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Running title: Cardiotoxicity after hematopoietic stem cell transplantation

Elevation of cardiac biomarkers after allogeneic transplantation: the impact of hematopoietic stem cell cryopreservation

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Hematopoietic stem cell transplantation (HSCT) represents a curative treatment modality for numerous hematologic malignancies. Advances in treatment protocols and supportive care have markedly enhanced post-transplant survival rates. Consequently, the number of long-term survivors has increased. However, HSCT may induce organ and tissue injury of varying severity, ranging from subclinical alterations to severe, potentially fatal complications. The aim of this study was to quantify plasma levels of the cardiac biomarkers: N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitive cardiac troponin T (hs-cTnT), and to evaluate cardiovascular (CV) complications in patients undergoing HSCT with and without cryopreservation of stem cells. During the COVID-19 pandemic, the use of cryopreserved allografts increased. Dimethyl sulfoxide (DMSO) is a cryoprotectant added to cell media to prevent ice formation and subsequent cell death during the freezing process of cryopreserved grafts. This study included 106 consecutive hematologic patients who underwent allogeneic HSCT. Serial measurements of plasma NT-proBNP and hs-cTnT concentrations were performed the day before conditioning regimen (baseline), on the day after HSCT (D+1), and subsequently on days D+2, D+7, D+14, and D+30, or at the onset of clinical symptoms. Newly diagnosed cardiac events post-HSCT were evaluated. NT-proBNP concentrations increased in all patients on D+1 after HSCT, reaching their peak at that time. Thereafter, NT-proBNP levels showed a gradual decline, but they did not return to baseline. A statistically significant increase in NT-proBNP values was observed in the group of patients who received cryopreserved grafts compared to those who received non-cryopreserved grafts. Hs-cTnT values during the early post-transplant period were comparable to pre-transplant levels in both groups - in patients who received cryopreserved grafts and also in patients who received non-cryopreserved grafts. After transplantation, we observed persistent hs-cTnT levels above the cut-off value in 28 % of patients. Clinically significant CV complications were identified in 10 (9.4 %) patients. Patients with manifest CV complications had significantly higher concentrations of both cardiomarkers compared with those without CV complications. In conclusion, persistent elevation in cardiac biomarkers may indicate a reduced functional myocardial reserve or diminished cardiac tolerance to cardiac stressors. Potential cardiac toxicity related to DMSO exposure in cryopreserved grafts should be considered in the differential diagnosis of cardiac events following HSCT involving cryopreserved stem cells.

Key words: hematopoietic stem cell transplantation; cardiotoxicity; cardial biomarkers; early cardiovascular complications

The number of hematopoietic stem cell transplantations (HSCT) performed in adult patients has increased substantially in recent years for both malignant and non-malignant indications. Advances in supportive care and accumulated clinical experience have contributed to a significant reduction in transplant-related mortality [1].

Cancer therapy-related cardiovascular toxicity (CTRCD) and cancer-radiation-induced cardiotoxicity (CRIC) are burgeoning clinical problems [2]. Atrial fibrillation is the most common form of CRIC, but this field also comprises heart failure, hypertension, arrhythmias, ischemia, thromboembolism, pericardial effusions, valvular heart disease, and sudden cardiac death [3-5]. Among HSCT survivors, cardiovascular disease (CVD) is one of the leading causes of non-relapse mortality [6]. HSCT survivors have a 4-fold increased risk of developing CVD when compared to the general population [7]. The median age at the first cardiovascular event is 53 years [8]. Patients after hematopoietic cell transplantation also have an increased risk of developing metabolic syndrome, which is an important risk factor for the development of cardiovascular diseases [9].

The prevention, diagnosis, and management of cardiovascular toxicity associated with cancer therapy remain significant clinical challenges. Recent guidelines have highlighted that novel echocardiographic parameters and circulating biomarkers may facilitate the early detection of cardiac injury [5]. The European Society of Cardiology, the European Society of Medical Oncology (ESMO), the International CardioOncology Society (IC-OS), the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the Cardio-Oncology Council of the American College of Cardiology (ACC) have published recommendations on the management of patients with cancer receiving cardiotoxic treatment [5, 10, 11].

Systematic evaluation of cardiac biomarkers in CTRCD is urgently needed to standardize assessment in patients undergoing oncologic treatment. Future studies should define optimal sampling time points, implement rigorously standardized assay methodologies, and establish evidence-based biomarker cut-off values that maximize diagnostic performance (sensitivity, specificity) and predictive validity.

The COVID-19 pandemic necessitated modifications to numerous treatment protocols. The European Group for Blood and Marrow Transplantation (EBMT) issued guidance recommending routine cryopreservation of stem cell allografts [12]. Cryopreservation had previously been used primarly for autologous grafts. Dimethyl sulfoxide (DMSO) is the most commonly employed

cryoprotectant agent for the long-term preservation of hematopoietic stem cells and cardiovascular DMSO toxicity may manifest as bradycardia, hypertension, and cardiac arrhythmias, and more rarely as coronary vasospasm or myocardial infarction. DMSO metabolites can induce direct endothelial irritation and oxidative stress.

In this study, we aimed to evaluate the levels of high-sensitive cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) and to investigate the potential role of DMSO in altering these cardiomarker levels and in the development of early cardiac events. This study was conducted during the peak of the COVID-19 pandemic, a period during which the number of transplants involving cryopreserved grafts increased substantially. To the best of our knowledge, this is the first study to assess the impact of DMSO on cardiovascular toxicity in allogeneic transplant recipients.

Patients and methods

Patients. The study cohort included 106 consecutive patients who underwent allogeneic stem cell transplantation from January 2020 to January 2022 at the Transplantation Centre of Department of Hematology, University Hospital Bratislava. The study was approved by the local Ethics Committee of University Hospital Bratislava. Written informed consent was obtained from all patients. Among the allogeneic transplant recipients, there were 45 women and 61 men, with a median age of 48 years (range: 18-68 years).

Patients underwent hematopoietic stem cell transplantation for hematologic disorders. The predominant indication was acute myeloblastic leukemia, accounting for approximately 50% of all transplants. All but one recipient received peripheral blood stem cell grafts. Sixty-eight patients received grafts from matched unrelated donors (MUD), and 38 received grafts from HLA-identical sibling donors.

Sixty-five patients received a myeloablative regimen (cyclophosphamide 120 mg/kg, either with busulfan 16 mg/kg or total body irradiation 12 Gy) as part of the conditioning treatment, while the remaining 41 patients received a reduced-intensity regimen (BuFlu: fludarabine 180 mg/m², busulfan 8 mg/kg; FluCy: fludarabine 150 mg/m² and cyclophosphamide 1,200 mg/m²; BuFluCy: fludarabine 100 mg/m², busulfan 6 mg/kg, cyclophosphamide 40 mg/kg; FluMel: fludarabine 150 mg/m² and melphalan 140 mg/m²; or FluTreo: fludarabine 150 mg/m² and treosulfan 30 mg/m²). Cryopreservation of hematopoietic stem cells was performed in 67 (63%) patients (cryo group), while the remaining 39 patients received non-cryopreserved transplants (non-cryo group). Cryopreservation was used in most unrelated transplants. The cryopreserved group contained a

substantially higher proportion of unrelated donors (56 unrelated vs. 11 sibling donors), whereas the non-cryopreserved group showed the opposite pattern, with more sibling donors (27 sibling vs. 12 unrelated donors) ($p < 0.001$). The average dimethyl sulfoxide concentration in the hematopoietic stem cell grafts was 7-8% DMSO, with an average volume of 21.6 ml (range: 17.6-26.3 ml).

Eighty-four patients were treated with an anthracycline (ANT) prior to stem cell transplantation, with a median cumulative doxorubicin dose of 180 mg/m² (range: 50-576 mg/m²). The cumulative dose of ANT was calculated as an equivalent dose of doxorubicin. Nine patients received pre-transplant radiotherapy, and total body irradiation was used as part of the conditioning regimen in 30 patients.

Graft-versus-host disease (GvHD) prophylaxis comprised a calcineurin inhibitor combined with a short course of methotrexate. Acute GvHD occurred in 36 patients and was treated with intensified immunosuppression, including systemic corticosteroids. Medical histories were reviewed for obesity, hypertension, dyslipidemia, diabetes mellitus, and cardiovascular disease (including coronary artery disease, stroke, and atrial fibrillation). Key demographic and clinical characteristics of the study cohort are summarized in Table 1.

Study design. Serial measurements of plasma concentrations of NT-proBNP and hs-cTnT were performed on the day before initiation of the conditioning regimen (baseline value), the day after stem cell transplantation (D+1), 2 days after transplantation (D+2), 7 days (D+7), 14 days (D+14), and 30 days after transplantation (D+30), or upon the onset of clinical symptoms.

Venous blood samples were obtained via an established central venous catheter during routine morning blood collections, yielding a total of 584 specimens for analysis. Plasma concentrations of the biomarkers were immediately measured using an electrochemiluminescent immunoassay on the Elecsys 2010 analyzer (Roche Diagnostics). Normal values for hs-cTnT were < 14 ng/l and for NT-proBNP < 125 ng/l. The evaluation of cardiac markers was conducted in collaboration with the Department of Clinical Biochemistry at the University Hospital Bratislava.

We further evaluated newly diagnosed early cardiac events occurring after stem cell transplantation. Cardiac events were defined as the occurrence of myocardial infarction, arrhythmias, electrocardiographic abnormalities, heart failure requiring pharmacological treatment or intervention, and cardiovascular mortality.

Statistical analysis. Baseline patient characteristics, such as age at the time of stem cell transplantation and cumulative anthracycline dose, were expressed as median and range. The values of NT-proBNP and hs-cTnT were reported as median and 95% confidence intervals (CI). We used the chi-squared test to compare parameters between groups. The Wilcoxon signed-rank test was

used to compare data between two dependent groups, and the Mann–Whitney U test was used to compare data between two independent groups. For comparisons of multiple groups or multiple measurements, we employed the Kruskal-Walli's test and repeated-measures ANOVA. Statistical analysis was conducted using MedCalc statistical software version 20. A two-sided p-value < 0.05 was considered statistically significant.

Results

Analyses of selected cardiac markers. We monitored abnormalities and the dynamics of changes in plasma concentrations of NT-proBNP and hs-cTnT as biochemical markers of cardiac injury. The values of NT-proBNP in individual measurements are detailed in Figure 1. When evaluating the dynamics of NT-proBNP concentrations, we observed a statistically significant difference between the measured values at different time intervals between the cryo and non-cryo groups (baseline: p=0.02; D+1: p=0.01; D+2: p=0.05; D+7: p=0.01). An increase in NT-proBNP concentrations was found in all patients as early as D+1 post-transplantation, with levels reaching their peak at this time point. In the entire patient cohort, the proportion of patients with NT-proBNP values above 125 ng/l at each time point was as follows: baseline 45% (median 83.3 ng/l), D+1: 100% (median 1,081 ng/l), D+2: 100% (median 764.4 ng/l), D+7: 93% (median 473.5 ng/l), D+14: 96% (median 562.6 ng/l), and D+30: 93% (median 409 ng/l).

A more significant increase was detected in the subgroup of patients who received cryopreserved grafts. The NT-proBNP values in the cryo group reached nearly double the values compared to the non-cryo group. Over time, NT-proBNP levels gradually declined in both groups; however, by D+30 post-transplantation, values had not yet returned to baseline (Figure 1).

Patients receiving reduced-intensity conditioning showed significantly higher baseline NT-proBNP levels (median 178 vs. 94 ng/l, p=0.003). Following transplantation, myeloablative conditioning was associated with significantly higher NT-proBNP concentrations on D+1 and D+7 (p=0.02 and p=0.02, respectively). These differences diminished by D+14 and D+30, when no significant effect persisted.

The results of biochemical analyses of hs-cTnT at various measurements (baseline, D+1, D+2, D+7, D+14, and D+30) in cryo and non-cryo groups are presented in Figure 2. During the early post-transplant period, hs-cTnT levels remained comparable to baseline pre-transplant values in both patient groups, irrespective of receiving cryopreserved or non-cryopreserved grafts.

Across all included patients, the proportion of patients with hs-cTnT values above 14 ng/l at each time point was as follows: baseline 20% (median 8.9 ng/l), D+1: 13.8% (median 8.9 ng/l), D+2:

19.7% (median 9.1 ng/l), D+7: 33.6% (median 11.2 ng/l), D+14: 33.6% (median 12.0 ng/l), and D+30: 28% (median 11.0 ng/l). The highest number of patients with abnormal hs-cTnT values was recorded at D+7 and D+14. However, with the passage of time after transplantation, we observed persistent borderline hs-cTnT values; even at D+30, hs-cTnT levels had not returned to baseline.
High-sensitivity cardiac troponin did not differ significantly between the myeloablative and reduced-intensity conditioning groups at any time point.

Clinically significant cardiovascular complications. In our patient cohort, we observed clinically significant cardiovascular complications in 10 patients, resulting in an incidence of 9.4%. Cardiotoxicity was defined as congestive heart failure occurring in five patients, newly diagnosed atrial fibrillation in two patients, supraventricular arrhythmia with ventricular ectopy in one patient, and prolonged QT interval corrected using Fridericia's formula (QTcF) in two patients (QT/QTcF: in the first patient 600/540 ms and in the second patient 460/510 ms); the first patient also had concomitant bradycardia.
The changes in NT-proBNP levels in patients with and without cardiovascular complications are depicted in Figure 3. An increase in NT-proBNP concentrations was observed in all patients following administration of the conditioning regimen and hematopoietic stem cell transplantation. However, patients with clinically manifest cardiovascular complications had significantly higher NT-proBNP values compared to those without cardiovascular complications, with the differences between groups being statistically significant ($p=0.013$). The highest NT-proBNP concentrations in patients with clinically significant cardiotoxicity were observed on days D+1 and D+2 after transplantation. In the group of patients with cardiovascular complications, elevated NT-proBNP levels persisted through D+30.
The hs-cTnT concentrations in patients with and without cardiovascular complications are depicted in Figure 4. Patients who developed cardiovascular complications had significantly higher hs-cTnT concentrations than those without cardiovascular complications. The highest hs-cTnT concentrations were observed on days D+7 and D+14 in patients with cardiovascular complications, and elevated levels persisted through D+30.
When evaluating clinically manifest cardiovascular complications between patients who received non-cryopreserved and cryopreserved grafts, we did not observe any statistically significant differences ($p=0.2$).
In the non-cryo group, we diagnosed clinically significant cardiotoxicity in two patients, while in the cryo group, cardiovascular complications were observed in eight patients (Table 2). Of the 10

patients with clinical cardiotoxicity, seven received a myeloablative regimen and three received a reduced-intensity regimen. The median time to onset of cardiovascular complications was 10 days relative to HSCT (range: D-3 to D+22).

Discussion

In the present prospective single-center study, we observed significant elevations in NT-proBNP and hs-cTnT concentrations following allogeneic hematopoietic stem cell transplantation. These elevations were significantly higher in patients who received cryopreserved grafts and in those who developed cardiovascular events. To the best of our knowledge, this is the first study to investigate the impact of allogeneic graft cryopreservation on cardiovascular complications.

A comprehensive cardiovascular evaluation, including natriuretic peptide assessment, has become a core component of the pre-HSCT assessment. NT-proBNP is currently used as a biomarker to guide pre-transplant cardiac assessment, but supporting evidence remains limited, and the optimal cutoff value for cardiology referral remains undefined [5]. Monitoring cardiac biomarkers offers a non-invasive and widely accessible method for patient surveillance, facilitating its implementation across a wide range of HSCT centers. Among the most widely studied biomarkers after HSCT are natriuretic peptides and cardiac troponins [13]. In our study, we observed significant elevations in NT-proBNP and hs-cTnT concentrations after HSCT. In contrast, Horáček et al. found significant elevations in NT-proBNP and GPBB (glycogen phosphorylase BB) in patients with acute leukemia during the conditioning regimen and HSCT, whereas other biomarkers, including cardiac troponins (cTnT and cTnI), creatine kinase-MB, and heart-type fatty acid-binding protein, remained within the reference range [14]. In another study by the same authors, the findings suggested that GPBB may be a sensitive biomarker for the detection of acute cardiotoxicity associated with conventional anthracycline-containing chemotherapy and high-dose chemotherapy followed by HSCT [15].

Natriuretic peptides are among the most extensively studied biomarkers after HSCT. Several studies have evaluated BNP and NT-proBNP levels in patients undergoing HSCT and have demonstrated elevated baseline natriuretic peptide levels in HSCT recipients [16, 17]. Se et al. observed that the median NT-proBNP level was 55 pg/ml at baseline and that 77.4% of patients had NT-proBNP concentrations > 125 pg/ml [18]. In our study, the median baseline NT-proBNP concentration was 83.3 ng/l, and only 45% of patients had elevated baseline values. These elevated baseline values may reflect subclinical myocardial dysfunction, renal impairment, advanced age, prior exposure to cardiotoxic chemotherapy, systemic inflammation, endothelial activation, or neurohormonal stress responses.

NT-proBNP rises very early during the conditioning phase. In our cohort, we observed abormal values of NT-proBNP above 125 ng/l on D+1 and D+2 after HSCT in all patients. Authors Horáček et al. found NT-proBNP elevations above 500 ng/l in 26.1% patients after conditioning regimen and in 39.1% after HSCT [14]. Similar findings were reported by Poreba et al., who found marked NT-proBNP elevations immediately after the conditioning, even in patients without pre-existing cardiac disease [16]. Roziaková et al. also demonstrated a significant increase in NT-proBNP already on day 1 after HSCT in 95% of patients [19]. This early rise in NT-proBNP may reflect myocardial injury caused by chemotherapy and/or radiotherapy administered as part of the conditioning regimen, or acute volume overload resulting from intensive hydration during conditioning. Further evaluation of the relationships among conditioning regimens, NT-proBNP levels, cardiotoxicity, and mortality may provide important prognostic information, particularly in frail patients, highlighting the need for future studies focused on individualized management strategies. The choice of conditioning regimen is largely influenced by patient comorbidities, and reduced-intensity conditioning represents an alternative for frail patients. In the cohort reported by Polomski et al., patients with NT-proBNP concentrations < 125 pg/ml were more frequently treated with myeloablative conditioning regimens, whereas non-myeloablative regimens were more commonly used in patients with higher NT-proBNP levels [20]. Similarly, patients in our cohort receiving reduced-intensity conditioning had significantly higher baseline NT-proBNP concentrations, consistent with a greater burden of pre-existing cardiac stress or comorbidity. After transplantation, however, the pattern appeared to reverse, and myeloablative conditioning was associated with significantly higher NT-proBNP concentrations on D+1 and D+7, suggesting more pronounced early hemodynamic or myocardial stress in this group. Cyclophosphamide is used in both myeloablative and non-myeloablative conditioning regimens, and its administration particularly at high doses or as post-transplant cyclophosphamide has been associated with clinically relevant cardiotoxicity. Elevated BNP levels (> 530 pg/ml) after post-transplant cyclophosphamide were significantly associated with a higher incidence of early cardiac events (63% vs. 3.9% for high versus low BNP levels, respectively) [21].

Abnormal NT-proBNP concentrations above 125 ng/l were still present in 93% of patients at D+7, 96% at D+14, and 93% at D+30, particularly among patients who developed cardiovascular events. Horáček et al. reported NT-proBNP elevations above 500 ng/l in seven patients (30.4%) on day 14 after HSCT [14]. In our previous study, we found persistent simultaneous elevations of NT-proBNP and hs-cTnT concentrations for more than 14 days after HSCT. In the present study, we confirmed persistently elevated NT-proBNP concentrations up to 180 days after HSCT. Such sustained

elevations of cardiac biomarkers may indicate reduced myocardial reserve or diminished cardiac tolerance to physiological stressors [22].

NT-proBNP elevations during the early post-transplant period can also be caused by the infusion of incompatible grafts and the use of cryopreserved grafts containing the potentially cardiotoxic cryoprotectant dimethyl sulfoxide (DMSO). Early work by Martino et al. demonstrated that major cardiac complications, including arrhythmias and blood pressure fluctuations, were associated with the total administered DMSO dose. The cardiotoxic potential of DMSO has been associated with exposure to approximately 100-150 ml of a 10% DMSO solution [23]. Despite the significantly lower DMSO volume in the grafts used in our cohort, we observed significantly higher NT-proBNP levels in patients who received cryopreserved grafts. Evidence regarding DMSO-related cardiotoxicity remains limited, as most published data consist of isolated case reports rather than systematic studies. Khawandanah et al. described DMSO-induced myocardial infarction during infusion of a cryopreserved allogeneic graft, implicating coronary vasospasm and endothelial irritation as plausible mechanisms [24]. Additional evidence from Horáček et al. showed that DMSO-preserved grafts caused significant increases in systolic and diastolic blood pressure, with the magnitude of the hemodynamic changes correlating with the administered DMSO dose, further supporting a direct vasoactive effect of DMSO on the cardiovascular system [25].

Chemotherapy-induced cardiotoxicity, which is typically associated with a non-ischemic etiology, is characterized by low-grade and persistent troponin elevations reflecting ongoing myocardial injury. These properties of troponin, together with the development of high-sensitivity assays, support its use as a biomarker for the early detection of cardiotoxicity. Because the majority of patients in our cohort had received cardiotoxic chemotherapy as part of induction or consolidation therapy, baseline high-sensitivity cardiac troponin T (hs-cTnT) levels prior to transplantation were elevated in 20% of patients. Notably, persistent low-grade elevations of hs-cTnT were observed for at least 30 days following HSCT in 28% of patients, suggesting sustained myofibrillar injury likely attributable to prior exposure to chemotherapy and/or radiotherapy. Among patients with clinically significant cardiovascular complications, we observed significantly higher hs-cTnT concentrations compared with asymptomatic patients. Comparable findings were reported by Rotz et al., who found that patients with newly diagnosed systolic dysfunction had significantly higher hs-cTnI concentrations than the control group, and that the first increase in cTnI appeared to occur on day+7 [26]. In contrast, the cohorts studied by Poręba et al. and Horáček et al. did not demonstrate any troponin elevations [14,16]. However, these findings were likely influenced by the exclusion of patients with pre-existing cardiac disease or other cardiovascular conditions that may predispose

them to cardiac complications [16]. In contrast, the cryopreserved-graft group in our cohort had a significantly higher prevalence of pre-transplant cardiovascular comorbidities, which may have contributed to the more pronounced elevations in cardiac biomarkers and the greater severity of cardiotoxicity observed after transplantation.

Comparison of clinically significant cardiovascular complications between recipients of non-cryopreserved and cryopreserved grafts revealed no statistically significant differences in the incidence of adverse cardiovascular events. However, cardiac biomarker concentrations differed significantly between the groups, with recipients of cryopreserved grafts exhibiting higher levels of both NT-proBNP and hs-cTnT. To date, no large-scale international studies have specifically evaluated cardiovascular toxicity associated with allogeneic hematopoietic stem cell transplantation using cryopreserved grafts or the potential cardiotoxic effects of DMSO.

Our study has some limitations. In some cases, cardiac biomarkers could not be assessed due to early post-transplant mortality or discharge from the hospital before day 30, after which follow-up continued at the referring hematology center. Consequently, extended follow-up was not feasible in several cases because follow-up data were no longer available.

In conclusion, persistent elevations in cardiac biomarkers may reflect reduced myocardial functional reserve or diminished cardiac tolerance to physiological stressors. DMSO-related cardiotoxicity should be considered in the differential diagnosis of cardiovascular events following hematopoietic stem cell transplantation involving cryopreserved grafts. Strategies aimed at reducing DMSO exposure without compromising graft viability, as well as increased use of non-cryopreserved stem cell products, should be considered, particularly in patients with pre-existing cardiovascular comorbidities. Further prospective studies with longer follow-up are needed to better define the incidence, underlying mechanisms, and long-term clinical consequences of cardiovascular toxicity associated with cryopreserved grafts and DMSO exposure.

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Figure Legends

Figure 1. NT proBNP concentrations in cryo vs non cryo groups before and after HSCT. The figure displays NT proBNP concentrations at baseline, D+1, D+2, D+7, D+14 and D+30. Dynamics

showed statistically significant differences across following timepoints (baseline: p=0.02; D+1: p=0.01; D+2: p=0.05; D+7: p=0.01). A statistically significant difference was observed across all measured time points ($p < 0.001$). NT proBNP increased in all patients by D+1, peaking at this time; the increase was greater in the cryopreserved graft group (cryo vs non cryo ≈ two-fold at its peak). Values declined thereafter but had not returned to baseline by D+30.

Figure 2. Hs-cTnT concentrations in cryo vs non cryo groups before and after HSCT. The figure presents hs cTnT levels pre transplant and at D+1, D+2, D+7, D+14, and D+30; early post transplant values were similar to baseline in both groups, abnormal hs cTnT peaked at D+14 and borderline elevations persisted through D+30 without return to baseline. A statistically significant difference was observed across all measured time points (p=0.001).

Figure 3. NT-proBNP values in patients with and without cardiovascular complications. Changes in NT proBNP in patients with and without cardiovascular complications at baseline, D+1, D+2, D+7, D+14 and D+30; NT proBNP rose in all patients after conditioning and HSCT, but levels were significantly higher in those with manifest cardiovascular complications, peaking at D+1 and D+2 and remaining elevated through D+30.

Figure 4. Hs-cTnT values in patients with and without cardiovascular complications. Figure 4 displays hs-cTnT values in patients with and without clinically significant cardiotoxicity at baseline, D+1, D+2, D+7, D+14 and D+30; patients with cardiotoxicity showed significantly higher hs cTnT (p=0.047), peaking at D+7 and D+14 and remaining elevated at D+30.

470 **Table 1.** Patient characteristics.

	All Patients	Cryo group	Non-Cryo group	p-value
Patients	106	67 (63.2%)	39 (36.8%)	
Gender				
Men	61	35 (57.4%)	26 (42.6%)	0.24
Women	45	32 (71.1%)	13 (28.9%)	0.0046
Diagnosis				
AML	50	30 (60%)	20 (40%)	0.15
ALL	17	11 (64.7%)	6 (35.3%)	0.22
MDS	14	11 (78.6 %)	3 (21.4 %)	0.03
Lymphoma, Myeloma	13	10 (76.9%)	3 (23.1%)	0.5
Other diagnoses	12	5 (41.7%)	7 (58.3%)	0.56
Source of blood cells				
Sibling donor	38	11 (28.9%)	27 (71.1%)	0.009
Unrelated donor	68	56 (82.4%)	12 (17.6%)	≤ 0.0001
Bone marrow	1	0	1	
Peripheral blood cells	105	67 (63.8%)	38 (36.2%)	0.004
Conditioning regimen				
Myeloablative regimen	65	41 (63.1%)	24 (36.9%)	0.03
Reduced intensity regimen	41	26 (63.4%)	15 (36.6%)	0.08
Age at the HSCT (Median-Range)	48 (16-68)	51.5 (18-68)	47 (21-67)	0.2
Total body irradiation	30	18 (60%)	12 (40)	0.27
Pre-transplant radiotherapy	9	6 (66.7%)	3 (33.3%)	0.3
Acute GvHD	36	23 (63.9%)	13 (36.1%)	0.09
Pre-transplant cardiovascular comorbidities	48	31 (64.6%)	17 (35.4%)	0.04
LVEF (%) before HSCT	64	64	65	0.44
LVEF (%) 1 month after HSCT	65	65	65	1.0

471 Notes: p-values comparing the cryopreserved and non-cryopreserved group
472 Abbreviations: AML-acute myeloblastic leukemia; ALL-acute lymphoblastic leukemia; MDS-
473 myelodysplastic syndrome; HSCT-transplantation of hematopoietic stem cells; GvHD-graft versus
474 host disease; LVEF-left ventricular ejection fraction
475

476 **Table 2.** CV complications in the cryo and non-cryo groups.

Clinical cardiotoxicity	**Group**		
	Non-cryo	Cryo	
No	37	59	96 (90.6%)
Yes	2	8	10 (9.4%)
	39 (36.8%)	67 (63.2%)	106

477

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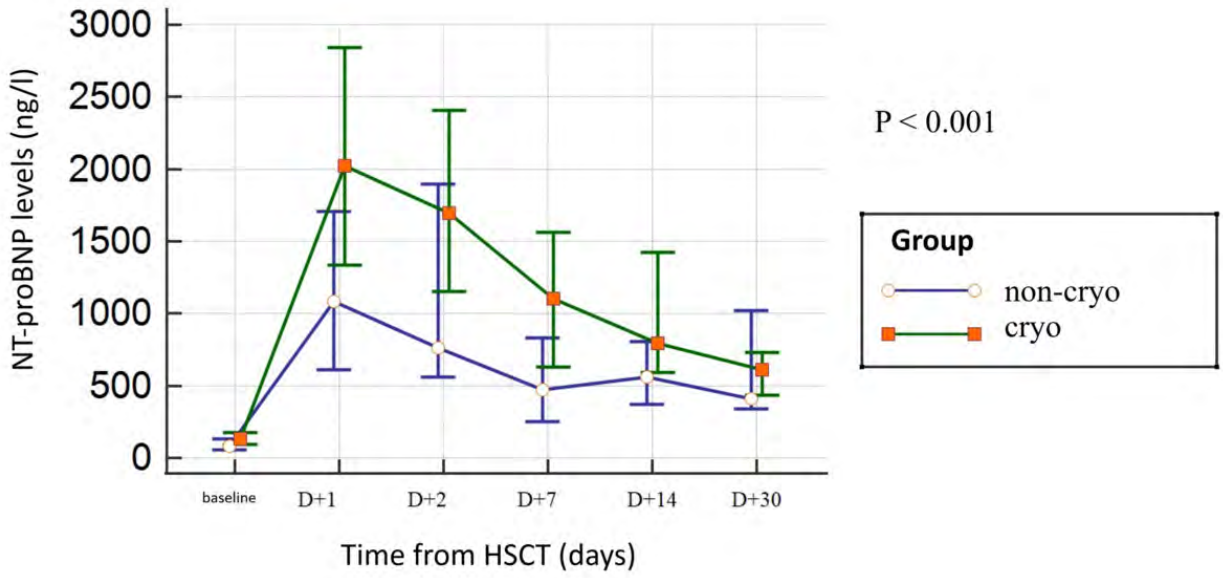

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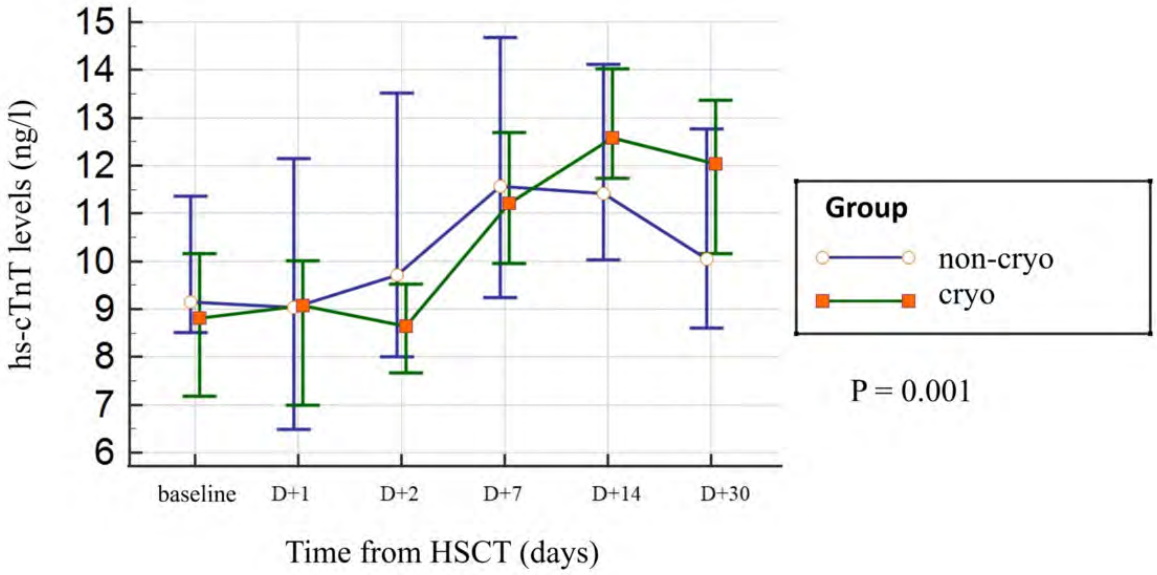

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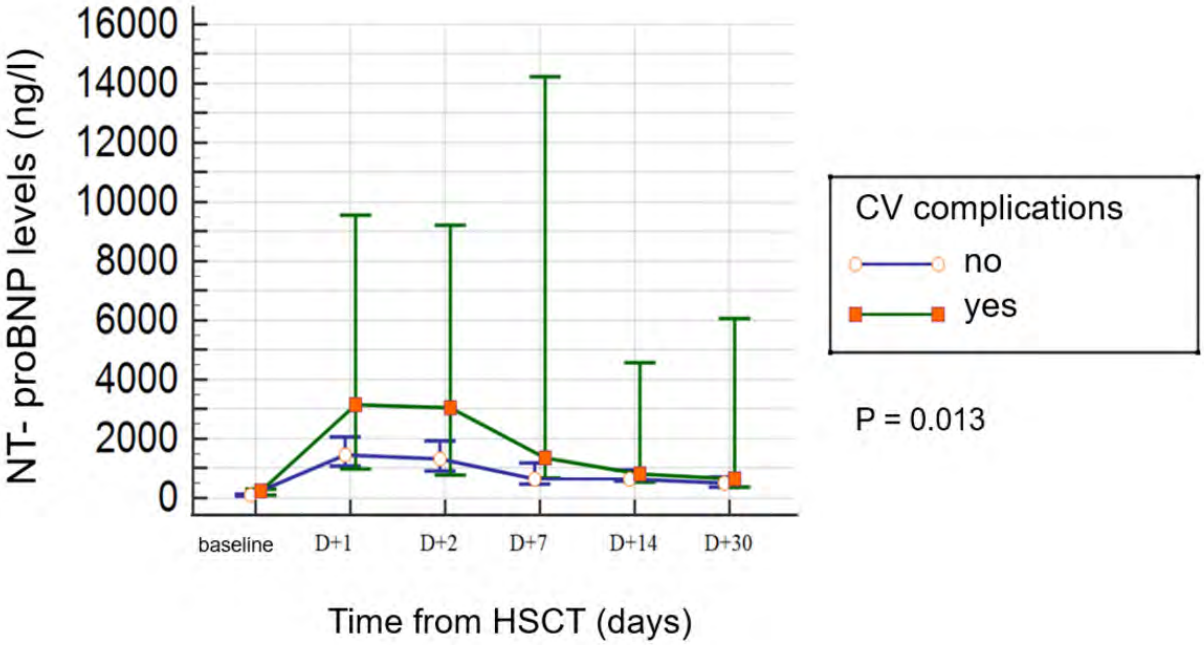

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