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Buffy coat-derived platelets cryopreserved using a new method: Results from a pivotal clinical trial on thrombocytopenic patients with acute leukaemia

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ABSTRACT

The administration of cryopreserved platelets (PLTs) may overcome the limits of platelet shortage and availability, especially during some seasons or in specific contexts like rural areas. After in vitro validation studies, ad hoc prepared buffy coat-derived pooled platelet concentrates (BC-PLTs), treated with dimethyl sulphoxide (DMSO) and cryopreserved (CRY BC-PLTs) at -80 °C with a modified Valeri method, were transfused in patients with severe thrombocytopenia secondary to chemotherapy for acute leukaemia (AL). Five inpatients were enrolled in the pivotal clinical trial NCT02032134: 4 males and 1 female with a mean age of 71 years (range: 65–80). Four patients were diagnosed with acute myeloid leukaemia and 1 had acute lymphoblastic leukaemia.Transfusion of one Unit of CRY BC-PLTs resulted effective in active bleeding control in two patients without any adverse reaction or concomitant antihaemorrhagic therapies. CRY BC-PLTs met the currently accepted criteria for cryopreserved PLTs, their transfusion in patients with AL was safe. (Clinical trial: NCT02032134).

1. Introduction

Platelet transfusion is frequently adopted for bleeding prophylaxis in patients with blood cancer or severe thrombocytopenia secondary to chemotherapies. Platelets are currently stored at room temperature (RT, 20°C-24°C) under constant agitation, they can be administered within 5 days after storage [1] according to current regulations. RT stored PLTs are administered to increase the PLT count in thrombocytopenic patients and to prevent severe bleeding. The short shelf life and agitation needed for RT PLT storage may however negatively impact their use in some contexts like military and isolated fields [2,3]. Most of the available in vivo studies on CRY-PLTs have been conducted on healthy volunteers or trauma patients needing massive transfusions. Studies on healthy volunteers showed a good viability of CRY-PLTs, even if slightly reduced compared to liquid stored PLTs [4–6]. Clinical studies on trauma patients requiring intensive transfusion support mainly in the military field have not reported major adverse events with improved overall survival and bleeding control [7]. Patients with cancer and chemotherapy related thrombocytopenia are the largest recipients of platelets; however, data on CRY-PLTs transfusion in these subjects are scant [8–10]. Only one study has been performed in haematological patients: autologous CRY-PLTs [9] treated with ThromboSol have been infused to control severe thrombocytopenia secondary to chemotherapy. From this premise, after the development and in vitro validation of a new kit for buffy coat derived platelet cryopreservation (CRY BC-PLT) with DMSO [11], we have evaluated in the pilot in vivo trial NCT02032134. the efficacy and safety of CRY BC-PLTs infused in haematological cancer patients with severe thrombocytopenia

2. Methods

After in vitro assays determination of CRY BC-PLT viability up to 9

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Table 1
Baseline characteristics of patients transfused with CRY BC-PLTs.

	1*	2	3*	4*	5
Sex	M	F	M	M	M
Age	67	77	66	65	80
Disease	AML	AML	ALL	AML	AML
Reason for PLT transfusion	Prophy	Bleed	Prophy	Bleed	Prophy
Basal PLT count (x 10^9/L)	4	7	6	6	2
PLT count (x 10^9/L) at 1 hour	8	7	21	6	3
PLT count (x 10^9/L) at 24 hours	12	4	17	6	2
CCI at 1 hour (x 10^9/L)	6800	0	5700	0	1700
CCI at 24 hours (x 10^9/L)	14400	0	18700	0	0

ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; CCI = corrected count increment; CRY BC-PLTs = cryopreserved buffy coat derived platelets; F = female; M = male; PLT = platelets; Prophy = prophylaxis; Bleed = mucosal bleeding (epistaxis). *Patients with low platelet count secondary to chemotherapy.

months after storage, CRY BC-PLT concentrates for in vivo use were prepared and handled with the same method adopted for the in vitro study [11]. A patented cryopreservation kit has been adopted to avoid one step of external manipulation of the BC-PLTs for cryopreservation. Volunteer blood donors met the requirements of the Italian transfusion centre's recommendations and took no medication known to affect PLT function for 10 days before donation. In detail, PLTs from 5 donors with the same blood group were assembled in a sterile kit and mixed with an InterSol solution (100 mL) so that at least 30% was constituted by plasma and 70% by InterSol. InterSol was adopted to wash the sacks containing buffy coats and PLT outflow by increasing the volume of supernatant for PLT suspension after centrifuge. The BC-PLTs combined with InterSol underwent soft centrifugation and, with an automated separator, were then leukoreduced with filtration. The PLT count was assayed from the BC-PLTs after leukodepletion. BC-PLTs were selected for storage only with a PLT count > 1250 × 103/μL. The final PLT concentrates were transferred to a 650-mL patented cryopreservation kit (Promedical). A total mean amount of 300 mL BC-PLTs was then transferred to one bag of the kit. After connecting a plastic bag containing 75 mL saline solution with 25% DMSO, under constant agitation, the DMSO solution was transferred to the bag containing the BC-PLTs in 5 min. After mixing with the DMSO solution, each bag underwent centrifugation (2000 *g* for 10 min). Using a manual separator, supernatant was then completely removed (leaving approximately 10 to 15 mL) and the BC-PLTs were re-suspended with plasma from 1 of the initial 5 PLT donors to a final volume of 200 mL. The BC-PLTs rested for approximately 45 min, they were then agitated for approximately 1 h to allow PLT disaggregation. CRY BC-PLTs were transfused in haematological in patients needing platelet transfusion for prophylaxis or treatment of bleeding as referred in NCT02032134. The main objectives of the trial were to evaluate the efficacy and safety of platelets, cryopreserved with DMSO by the above reported method, in patients with severe thrombocytopenia. Haematological in-patients falling into one of the following categories were eligible:: Stable clinical conditions and no complications with platelets ≤ 10 × 10^9/L; active bleeding and platelets ≤ 20 × 109/L; high blood pressure, high fever, rapid drop in platelets, infection, chemotherapy, coagulation abnormalities with platelets ≤ 20 × 10^9/L. Patients suffering from congenital immunodeficiency, Hodgkin's disease treated with purine analogues (Fludarabine, cladribine) and bone marrow transplant candidates, either recipients or donors, were excluded.

To evaluate the availability of CRY-BC PLTs after transfusion, the corrected count increment (CCI) of the platelets was determined at 1 h and 24 h' post-transfusion, respectively. CCI was determined according to the following formula: (platelet increment per ul) x (body surface area in m2)/number of platelets transfused (x 10^{11}). Pre-treatment and 24 h after treatment, whole blood from patients transfused with CRY BC-PLTs was tested for thromboelastography (TEG) and thrombin generation potential (CAT). The patients were observed for up to 7 days after infusion and the occurrence of any side effect was registered. The study was carried out in accordance with the provisions of the Declaration of Helsinki and local regulations. Informed consent was obtained from each participant. The protocol was approved by the institutional review board at our institution and recorded on ClinicalTrials.gov. Statistical analysis of quantitative and qualitative data, including descriptive statistics, was performed for all the items. Repeated measures analysis of variance (ANOVA) was adopted, sphericity test [12] to determine the equality of variances of the differences between measurements was also applied. Pairwise comparisons table was adopted to compare different measurements. Bonferroni correction for multiple comparisons was applied for P-values and confidence intervals. For P-value < 0.05, it is concluded that there is a significant difference between the different measurements. Data were analysed using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium).

3. Results

From June 2014 to January 2015, 5 inpatients were enrolled in clinical trial NCT02032134: 4 males and 1 female (Table 1). with a mean age of 71 years (range: 65–80). Four patients were diagnosed with acute myeloid leukaemia (AML) and 1 had acute lymphoblastic leukaemia (ALL). All enrolled patients received 1 unit (170–200 mL final volume) of CRY-BC PLTs: three patients were treated prophylactically for very low PLT counts (mean basal PLT count = 5 × 10^9/L, range: 2–7) secondary to chemotherapy; two patients had a very low platelet count with active mucosal bleeding (epistaxis) at the time of CRY BC-PLTs transfusion; four subjects had previusoly received, standard PLT concentrates from apheresis (mean PLT Units transfused in each patient: 6, range:3-13) with a CCI of more than 10 × 10^9 /l (calculated one hour after transfusion), after chemotherapy. Two patients, aged 65 and 67 years, affected by AML were treated in induction with cytarabine plus daunorubicin, one 77 years old patient with AML received azacitidine, one 66 years old patient with ALL was treated with cyclophosphamide and daunorubicin. All enrolled subjects were not under any concomitant systemic anti-haemorrhagic treatment (such as tranexamic acid) at the time of CRY-BC PLT transfusion. The mean residual amount of DMSO in the CRY BC-PLTs was approximately

Table 2
TEG parameters before and up to 24 h after infusion of CRY BC-PLTs.

	T0 (N = 5)	H1 (N = 5)	H4 (N = 5)	H24 (N = 5)	P value
TEG-R (Mean ± SD)	17.68 ± 1.39	17.58 ± 2.31	16.50 ± 2.53	24.39 ± 1.85	NS
TEG-K (Mean ± SD)	27.63 ± 18.71	14.02 ± 0.90	25.58 ± 20.92	27.18 ± 12.5066	NS
TEG-MA (Mean ± SD)	36.08 ± 16.16	37.20 ± 14.17	27.45 ± 17.51	30.22 ± 17.31	NS
TEG-MRTG (Mean ± SD)	11.46 ± 4.98	16.95 ± 3.42	13.53 ± 4.44	12.29 ± 4.31	NS

T0 = basal values before the infusion of 1 unit of CRY BC-PLTs; H1 = 1 h after infusion; H4 = 4 h after infusion; H24 = 24 h after infusion; TEG-MRTG = maximum rate of thrombus generation; NS: Not significant.

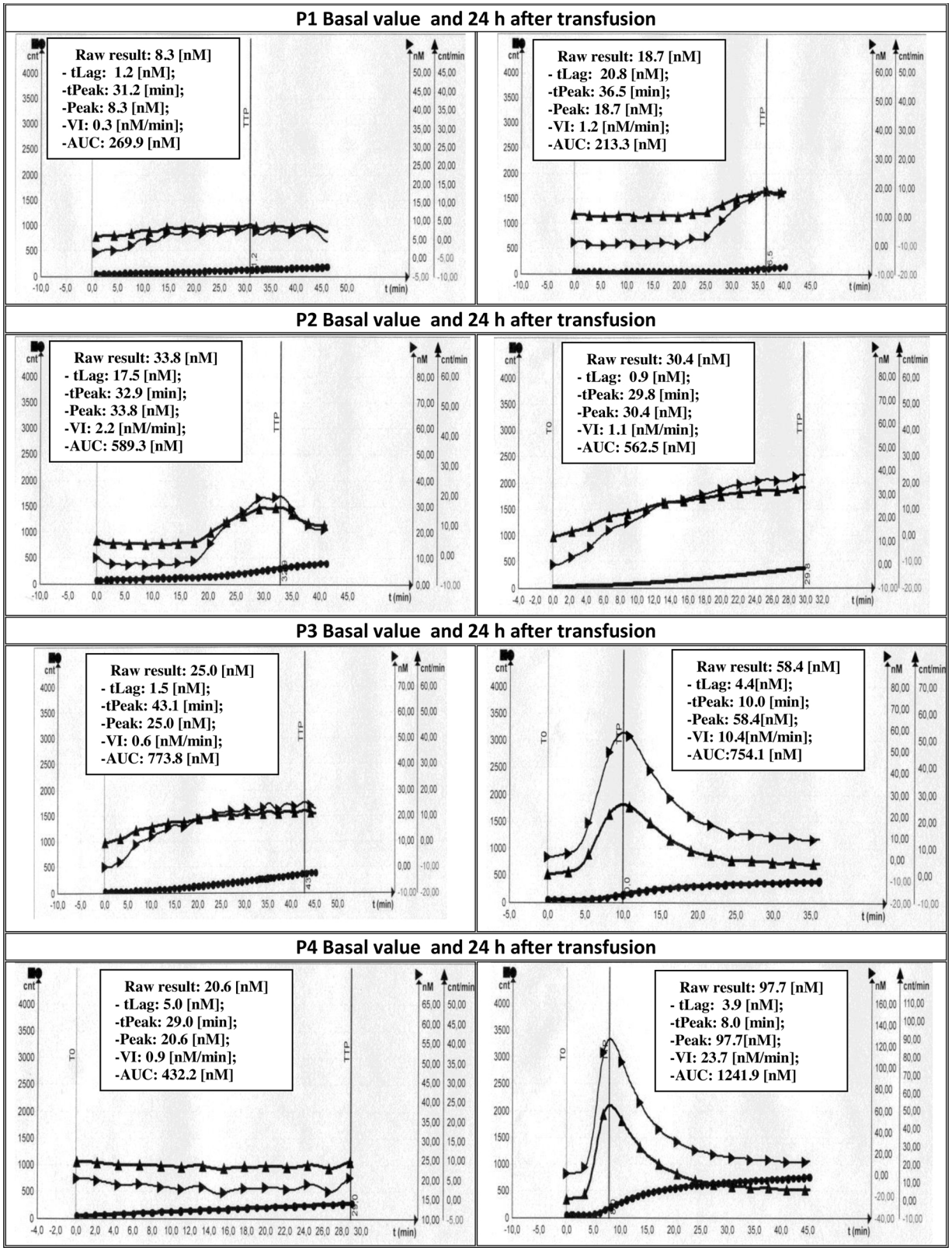

Fig. 1. Thrombin generation curves before and 24 h after CRY BC-PLTs transfusion.

400 mg per transfused unit. One patient showed a good response to the CRY BC-PLTs with an increase in the PLT count after 1 h, stable up to 24 h (Table 1). Bleeding was effectively prevented in all cases. Epistaxis ceased after CRY BC-PLT transfusion without any concomitant additional anti-haemorrhagic medication, even if thrombocytopenia was not overcome. In detail, epistaxis was completely controlled within two hours after CRY BC-PLTs transfusion, in both cases. Three patients treated with CRY BC-PLT required also, on the same day, red bood cells (RBC, 1U) transfusions for severe anemia. RBCs were given 12 h after CRY BC-PLTs in all cases. One patient transfused with CRY BC-PLT for

epistaxis, had received RBC transfusion (1U), twenty-four hours before CRY BC-PLTs. Fresh frozen plasma was not administered in any of the enrolled patients at the time of CRY BC-PLTs and during the observation period. TEG parameters, assayed on whole blood, from baseline up to 24 h after transfusion are illustrated in Table 2 (not statistically significant difference was observed among the reported parameters). Thrombin generation potential was stably maintained up to 24 h after the infusion of 1 unit of CRY-BC PLTs without any adverse effect (mean ETP pre-treatment was 414.13 ± 160.60, and 24 h after transfusion was 326.95 ± 152.54). Fig. 1 illustrates CAT results from four enrolled patients. CRY-BC PLTs could significantly decrease the time to clot formation and clot strength as measured also by thromboelastography. CRY-BC PLT activation/deterioration was accompanied by an effective haemostatic in vivo function. The primary aim of the trial was to evaluate the efficacy of CRY BC-PLTs (an increase in the platelet count after transfusion); the trial was however terminated because of further recruitment failure within the interval of available CRY BC-PLTs for the study. CRY BC-PLT transfusion did not cause any adverse reaction, acute or late.

4. Discussion

We have here transfused CRY BC PLTs in five patients with acute leukaemia, the current study could thus be considered as a pivotal trial. TEG on whole blood from patients transfused with CRY BC-PLTs was also performed. The adequate haemostasis achieved at TEG in in vivo studies supports the hypothesis that in vitro CRY BC-PLT activation/deterioration does not necessarily mirror an impaired haemostatic in vivo function. Storage-associated defects identified in vitro may be reversible upon transfusion [13]. In cases of massive or active bleeding, the haemostatic function becomes essential to allow fast blood clot formation. On a clinical point of view, haematologists on the contrary ground the transfusion management of patients with severe thrombocytopenia on the expected increase in PLTs count; platelet circulating time is the first issue usually considered when administering PLTs concentrates for prophylaxis, aiming to stably maintain a “safe” platelets count value (PLTs $> 10 \times 10^9$/L). For the above reported expected outcome, it was difficult to further enrol patients in the clinical trial NCT02032134.We must however acknowledge that universally recognised criteria for the transfusion of cryopreserved platelets are currently lacking. The wide interindividual variability of global haemostatic assay indicators and platelet characteristics might negatively affect the clinical validity of laboratory assays performed on cryopreserved products. Currently available assays have been often adopted to evaluate only specific platelets properties such as recovery or survival, but not for others such as haemostatic functions.

The post-transfusion effects of released platelet cytokines are still uncertain and have been explored mainly in emergency settings. Our clinical results, even if on a quite small number of subjects enrolled, in part mirror those observed in the context of autologous CRY-PLT administration in patients with severe thrombocytopenia secondary to high-dose chemotherapy, where CRY-PLTs directly frozen at -80 °C did not overcome thrombocytopenia [9].

In vivo recovery of cryopreserved platelets in thrombocytopenic patients was half that of fresh platelets [8]. Similarly, CRY-PLT infusions in cardiopulmonary surgery settings [14,15] showed a good control of blood loss but lower post-transfusion increase in the PLT count and impaired platelet survival. To the best of our knowledge, this is the first prospective trial to evaluate the clinical efficacy and safety of cryopreserved buffy coat-derived platelet transfusion in haematological patients. Patients enrolled in the reported trial were frail subjects with acute leukemia; however, they did not experience any immediate or late adverse events secondary to transfusion. DMSO is infact known to determine cardiac, neurological, and renal side effects [8] but CRY BC-PLT units obtained with the previously reported method presented a low final DMSO concentration, so they resulted safe and well tolerated. Our findings do not allow any firm conclusion on the administration of CRY BC-PLTs in haematological patients with acute leukaemia mainly due to the small number of enrolled patients. The efficacy and safety of CRY BC-PLTs in patients with acute leukaemia needs to be better assessed in larger prospective ad hoc studies. Feasibility of CRY-BC-PLTs also in haematological settings, besides military and trauma, may be of great support.

Author’s contribution

MN and SS conceived the study. MN was the principal investigator in the clinical trial and wrote the paper.SM and SR enrolled patients in the clinical trial. GDeF and RA collected blood from healthy volunteers and prepared platelets concentrates. AR contributed kits for cryopreservation. LL and PSA performed the coagulation assays. FD and NC performed flow cytometry assays. AD analyzed the data and performed the statistical analysis.

Declaration of Competing Interest

AR was a Promedical® employer. G DeF acted as a consultant for Promedical®. All other authors have no relevant conflicts of interest to declare.

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