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Autologous platelet transfusion in patients receiving high-dose chemotherapy and circulating progenitor cell transplantation for stage II/III breast cancer

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Abstract

Background and Objective. **Concerns about the risk of transfusion therapy are driving towards new strategies which are designed to minimize exposure to allogeneic blood products. We aimed to find out whether it is possible to support the phase of thrombocytopenia following high-dose chemotherapy (HDC) and circulating progenitor cells (CPC) transplantation by autologous platelet concentrates (PC).**

Design and Methods. **PC were collected from 32 patients undergoing HDC and CPC transplantation for stage II/III breast cancer. A single plateletpheresis was performed at rebound after high-dose cyclophosphamide, when platelet count exceeded 250×10^9/L. PC were cryopreserved in 5% DMSO after controlled-rate freezing and stored in liquid nitrogen. *In vitro* studies of cryopreserved platelets (aggregation, ATP release and change of mean platelet volume induced by EDTA) were performed. When platelet counts dropped below 20×10^9/L following HDC (thiotepa 600 mg/m², L-PAM 160 mg/m²) and CPC transplant ($CD34^+$ cells $>5\times10^6$/kg), PC were thawed in a 37°C water bath, centrifuged to remove DMSO, resuspended in autologous plasma and reinfused within one hour.**

Results. **Large quantities of platelets were harvested in all patients (median 6.6×10^{11}, range 4.8-12.2). *In vitro* studies showed preserved platelet function as compared to both fresh platelets and standard PC. Twenty-eight out of 32 patients received autologous PC. At the time of transfusion most of the patients were febrile (>38°C) and had mucositis >G2. The median number of platelets reinfused was 3.8×10^{11} (range 2.0-8.1) with a median loss during the freeze-thaw-wash procedure of 37%. Autotransfusion was able to maintain platelet count above 20×10^9/L in most patients, with a corrected count increment > 7.5 in 20 cases. Four patients required one additional allogeneic transfusion, two because of a poor increment and two due to a late-occurring epistaxis. No side effects related to PC infusion were recorded. Sixteen control patients who received the same HDC and a similar number of $CD34^+$ cells required a total of 17 allogeneic PC units (1 patient did not require platelet transfusion).**

Interpretation and Conclusions. **Our data demonstrate that large doses of autologous platelets can easily be collected and safely administered to support the period of thrombocytopenia in patients undergoing HDC and CPC transplantation. Autologous PC in these patients can abrogate the risks deriving from allogeneic platelet transfusion.**

Key words: high-dose chemotherapy, platelet transfusion, autologous, transplantation

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The use of autologous circulating progenitor cells (CPC) following high-dose chemotherapy (HDC) determines faster hematopoietic recovery than bone marrow (BM) infusion,[1] but platelet transfusions are still required in most cases for the prophylaxis and treatment of hemorrhage. The tempo of platelet engraftment, and therefore the platelet transfusion requirement, depends mainly on the amount of CPC transplanted and is faster when more than 5×10^6 $CD34^+$ cells/kg body weight are reinfused.[2] The main problems associated with platelet transfusion are refractoriness due to alloimmunization against platelet-specific and/or HLA class I antigens, the risk of transmitting infections (CMV, EBV, HTLV, HCV, HBV), transfusion-associated graft-versus host disease (GvHD) and febrile non-hemolytic reactions.[3-7] Transfusion of leukodepleted, HLA-matched platelets reduces (but does not eliminate) the risk of both refractoriness and viral infection,[8,9] but this strategy is expensive, filtration is not entirely effective because of platelet loss and cytokine activation, and HLA-matched platelets are not always available. Ideally, the transfusion of autologous platelets is the best option for avoiding alloimmunization and infection. This approach has been successfully employed in alloimmunized patients treated for acute leukemia.[10,11] However, in such cas-

es support with blood transfusions is necessary for several periods of treatment-induced BM aplasia, which can last two-three weeks. This makes full support of the thrombocytopenic phases with autologous PC hard to get.

This policy can, however, be considered in patients with solid tumors or lymphomas receiving HDC and autologous CPC transplantation, in whom platelet recovery is rapid and platelet transfusion requirement is often limited.

We recently reported that platelet concentrates (PC) can be collected from the stem cell harvest, stored and safely reinfused in patients receiving HDC and CPC support.[12] In the light of these results a new PC collection and transfusion protocol was performed in 32 additional patients undergoing high-dose sequential (HDS) adjuvant chemotherapy[13] for high-risk breast cancer (BC). Large quantities of platelets were collected through a single plateletpheresis, taking advantage of the thrombocytosis which follows recovery after high-dose cyclophosphamide (HDCY) administration. This report gives the results of the in vitro studies performed on cryopreserved platelets as well as the clinical outcome of the patients to whom PC were transfused.

Materials and Methods

Patients

Thirty-two consecutive women receiving HDC and CPC transplantation for stage II-III high-risk breast cancer entered the study. None showed histologic evidence of neoplastic bone marrow involvement or hypocellular marrow at the time of chemotherapy administration. All patients underwent HDS chemotherapy[13] which included HDCY (7 g/m^2) plus G-CSF support, high-dose methotrexate (HDMTX, 8 g/m^2) with leucovorin rescue plus vincristine (1.5 mg/m^2), two epirubicin doses (120 mg/m^2) and, as the final myeloablative regimen requiring CPC support, thiotepa (600 mg/m^2) and L-PAM (160 mg/m^2). More than 5×10^6 CD34$^+$ cells, collected following HDCY and G-CSF administration, were reinfused in all patients 24 hours after the completion of HDC.

As a control group we considered 16 high-risk breast cancer patients who were treated consecutively in the same Institution just before the beginning of the present study according to the same protocol but who were supported with allogeneic PC.

Platelet transfusions were given on a prophylactic basis during the period of bone marrow aplasia following HDC when the platelet count dropped below 20×10^9/L or in the presence of bleeding episodes.

Relevant patient characteristics are shown in Table 1. No patient had received previous blood transfusions. Written informed consent was obtained from all patients before participating in the study.

Table 1. Patient characteristics.

	Study group	*Control group*
Number	32	16
Age, yrs (median, range)	47 (31-56)	44 (33-52)
Previous chemotherapy*	3	2
Chest wall irradiation	none	none
CD34$^+$ cells reinfused (x10^6/kg bw); (median, range)	7.3 (5.1-10)	7.6 (5.2-12.1)
Days to reach platelet count > 50x10^9/L after transplantation (median, range)	11 (11-15)	11 (10-14)

**Neoadjuvant epirubicin (120 mg/m^2) for 3 cycles.*

Platelet collection and storage

A single plateletpheresis was performed by Cobe Spectra and CS 3000 Plus Baxter cell separators in all patients at rebound after HDCY, when circulating platelets exceeded 250×10^9/L. To maximize platelet yields we took advantage of the thrombocytosis that frequently follows recovery from profound BM aplasia. Harvesting was performed by processing 1-1.5 blood volumes within 90 minutes time, with a blood flow rate of 50-75 mL/min and an ACD-A/blood flow rate ratio of 1:9 or 1:11. Supplementary autologous plasma was collected during the procedure and was used for preparing the cryopreserving solution and resuspending platelets after thawing. After collection, PC were maintained at 20±2°C for 1 hour before manipulation. PC were transferred without further processing to a 200 mL Teflon freezing bag (NPBI, Emmer-Compascuum, The Netherlands) and the cryopreserving solution (DMSO in autologous plasma) was slowly added over 15-20 minutes to ensure a final concentration of 5% DMSO (Sigma, St Louis, MO, USA). Each bag, containing a maximum volume of 200 mL, was frozen with a computer-controlled cryopreservation device (Icecube 1600, Sy-Lab, Austria) and stored in liquid nitrogen.

Platelet concentration before freezing ranged between 1.2 and 2.76×10^9/mL (median 1.84). When required, control patients received allogeneic platelet transfusion from a single donor collected by the same device and gamma irradiated with a dose of 25 Gy as recommended by the FDA.[14]

Platelet transfusion

When required cryopreserved platelets were rapidly thawed by immersion in a 37°C water bath and centrifuged at 2500 rpm for 12 minutes to remove DMSO upon addition of 10% ACD-A to prevent clumping. The PC was then resuspended in a transfer bag (Terumo, Tokyo, Japan) with approximately 100-150 mL autologous plasma and reinfused within one hour of thawing using a standard tranfusion device over 15-30 minutes. The total platelet count of the final bag was performed and compared with the total number of platelets before freezing.

Table 2. *In vitro* characteristics of platelets from autologous cryopreserved PC (n=25) and allogeneic PC stored at 20±2°C for two-three days (n=17). For comparison, the results obtained with fresh platelets are reported. Results are given as mean±SD.

	Autologous PC	*Allogeneic PC*	*Fresh platelets*
Maximal extent of aggregation (%) by			
ADP 10 µM	11.8±9.6	25.5±24.8*	49.6±26.7**
Collagen 10 µg/mL	41.1±26.3	57.5±27.0	77.6±20.5**
Collagen 10 µg/mL + epinephrine 10 µM	52.1±21.9	76.4±17.4**	84.4±10.1**
Ristocetin 1.5 mg/mL	47.0±27.0	61.9±30.5	84.9±20.3**
ATP release (µM) by			
Collagen 10 µg/mL + epinephrine 10 µM	0.87±0.58	1.53±0.72**	2.39±1.26**
MPV (fL)			
MPV-EDTA	8.49±0.64	8.16±0.99	7.76±0.48*
MPV+EDTA	8.72±0.61	8.38±1.00	8.04±0.34*
dMPV	0.23±0.19	0.24±0.17	0.28±0.15

**p<0.05, **p<0.01 with respect to autologous PC.*

Platelet counts were obtained before and one hour after transfusion and every day thereafter. Post-transfusion results were expressed as corrected count increment (CCI).[10] Clinical factors of higher platelet consumption (fever, mucositis, etc.) were recorded.

In vitro studies

To test the quality of cryopreserved PC *in vitro*, we studied platelet aggregation and ATP release induced by single and paired agonists, and changes in mean platelet volume (dMPV) induced by EDTA. For comparison, the same studies were performed on fresh platelets and on irradiated PC obtained by apheresis and stored at 20±2°C for two-three days.

Platelet aggregation and ATP release were studied in platelet rich plasma (PRP) according to the method of Born and the luciferase-luciferin procedure, respectively, as previously reported.[15] Briefly, platelets from PC were centrifuged to pellet at 350 g for 15 min. and resuspended in citrated platelet poor plasma (PPP) to a final concentration of 250×10^9/L. For the study of fresh platelets, PRP was obtained by centrifugation at 250 g×10 min. of ACD-A anticoagulated blood from 20 healthy volunteers; platelets were then pelletted and resuspended in autologous citrated PPP (250×10^6/L, final concentration). After an undisturbed 30-minutes period, platelet aggregation and ATP release were simultaneously studied in a Lumi-Aggregometer (Chrono-Log Corporation, Havertown, PA, USA). Platelet aggregation was investigated after stimulation with collagen (Mascia Brunelli, Milan, Italy), ADP and ristocetin (both from Sigma), and coupled agonists [epinephrine (Mascia Brunelli) + collagen]; ATP release was investigated after the addition of coupled agonists.

For the evaluation of dMPV,[16] ACD-A anticoagulated PRP samples containing 100×10^9/L fresh or PC platelets were incubated either in the presence or absence of 4.8 mM K2-EDTA (final concentration) at room temperature for 1 hour and analyzed in a Sysmex NE-1500 cell counter (Dasit, Milan, Italy). The difference between the MPV of the sample in EDTA and that in the plain tube was calculated.

Data analysis

Statistical comparisons were performed with Statview (Abacus Concepts, Berkeley, CA, USA) software run on a Macintosh SE Personal Computer (Apple, Cupertino, CA, USA), and the ANOVA, Scheffé and Student's t-tests were used. Values of *p* lower than 0.05 were considered statistically significant.

Results

Platelet collection

All patients achieved a platelet count greater than 250×10^9/L and underwent a single plateletpheresis at a median of 21 days (range 17-30) after HDCY. Before platelet collection 3 patients were also given HDMTX plus vincristine which is the second step of the HDS chemotherapy program.

On the day of harvesting the platelet count ranged between 251 and 582×10^9/L (median 365). The median number of platelets collected per procedure was 6.6×10^{11} (range 4.8-12.2).

In vitro studies

Table 2 shows the results of *in vitro* quality tests of cryopreserved PC and compares them to the results from fresh platelets and PC stored at 20±2°C for two-three days. While the maximal extent of aggregation after a weak agonist (ADP) was much lower in cryopreserved platelets than in fresh ones and traditional PC, clumping of cryopreserved platelets after ristocetin, a strong agonist (collagen) and paired agonists was greater than 50% that of controls. Cryopreserved platelets were still able to undergo a release reaction, although the amount of ATP

Table 3. Summary of clinical results; only patients receiving platelet transfusion are reported.

	Study group	Control group
Number of patients	28	15
Platelet count before transfusion* ($x10^9$/L)	16 (8-19)	17 (10-19)
Platelet count after 1 hour ($x10^9$/L)*/**	36 (17-61)	50 (24-63)
Platelet count after 24 hours ($x10^9$/L)*	30 (17-91)	37 (20-58)
CCI at 1 hour post-transfusion* ($x10^9$/L)*	8.7 (2.4-19)	13.3 (4-16)
Patients achieving CCI > 7.5x10^9/L	20 (71%)	12 (80%)
Allogeneic transfusions	4	17
#patients having mucositis > G2	25/28	13/15
#patients having fever > 38°C	23/28	11/15

**Median (range); **p=0.062.*

released was low.

Platelet size was larger in cryopreserved platelets than in control samples but the dMPV induced by exposure to EDTA was very low in all cases, indicating good platelet quality.

Clinical results

Table 3 and Figure 1 summarize the clinical results of our study. At the time of transfusion most of the patients were febrile (>38°C) and had mucositis > G2. No side effects related to autologous PC infusion were recorded. Twenty-eight patients received their autologous PC. Two patients did not require platelet support (platelet nadir=22 and 25×10^9/L, respectively) and two were not transfused, because of the detection of CK19 mRNA in the apheretic product by RT-PCR.[17] This unexpected result could reflect either the presence of neoplastic epithelial cells or, more likely, a lack of specificity of our detection method since 3/25 plateletpheresis from normal donors were also found to be CK19 positive. However, at the time of analysis, we could not rule out the possibility of even a minimal risk of tumor cell contamination and decided not to retransfuse the two positive PC. A subsequent analysis, using a more sensitive approach developed in our laboratory[18] showed that none of the collected PC were CK19 positive.

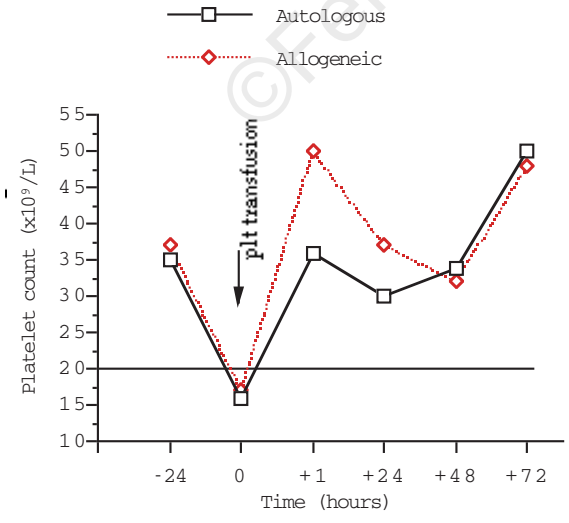

Figure 1. Median platelet count before and after autologous (n=28, straight line) or allogeneic (n=15, dotted line) platelet transfusion.

The median number of autologous platelets reinfused was 3.8×10^{11} (range 2.0-8.1), with a median loss during the freeze-thaw-wash procedure of 37%. All patients receiving autologous PC showed an increment in platelet count at 1 and 24 hours with a CCI >7.5 at 1 hour in 20. Hemorrhage was not recorded in any patient. Four patients required an additional allogeneic transfusion, two for epistaxis (one related to a pre-existing nasal varix) occurring 24 and 48 hours after autologous transfusion, when the platelet count was greater than 20×10^9/L and two because of a poor increment in platelet count (plt <20×10^9/L 1 hour post-transfusion).

Among the 16 control patients, one was not transfused (platelet nadir: 27×10^9/L) and 15 required a total of 17 allogeneic platelet transfusions (median number of platelets/transfusion: 3.9×10^{11}). Platelet counts before and after transfusion are shown in Figure 1. One patient was suffering from mild epistaxis which partially resolved after transfusion. Three patients in the control group showed poor response to platelet transfusion (CCI < 7.5). Anti-HLA or platelet antibodies, that may have contributed to refractoriness in these patients, were not investigated. However, it has to be taken into account that all patients receiving high-dose L-PAM and thiotepa have detrimental factors (in particular severe mucositis) which can per se account for the poor response to platelet transfusion in some cases.

Discussion

Although hematopoietic reconstitution after HDC is most rapid when support with CPC is given, there is still a delay in reaching safe platelet levels. Patients with severe thrombocytopenia consequent to myelosuppressive therapy may benefit from prophylactic platelet transfusion, even if there is no universal consensus about a platelet threshold for prophylactic transfusion.[19,20] Hemorrhagic episodes are more frequent in intensively treated patients who show a rapid fall in platelet count, especially when other detrimental factors such as fever and mucositis are present.[20,21]

Because patients receiving high-dose thiotepa and L-PAM are likely to have all the above mentioned features, we considered the 20×10^9/L trigger value to be reasonable for prophylactic platelet transfusion in such patients.

The use of allogeneic PC contributes to reducing morbidity and mortality from hemorrhage in these patients but, despite major advances in recent years, this practice still carries the risk of alloimmunization,[8,9,22] transmission of infectious diseases,[7,23,24] non-hemolytic febrile reactions[5] and graft-versus-host disease.[3] Furthermore, prevention of alloimmunization by centrifugation or filtration is associated with platelet loss and, occasionally, severe reactions.[6,25]

In this study we report for the first time the possibility of supporting, in a high percentage of patients undergoing CPC transplantation for high-risk breast cancer, the phase of thrombocytopenia following HDC with previously collected and stored autologous PC. Patients receiving HDC and support with adequate amounts of hematopoietic progenitors, namely more than 5×10^6 CD34-positive cells/kg bw, are ideal candidates for this approach. In fact, the thrombocytopenic phase following myelosuppression is short, in most cases one allogeneic PC unit from a single donor is needed, and blood components other than platelets are not frequently required.

Taking advantage of the platelet rebound after myelosuppressive chemotherapy we were able to harvest adequate amounts of platelets from all patients with a single apheresis procedure that was exactly the same as that performed in normal donors.

Significant platelet loss during the freeze-thaw-wash procedure was documented in most patients. This disappointing observation, which, however, did not compromize the clinical results of the study, could partially be related to the concentration of frozen platelets[26,27] since in our study group platelet loss was more evident when this amount was higher than 2×10^9/mL (data not shown).

The quality of cryopreserved PC was assessed *in vitro* by different established tests.[16] The scanty platelet aggregation seen following a weak agonist (ADP) and the severely reduced release of ATP seem to indicate platelet lesions due to cryopreservation. However, cryopreserved platelets still retained an ability that was more than 50% that of both fresh platelets and traditional PC to aggregate after strong and paired agonists. Moreover, platelet agglutination response to ristocetin was consistent with good preservation of the membrane glycoprotein Ib, which is known to play an essential role in platelet adhesion to the subendothelium. The good quality of the cryopreserved platelets was also indicated by the slight increase in MPV after exposure to EDTA. Taken together, these results indicate that the cryopreserved platelets exhibited the well known *storage-lesion* deriving from previous *in vitro* activation,[28] but they also suggest that the machinery for platelet adhesion and aggregation was still operative. Furthermore, the good *in vitro* quality of cryopreseved platelets as compared with that of standard apheresis PC was confirmed by morphologic profile, pH level and evaluation of hypotonic shock response (data not shown).

Concerning the *in vivo* effectiveness of the PC, an adequate post-transfusion response requires a CCI greater than 7.5×10^9/L 1 hour after transfusion. In our study this postulate was fulfilled by 71% and 80 % of the autologous and allogeneic platelet transfusions, respectively. The mean CCI 1 hour after autologous transfusions was 35% lower than the increments achieved with fresh allogeneic PC; however, autologous PC were able to avoid the need for allogeneic transfusion in the vast majority of our patients.

In conclusion, cryopreserved autologous PC produced increments in platelet count similar to those seen with allogeneic transfusion and there were no serious bleeding episodes in the patients treated. The advantages of using autologous blood products are the reduction of transfusion-associated risks and the possibility of alleviating the logistical problems imposed by fluctuations in platelet supply and demand. We suggest, therefore, that this approach should be made available for all patients undergoing HDC and CPC support, and not only for alloimmunized ones as was previously recommended.[11,26] Platelet rebound after cytotoxic treatment can be exploited to obtain large PC collections. Further improvement in platelet autotransfusion is expected in the near future, when the availability of thrombopoietin[29] and new cryopreservants[30] in clinical practice will favor maximal platelet harvest and allow a higher retention of functional activity.

Contributions and Acknowledgments

PP was the principal investigator and formulated the design of the study with CP. Both took part in the assessment of patients. PN did the platelet functional studies. GADP, PPr and LP followed the patients clinically. CZ and NG were responsible for platelet processing and storage MB did the PCR analysis on the apheresis products. LT and CP performed the plateletphereses. CLB, LS and GRDC supervised the whole study and participated in writing the paper.

The order in which the names appear is based on the conceptual contribution given to the paper and the time spent for performing experiments, looking after the patients and interpretating the data. The first author gave a major contribution for carrying on the study while the last author is the chief of the institution where most of the work was performed.

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Disclosures

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