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 Full-Length Article
 Stem Cell Therapy

A French single-center experience on allogeneic stem cell transplant cryopreservation during severe acute respiratory syndrome coronavirus 2 pandemic

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ARTICLE INFO

Article History:

Received 18 November 2022

Accepted 11 April 2023

Available online xxx

Key words:

COVID-19

cryopreservation

hematopoietic stem cell transplantation

matched unrelated donors

SARS-CoV2

ABSTRACT

Background aims: Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a curative treatment for chemo-resistant hematological malignancies. Because of transport restriction imposed by the coronavirus disease 2019 pandemic, regulatory bodies and societies recommended graft cryopreservation before recipient conditioning. However, the freezing and thawing processes, including washing steps, might impair CD34+ cell recovery and viability, thereby impacting the recipient engraftment. Over 1 year (between March 2020 and May 2021), we aimed to analyze the results of frozen/thawed peripheral blood stem cell allografts in terms of stem cell quality and clinical outcomes.

Methods: Transplant quality was evaluated by comparing total nucleated cells (TNCs), CD34+ cells and colony-forming unit–granulocyte/macrophage (CFU-GM)/kg numbers as well as TNC and CD34+ cell viabilities before and after thawing. Intrinsic biological parameters such as granulocyte, platelet and CD34+ cell concentrations were analyzed, as they might be responsible for a quality loss. The impact of the CD34+ cell richness of the graft on TNC and CD34 yields was evaluated by designing three groups of transplants based on their CD34/kg value at collection: $>8 \times 10^6$ /kg, between 6 and 8×10^6 /kg and $<6 \times 10^6$ /kg. The consequences of cryopreservation were compared in the fresh and thawed group by evaluating the main transplant outcomes.

Results: Over 1 year, 76 recipients were included in the study; 57 patients received a thawed and 19 patients a fresh allo-SCT. None received allo-SCT from a severe acute respiratory syndrome coronavirus 2–positive donor. The freezing of 57 transplants led to the storage of 309 bags, for a mean storage time (between freezing and thawing) of 14 days. For the fresh transplant group, only 41 bags were stored for potential future donor lymphocyte infusions. Regarding the graft characteristics at collection, median number of cryopreserved TNC and CD34+ cells/kg were greater than those for fresh infusions. After thawing, median yields were 74.0%, 69.0% and 48.0% for TNC, CD34+ cells and CFU-GM, respectively. The median TNC dose/kg obtained after thawing was 5.8×10^8 , with a median viability of 76%. The median CD34+ cells/kg was 5×10^6 , with a median viability of 87%. In the fresh transplant group, the median TNC/kg was 5.9×10^8 /kg, and the median CD34+ cells/kg and CFU-GM/kg were 6×10^6 /kg and 276.5×10^4 /kg, respectively. Sixty-one percent of the thawed transplants were out of specifications regarding the CD34+ cells/kg requested cell dose (6×10^6 /kg) and 85% of them would have had this dose if their hematopoietic stem cell transplant had been infused fresh. Regarding fresh grafts, 15.8% contained less than 6×10^6 CD34+ cells/kg and came from peripheral blood stem cells that did not reach 6×10^6 CD34+ cells/kg at collection. Regarding the factor that impaired CD34 and TNC yield after thawing, no significant impact of the granulocyte count, the platelet count or the CD34+ cells concentration/ μ L was observed. However, grafts containing more than 8×10^6 /kg at collection showed a significantly lower TNC and CD34 yield.

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Conclusions: Transplant outcomes (engraftment, graft-versus-host disease, infections, relapse or death) were not significantly different between the two groups.

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Introduction

The coronavirus disease 2019 (COVID-19) epidemic started in late January 2020 in France. In March 2020, France reached stage 3 of the epidemic and the first lockdown was decided. The rapid increase in COVID-19 cases led to a major reorganization of the French Health system [1]. Coordinated and multidisciplinary care pathways for suspected or screened patients with COVID-19 impacted the global care pathway, with cancellations or delayed care and surgeries. Hematology and stem cell transplant departments also were impacted by this reorganization. However, allogeneic hematopoietic stem cell transplantation (allo-SCT) cannot be delayed for patients with hematological malignancies, especially acute leukemias, which have a very high risk of relapse. Maintaining safe hematopoietic stem cell donations from related or unrelated donors appeared imminently mandatory [2,3]. For unrelated donors, transplant centers and registries had to solve many challenges, such as the graft traveling and testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [4]. Depending on the donor's country of origin, low availability of flights or closed borders can impact the graft's prompt delivery to transplant centers (which is usually expected to be less than 48 hours after collection to avoid a decrease in stem cell viability) [5].

Moreover, because the precise risk of transmission of COVID-19 infection from an infected donor remained unclear at this time, donor SARS-CoV-2 real-time polymerase chain reaction (PCR) testing from the upper respiratory tract was required at donation by regulatory agencies such as the High Committee of Public Health in France, on behalf of the Biomedicine Agency and was published by the European Society of Blood and Marrow Transplantation [6]. However, significant disparities regarding SARS-CoV-2 testing existed between countries in Europe. For example, Germany and Poland did not require any SARS-CoV-2 testing for asymptomatic donors, defined by a specific questionnaire identifying the risks of SARS-CoV-2 infection before donation. Consequently, transplant centers that took care of the recipients could request an additional ethylenediaminetetraacetic acid tube (intended to accompany the graft on arrival) or a few milliliters of the graft to carry out a SARS-CoV-2 real-time PCR. The implementation of this circuit has compelled clinicians to wait for the test result before starting the conditioning regimen of the recipient, and therefore having to freeze the graft collected upon arrival at the transplant center. Freezing hematopoietic cells is usually performed for autologous SCT [7]. So far, this practice has been exceptional for allo-SCT, usually performed with freshly infused HSCs to ensure their greatest viability and functionality. To date, exceptional reasons for freezing allografts are inability to synchronize recipient and donor agendas or unpredictable events in recipients such as relapse, infection or a defective catheter. However, the freezing process can affect cell viability. The entire freezing–thawing and washing process may impact the richness of the graft and have the clinical consequence of delaying the patient's aplastic recovery. Therefore, in this study, we aimed to analyze the results of 1 year of recommended frozen/thawed allogeneic transplants in terms of graft quality after thawing and clinical outcomes.

Methods

Study design

This is a retrospective monocentric study of 76 consecutive allogeneic peripheral blood stem cell transplants performed between

March 15, 2020 (start of the first lockdown in France), and May 15, 2021 (end of the second lockdown), at the University Hospital of Nancy. Informed consent was obtained from the patients for the clinical and biological data analyses. This study was independently reviewed and approved by the ethics committee of the Nancy University Hospital (decision number: CO-28) and was registered to the clinicaltrials.com website (NCT05088941).

Patient (recipient) characteristics and outcomes

Recipient characteristics were age at transplant, sex, weight, the hematological diagnosis, conditioning regimens, type of graft-versus-host disease (GVHD) prophylaxis, use of growth factors (granulocyte colony-stimulating factor or N-plate) after transplant, hematological recovery (number of days between [i] transplant and absolute neutrophil count $>0.5 \times 10^9/L$, [ii] transplant and platelets $>20 \times 10^9/L$, and [iii] transplant and platelets $>50 \times 10^9/L$). Acute GVHD [8] and chronic GVHD [9] were defined as previously described. Recipient follow-up was between 98 and 116 days for the thawed and fresh groups, respectively.

Donor and graft data collection

Donor characteristics collected were age at collection, sex, HLA compatibility (match-related [MRD], haploidentical, match-unrelated [MUD]), graft type (bone marrow or peripheral blood stem cells [PBSC]), ABO compatibility (major, minor, both, none) and country origin.

Freezing and thawing processes

The freezing process involved a cryoprotectant, a solution made of 80% albumin 4% (Vialebex, LFB, France) and 20% dimethyl sulfoxide (Wak Chemie, Steinbach [Taunus], Germany) [10] and a control rate freezer (Planer; Cryo Products, Hertogenbosch, Netherlands). Cell bags were stored in vapor nitrogen. The number of bags cryopreserved and thawed during the period was evaluated as well as the delay between graft cryopreservation and thawing.

Upon physician's request, cells were thawed in a 37°C water bath, and washed twice with a solution composed of 50% NaCl 0.9% (Frese-nius Medical Care, Bad Homburg v. d. Höhe, Germany), 40% human albumin 4% (Vialebex, LFB, France) and 10% ACD (Macopharma, Tour-coing, France). Total nucleated cell (TNC) numeration was performed using the Pochi device (Sysmex, Kobe, Japan), CD34 enumeration (single platform method), viability (7-aminoactinomycin D staining) and colony-forming unit–granulocyte/macrophage (CFU-GM) seeding were performed as previously described [11,12].

Statistical analysis

The endpoints considered were (i) the quality loss of the transplant after thawing, defined as CD34+ cells $<6 \times 10^6 /kg$ or CD34 + cell recovery $<50\%$, to evaluate the impact of freezing on hematopoietic stem-cell transplantation (HSCT) quality; (ii) the clinical consequences: first, the delay of hematological recovery and the percentage of patients with a delayed hematological recovery and second, the following events: acute GVHD, chronic GVHD [13], transplant rejection, infections, relapse, the necessity of a second HSCT and death. A delayed hematological recovery was defined as a day of achievement of neutrophil cell count $>0.5 \times 10^9/L$ and/or platelet count $>20 \times 10^9/L$ or $>50 \times 10^9/L$ later than day 30 after HSCT.

Characteristics of patients and graft numbers and percentages were considered as categorical variables and median, first and third quartiles and min/max values as continuous variables. We first compared baseline characteristics of patients and grafts between the fresh and cryopreserved groups to detect any covariate imbalance between groups. To evaluate the impact of cryopreservation on the graft quality, we compared graft characteristics (TNC, CD34, CFU-GM) before and after cryopreservation of PBSC in the thawed group, as well as graft characteristics of fresh and thawed HSCT after transplant. Then, we aimed to identify factors associated with transplant quality loss among: granulocyte percentage, platelets count and CD34 count/ μL in grafts at collection. We also analyzed the impact of baseline CD34 richness before cryopreservation on CD34 and TNC yields and viability after thawing, by categorizing CD34 richness into three groups: high CD34 (more than 8×10^6 CD34/kg before freezing), medium CD34 (between 6 and 8×10^6 CD34/kg before freezing) and low CD34 group (less than 6×10^6 CD34/kg before freezing). To evaluate the impact of cryopreservation on clinical outcomes, we first compared the median delay of hematological recovery (neutrophil and platelet recoveries) between fresh and cryopreserved groups. Then, we compared the occurrence of each of the six clinical events (acute and chronic GVHD, transplant rejection, infections, relapse, the necessity of a second HSCT and death) between groups, and the occurrence of at least one of the six clinical events considered together. All comparisons were made using the signed Wilcoxon rank tests, Fisher test or Kruskal–Wallis test, except for the occurrence of at least one event for which a log-rank test was used to compare survival curves generated by the Kaplan–Meier method.

The significance threshold is set at 5%. These statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC). The figures were made using GraphPad Prism (San Diego, CA).

Results

Recipient and donor characteristics

Recipient and donor characteristics are summarized in [Table 1](#). Seventy-six patients were transplanted between March 15, 2020, and May 15, 2021, including 41 men and 35 women, with a median age of 56.5 years (range 2–74 years). Acute leukemias (myeloid or lymphoid) represented, respectively, 36.8% and 13.2% of allo-SCT indications, followed by myelofibrosis (10.5%), aplastic anemia (10.5%) and lymphomas (9.2%). Myelodysplastic syndrome and other myeloproliferative neoplasms represented, respectively, 9.2% and 5.3%. Conditioning regimens were mainly reduced-intensity conditioning based on cyclophosphamide and fludarabine (51.31%), followed by reduced-toxicity conditioning based on fludarabine (30.6%). GVHD prophylaxis was based either on cyclosporinA (CSA) and mycophenolate mofetil (MMF) with or without anti-Janus kinases, or CSA alone or with methotrexate.

Among donors, we observed 55 men and 21 women. Median age was 30.2 years old (range 19–69.6 years). We observed 32.9% related donors with 15.8% MRD and 17.1% haploidentical donors and 67.1% unrelated donors with 90% MUD (10/10 HLA-compatibility) and 10% mismatched unrelated donors (9/10 HLA-compatibility). Concerning ABO blood group compatibility, no ABO incompatibility was found for 68.4% allo-SCT, whereas minor, major and both incompatibilities concerned 14.5%, 9.2% and 7.9%, respectively. Overall, 19 (25%) patients received a fresh transplant and 57 patients (75%) a thawed transplant. No significant differences were observed between the two groups concerning age, sex or weight. Regarding the repartition of diagnosis between the two groups, the main difference concerned the percentage of recipients with acute myeloid leukemia, twice more represented in the thawed group than in the fresh group.

As expected, we observed a significant difference between the two groups in terms of HLA-compatibility between donor and

recipient: 57.9% versus 24.5% RD (MRD and HD) in the fresh versus cryopreserved group and 42.1% versus 75.4% MUD in the fresh versus cryopreserved groups, respectively ($P = 0.016$). Regarding MUD, PBSCs collected were mainly freeze-thawed. MUD origin was mainly European Countries with 31% coming from Germany and 12.6% from countries outside of Europe (UK, USA, Canada, Argentina, Israel). No HSCT donors were SARS-CoV-2 positive during the period.

Imbalanced characteristics of fresh and cryopreserved transplants at collection

PBSC (N = 76) was the only stem cell source used in this study ([Table 2](#)). No significant difference was observed between the fresh and the thawed group at collection, regarding the mean TNC and TNC/kg values. Mean CD34 value was significantly greater in the fresh group than in the thawed group ($P = 0.01$). This was probably due to the fact that multiple collections can be performed to reach the requested dose in the case of a related transplant. Also, this can be due to the fact that collections obtained from MUD were greater in the thawed group and many foreign centers do not plan to collect CD34 /kg dose higher than 5×10^6 in that case.

Storage times of cryopreserved transplants

Mean storage time of bags dedicated to allo-SCT was 14 days, with a minimum of 6 days and a maximum of 109 days. The cryopreservation of HSCT led to 350 frozen stem cell bags. Among them, 309 were thawed to perform HSCT (mean 4.3 bags thawed /transplant [min: 3; max: 11]) and 41 kept frozen for future donor lymphocyte infusion (DLI) ([supplementary Figure 1](#)). In the fresh transplant group, the excess of collected cells was cryopreserved in 41 bags dedicated for future DLI (mean 1.5 bags frozen per transplant).

HSCT characteristics after thawing and comparison with the fresh transplant group

After thawing and washing, median yields were 74.0%, 69.0% and 48.0%, respectively, for TNC, CD34 and CFU-GM ([Figure 1](#)). Of note, no significant differences were observed between thawed allograft yields compared with those observed for thawed autologous grafts performed in our center at the same period (data not shown) (n = 76) ([Figure 1](#)). The median TNC dose/kg obtained after thawing was 5.8×10^8 /kg (min: 2, max: 14.3×10^8) with a median viability of 76% (min: 44, max = 89). The median CD34+ cells/kg obtained after thawing was 5.2×10^6 (min: 2.1, max: 9.5×10^6) and the median of CD34 viability was 87% (min: 37, max: 95) ([Table 3](#)).

A significant difference was observed between the values at collection and after thawing for TNC, CD34+ cells and CFU-GM ($P < 0.001$ for all). Of note, among the 57 thawed allografts, 22 (38.5%) reached after thawing the 6×10^6 /kg CD34 quantity usually requested for transplantation. Among them, two transplants were partially infused to recipients on physician's request, due to the adjustment of the CD34 cell dose /kg to the prescription. Among the 35 allografts that didn't reach 6×10^6 /kg after thawing (61%), 30 recipients would have had this dose if their HSCT had not been frozen but infused fresh. The CD34+ cells/ kg were below 6×10^6 /kg for five recipients at collection (8.7%; minimal value of CD34+ cells/kg at 4.45×10^6 /kg).

Concerning the 19 fresh HSCT transplants ([Table 4](#)), four had a transformation step due to minor ABO incompatibility. Only 15.8% (n = 3/19) of the grafts infused contained less than 6×10^6 CD34+ cells/kg and came from PBSC that did not reach 6×10^6 CD34+ cells/kg at collection. Thirteen transplants were partially infused due to an adjustment of the CD34+ cells/kg cell dose to the 6×10^6 /kg required and the additional cells left were frozen for future DLI.

Table 1
Recipients and donors baseline characteristics and repartition between the fresh and thawed transplant group.

	All					Fresh, N = 19 (25.0%)				Cryopreserved, N = 57 (75.0%)					P ^a
	N	%	Median	Q1	Q3	N	%/me*	Q1	Q3	N	%/me*	Q1	Q3		
Recipient age	76		56.5	47.7	62.5	19	59.2	54.0	62.4	57	55.8	44.1	62.6	0.1045	
Recipient sex															
Male	41	53.9				12	63.2			29	50.9			0.3523	
Female	35	46.1				7	36.8			28	49.1				
Weight, kg	76		69.0	60.0	81.5	19	74.0	64.0	84.0	57	65.0	58.0	80.0	0.1539	
Diagnosis														0.2835	
Medullary aplasia	2	2.6				0	0.0			2	3.5				
Aplastic anemia	8	10.5				1	5.3			7	12.3				
Acute myeloid leukemia	28	36.8				5	26.3			23	40.4				
Acute lymphoblastic leukemia	10	13.2				3	15.8			7	12.3				
Lymphoma	7	9.2				3	15.8			4	7.0				
Myelofibrosis	8	10.5				5	26.3			3	5.3				
Myelodysplastic syndrome	7	9.2				1	5.3			6	10.5				
Other acute leukemias	2	2.6				0	0.0			2	3.5				
Chronic myeloproliferative disease	4	5.3				1	5.3			3	5.3				
Conditioning regimen														0.2116	
Myeloablative	7	9.21				0	0			7	12.5				
Non-myeloablative	7	9.21				2	10.5			5	8.8				
RIC	39	51.31				13	68.4			26	45.6				
RTC	23	30.26				4	21.1			19	33.3				
GVHD prophylaxis														0.0347 ^b	
CSA+MMF	52	68.42				12	63.2			40	70.2				
CSA+MMF+anti-JAK	5	6.57				4	21.1			1	1.8				
CSA+MTX	15	19.73				2	10.5			13	22.8				
CSA	4	5.26				1	5.3			3	5.3				
Growth factors														1.000 ^b	
G-CSF															
Yes	24	63.2				6	31.6			18	31.6				
No	52	136.8				13	68.4			39	68.4				
N-Plate														0.1611 ^b	
Yes	6	7.89				3	15.8			3	5.3				
No	70	92.10				16	84.2			54	94.7				
Donor age	76		30.2	23.8	41.6	19	40.6	25.8	51.7	57	29.6	23.6	40.2	0.0904	
Donor sex														0.4590	
Male	55	72.4				15	78.9			40	70.2				
Female	21	27.6				4	21.1			17	29.8				
HLA compatibility of the donor														0.0163	
MRD	12	15.8				6	31.6			6	10.5				
MUD	51	67.1				8	42.1			43	75.4				
HD	13	17.1				5	26.3			8	14.0				
Donor versus recipient ABO blood group incompatibility														0.5001	
None	52	68.4				12	63.2			40	70.2				
Minor	11	14.5				2	10.5			9	15.8				
Both	6	7.9				3	15.8			3	5.3				
Major	7	9.2				2	10.5			5	8.8				

Anti-Jak, Janus kinase inhibitors; CSA, cyclosporin A; G-CSF, granulocyte colony-stimulating factor; GVHD, graft-versus-host disease; HD, haploidentical donor; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; MRD, match-related donor; MTX, methotrexate; MUD, match-unrelated donor; RIC, reduced-intensity conditioning; RTC, reduced-toxicity conditioning.

^a Wilcoxon tests.

^b Fisher test.

Table 2
Baseline characteristics of fresh and cryopreserved transplants at collection.

Graft characteristics at collection	Fresh N = 19 (25.0%)				Cryopreserved N = 57 (75.0%)				P ^a
	N	%/median	Q1	Q3	N	%/median	Q1	Q3	
TNC, 10 ⁹	19	65.0	52.7	73.8	57	55.7	41.9	71.2	0.0882
TNC per recipient weight, 10 ⁸ /kg	19	8.4	7.0	11.7	57	8.9	6.7	10.5	0.8671
CD34+ stem cells, 10 ⁶	19	623.7	548.7	738.1	57	541.2	458.3	630.9	0.0195
CD34+ stem cells/kg, 10 ⁶ /kg	19	9.1	6.4	10.4	57	8.1	7.4	9.0	0.3343
CFU-GM/kg, 10 ⁴ /kg	19	276.5	114.5	373.3	56	236.2	144.1	318.4	

CFU-GM
colony-forming unit–granulocyte/macrophage

TNC
total nucleated cells.

^a Wilcoxon test.

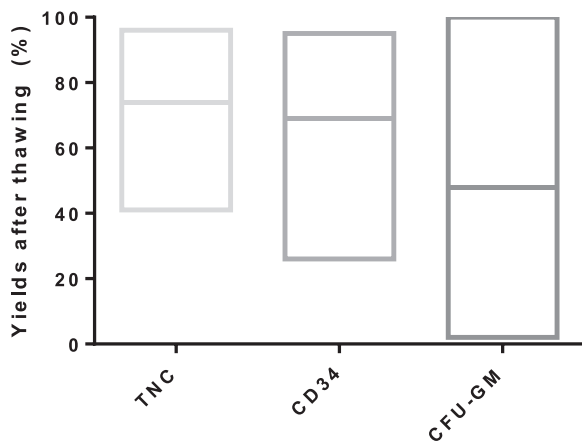


Figure 1. Yields observed after allogeneic (n = 57) thawing between March 15, 2020, and May 15, 2021. Results expressed in median and min to max. CFU-GM, colony-forming unit–granulocyte/macrophage; TNC, total nucleated cells.

The comparison of the fresh and thawed HSCT before transplant (after adjustment of the CD34+ cell dose to 6×10^6 /kg upon physician request) showed no significant difference regarding CD34 /kg or TNC/kg infused. A significant difference was observed regarding the number of CFU-GM/kg infused ($P = 0.0005$) (Table 4), with lower values obtained in the thawed transplant group, suggesting a loss of function of CD34+ cells after thawing.

Factors that may influence thawing results

On a global analysis of the 57 thawed transplants, 35 (61.4%) were concerned either by a thawing CD34 yield below 50% or a CD34 cell dose/kg infused below 6×10^6 /kg, which define a quality loss of the transplant. Based on this finding, we next tried to identify, among the granulocyte percentage, the platelet count, the CD34 count / μ L and the CD34 richness of transplant, which factors may have had an impact on transplant quality (Table 5).

In the transplant group showing no quality loss, the median granulocyte percentage was 14.5% versus 16.0% in the group showing a quality loss ($P = 0.6013$). The median platelet count in the group without quality loss was 1585×10^6 /mL versus 1475×10^6 /mL in the other group ($P = 0.2271$). The median value of CD34+ cells/ μ L observed in the transplant group without quality loss was higher than the median value measured in the group with quality loss (1941 versus 1914, respectively), but the difference was not significant ($P = 0.5851$).

Next, to determine whether the CD34 richness before cryopreservation may influence the CD34 and TNC yields and viability after thawing, three groups of transplants were defined: high CD34, moderate CD34 and low CD34, as described in the Methods section. Twenty-seven of 50 (54%) adult cryopreserved grafts contained more than 8×10^6 CD34 /kg before freezing (high CD34/ kg group), 19 of 50 (38%) contained between 6 and 8×10^6 CD34 /kg before freezing (moderate CD34/ kg group) and 4 (8%) grafts contained less than 6×10^6 CD34 /kg before freezing (low CD34/kg group). Surprisingly, the transplants belonging to the high CD34 rich groups showed a significantly lower CD34 yield (62%) after thawing than the moderate (73%) and low (67.5%) CD34 groups ($P < 0.05$) (Figure 2a). The CD34

Table 3
Graft characteristics after thawing.

	N	Median	Q1	Q3	Min	Max
Total nucleated cells after thawing, 10^9	57	39.6	30.7	51.8	8.0	105.0
Total nucleated cells per recipient weight after thawing, 10^8 /kg	57	5.8	4.9	7.9	2.0	14.3
Total nucleated cell viability, %	57	76.0	66.0	81.0	44.0	89.0
CD34+ stem cells after thawing, 10^6	57	358.0	278.2	434.3	78.3	656.9
CD34+ stem cells after thawing per recipient weight, 10^6 /kg	57	5.2	4.4	6.4	2.1	9.5
CD34+ stem cells viability after thawing, %	57	87.0	81.0	90.0	37.0	95.0

Table 4
Characteristics of fresh and thawed HSCTs before transplant.

	Fresh N = 19 (25.0%)				Thawed N = 57 (75.0%)				P^a
	N	Median	Q1	Q3	N	Median	Q1	Q3	
Total nucleated cell per recipient weight, 10^8 /kg	19	5.9	4.6	7.6	57	5.7	4.7	7.4	0.9809
CD34+ stem cells per recipient weight, 10^6 /kg	19	6.0	6.0	6.0	57	5.2	4.4	6.2	0.0882
CFU-GM per recipient weight, 10^4 /kg	19	276.5	114.5	373.3	57	100.6	46.6	142.9	0.0005

CFU-GM
colony-forming unit–granulocyte/macrophage
HSCT
hematopoietic stem-cell transplantation.

^a Wilcoxon tests.

Table 5
Factors associated with thawed transplant's quality.

	Transplants with quality loss (CD34 yield <50% or CD34/kg < 6×10^6 /kg)								
	No, N = 22 (38.6%)				Yes, N = 35 (61.4%)				P^a
	N	Median	Q1	Q3	N	Median	Q1	Q3	
Granulocyte percentage, %	22	14.5	12.0	19.0	35	16.0	11.0	27.0	0.6013
Platelets count, 10^6 /mL	22	1585.0	1330.0	2200.0	35	1475.0	1100.0	1880.0	0.2271
CD34 count per μ L, CD34/ μ L	22	1941.0	1517.0	2926.0	35	1914.0	1298.0	2469.0	0.5851

^a Wilcoxon rank test.

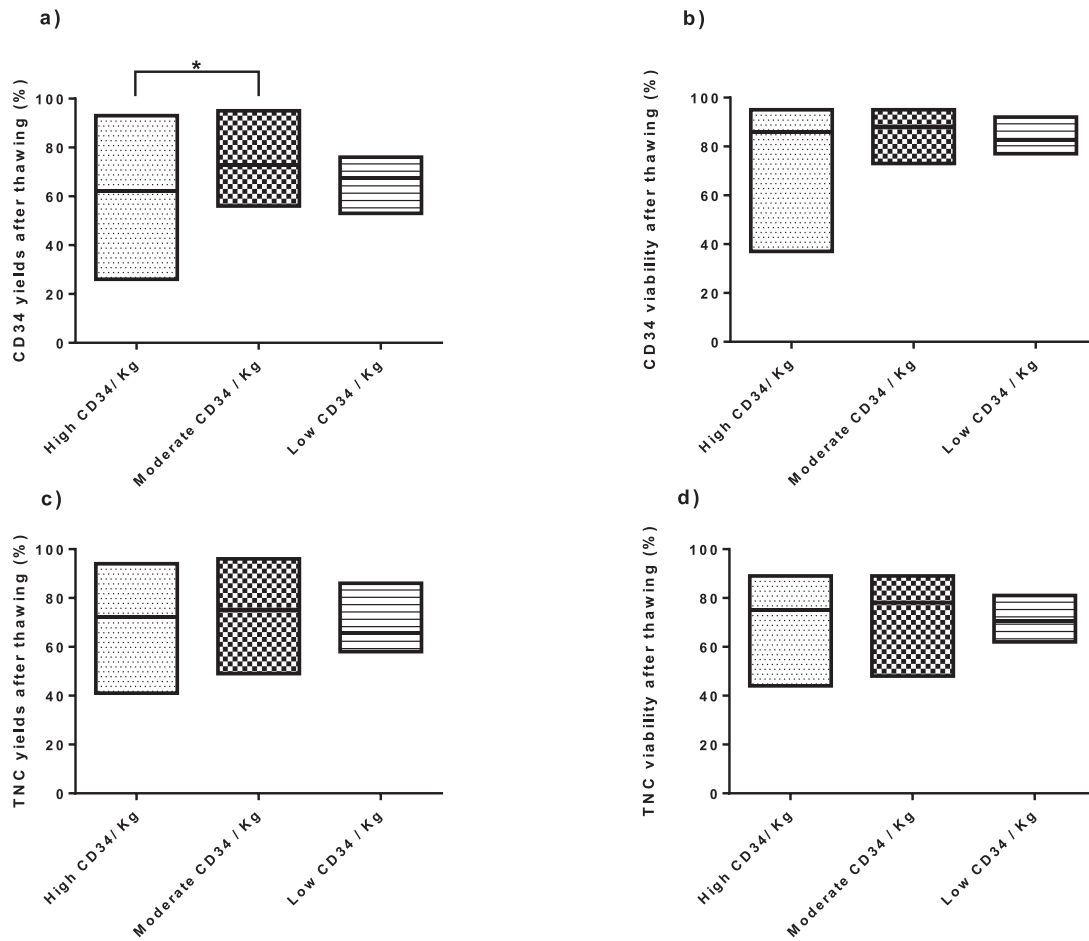


Figure 2. Yield (a) and viability (b) of CD34 and yield (c) and viability (d) of TNCs after thawing obtained for the high (n = 27), moderate (n = 19) and low group (n = 4). Results expressed in median and min to max. * $P < 0.05$. TNC, total nucleated cells.

viability in the high CD34 group (86%) was similar to that observed in the moderate group (88%) and the low group (82.5%) (Figure 2b). Similar results were found for TNC yields (72%, 75% and 65.5%, respectively) and viability (75%, 78% and 70.5%) (Figure 2c,d).

In summary, the factor that had a significant impact on the CD34 and TNC yields after thawing was the CD34 richness at collection. No significant impact was observed regarding the granulocyte count, the platelet count or the CD34 concentration/ μL .

Clinical consequences of cryopreservation

We next analyzed whether the cryopreservation of the transplant might have an impact on patient outcomes (Table 6). Hematological recovery was defined on neutrophil count above $>0.5 \times 10^9/\text{L}$ and/or on platelet count above $>20 \times 10^9/\text{L}$ and $>50 \times 10^9/\text{L}$. In the thawed transplant group, the median time for neutrophil recovery was

18 days (range 11–42 days) and 15 days (range 1–90 days) and 18 days (range 6–95 days) for platelet recovery $>20 \times 10^9/\text{L}$ and $>50 \times 10^9/\text{L}$, respectively. Among the 57 patients receiving a thawed graft, 10 (17.5%) showed a delayed hematological recovery. In the fresh HSCT group, the median neutrophil reconstruction time was 18 days (range 12–31 days) and the platelet reconstitution time was 12.5 days (range 1–60 days) and 22 days (range 10–70 days) for platelet count $>20 \times 10^9/\text{L}$ and $>50 \times 10^9/\text{L}$ respectively. Among the 19 patients receiving a fresh graft, 4 (21.05%) experienced a delayed hematological recovery. There was no significant difference between the thawed and fresh group considering hematological reconstitution (Table 6).

Regarding the clinical events occurring during the post-transplant period, acute and chronic GVHD, transplant rejection, infections, death, relapse, and the need of a second HSCT were considered.

Table 6
Hematological recovery in the fresh and thawed group.

	Fresh, N = 19 (25.0%)				Thawed, N = 57 (75.0%)				P^a
	N	%/median	Q1	Q3	N	%/median	Q1	Q3	
Day of achievement of an ANC = $0.5 \times 10^9/\text{L}$, days	19	18.0	15.0	22.0	55	18.0	15.0	21.0	0.3703
Day of achievement of a platelet count = $20 \times 10^9/\text{L}$	18	12.5	10.0	18.0	54	15.0	11.0	21.0	0.6969
Day of achievement of a platelet count $>50 \times 10^9/\text{L}$	18	17.5	14.0	27.0	52	18.0	13.5	25.5	0.4955

ANC
absolute neutrophil count.

^a Wilcoxon test.

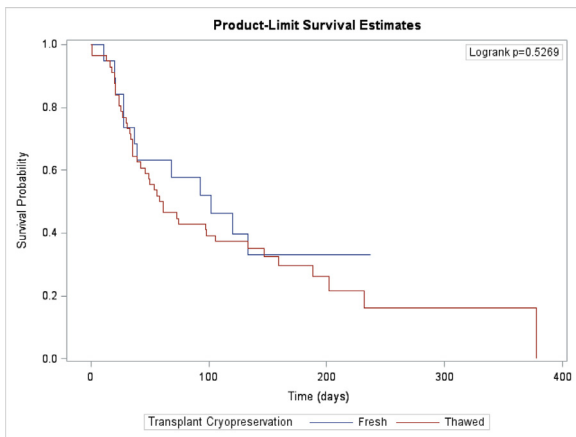


Figure 3. Survival without relapse or graft-versus-host disease in the fresh (blue) and cryopreserved (red) transplant groups (Kaplan–Meier method).

Regarding GVHD, 31 patients showed an acute GVHD and 11 a chronic GVHD in the thawed transplant group whereas 15 and two patients presented acute and chronic GVHD, respectively, in the fresh transplant group (acute GVHD, $P = 0.92$; and chronic GVHD, $P = 0.20$).

Transplant rejection occurred for two patients diagnosed for medullary aplasia in the thawed transplant group. One received less than 2×10^6 CD34/kg after thawing and the other received 4.3×10^6 /kg. One transplant rejection occurred in the fresh transplant group, concerning a patient diagnosed with a myelomonocytic leukemia, despite a transplant containing the requested CD34 cell dose (6×10^6 /kg).

Infections were observed in 22 patients in the thawed group and eight in the fresh group ($P = 0.60$), and relapse was observed in seven and two patients, respectively ($P = 0.71$). Death occurred for one recipient in the fresh group, whereas five deaths were observed in the thawed group ($P = 0.65$).

Taken together, the number of recipients concerned by at least one outcome in the six analyzed was 14 of 19 (73.7%) in the fresh group and 48 of 57 (84.2%) in the thawed group. The Kaplan–Meier curves of occurrence of clinical events by groups are presented in Figure 3, showing no statistical difference between the fresh and the thawed groups ($P = 0.526$).

Discussion

The implementation of cryopreservation of allografts has upset the organization of the transplant circuit in the hematology departments. This has increased the cryopreservation activity of cell therapy units, an activity carried out without increasing the technical staff. It was economically expensive with more materials and reagents used; a cost linked to nitrogen storage. This caused storage difficulties in cell therapy units, where the space available for the grafts collected is becoming scarce [14]. In our case, the equivalent of one filled liquid nitrogen container was stored during the period. Considering the freezing process from an organizational point of view, the benefit of freezing HSCT for patients remained limited. Despite waiting for the results of the SARS-CoV-2 PCR tests has secured the donation, it should be noted that very few donations have been collected from positive donors and none in our center. In addition, to date, no data suggested that the SARS-CoV-2 may infect the blood [15] and be a transfusion transmissible disease. Moreover, a negative SARS-CoV-2 collection can be obtained from a positive donor. In our study, no donor was positive for SARS-CoV-2 at the time of collection. Moreover, although the median time between collection and transplant was 14 days, some patients experienced a very delayed infusion (>100 days after collection) due to unexpected events such as infections.

In this study, the impact of cryopreservation on 57 HSCTs was examined and compared with 19 fresh HSCTs performed at the same period considering graft quality. Graft characteristics after thawing such as CD34/kg, TNC/kg, CFU-GM/kg and the CD34 and TNC viability. The requirement of larger collections from the donor to overcome the potential loss after thawing was confirmed in our study, although some donor centers were opposed to collecting more than 5×10^6 CD34/kg. The median CD34/kg at collection in the thawed group was greater than in the fresh group, although the difference was not found significant. The three parameters (TNC, CD34 and CFU-GM) were significantly different at collection and after thawing. The median CD34 recovery was 69%, a value close to the results obtained by Purtill *et al.* [5], 74% but far from the 90% recovery published by Berens *et al.* [16]. This is probably due to discrepancies in the freezing method and flow cytometry analyses.

Of note, in our study, 35 of 57 recipients received a HSCT out of specifications (less than 6×10^6 CD34+ cells/kg). Thirty of them would have had the requested CD34 cell dose if their HSCT had not been frozen. Kanda *et al.* [17] also analyzed the impact of cryopreservation on allogeneic HSCT. In their study cohort, they compared the thawing results of 29 PBSC grafts and 83 bone marrow but unlike our study, didn't compare the results to fresh HSCT. The median time from cryopreservation to infusion of 231 hours (9.6 days) was shorter than in our study (14 days). The authors did not report any significant difference regarding hematologic recovery after PSBC or BM thawing.

Then, to identify a parameter that may influence thawing results, the CD34 richness, platelet count, granulocyte percentage or CD34+ cells/ μ L were analyzed in HSCT presenting a quality loss defined by a yield below 50% or CD34+ cells/kg dose below 6×10^6 . We observed that after thawing, results could be random, sometimes with poor performance, without really identifying a parameter that could be responsible among the platelet count, the percentage of granulocytes or the concentration of CD34+ cells / μ L, as no significant differences were observed between the two groups. However, the richness of the stem cell transplant seemed to have an impact on the thawing results, especially as CD34 yield was significantly lower in the CD34 group containing more than 8×10^6 /kg. Purtill *et al.* [5] also observed an impact of the delay between collection and thawing and the WBC concentration at collection. Based on our thawing results, showing a mean recovery of 69% of CD34+ cells after thawing, we can recommend to ask for 30% more cells to be collected by the collection center, if a cryopreservation is planned.

Regarding the outcome of recipients having a thawed HSCT, no significant difference was observed regarding acute or chronic GVHD, relapse, rejection, infections or death.

In summary, the strength of our study was to compare thawed and fresh HSCT performed on the same period, considering biological parameters including graft characteristics (TNC, CD34 and CFU-GM) but also clinical outcomes observed in the patients. We highlight the impact of freezing on HSCT that dropped under the requested cell dose after thawing. This impact was also mainly seen on HSCT containing a high amount of CD34 before freezing. However, when looking at the results on a global analysis, many parameters didn't reach significant difference between the fresh and thawed groups probably because of the low number of patients in each group. Therefore, this needs to be confirmed on a larger number of patients.

In conclusion, allogeneic HSCT cryopreservation was required to mitigate donor related risk and travel perturbation due to the COVID-19 pandemic. However, such practice increased the collected cells number for the donor and complicated the transplant circuit. Regarding the graft quality, cryopreservation caused a cell loss that led to HSCT out of specifications. This also led sometimes to a delayed infusion of HSCT to the recipient, which might increase relapse risk. All these considerations may raise the question if cryopreservation is still appropriate, considering donor's vaccination.

Funding

No funding was received.

Declaration of Competing Interest

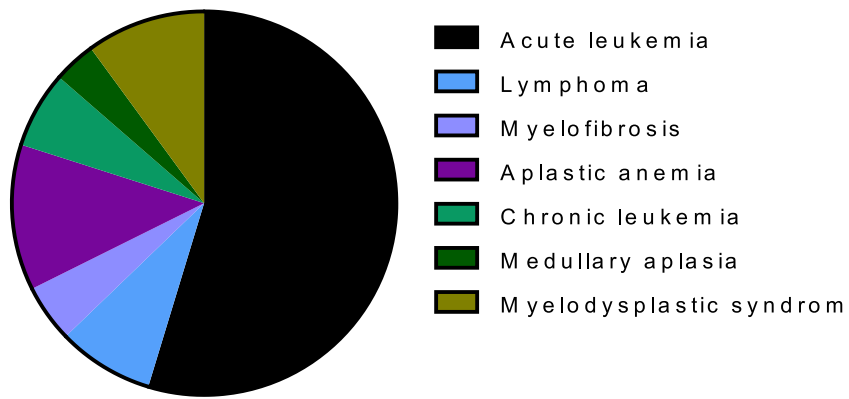
The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

Conception and design: V. DECOT. Acquisition of data: C. LAROYE. Analysis and interpretation: N. Thilly and A. Luc. Drafting and revising the manuscript: V. DECOT and M. D'AVENI. All authors have approved the final article.

References

- [1] Lefrant J-Y, Pirracchio R, Benhamou D, Dureuil B, Pottecher J, Samain E, Joannes-Boyou O, Bouaziz H. ICU bed capacity during COVID-19 pandemic in France: From ephemeral beds to continuous and permanent adaptation. *Anaesth. Crit. Care Pain Med.* 2021;40:100873.
- [2] Auletta JJ, Novakovich JL, Stritesky GL, Newman J, Fridy-Chesser ST, Hailperin K, Devine SM. Meeting the demand for unrelated donors in the midst of the COVID-19 pandemic: rapid adaptations by the national marrow donor program and its network partners ensured a safe supply of donor products. *Transplant. Cell. Ther.* 2021;27:133–41.
- [3] Xu L-P, Lu D-P, Wu D-P, Jiang E-L, Liu D-H, Huang H, Sun Z-M, Li N-N, Liu Q-F, Zhang X, Lai Y-R, Song Y-P, Song X-M, Liu S-X, Zhang Y-C, Luo C-J, Xia L-H, Niu T, Yu Y, Zhang X-H, Tang X-W, Luo Y, Huang X-J. Chinese Blood and Marrow Transplantation Registry Group. Hematopoietic stem cell transplantation activity in China 2020–2021 during the SARS-CoV-2 pandemic: a report from the Chinese Blood and Marrow Transplantation Registry Group. *Transplant. Cell. Ther.* 2023;29:136.e1–7.
- [4] Szer J, Weisdorf D, Querol S, Foeken L, Madrigal A. The impact of COVID-19 on the provision of donor hematopoietic stem cell products worldwide: collateral damage. *Bone Marrow Transplant* 2020;55:2043–4.
- [5] Purtil D, Antonenas V, Chiappini P, Tong D, O'Flaherty E, Bajel A, Kabani K, Larsen S, Tan S, Hutchins C, Curtis DJ, Kennedy GA, Watson A-M, Bai L, Greenwood M, Gottlieb DJ, Hamad N. Variable CD34+ recovery of cryopreserved allogeneic HPC products: transplant implications during the COVID-19 pandemic. *Blood Adv* 2020;4:4147–50.
- [6] Ljungman P, Mikulska M, de la Camara R, Basak GW, Chabannon C, Corbacioglu S, Duarte R, Dolstra H, Lankester AC, Mohty M, Montoto S, Murray J, Peffault de Latour R, Snowden JA, Yakoub-Agha I, Verhoeven B, Kröger N, Styczynski J. European Society for Blood and Marrow Transplantation. The challenge of COVID-19 and hematopoietic cell transplantation; EBMT recommendations for management of hematopoietic cell transplant recipients, their donors, and patients undergoing CAR T-cell therapy. *Bone Marrow Transplant* 2020;55:2071–6.
- [7] Parrondo RD, Ailawadhi S, Sher T, Chanan-Khan AA, Roy V. Autologous stem-cell transplantation for multiple myeloma in the era of novel therapies. *JCO Oncol. Pract.* 2020;16:56–66.
- [8] Harris AC, Ferrara JLM, Levine JE. Advances in predicting acute GVHD. *Br. J. Haematol.* 2013;160:288–302.
- [9] Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers MED. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol. Blood Marrow Transplant. J. Am. Soc. Blood Marrow Transplant.* 2005;11:945–56.
- [10] Appelbaum FR, Herzig GP, Ziegler JL, Graw RG, Levine AS, Deisseroth AB. Successful engraftment of cryopreserved autologous bone marrow in patients with malignant lymphoma. *Blood* 1978;52:85–95.
- [11] Decot V, Alla F, Latger-Cannard V, Visanica S, Witz B, Stoltz J-F, Bensoussan D. Thawed autologous peripheral blood stem cells require modified quantification methods for hematopoietic progenitor cell evaluation. *Biomed. Mater. Eng.* 2012;22:57–67.
- [12] Massin F, Huili C, Decot V, Stoltz J-F, Bensoussan D, Latger-Cannard V. Validation of a single-platform method for hematopoietic CD34+ stem cells enumeration according to accreditation procedure. *Biomed. Mater. Eng.* 2015;25:27–39.
- [13] Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J. Clin. Oncol.* 2001;19:3685–91.
- [14] Valentini CG, Chiusolo P, Bianchi M, Metafuni E, Orlando N, Giammarco S, Bacigalupo A, Sica S, Teofili L. Coronavirus disease 2019 pandemic and allogeneic hematopoietic stem cell transplantation: a single center reappraisal. *Cytotherapy* 2021;23:635–40.
- [15] Chang L, Yan Y, Wang L. Coronavirus disease 2019: coronaviruses and blood safety. *Transfus. Med. Rev.* 2020;34:75–80.
- [16] Berens C, Heine A, Müller J, Held SAE, Mayer K, Brossart P, Oldenburg J, Pöttsch B, Wolf D, Rühl H. Variable resistance to freezing and thawing of CD34-positive stem cells and lymphocyte subpopulations in leukapheresis products. *Cytotherapy* 2016;18:1325–31.
- [17] Kanda Y, Inoue M, Uchida N, Onishi Y, Kamata R, Kotaki M, Kobayashi R, Tanaka J, Fukuda T, Fujii N, Miyamura K, Mori S-I, Mori Y, Morishima Y, Yabe H, Koderia Y. Cryopreservation of unrelated hematopoietic stem cells from a blood and marrow donor bank during the COVID-19 pandemic: a nationwide survey by the Japan Marrow Donor Program. *Transpl. Cell Ther* 2021;664. e1-664.e6.



Total = 309

Figure S1. Number of HSCB bags frozen between March 15th 2020 and May 15th 2021 and repartition regarding diagnosis.