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Dear transplant colleagues

In 2019 we celebrated the 40th anniversary of the first bone marrow transplant (BMT) 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 BMT-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 this volume of JBMCT, Journal of Bone Marrow Transplantation and Cellular Therapy, 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*

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CURRENT USE AND OUTCOMES OF HEMATOPOIETIC STEM CELL TRANSPLANTATION: BRAZILIAN SUMMARY SLIDES

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ABSTRACT

The first HSCT program in Latin America started in 1979 at the Federal University Hospital (Curitiba, Paraná). Over the years, the number of centers performing transplants in the country increased, generating the need to know the results of this modality of treatment. Understanding the HSCT scenario in Brazil is still challenging since not all Brazilian centers report data to the Center for International Blood and Marrow Research (CIBMTR). Although it has been improving over the last years, infrastructure and trained data managers are still lacking. The partnership between the Brazilian Cellular Therapy and Bone Marrow Transplant Society (SBTMO) and the CIBMTR, allowed the return of Brazilian data registered in the CIBMTR, through the Data Back to Center (DBtC), in a standardized and organized way. With this database it was possible to know the demographic data and the outcomes of transplants performed in Brazil. Between 2012 and 2021, complete information of 7,982 transplants were reported to the CIBMTR from 31 Brazilian transplant centers. The consolidation of the Hematopoietic Stem Cell Transplantation Brazilian Registry (HSCTBR) using CIBMTR infrastructure, allowed the Brazilian Summary slides development and update. Despite the difference in the number of cases and of follow-up time, the results in this study were similar to those presented in the US Summary Slides.

Keywords: Data Management. Hematopoietic Stem Cell Transplant. Research Report.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a therapy that can be the only option for curing many malignant and non-malignant hematological diseases, as well as extending the survival of many patients[1]. Brazil has a large HSCT program, with 126 teams in 86 transplant centers recognized by the Brazilian Ministry of Health.

The first national results on this treatment modality were published in 1985[2]. In 1997, a Brazilian center took part for the first time in an international multicenter study[3]. Over the following years, some national multicenter studies were developed. Back then, the initiatives for the creation of the Hematopoietic Stem Cell Transplantation Brazilian Registry (HSCTBR) had already begun[4].

Until the publication of the First Brazilian Summary Slides in 2021[5], the Brazilian Association of Organ Transplants (ABTO), created in 1995, was the only source of information about the number of Brazilian HSCT performed every year. According to ABTO, 3,826 transplants were performed in 2021: 1,547 allogeneic and 2,279 autologous[6].

According to the CIBMTR, a total of 295,682 autologous and 287,972 related and unrelated allogeneic transplants were reported around the world between 1970 and 2021[7]. Despite the existence of the first summary slides[8], the HSCT scenario in Brazil is still challenging, because not all Brazilian centers report data to the CIBMTR and there is a lack of infrastructure and trained data managers (DM). Therefore, over the years, through a working group composed of physicians and DM and with the collaboration of the CIBMTR and the SBTMO, strategies such as continuing education in data management and communication channels were developed to support DM and centers in affiliation process. These actions favor the increasing numbers of registered and active Brazilian centers in the CIBMTR[9].

The partnership between SBTMO and CIBMTR allowed access through the tools available in the registry, such as the DBtC, which allows the return of the data sent by the Brazilian transplant centers to CIBMTR. Part of the data inserted can return to the centers registered in a standardized and codified way, allowing the analysis of the outcomes of transplants performed in the country. The consolidation of the HSCTBR using CIBMTR infrastructure and the accessibility to these data is fundamental for public health administration.

OBJECTIVE

Our objective is to understand the demographic data and the outcomes of transplants performed in Brazil using the DBtC tool to retrieve the data registered in the CIBMTR in a standardized and organized way. Furthermore, make the data available to HSCT centers and maintain a routine to update the results.

METHODS

Data from 8,197 transplants performed between 2012 and 2021 were extract from the CIBMTR portal using the DBtC, with information from transplanted patients in 31 Brazilian centers that sent their data to the CIBMTR. However, only 7,982 transplants had completed data for analysis (3,459 autologous and 4,523 allogeneic). For this reason, this was the total of HSCT considered in the analyses. The spreadsheet was imported into Power BI Desktop (PBI). Functions were updated to count the number of transplants performed and the number of participating centers, to translate some columns into Portuguese, to categorize disease classification, to group variables, and for calculating global survival analyses, and sheet relationships.

Patients were classified in pediatric (0-17 years of age) and adults (≥ 18 years of age). Allogeneic transplants were categorized as matched related donor, mismatch related donor (including haploidentical and related donors with one mismatch), and unrelated donor. Grafts were classified as Bone Marrow (BM), Peripheral Blood Stem Cells (PBSC) and umbilical cord blood (CB). The disease stage for acute leukemias was classified as 1st remission, 2nd or further remission and patients who underwent HSCT with active disease.

Patients with Myelodysplastic Syndrome (MDS) were divided into Early Stage, which is subdivided into refractory anemia (RA); refractory anemia with ring sideroblasts (RARS); refractory cytopenia with multilineage dysplasia (RCMD); and with MDS with del(5q) alone, or Advanced Stage, including refractory anemia with excess blasts (RAEB) and Chronic Myelomonocytic Leukemia (CMML). Patients with Lymphoma were categorized as chemosensitive and chemoresistant disease by the response to treatment prior to HSCT.

The classification of conditioning was based on the agents and doses used, Myeloablative Conditioning (MAC) for patients who received total body irradiation (TBI) ≥500 cGy in a single dose or >800 cGy in fractionated doses; busulfan >9 mg/kg oral or ≥7.2 mg/kg IV or melphalan >150 mg/m^2 as a single agent or in combination with other drugs. The other conditionings that did not fill the criteria for MAC were classified as Reduced Intensity/Non-Myeloablative (RIC/NMA)[10,11]. The causes of death were classified using the standard classification from DBtC. The main causes of death between 2017-2021 were separated between deaths 0-100 days and deaths >100 days up to 3 years after HSCT. For the analysis of overall survival (OS), patients who underwent 1st HSCT were selected, and those who were without follow-up update after transplantation or had error in survival time were excluded (table 1).

The charts were generated in the PBI and exported to PowerPoint for publication. Global survival analyses were performed by the Kaplan Meier method (Comparison between groups by long-rank test) using the R program (Version 4.1.0).

The use of this data was ethically enabled by the national Institutional Review Board (IRB) approval in 2019 (Conep CAAE: 65575317.5.1001.0071, principal investigator Dr. Nelson Hamerschlak).

RESULTS

Between 2012 and 2021, 7,982 transplants were reported from 31 transplant centers in Brazil (table 2), 16 (52%) located in the state of São Paulo; 4 in Paraná, 2 in Rio de Janeiro; 2 in Rio Grande do Sul; 2 in Minas Gerais and 1 center in each state: Ceará, Distrito Federal, Rio Grande do Norte, Pernambuco and Santa Catarina.

The number of CIBMTR active centers keeps increasing along the last years, reaching 26 active centers in 2020 (figure 1), which have contributed to the increase in the total number of Brazilian transplants registered in the CIBMTR since 2016, reaching 1,177 transplants in 2019. However, there was a decrease in the number of HSCT registered in 2020 and 2021, because of the Sars-CoV-2 pandemic (figure 2).

Between 2012 and 2021, 43.2% of the allogeneic transplants performed in Brazil used a matched related donor, followed by an unrelated donor (31.2%), and a mismatch related donor (25.6%). In the last 2 years, the main type of allogeneic transplant performed in the country used a mismatched related donor (figure 3).

Regarding the graft source for allogeneic transplants, BM was used in most pediatric transplants, while in adults the main source was PBSC from 2018 on (table 3).

Mismatched related donors were used to treat acute myelogenous leukemia (AML; 30.2%), followed by non-malignant diseases (25.7%) and acute lymphoblastic leukemia (ALL; 23.1%); 50.6% of them used MAC and 49.4% used RIC/NMA.

The main global indications for HSCT in Brazil between 2019-2021 were Multiple Myeloma (861;

26%), followed by AML (536, 16%), ALL (405; 12%), non-Hodgkin lymphoma (NHL; 383; 11%) and Hodgkin disease (HD; 336; 10%) (figure 4). In pediatric allogeneic HSCT, the main diseases were ALL (36%), other Non-Malignant (22%) and AML (18%). In adults, the main indications for allogeneic transplants were AML (35%), ALL (18%) and MDS (11%).

Acute leukemias continue to be the main indication for allogeneic transplantation, but from 2016 on, there was an increase in indications for MDS/MPN and Lymphomas. The main indications for autologous HSCT remain Multiple Myeloma and Lymphomas.

In patients with acute leukemias, 50.5% of those with AML and 46.7% with ALL were in the 1st remission. Most HSCT were from matched related donor in both AML (48.4%), as well as in ALL (38.5%) (table 4).

Infections were the leading cause of death in the first 100 days after all transplants: autologous (68%), matched related donor (54%), unrelated donor (57%), and mismatch related donor (61%). The most common cause of death more than 100 days after HSCT was the primary disease: autologous (67%), matched related donor (46%), unrelated donor (43%) and mismatch related donor (49%).

For the analysis of OS, the median follow-up was 23 months in allogeneic and 13 months in autologous HSCT. Patients with acute leukemia who underwent transplantation with advanced stage had lower survival rates compared to the other stages (table 5).

Adults had a better survival after HSCT from matched sibling donors when having HSCT for AML (p=0.085; figure 5) and ALL (p=0.008; figure 6), but donor source had no impact in pediatric patients with acute leukemias.

The 2-year survival for MDS was similar despite disease risk and donor source (figure 7). Patients with CML had a 2-year OS of 60.4% with a matched related donor, 51.0% with a mismatch related donor and 60.5% with an unrelated donor (p=0.712) (figure 8). Patients with Myelofibrosis had a survival of 61.4% in 2 years (figure 9). Donor source had no impact in children with Aplastic Anemia, different from adults who had a better survival after HSCT from matched sibling donors (p=0.002) (figure 10).

Patients undergoing autologous HSCT to treat chemosensitive Lymphomas had a significantly better 2-year OS than chemoresistant disease: 88.2% versus 74.7% in HD (p=0.038) and 75.3% versus 52.8% in NHL (p<0.001) (figure 11). In Multiple Myeloma, the 2-year OS was 82.0% (figure 12).

DISCUSSION

Our study, using DBtC data, demonstrated a greater number of allogeneic than autologous transplants reported to the CIBMTR, but according to ABTO there is a greater number of autologous transplants in the country. The explanation for this difference is due to the larger number of affiliated centers in the CIBMTR that perform allogeneic transplants.

We observed an increase in the number of transplants with mismatch related donor since 2012, and a decrease in unrelated CB transplants in the same period, probably due to the use haploidentical donors with cyclophosphamide after transplantation.

Comparing our data with the American summary slides published in the CIBMTR website[12], the matched related donor is the main type of transplants performed in Brazil, while in the United States (USA), it is unrelated BM/PBSC.

In pediatric patients, the main source was BM in Brazil, following the same trend in the USA; in adult, while in Brazil the use of PBSC has been increased over the years and has become the main source used since 2018, in the three modalities of allogeneic donors, in the USA the main source was PBSC since 2000.

In Brazil, in recent years, the main indications for HSCT were MM, AML, ALL, NHL, and HD, while in the USA in 2020 were MM, AML, NHL, MDS/MPN and ALL.

Another important comparison was the cause of early death, 0 to 100 days after transplantation: in Brazil, the main cause of early mortality was infection for autologous, matched related donor, mismatch related and unrelated donors, while in the USA, it was the primary disease for autologous and unrelated donors, and organ failure to matched and mismatch related donor.

Comparing the 2-year OS in our study with the 3-year OS in the US Summary Slides, the Brazilian data is similar to the survival rates reported by American centers (table 6), despite the socioeconomical differences.

The Brazilian Summary slides can be fully accessed by active centers in the HSCTBR, through the SBTMO data request flow (figure 13).

CONCLUSION

The partnership between SBTMO and CIBMTR made the HSCTBR possible through the availability of the DBtC. The analysis of the data from Brazil, allowed us to develop a Brazilian Summary Slides to better understand the transplants outcomes, making them available to centers as a national and international benchmarking. The Brazilian Summary is updated twice a year and published at the SBTMO website. Despite the difference in the number of cases and follow-up time, the results in this study were similar to those presented in the US Summary Slides.

The initiatives for the HSCTBR consolidation had positive results, such as the increase in the number of Brazilian centers affiliated to the CIBMTR and the qualification of DM. However, there is still a lot to be done. It is necessary to upgrade the commitment of the HSCT centers, in order to improve the registry of transplants, the accomplishment of long-term follow-up and the DM continuing education, stimulating the data quality improvement in the national registry. It is also essential to receive the support of the government (resources, infrastructure and qualification). The union of strength and perseverance will allow the consolidation of the HSCTBR, allowing the provision of better care to patients.

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TABLE 1. Exclusion criteria for overall survival

Exclusion criteria	n
Patients without follow-up update	1,186
Error in survival time	34
2nd HSCT or more	706

TABLE 2. HSCT centers

Participants Centers
A.C. Camargo Cancer Center
Albert Einstein Hospital
Associação Hospitalar Moinhos de Vento
Bio Sana's Serviços Médicos
Bio Sana's São Camilo
Centro De Pesquisa Clinica Hospital 9 De Julho
Centro de Pesquisas Oncológicas Dr. Alfredo Daura Jorge (CEPON)
Complexo Hospitalar de Niterói
CTMO-HCFMUSP
Fundação Pio XII - Hospital de Câncer de Barretos
Hospital Amaral Carvalho
Hospital de Clínicas - UFPR
Hospital de Clínicas de Porto Alegre
Hospital Erasto Gaertner
Hospital Leforte Liberdade
Hospital Nossa Senhora das Graças - IP
Hospital Pequeno Príncipe
Hospital Samaritano
Hospital Sírio Libanês
Hospital Universitario da Universidade Federal de Juiz de Fora
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Instituto da Criança - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ITACI)
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TABLE 3. Source of cells used by donor type, age and year of HSCT

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Patients <18 Years										
Matched Related Donor										
PBSC	2%	4%	2%	3%	9%	5%	9%	8%	3%	15%
BM	89%	80%	93%	94%	91%	93%	83%	90%	97%	85%
CB	9%	16%	5%	3%	0%	2%	8%	2%	0%	0%
Unrelated Donor										
PBSC	5%	3%	16%	13%	8%	8%	12%	4%	26%	28%
BM	55%	74%	78%	74%	84%	87%	80%	88%	70%	62%
CB	40%	23%	6%	13%	8%	5%	8%	8%	4%	10%
Mismatch Related Donor										
PBSC	24%	10%	28%	14%	29%	22%	33%	26%	23%	22%
BM	76%	90%	72%	86%	71%	78%	67%	74%	77%	78%
Patients ≥18 Years										
Matched Related Donor										
PBSC	49%	47%	43%	52%	46%	53%	53%	56%	64%	65%
BM	51%	53%	57%	48%	54%	47%	47%	44%	36%	35%
CB	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Unrelated Donor										
PBSC	40%	31%	39%	53%	50%	47%	58%	55%	57%	80%
BM	43%	62%	61%	43%	50%	53%	42%	44%	39%	20%
CB	17%	7%	0%	4%	0%	0%	0%	1%	4%	0%
Mismatch Related Donor										
PBSC	18%	33%	40%	36%	40%	42%	59%	67%	74%	73%
BM	82%	67%	60%	64%	60%	58%	41%	33%	26%	27%

TABLE 4. Acute Leukemia by disease stage, donor type and HSCT year

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
AML										
Disease Stage										
1st complete remission	36%	46%	48%	45%	59%	50%	52%	55%	52%	55%
2nd or subsequent complete remission	36%	26%	38%	41%	31%	30%	29%	25%	31%	25%
Relapsed disease/Never in CR	28%	28%	14%	14%	10%	20%	19%	20%	17%	20%
Donor Type										
Matched Related Donor	51%	58%	68%	48%	50%	50%	48%	46%	44%	38%
Mismatch Related Donor	16%	7%	8%	17%	22%	23%	29%	29%	40%	46%
Unrelated Donor (BM/PBSC)	28%	26%	20%	34%	28%	27%	22%	25%	16%	15%
Unrelated Donor (CB)	5%	9%	4%	1%	0%	0%	1%	0%	0%	1%
ALL										
Disease Stage										
1st complete remission	45%	42%	52%	59%	53%	42%	51%	39%	41%	46%
2nd or subsequent complete remission	51%	52%	40%	40%	37%	50%	34%	48%	49%	44%
Relapsed disease/Never in CR	4%	6%	8%	1%	10%	8%	14%	13%	10%	10%
Donor Type										
Matched Related Donor	44%	56%	50%	45%	42%	37%	38%	31%	33%	26%
Mismatch Related Donor	7%	2%	3%	7%	15%	26%	26%	29%	40%	51%
Unrelated Donor (BM/PBSC)	31%	34%	46%	42%	42%	37%	34%	35%	25%	22%
Unrelated Donor (CB)	18%	8%	1%	6%	1%	1%	2%	5%	2%	1%

TABLE 5. Overall survival of AML/ALL patients

	N	OS in 2 years (%)	p
AML			
Patients Age 0-17 Years			
Donor Type			
Matched Related Donor	69	48,9% (35,0-61,4)	
Mismatch Related Donor	56	63,3% (45,3-76,7)	0.440
Unrelated Donor	70	55,7% (42,2-67,2)	
Patients Age ≥18 Years			
Donor Type			
Matched Related Donor	439	54,6% (49,3-59,5)	
Mismatch Related Donor	188	43,2% (33,2-52,9)	0.085
Unrelated Donor	187	53,3% (45,0-60,9)	
Matched Related Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	34	54,4% (33,7-71,2)	
2nd or subsequent complete remission	23	50,6% (27,0-70,2)	0.756
Relapsed disease/Never in CR	12	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	294	63,7% (57,4-69,3)	
2nd or subsequent complete remission	82	37,2% (25,0-49,4)	<0.001
Relapsed disease/Never in CR	63	31,0% (18,4-44,4)	
Mismatched Related Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	20	74,8% (45,4-89,9)	
2nd or subsequent complete remission	25	70,3% (40,6-87,1)	0.992
Relapsed disease/Never in CR	11	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	107	49,0% (34,4-62,1)	
2nd or subsequent complete remission	53	47,1% (30,6-62,0)	0.003
Relapsed disease/Never in CR	28	10,5% (0,8-35,0)	
Unrelated Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	28	73,1% (48,4-87,3)	
2nd or subsequent complete remission	26	59,3% (37,1-75,8)	0.133
Relapsed disease/Never in CR	16	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	73	67,7% (53,9-78,2)	
2nd or subsequent complete remission	77	55,6% (42,8-66,7)	<0.001
Relapsed disease/Never in CR	37	18,3% (6,4-35,1)	

	N	OS in 2 years (%)	p
ALL			
Patients Age 0-17 Years			
Donor Type			
Matched Related Donor	105	60,4% (48,9-70,2)	
Mismatch Related Donor	93	46,1% (32,6-58,6)	0.149
Unrelated Donor	208	60,7% (53,0-67,5)	
Patients Age ≥18 Years			
Donor Type			
Matched Related Donor	260	57,0% (50,2-63,2)	
Mismatch Related Donor	110	47,4% (35,7-58,2)	0.008
Unrelated Donor	143	44,0% (34,8-52,7)	
Matched Related Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	32	71,9% (52,9-84,3)	
2nd or subsequent complete remission	58	51,9% (36,1-65,5)	0.405
Relapsed disease/Never in CR	15	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	194	66,0% (58,2-72,6)	
2nd or subsequent complete remission	54	30,1% (17,5-43,8)	<0.001
Relapsed disease/Never in CR	12	-	
Mismatched Related Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	17	75,5% (46,9-90,1)	
2nd or subsequent complete remission	67	42,7% (27,7-57,0)	0.232
Relapsed disease/Never in CR	9	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	65	57,2% (41,7-69,9)	
2nd or subsequent complete remission	38	39,3% (21,0-57,1)	0.233
Relapsed disease/Never in CR	7	-	
Unrelated Donor			
Patients Age 0-17 Years			
Disease Stage			
1st complete remission	62	73,7% (59,9-83,4)	
2nd or subsequent complete remission	127	57,1% (47,2-65,8)	0.021
Relapsed disease/Never in CR	19	-	
Patients Age ≥18 Years			
Disease Stage			
1st complete remission	84	48,0% (35,9-59,2)	
2nd or subsequent complete remission	49	40,3% (25,6-54,5)	0.233
Relapsed disease/Never in CR	10	-	

TABLE 6. Comparison overall survival – Brazil and USA

	Brazilian Registry (2012-2021)		US Summary Slides (2009-2019)	
	N	OS in 2 years (%)	N	OS in 3 years (%)
AML				
Matched Related Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	34	54.4% (33-71)	391	69% (65-74)
2nd or subsequent complete remission	23	50.6% (27-70)	133	68% (60-77)
Relapsed disease/Never in CR	12	-	75	30% (21-43)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	294	63.7% (57-69)	5,317	58% (57-60)
2nd or subsequent complete remission	82	37.2% (25-49)	1,226	54% (51-57)
Relapsed disease/Never in CR	63	31.0% (18-44)	1,721	31% (29-33)
Unrelated Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	28	73.1% (48-87)	368	66% (61-71)
2nd or subsequent complete remission	26	59.3% (37-75)	212	64% (57-71)
Relapsed disease/Never in CR	16	-	118	34% (26-44)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	73	67.7% (53-78)	7,441	56% (55-57)
2nd or subsequent complete remission	77	55.6% (42-66)	1,940	54% (52-57)
Relapsed disease/Never in CR	37	18.3% (6-35)	2,463	31% (30-33)
Mismatched Related Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	20	74.8% (45-89)	172	63% (56-72)
2nd or subsequent complete remission	25	70.3% (40-87)	99	61% (51-73)
Relapsed disease/Never in CR	11	-	71	37% (27-50)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	107	49.0% (34-62)	1,977	53% (50-55)
2nd or subsequent complete remission	53	47.1% (30-62)	572	55% (51-60)
Relapsed disease/Never in CR	28	10.5% (0,8-35)	706	28% (25-32)
ALL				
Matched Related Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	32	71.9% (52-84)	317	79% (74-84)
2nd or subsequent complete remission	58	51.9% (36-65)	464	70% (66-74)
Relapsed disease/Never in CR	15	-	38	57% (43-76)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	194	66.0% (58-72)	2,302	64% (62-66)
2nd or subsequent complete remission	54	30.1% (17-43)	640	45% (41-49)
Relapsed disease/Never in CR	12	-	249	37% (31-44)
Unrelated Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	62	73.7% (59-83)	312	80% (75-84)
2nd or subsequent complete remission	127	57.1% (47-65)	421	64% (60-69)
Relapsed disease/Never in CR	19	-	40	68% (54-84)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	84	48.0% (35-59)	2,425	64% (62-66)
2nd or subsequent complete remission	49	40.3% (25-54)	765	46% (43-50)
Relapsed disease/Never in CR	10	-	253	36% (30-42)
Mismatched Related Donor				
Patients Age 0-17 Years				
Disease Stage				
1st complete remission	17	75.5% (46-90)	137	75% (67-83)
2nd or subsequent complete remission	67	42.7% (27-57)	233	63% (57-70)
Relapsed disease/Never in CR	9	-	23	28% (14-57)
Patients Age ≥18 Years				
Disease Stage				
1st complete remission	65	57.2% (41-69)	771	69% (65-73)
2nd or subsequent complete remission	38	39.3% (21-57)	344	47% (42-54)
Relapsed disease/Never in CR	7	-	99	28% (20-39)
MDS (Adults)				
Matched Related Donor				
Disease Stage				
Low risk	91	58,9% (47-68)	677	52% (48-56)
High risk	90	55,8% (43-66)	1,693	46% (44-49)
Unrelated Donor				
Disease Stage				
Low risk	43	52,3% (35-66)	1,133	49% (46-52)
High risk	40	43,1% (25-59)	2,997	46% (44-48)
Aplastic Anemia				
Patients Age 0-17 Years				
Donor type				
Matched Related Donor	54	80,8% (67-89)	504	98% (96-99)
Mismatched Related Donor	49	70,5% (54-82)	110	86% (80-93)
Unrelated Donor	65	84,2% (72-91)	337	90% (95-99)
Patients Age ≥18 Years				
Donor type				
Matched Related Donor	133	84,3% (76-89)	625	84% (81-87)
Mismatched Related Donor	42	75,2% (58-85)	177	80% (74-86)
Unrelated Donor	69	58,1% (44-69)	581	77% (74-81)

FIGURE 1. Brazilian active centers in the CIBMTR by year

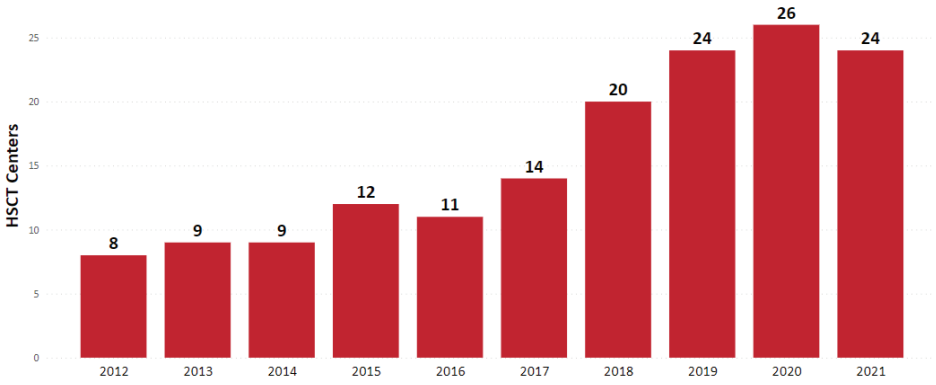


FIGURE 2. Transplants performed in Brazil and reported in the CIBMTR

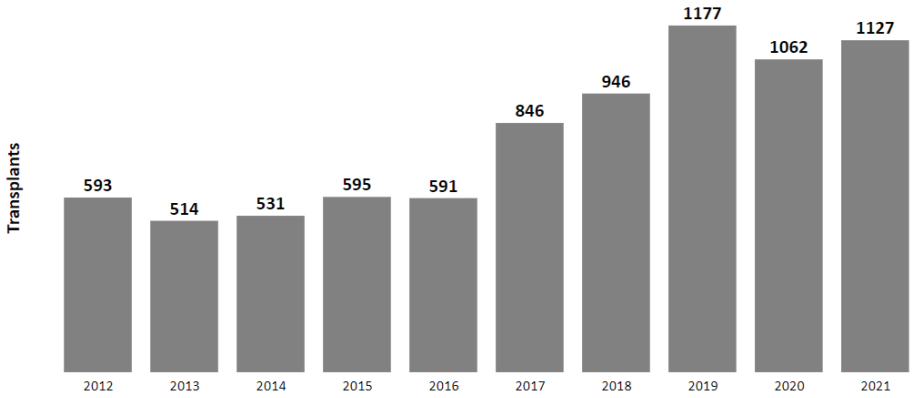


FIGURE 3. Relative proportion of allogeneic HSCT in Brazil by donor type

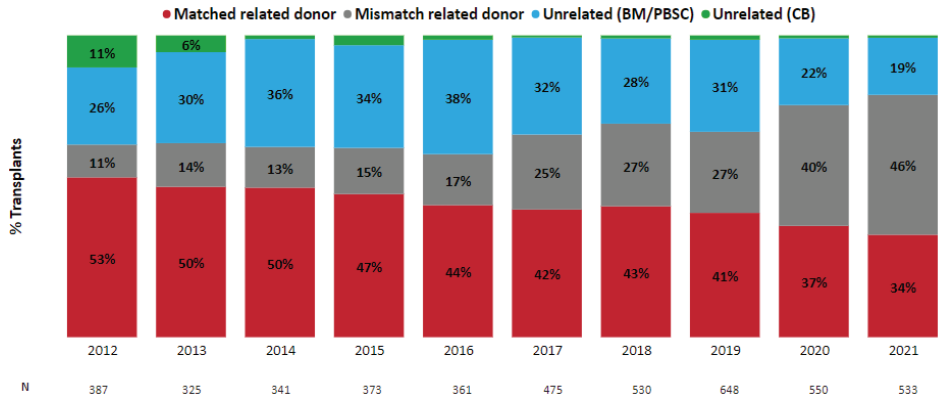


FIGURE 4. **Global indications for HSCT in Brazil, 2019-2021 (n=3,366)**

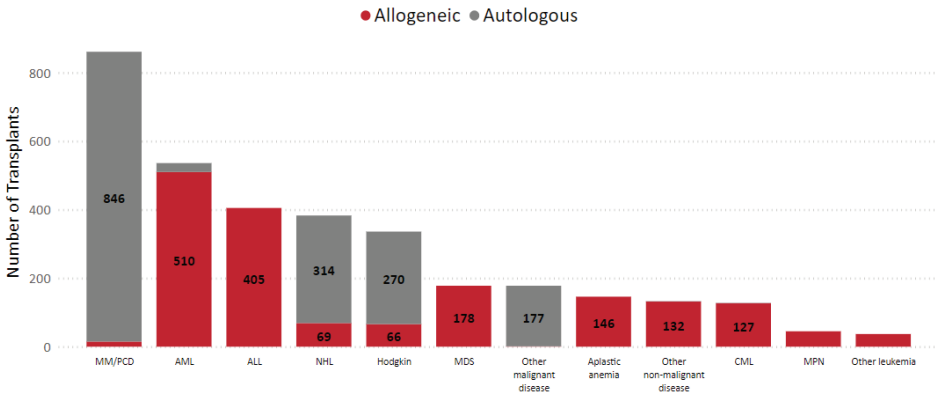


FIGURE 5. **AML, overall survival after 1st allogeneic HSCT by donor type**

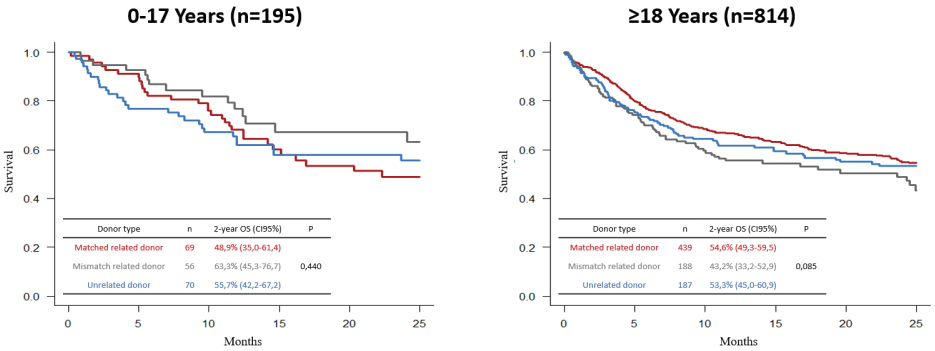


FIGURE 6. **ALL, overall survival after 1st allogeneic HSCT by donor type**

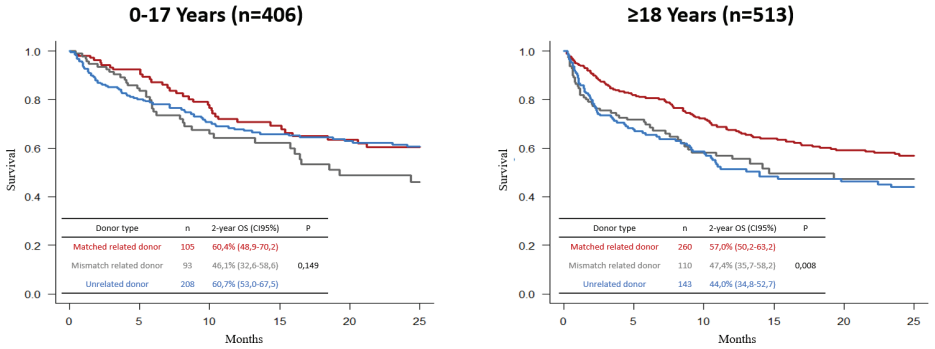


FIGURE 7. **MDS, overall survival after 1st allogeneic HSCT by disease stage**

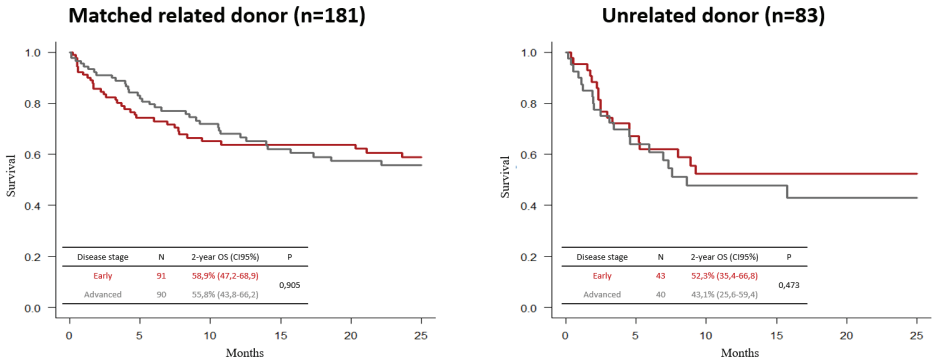


FIGURE 8. **CML, overall survival after 1st allogeneic HSCT by donor type**

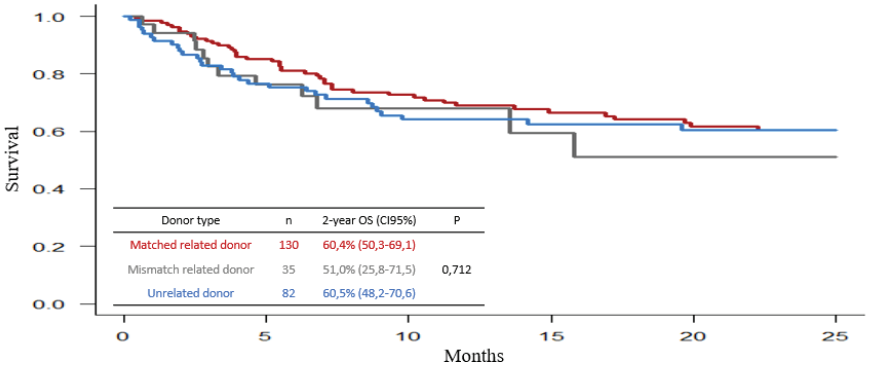


FIGURE 9. **Myelofibrosis, overall survival after 1st allogeneic HSCT**

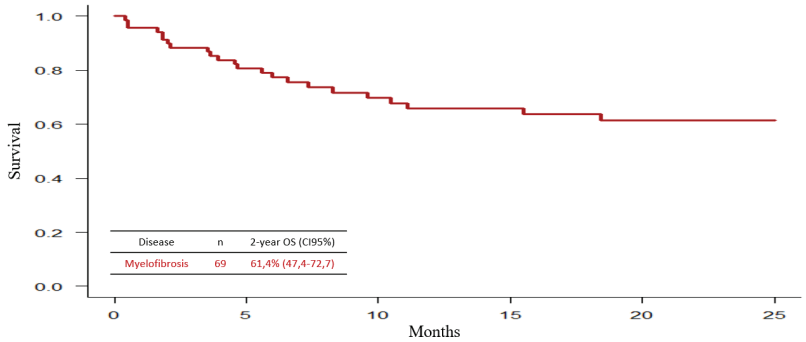


FIGURE 10. **Aplastic Anemia, overall survival after 1st allogeneic HSCT by donor type**

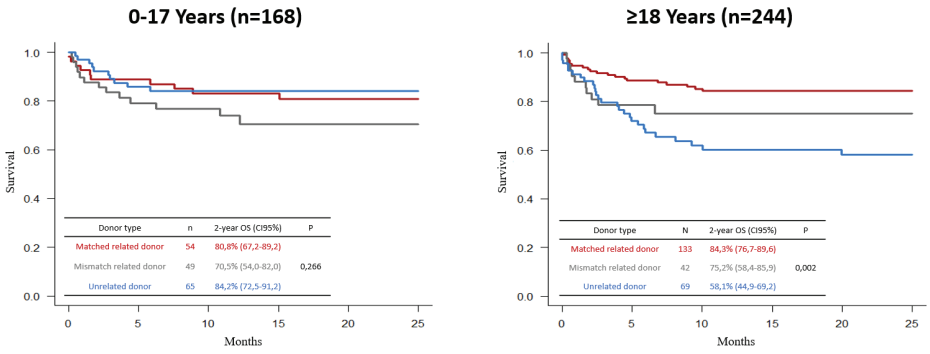


FIGURE 11. **Lymphomas, overall survival after 1st autologous HSCT**

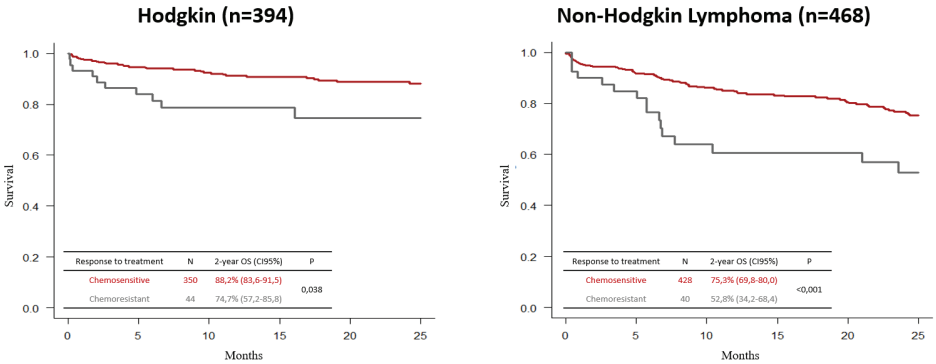


FIGURE 12. **Multiple Myeloma/ Plasma Cell Leukemia, overall survival after 1st autologous HSCT**

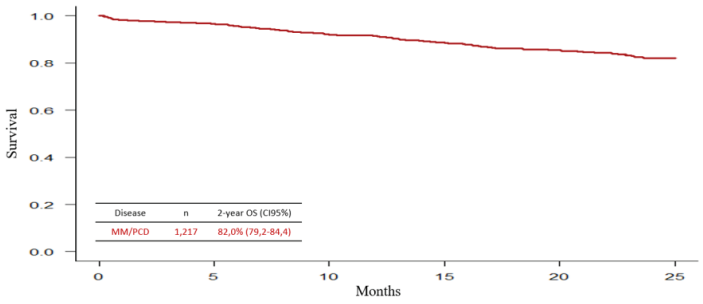
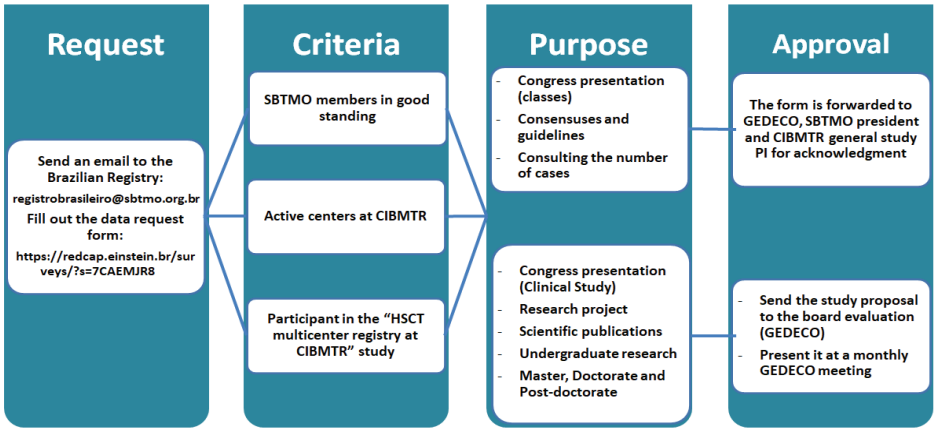


FIGURE 13. **Data requesting flow**



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LYMPHODEPLETION IN CELL THERAPY

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy has become a factible therapy for hematologic neoplasms. Prior to infusion, strategies as lymphodepletion and bridge therapy are frequently performed to prolong the persistence of infused cells and increase the effectiveness of the treatment. The aim of this review is to investigate the use of Lymphodepletion and bridge therapy, protocols available, indications, advantages, negative effects, agent associated toxicity, applicability for specific onco-hematological diseases and how to optimize the procedure, guarantying security and efficacy of this approach.

Keywords: Lymphodepletion. Bridge Therapy. Cell Therapy.

OBJECTIVE

To describe the importance and applicability of Lymphodepletion and bridge therapy, specifying the indication and its types, considering the appropriate time for both.

INTRODUCTION

"Adoptive" cell therapy (ACT) is a therapeutic option already available for cancer patients. T cells genetically modified to express a chimeric antigen receptor (CAR) against CD-19 antigens have been approved by the US Food and Drug Administration (FDA) for the treatment of acute lymphoblastic leukemia and non-specific lymphoma. Hodgkin in 2017 and 2018[1,2]. Currently, TCA studies with tumor-infiltrating lymphocytes (TILs) are ongoing in patients with melanoma metastatic[3-6] and other solid tumors. Previous studies have shown that the success rate for obtaining adequate amounts of TILs and the adequate time for their preparation can be obstacles to large-scale use.

Studies performed over a decade ago in patients with metastatic melanoma showed that a conditioning regimen of lymphodepletion prior to adoptive cell transfers significantly improved the efficacy of treatment with expanded TILs "in vitro"[7]. A conditioning regimen of lymphocyte depletion likely acts through multiple mechanisms, including the elimination of consuming structures ("sinks") of homeostatic cytokines, such as interleukins 2 (IL-2), IL-7 and IL-15; the eradication of immunosuppressive agents such as regulatory T cells and myeloid-derived suppressor cells, the induction of costimulatory molecules and the inhibition of indoleamine 2,3-deoxygenase in tumor cells; promoting the expansion, function and persistence of transferred T cells[7-9]. These experiments resulted in the use of conditioning of lymphocyte depletion in clinical trials with treatment with CAR-T cells. Studies have shown the association between an increased serum level of IL-15 after lymphodepletion and better clinical response in the treatment of lymphomas with anti-CD19[10] CAR-T cells and an increased expansion

and persistence of anti-CD19 CAR-T cells and better outcomes. Clinical trials on lymphocyte-depleting conditioning regimens that combined fludarabine with cyclophosphamide compared to regimens without fludarabine in patients with non-Hodgkin lymphomas[11].

Lymphodepletion causes lymphopenia and affects subpopulations of T, B, and NK cells, having several positive effects:

- **Tumor burden reduction**

- Changes in tumor phenotype:
  - Decreased production of tumor cell metabolites: adenosine, kynurenines (indoleamine 2,3-deoxygenase and tryptophan 2,3-deoxygenase), prostaglandin E2, norepinephrine and epinephrine; metabolites that inactivate tumor-infiltrating immune cells and polarize them to anti-inflammatory phenotypes.

  Changes in the expression of costimulatory molecules.

- **Changes in the tumor microenvironment:**

  - Reduction of regulatory T cells and vascular endothelial cell damage making the environment more favorable for CAR-T cells.

- **Removal of cytokine "sinks":**

  - Greater availability of IL-2, IL-7 and IL-15, associated with optimized response to CAR-T cells.

- **Suppression of the host's immune system:**

  - Decreased immunogenicity and increased persistence of infused CAR-T cells.

  - The negative effects of lymphodepletion can be:

- **Pancytopenia and immunosuppression, increasing the risk of infections.**

- **Specific toxicities of cytotoxic agents:**

  - Fludarabine: fever and neurotoxicity.

  - Cyclophosphamide: hemorrhagic cystitis, pericarditis and neurotoxicity.

  - Increased risk of secondary neoplasms.

A broad spectrum of conditioning regimens are used to improve response rates to adoptive cell therapies, but no more consistent approach has been documented. Comparative studies between different regimens are scarce and with a small number of patients recruited, making it difficult to conclude which are the best agents and dosages, given that both response rates and toxicity seem to be dependent on the disease and its stage of each patient. and each specific cellular product.

Pre-immunotherapy CAR-T-cell lymphodepletion in hematologic malignancies: The use of pre-CAR-T-cell therapy lymphocyte depletion conditioning regimens is almost unanimous. Despite this, comparative studies between regimens are very limited, making it difficult to conclude which is the best approach between different treatments. The table 1 below summarizes some of these studies:

Other early-stage studies seek to optimize pretreatment lymphodepletion with CAR-T cells in patients with B-cell malignancies. The table 2 lists some of these studies:

Pretreatment Lymphodepletion of Solid Tumors with CAR T Cells: Although CAR-T cells were initially evaluated in the context of solid tumor treatment, the results were poor; with the emergence of the importance of lymphodepletion, new studies, although limited, were carried out and are presented in the table 3:

- **Pre-infusion CAR-T cell bridging therapy**

In the process between leukopheresis, processing and infusion of CAR-T cells, disease progression can occur. Clinical management during this period is a challenge. Intervention strategies are known as bridging therapy and are usually performed with high doses of chemotherapy, immunochemotherapy and/or radiotherapy.

Clinical studies on the impact of bridging therapy and how it should be performed are scarce. Luft et al., retrospectively reviewed 75 cases of patients with relapsed/refractory large B-cell lymphoma who received CAR-T therapy. Of these, 52 received bridging therapy (BT) and 23 did not (NBT). BT included high-dose corticosteroids (HD, n=10), chemotherapy-based regimen (CT, n=28) and radiotherapy (RT, n=14). CT included cytotoxic chemotherapy, immunotherapy and targeted therapy. There was no significant difference in overall response rate, overall survival, and progression-free survival between groups and subgroups of BT[39].

The development of cytokine release was similar in the groups, but there was a tendency towards an increase in the average level of neurotoxicity syndrome associated with immune effector cells in the

group submitted to BT. The development of cytopenias on day +180 after CAR-T therapy was significantly higher in the BT (50%) vs NBT (13.3%) group and was statistically significant (p = 0.038). Subgroup analysis also showed significantly greater cytopenias at day +180 in the CT (58.3%) and RT (57.1%) subgroups (p = 0.04).

Recently, Liebers et al.[40] analyzed 105 patients with relapsed/refractory large B-cell lymphoma (LGCB) who received the monoclonal antibody polatuzumab vedotin with bendamustine and rituximab (pola-BR) as salvage therapy (n=54) or bridging therapy (n=51) for CAR-T infusion (n=41) or for allogeneic bone marrow transplantation (n=10). Overall survival (OS) at six months was 49.6% and 77.9% for the rescue and bridging therapy groups, respectively.

Kuhnl et al. presented the profile of 250 patients with high-grade relapsed/refractory (LGCB) from the CAR-T program in England, where 174 patients were selected for therapy with (axicabtagene ciloleucel (axi-cel) and 76 for use of tisagenlecleucel (tisagen). Regarding the severity of the disease, 79% of the cases were in an advanced stage, 31% had bulky disease and 66% had extranodal involvement. In relation to previous treatment, (39%) of the patients had received 3 or more lines of treatment previous studies, 33 patients were previously submitted to auto HSCT, and 5 to allo HSCT; 77% of patients had stable or progressive disease as a better response to the last line of treatment[41].

In a retrospective study of patients with relapsed/refractory B-cell acute lymphoblastic leukemia undergoing CAR T-cell (tisagenlecleucel) infusion after cyclophosphamide/fludarabine lymphodepleting chemotherapy Fabricio et al. 2022 estimated the fludarabine exposure as area under the curve (AUC; mg × h/L) using a validated population pharmacokinetic (PK) model. The optimal fludarabine exposure was found to be ≥ 13.8 mg × h/L and was associated with reduced disease relapse and a clinically relevant composite end point of relapse or loss of B-cell aplasia. No increase in toxicity was noted in the analysis, but according to the authors, this is an important consideration for prospective studies. Fludarabine exposure before CD19- specific CAR T-cell therapy (tisagenlecleucel) in pedaytric and young adult patients with R/R B-ALL was associated with lower relapse probability. Similar analysis with other CAR T-cell products that use fludarabine-based lymphodepleting chemotherapy will be useful to identify the optimal fludarabine exposure for individual products[42].

The need and intensity of bridging therapy must be evaluated in each case in a specific way and depends on factors such as the aggressiveness of the disease, response to previous treatments, related toxicity, among others. However, studies have shown promising results with bridge therapy for the use of CAR-T treatment in diseases such as lymphomas and ALL. New prospective studies are needed to better assess the role of different BT strategies in the use of CAR-T cells.

CONCLUSION

-Lymphodepletion improves the expansion, persistence and migration of CAR-T cells, enhancing their antitumor effect and available homeostatic cytokines, depleting inhibitory molecules and cell populations. Beneficial actions on the microbiome have also been reported.

- The scarcity of comparative studies between different lymphodepletion regimens does not allow a consensus on the best approach to obtain it.

- It is related to a number of toxicities, including varying degrees of cytopenias and even, in more severe cases, the cytokine release syndrome.

- Higher intensity and inclusion of Fludarabine in their protocols are associated with greater efficacy but also more toxicity.

- The addition of intermedayte doses of Fludarabine to conditioning regimens is increasingly used to improve the expansion and persistence of infused cells, in addition to reducing the immunogenicity of transgenic products.

- A number of alternatives to lymphodepletion are under development, including the addition of stimulatory cytokines to the infused cells.

- Regarding Bridge Therapy, it can be essential, in cases where the disease activity does not allow waiting the necessary time for the production of CAR-T cells.

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TABLE 1: Comparative studies of lymphocyte depletion conditioning regimens for Hematologic malignancies

Study	Neoplasm	Cell's	Lymphodepletion	Results
MMSKCC12	LLC R/R	CD-28 2ª g CAR T	CY (1,5 ou 3 g/m2) X No LD	- Increased persistence of CAR-T cells. - Better effectiveness
Geyer et al.13	LLC R/R	CD-28 2ª g CAR T	FLU/CY X CY	FLU/CY: - Higher lymphocyte nadir - Higher peak cell expansion. circulating CAR-T
Curran et al.14	LLA-B R/R	CD-28 2ª g CAR T	CY 3 g/m2 X CY 1,5 g/m2	CY 3 g/m2: - Higher CR rates - Greater depletion of lymphocytes and greater peak of CAR-T cell expansion.
UPENN15	Neoplasias of células B	4-1BB- 2ª g CAR (CTL-019)	FLU/CY X Pentostatin/CY X Bendamustina	No differences
ELIANA16	LLA-B R/R	Tisagenlecleucel (CTL-019)	FLU 30 mg/m2 x 4 days e CY 500 mg/m2 x 2 days	66% SFR in 18 m
JULIET17	LNHDGCB R/R	Tisagenlecleucel (CTL-019)	FLU 25 mg/m2 x 3 days e CY 250 mg/m2 x 3 days X Bendamustina 90 mg/m2 x 2 days X No LD	FLU/CY: - Higher overall response rate18
NCI19	Neoplasias of células B	CD19 específico CD28 2ª g CAR	FLU 25 mg/m2 x 5 days e CY 60 mg/Kg x 2 days X FLU 30 mg/m2 x 3 days e CY 300 – 500 mg/m2	Higher neurotoxicity in the group with higher doses of CY
ZUMA-120	Primary LNHDGCB and LNH of mediastinum R/R	CD19 específico CD28 2ª g CAR axicabtagene ciloleucel (Axi-cel)	FLU 30 mg/m2 e CY 500 mg/m2 x 3 days	40% RC in 14,5 m
Wang et al.21	CML R/R	KTE-X19 brexucabtagene autoleucel	FLU 30 mg/m2 e CY 500 mg/m2 x 3 days	61% SLR in 12 m
FHCRC22	ALL B R/R	4-1BB-based 2ª g CAR céls. CD4+ e CD8+ of memória purificadas - lisocabtagene maraleucel (liso-cel)	CY (3 differents doses) X FLU 25 mg/m2 x 3 or 5 days e CY 60 mg/Kg x 1 day	FLU/CY: - Increase in the area under the CAR-T cells curve. - Better evolution
FHCRC23	LNH-B R/R	lisocabtagene maraleucel (liso-cel)	CY (3 differents doses) X FLU 25 mg/m2 x 3 ou 5 days e CY 60 mg/Kg x 1 day	FLU/CY: - Higher overall response rate and CR - Higher rates of CAR-T cell expansion and persistence.

PLAT-0224	LLA-B R/R CYA	lisocabtagene maraleucel (liso-cel)	CY (2 – 4 g/m2) X FLU 30 mg/m2 x 4 days e CY 500 mg/m2 x 2 days.	FLU/CY: - Largest CAR-T cell peaks and the area under the curve
TRANSCEND25	LNH-B R/R	lisocabtagene maraleucel (liso-cel)	FLU 30 mg/m2 e CY 300 mg/m2 x 3 days	53% RC in 18,8 m
CARTITUDE-126	MM R/R	Céls. BAR-T anti-BCMA 2ª g CD28/CD3ζ	FLU 30 mg/m2 e CY 300 mg/m2 x 3 days	77% SLP in 12 m
Ramos27	LH R/R	Céls CAR-T anti CD-30 CD28ζ 2ª g	FLU 30 mg/m2 x 3 days, CY 500 mg/m2 x 3 days, bendamustina 90 mg/m2 x 2 days ou FLU 30 mg/m2 x 3 days e bendamustina 70 mg/m2 x 3 days	36% SLP in 12 m
CALM28 e PALL29	LLA-B R/R	UCART19/ALLO-501	FLU 90 mg/m2, CY 1500 mg/m2, Alemtuzumab 1 mg/Kg (máx. 40 mg) – (CALM) FLU 150 mg/m2, CY 120 mg/Kg, Alemtuzumab 1 mg/Kg (máx. 40 mg)	Phase I Allogeneic CAR-T cells

TABLE 2: Strategies to optimize lymphodepletion with CAR-T cells in patients with B-cell malignancies

Method	Study	Objective
Add a inhibitor of "checkpoint"	ALEXANDER (AUTO-330)	Increase activity and persistence of CAR-T
Add of Rituximab	ZUMA-14 (axi-cel)31	Increase the anti-lymphoma effect and persistence of CAR-T
Add of monoclonal antibody anti-CD52	ALPHA (Allo-501)32	Increase the anti-lymphoma effect and persistence of CAR-T
Add radioimunotherapy with antibody anti CD45 conjugated to I31	Ludwig33	Increase the specificity of lymphodepletion.

TABLE3: Recent studies of Lymphodepletion in different Neoplasms

Study	Neoplasm	Cell's	Lymphodepletion	Results
Christie Cancer Centre34	Neoplasias that expressed Carcioembryogenic Antigen (CEA)	1ª g CAR-T directed to Carcioembryogenic Antigen (CEA) + systemic IL-2	FLU 25 mg/m2 x 5 days X FLU 25 mg/m2 x 5 days e CY 60 mg/Kg x 2 days	FLU/CY: - Longer duration of lymphopenia - 3 in 4 patients reached stable disease - Pulmonary toxicity peak-associated to CAR-T
Baylor35	Rhabdomyosarcoma that expressed HER2	CAR-T cells with CD-28 against HER2	FLU/CY	CC after reinfusion of CAR-T post relapse
Heczey36	Neuroblastoma R/R that expressed Disialoganglioside (GD2)	CAR-T cells of 3ª generation against GD2	FLU 30 mg/m2 x 2 days, CY 500 mg/m2 x 3 days +/- inhibitor of PD-1	- Increase in homeostatic cytokines - Increased persistence of CAR-T - Limited efficacy even in the anti-PD-1 group
Adaptimmune37,38	Synovial Sarcoma	CAR-T cells against NY-ESO-1 peptide	CY 1800 mg/m2 x 2 days X FLU 30 mg/m2 x 4 days e CY 600 mg/m2 x 2 days X FLU 30 mg/m2 x 4 days e CY 1800 mg/m2 x 2 days	- Better results in the group with more intensive conditioning - FLU/CY: increase of circulating homeostatic cytokines, grafting and persistence of CAR-T - Grade 4 adverse effects in all patients

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MANAGEMENT OF INFECTIONS AND VACCINATION IN CAR-T CELL THERAPY

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GOALS

1. Describe the procedures necessary for the clinical and epidemiological evaluation of the chimeric antigen receptor T-cell therapy (CAR-T cell therapy) candidate, classify the infectious risk, define the criteria for the implementation of prophylactic, empirical and preemptive antimicrobial strategies and guide laboratory and clinical monitoring of the infectious events.

2. Define the indications and contraindications of inactivated and attenuated vaccines and propose a vaccination schedule before and after CAR-T therapy.

INTRODUCTION

As a result of the underlying disease and previous cytotoxic treatments, candidates for CAR-T cell therapy are at increased risk of infections due to the high degree of pre-existing immunosuppression. This immunosuppressive state is aggravated by the lymphodepleting chemotherapy given prior to CAR-T infusion, and later by prolonged cytopenias, due to the "on target, off-tumor" depletion of normal CD19-expressing B cells in most patients, which contributes to hypogammaglobulinemia[1]. Furthermore, despite encouraging results, the currently approved CAR-T cell therapy products have severe toxicities, including cytokine release syndrome (CRS) and immune-effector cell associated neurological syndrome (ICANS)[2,3].

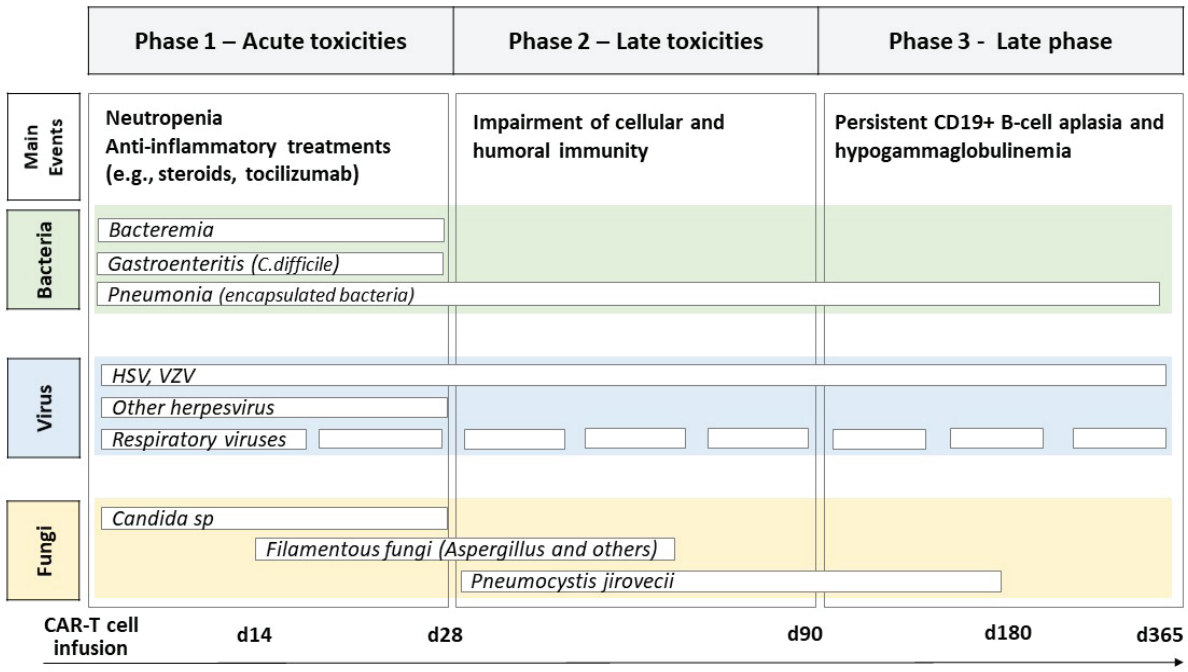
The treatment of these acute complications in addition to the cytopenias may result in deep and long-term immunological deficits, as the CAR-T cells can persist for years[4-6]. Approximately 18% to 34% of patients develop infections within the first 2 months after CAR-T therapy despite antimicrobial prophylaxis[7].

The rapidity of commercialization and use of CAR-T therapies has revealed an unexplored gap in the management of infections in these patients. The recommendations suggested in this manual are based on data from retrospective studies, expert opinion and approaches used in other relevant settings, since to date, there are no randomized controlled trials on infections in recipients of CAR-T therapy.

PREVALENCE OF POST-CAR-T THERAPY INFECTIONS AND RISK FACTORS

Infections post-CAR-T therapy are distributed with varied frequency in 3 distinct periods, namely: phase 1, up to 30 days after cell therapy infusion, considered the critical period when acute toxicities are expected (CRS, ICANS); phase 2, between 30 and 90 days after infusion, characterized by infectious events that reflect the slow reconstitution of cellular and humoral immunity; and phase 3, after 90 days of CAR-T therapy with infections mainly due to hypogammaglobulinemia and persistent B-cell aplasia. Figure 1 shows the main infectious agents according to the period after CAR-T therapy.

FIGURE 1. **Relevant infections according to period after CAR-T therapy[4].**



A growing number of publications has emerged on infections in CAR-T therapy and, therefore, the recommendations described in this manual should be updated as information with a higher level of evidence becomes available.

To date, most infections are caused by bacteria and almost all cases of bacteremia occur within the first 2 weeks after CAR-T cell infusion. Respiratory virus infections are the second in frequency. There are case reports of herpes simplex virus (HSV) and varicella-zoster (VZV) reactivation in patients with poor compliance to acyclovir prophylaxis but infections due to other herpesviruses and double-stranded DNA viruses (adenovirus, polyomavirus BK) seem to be rare, as well as invasive fungal infections[4].

The risk factors for infections reported in some recently published studies are related to both the host and CAR-T therapy. Factors related to the host include the status of underlying disease, previous chemotherapy, cumulative immunosuppression by previous therapies, previous HCT (allogeneic or autologous), basal cytopenia, presence of comorbidities, previous infections and antimicrobial prophylaxis. Regarding to the factors associated with CAR-T therapy are relevant the type of CAR-T therapy (dose, administration schedule, resulting cytopenias and other hematological side effects), the occurrence of serious adverse events that require additional immunosuppression such as, CRS, ICANS, hemophagocytic lymphohistiocytosis and macrophage activation syndrome, the conditioning regimen and the resulting hypogammaglobulinemia[8].

Lower infection rates are observed in patients who used the optimized dose of CAR-T cells, determined by the underlying disease and tumor burden[1]. According to previous studies, the optimized dose of CAR-T cells maintains the same antitumor activity with a reduced risk of severe CRS[9,10]. Therefore, the use of optimized dose of CAR-T cells is an important step in the management of infections in this setting. Table 1 shows some studies published to date reporting infection rates.

TABLE 1. Recent publications reporting prevalence rates of infections in CAR-T therapy.

Author (year)	Time of occurence	N	Frequency	Agents			Reference
				Bacteria	Virus	Fungi	
Hill (2018)	Early (□28 dias)	133	23%	17%	8%	3%	(1)
	Late (>28 dias)	119	14%	7%	9%	2%	
Park (2018)	Early (□ 30 dias)	53	42%	30%	9%	8%	(6)
	Late (>30 dias)	32	31%	16%	28%	3%	
Luo (2019)	Up to 30 days	109	17%	13%	3%	2%	(11)
Wudhikan (2020)	Up to 12 months	60	63%	57%	44%	4%	(12)
Vora (2020)	Early (□28 days)	83	40%	18%	19%	1%	(13)
	Late (>28 days)	48	17%	6%	11%	0%	
Cordeiro (2020)	After 90 days	54	61%	12%	11%	3%	
Logue (2021)	Early (£ 30d)	85	37%	-	-	-	
	Late (>30d)	70	44%	-	-	-	
Strati (2021)	Up to 24 months	31	77%	14%	24%	6%	(16)

For a better understanding of the actions to control infections in CAR-T therapy, the steps will be described according to the longitudinal follow-up of patients, namely: 1) Infectious assessment before CAR-T therapy; 2) Antimicrobial prophylaxis and monitoring of infections and 3) Vaccination in candidates and recipients of CAR-T therapy.

1. CLINICAL AND EPIDEMIOLOGICAL EVALUATION BEFORE CAR-T THERAPY

1.1. Serological tests

Serology for HIV, HBsAg (HBV surface antigen), anti-HBs (HBV surface antibody), anti-HBc (HBV core antibody), anti-HCV (antibody against the virus of hepatitis C) are mandatory. In case of any positive marker, the nucleic acid tests (NAT) must be carried out. Prior HIV serology is important because some PCR-based screening tests may have false-positive results in post-CAR-T follow-up if lentiviruses were used as vectors to produce the CAR-T cells[17].

Other recommended serologies are: cytomegalovirus (CMV), human T-cell lymphotropic virus type 1 (HTLV-1), Toxoplasma gondii (toxoplasmosis) and Treponema pallidum (syphilis). In children, additional serological screening for herpes simplex virus 1/2 (HSV1/2) and varicella-zoster virus (VZV) may be considered. Candidates who are seropositive for HSV1/2 and/or VZV should receive prophylaxis with acyclovir or valaciclovir. Given the high seroprevalence of HSV and VZV in adults, universal prophylaxis with acyclovir is recommended, without the need for prior serological screening (see prophylaxis).

1.2. Assessment of vaccination cards

As part of the pre-CAR-T therapy evaluation, it is recommended to review the patient's vaccination history, and check the status of influenza and pneumococcal vaccines. Influenza vaccine should be administered after leukapheresis and at least 2 weeks before lymphodepletion chemotherapy, and then annually, before the influenza season[4]. The seasonality of influenza in Brazil depends on the latitude, with the highest concentration of cases from January to April in the tropical zone and from May to September in the south temperate zone.

1.3. Active infections in the pre-infusion period

Active or uncontrolled infections should be treated prior to infusion of CAR-T therapy. Active infections can be aggravated by lymphocyte-depleted chemotherapy including fludarabine, and by the severe suppression of humoral immunity driven by CAR-T cells. Infections can also result in more severe toxicities due to elevated levels of inflammatory cytokines. About 10% of serious or life-threatening infections after CAR-T therapy were cases of progression of infections diagnosed in the pre-infusion period of cell therapy[1]. Patients with respiratory symptoms

should collect a respiratory panel and await resolution of symptoms to initiate lymphocyte depletion, especially in cases of infection by SARS CoV-2, RSV, PIV 1, 2, 3 and 4, INF A and B, hMPV and ADV[4].

1.4. Empirical treatment of strongyloidiasis

Countries in tropical and subtropical regions may have a high prevalence of strongyloidiasis, which may be severe in immunocompromised patients. Given the low sensitivity of serological diagnosis, empirical treatment of *Strongyloides stercoralis* with ivermectin 200mg/kg/day on 2 consecutive days should be considered[18].

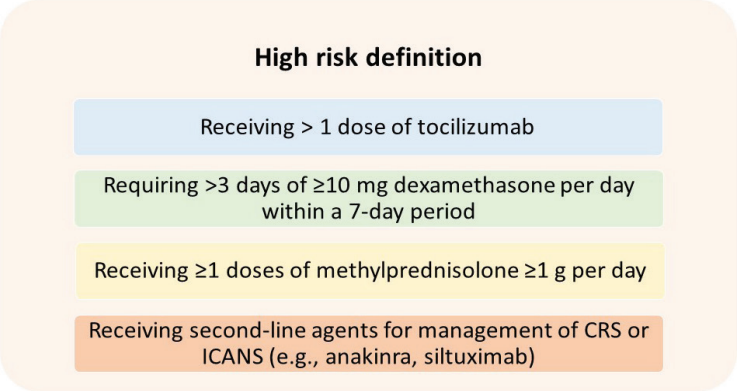
1.5. Tuberculosis investigation

In addition to the increased risk of reactivation of latent *Mycobacterium tuberculosis* infection (LTBI) in cancer patients, recipients of CAR-T therapy may need treatment with tocilizumab, an interleukin 6 (IL-6) receptor antagonist that is independently associated with an increased risk of active *M. tuberculosis* infections[19]. Given the high prevalence of tuberculosis in Brazil, investigation of previous tuberculosis in the patient or in close contacts is mandatory. A recent Brazilian study showed LTBI prevalence of 8.7% in HCT candidates. About 10% of the patients reported cases of tuberculosis in their family[20]. Laboratory investigation of LTBI is recommended preferably by the interferon gamma release assay (IGRA) or by the tuberculin skin test (TST). Both tests may be indeterminate in patients with severe lymphopenia. It is recommended to maintain a high level of suspicion for prompt investigation of active TB. In case of proven LTBI, and after exclusion of active TB, prophylaxis with INH should be considered for 6 to 9 months, at a dose of 5 to 10 mg/kg/day up to a maximum dose of 300 mg/day, especially in patients who used tocilizumab.

2. ANTIMICROBIAL PROPHYLAXIS AND INFECTION MONITORING

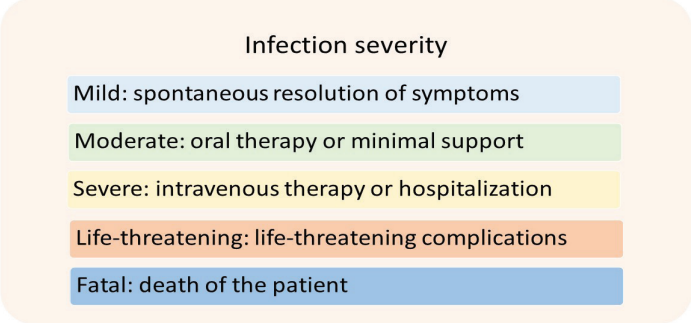
For a better management of infections, it is very important to classify the patient's infectious risk. High-risk patients meet at least one of the criteria described in Figure 2. Evaluation by an infectious disease specialist is recommended for all high-risk patients.

FIGURE 2. High risk definition



Another important parameter is the classification of infection severity. For the analysis of outcomes, it is important to use the same definitions. The infection is considered: a) mild, if no treatment is needed; b) moderate, if only oral treatment or minimal supportive care is needed; c) severe, if intravenous therapy or hospitalization is required; d) life-threatening; or e) fatal, in the event of the patient's death[21]. The classification of infection severity can be seen in Figure 3.

FIGURE 3. Classification of infection severity[21].



2.1. Bacterial infections

2.1.1. Prophylaxis: Levofloxacin 750 mg PO daily should be given during neutropenia (if absolute neutrophil count <500 cells/mm3). Alternative drugs may be considered for patients with contraindications to the use of fluoroquinolones.

2.1.2. Empirical antibiotic therapy: In patients with fever, with or without neutropenia, empirical antibiotic therapy should be initiated according to the center guidelines and blood culture routines. If cefepime is the first choice, vancomycin should only be introduced if there is a clear indication for its use, as cefepime has a broad spectrum of activity for gram-positive and gram-negative agents. As cefepime is associated with neurotoxicity in the setting of advanced age and acute kidney injury, alternative drugs with similar spectrum such as ceftazidime plus vancomycin may be considered. The treatment of persistent or refractory CRS or ICANS may mask typical signs and symptoms of infection. In case of doubt whether CRS/ICANS or infection, escalation of therapy to include meropenem and vancomycin should be considered independent of fever. De-escalation of antibiotics can be considered from 48 hours after defervescence and significant improvement in symptoms, always in consultation with the infectious disease specialist.

2.1.3. Monitoring of bacterial infections: Blood cultures must be taken according to local recommendations. For patients with CRS or grade ≥2 neurotoxicity, aerobic and anaerobic blood cultures should be taken twice a week, regardless of the occurrence of fever.

2.2. Viral infections

2.2.1. Prophylaxis: To control HSV-1, HSV-2 and VZV reactivations, prophylaxis with acyclovir/valaciclovir should be started with lymphodepletion chemotherapy and continued for up to 1 year after infusion of CAR-T therapy. Prophylaxis should be discontinued during preemptive use of ganciclovir (GCV) or valganciclovir (vGCV), and resumed after its end.

Few information is available regarding the management of persistent hepatitis B (HBV) and C (HCV) infections because patients infected with these viruses were excluded from the initial CAR-T studies. Data reported to date suggest that CAR-T therapy is safe in patients with HBV as long as they receive prophylaxis with antivirals such as entecavir. Rare reports of fulminant hepatitis and death have occurred in patients who have stopped entecavir[22].

In a case-control study in China, including 41 HBV-infected patients and 29 controls, no difference was observed in toxicity (CRS or ICANS) or response to CAR-T therapy between groups. HBV reactivation was observed in 17% of HBsAg positive patients and in 3.4% of patients with past HBV infection (anti-HBc positive). Exacerbation of hepatitis was not observed and only one case had an elevation of alanine aminotransferase (ALT)[23]. Strati and colleagues reported two cases of HBV and one case of HCV infected patients who received CAR-T therapy. No patient developed fulminant hepatitis or had viral reactivation or abnormal liver function tests during CAR-T therapy[24].

Based on available information, the risk-benefit of CAR-T therapy in patients with HBV or HCV infection should be evaluated on a case-by-case basis. If CAR-T cell therapy is the option, patients should receive prolonged prophylaxis or suppressive treatment in consultation with an infectious disease specialist or hepatologist.

2.2.2. Preemptive therapy (CMV): The introduction of preemptive therapy should be done with GCV or vGCV according to the established qPCR cut-off, or a positive pp65 antigenemia. vGCV should be administered along with food for better drug absorption, in patients without diarrhea and without significant alterations in liver tests. The duration of preemptive therapy should be at least 14 days, followed or not by maintenance therapy (half dose, once daily for another 2 weeks). In case of neutrophil counts below 1,000/mm^3 before starting preemptive therapy, the alternative drug is foscarnet (FOS) 90mg/kg every 12 hours[18].

2.2.3. Monitoring of CMV reactivation: Patients with a previous history of HCT should be monitored weekly by CMV quantitative PCR (qPCR) or pp65 antigenemia followed by preemptive treatment with GCV, vGCV or FOS, if necessary, according to SBTMO recommendations for allogeneic HCT recipients[18]. In patients without a history of HCT, there is no need for monitoring, except by high-risk patients (as defined above).

High-risk patients should be monitored for CMV. Monitoring for CMV reactivation should be done weekly for up to 30 days after the last day of dexamethasone (or equivalent) ≥ 10 mg, or other cytokine therapies such as tocilizumab and anakinra, whichever occurs later. In case of reactivation and preemptive therapy, monitoring should be done weekly up to 30 days after discontinuation of preemptive therapy or 2 consecutive negative tests

(whichever occurs later). Differential blood count should be evaluated within 24 hours of starting treatment, and repeated 2-3 times a week during treatment with vGCV, GCV, or FOS. Renal function tests should be measured at least once a week for proper adjustment of antiviral doses.

2.3. Fungal infections

2.3.1. Prophylaxis: Fluconazole should be administered during neutropenia (absolute neutrophil count <500/mm3) at a dose of 200 mg orally or IV daily. Treatment should be continued until neutropenia resolves. Micafungin 50 mg IV daily can be used as an alternative for patients with contraindications to fluconazole therapy. Posaconazole prophylaxis should be used in high-risk patients (defined above), or if the patient remains neutropenic (<500/mm3) for more than 20 days. The dose of posaconazole on the first day is 300mg PO twice a day, followed by 300mg PO once a day. Prophylaxis should be continued for up to 30 days after the last day of dexamethasone (or equivalent) above 10 mg, or other cytokine therapies such as tocilizumab and anakinra, whichever occurs later. Posaconazole can be discontinued if the absolute neutrophil count is ≥ 500/mm3 without G-CSF for 3 consecutive days. If possible, monitoring the serum level of posaconazole is recommended 7 to 10 days after starting prophylaxis, then weekly, maintaining therapeutic posaconazole levels above 0.7 mg/mL. Voriconazole should be avoided after CAR-T therapy if possible, due to the risk of neurotoxicity. Isavuconazole is not routinely used for prophylaxis[4].

2.3.2. Pneumocystis jirovecii prophylaxis: Patients taking trimethoprim-sulfamethoxazole for *P.jirovecii* prophylaxis can maintain prophylaxis during neutropenia post-CAR-T therapy. One double-dose tablet is recommended orally, 3 times a week. If there is concern about potential myelosuppression, trimethoprim-sulfamethoxazole can be started after absolute neutrophil counts above 0.5 x 10^9/L is reached and maintained for at least 6 months[25]. *P.jirovecii* prophylaxis should be started in all patients on the 28th day after CAR-T cell infusion. Alternative drugs in case of sulfa allergy or prolonged cytopenias are aerosolized pentamidine (300 mg once a month), dapsone (100 mg PO/day) or atovaquone (1500 mg PO/day).

3. VACCINATION IN CAR-T THERAPY CANDIDATES AND RECIPIENTS

CAR-T therapy targets cells that express CD19, present on both malignant and non-malignant B cells. However, terminally differentiated B cells such as long-lived plasma cells have low expression of CD19 and can survive after lymphodepletion chemotherapy and CAR-T therapy[26]. Experimental studies have shown that, unlike memory B cells, mature plasma cells in general do not participate in the processing and presentation of antigens, and their main function is to secrete large amounts of specific antibodies for long periods[26].

In adults who had sustained complete response for 6 months after CAR-T therapy, Hill et al. demonstrated a small decrease in serum total IgG concentrations, with preservation of virus-specific antibody concentrations[27]. These data suggest a small impact of CAR-T therapy on preexisting humoral immunity for up to 1 year in adults, and raise an important question about the current recommendation for intravenous immunoglobulin prophylaxis. It is noteworthy that these observations may not be valid for children due to the lower number of established plasma cell clones.

Therefore, it is still unclear whether there is a need for vaccination after CAR-T therapy[7]. Given the paucity of evidence for or against, vaccination is recommended in patients with a complete response for ≥ 6 months (28).

3.1. General recommendations

In general, priority is given to the inactivated influenza vaccine, 13-valent pneumococcal conjugate vaccine, and *Haemophilus influenzae* type b conjugate vaccine. According to expert opinion (EBMT and ASTCT), vaccination schedules similar to post-HCT revaccination program may be necessary[28].

Inactivated vaccines can be administered after ≥ 6 months of CAR-T therapy and ≥ 2 months after the last dose of IVIg. Attenuated vaccines can be administered after ≥ 1 year of CAR-T therapy. Figure 4 summarizes the contraindications for the use of inactivated and attenuated vaccines.

FIGURE 4. **Contraindications for the use of inactivated and attenuated vaccines.**

Contraindications for inactivated vaccines	Contraindications for attenuated vaccines
• Supplemental immunoglobulins within the past 2 months • Receiving immunosuppressive therapy that reduces T-cell or B-cell function or active symptoms of graft-versus-host-disease that requires treatment • Administration of anti-CD20 or anti-CD19 agent within the past 6 months • Actively receiving chemotherapy*	• Administration of anti-CD20 or anti-CD19 agent within the past 6 months • ≤1 year post CAR–T-cell therapy • ≤2 years post autologous or allogeneic HSCT • ≤1 year off systemic immunosuppressive therapy • ≤8 months after last dose of supplemental immunoglobulins • Absolute $CD4^+$ T-cell ≤200 cells/mm^3 • Absolute $CD19^+$ or $CD20^+$ B-cell ≤20 cells/mm^3 • Actively receiving chemotherapy*

(*Vaccination may be considered in certain therapies that do not suppress T-cell or B-cell responses, such as checkpoint inhibitors, immunomodulatory agents (eg, lenalidomide), tyrosine kinase inhibitors, and select other agents)

TABLE 2. **Proposal for a vaccination schedule in CAR-T therapy for adults.**

Vaccines	Time after CAR T therapy						Obs
Inactivated	Pre CAR-T	>6mo	>7mo	≥8moo	>12m	>18m	
Influenza	INF	INF					Annually
PCV13		PCV13	PCV13	PCV13			
PPV23						PPV23	
HiB		HiB	HiB	HiB			
DTaP		DTaP	DTaP	DTaP			
Hepatitis A					HAV	HAV	Serology before
Hepatitis B		HBV	HBV		HBV		
MCV ACWY*		MCV		MCV			
IPV*		IPV	IPV	IPV			
HPV*		HPV		HPV	HPV		9-45 years
Live attenuated	≥12-24 mo						
MMR	MMR						Serology before
VZ (Shingrix®)	VZ						≥50 years, VZVÅ

3.2. Vaccination Schemes in CAR-T Therapy

Many centers wait for the resolution of B cell aplasia before restarting vaccination. The decision to initiate vaccination should be made individually or based on institutional guidelines. Tables 2 and 3 show suggested vaccination schedules for adults and children, respectively.

TABLE 3. Suggested vaccination schedule in CAR-T therapy for children.

Vaccines	Time after CAR T therapy						Obs
Inactivated	Pre CAR-T	>6mo	>7mo	≥8mo	>12mo	>18mo	
Influenza	INF	INF					Annually
PCV13		PCV13	PCV13	PCV13			
PPV23						PPV23	
HiB		HiB	HiB	HiB			
DTaP		DTaP	DTaP	DTaP			
Hepatite A					HAV	HAV	Serology before
Hepatite B		HBV	HBV		HBV		
MCV ACWY*		MCV		MCV			
IPV*		IPV	IPV	IPV			
HPV*		HPV		HPV	HPV		9-45 years
Live attenuated	≥24 mo	≥25 mo					
MMR	MMR	MMR					Serology before
VV (Varivax®)	VV	VV					VZV negative

(*Can be postponed after 12 months. PCV13=13-valent conjugate pneumococcal vaccine; PPV23=23-valent polysaccharide pneumococcal vaccine; HiB=Hemophilus influenza type B vaccine; DTaP=Diphtheria, tetanus and acellular pertussis vaccine; MCV=tetravalent conjugate meningococcal vaccine; IPV=inactivated poliomyelitis vaccine; HPV=papillomavirus vaccine; MMR=measles , mumps, rubella vaccine; VV=varicella vaccine; VZ=recombinant herpes zoster vaccine).

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CITOKINE RELEASE SYNDROME MANAGEMENT IN ADULTS AND PEDIATRIC PATIENTS

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ABSTRACT

Chimeric Antigen Receptor (CAR) T cell therapy has demonstrated efficacy in B cell malignances. However, the treatment is not harmless and, in some patients, can lead to a fatal endpoint. For this reason, the knowledge and the early recognition and management of the side effects related to CAR-T cell therapy for the multidisciplinary team is essential. In this article, we have summarized the current recommendations for identification, gradation and management of cytokine release syndrome related to CAR-T cell therapy.

Keywords: cytokine release syndrome. CRS. CAR T complications. Anti IL 6. Tocilizumab. Toxicity.

INTRODUCTION

Treatment with CD19 or CD22-targeted chimeric antigen receptor-engineered T (CD19/CD22 CAR-T) has demonstrated efficacy in B cell malignances, especially in acute lymphoblastic leukemia (ALL) and non-Hodgkin Lymphoma. Currently, tisagenlecluce is approved by ANVISA in Brazil for relapsed and/or refractory pediatric B-ALL up to the age of 25 years and for non-Hodgkin lymphomas.

CRS represents a potentially serious complication of CART therapy. It is a cytokine-mediated systemic inflammatory response that occurs after CAR T cell infusion when cytokines (interleukin 6 (IL-6), interferon gamma (IFNg) and tumor necrosis factor (TNF)) are released by activated T cells or other immune cells, such as monocytes/macrophages[1]. Clinical presentation is variable and depends on the CAR T product and patient characteristics, such as underlying disease and tumor burden. The peak incidence is between 2 and 7 days after infusion up to 3 weeks (median 1 to 3 days)[2]. The incidence varies from 57 to 93% of adult patients receiving cell therapy[3] and up to 77% of children, as reported in the phase 2 clinical trial, ELIANA[4].

Symptoms related to CAR-T-cell-induced CRS may include fever, tachycardia, hypoxia, nausea, headache, skin rash, hypotension requiring administration of vasopressors or not, acute respiratory failure, coagulopathy secondary to disseminated intravascular coagulation and/or multiple organs disfunction / failure[5].

Although the toxicities are mostly reversible with appropriate supportive care and specific treatment, some cases can be fatal. Early recognition of these toxicities and prompt intervention reduces related morbidity and mortality.

To achieve this goal, it is mandatory training healthcare professionals involved in these patients clinical care. The education of patients and their caregivers is also extremely important.

OBJECTIVES

Provide comprehensive direction for the diagnosis, classification, and management of cytokine release syndrome (CLS) related to CAR-T cell treatment in adult and pediatric patients.

PROCEDURE DESCRIPTION

As described above, CRS has a wide variety of signs and symptoms. After its diagnosis, CRS must be classified according to its severity.

GENERAL RECOMMENDATIONS:

The classification proposed by the Consensus of the American Society for Cell Transplantation and Therapy (ASTCT)[5] is the most used and is also recommended by the Brazilian Society for Cell Therapy and Bone Marrow Transplantation (SBTMO). This classification considers only three vital signs (temperature, blood pressure and oxygen saturation), which facilitates their grading (Table 1). Organ toxicities associated to CRS can be categorized according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0[6] and will not influence its classification.

CRS assessment should be performed at least every 12 hours, or more often if the patient clinical status change.

The Intensive Care Unit (ICU) team will consult theses patients as needed. Indications for ICU transfer are: grade 2 CRS not responsive to intravenous (i.v.) fluid bolus, decreased urine output, or other patient-specific clinical factors and grade 3 or 4 CRS.

As many symptoms of CRS can mimic other medical conditions such as sepsis, infection, or adrenal insufficiency, it is very important that a thorough workup is performed to rule them out.

Laboratory tests suggestion: complete blood count, liver profile, renal function, sodium, potassium, magnesium, phosphorus daily. Consider monitoring C-reactive protein (CRP) and ferritin levels daily during the phase when CRS is likely to occur (first 10 days) and continuing monitoring until CRS resolves.

Coagulation profile (APTT, PT, fibrinogen) at least twice a week or more often if clinically indicated.

Consider cytokines dosage panel (such as IL-6) only if indicated for some specific monitoring (e.g., if the patient is not responding to interventions). It is not routinely recommended.

Avoid granulocyte colony-stimulating factor (filgrastim) in the first few weeks after infusion during the period when CRS may occur.

Do not administer corticosteroids unless approved by the hematologist.

If the patient has grade I CRS, levetiracetam 500mg orally 12/12h or 10mg/kg 12/12h for children should be started as prophylaxis.

Anti-cytokine therapy:

In general, first-line therapy for patients diagnosed with CRS is done by IL-6 blockers such as the anti-IL 6 antibody, tocilizumab. Its administration may be considered in patients with CRS grade 1 who persist with fever for 72 hours with no other defined cause or who persist with fever above 39°C for 48 hours, as well as in patients with CRS grade 2. Tocilizumab should be administered for patients with grade 3 and 4 CRS[7,8].

It is important to know that initiating therapy with anti-IL-6 antibodies and/or corticosteroids within 24 hours of the beginning of symptoms was associated with reduced CRS severity without compromising the effect of CAR T cells[9].

The recommended dose for tocilizumab is 8mg/kg for patients weighing over 30kg and 12mg/kg for patients weighing less than 30kg, with a maximum dose of 800mg/dose[10]. The minimum interval between the first dose and subsequent doses is 8 hours, and in practice, a dose of tocilizumab has been given every 24 hours in patients who do not experience progressive clinical deterioration requiring faster intervention.

Although it is established that up to 4 doses of tocilizumab are allowed and possible, it is believed that after two doses the drug has already achieved as much blockade as possible in the T cell receptors.

In rare situations in which the patient does not improve with the proposed measures (estimated at less than 10% of cases), the therapy must be modified[11,12], either by the combination of corticosteroids or by changing the anti-IL-6 antibody (for example, Siltuximab, with a different mechanism of action) or by choosing another target of action such as the interleukin 1 (IL-1) receptor antagonist, Anakinra (not yet approved in our country) or the monoclonal antibody that blocks IL 1 beta (Canakinumab, the only anti IL 1 approved and available in Brazil at this time)[11,12]. More serious situations may require the use

of cyclophosphamide or even anti-immunoglobulin to control the inflammatory condition and must be discussed individually.

CORTICOSTEROIDS

For grade 2 hypotension, methylprednisolone 1mg/Kg in a single dose or dexamethasone 0.5mg/Kg (maximum dose 10mg) also in a single dose should be associated.

For grade 3 hypotension, if the patient is using 1 vasopressor, the use of methylprednisolone 1mg/kg/day divided every 12 hours or dexamethasone 0.5mg/kg per IV dose divided every 6 hours is indicated (maximum dose 10mg). If the patient is using 2 vasopressors, methylprednisolone 2mg/kg/day divided every 12 hours or dexamethasone 1mg/kg per IV dose (maximum dose 20mg) divided every 6 hours.

For grade 2 hypoxia, methylprednisolone 1mg/Kg in a single dose or dexamethasone 0.5mg/Kg (maximum dose 10mg) also in a single dose is associated.

For grade 3 hypoxia, methylprednisolone 1mg/kg/day divided every 12 hours or dexamethasone 0.5mg/kg (maximum dose 10mg) divided every 6 hours is used. If hypoxia does not improve within 24 hours or if pulmonary infiltrates progress rapidly or if the need for oxygen increases rapidly, the corticosteroid dose should be increased to 2mg/kg/day of methylprednisolone divided every 12 hours or of dexamethasone to 1mg /kg per dose divided every 6 hours (maximum dose 20mg).

For grade 4 hypoxia or hypotension, we will use pulse methylprednisolone, 30mg/kg/day for 3 days (maximum 1.000mg/dose).

Once the patient has started corticosteroid use, gradual withdrawal or complete discontinuation is recommended once SRC improves to grade < or = 1.

PEDIATRIC PATIENTS PARTICULARITIES[7]:

Hypotension is defined by age-specific physiological normal ranges for age and/or by comparison with the patient's baseline values. In table 2 we can see the 5% percentile of systolic pressure by age group.

The i.v. fluid bolus should be done with 10ml/kg (maximum of 1000ml) and can be repeated once to maintain the normal blood pressure defined by age. After this attempt, if the child still needs fluid resuscitation, the use of colloids should be considered, especially if the patient has hypoalbuminemia.

The use of colloids in this situation is recommended because i.v. albumin can reduce the duration of vasopressors support and decrease the degrees of respiratory, cardiac and neurological failure. In very critically ill patients, albumin administration is associated with a reduction in endothelial dysfunction during the inflammatory processes similar to those seen after CAR T cell therapy.

In addition, acute fluid overload in patients with capillary leaky (especially infants and children weighing < 20 kg, who may be less able to tolerate substantial volume changes) is a major concern as it may contribute to respiratory failure.

Vasopressors and cytokine blockade should start in the time of hypotension and should not be delayed in favor of more than two consecutive i.v. fluid bolus.

Assessment of cardiac function by Doppler echocardiography should be performed in pre cell therapy clinical evaluation to obtain the patient's baseline function. At the time of CRS (from grade 2) it will be very important that the exam be repeated in order to determine which vasopressor will be the most suitable for the child.

In patients with grades 2 to 4 CRS who may have adrenal insufficiency (e.g., patients treated with pediatric ALL protocols), administration of stress-dose hydrocortisone (25mg/m2 6/6h for 24 hours or 100mg/m2) or even Fludrocortisone (0,1mg/dose once a day) may precede initiation of vasopressor therapy and/or cytokine blocking therapy.

In grade 3 CRS, for whom the amount of oxygen at high flow is not sufficient, noninvasive continuous positive pressure ventilation may be a viable option. If indicated, it should be performed in the ICU and not delay intubation.

According to the first classification of CRS proposed by Lee et al[13], if the patient needs high doses of vasopressor, he/she should be classified with CRS grade 4. It's worth mentioning that there is no consensus to define high dose vasopressor in children as there is for adults[13]; thus, this evaluation must be carried out in an individualized and dynamic way by the ICU team who will take care of the patient so that they can promptly inform the hematologist about the progressive increase in dose and/or association of vasopressors for the pediatric patient, because in this context the CRS will be classified in grade 4.

In **Figure 1**, there is a flowchart with the steps to be followed for the pediatric and adult patients experiencing CRS according to their classification.

Critical points and risks of the process
Emergency medical equipment (eg, complete and checked emergency car) must be present throughout the procedure. At least four doses of tocilizumab must be readily available to each patient before initiating lymphodepletive chemotherapy.

Access to corticosteroids should also be easy and quick, but their prescription must always be done by the physician responsible for the patient receiving CAR-T.

Keep ICU and neurology staff trained and aware of patients at risk.

Practice Standard
Not applicable

Training and Competence Assessment Frequency
Annual training for medical and multidisciplinary staff (including ICU and neurology unite), and whenever there is a new member in the team.

Quality Indicators
Incidence of cytokine release syndrome.
Response to treatment.
Mortality.

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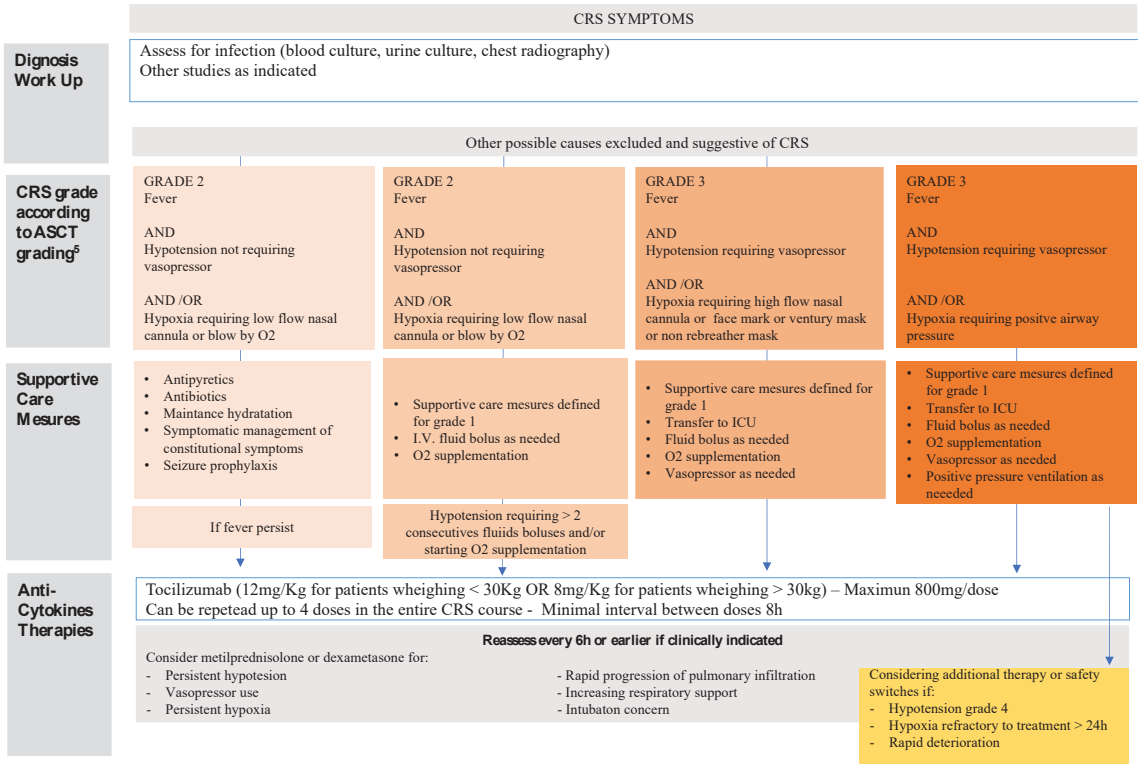
TABLE 1: **ASTCT Consensus Grading for Cytokine Release Syndrome adapted**[5]

CRS PARAMETERS	GRADE 1	GRADE 2	GRADE 3	GRADE 4
FEVER	Axillary temperature > = 37.8ºC (no other cause) In patients receiving antipyretic and/or anti-cytokine therapy such as tocilizumab or corticosteroids, fever is no longer needed to classify the CRS severity. In this case, the CRS classification will consider hypotension and/or hypoxia. The degree of CRS is determined by the most severe event between hypoxia and hypotension.			
WITH				
HYPOTENSION	Absent	No vasopressor needed	Need 1 vasopressor with or without vasopressin	Need of multiples vasopressors (excluding vasopressin)
AND / OR				
HYPOXIA	Absent	Low-flow O2 supplementation (O2 nasal catheter or blow-by oxygen or venturi mask)	O2 supplementation with high-flow nasal cannula or non-rebreather mask or face mask	O2 Supplementation with Positive Pressure (non-invasive or IOT with mechanical invasive ventilation)

TABLE 2: **Percentile 5% Hypotension by Age**

	Systolic pressure (mmHg)
Age	**Percentile 5%**
0-1 month	< 60
>= 1 month – 1 year	< 70
2 years	< 74
3 years	< 76
4 years	< 78
5 years	< 80
6 years	< 82
7 years	< 84
8 years	< 86
9 years	< 88
> = 10 years	< 90

FIGURE 1: Grading and Management of Cytokine-Released Syndrome5,12



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INDICATION AND SELECTION OF PEDIATRIC PATIENTS FOR GENETICALLY MODIFIED T CELLS – FOR THE SBTMO TECHNICAL MANUAL OF CELLULAR THERAPY

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ABSTRACT

The use of CAR-T cells will completely change the treatment of many hematological diseases, including relapsed pediatric B-lineage acute lymphoblastic leukemia. This article is part of the Brazilian Society of Bone Marrow Transplantation (SBTMO)'s "Technical manual of cellular therapy" to guide physicians to select patients and indicate the use of the CAR-T cells in pediatric patients with acute lymphoblastic leukemia. Other aspects of CAR-T cell therapy and management of important toxicities are included on other articles of this volume.

Keywords: chimeric antigen receptor; acute lymphoblastic leukemia; pediatric; hematopoietic stem cell transplantation

OBJECTIVE

To assist pediatricians, hematologists and/or oncologists in the selection of patients for early and assertive referral to CAR-T cell therapy to achieve the best results with treatment.

INTRODUCTION

• CAR-T cells are the patients' own T lymphocytes genetically modified in the laboratory to express receptors that recognize the targets of interest, present in the tumor we want to treat.

• A fragment of DNA that encodes these receptors is artificially constructed in the laboratory; it produces a receptor with high affinity to the antigens that are present in the target cells and, at the same time, provides "co-stimulation," for lymphocyte activation.

• The most widely used receptor for the treatment of B lineage acute lymphoblastic leukemia (B-ALL) was designed to recognize the **CD19** antigen on lymphoblasts.

• The most frequently used co-stimulatory molecules are CD28 and 4-1BB.

• As these molecules are constructed, *i.e*, they do not exist in nature, they are called "**chimeric**."

• The patient's leukocytes are collected by leukapheresis, cryopreserved, sent to reference laboratories. Then, the T lymphocytes are separated from the total leukocytes (*buffy coat*).

• The DNA encoding the receptor of interest and the co-stimulatory molecules is introduced into the lymphocyte nucleus and incorporate into the DNA; from the nucleus, it orders the production of the chimeric receptor. For this genetic incorporation ("**transduction**"), it is usually used a retrovirus ("**viral vector**") artificially bound to DNA, which was built to encode the chimeric receptor and co-stimulator molecules. This retrovirus "infects" the T lymphocytes that have already been separated from the patient's blood.

• The T lymphocytes, after the incorporation of the artificial DNA into their genetic material, produces the chimeric receptor. The receptor migrates to the membrane of the mature lymphocyte and there remains ready to recognize the enemy's CD19.

• The T lymphocytes resulting from this process are called **T** lymphocytes *with* ***chimeric antigen receptors*** (**CAR-T**).

• CAR-Ts are led to multiply in the laboratory to the desired amount to treat the patient (they undergo "**expansion**").

• The cells are then frozen again ("**cryopreserved**") and sent to the patient's treatment center, remaining ready to be subsequently thawed and infused into the patient's bloodstream.

• As this therapy uses genetically modified lymphocytes, it is considered a type of **Gene Therapy**.

• Patients should be treated in highly trained and certified **transplant centers** for the management of toxicities associated with cell therapy.

• The time required between the end of other therapies and leukapheresis performed for the collection of lymphocytes that will be treated in the laboratory is called "***wash out***" and it is quite variable, depending on the potential for destruction of normal T lymphocytes of each type of therapy.

• Therapy administered to the patient after the initial leukapheresis is performed, and before the time of receiving the CAR-T cells, it is called "**bridging therapy**." The objective of the bridging therapy is to keep the patient as healthy as possible, with minimal toxicities *and* tumor load control, without necessarily aiming for remission of the disease.

• Normal lymphocytes of the patient should be destroyed with chemotherapy prior to infusion of CAR-T lymphocytes for them not to offer competition. This **lymphodepletion** chemotherapy recommended for children with B-ALL is performed with fludarabine and cyclophosphamide.

• CAR-T cells are usually sent frozen to the treatment center and thawed at the bedside at the time they will be infused through the catheter, without any manipulation, only introducing saline into the bag at the end of the infusion to ensure that the bag is washed, and the entire product is infused.

• After the cells are injected into the patient's bloodstream, it is essential that they multiply again (undergo "**clonal expansion**") and remain for long periods in the patient (have "**persistence**").

• CAR-T cells are considered "living drugs," as they tend to remain viable in the patient's body for long periods. Because CAR-Ts to treat B-ALL also destroys normal B cells (cause "**B-cell aplasia**"), the patient remains dependent on **immunoglobulin replacement** for infection prevention.

• Adequate levels of immunoglobulins in patients who received anti-CD19 CAR-T or the presence of circulating normal B lymphocytes are indicative of loss of the CAR-T cells, so the options of a new CAR-T infusion, effective in approximately half of patients, or the indication of an allogeneic bone marrow transplantation should be discussed with the team, even before the leukemia relapses.

• The only international centers that offer treatment with (in house) CD19 CAR-T cells for ALL at a fixed price is Barcelona (150,000 Euros for everything in the treatment), according to a personal verbal communication from Dr. Alvaro Urbano-Ispizua *at the European School of Haematology meeting on* CAR-T cells in 2021.

• As the treatment still has a very high cost (often higher than a hematopoietic stem cell transplant), its clinical use outside a research protocol remains restricted to patients who do not have the possibility of curing the disease with other therapeutic strategies.

• To the best of our knowledge, the only **commercially available** therapy worldwide in 2022 for treating children with B-ALL is **Tisagenlecleucel, Kymriah®, Novartis**, which has the CD19 antigen as therapeutic target.

• In **Brazil**, the Tisagenlecleucel, Kymriah® was used as a Class II Advanced Therapy Product for a BELINDA clinical trial sponsored by Novartis Biociencias S.A. for the treatment of adults with relapsed or refractory lymphomas according to Resolution-re No. 1,105 of April 15, 2020.

• The Tisagenlecleucel was submitted to the National Health Surveillance Agency (Anvisa), included in the list of Brazilian Common Denominations of Brazilian Pharmacopoeia, according to RDC No. 480 of March 15, 2021, and it was **approved by Anvisa** in February, 2022 but up to September, 2022, has not been granted a price to be commercialized in the country and, therefore, is not yet available as we write this manual. The prescribing information is already available in Portuguese at the Novartis website

• These commercial CAR-T cells can only be administered in **centers with teams trained and approved by the Novartis laboratory** for the use of CAR-T cells, recognition and treatment of their toxicities, and immediate availability of interleukin-6 inhibitor (IL-6), **Tocilizumab** – Actemra®, Roche.

• There are hundreds of experimental products and protocols abroad for treating children and adults with B-ALL (including the CD22 therapeutic target), T-ALL, acute myeloid leukemia, lymphomas, myeloma, neuroblastoma, brain tumors and various other solid tumors, in addition to HIV/AIDS, but only one trial opened in Brazil for newly diagnosed multiple myeloma "A Study of Bortezomib, Lenalidomide and Dexamethasone (VRd) Followed by Cilta-cel, a CAR-T Therapy Directed Against BCMA Versus VRd Followed by Lenalidomide and Dexamethasone (Rd) Therapy in Participants With Newly Diagnosed Multiple Myeloma for Whom ASCT is Not Planned as Initial Therapy (CARTITUDE-5)" enrolling patients at the Hospital Sao Rafael, Salvador and AC Camargo Cancer Center and Hospital Israelita Albert Einstein in Sao Paulo, Brazil

INDICATIONS OF CAR-T CELL TISAGENLECLEUCEL:

Indications in pediatric B-ALL as of today are restricted to the commercially approved product Tisagenlecleucel, according to the Brazilian prescribing information:

• Children and young adults up to (including) 25 years of age with ALL:

- refractory to therapy *or*
- in second or subsequent relapse *or*
- relapsed after hematopoietic stem cell transplantation.

• Adults with diffuse large cell lymphoma that is refractory of relapsed after two or more lines of therapy.

SELECTION OF PATIENTS WITH B-ALL FOR TISAGENLECLEUCEL:

• Expression of the CD19 in the B-ALL blasts;

• Primary refractoriness *or*

• any relapse after allogeneic hematopoietic stem cell transplantation *or*

• second or further relapse *or*

• patients with relapsed disease ineligible for hematopoietic stem cell transplantation due to comorbidities, impossibility of tolerating a myeloablative conditioning regimen, with not HLA-compatible or partially compatible donor *and*

• possibility to wait for the minimum period without the prohibited medications ("wash-out") *and*

• clinically stable enough to tolerate leukapheresis, a bridging therapy until cells are prepared, lymphodepletion therapy, prolonged aplasia, cytokine release, neurotoxicity and hypogammaglobulinemia.

Observations:

- Age **below 3 years *does not*** contraindicate therapy

- The presence of **Down's Syndrome *does not contraindicate*** therapy

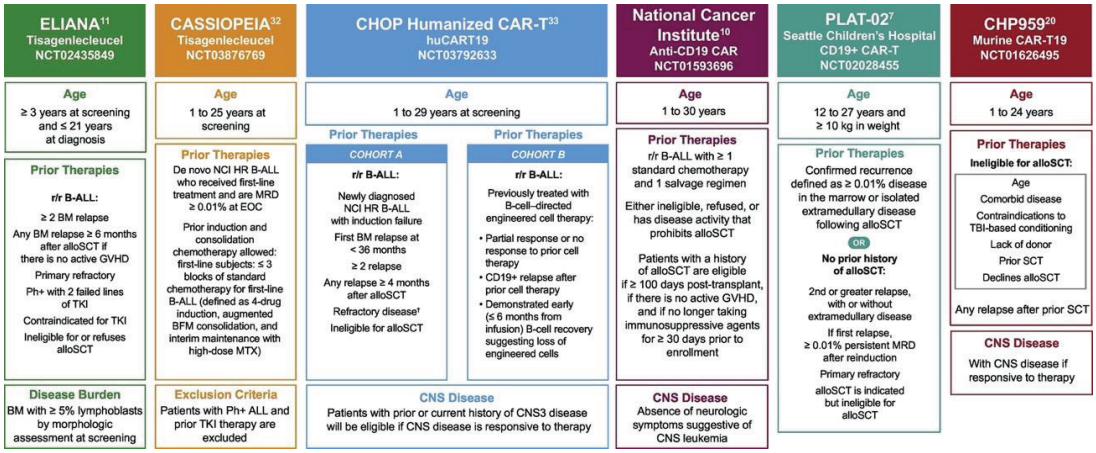
- The various **adverse genetic risk factors *do not*** contraindicate therapy

- The presence of **extra-medullary disease *does not*** modify the indication of therapy. It is clear CAR-T cells can enter the central nervous system and spinal fluid, but their ability to reach other immunoprivileged sites, such as testicles, optic nerve or eye globe, is still less clear.

- The disease being in **activity** or in **remission** at the time of infusion ***does not*** modify the patient's eligibility, although the results of the therapy are superior when patients have chemossensitive disease and achieve remission with the bridging therapy.

- The use of **blinatumomab** before CAR-T cell therapy ***does not*** contraindicate its use. Patients who respond to blinatumomabe generally also respond to CAR-T. However, if a negative CD19 clone emerges, the CAR-T will be totally inactive. The use of pre-CAR-T **inotuzumab** is not considered ideal due to the potential to decrease the chance of response to CAR-T, but it is not a contraindication.

FIGURE 1: Clinical eligibility for children when included in research protocols[11]



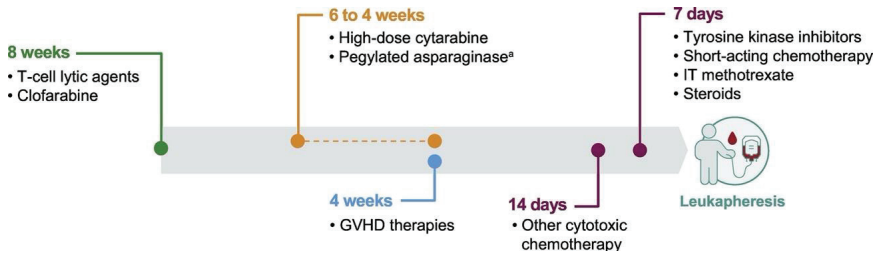
Patient eligibility to perform leukapheresis for T-cell collection and CAR-T cell manufacturing**:**

Absolute **lymphocyte count of >500/µL** (although some studies require ≥100/µL) ***or*** peripheral blood CD3 count of >150/µL

Age below 3 years and weight ***does not*** contraindicate leukoapheresis but indicates that it must be performed in a pediatric centers experienced with leukapheresis in these patients.

Wait for proper *wash-out time* (Figure 2)

FIGURE 2: Waiting time (wash out) until the patient is released for the collection of autologous lymphocytes by leukapheresis[11]



Center eligibility to use Kymriah in the United States (still unclear in Brazil)**:**

To receive authorization for the use of Kymriah hospitals must have:

Risk Evaluation and Mitigation Strategies **(REMS)** program approved by Novartis with a trained and authorized representative to:

• Carry out the certification process and supervise the implementation and compliance of the REMS Program in the hospital;

• **Train and evaluate** the knowledge of **all relevant employees** involved in the **prescription, dispensation, or administration** of Kymriah;

• Establish processes and procedures for:

- ensure that **new employees** involved in the prescription, dispensation, or administration of Kymriah are trained and complete the Knowledge Assessment.

- check for at least **two doses of tocilizumab** available at the site for each patient and are ready for immediate administration (within 2 hours).

- provide patients with the **Patient Card**.

Before the first infusion the center shall:

- Check for at least **two doses of tocilizumab** available on site for each patient and that they will be ready for **immediate administration (within 2**

hours) through the processes and procedures established in the Service's REMS Program

Before the patient is discharged the center shall:

- Provide **Pocket Card** to the patient (according to your REMS program)
- To maintain certification to dispense, if there is change in authorized representative:
- Have the new authorized representative enrolled in the REMS Program.

To maintain certification to dispense Kymriah, if the center has not dispensed at least once a year from the date of certification in the REMS Program:

- Train all relevant employees involved in the prescription, dispensing or administration of Kymriah according to the REMS Program
- Assess the knowledge of the relevant team involved in the prescription, dispensing or administration

At all times the center shall:

- **Report any adverse events** suggestive of cytokine release syndrome or neurological toxicities to the REMS Program.
- **Keep personnel training records**.
- **Keep records that all processes and procedures are in place and being followed**.
- **Comply with audits** conducted by Novartis or a third party working on behalf of Novartis to ensure that all processes and procedures are in place and are being followed.

Recommended bridging therapy:[11]

Tumor load	Recommended bridging therapy	Outpatient
Low	Maintenance pulses with vincristine/ corticosteroids Targeted drugs, for example, tyrosine kinase inhibitors Maintenance oral mercaptopurine and methotrexate Immunotherapy (?)	Yes
Slow progression	Capizzi style Methotrexate in (with or without PEG asparaginase) Low doses of Cytarabine (300 mg/m^2) and etoposide (150 mg/m^2) every 2–3 weeks Maintenance rotating drug pairs (St. Jude) Immunotherapy (?)	Yes
Rapid progression	Etoposide (100 mg/m^2/day) and cyclophosphamide (440 mg/m^2/day) for 3–5 days FLAG High-dose Cytarabine (3 g/m^2 every 12 h × 4 doses) D1,D2	No
Extra-medullary disease	Radiotherapy can be performed	Yes

Recommended lymphodepleting therapy:

• **Fludarabine** (30 mg/m^2 intravenously daily for 4 days) and **cyclophosphamide** (500 mg/m^2 daily for 2 days, starting with the first dose of fludarabine).

• If the patient has previously had a grade 4 hemorrhagic cystitis with cyclophosphamide or demonstrated a chemo refractoriness to a cyclophosphamide-containing regimen administered just before the lymphodepletion chemotherapy, the following regimen may be used: Cytarabine (500 mg/m^2 intravenously daily for 2 days) and etopoide (150 mg/m^2 intravenously daily for 3 days, starting with the first dose of cytarabine).

Critical points:

• Avoid the patient's elective exposure to anti-CD19 immunotherapy (BiTE - Blinatumomabe - Blincyto®) when the patient is being considered for CAR-T cell therapy to prevent tumor cell escape to anti-CD19 CAR-T.

• Also avoid exposure to anti-CD22 (Inotuzumab) as it decreases response to anti-CD19 CAR-T.

• Check availability of centers offering CAR-T cell therapy.

• Check how the CAR-T cell will be paid.

• Check the availability of a **programmable freezer**, and **nitrogen cryopreservation**.

• **Team training** for all stages of therapy, especially the management of toxicities.

• Immediate availability of **tocilizumab**.

• Availability of **infrastructure** and **intensive care**.

• Availability of **data manager** to report treatment results and patient follow-up for **at least 15 years.**

Risks involved in the process:

• **Frustration** of the family members and physicians due to the inability to offer treatment due to medical, infrastructure or financial reasons.

• Severe **cytokine release syndrome**.

• Severe **neurotoxicity**.

• Prolonged **cytopenias**, and subsequent **infections**.

• Long-term **B lymphocyte aplasia** requiring **immunoglobulin replacement** and increased **susceptibility to viral infections**, already so common in our country.

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EVALUATION OF PREDICTABILITY OF BONE MARROW CELL CELLULARITY FROM RELATED AND UNRELATED DONORS

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ABSTRACT

Introduction: For successful hematopoietic stem cell transplantation, a minimum number of total nucleated cells should be obtained (TNC) during the bone marrow (BM) harvest procedure. Objectives: Evaluate the predictability of the BM TNC collected from an interim sample collected during the procedure and the factors related to high-cellular harvest. Methodology: This is a retrospective observational study including BM donors and recipient from 2017 to 2019. The final TNC was based on an interim quantification of TNC and was compared with the actual final TNC obtained. Results: 81 donors were included and interim TNC of 53 donors were available. Based on linear regression, a significant correlation was found between the volume of BM collected and the interim and final TNC (n=53; R2=0.83; P<0.001). The relationship between donor and recipient weight significantly influenced the collection yield. There was also a positive correlation between the volume of BM collected and the interim and final count of TNC (n=53; r=0.88; p<0.001). The difference in donor and recipient weight also had a positive correlation (p<0.02). Conclusion: Our results showed that interim TNC quantification can help to achieve a better performance during the procedure, allowing real-time re-estimation of the volume needed to be harvested.

Keywords: Hematopoietic Stem Cell Transplantation; Bone Marrow Harvest; Donors.

INTRODUCTION

Stem cells are probably the best-known cell type and has been used for over 50 years, mainly in diseases related to the hematopoietic and immune systems. Over the last decades, the method of collecting these cells has been refined, incorporating multiple steps to ensure the safety of donors and the best results for recipients[1].

A minimum number of total nucleated cells (TNC) during bone marrow (BM) collection procedure is necessary for a successful hematopoietic stem cell transplantation (HSCT). Some authors[2–4] state that the minimum TNC should be between 3 and $5x10^8$/kg and that values below $2x10^8$/kg are considered inadequate. Other studies[5,6] suggest that a higher dose of TNC may improve overall survival and reduce transplant-related mortality. There is a trend towards incorporating $CD34^+$ counts in the bone marrow harvest routine; some authors suggest that the minimum $CD34^+$ count should be between 2 and 4 x 10^6/kg for BM[2,7,8].

To improve the harvested TNC and $CD34^+$, there are mainly two possibilities: (1) to increase the harvested volume or (2) to increase the TNC concentration. The target volume of BM collection is empirically based on recipient weight, with a target of 15 to 20 mL/kg (usually limited to 20 mL/kg of the donor's weight). Although this method is widely used, a significant percentage (27%-50%) of recipients receive a relatively low dose of TNC ($<2.4x10^8$/kg)[6]. To obtain a higher dose of TNC, clinicians generally tend to collect as much BM as possible through multiple small-volumes BM aspirates and/or by using fenestrated needles. The former strategy can prolong anesthesia time, increase the number of puncture sites and increase blood loss in donors[5,6,9].

A large volume of BM can also be harmful for recipients, due to the risk of fluid overload. The most effective and safest way to reach the target dose is to increase the amount of TNC collected[6,10]. Therefore, this study aimed to evaluate the predictability of the total cellularity of the collected bone marrow from an interim sample collected during the procedure and to evaluate factors related to a bone marrow collection with a total nucleated cells (TNC) greater than $4x10^8$/kg of recipient's weight.

METHODOLOGY

This is a retrospective observational study, including related and unrelated hematopoietic stem cell transplant (HCT) donors from a public hospital in the Rio de Janeiro, Brazil. The data collection was from 2017 to 2019. The stem cell collection procedure was performed in the operating room, under general anesthesia, and the bone marrow was aspirated from the posterior iliac crests, bilaterally, using a needle with lateral holes and fenestrated (Argon Medical Devices). On average, 3 to 5 ml were aspirated per puncture in each iliac crest to avoid sample dilution.

The BM collection teams consisted of two physicians and two experienced nurses, two nursing technicians, and an anesthesiologist. The average time for each collection was estimated at 90 minutes, and the maximum time was 120 minutes. The targeted final collected BM volume was estimated by 20 mL per kg of the recipient's body weight, with a maximum amount of 20 mL per kg of the donor's body weight.

The aspirated BM was homogenized with an anticoagulant solution of heparin 5000UI/mL and 0,9% saline. The final concentration was 100 UI/ml (heparin and saline solution). The total volume of the solution used was 10% of the estimated volume for each BM collected. Closed-system (BioAccess®) collection bags and filters were used. The total volume of the harvested BM was obtained by subtracting the volume of anticoagulant from the volume of the mix.

Based on the study by Wang and Cols[6], our bone marrow transplant team in 2017 instituted an intermediate collection of TNCs when half of the total target volume (20 kg/mL of the recipient's weight) was reached. A 2 mL sample of the concentrate was withdrawn from the BM collection bag and placed in an EDTA tube. This sample was sent at the end of the procedure with the collection bag to the lab for leukometry analysis. The collection team was not aware of the interim count during the procedure.

$$Estimated\ final\ TNC = Interim\ TNC \times \frac{Final\ collected\ volume}{Interim\ collected\ volume}$$

To analyze the correlation between final and intermediate TNC, we included all donors with interim and final quantification (Spearman correlation). For analysis of factors associated with the amount of TNC collected, we included all adult donors and recipients. We did not include children, who are usually underweight, to avoid a biased results. For the analysis of factors associated with the amount of TNC collected, linear regression (TNC as a continuous variable) and logistic regression (TNC > $4.0x10^8$/kg being the outcome) were performed. We considered statistically significant two-sided P-values less than 0.05. Analyzes were performed using the R statistical program, version 4.1.0.

The study was approved by the local Research Ethics Committee and all donors signed an informed consent form, following the ethical precepts of research with human beings.

RESULTS

The characteristics of the 81 donors was separated in two groups (Table I). Group A included the donors with a TNC count ≥ $4x10^8$/kg and group B with a TNC count $<4x10^8$/kg. In both groups, there was a male predominance, the mean age of the groups was equivalent. Unrelated donors had a higher TNC count than related ones (P = 0.126).

The harvested volume was between 6.9 and 22.7 mL/kg, with a median of 15.4 (receiver weight), when recipients were heavier than donors. All harvested bone marrow had <20mL/kg of the donors' weight, following the standards of the National Marrow Donor Program (NMDP). The collected BM volumes

also did not have a statistical significance (P = 0.436) when group A (CNT ≥ 4 x10^8/kg) was compared with group B (CNT <4 x10^8/kg).

Regarding ABO compatibility, 66.7% of compatible donors had a TNC count ≥ 4x10^8/Kg, while 68.8% of non-compatible donors had a TNC count <4x10^8/Kg (P= 0.792). Among the 81 included donors, 53 had interim TNC quantification by the half-time of the collection. Based on linear regression, a significant correlation was found between the volume of BM collected and the intermediate and final count of TNC (n = 53; R2 = 0.83; P <0.001; Figure 1).

The relationship between donor and recipient weight significantly influenced the collection yield; only 7.7% of BM collections from donors who weighed less than their recipients achieved a TNC count ≥ 4×10^8/kg, compared with 84% of collections from donors heavier than their recipients (P<0.003). Figure 2 show that the weight difference between the donor and recipient achieved a positive relationship with the number of TNC. Donors weighing less than the recipient's weight had a lower count of 4x10^8/Kg TNC. Figure 3 shows a positive but weak correlation between the measurement of TNC and $CD34^+$ Cells (n = 53; R2 = 0.44).

DISCUSSION

Our results showed a positive correlation between the volume of bone marrow collection and the intermediate and final count of total nucleated cells (n = 53; r = 0.88; p <0.001). This suggests that if the intermediate count is implemented during the procedure in the operating room, it may contribute to achieving ideal cellularity (TNC ≥ 4x10^8/kg) in donors with lower-than-expected interim counts, in addition to saving donors from possible risks and side effects in those with higher-than-expected interim counts.

Other authors[6] who have implemented the interim quantification of TNC also found a positive correlation between interim and final total counts (r= 0.8774; p<0.001), corroborating our results and demonstrating that this strategy can be effective. However, we know that other issues can also influence the achievement of good cellularity, such as, the selection of the ideal donor (younger men and donor who weigh more than their recipients) as well as experienced centers and operators[3,5,9–12].

In our study, we did not find a significant correlation between TNC and donor gender (P = 0.722), despite the predominance of males. This brings us to other factors that can also influence BM collection, such as age; however, we didn't find a correlation between age and the TNC of donors (p=.094). A study[13] carried out in a cohort of donors from the National Marrow Donor Program identified a significant decrease in the quality of BM collections over time associated with older ages, male gender, and race, reinforcing the importance of care in donor selection. Unfortunately, data regarding donor race were incomplete, and thus comparisons of this variable were not possible.

There was a statistical positive correlation between donor and recipient weight (p<0.02); 84.6% of donors with a weight higher than recipients reached an ideal cellularity (>4x10^8/kg). Among the donors who weighed equal or less than the recipients, only 15.4% achieved this ideal cellularity. A study[10] with 110 unrelated donors revealed a significant impact on the discrepancy between donor and recipient weights on the amount of TNC collected: only 18% of collections from donors who were lighter than their recipient achieved an ideal TNC/kg. Whenever possible, donors with equivalent weight to or greater than the recipient should be selected. When this is not possible, the transplant center should consider the possibility of using peripheral blood stem cells instead of BM[5,9,10].

As expected, the correlation between $CD34^+$ and TNC at the end of BM collection was positive, but weak, preventing us from stating an increase in the number of $CD34^+$ cells with increasing number of TNC. In our study the volume collected did not influence TNC (P=0.436); smaller volumes had a higher TNC when compared with longer ones. Other authors[2,13,14] suggest that large volumes and longer collection times may result in a lower chance of obtaining the target dose of TNC. One hypothesis is that the low-volume bone marrow comes with less peripheral blood contamination, reducing the dilution of the final product[4,10].

In summary, our results showed a positive correlation between the TNCs in the middle and the TNCs at the end of bone marrow collection, which reminds us of the importance of quantifying these cells during the harvest period, possibly decreasing the procedure time and the risks for the donor or increasing the amount of BM harvested from donors that can tolerate larger volumes. However, we must carry on further research to assess at which point the collection can be safely interrupted for the donor, with enough number of TNC for the recipient to engraft.

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TABLE 1. Donor Characteristics and Results of BM Harvesting

Characteristic	Group A CNT ≥ 4 x108	Group B CNT <4 x108	P value
Total, n	45	36	
Age			0.094
Yr, median (SD)*	34 (11.5)	38.8 (13.6)	
Sex, n (%)			0.722
Male	28 (62.2)	21 (58.3)	
Female	17 (37.8)	15 (41.7)	
Types of donars n (%)			0.126
Related	15 (33.3)	20 (55.6)	
Unrelated	23 (51.1)	13 (36.1)	
haploidentical	7 (15.6)	3 (8.3)	
BM harvest volume (mL) n (%)			0.436
mean(SD)	1006.4 (371.3)	1066.6 (306.5)	
164 – 998	15	12	
1000 -1259	18	15	
1318 - 1548	11	9	
ABO compatibility n (%)			0.792
ABO match	26 (66.7)	22 (68.8)	
ABO major mismatch	6 (15.4)	6 (18.8)	
ABO minor mismatch	7 (17.9)	4 (12.5)	
Weight difference n (%)			0.003
Recipient heavier	3 (7.7)	6 (17.1)	
Equal weight, up to 5 kg	3 (7.7)	12 (34.3)	
Donor heavier	33 (84.6)	17 (48.6)	

*SD- Standard deviation

FIG.1- Correlation between midway and final BM cell density (n = 53; r = 0.83; P<0.001).

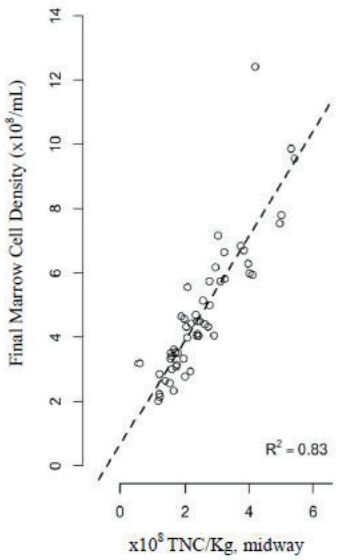


FIG.2 - Correlation between the difference weight of the donor and the recipient and the number of total nucleated cells.

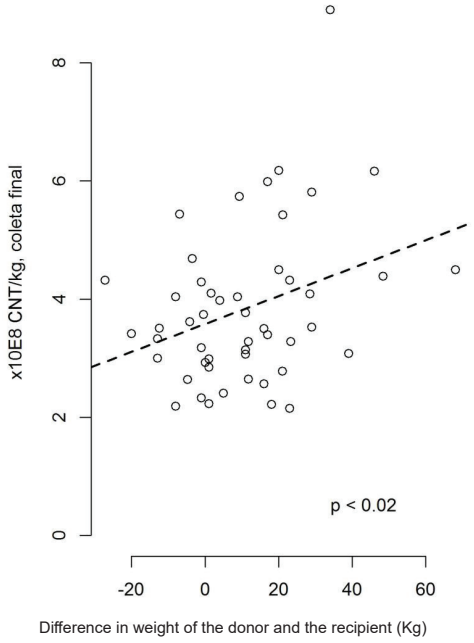
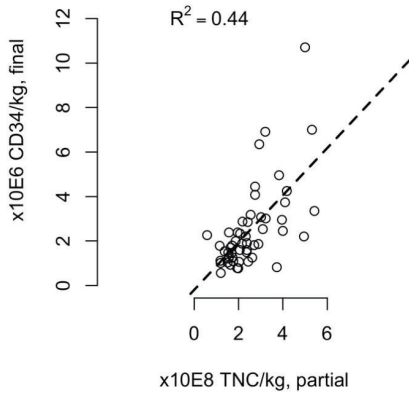


FIG.3 - Correlation between CD34+ and CNT at final of CTH collection.



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REGISTRATION OF NEW POTENTIAL BONE MARROW DONORS: EXPERIENCE IN THE INTERIOR OF PERNAMBUCO

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ABSTRACT

Objective: To report the experience of nursing students in an extension project to capture and raise awareness of potential bone marrow donors. Methods: This is an experience report resulting from actions carried out by an extension project. The actions took place in Pesqueira, Pernambuco, Brazil, held in three events between March and May 2019. The sample consisted of 369 new potential bone marrow donors. Results: The first intervention totaled 54 health professionals, in the second, 248 records were obtained in the general population. The last intervention provided a total of 67 student registrations. Final Considerations: The actions promoted reflections, in addition to raising awareness and attracting new potential donors, for doing something not yet seen in the city, showed that extension is a fundamental tool to carry out the exchange of values between the university and the community.

Keywords: Hemotherapy Service. Health Education. Bone Marrow. Bone Marrow

INTRODUCTION

Cancer is in the second position as the most relevant cause of death in the world, in every six conditions one death is due to the disease. In Brazil, it is estimated the occurrence of 600 thousand new cases of cancer in the 2018 to 2029. Of these, the hematological ones stand out, which in Brazil are estimated in 22,780 cases and of these 12,210 affect man and 10,570 women[1, 3].

Leukemia is a disease that originates in the bone marrow (BM), the region in which blood cells are produced and its incidence occurs between 2 and 3 years of age, present in 17% in the first year of life. This disease is present in 80 per million people and is responsible for the largest number of childhood deaths related to cancer in Brazil, however, if diagnosed and treated early, it has an 80% chance of cure from the very therapeutic measure. successful as OM transplantation, a process in which the patient undergoes invasive technologies, highly complex medical procedures and immunosuppression[4,5].

Stem Cell Transplant (SCT) is a therapy that consists of replacing a diseased tissue with healthy cells between compatible individuals, related or not, with the aim of cellular reconstitution. When the option for transplantation is defined, the search for compatible donors in a specific database begins. In Brazil, this donor base is called REDOME, Brazil's Bone Marrow Registry, which is in the third position as the largest donation bank in the world and contains necessary information on the potential donor

who registers to try to achieve compatibility with potential recipients[6].

The limitations of publication in open advertisement, as well as the lack of available blood centers in cities, can directly impact the knowledge and registration of the possible donor, and these factors influence the adherence to the registration as an BM donor. Thus, it is necessary to offer the population information about REDOME in order to increase the registration of voluntary MB donors, through dissemination, campaigns and social media in order to develop a sense of solidarity[7,8].

The process of training university students in the health area can contribute to the development and direction of remedial measures and educational campaigns, with educational interventions to inform the population about BMT through extension projects. In this way, the number of entries in REDOME can be further increased, as it is still insufficient for the need of countless patients who only have transplantation as a treatment[9,10].

The objective of this study was to report the experience of nursing students in an extension project to raise awareness and register potential bone marrow donors.

METHODS

This is an experience report resulting from actions carried out by the extension project entitled Minha Vida na Sua Vida: Community Awareness on the Registration in the Bone Marrow Donor Bank carried out at the Federal Institute of Education, Science and Technology, IFPE Campus Pesqueira in partnership with the Blood Center of Arcoverde, Pernambuco, Brazil and the Health Department of Pesqueira, State of Pernambuco, Brazil. The actions were carried out in three events between March and May 2019. The study was approved by the Research Ethics Committee, under Ruling No. 3.549.307.

The materialization of the project took place in Pesqueira, Pernambuco, Brazil, estimated at 67,395 inhabitants in 2019 according to the Brazilian Institute of Geography and Statistics (IBGE)[11]. The city has basic health units, hospital, Emergency Care Unit (UPA) and private clinics, but it does not have a blood center, which makes it difficult for the population to access both information and the possibility of becoming a donor. In this context, it was decided to sensitize this population to increase the number of people registered in REDOM.

The study included individuals who met the criteria established as recommended by the REDOME: People who attended the events held to attract new potential donors who were aged between 18 and 55 years; in good general health; no infectious or disabling diseases; who did not have neoplastic (cancer), hematological (blood) or immune system diseases. Exclusion criteria were: Do not wait for the mandatory lecture from the blood center; away from the site. The study sample consisted of 369 new potential MB donors, numbers that exceeded the half-yearly goal of registered at the Pernambuco Hematology and Hemotherapy Foundation (HEMOPE) in the city of Arcoverde, Pernambuco.

For the capture of possible donors, the nearest blood center located in Arcoverde, Pernambuco, which made available the presence of its team on pre-scheduled dates, to carry out the registration and collection of blood material that are necessary for the insertion of the citizen in the donor bank.

The project had four extensionists who underwent training in the collection of blood material and more than 30 volunteers who helped in the dissemination and organization of the actions that took place in the community, through the delivery of pamphlets and posters in the public health services sectors of the city: in the hospital, ten health posts, health secretary and public health polyclinic. The extension team, together with the coordination of the nursing course and the direction of the Pesqueira, Pernambuco campus, also participated in interviews to publicize the actions on the city's local radio, so that the news reached and sensitized the greatest possible number of citizens in the region.

RESULTS

In the initial period of carrying out the actions, the general population adhered to the proposal offered by the extension. As the city of Pesqueira, Pernambuco does not have a blood center, the project made this access possible through the coming of the HEMOPE team to the city to carry out the registration of people who showed interest in being a donor, from the dissemination of the theme carried out by broad way.

Candidates who met the criteria established by REDOME filled out the free and Informed Consent Form (ICF) and a questionnaire about prior knowledge about BM donation, after that, they underwent a mandatory lecture, held by the coordination of HEMOPE, with the information necessary for the poten-

tial donor to be aware of the decision and how the entire donation process takes place. For registration, a specific form established by the blood center was used, which contained sociodemographic data and personal contact information, and after filling it out, the potential donor went to the collection site for 5ml of blood material to complete their registration.

On March 22, 2019, the first intervention took place at the IX Municipal Health Conference of Pesqueira, as shown in Figure 1, this choice was due to the concentration of a greater number of health professionals in a single event. It is understood that health professionals are more sensitive to the cause due to the higher level of knowledge about BM transplantation. At the time, a total registration of 54 health professionals, potential bone marrow donors, was obtained.

FIGURE 1: IX Municipal Health Conference of Pesqueira

Then, on April 29, 2019, the second intervention took place, this time at the IFPE Campus Pesqueira and the target audience was the general population informed of the event from the local radio. This action was attended by people from the locality and neighboring towns, soldiers from the Pesqueira, Pernambuco army and students. The choice of the place of action was due to the infrastructure facilitating the meeting of a significant number of people, as shown in Figure 2. It was possible to register 248 new potential donors.

FIGURE 2: Federal Institute of Education, Science and Technology, IFPE - Campus Pesqueira

Subsequently, on May 14, 2019, the third intervention developed in an event during the nursing week of the IFPE, Campus Pesqueira took place, as shown in Figure 3. The location was chosen due to the high concentration of nursing students, which provided a total of 67 registrations.

FIGURE 3: Federal Institute of Education, Science and Technology, IFPE - Campus Pesqueira

There is a large number of records in Brazil, however the levels of incompatibility are alarming due to the country's miscegenation[5]. This fact leads to a greater need for the registration number to be even more relevant to reduce the rates of deaths in the waiting list for a match. Due to the difficulty of compatibility and the number of people registered in the donation banks, it was decided to sensitize the population about bone marrow donation in order to encourage the feeling of collaboration and solidarity with others in the community. A minority of the population in the aforementioned study site has access to knowledge generated at universities, especially on issues related to health, and for this reason there are questions about bone marrow donation and how it is performed.

Thus, extension is an essential tool for the democratization of this access to knowledge, which makes interventions by academics from extension projects essential, as it provides opportunities for the provision of services that benefit communities, as well as enabling a reflection about the existing social difficulties. Activities of this nature contribute positively to the search for social solutions, in addition to preparing the student for the professional environment. These health education practices end up including a greater diversity of knowledge to society and promote greater adhesion of the population[12,13].

Repercussions obtained within the community are perceived as positive not only in the context of learning, but in the perspective of contributing to the process of developing the population's awareness and promoting change.

FINAL CONSIDERATIONS

The actions provide an opportunity for a process of sensitization of the population that presented itself in a satisfactory way, as these actions promoted reflections regarding the performance of blood centers, bone marrow donation and transplantation in the municipality of Pesqueira, Pernambuco. In three actions, it was possible to sensitize the entire population of Pesqueira, Pernambuco, as well as neighboring cities, and promote the registration of 369 new potential donors, which contributed to the growth in the number of registered in REDOME.

In this report, it was possible to observe that the project, in addition to raising awareness and attracting new potential donors, as it does something not yet seen in the city, showed that extension is a fundamental tool to exchange values between the university and the community. In addition, it significantly impacted lives, sensitized and disseminated knowledge to a large portion of the population, while students learn with the knowledge of the communities to obtain performances that meet the characteristic needs of that environment, thus representing a successful experience to everyone involved.

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ANALYSIS OF THE IMPACT OF COMORBIDITIES ON THE MORTALITY OF PATIENTS HOSPITALIZED WITH COVID-19

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ABSTRACT

Introduction: SARS-CoV-2 has resulted in a pandemic since March 2020. The knowledge of the impact of risk factors is fundamental for its adequate treatment. This study aims to analyze the impact of comorbidities and transplant of solid organs and tissues in patients who were hospitalized because of Covid-19. Methods: There were collected data from 457 patients that had been diagnosed with Covid-19 who were hospitalized in a ward or in an intensive care unit (ICU) at a university hospital. All the patients were sorted for history of transplant of solid organs and tissues. The laboratory results of admission, place of hospitalization and outcome were compared among the group of transplanted and non-transplanted patients. Results: In total, there were collected 457 patient's data that had been diagnosed with Covid-19. The lethality in our service was 17,94%. The mortality of patients hospitalized in ICUs was 57,14%. The patients that presented hypertension (48,36%) showed a mortality level of 23,53% versus 12,71% who did not. Differently from the previous comorbidity, DM-2 showed no statistical significance. Transplanted patients had 2,13 more chances of being hospitalized in the ICU than in the ward. Also, transplanted patients had 2,21 more chances of death. The mortality in kidney transplant patients was 35.29%, liver transplant was 23.08% and allogenic bone marrow transplant 33.33%. Conclusion: In our sample of patients that had been hospitalized with COVID-19, the prior diagnosis of hypertension and transplant showed higher levels of mortality, mainly kidney transplantated patients (35.29%).

INTRODUCTION

The disease caused by SARS-CoV-2 has resulted in a pandemic since March 2020, and many studies have been done in order to understand the broad spectrum of the seriousness of this disease. The knowledge of the impact of risk factors is fundamental for its adequate treatment. We, hereby, present the analysis of 457 patients that were hospitalized with COVID-19, at the University Hospital Walter Cantídio, in Fortaleza, Ceará, Brazil.

OBJECTIVES

This study aims to analyze the impact of transplant in solid organs and tissues, in patients who were hospitalized because of Covid-19. As a secondary objective, we studied the impact of comorbidities (hypertension, diabetes and chronic renal disease) in patients' mortality.

METHODS

There were collected data from 457 patients that had been diagnosed with Covid-19 who were hospital-

ized in a ward or in an intensive care unit (ICU) at the University Hospital Walter Cantídio. There were only included patients with confirmed diagnosis through RT-PCR or a fast molecular test for COVID-19.

All the patients were sorted for history of transplant of solid organs and tissues (renal, hepatic and of bone marrow, as well as comorbidities as systemic arterial hypertension (SAH), diabetes mellitus type 2 (DM-2) and chronic kidney disease (CKD). The laboratory results of admission, including inflammatory marker, place of hospitalization and outcome, were compared among the group of transplanted and non-transplanted patients. All data were extracted from an electronic record.

The Mann-Whitney test was used to compare central trends of two independent samples, and the Chi – Square Test of Independence, in order to analyze the joint distribution of two variables, being used as a significant, $p<0,05$.

The statistical analyses were held with the support of software Microsoft Excel 2019 and R version 4.1.0. This paper was previously approved by the Institutional Review Board by Universidade Federal do Ceará (Hospital Universitário Walter Cantidio) by the number 4.023.458, CAAE: 31511620.6.0000.5045.

RESULTS

In total, there were collected 457 patients that had been diagnosed with Covid-19 and were hospitalized in wards or ICUs, being that 73,96% were in wards, and the other ones - 26,04% - in ICUs.

Among these patients, 57,33% were males. Regarding the presence of the main comorbidities, 48,36% had previous diagnosis of hypertension, 33,26% DM-2 and 12,47% CKD. In total, 8,53% of patients had undergone a certain type of transplant, being that: 3,72% kidney transplant, 2,84% liver transplant and 1,97% allogeneic bone marrow transplant.

Patients' mean age was 57 years old; with quartile deviation of 22 years [18 -103 years old]. The mean body mass index (BMI) was 26,9 kg/m^2, with deviation of 8,35.

Of all, 17,94% progressed to death. Regarding the mortality of patients hospitalized in ICUs, the percentage was 57,14%. In order to understand the patients' risks of death, and their associations with other factors, a cross tabulation was done between the studied variables and outcomes.

Firstly, on Table 1, it is shown the outcome associations, with the main socio-demographic variables. It is noticed that:

The percentage of deaths in male patients was 19,47%, while that in women this percentage was a little lower, 15,9%. Despite the difference, there wasn't statistical significance among the sexes.

The patients that presented SAH (48,36%) showed mortality level of 23,53%, and the ones who didn't, showed mortality level of 12,71%, with statistical significance. The patients with SAH showed 2,11 more chances of death.

Differently from the previous comorbidity, DM-2 didn't show significant value in the chi-square test, p value = 0,335 > 0,05. Out of the patients with DM-2, 20,39% died versus 16,72% of those without DM-2. Out of the patients without DM-2, 24,9% needed a bed in the ICU, versus 29,86% of diabetic patients, also without statistical significance.

The proportion of death of patients with CKD was 26,32%, and patients without CKD was 16,75%. Despite this difference, the obtained p value was 0,078 > 0,05.

We can see the association of the holding of any kind of transplant with the place of hospitalization, the outcome and the values of some laboratory variables.

The number of patients that did any type of transplants was 39, which corresponds to 8,53% of the total of analyzed patients, being that 3,72% was kidney transplant, 2,84% liver transplant and 1,97% bone marrow transplant.

On Table 2, it is presented the laboratory variables, according to the groups that had or had not done a transplant. We concluded that the values of white blood cells (WBC), neutrophils, segmented neutrophils, lymphocytes, monocytes and platelets were lower in the groups of transplanted patients, with statistical significance. The amount of band neutrophils, although higher in the group of non-transplanted patients, did not show any statistical significance.

Beyond the laboratory variables, it was also crossed with the holding of transplant, the place of hospitalization – ward or ICU- and the outcomes of death or cure, which follows:

There were hospitalized in the ward, a total of 338 patients, and in the ICU, 119 patients, being that out

of the total number of patients that had been hospitalized in the ward, 6,8% had done a certain type of transplant, and regarding the patients who were in the ICU, 13,45% of them had done a transplant.

The percentage of transplanted patients is higher in the ones in the ICU than in a ward, with statistical significance.

With the aim of quantifying the identified association, it calculated the odds ratio considering the chances of a transplanted patient in the ICU, by the chances of transplanted patients in the ward, and the obtained result was 2,13, with confidence interval of 95% between 1,08 and 4,18. We conclude there are 2,13 more chances of transplanted patients being hospitalized in the ICU, than in the ward and 2,21 more chances of death in the transplanted patients group.

ASSOCIATION BETWEEN LABORATORY VARIABLES AND OUTCOME, IN THE TRANSPLANT GROUP

With the purpose of knowing if the holding of a transplant influences the patient's risk of death, it was done the analysis of the crossing between the outcome and the laboratory variables, for each group of patients, the ones that had done a transplant and the ones who had not.

Table 3 shows the mean values and the interquartile deviation, together with the p value for each comparison between the variable and the outcome, for each transplant group.

Out of the people who **did** any kind of transplant, the amount of lymphocytes statistically differ, with 95% confidence interval, between the ones that died and the ones who were cured.

And among the patients who **did not** do a transplant, the amount of lymphocytes was also significantly different among the ones who died and the ones who were cured, the amount of lymphocytes was also higher in the group of patients who were cured.

DISCUSSION

In this study, we analyzed the data of 457 patients who were hospitalized with COVID-19 in a tertiary hospital in Fortaleza, Ceará, Brazil. The mean age was 57 years old, and there was a higher prevalence of the male sex, in a consensus with other cohorts[1,2,3]. The mortality rate in our service was 17,94%.

Among the total of hospitalized patients, 48,36% had the previous diagnosis of SAH. It was observed 2,11 more chances of death than in patients who did not show this disease, in consensus with what was found by M. Salazar et al., they considered as possible causal nexus the myocardial damage and myocardial dysfunction supported by frequent findings of high levels of troponin and electrocardiographic anomalies[4].

Huang et al, in a meta-analysis of 6452 patients, found that DM-2 is associated with mortality, severity of COVID-19 and respiratory distress syndrome[5]. However, in our sample, although the number of diabetic patients that died and had needed an ICU was higher, in relation to the non-diabetic patients, there was not a statistical significance.

A meta-analysis with 15017 patients, identified that CKD was associated with the severity of COVID-19[6], in contrast to what had been found in our sample.

The divergences that were found in literature may be due to the absence of a previous diagnosis and appropriate treatment of the underlying disease.

The immune response of the organ receptors, particularly the immune response of T cells, is suppressed because of the prolonged use of immunosuppressive agents. In addition, transplanted patients showed higher prevalence of comorbidities, such as hypertension, DM-2 and CKD, which increase the severity and mortality. We identified that transplanted patients showed 2,21 more chances of death than non-transplanted patients. According to Guangyu et al, the innate and adaptive immunity can be altered in receptors of transplant of solid organs who make use of immunosuppressive drugs for a prolonged period, causing a risk of infection. Moreover, the use of immunosuppression makes these patients more susceptible to viral respiratory infections and they are more prone to develop bacterial and fungal co-infections[7].

CONCLUSION

We conclude that the prior diagnosis of arterial hypertension and transplant of solid organs and tissues, showed higher levels of mortality, if compared to the population who did not show these co-morbidities in patients with COVID-19.

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TABLE 1 – Frequency of sociodemographic characteristics by hospitalization outcome.

Variables	Outcome		Total	Value p[a]	RC (CI 95%)
	Death (%)	Cure (%)			
Sex					
Female	31 (15,9%)	164 (84,1%)	195 (100%)	0,325	
Male	51 (19,47%)	211 (80,53%)	262 (100%)		
Place of Hospitalization					
Ward	14 (4,14%)	324 (95,86%)	338 (100%)	<0,001	
ICU	68 (57,14%)	51 (42,86%)	119 (100%)		30,86 (16,16-58,91)
Previous Hospitalization due to Other Reasons					
No	64 (16,62%)	321 (83,38%)	385(100%)	0,089	
Yes	18 (25%)	54 (75%)	72(100%)		
Diagnosis Means					
Fast Test	10 (19,61%)	41 (80,39%)	51 (100%)	0,003	
PCR	12 (17,73%)	334 (82,27%)	406(100%)		2,11(1,29-3,46)
Presence of DM-2					
No	51 (16,72%)	254 (83,28%)	305 (100%)	0,335	
Yes	31 (20,39%)	121 (79,61%)	152 (100%)		
Presence of CKD					
No	67 (16,75%)	333(83,25%)	400(100%)	0,078	
Yes	15 (26,32%)	42 (73,68%)	57(100%)		
Transplant					
Renal	6 (35,29%)	11 (64,71%)	17 (100%)	0,843[b]	
Hepatic	3 (23.08%)	10 (76,92%)	13 (100%)		
Bone Marrow	3 (33,33%)	6 (66,67%)	9 (100%)		
Nor applicable[c]	70 (16,75%)	348 (83,25%)	418 (100%)		
Total	829 (17,94%)	375 (82,06%)	457(100%)		

Chi Square Test of Independence; b. Fisher's Exact Test; c. Category is not considered in the significance test.

TABLE 2 – Descriptive statistics of laboratory variables

Variables	Transplant	Mean	IQI	Minimum	Maximum	p Value
White blood cells	No	9129	4961,75	23,55	214500	< 0,001
	Yes	4997	4884,5	104	28270	
	Total	8919	5239,5	23,55	214500	
Neutrophils	No	7189	4783,5	0	47636	< 0,001
	Yes	3780	4348	0	25725	
	Total	6953	4918,5	0	47637	
Band Neutrophils	No	204,5	308,5	8	3928	0,173
	Yes	770,5	1142,75	186	1400	
	Total	212,5	332,5	8	3928	
Segmented Neutrophils	No	7197	4537	0	40761	< 0,001
	Yes	3780	4442	39	29594	
	Total	7011	4821	0	40761	
Lymphocyte	No	1036	874,25	0	13370	0,015
	Yes	730	1024,5	38	2450	
	Total	1005	876	0	13370	
Monocyte	No	504,5	428,75	0	3189	0,002
	Yes	356	381,5	2	1960	
	Total	487	431,5	0	3189	
Platelet	No	245250	156725	7660	673300	< 0,001
	Yes	110400	127230	8847	465200	
	Total	237900	155100	7660	673300	
PT (INR)	No	1,01	0,18	0,8	2,28	< 0,001
	Yes	1,09	0,23	0,89	1,62	
	Total	1,02	0,19	0,8	2,28	
APTT	No	1	0,3	0,03	7	< 0,001
	Yes	1,2	0,36	0,72	2,57	
	Total	1,01	0,32	0,03	7	
D-Dimer	No	1134,5	1673,5	0,03	113023	0,039
	Yes	4,26	1325,75	0,72	42104	
	Total	1069	1612	0,03	113023	
Fibrinogen	No	478	173	1,45	848	0,478
	Yes	484	284	231	875	
	Total	481	212	1,45	875	
PCR	No	5,3	9,85	0,01	23,2	0,261
	Yes	6,07	11,09	0,04	20,61	
	Total	5,34	9,93	0,01	23,2	
Ferritin	No	852,5	380,25	44	14351	0,026
	Yes	1309,5	1400	73,6	5667	
	Total	886	944,5	44	14351	
LDH	No	678,5	395,75	248	7060	0,736
	Yes	701	404	284	5282	
	Total	681	398	248	7060	

TABLE 3 – Association between laboratory variables and outcome

Variables	Did Transplant		p value	Did not do Transplant		p value
	Death	Cure		Death	Cure	
White blood Cells	5546 (6933,5)	4997 (3870)	0,642	11270 (7490)	8905 (4524)	< 0,001
Neutrophils	5086 (6222,75)	3659 (3249,5)	0,191	9916 (6731)	6782(4180,5)	< 0,001
Band Neutrophils	1330 (607)	211 (0)	1	463,5 (496,5)	174,5 (227)	0, 075
Segmented Neutrophils	5086 (6231,5)	3659 (3249,5)	0,224	9570 (6731)	6937 (4068)	< 0,001
Lymphocytes	445 (639,5)	907 (840,5)	0,045	907 (735)	1071 (845)	0,01
Monocytes	158,5 (365,25)	405 (287,5)	0,132	509 (551)	494 (399)	0,646
Platelets	102960(108967,5)	129100(127685)	0,578	1,14 (0,2)	0,99 (0,14)	< 0,001
PT	1,13 (0,23)	1,08 (0,22)	0,129	1,14 (0,2)	0,99 (0,14)	< 0,001
APPT	1,29 (0,66)	1,18 (0,3)	0,75	1,18 (0,48)	0,97 (0,25)	< 0,001
D DIMER	706,13(11578,47)	4 (974,25)	0,527	1462 (3891)	1049(1558)	0,161
Fibrinogen	577,5(284,25)	484 (284)	0,8825	414 (151,5)	493,5(209,75)	0,306
PCR	14,93 (4,58)	4,8 (7,43)	< 0,001	11,56 (8,88)	4,36 (8,84)	< 0,001
Ferritin	109,75(1446,75)	1432,5(1281,75)	0,792	999 (1218,62)	814 (835,75)	0,128
LDH	787,5 (235,5)	566 (418)	0,031	768 (501,25)	658,5 (372,25)	0,047

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HEMATOPOIETIC STEM CELL TRANSPLANTATION AND GUT MICROBIOTA: OUTCOMES AND USE OF PROBIOTICS, A NARRATIVE REVIEW

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is a complex procedure used to treat several onco-hematological neoplasms, benign hematological diseases, and some types of solid tumors. In recent years, the role of the gut microbiota in HSCT has been studied, revealing that the microbiota has a direct interaction with the immune system and the microbial balance within the body (eubiosis), providing beneficial health effects, and changes in such state result in dysbiosis, which has been associated with several pathological states. The process in which the patient undergoes HSCT can cause microbiota imbalance with reduced diversity, which would be related to negative post-HSCT outcomes, including increased mortality and development of graft-versus-host disease (GVHD). The modulation of the gut microbiota through methods such as the use of probiotics has been explored as an alternative for the recovery and/or maintenance of the gut microbiota.

Keywords: Hematopoietic stem cell transplantation. Microbiota. Gut microbiota. Probiotics.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a complex procedure used to treat several onco-hematological neoplasms, benign hematological diseases, and some types of solid tumors. The conditioning step for HSCT consists of chemotherapy, with or without radiotherapy, with the objective of immunosuppression and eradication or reduction of the disease. Subsequently, an intravenous infusion of hematopoietic progenitor cells is performed, to restore the patient's bone marrow function[1,2].

The cells used for HSCT can be from the patient (autologous HSCT) or from a donor, who can be related or unrelated (allogeneic HSCT). In this last type of transplant, graft-versus-host disease (GVHD) can occur, which results from an alloreactivity reaction of the graft's lymphocytes against the histocompatibility antigens of the host, which is one of the main causes of post-transplant morbidity and mortality[3].

In recent years, the role of the gut microbiota in HSCT and its outcomes has been studied. The relationship between the microbiota and the pathogenesis of GVHD was suggested many years ago after a study with germ-free mice[4]. It should be noted that GVHD occurs very frequently in the gastrointestinal tract (GIT), one of the main sites of bacterial colonization.

The gut microbiota can be considered as a virtual and metabolic organ[5], comprising an ecosystem formed by microorganisms synergistically adjusted to human physiology. It performs essential functions

for the organism as a physical, functional, and immunological barrier of the GIT[6,7].

The microbiota interacts directly with the immune system, and the intestinal defense barrier is composed of the microbiota, the mucosal barrier, and the gut-associated lymphoid tissue (GALT), the latter being responsible for communication of T and B lymphocytes with cells from other tissues and production of immunoglobulin A[8].

The balance state of the microbiota (eubiosis) promotes beneficial health effects, and changes in such state result in dysbiosis[9]. The process to which the patient undergoes HSCT can cause an imbalance of the microbiota[10], since, in addition to chemotherapy and radiotherapy, which cause gastrointestinal toxicity effects, there may be a breakdown of the epithelial barrier with consequent bacterial translocation, in many cases influenced by the prophylactic or therapeutic use of broad-spectrum antibiotics[11].

Holler *et al*. demonstrated that, at the time of admission for transplantation, patients have a predominance of commensal bacteria while, after transplantation, there is a tendency for an increase in Enterococcus, whose prominence is facilitated by the use of prophylactic antibiotics or in the treatment of febrile neutropenia and, in particular, among patients who develop GIT GVHD[12].

The decrease in gut microbiota diversity at the time of grafting appears to have a strong relationship with mortality[10]. Thus, the assessment of microbiota diversity through methods such as next generation 16S rRNA gene sequencing[13], with the purpose of taxonomic and phylogenetic assessment and, later, interventions with the aim of preserving the microbiota, such as the use of probiotics, could help reduce morbidity and mortality in HSCT patients.

According to the National Consensus on Oncology Nutrition in Brazil, the use of probiotics for neutropenic patients is not indicated[14]. However, studies using some types of probiotics have shown that their use can be safe in HSCT[15,16].

According to the World Health Organization (WHO), probiotics can be defined as "live microorganisms capable of improving the intestinal microbial balance, producing beneficial effects on the health of the individual." Some of the main benefits are increased immune defense with activation of T lymphocytes, NK cell activity, and acting on inflammatory mediators with a decrease in pro-inflammatory cytokines (interleukins 12, 6, and 4) and an increase in interleukin 10, which has anti-inflammatory action[17].

Evidence shows that, in healthy individuals, the use of probiotics can, in addition to improving the immune response, help with bowel movements and stool consistency. Thus, they act against the colonization and translocation of pathogenic microorganisms and could also help to reduce the risk of antibiotic resistance. And, although colonization by probiotics may not occur upon their use, the passage of the probiotic through the intestine seems to be sufficient to reduce colonies of pathogenic bacteria due to reduced adhesion and competitive nature[17].

The bacteria most used as probiotics and with the most widely known effects are Lactobacilli and Bifidobacteria, and they are also the most tested in the context of HSCT, as seen in an experimental study with animals, in which the consumption of *Lactobacillus rhamnosus GG*, before and after transplantation, was evaluated. The use of such probiotic improved the survival of the animals and reduced the incidence of acute GVHD[19]. This same lactobacillus was used in a sample of allogeneic HSCT patients at the time of grafting and showed no effect on the severity or incidence of GVHD[16]. However, more studies are needed regarding the use of probiotics in such patients, with different moments of use, dose, and strains.

NORMAL GUT MICROBIOTA AND CHANGES IN HSCT

The human gut microbiota contains several microorganisms that colonize the surfaces of the GIT with diverse composition throughout the digestive tract[19]. In healthy individuals, the composition of the microbiota is relatively stable, with six phyla of bacteria dominating the microbiota: Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria, and Verrucomicrobia. Among them, there is a predominance of Gram-positive Firmicutes followed by Gram-negative Bacterioidetes[20,21].

There are already known factors related to changes in the gut microbiota, considering its malleability and/or fragility in the face of environmental and diet changes, which are the use of antibiotics, geographic location, pathologies, lifestyle, fiber supply, aging, type of delivery, among others[6,19,21,22].

The microbiota performs essential functions for the human organism such as nutrients digestion, protection against pathogens, and interaction with the immune system, as well as production of metabolites[23,24,25]. For some time, it has been considered a

virtual and metabolic organ[5] that, in general, will act as a physical, functional, and immunological barrier of the gastrointestinal tract[26]. Therefore, understanding that the alteration of the microbiota balance state, in which eubiosis can become dysbiosis, a state of unbalance that can result in the loss of beneficial health effects and the initiation of a potentially pathological state, is essential [9].

Several studies show that there is a decrease in microbiota diversity in HSCT[12,27-31] with losses of beneficial bacteria such as Faecalibacterium and Ruminococcus[28]. In the study by Montassier *et al*. with Non-Hodgkin Lymphoma patients admitted for HSCT, it was seen that there was a significant decrease in Firmicutes and Actinobacteria and an increase in Proteobacteria after conditioning [32].

One of the causes for the loss of diversity may be the chemotherapy used in conditioning, which has several effects on the patient, including GIT mucositis, which leads to alteration of intestinal villi and loss of enterocytes. The inflammatory process could partly explain changes in the taxonomic composition and metabolic capacity of the gut microbiota[32].

In addition to chemotherapy, the use of antibiotics required during the transplantation process also affects the gut microbiota[27,29,33,34] although different types of antibiotics have different impacts on the diversity of the microbiota[35].

A study with a large cohort of patients showed that early antibiotic treatment in transplant patients is associated with significant changes in the microbiota[34]. Such study found a lower overall survival and a higher transplant-related mortality in patients who started using antibiotics earlier than in those who started after transplantation, and the lowest transplant-related mortality was found in the group that did not receive additional antibiotics[34]. Such data are in line with the idea that changes in the microbiota are related to worse outcomes for these patients.

Other studies have also shown that changes and loss of diversity may be related to negative outcomes after HSCT, such as increased mortality[10], decrease in survival[36], pulmonary complications[37], and bacteremia related to the predominance of certain types of bacteria in this context [27, 38]. In addition, there is a relationship to decrease in overall survival [10], which may be influenced by the colonization of the gut microbiota by antibiotic-resistant bacteria [39,40].

There also seems to be a relationship between gut microbiota composition and post-transplant relapse/progression, as seen in a study that found a lower cumulative incidence of progression/relapse in patients with an abundance of a group of bacteria composed mostly of *Eubacterium limosum*, when compared to a group that did not have such bacteria [41].

On the other hand, a study published in 2017 analyzed the composition of the preconditioning gut microbiota and found no differences in outcomes such as mortality and post-transplant survival among groups with low, moderate, or high diversity. However, it did find differences in the composition of the microbiota of those who had GVHD, when compared to those who did not [42]. Similarly, another study that analyzed pre-transplant stool samples found that there seems to be less diversity in the microbiota of those patients who developed bacteremia when compared to those who did not [43], which suggests the importance of eubiosis pre-transplant.

Another important aspect regarding the gut microbiota is the production of short-chain fatty acids (SCFA), which can also be compromised in HSCT [28,44]. SCFA can be produced from bacterial fermentation of carbohydrates in the intestine and serve as a source of energy, have anti-inflammatory action, and stimulate the production of some hormones, among other important functions for the host's health.

In the study by Biagi *et al*., it was found from stool samples from post-HSCT patients, who developed acute GVHD, that there is a decrease of about 76% in the production of SCFA post-transplantation[28]. Moreover, it appears to take about two months post HSCT to recover the microbiota ecosystem and its metabolic capacity[28]. In some cases, dysbiosis remains up to one year post-transplantation [45].

There are already ways to assess the "health" of the gut microbiota, through biomarkers such as urinary *3-indoxyl sulfate* (3-IS). 3-IS is a product of tryptophan degradation by commensal bacteria that inhabit the intestine and appears to be a predictor of intestinal GVHD[10,46]. Furthermore, low levels of 3-IS up to 10 days post-HSCT are associated with higher HSCT-related mortality and worse overall survival, with high levels of 3-IS being correlated with Clostridiales while low levels are associated with the Bacilli class [47].

As for the strategies that could help in the maintenance or recovery of the microbiota during transplantation, studies suggest the rational use of antibiotics, as well as the possibility of fecal transplantation and use of probiotics [29,35,44,48-50].

MICROBIOTA AND GVHD

There seem to be differences in the composition and diversity of the microbiota of patients who develop GVHD when compared to those who do not[28,51,52], and GVHD may be related to the loss of the protective effect of commensal bacteria[10,12].

One of the possible mechanisms for the alteration of the microbiota in GVHD is via Paneth cells. These cells, located in the intestine, which have a regulatory function through the expression of alpha-defensins, which result in the death of non-commensal bacteria and preservation of commensal bacteria, seem to be the target of GVHD[53]. The damage caused to Paneth cells would lead to the reduction of alpha-defensins, altering the normal intestinal environment[53,54], making such cells another focus for approaches to preserve or recover the microbiota[55]. It is known that the intestinal expression of several antimicrobial peptides is reduced in the presence of acute GIT GVHD and is associated with dysbiosis[56].

With the microbiota in dysbiosis, there is a growth in pathogenic bacteria such as the *Enterococcus* spp. And, consequently, a higher risk of bacteremia [10,12]. In the presence of GIT GVHD, there appears to be an even greater risk of bacteremia caused by enteric bacteria[57]. In addition, a study showed a higher incidence of transplant-related mortality in patients with acute GI GVHD who developed blood infection by enteric bacteria[58]. Relatively recent studies have indicated that the diversity of the gut microbiota during the grafting period is associated with acute GVHD[59,60].

A study with pediatric patients found in the pre-transplant analysis that patients who did not develop GVHD had a greater abundance of propionate-producing Bacteroidetes (a SCFA), that were persistent after HSCT-induced microbiota changes [28]. In a study with adult patients, the pre-transplantation analysis of those who had GVHD had significantly greater abundance of the Firmicutes phylum and a lower tendency for Bacteroidetes when compared to those who did not have GVHD [42]. Studies also suggest the influence of the donor's microbiota on the development of GVHD [59,61].

An animal model study showed that, in the acute phase of intestinal GVHD, there is a shift in favor of bacteria from the most pro-inflammatory species, the Enterobacteriaceae family, while there is a decrease in *Lactobacilli*, *Clostridia*, *Bifidobacteria*, and *Bacillus spp*., indicating that, in acute intestinal inflammation, there is an alteration in the intestinal flora, as well as a decrease in its diversity [62].

In humans, an association of bacterial microbiota diversity with the development of GVHD in pediatric patients has already been found[63], with GVHD-related mortality in adult patients[64]. Also, the gender *Blautia* would be associated with the development of GVHD when in small quantity[63], with lower GVHD-related lethality and better overall survival when in abundance[64].

Regarding genetic aspects, the *fucosyltransferase-2* (FUT2) gene, which regulates the expression of the H antigen, was evaluated in HSCT patients for its relationship with the gut microbiota, since the ABH antigens in the mucosa serve as a source of energy for the bacteria and adhesion receptors for many microbes. FUT2 genotype seems to influence the risk for bacteremia and GVHD in such patients. However, the authors emphasize that there are several other factors that influence the diversity of the microbiota and can interfere with post-HSCT outcomes, such as the use of antibiotics[65] previously mentioned.

The use of antibiotics that target intestinal bacteria as prophylaxis in HSCT has already been associated with the severity of acute GVHD of GIT organs and liver, as well as impacted on overall survival in a retrospective study with 500 patients[66]. In this same study, the incidence of GIT GVHD was twice as high in the group that received antibiotics compared to those that did not[66].

Differences in the activity spectrum of antibiotics could influence the frequency and severity of GVHD, and the use of antibiotics that preserve anaerobic commensal bacteria could reduce the risk and incidence of GVHD[67,68]. However, there may be differences in antibiotic use and between populations in terms of microbiota, since, in a study with Japanese patients undergoing allogeneic HSCT, the use of fourth generation cephalosporins was associated with the development of GVHD, while piperacycline tazobactam was not[69], a result that is different from the one found in a sample of American patients[67]. In addition, use of carbapenem for more than seven days has also been associated with risk of intestinal GVHD[70].

Routy *et al*. point out that, in addition to the epithelial damage caused by conditioning, the use of prophylactic antibiotics or in episodes of febrile neutropenia, fasting and the use of parenteral nutrition also influence the change in the composition and di-

versity of the microbiota[66]. The stimulus for oral and enteral ingestion, as well as the use of less intense conditioning, when possible, could help in the preservation of bacteria that seem to be favorable, such as those of the genus Blautia, as discussed by Jenq *et al*. in their paper published in 2015[64].

PROBIOTICS AND HSCT

The use of probiotics has already been shown to be beneficial in several clinical situations, such as in the prevention and treatment of diarrhea associated with the use of antibiotics, inflammatory bowel diseases, and *Clostridium difficile* infection. Its use seems to favor the intestinal-related immune response. But the safe use of probiotics in immunosuppressed patients is still uncertain.

Cases of negative events in post-HSCT patients related to microorganisms that are used as probiotics are described in the literature. There is a case report of meningitis in a pediatric patient with acute lymphoblastic leukemia after allogeneic transplantation whose microorganism was identified as *Lactobacillus rhamnosus*. In such case, there was no known probiotic consumption. However, the authors are aware that the use of some antibiotics and the presence of such microorganisms as part of the normal microbiota may be related to the development of infections in these patients, even if there is no consumption of the probiotic itself[71].

On the other hand, there are cases of sepsis in HSCT patients directly associated with the consumption of probiotic yogurts with *Lactobacillus acidophilus* [72] and *Lactobacillus rhamnosus*[73]. However, in both cases the patients consumed the probiotic post-transplantation, in large amounts in the first case (6-8 units of yogurt daily) and at times of severe neutropenia, in addition to being two isolated case reports.

However, infections by microorganisms that are used as probiotics seem to be less frequent in HSCT patients[74]. A retrospective study with a cohort of 3796 patients evaluated episodes of bacteremia/sepsis caused by *Lactobacillus*, *Bifidobacterium*, *Streptococcus thermophiles,* and *Saccharomyces* in up to one year post-transplantation, without evaluating whether there was consumption of probiotics, and found only 0.5% of cases, most of them in allogeneic HSCT patients (71%) caused by *Lactobacillus* and within the first 100 days post-transplant.

The safety of using probiotics in such patients, as can be seen, is still controversial. In this sense, Ladas *et al*. carried out a pilot study to verify the safety and viability of the probiotic *Lactobacillus plantarum* in children and teenagers undergoing allogeneic HSCT from D-7 or D-8 to D+14, and found that there were no adverse effects related to the use of the probiotic, suggesting that this probiotic would be safe to use for these patients[15].

But an observational study published in 2012 evaluated nutritional habits of patients before transplantation and found a negative correlation between yogurt intake and episodes of febrile neutropenia[75].

Regarding the relationship between the use of probiotics and GVHD, this review found a randomized clinical trial that used *Lactobacillus rhamnosus* GG in capsules at the time of grafting and followed the development of GVHD in such patients. This study showed that the use of the probiotic was safe, but there was no difference in the incidence or degree of GVHD, nor evidence of significant changes in microbiota diversity[76].

Therefore, we can conclude that there is a need for further studies to understand how changes in the microbiota can interfere with the host's health and alter the development of GVHD[77], in addition to the importance of testing other probiotics in different moments of transplantation, since there are still no validated methods or approaches for the effective preservation of the microbiota[78].

However, there is a lot of evidence, including genomic ones, that demonstrate the predictive role of the gut microbiota as a biomarker for GVHD, in addition to the significant relationship with other negative outcomes in the presence of dysbiosis. Thus, the modulation of the gut microbiota through methods such as the use of prebiotics, adequate use of antibiotics, fecal microbiota transplantation when applicable, and the use of probiotics are still controversial approaches, whose possible positive results deserve to be explored due to the potential for improvement in post-HSCT results and due to their relationship with the development of GVHD[46,79-83].

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FOLLOW–UP BEYOND 1 MONTH AFTER AUTOLOGOUS CAR T CELL THERAPY

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ABSTRACTS

The incidence of medium-term and long-term adverse events are critical factors determining the utility of CAR T-cell therapy and research of risk factors and timeline of late effects will be critical for optimal survivorship care. The most commonly reported toxicities during long-term follow-up after anti-CD19 CAR T-cell therapy are decreased blood B-cell counts, hypogammaglobulinemia, prolonged cytopenias and infections. Common determinants of late toxicities are age, underlying tumor type, previous therapy and CAR construct. Here we will provide some recommendations for patient monitoring during medium-term and long-term follow-up and management of the late adverse effects.

OBJECTIVES

- Describe the clinical, laboratory and radiological follow-up after CAR Cell Treatment.
- Standardize and optimize medical care with screening and therapeutic and preventive interventions of the main complications related to CAR T cell treatment.
- Monitor immune reconstitution during the follow-up and follow-up period.
- Monitor the persistence and expansion of CAR T Cells.

INTRODUCTION

Long-term follow-up of patients who have received treatment with CAR T cells involves collaboration between the hematology team that referred the patient and the cell therapy multi-disciplinary team. Therefore, the center that referred the patient must receive specific instructions and contact information so that the necessary support can be maintained after the patient returns. After CAR T cell therapy, the patient will require close monitoring to deal with possible late complications of therapy: prolonged cytopenias; late neurological toxicity such as tremors, memory changes, increased risk of infection, etc.

Cytopenias

Haematological toxicity has a cumulative 1-year incidence of 58% post-CD19-CAR-T, is often prolonged and can follow an biphasic temporal course. The first phase is attributed to the lymphodepletion regimens, bridging therapy before CAR-T infusion, severe CRS, etc.

An analysis of hematological reconstitution in CAR T-treated patients published in 2020 showed that blood count normalization occurs in only about 15% of patients after 3 months and about 60% after 9 months of CAR T cell infusion. In ZUMA-1, about 17% remained with grade 3 or greater cytopenias after 3 months of infusion[1]. About 30% of patients may have late, beyond D+30, severe neutropenia, and

20% late thrombocytopenia. In addition, up to 20% of patients have neutropenia lasting, longer than 90 days. The pathogenesis of the prolonged cytopenia is not yet well understood, but is likely contributed to by multiple factors, such as the occurrence of a more severe cytokine release syndrome, large tumor mass and low marrow reserve. Furthermore, the cytokine profile in these patients with prolonged cytopenia, revealed elevations in serum levels of IFNγ, IL-6 and IL-8 in patterns similar to those seen in acquired bone marrow failure states. Cytopenias after CAR T anti-BCMA, are intense after lymphodepletion: 97% grade 3 or greater, and tend to recover within 1 month. Rejeski and in a multicenter, retrospective analysis of 258 patients, found a positive correlation between baseline thrombocytopenia and hyperferritinemia and day+60 cytopenia. They developed a score: CAR-HEMATOTOX, which included hemoglobin, platelet count, absolute neutrophil count and baseline inflammatory markers: C-reactive protein, and ferritin. This score can predict the incidence of severe neutropenia more, or less than 14 days.

B-cell aplasia and hypogammaglobulinemia

In a long-term follow-up study of CD19-directed CAR T therapy in relapsed/refractory (R/R) B cell NHL or CLL, 67% of patients had hypogammaglobulinemia beyond 90 days and can persist for months or even years[2]. Updated results of the ZUMA-1 trial, which tested axi-cell in R/R aggressive B cell NHL, show that 31% of patients received intravenous immunoglobulin (IVIG), Generally, polyclonal CD19+ B cells recover in 50% of cases in remission around 6 to 12 months after infusion and can mean an increased chance of relapse. On the other hand, persistence of CAR T cells can result in profound hypogammaglobulinemia or agammaglobulinemia.

Due to immunological immaturity, immunoglobulin replacement is routine in paediatric CAR-T cell patients to obtain a serum level > 400 mg/dL regardless the clinical picture[3]. In adults, some specific anti-pathogen antibodies may remain, due to CD19 negative plasma cells, and in this population, the Immunoglobulin replacement can be titrated by the incidence of breakthrough infection.

Immune Reconstitution

Several studies with anti-Cd19 CAR T show that CD8+ counts recover quickly, while CD4+ T cells may persist low, with a count less than 200 cells/µL in about 33% in those who remained on remission 1 year after treatment.

Cardiovascular toxicity

Cardiovascular events are common in adult patients after CAR-T, with a correlation between the occurrence of CRS >2, elevated troponin and a longer time between the onset of CRS and the administration of tocilizumab[4].

Secundary Malignancies

Most patients treated with CAR T cells have received several previous oncological treatment lines, and therefore are more susceptible to secondary neoplasms, mainly myelodysplasia or acute myeloid leukemia. A study with 86 patients by Cordeiro et al (2020)[3] showed that 15% developed secondary neoplasms. This percentage rose to 29% in the population that achieved prolonged complete remission. It is noteworthy that 62% of these patients had already undergone hematopoietic stem cell transplantation.

The follow-up after infusion of CAR T cells should also include analysis of persistence and expansion of CAR T cells, either by direct methods, such as flow cytometry or PCR, or by indirect methods, such as recovery of lymphopenia after infusion of CAR cells T. The ELIANA study (CAR T cells for ALL) showed that recovery of B lymphocyte count: >1%/total leukocytes or >3% of lymphocytes before the 6th month was related with lower relapse-free survival.

PROCEDURE

In the pre-treat possibility of evaluation by specialists from other areas, such as: psychology, neurology, infectology and specific exams (depending on the underlying disease), during the follow-up period after hospital discharge.

The frequency of evaluations will vary according to the status of the underlying disease, associated complications, risk of infections and possible need for transfusion. Some patients with prolonged cytopenia may require the use of growth factors or even spinal cord evaluation.

Up to 30 days from the infusion of CAR T cells, the patient must stay at a place for a maximum of 30-45 minutes from the hospital with a companion who knows how to identify signs of treatment toxicity: CRS (cytokine release syndrome, ICANS (Immune effector cell-associated neurotoxicity Syndrome) . Patient should not drive until 8 weeks after infusion of CAR T cells.

In addition to cytopenias and infections, other late adverse events that should be monitored after CAR

T treatment: immune events (pneumonitis, dermatitis); neurological, psychiatric, secondary neoplasms. The proposal assessment after CAR T cell therapy should be individualized for each patient, according to the disease of: CLL, ALL or non-Hodgkin's lymphoma; and patient characteristics: co-morbidities, toxicities, infectious history and risk, etc.

Management Recommendations

- Cytopenias

Granulocyte growth factor (G-CSF) has been used for the treatment of neutropenia, however, to avoid interaction with the risk of peak CRS and CAR T cell expansion, its use is avoided until 14 days after cell infusion. T CAR. Up to 28 days after infusion of CAR T Cells, bone marrow evaluation is not indicated, only follow-up with blood count is sufficient. Clinically, stable patients can be discharged from the hospital, even with cytopenia, using antimicrobial and antifungal prophylaxis.

If cytopenias persist after 28 days, a myelogram and/or bone marrow biopsy are indicated, as well as investigation of infection by viral pathogens[5]. In cytopenias grade 3: anemia: 10-8.0/dL; neutropenia: 500-1000/mm3; thrombocytopenia: 25.000-50,000 per mm3, the use of G-CSF should be considered, as well as the use of corticosteroids. In grade 4 cytopenias: neutropenia less than 500/mm3 and thrombocytopenia < 25,000/mm3, high-dose corticosteroids and the use of granulocytic growth factor may be considered, besides Blood product transfusion and prophylactic antibacterial and antifungal agents in patients with prolonged neutropenia.

Hypogammaglobulinemia

Patients with serum IgG levels <400 mg/dL prophylactic replacement should be considered and when the patient has recurrent infections replacement is indicated.

Replacement: IVIG or s.c. formulation, dosing every 3-4 weeks at 400-600 mg/kg body weight to maintain an IgG through level of >400 mg/dL and continuing until B cell recovery with spontaneous.

Monitoring Recommendations:

From hospital discharge to D+100

- Weekly visits until D+60, which can be fortnightly after D+60.

- Weekl y exams up to D+60, which can be biweekly: Blood count, reticulocytes, biochemistry, DHL, Liver Profile, Blood glucose, C-reactive protein, Creatinine, urea, fibrinogen.

- Quantitative CMV blood PCR in cases of cytopenias, fever or other clinical indication in patients previously submitted to HSCT. PCR for EBV or adenovirus only on clinical suspicion.

- Tests requested monthly: ferritin, Immunoglobulins (IgA, IgM, IgG); peripheral blood immunophenotype: CD3/CD4/CD8/CD16/CD19/CD56.

- Assessment for persistence of CAR T. From D+100 a D+365

- Monthly visits up to D+180, which can be every 2 months after D+180.

- Exams requested monthly: Blood count, reticulocytes, biochemistry, DHL, AST, ALT, bilirubin, Blood glucose, C-reactive protein, Creatinine, Urea, fibrinogen, ferritin.

- Monthly visits up to D+180, which can be every 2 months after D+180.

- Every 2 months up to D+180: Quantitative immunoglobulins or serum protein electrophoresis and peripheral blood immunofenotyping: CD3/CD4/CD8 /CD16/CD19/CD56. After the 6th month, exams can be done every 3 months.

Monitoring the loss of B cell aplasia is useful to assess the risk of CD19+ disease relapse, definitions vary, but an increase of 50% of CD19 cells or 3% of the B cell population can mean recovery. The loss of B cell aplasia before 6 months of infusion is associated with an increased risk of relapse.

- Other tests according to age and patient: Iron Profile, Hormone Profile (TSH, T4, FSH, LH, estradiol, progesterone, ACTH, cortisol, total and free testosterone, PTH, GH, IGF-1, Prolactin). Lipid profile, Bone assessment, Autoantibody profile (ANA, ENA, Rheumatoid factor, ANCA, anti-TPO, anti-Thyroglobulin) in cases with suspected autoimmunity diseases.

- Transthoracic echocardiogram and high-resolution chest CT (depending on age, symptoms and previous treatments).

In case of persistent grade 3 or 4 cytopenias, bone marrow aspiration or biopsy should be performed to assess cellularity and rule out hematophagocytosis, myelodysplasia, or leukemia recurrence.

After 1 year: Visits at least every 3 to 6 months. - Start screening for secondary neoplasms after 1 year

(D+365) and according to general age recommendations (total/free PSA, Mammography, Pap smear, Colonoscopy and Endoscopy).

Training and updates should be defined by institution decision. As CAR T cells is a therapy with increasing and continuous advances, we suggest training and annual assessment of the competence of teams annually.

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