the return of the CIBMTR database, through a business intelligence tool (BI), the Data Back to Center (DBtc). Through this tool provided by CIBMTR, it was possible to extract a large volume of data in Excel format, ready for analysis, in a short period of time. The analysis was made joining the spreadsheets extracted from the DBtC by each of the 7 participating centers and, even with a modest number of centers, there was an expressive number of transplants. The HAC GD unified the worksheets and analyzed the data using the SPSS software (version 15.0 for windows). Patients undergoing the 1st HSCT from 2008 to 2018, a total of 3,994 patients, were included. This analysis showed the diseases most frequently transplanted in Brazil, as well as the increasing number of transplants from HLA mismatched related donors in recent years; stem cell sources and overall survival (SG) by diagnoses were described for adult and pediatric patients [7]. This analysis resulted in two abstracts selected for oral presentation at the Tandem Meetings and SBTMO Annual Meeting in 2019. In the latter, the GDs received the "Young Scientist Award - Dr.Ricardo Pasquini".

In 2019, this project was approved by the National Research Commission (CONEP), the Brazilian Central IRB. Today we have 20 participating centers and eight more are being included. Although the number of participating centers is modest, they represent a significant part of the transplants performed in the country.

Over time, the actions of these DGs have highlighted the fundamental importance of this profession in the HSCT scenario and, although it is not formally recognized in the country, SBTMO officialized the creation of the GTGD in 2019 to continue and further expand the participation in national and international registries (ABTO, WBMT/LABMT and CIBMTR).

To effectively start the work, the GTGD created a small executive committee: Anderson João Simione from HAC as president, Cinthya Corrêa da Silva from HIAE as vice-president, and Heliz Regina Alves das Neves from HC-UFPR as scientific coordinator. The identity of the group was defined, establishing its mission, vision and value, in addition to the elaboration of a logo (in Figure 2). Immediately thereafter, with the need of more professionals to contribute, Bruna Leticia S. S. Geraldo from Bio Sana's - IBCC was added for administrative and scientific support and Monique S. Ammi, as a representative from the CIBMTR.

Currently there are monthly meetings promoted by GTGD, where issues are addressed in the area of HSCT by the GDs themselves and expert guests.

Other initiatives were added to the actions in the preparation and consolidation of the Brazilian GDs, such as the start of the data manager training project in 2018, by AMEO. In 2019, AMEO and the GD of Bio Sana's visited the CIBMTR and received specific training to fill in the forms and train the professionals. AMEO, in partnership and financial support from the Brazilian government through the National Program for Support to Oncologic Care (PRONON) has developed an innovative strategy to empower and encourage new GD in the country. Of the 36 HSCT centers perform unrelated donor transplants, 30 participated in a 14-month training for new GDs. The program provided notebooks and financial support to the participants. The training was performed with online classes three times a week in three cycles, followed by a three-day visit to each center by one of the two AMEO nursing instructors. Of the total institutions participating in this training, 57% were public and 83% of the new GDs received financial assistance, 60% of whom were TCTH nurses. In addition, 90% of the new GDs completed the first of the three modules with a frequency above 75%. According to the new GDs evaluation, the program is excellent and of high importance to 100% of them.

These actions resulted in an increase from 24 to 41 transplant centers registered at the CIBMTR from 2016 to 2019 (Figure 1). Actually, there are 32 Brazilian transplant centers reporting data to CIBMTR (Table 1).

In 2019, the SBTMO signed a contract of partnership with the CIBMTR to have a HSCT registry with good quality and accuracy of data that are necessary to generate indicators and outcomes of HSCT performed in Brazil.

DISCUSSION

The issue behind all the above described initiatives is the lack of a national outcomes registry that may allow data analyses and multicenter studies. The process of developing a system for the HSCT is complex, as it requires planning, investment, infrastructure, time, professional training, awareness of transplant teams and support from government entities. The CIBMTR offers many tools such as QlikView free of charge, in addition to the system for data entry, which enables data analysis. However, there are some limiting factors of this toll, such as the impossibility of overall survival analysis comparing more than two groups.

Another benefit of reporting to the CIBMTR is the use of the data devolution tool, the DBtC, where each center can extract spreadsheets with its data and develop analyses through other statistical software. CIBMTR also supports GDs, such as content for guidance on filling in forms, help desk service, online question shift (ServiceNow). In addition, centers registered as research have a refund after filling out Comprehensive Research Forms (CRF), what can help to finance data management, and there is also a scholarship to non-U.S. GDs to participate in the annual TCT Meeting.

Through the approval by the Ethics Committee of the multicenter trial to report to the CIBMTR made it possible to legally send data to North America and made it easier to new centers to join. The first Brazilian multicenter study using the CIBMTR database, demonstrated the effectiveness of BI tools, used to have the center data and analyze it, DBtC and QlikView, respectively. The use of these tools allowed an analysis, in a short period of time, and to have relevant results from Brazilian transplant centers.

There are some limitations when using DBtC, as incomplete data retrieval, lack of information on relapse, the categorization of haploidentical donors, and the delay to have data from the CIBMTR portal, which is not updated in real time. However, CIBMTR is receptive to discuss problems brought by the Brazilian teams and to help finding solutions.

The training in the CIBMTR of the GDs brought new perspectives to the professionals, because in addition to learning, demonstrated the importance of the category for the HSCT, as already seen in the USA. The education of this professional, either through fast courses (EAD) or intensive training, as promoted by AMEO, decreased the gap between different professionals (nurses, biomedicals, system analysts, secretaries, and others) and brought the GDs closer to each other as a group. Since the recognition of the GTGD by the SBTMO, their work has been officialized and their responsibility has increased in gathering HSCT data for the county and designing future guidelines. The agreement signed between SBTMO and CIBMTR formalized the use of Registry, promoting greater adherence of the centers in sending data. Currently, the interface of the system is in English and the translation and adaptation to the Brazilian reality is being discussed, as happened with Canada and Japan.

CONCLUSION

Our initiatives have yield positive results, such as the better qualification of the professionals and the increasing number of centers affiliated to CIBMTR. There is still much to be done. Now, one should continue and improve the qualification of the GDs and maintain the commitment of the HSCT centers to include new patients and complete their long-term follow-up. Next, to have support from HSCT centers and government to provide infrastructure, training and awareness of the multidisciplinary team to this activity. The future challenges are the development of the SBTMO website about data management, including support (already under construction), the Continuous Process Improvement (CPI) infrastructure to ensure the quality of data from affiliated centers, the creation of a commission for the organization and regulation of scientific production, and many other projects.

It is clear to us that reaching most of the affiliated and active centers we will be able to better understand the Brazilian HSCT scenario. Afterall, research based on data captured with quality, accuracy and security, it is possible to enable multicenter studies, benchmarking and, consequently, improve the care of patients undergoing HSCT.

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To all multidisciplinary teams of TMO that directly or indirectly enabled the development of the work of the GDs.

Patients who undergo this treatment modality and contribute to scientific research by making their data available.

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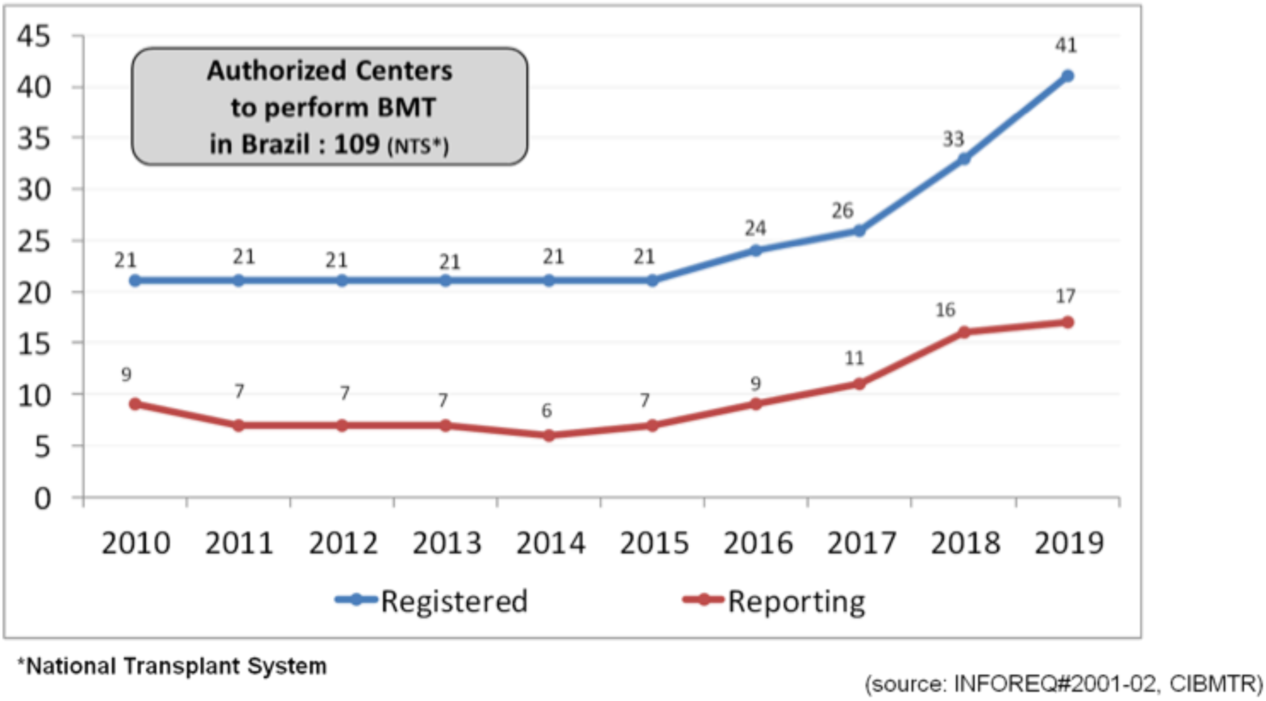
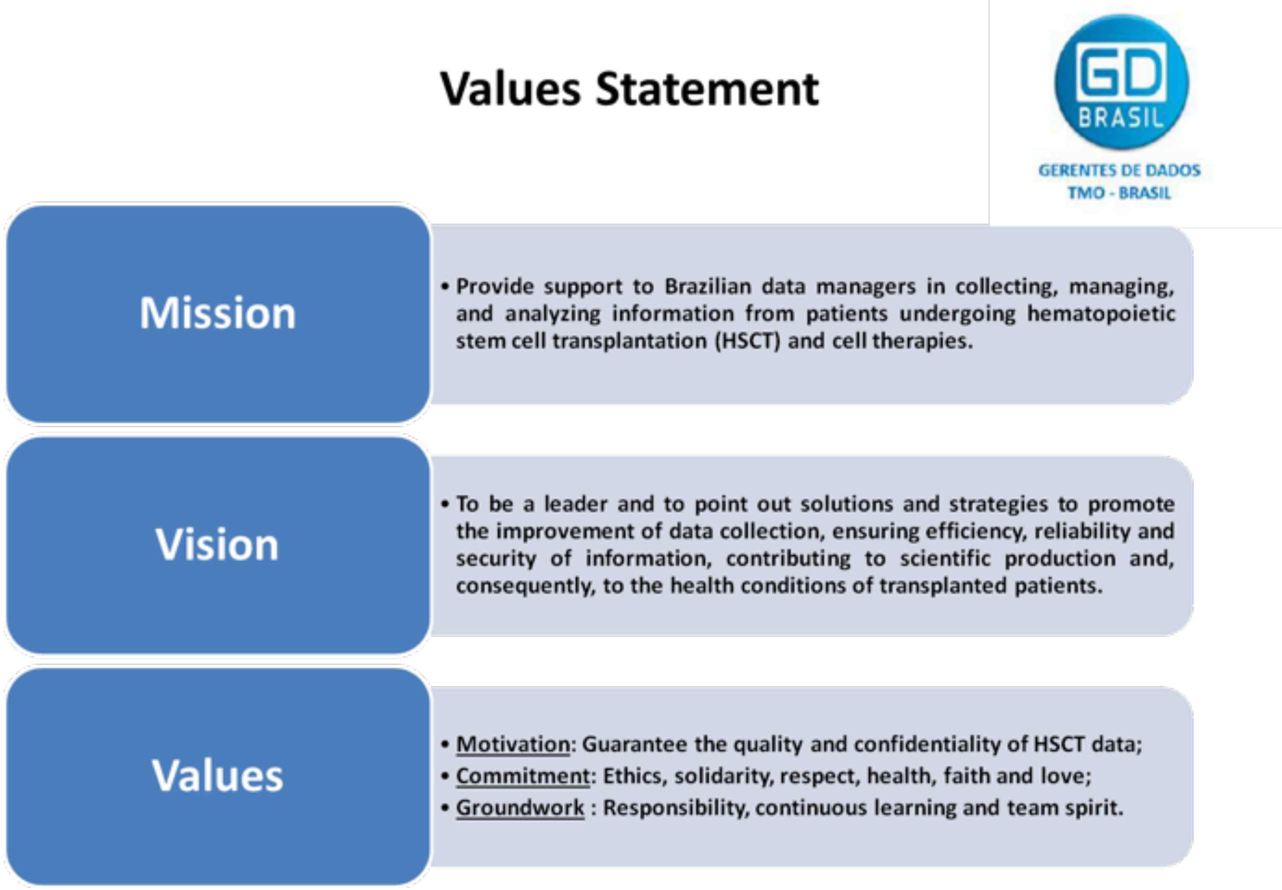


Figure 1 - Number of Centers Registered and Actively Reporting to the CIBMTR

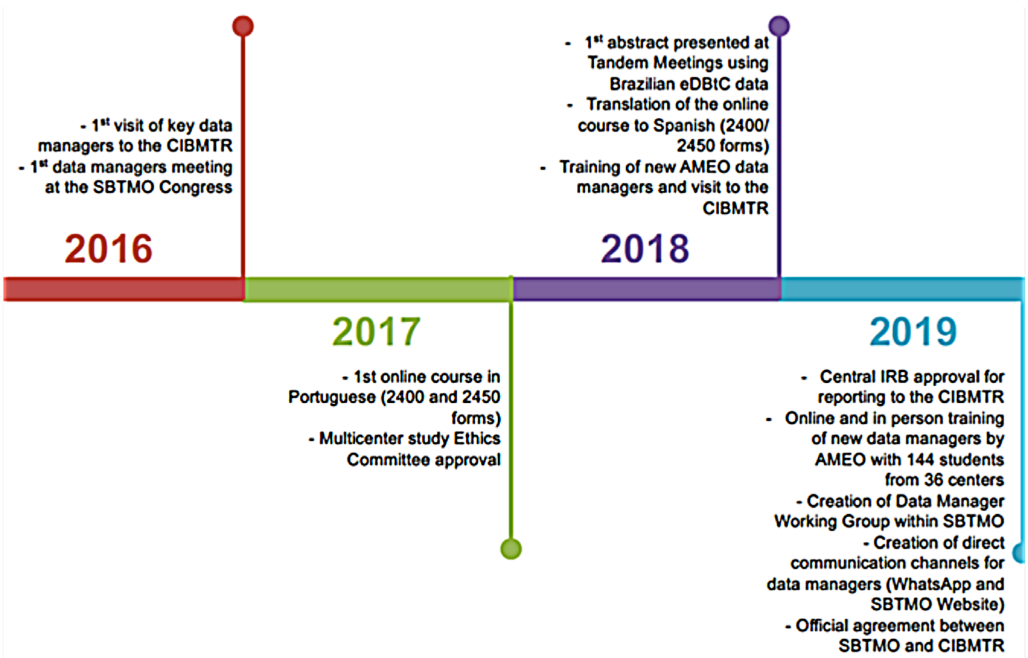
Table 1 - Brazilian transplant centers that currently report data to CIBMTR

NAME	CITY	COUNTRY
Hospital Nossa Senhora das Graças – IP	Curitiba	Brazil
Hospital Nossa Senhora das Graças – IF	Curitiba	Brazil
Universidade Federal de São Paulo - Hospital São Paulo	São Paulo	Brazil
Hospital e Maternidade Brasil	Santo André	Brazil
Associação Hospitalar Moinhos de Vento	Porto Alegre	Brazil
Bio Sana's São Camilo	São Paulo	Brazil
A.C. Camargo Cancer Center	São Paulo	Brazil
UNICAMP – HEMOCENTRO	Campinas	Brazil
Hospital Amaral Carvalho	Jau	Brazil
UFMG Hospital das Clínicas Servico de Transplante de Medula Óssea	Belo Horizonte	Brazil
Hospital Leforte Liberdade	São Paulo	Brazil
Hospital Erasto Gaertner	Curitiba	Brazil
Hospital de Clínicas de Porto Alegre	Porto Alegre	Brazil
Instituto de Oncologia Pediátrica – GRAACC	São Paulo	Brazil
Instituto de Cardiologia do Distrito Federal - Unidade de TMO Pietro Albuquerque	Brasilia	Brazil
Natal Hospital Center	Natal	Brazil
Hospital Universitario da Universidade Federal de Juiz de Fora	Juiz de Fora	Brazil
Instituto da Criança - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ITACI)	São Paolo	Brazil
Instituto Nacional de Câncer	Rio de Janeiro	Brazil
Hospital de Clínicas – UFPR	Curitiba	Brazil
Fundação Pio XII - Hospital de Câncer de Barretos	Barretos	Brazil
Hospital Samaritano	São Paulo	Brazil
Albert Einstein Hospital	São Paulo	Brazil
Hospital Sírio Libanês	São Paulo	Brazil
Hospital São Camilo	São Paulo	Brazil
Federal University of Ceará	Fortaleza	Brazil
Complexo Hospitalar de Niterói	Niterói	Brazil
Centro de Pesquisas Oncológicas Dr. Alfredo Daura Jorge (CEPON)	Florianopolis	Brazil
IBCC - Instituto Brasileiro de Controle do Câncer	São Paulo	Brazil
CTMO-HCFMUSP	São Paulo	Brazil
Real e Benemérita Sociedade de Beneficiência Portuguesa de São Paulo	São Paulo	Brazil
Hospital Universitario Clementino Fraga Filho, Univ. Fed. RJ	Rio de Janeiro	Brazil



Source: https://www.cibmtr.org/Meetings/Materials/CRPDMC/Pages/2020-Clinical-Research-Professionals--Data-Management.aspx
Source: https://www.cibmtr.org/About/WhoWeAre/Centers/Pages/index.aspx?country=Brazil

Figure 2 - Statement Values: Mission, Vision and Values



Source: https://www.cibmtr.org/Meetings/Materials/CRPDMC/Pages/2020-Clinical-Research-Professionals--Data-Management.aspx

Figure 3 - Methods: Timeline of Actions, 2016 - 2019

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ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANT

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ABSTRACT

Graft versus host disease is one of the main complications of Hematopoietic stem cell, involving about 50% to 80% of the patients. Acute GVHD clinical manifestations and therapy is discussed, as well as new NIH criteria for the diagnosis and classification of chronic GVHD. Therapy for both refractory chronic and acute GVHD is an important field of discussion once there is no superiority for the majority of the agents after primary therapy has failed. Hence, this review is meant to be a useful tool of consultation for clinicians who are dealing with this complex complication.

Key words: graft versus host disease, hematopoietic stem cell transplant

INTRODUCTION

Nearly 50% of Hematopoietic Stem Cell Transplant (HSCT) patients develop graft versus host disease (GVHD), some of them severe, leading to mortality of up to 20% 1. Published data on incidence and severity of GVHD are heterogenous. However, there are estimates that 60-80% of long-term survivors have some GVHD activity and need long time immunosuppressive therapy after HSCT2.

ACUTE GRAFT VERSUS HOST DISEASE (AGVHD):

Diagnosis

In 2005, National Institute of Health (NIH) Consensus document on cGVHD was published with the objective of standardize diagnosis and classification of this disease. [3] The new classification proposed the categories of classic acute GVHD for those patients who developed the characteristic inflammatory syndrome generally before day +100 after HSCT, and the category of late, persistent or recurrent acute GVHD for those patients with no clinical signs of chronic GVHD who developed acute GVHD symptoms after day +100 [3].

Epidemiology and risk factors

Risk factors for aGVHD were identified by several studies as follows: HLA mismatch, alternative donors (unrelated or haploidentical donors), sex disparity (especially female donor to male patient), myeloablative conditioning regimen, immunophrophylaxis, stem cell source (peripheral blood) [4].

Acute GCHD Clinical Picture

Skin, gastrointestinal tract (GI) and liver are the main involved sites affected by aGVHD. Skin is usually the first organ noticed, presenting as a maculopapular erythema frequently involving palms, soles and ear pavilions as well as nape and shoulders. It can disseminate by all over the body surface becoming confluent and pruriginous, sometimes even painful. On severe forms it looks like Stevens-Johnson syndrome, with epidermal necrosis and bullae formation. Superior (esophageal and gastric mucosa) or lower GI tract (bowel) can be involved. Clinical presentation varies from anorexia, nausea and vomiting to severe diarrhea usually accompanied by abdominal pain, and sometimes bleeding. [5] Severe lower GI tract is associated to mortality and lower overall survival in those patients. Stool volume can be higher than 10 liters in 24 hours. 6 Hepatic aGVHD occurs generally in patients with skin or GI involvement, but it can be rarely isolated. Hepatic and canalicular enzymes (AST, ALT, predominantly conjugated bilirubin, alkaline phosphatase and gamma glutamyl transferase) are elevated. [7]

Acute GVHD staging and classification

The first staging system for acute GVHD was published in 1974 by Glucksberg *et al.* [5] Each organ was evaluated according to the severity of involvement and resultant data were globally graded. [5,7] After two decades this classification was reviewed by Keystone aGVHD Consensus, which confirmed the predictive value of severe acute GVHD (II to IV) for mortality rate at day 100. Furthermore, upper GI acute GVHD was recognized and for lower GI involvement, bleeding and severe pain were included as grade IV aGVHD together with stool volume (table 1). [8]

AGVHD staging is important for evaluating therapy response and correlates to overall survival after HSCT as mentioned before. [9] Patients who develop grade II-IV aGVHD have lower overall survival compared to mild ones (gradeI). Upper GI involvement has better prognosis and better response to low dose steroids than lower GI acute GVHD. Moderate and severe GVHD are seen in up to 40% of allogeneic HSCT. [10] Recently an international Consortium for diagnosis and staging of acute GVHD, Mount Sinai Acute GvHD International Consortium (MAGIC), revised those criteria based on a very robust database. Main changes were definition of GI Grade IV acute GVHD and upper GI acute GVHD. This turned out to be a very important instrument for multicentric transversal and prospective studies and is now recommended by CIBMTR, EBMT and NIH for the classification of acute GVHD. Correlative studies with biomarkers were also possible using this platform. [11,12]

ACUTE GVHD THERAPY

The choice of initial therapy for acute GVHD depends on organs involved, severity of symptoms, and weight of graft versus leukemia effect on the individual clinical context.

Grade I acute GVHD

Mild acute GVHD (grade I) therapy consists mainly of using topical agents along with optimization of phrophylaxis (serum level of calcineurin inhibitor adjustment). Adjuvant therapy as antihistaminic medications can also be used for pruritus control. The is no indication for systemic therapy. [13]

Grade II-IV acute GVHD

Patients with moderate acute GVHD (grade II-IV) should receive methylprednisolone (MP) or prednisone at a dose of 2mg/kg/day. This has been the standard therapy for decades and its effect is related to both lymphocytic and anti-inflammatory effect. [13] At the same time, drugs used as immunophrophylaxis as cyclosporine or tacrolimus should not be discontinued. In a retrospective study of 733 patients, use of MP at lower doses as 1mg/kg/day was effective and not harmful for those patients with acute GVHD grade I-IIa. Dose could be escalated to 2mg/kg/day when necessary, if symptoms worse within 72 hours. For this subgroup survival was comparable and this approach allowed the use of 50% steroid doses.14 Use of non-absorbent steroids (beclomethasone e budesonide) has also been used as adjuvant therapy together with systemic steroids for upper or lower acute GVHD. [15,16] About 60% of patients respond to initial therapy but some of these responses are not durable. [17]

Secondary therapy for acute GVHD grade II-IV

Progression after three days, no improvement after seven days or no resolution after 14 days of therapy with MP 2mg/kg/day associated to calcineurin inhibitor defines steroid refractory acute GVHD and a second line therapy is indicated. Refractory acute GVHD has poor prognosis as secondary therapy is unsuccessful. One year overall survival for this population was only 20-30%. [18]

Few prospective studies have been published with second line agents, but results are hardly comparable due to high heterogeneity among drugs, centers and approaches. Bad quality historical controls are additional obstacles, partially due to low accuracy on grading initial acute GVHD. As there is no superiority of any agent, choice should be guided by factors as previous therapy effects, drug interaction, availability, costs and team experience. Generally, mean response rate is about 50%, and median survival of about 60% at six months after therapy. [18] Main results published with these agents are summarized below.

Mycophenolate Mofetil (MMF)

MMF acts through inhibition of guanosine triphosphate synthesis, which is important for lymphocyte proliferation. MMF was one of four drugs tested as initial therapy added to MP at BMT CTN 0302, a randomized phase II trial. [19] However this association was not superior and had more adverse events when compared to standard therapy at a blinded phase III randomized trial (BMT CTN 0802). There was no difference in GVHD free survival or cumulative incidence of chronic GVHD in 12 months. [20] Retrospective studies using MMF as second line therapy show rates of complete and partial remission of about 77% in six months and is a clinical option in this scenario. [21, 22]

Extracorporeal Photopheresis (ECP)

ECP consists of irradiation of circulating lymphocytes collected by apheresis, incubated with the sensitizing agent 8-methoxypsoraleno, and exposed to UVA phototherapy. It induces apoptosis of all cells, including activated T-cells after 24 hours. Reinfusion of these apoptotic cells stimulates antigen presenting cells (APCs), which regulate immune homeostasis through production of anti-inflamatory cytokines and regulatory T cell recruitment and expansion, inducing immune tolerance. [23]

In 2006 a prospective phase II study was published evaluating ECP and included 59 patients with severe steroid refractory or steroid dependent acute GVHD. Complete responses were seen in 82% of patients with cutaneous involvement, 61% of those with liver GVHD and 61% of patients with GI GVHD. [24] No opportunistic infections were observed and there was no increment of relapse rate. [24]

Antithymocyte globulin (ATG)

These polyclonal antibodies are the most frequently used all over the world for acute GVHD second line therapy. Although there is large experience with this agent for more than three decades, response rates are achieved in only 20 to 30% of the patients, with poor overall survival of less than 10%. Better responses are seen in cutaneous acute GVHD. [25,26]

Anti IL-2 receptor monoclonal antibodies (basiliximab, daclizumab)

These drugs bind to alpha subunit of interleukin 2 receptor (CD25) at activated T lymphocytes. Basiliximab is a chimeric antagonist of IL-2 receptor and has been successfully used as secondary therapy for acute GVHD, achieving up to 71% of overall response rate in a phase I trial published in 2002 with 17 patients 27. Funke et al published in 2005 a single center experience in 34 patients with severe acute GVHD (grade III-IV) refractory to steroids. Overall response rate was 80%, and five years overall survival was 30%.28

Tumoral Necrosis Factor Antagonists (Infliximab, Embrel)

These drugs are frequently used as therapy for severe acute GVHD refractory to steroids and involving lower GI tract18. The biggest experience was published by Couriel and cols29, who studied 37 patients diagnosed with refractory acute GVHD and achieved an overall response rate of 70%.

Ruxolitinib

Formely used as therapy for chronic myeloproliferative disorders (primary myelofibrosis and Policytemia Vera), ruxolitinib inhibits γ-interferon receptor signalizing. With this rational, it started also to be tested in GVHD patients. Janus kynases. (JAKs) are important effectors in all three recognized acute GVHD pathogenetic phases. Ruxolitinib blocks cytokine production and signalization and regulate development and function of T cells and APCs. Furthermore, Jak-STAT inhibition in pre-clinical models reduced GVHD without compromising GVL. [30, 31]

Two studies were performed sequentially using ruxolitinib as a 2-line therapy for refractory GVHD (grade II-IV). The first called REACH1 (phase II, prospective, single arm) reported a global response (RC + RP) of 54.9% at D28 in a cohort of 49 patients. The dose ranged from 10 to 20mg / day, with cytopenias and viral reactivation being the main toxicities to the drug. still in need of treatment 31. This study also evaluated overall survival at D180 and reached the percentage of 73% with only 11 patients (15%) still in need of treatment after six months. [32]

In 2019, ruxolitinib became the first (and only to date) treatment approved by the FDA (Food and Drug Administration) for corticosteroid refractory aGVHD.

The second study, called REACH 2, was published in April 2020 and was the third phase 3 study described in the literature. It consisted of a multicenter, randomized trial comparing the efficacy and safety of the use of ruxolitinib in the oral dose of 20mg with a control arm that consisted of one of nine therapies frequently used to rescue corticosteroid disease (this treatment was of the investigator's choice). A total of 309 patients were randomized and the overall response rate at D28 was significantly higher in the ruxolitinib group compared to the control group (62% vs 39%, odds ratio, 2.64, 95% CI 1.65 to 4.22, P <0.0001). The lasting global response in D56 was also significantly higher in the inhibitor group (40% X 22%). When extending the follow-up period to 6 months (180 days), a 10% loss of therapeutic response can be observed in the ruxolitinib group compared to 39% in the control. [33]

4. CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD):

GVHD is the major cause of late allogeneic HSCT morbidity and mortality, occurring in 30-70% of patients. [34] The 2-year cumulative incidence of cGVHD, as defined by the National Institute of Health (NIH) criteria, after allogeneic HSCT with bone marrow or peripheral blood from related or unrelated donors, in a study that assessed risk factors for aGVHD and cGVHD was 34% (range 32% -35%). [35] The clinical manifestations of cGVHD can be restricted to a single organ or can be disseminated, with a profound impact on quality of life. The pathophysiology of cGVHDc involves inflammation, cellular and humoral immunity and fibrosis. [36] This immunological complication resembles autoimmune diseases with clinical manifestations of collagen vascular diseases, such as oral lichen planus, sicca keratoconjunctivitis, xerostomia, polyserositis, esophagitis and esophageal stenosis, ulceration and vaginal stenosis, intrahepatic obstructive liver disease, obstructive lung disease, scleroderma, fasciitis and myositis. Clinical manifestations almost always appear in the first two years after transplantation. [36]

Chronic GVHD diagnosis and differentiation from acute GVHD

There were few changes from 2005 NIH consensus to 2014 NIH Consensus on chronic GVHD. [4,37] Acute GVHD includes two categories : (1) Classic acute GVHD, usually within 100 days of HSCT and with no diagnostic or distinctive signs of chronic GVHD; (2) Late, persistent or recurrent acute GVHD, also without signs of chronic GVHD (cGVHD) but occurring later than 100 days of HSCT. For both 2005 and 2014 Consensus documents, chronic GVHD consisted of one of two categories: (1) classic cGVHD, with no signs of acute GVHD; (2) Overlap syndrome, where the patient has concomitant diagnostic or distinctive signs of cGVHD and inflammatory manifestations (liver or GI activity, skin erythema). This entity was better defined at 2014 consensus. [37] Clinical manifestations, either than timing, will determine the diagnosis of acute or chronic GVHD. Diagnostic symptoms or signs of chronic GCHD are enough for diagnosis, without the need for biopsy or other tests from other organs involved. Lichenoid changes and sclerosis are diagnostic for chronic GVHD. Distinctive signs are defined as those commonly found in chronic GVHD but there is the requirement of a biopsy for confirmation (e.g ocular sicca, vitiligo). [37] For cGVHD diagnosis is necessary at least one diagnostic manifestation or at least one distinctive manifestation confirmed by biopsy or lab tests, specialist evaluation (ophthalmologist, gynecologist) or radiology images, at the same or in another organ, except when contra-indicated. [37]

Clinical organ scoring system

The scoring system of the 2005 consensus 4 was modified based on the available evidence, or lack of it, and the doubts generated by the researchers and the clinical practice. [37] The organs considered for scoring include skin, mouth, eyes, GI tract, liver, lungs, joints, fascia and genital tract. Each organ or location is scored on a 4-point scale (0-3) with 0 representing non-involvement and 3 reflecting serious impairment. Several studies have shown that the global severity, by 2005 NHI criteria, at diagnosis, is associated with overall survival and TRM. Some elements of the score were validated as measures of quality of life. [37] The mild, moderate, and severe description of reflects the degree of impact and functional impairment, in each organ or location, due to cGVHD. Tables 2 and 3 summarize NIH consensus clinical score system and global scoring for GVHD.

Chronic GVHD therapy

Mild asymptomatic GVHD can often be managed with local treatment (eg, topical corticosteroids for skin involvement). In patients with three or more organs, or with a score of 2 or higher, in any organ, systemic treatment should be considered. Although it is associated with a lower relapse rate, cGVHD remains the main cause of late morbidity and mortality in HSCT recipients. [38] The frequent involvement of several organs and the pleomorphic clinical picture of this complication require multidisciplinary management, which includes, in addition to several medical specialties, nutrition, physiotherapy, psychological, dental, social and occupational therapy. [39] Periodic assessment of quality of life is recommended in patients with cGVHD, representing an efficient instrument for response to treatment. [40]

Mild chronic GVHD therapy

The mild symptomatic form should generally only be treated with topical agents, but data as the underlying disease (malignant or non-malignant) and its status at transplant, presence of high risk factors for mortality associated with GVHDc (thrombocytopenia, progressive onset of the disease) should be considered. [39] In addition, manifestations of mild GVHD that do not respond satisfactorily to topical treatment, such as hepatic GVHD or fasciitis, can be treated with isolated corticotherapy. [39]

MODERATE AND SEVERE CHRONIC GVHD THERAPY

First line therapy

The criteria defined by NIH Consensus for systemic treatment include: scores >2 in one organ, involvement of three or more organs and mild GVHD with high risk characteristics (platelet counts <100,000 / mm3 and use of immunosuppressants for the diagnosis of GVHD). [37]

The standard initial systemic treatment consists of prednisone 1 mg / kg / day and cyclosporine (CSA) 10 mg / kg / day divided into 2 doses, administered orally, with a dose of CSA adjusted by the plasma level. [41] Tacrolimus has also been used to replace cyclosporine with similar responses. Withdrawal should be initiated, if there is a response or stable manifestations, after two weeks of treatment, reducing the dose of prednisone by 25% each week until reaching, in 6 to 8 weeks, the target dose of 1 mg / kg every other day, which should be maintained for 2 to 3 months in cases of non-complete response, severe forms or the presence of risk factors. Then, reduce 10 to 20% per month until total suspension in 9 to 12 months according to tolerance. [41]

Steroid refractory GVHD is defined by disease progression after 2 weeks of therapy (prednisone at a dose of 1 mg / kg / day); stable disease using prednisone (> 0.5 mg / kg / day) for 4-8 weeks or inability to reduce the prednisone dose below 0.5 mg / kg / day.49 Indications for second-line treatment include worsening manifestations of GVHD in an organ primarily involved, absence of any response after one month of treatment, or inability to reduce the dose of prednisone below 1 mg / kg / day within 2 months. [41]

Second line therapy

Several therapeutic options have been tested in patients with GVHD refractory to first-line treatment. The choice of treatment, therefore, will depend on the toxicity pattern of the medication chosen, the organs involved, the patient's preference and the availability of the transplant center. [41]

The main agents used in the treatment of refractory GVHD are selected in table 4 and summarized below.

Extracorporeal Photopheresis (ECP)

ECP has been widely used as a second-line therapy for muco-cutaneous cGVHD, with complete response rates above 80% and significant improvement in cGVHD with sclerosis. Recently, Flowers et. Al42 reported results of a prospective randomized double-blind phase II study in 95 patients with steroid refractory, dependent, or intolerant cGVHD treated with FEC in combination with conventional immunosuppressants. There was no significant difference in the improvement of the total skin score (TSS) at week 12, however, a higher rate of complete and partial responses of GVHD in the skin was observed in the ECP arm compared to the control arm; more patients in the ECP arm had at least a 50% reduction in the dose of steroids and at least a 25% reduction in total skin score (TSS) in week 12.42 In the extension study, the group undergoing FEC had a significant improvement in skin score at week 24 when compared to the group without intervention. [43] FEC has the advantage of not increasing the risk of infections and having few adverse effects.

Mycophenolate Mofetil (MMF)

This immunosuppressant, whose prodrug, mycophenolic acid, interferes with purine synthesis and produces cytostatic effect on T and B lymphocytes, is frequently used in rescue therapy for refractory cGVHD. Global response rates vary between 23 and 79% of patients in several case series.44 Lopez et. Al45 reported in 2005 the largest series of cases with 35 patients with steroid refractory cGVHD. There was 79% overall response and 35% complete responses. Seventy-three percent of patients were able to discontinue immunosuppression after adding this drug and only 3% of treated patients discontinued due to toxicity.

Rapamicin (mTOR) mammalian receptor inhibitors: sirolimus

These drugs combine immunosuppressive effects and antiproliferative properties in fibroblasts and smooth muscle fibers. There are reports of antineoplastic effects. Sirolimus and everolimus, bind to mTOR forming a complex that induces the cell cycle to stop in G1 by inhibiting transcription, DNA translation and protein synthesis. In contrast to calcineurin inhibitors, these drugs promote the generation of regulatory T cells. [46]

Jurado et. Al47 published a case series in 2007 of 47 patients who used sirolimus as a secondary treatment in combination with other drugs. The overall response rate was 81% with 38% complete responses; 47% of these patients discontinued immunosuppression and the overall survival was 57% in three years. Couriel et. Al48 also reported their experience with sirolimus as rescue therapy in 35 patients with cutaneous and visceral GVHD. There was a 63% global response, 17% of which were complete and 34% of patients discontinued immunosuppression. The overall 2-year survival was 41%.

Rituximab

Rituximab binds to the extracellular portion of the CD 20 surface molecule and induces complement-mediated apoptosis and cell death, either directly or through normal or neoplastic B cells. [49] Cutler et. Al50 carried out the first prospective phase I-II study reporting the efficacy of rituximab (375 mg / m2) in 21 patients receiving a total of 38 cycles. Objective responses were observed in 70% of patients allowing for a significant reduction in the steroid dose. Patients with cutaneous or musculoskeletal manifestations of cGVHD showed a better response. VonBonin et. Al51 used lower doses of 50 mg / m2 / week for 4 weeks in 11 patients with refractory GVHD and 2 with post-transplant autoimmune disorders (immune glomerulonephritis and thrombocytopenia), observing an overall response rate of 69%, including 3 patients (23 %) with complete remission (CR). Recently, Arai et. Al52 published a prospective randomized study comparing imatinib and rituximab. Significant clinical response was observed in 9 of 35 (26%, 95% CI: 13-43%) participants randomized to imatinib and 10 of 37 (27%, 95% CI: 14-44%) randomized to rituximab.

Imatinib

Imatinib, an inhibitor of several kinases used successfully in BCR-ABL positive malignancies, has recently been used to treat cGVHD based on its antifibrotic activity by blocking the platelet-derived growth factor (PDGFR) receptor and growth factor beta transformation (TGFß). [53] The main adverse events with this drug include hematological toxicity, water retention, dyspnea, leading to discontinuation of the drug in 15 to 25% of patients. Responses were observed in 50% to 80% of patients with cutaneous, occular and intestinal involvement by cGVHD over a six-month period. In cases of pulmonary involvement, the best responses were seen in mild bronchiolitis. [53]

Low dose Methotrexate

MTX is an antimetabolite that in low doses has immunomodulatory and anti-inflammatory properties. Giaccone et al54 reported 71% (10/14) of refractory GVHD control, with a prednisone reduction to dose <1 mg / kg / alternate days, with a 7.5 mg / m2 / week MTX regimen in patients with refractory, long-term GVHD with 5 sites affected on average. At a median follow-up 25 weeks, overall survival rate was 92.8%, and no grade III / IV toxicity was observed. A more recent series of 27 children with refractory GVHD (17 with chronic form) treated with MTX doses of 3-10 mg / m2 / week showed 58.8% overall response, with prednisone suspension on 7/17 and reduction (dose <0.4 mg / kg) in 9/17 patients. [55]

Ibrutinib

Ibrutinib is a drug of the tyrosine kinase enzyme inhibitor class, with recognized activity in the Bruton's tyrosine kinase (BTK) pathway which is predominantly expressed in the B lymphocytes. It inhibits signal transduction from the B cell receptor leading to blockade of the B cell receptor activation, interruption of the cell cycle and apoptosis. Its role in the treatment of lymphoproliferative malignancies of lineage B had already been recognized through several clinical studies and its use had been approved since 2017 by the FDA.

In addition to the BTK inhibition described above, ibrutinib is also able to alter the function of T lymphocyte through the inhibition of tyrosine kinase stimulated by IL-2 (ITK) which comes to reach T lymphopoiesis. Thus, it leads to decreased cell activation and proliferation of T lymphocytes, in addition to a decrease in the release of inflammatory cytokines. [56]

The rationale for the use of ibrutinib has led to its applicability as rescue therapy in refractory GVHD. To date, the only therapy approved for GVHD as 2-line by the FDA and recently also approved by ANVISA. A phase 1b / 2 study published by Miklos *et al* in 2017 used the daily dose of 420mg in a cohort of 42 patients with a median time to initial response of 87 days. The overall response rate was 67% and 21% had CR therapy. About 70% of patients showed sustained clinical response for> 5 months after starting the drug. In general, 26 patients in the cohort (62%) achieved a reduction of corticosteroids dose to <0.15mgkg per day during the study. [56]

Ruxolitinib

The good results observed in GVHD with this agent has stimulated the industry and the results of a prospective phase III trial currently being recruited are awaited. The Spanish Group of Hematopoietic Transplantation and Cell Therapy (GETH) published in 2020 the results of a retrospective study with 56 heavily treated patients, with median of three previous therapies (1-10). The overall response rate was 57.1% in this adverse clinical group. There was gradual steroid taper and OS was 81% after 1 year of follow-up. [57]

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Table 1 - Comparison of the different guidelines available for acute GvHD assessment: individual organ severity staging12

Organ Severity Stage	Original Glucksberg criteria	"Modified Glucksberg" or "Keystone" criteria and IBMTR criteria	MAGIC criteria
Skin 0 1 2 3 4	No rash Rash < 25% of BSA Rash 25% to 50% of BSA Rash > 50% of BSA Generalized erythroderma with bullous formation	No rash Rash < 25% of BSA Rash 25% to 50% of BSA Rash > 50% of BSA Generalized erythroderma with bullous formation	No rash Rash < 25% of BSA Rash 25% to 50% of BSA Rash > 50% of BSA Generalized erythroderma with bullous formation
Liver 0 1 2 3 4	Total serum bilirubin < 34 μmol/L (< 2 mg/dL) or AST/SGOT 150-750 IU Total serum bilirubin 34-50 μmol/L (2 to 3 mg/dL) Total serum bilirubin 51-102 μmol/L (3.1 to 6 mg/dL) Total serum bilirubin 103-255 μmol/L (6.1 to 15 mg/dL) Total serum bilirubin > 255 μmol/L (> 15 mg/dL)	Total serum bilirubin < 34 μmol/L (< 2 mg/dL) Total serum bilirubin 34-50 μmol/L (2 to 3 mg/dL) Total serum bilirubin 51-102 μmol/L (3.1 to 6 mg/dL) Total serum bilirubin 103-255 μmol/L (6.1 to 15 mg/dL) Total serum bilirubin > 255 μmol/L (> 15 mg/dL)	Total serum bilirubin < 34 μmol/L (< 2 mg/dL) Total serum bilirubin 34-50 μmol/L (2 to 3 mg/dL) Total serum bilirubin 51-102 μmol/L (3.1 to 6 mg/dL) Total serum bilirubin 103-255 μmol/L (6.1 to 15 mg/dL) Total serum bilirubin > 255 μmol/L (> 15 mg/dL)
Upper GI 0 1	NA NA	No persistent nausea and no histologic evidence of GvHD in the stomach or duodenum Persistent nausea with histologic evidence of GvHD in the stomach or duodenum	No or intermittent anorexia or nausea or vomiting Persistent anorexia or nausea or vomiting
Lower GI 0 1 2 3 4	Diarrhea < 500 mL/day Diarrhea > 500 mL/day Diarrhea > 1000 mL/day Diarrhea > 1500 mL/day Diarrhea >2000 mL/day	Diarrhea < 500 mL/day Diarrhea > 500 mL/day Diarrhea > 1000 mL/day Diarrhea > 1500 mL/day Severe abdominal pain with or without ileus	Diarrhea < 500 mL/day or < 3 episodes/day for adultsb,c Diarrhea 500-999 mL/day or 3-4 episodes/day for adultsb,d Diarrhea 1000-1500 mL/day or 5-7 episodes/day for adultsb,e Diarrhea >1500 mL/day or >7 episodes/day for adultsb,f Severe abdominal pain with or without ileus or grossly bloody stools (regardless of stool volume)
Karnofsky Index	>30% <30%		

AST (Aspartate transaminase); BSA (Body surface area); GI (Gastro-intestinal tract); GvHD (Graft versus Host Disease); IBMTR (International Bone Marrow Transplantation Registry); IU (International units); MAGIC (Mount Sinai Acute GvHD International Consortium); NA (Not applicable); SGOT (Serum glutamic oxaloacetic acid transaminase)
aTo be suggestive for GvHD: anorexia should be accompanied by weight loss, nausea should last at least 3 days, or be accompanied by at least 2 vomiting episodes per day for at least 2 days [16]
bOne episode of diarrhea is considered to be about 200 ml for an adult and 3 ml/kg for a child (< 50 kg) [16]
cDiarrhea <10 mL/kg/day or <4 episodes/day for children
dDiarrhea 10-19.9 mL/kg/day or 4-6 episodes/day for children
eDiarrhea 20-30 mL/kg/day or 7-10 episodes/day for children
fDiarrhea > 30 mL/kg/day or >10 episodes/day for children

Table2 - Scoring system for chronic GVHD37

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** Maculopapular rash/erythema Lichen planus-like features Sclerotic features Papulosquamous lesions or ichthyosis Keratosis pilaris-like GVHD	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
SKIN FEATURES SCORE:	No sclerotic features		Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** Deep sclerotic features "Hidebound" (unable to pinch) Impaired mobility Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** Hyperpigmentation Hypopigmentation Poikiloderma Severe or generalized pruritus Hair involvement Nail involvement *Abnormality present but explained entirely by non-GVHD documented cause (specify):*________________				
MOUTH *Lichen planus-like features present:* **Yes** **No** *Abnormality present but explained entirely by non-GVHD documented cause (specify):*________________	No symptoms	Mild symptoms **with** disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major limitation of oral intake

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* **Yes** **No** **Not examined**	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*__________				
GI Tract ***Check all that apply:*** Esophageal web/ proximal stricture or ring Dysphagia Anorexia Nausea Vomiting Diarrhea Weight loss ≥5%* Failure to thrive	No symptoms	Symptoms without significant weight loss* (<5%)	Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*__________				
LIVER	Normal total bilirubin and ALT or AP < 3 x ULN	Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	Elevated total bilirubin > 3 mg/dL
Abnormality present but explained entirely by non-GVHD documented cause (specify):				
LUNGS** **Symptom score**:	No symptoms	Mild symptoms (shortness of breath after climbing one flight of steps)	Moderate symptoms (shortness of breath after walking on flat ground)	Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: % FEV1 ☐	FEV1≥80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤39%
Pulmonary function tests Not performed *Abnormality present but explained entirely by non-GVHD documented cause (specify):*__________				

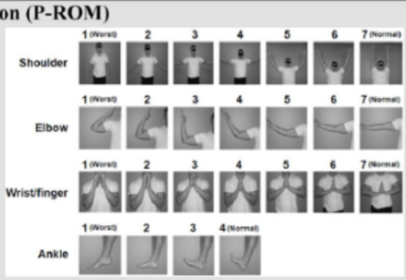
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*___				
GENITAL TRACT (*See Supplemental figure*‡) Not examined *Currently sexually active* Yes No	No signs	Mild signs‡ and females with or without discomfort on exam	Moderate signs‡ and may have symptoms with discomfort on exam	Severe signs‡ with or without symptoms
Abnormality present but explained entirely by non-GVHD documented cause (specify):				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

Ascites (serositis)___	Myasthenia Gravis___	
Pericardial Effusion___	Peripheral Neuropathy___	Eosinophilia > 500/µl___
Pleural Effusion(s)___	Polymyositis___	Platelets <100,000/µl ___
Nephrotic syndrome___	Weight loss>5%* without GI symptoms ___	Others (specify):___________

Overall GVHD Severity
(Opinion of the evaluator)
❑ **No GVHD** ❑ **Mild** ❑ **Moderate** ❑ **Severe**

Photographic Range of Motion (P-ROM)



† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

* Weight loss within 3 months.

**Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); ULN (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Figure).

Table 3 - GVHD severity

Mild chronic GVHD 1 or 2 organs involved plus Score 1 of involved sites plus Lung score 0 Severe chronic GVHD At least one organ with score 3 or Lung score 2 or 3	**DECH crônica moderada** 3 or more site plus score 1 of involved sites or At least one site (except lung) with score 2 or Lung score 1
Key Points: 1. On the skin: The highest score will be used to calculate the overall severity. 2. In the lung: FEV1 is used in place of the clinical score for the calculation of overall severity. 3. If an organ abnormality is unequivocally explained by a cause not associated with GVHD, the score of this organ will be zero for the calculation of global severity. 4. If an organ abnormality is attributed to multifactorial causes (GVHD plus other causes) the organ's score will be used to calculate the overall severity regardless of the contributing causes (the organ's score will not be disregarded).	

Table 4 - Secondary therapy for chronic GVHD

TREATMENT	% OVERALL RESPONSE	SURVIVAL
ECP	65-70	70%-78% at 1 y
Rituximab	66-86	72% at 1 y
Imatinib	22-79	75%-84% at 1.5 y
Mycophenolate mofetil	26-64	67%-96% at 1 y
MTOR inhibitor	76	72% at 3 y
Low dose methotrexate	71%	92,8% in 25 weeks
Ruxolitinib	57%	81% at 1 y
Ibrutinib	67%	Not reported

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COVID-19 AND HEMATOPOIETIC STEM CELL TRANSPLANTATION: RECOMMENDATIONS FROM THE BRAZILIAN SOCIETY OF BONE MARROW TRANSPLANTATION (SBTMO)

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INTRODUCTION

In December 2019, the World Health Organization (WHO) was notified about an outbreak of pneumonia of unknown etiology occurring in the city of Wuhan, China. DNA sequencing of clinical samples from Wuhan patients revealed a new coronavirus showing 82% sequence homology with severe acute respiratory syndrome coronavirus (SARS CoV) [1]. On January 31st 2020, the WHO declare the situation as a "public health emergency of international interest" due to the rapid expansion of the outbreak in China [2].

On February 12th, the novel coronavirus was named "severe acute respiratory syndrome coronavirus 2" (SARS- CoV-2) by the International Committee on Taxonomy of Viruses, and "COVID-19" the disease caused by SARS CoV-2 [3]. Initially limited to Wuhan, COVID-19 cases were increasingly reported in several Chinese cities and later in several countries. On February 26th, the first case of COVID-19 was identified in the city of São Paulo, in a 61-year-old patient returning from Italy, one of the countries hard hit by COVID-19 [4]. On March 12, 2020, WHO declared the outbreak as a pandemic [5].

Up to July 2nd, 2020, more than 10.7 million cases of COVID-19 have been confirmed worldwide, with over 515 thousand deaths. Currently, the United States, Brazil and Russia are the countries with the highest number of cases, with the USA being the country with the highest absolute number of deaths (https://coronavirus.jhu.edu/map.html).

Hematopoietic stem cell transplant (HSCT) recipients are beginning to be affected by COVID-19 in Brazil and worldwide. So far, it is not clear whether the disease is similar to that seen in immunocompetent individuals. Likewise, the risk factors for unfavorable outcomes have not been established yet. Data from the EBMT COVID-19 registry suggest less severity in children compared to adults and lethality rates of up to 30%, therefore higher than in the general population (https://www.ebmt.org/covid-19-webinars).

SARS COV-2 INFECTION AND COVID-19

In the general population, SARS CoV-2 infection may present asymptomatically (up to 30% of cases) [6], or as a mild, moderate, or severe disease [7]. Symptomatic disease develops in 3 phases: acute or early phase (2 to 6 days), progressive phase (7 to 19 days) and convalescence (10 to 33 days) [8]. The mean incubation period is 5 days, varying from 1 to 14 days, being around 12 days in 95% of cases. The most frequent symptoms are fever (89%), cough (72%), loss of taste (71%) and smell (68%), and myalgia (43%), among others. In general, COVID-19 cases present mild forms of the disease (80%), 15% may require hospitalization and 5% will need treatment in an intensive care unit (ICU) [11].

After the first week, patients with COVID-19 may enter the convalescent phase with progressive fading of symptoms, or walk into the progressive phase, with worsening of the clinical picture. Shortness of breath with decreased O2 saturation and increased respiratory rate are indicators of severe COVID-19 and were observed in 23% to 35% of the cases initially reported in China [7,9]. Severe disease can quickly evolve to critical conditions requiring mechanical ventilation. In patients who enter the critical phase of the disease lethality rates of 50% have been observed [12].

Overall lethality rates are around 6%, but exceeded 10% in some countries such as France (17.9%), Italy

(14.4%), and United Kingdom (14%) (https://coronavirus.jhu.edu/map.html). In adults requiring hospitalization, the risk factors for COVID-19 mortality are age over 60 years (OR = 1.1; p = 0.004); high SOFA score (OR = 5.7; p <0.01) and D-dimer> 1mg/mL (OR 18.4; p = 0.003) (13). According to some authors, SARS CoV-2 viral load can be up to 60 times higher in severe cases compared to mild ones, and could be used as a prognostic biomarker [13)]

Although still uncertain, some preliminary information from early cases of COVID-19 in HSCT recipients suggests that immunocompromised patients may develop a different form of the disease [14].

RESPIRATORY VIRUS AND HSCT

Although undesirable, respiratory viruses (RV) are well-known seasonal visitors in the HSCT scenario. RV are a major cause of morbidity and mortality in HSCT recipients and a set of preventive measures is necessary to control transmission.

HSCT staff and patients are familiar with the RV polices in HSCT wards and outpatient units. Among others, information about hand hygiene, cough etiquette, proper use of masks, and self-report of respiratory symptoms are part of continued education programs in most HSCT centers. This fact has represented an advantage over other health services during the COVID-19 pandemic, regarding the compliance with prevention practices.

The present document summarizes the Brazilian Society for Marrow Transplantation (SBTMO) recommendations for management of COVID-19 in the setting of HSCT.

HSCT CENTERS

In the absence of an effective vaccine, the main prevention strategy is to avoid exposure to SARS CoV-2. HSCT recipients, candidates and donors should limit their exposure to infected individuals as much as possible and strictly follow the prevention practices.

At this point, reinforcement of educative actions is recommended for staff, donors, candidates, relatives and recipients. Continued education staff is recommended to prepare educative materials with written information about COVID-19 pandemic, reminding the measures to control RV transmission, such as hand hygiene, cough etiquette, wearing masks and social distancing.

HSCT centers should have separate areas and teams for COVID-positive and COVID-negative patients, and follow the policies and procedures defined by national authorities, as well as local or institutional policies.

Non-urgent transplants should be postponed, especially for non-malignant diseases. Similarly, non-essential appointments should be canceled or use telemedicine, if possible. Visitors should be prohibited or restricted as much as possible.

HSCT centers should ensure availability of hematopoietic stem cells (SC) by freezing the product before the start of conditioning regimen. If not possible, have an alternative donor as a back-up. Peripheral blood should be preferred as a SC source, unless there is a strong indication for bone marrow.

STAFF

Health professionals should follow local, institutional and national COVID-19 protocols. The HSCT center should provide personal protective equipment (PPE) and have a trained staff on the management of suspected or confirmed cases of COVID-19.

Staff with respiratory symptoms should be tested for SARS CoV-2. In the event of a diagnosis of COVID-19, the health professional must be away for 14 days, and retested after this period. Resolution of symptoms and two negative PCR tests within a 7-day interval are necessary to return to work. Testing is also recommended in the event of contact with a suspect or proven case of COVID-19. Given the high frequency of asymptomatic infections, periodic testing of staff is recommended to assess the risk of transmission and to better organize the shifts.

Staff should consider wearing uniforms and change street clothes for hospital clothes once arriving in the HSCT unit. Masks are important to limit SARS CoV-2 spread and to reduce the risk for HCW to become infected. Surgical masks are recommended in the hospital, and N95 masks or similar and eye or face shields are mandatory in COVID-19 positive areas [15]. .

Due to the workload in stressful circumstances, the psychological impact of COVID-19 on staff should not be underestimated [16]. The health professional should have regular rest intervals, and receive support from the team regarding stress management to avoid caregiver fatigue. Staff should receive guidance on how to prioritize sleep, maintain good communication with their colleagues, contact with family members, and maintain physical and leisure activities.

DONORS

Donors within 28 days prior to donation should practice good hygiene, avoid unnecessary travels, crowded places and large group meetings. It is recommended that donors are tested for COVID-19 before starting the mobilization procedure.

Donors with COVID-19 must be excluded from donation for 3 months. In case of urgency, earlier collection can be considered if the donor is well, with a negative test and if there is no appropriate alternative donor. Careful risk assessment must be done on a case-by-case basis.

In the event of close contact with a person diagnosed with COVID-19, the donor should be deferred from donation for at least 28 days, and closely monitored for the development of COVID-19 [14].

HSCT CANDIDATES

Transplant candidates should minimize the risk of SARS CoV-2 infection, ideally through home isolation 14 days prior to conditioning. Avoid unnecessary hospital appointments. Candidates need to be tested for SARS CoV-2 pre-admission, regardless of symptoms. Result must be negative before starting conditioning.

HSCT should be postponed for 3 months in candidates with SARS CoV-2 infection or COVID-19. In case of high-risk disease, the transplant should be postponed 21-28 days and have 2 negative tests with an interval of 24 hours before admission.

In case of contact with a suspect or confirmed case of COVID-19, any procedure (mobilization, collection, conditioning) should be postponed for 14 days (preferably 21), and the candidate monitored for the appearance of symptoms. PCR test must be negative before transplantation.

HSCT RECIPIENTS

HSCT recipients should avoid exposure to infected individuals, and comply with prevention practices such as hand hygiene and social distancing. If travel is necessary, preference should be given to particular car, avoiding public transportation (metro, bus, train and flights).

All patients, regardless the presence of symptoms, should be tested for SARS CoV-2 before entering HSCT ward. Test for SARS CoV-2 should also be tested in case of contact with a suspected or confirmed case of COVID-19, and whenever respiratory symptoms are present. The PCR test should be repeated if there is a strong suspicion of COVID19 and the test is negative (false negative).

Patients with a positive test for SARS CoV-2 or other respiratory virus should be removed from rooms with laminar flow or rooms with HEPA filter and positive pressure, unless the ventilation can be turned off.

Patients who test positive for SARS Cov-2 in an upper respiratory tract sample should undergo chest CT and evaluation of oxygenation impairment. Due to the risk of transmission to the healthcare professional, bronchoalveolar lavage (BAL) is not recommended in case of COVID-19, unless co-infection is suspected.

Patients with COVID-19 should comply with contact and droplet precautions, and be kept in isolation for at least 14 days after the disappearance of symptoms. PCR should be repeated weekly to assess duration of shedding and possibility of precautions' clearance.

Diagnosis

Acute or early phase: The gold standard in the diagnosis of acute SARS CoV-2 infection is RT-PCR. Sensitivity is greatest if respiratory samples are taken between 4 to 10 days from the onset of symptoms. Sampling at the very beginning of the symptoms can lead to false negative results and the test must be repeated after 72 hours in suspected cases. SARS CoV-2 is more frequently detected in bronchoalveolar lavage fluid (93%). However, due to the high risk of professional exposure, the procedure has not been recommended. Other respiratory samples which show greatest positivity are sputum (72%), nasal swab (63%) and saliva (87%) [17,18])

Convalescence phase: RT-PCR may turn negative after the first week of symptoms, and the detection of SARS CoV-2 antibodies can help in the diagnosis of COVID-19. To date, few studies have evaluated the dynamics of specific IgM and IgG antibodies and the duration of immunity is not well known. IgG antibodies may be detected before the appearance of IgM, or decrease faster than IgM levels [19] , around the eighth week of the disease. Although sensitivity and specificity rates of commercial serological tests around 90% have been reported [18], it is important to highlight that this is a rapid evolving field and better second-generation tests are awaited.

TREATMENT

At this point, no clear recommendations can be made about specific therapies in severe cases of COVID-19 due to limited data and unknown risk versus benefit. Also, it is not known whether HSCT recipients with asymptomatic infection or mild cases of COVID-19 can benefit from any specific treatment.

Immunosuppression and treatment of bacterial, fungal or viral co-pathogens should be maintained.

So far, the management of COVID-19 in HSCT recipients should be based on:
1) Antiviral, preferably within a prospective controlled study. So far, no antiviral drug has shown significant clinical improvement when used in severe cases of COVID-19 [20,21], or has been studied in transplant recipients. Thus, no recommendation can be made at this point. 2) Monitoring of d-dimer for the possible occurrence of endothelial damage, and prophylactic use of heparin (if not contraindicated) to prevent thrombosis. 3) Diagnosis and management of the cytokine storm, by monitoring C-reactive protein, interleukin-6, ferritin, and having established protocols for the introduction of tocilizumab, corticosteroids, immunoglobulins or other drugs for this purpose (https://www.ebmt.org/covid-19-webinars).

Therapy with COVID-19 convalescent plasma has been recently approved in several countries. Convalescent plasma therapy has been shown effective in the influenza H1N1 pandemic, as well as in the 2003 SARS CoV-1 and 2012 MERS CoV epidemics. However, in severe or life threatening cases of COVID-19, a small open-label randomized trial of convalescent plasma did not show improvement in time for clinical response [22]

COVID-19 pandemic evolution

Leaving China, the pandemic initially reached large urban centers mainly through air transport and major highways. The lack of knowledge about the disease and the high transmissibility of SARS CoV-2, associated with lack of diagnostic tests, occurrence of asymptomatic cases, delay in the implementation of stricter preventive measures, shortage of personal protective equipment, among others, led to an unprecedented increase of COVID-19 cases worldwide.

In Brazil, once local transmission of SARS CoV-2 was established, a migration of COVID-19 cases to the periphery of large cities was observed, and then to cities in the interior of the states, where the number of hospital and ICU beds is smaller. Despite the actions developed to control viral transmission and prevent the overload of the health system, Brazil failed to contain the pandemic and is currently in second place in relation to the absolute number of cases and deaths by COVID-19 (https://coronavirus.jhu.edu/map.html).

Due to the great economic difficulties caused by the pandemic, many states have gradually resumed activities, which invariably leads to localized reappearance of COVID-19 cases.

HSCT centers must prepare for the resumption of activities gradually, always taking into account the local situation. Some items need to be checked before resuming HSCT activities (table1).

COVID 19 and main changes for HSCT centers in Brazil

We are experiencing a new reality. Many of our routines, staff, physical areas have been modified. Time will tell if forever, or until an effective vaccine or treatment for COVID-19 appears.

In the short term, the main changes for Brazilian HSCT centers are the testing of asymptomatic candidates, donors, patients and companions entering the HSCT unit; ensure increased testing to follow up COVID-19 cases; testing algorithms in case of exposure to suspected or confirmed cases; define protocols for the treatment and management of COVID-19 complications; ensure social distance in outpatient areas; review the need of having patients in support homes; development or improvement of continued education programs on respiratory virus.

Difficult times create opportunities for change. Each center has its peculiarities and should use creativity to adapt these proposals rationally and according to their reality and needs.

These recommendations may be modified at any time based on new information that emerges on the prevention of SARS CoV-2 and the management of COVID-19.

Table 1- Check list to resume HSCT center activities during COVID-19 pandemic

Decreasing rates of hospitalization and deaths due to COVID-19 in the region for ≥ 14 days	✓
Enough number of ICU and hospital beds in case of an eventual increase of COVID-19 cases	✓
Sufficient supply of PPE and diagnostic tests	✓
Staff trained to manage COVID-19 cases;	✓
Definition of priorities for patients' admission	✓
Ensure point of entry screen and social distancing in common areas	✓
Revisit hospital visitation policies	✓
Definition of telemedicine policies	✓
Ensure continued education on RV preventive measures to the staff, patients, candidates and household contacts	✓

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