Ann Hematol (2002) 81:454–461
DOI 10.1007/s00277-002-0494-z

ORIGINAL ARTICLE

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Platelet flow cytometric findings in patients undergoing conditioning therapy for allogeneic hematopoietic stem cell transplantation

Received: 20 March 2002 / Accepted: 3 June 2002 / Published online: 10 August 2002

Abstract The conditioning regimen preceding hematopoietic stem cell transplantation (HSCT) causes a rapid decrease in the platelet count and signs of disseminated intravascular coagulation, possibly indicating platelet activation. As impacts during the conditioning regimen may predict later transplantation-associated complications, we investigated changes in platelet membrane glycoproteins (GP) and the liberation of microparticles. Platelet receptors and granules of 49 patients undergoing HSCT were evaluated by flow cytometric analysis before and after the different phases of the conditioning regimen [chemotherapy, total body irradiation (TBI), therapy with antithymocyte globulin (ATG)] and final transplantation. Following chemotherapy a high surface expression of CD62P, a low mepacrine staining, and a reduced surface expression of CD42b (part of the GP Ib/V/IX complex) were found, indicating an irreversible activation of platelets. In addition, elevated levels of circulating microparticles were observed, which may reinforce the thrombosis risk in these patients. Treatment with ATG leads to an elevated surface expression of PAC-1 epitopes, which are neoepitopes appearing after activation of GP IIb/IIIa. However, a significant degranulation was not detectable, which may be the consequence of inhibitory influences on platelets during ATG-induced cytokine release syndrome. TBI and transplantation itself had no influence on platelets. This study was able to demonstrate activating effects on platelets by certain phases of the conditioning regimen in patients receiving HSCT. Chemotherapy, in particular, leads to a strong and irreversible platelet activation and a generation of microparticles, which may cause an increased thrombosis risk. Our findings underline the impact of platelets on the pathogenesis of hemostatic complications during HSCT.

Keywords Platelets · Transplantation · Conditioning regimen · Microparticles

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Introduction

During allogeneic hematopoietic stem cell transplantation (HSCT), pathological changes in platelet hemostasis are common and may strongly influence the course of transplantation. A long period of thrombocytopenia is observed that requires regular platelet transfusions and may cause severe hemorrhaging [23]. Alterations of platelet function have also been observed in HSCT patients, especially during the post-transplant period [29]. In addition, hepatic veno-occlusive disease (VOD) and microangiopathic hemolytic anemia (MAHA), both life-threatening complications of HSCT, are regularly accompanied by a severe and transfusion-resistant thrombocytopenia [11, 30], which is independent from known clinical and blood bank factors normally influencing the success of platelet transfusions. These phenomena have not been clearly explained so far and seem to be due to an increased platelet sequestration in the circulation. Therefore, an involvement of an altered platelet function in the pathogenesis of these disorders has been discussed.

Platelet function is strongly dependent on glycoproteins (GPs) exposed on the surface of activated platelets [7, 9]. The GP IIb/IIIa complex is the inducible fibrinogen receptor on platelets and plays a central role in platelet aggregation. The GP Ib/V/IX complex is the von Willebrand's factor receptor responsible for adhesion of platelets to a damaged vessel wall, whereas the glycoprotein complex Ia/IIa (GP Ia/IIa) is a major collagen receptor on platelets [32]. During aggregation platelets degranulate and express multiple granule-stored

GPs on their surface that mediate platelet interactions with other vascular cells including leukocytes and the endothelium. Following intensive activation, platelets liberate minute membrane vesicles. These microparticles can be detected in the circulation where they may play a role in the hemostatic response to vascular injury by providing a large phospholipid surface serving as a substrate for plasmatic coagulation [39]. These particles are present in various clinical situations associated with platelet activation [14, 19, 38]. Following platelet concentrate storage, aging platelets may also form microparticles, which may contribute to the hemostatic effect of transfused platelets.

The conditioning regimen irreversibly damages hematopoiesis of the transplant recipient and causes a rapid fall in platelet count. Especially antithymocyte globulin (ATG), which is used as prophylaxis for graft-versus-host disease (GVHD), may cause an intense cytokine release with signs of therapy-induced disseminated intravascular coagulation [28], possibly indicating platelet activation. As impacts during the conditioning regimen may predict later transplantation-associated complications [12], we evaluated changes in membrane GPs on circulating platelets and the liberation of microparticles following different phases of the conditioning regimen.

Material and methods

Patients

Between January 2000 and July 2001, a random series of 49 patients who had undergone conditioning therapy for allogeneic hematopoietic stem cell (HSCT) transplantation were evaluated. None of the patients had any bacterial, fungal, or viral infections within 2 months prior to the beginning of the study and during the course of the study, nor did any have implanted stents, a history of antirheumatic medication, or active promyelocytic leukemia. No patient was refractory to platelet transfusions during the conditioning therapy (defined as an increase of platelet count by less than 20×10^9/l 1 h after transfusion of a platelet concentrate) or had developed platelet antibodies (HLA/HPA) during the study. All patients had a central line (Hickman catheter) inserted 1 week prior to the start of the conditioning therapy. The patient and transplant characteristics are given in Table 1. At the beginning of conditioning therapy, 26 (53.1%) of the patients had a platelet count over 100×10^9/l, 16 (32.6%) had a platelet count between 100×10^9/l and 20×10^9/l, and 7 (14.3%) patients had a platelet count below 20×10^9/l.

Medication

During conditioning therapy all patients were on orally administered acyclovir (4×400 mg) and ranitidine (1×300 mg). All patients received unfractionated heparin intravenously (daily dose 6000 IU) and, depending on the fluid balance, furosemide (daily dose 0–120 mg). Prior to chemotherapy or irradiation therapy, ondansetron (8 mg) was applied intravenously. Before and 6 h after the application of antithymocyte globulin (ATG, Fresenius Haemocare, Germany), 250 mg prednisolone were given intravenously. Along with cyclophosphamide, a similar dose of mesna was infused continuously. Parallel to busulfan therapy patients received oral doses of diazepam (10 mg).

Table 1 Demographic and transplantation data of the study population. If not stated otherwise, numbers give cases (percentage). *FLAMSA* intravenous fludarabine 30 mg/m^2 for 4 days in parallel with cytarabine 2000 mg/m^2 for 4 days and amsacrine 100 mg/m^2 for 4 days, *TBI* total body irradiation fractionated with 4 Gy on 1–3 consecutive days at a dose rate of 4.5 cGy/min, *Cy/ATG* intravenous cyclophosphamide 50 mg/kg for 3 days in parallel with intravenous ATG 10–30 mg/kg for 4 days, *Flu* intravenous fludarabine 30 mg/m^2 for 4 days, *Bu* orally administered busulfan 4 mg/kg for 4 consecutive days

n	Patients 49
Gender (male/female)	32/17
Age [median years (range)]	49 (20–68)
Underlying disease	
Chronic myelogenous leukemia	12
Acute myelogenous leukemia	14
Acute lymphatic leukemia	5
Non-Hodgkin's lymphoma	11
Myelodysplastic syndrome	2
Osteomyelofibrosis	1
Severe aplastic anemia	4
Conditioning regimen	
FLAMSA-TBI-Cy/ATG	16 (32.7%)
Flu-TBI Cy/ATG	16 (32.6%)
TBI-Cy/ATG	6 (12.2%)
Cy/ATG	5 (8.2%)
Busulfan	6 (12.2%)

Platelet concentrates

Transfusion of platelet concentrates (no HLA match) was routinely performed if the platelet count was below 20×10^9/l. These concentrates were obtained from the peripheral blood of single donors with a Cobe Spectra LRS-System (Cobe BCT Inc., Lakewood, Colo., USA) and stored at 18°C on a shaker for a mean time of 2 days (range: 0–4 days). Before transfusion, the concentrates were irradiated with 30 Gy.

Laboratory and clinical parameters

Laboratory investigations included a routine platelet count (H 6000 analyzer, Coulter, Fullerton, Calif., USA) and platelet flow cytometry. Platelet flow cytometry is an in vitro technique, which allows analysis of surface epitopes on platelets with specific fluorochrome-tagged antibodies [20]. The method used was based on that of the European Working Group on Clinical Cell Analysis [32] with additional in vitro stimulation of platelets to gain information on the platelet function. In addition to laboratory parameters, maximum body temperature and the number of platelet transfusions needed during the therapy block were registered.

Sampling times

The investigations were performed on the 1st day of the different blocks of conditioning therapy (chemotherapy, total body irradiation, ATG therapy, transplantation) and on the following day after completion of the block (Fig. 1). The first chemotherapy block was omitted from the conditioning regimens of 28 (57.1%) patients; 6 (12.2%) patients had only chemotherapy. Blood samples were taken between 6 and 8 a.m. in order to maximize the time difference to that of the platelet transfusions, which were regularly administered in the afternoon.

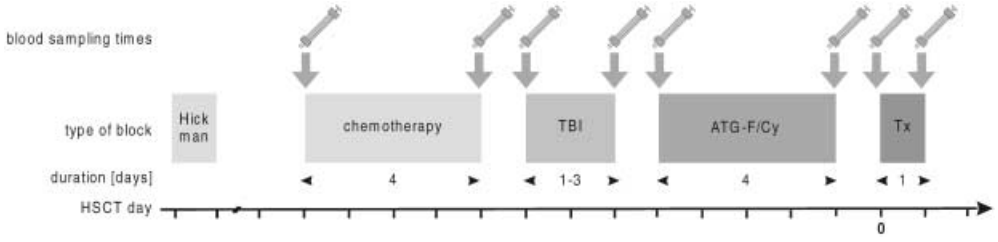

Fig. 1 Time points of blood sampling and duration of the particular conditioning blocks. Blood was taken in the morning immediately before/after the conditioning blocks. The first chemotherapy block was omitted from the conditioning regimens of 28 (57.1%) patients; 6 (12.2%) patients had only chemotherapy. In addition, the duration of the distinctive blocks is given. The Hickman catheter was implanted about 1 week before start of the conditioning regimen. *TBI* total body irradiation (4–12 Gy), *ATG-F/Cy* therapy with ATG and cyclophosphamide, *Tx* transplantation

Blood sampling and processing

Citrated blood was collected from the patients in 4.3-ml Primavette S coagulation tubes (Kabe Labortechnik, Nuembrecht-Elsenroth, Germany). To avoid platelet activation by shear stress during blood sampling, the blood was taken from the cubital vein using a cuff pressure of 40 mmHg with a 21-gauge cannula thoroughly avoiding negative pressure in the tube. Immediately after sampling, the blood was processed in the cytometry laboratory. Samples were diluted to a platelet density of 10×10^9/l with warm phosphate-buffered saline (pH 7.4, 37 C). After standardized incubation (5 min at 37 C) with the specific, fluorescein-tagged primary antibody and a phycoerythrin-tagged CD41-antibody (P2, 5 µg/ml, Immunotech, Hamburg, Germany) for platelet identification, the reaction was stopped by the addition of 2 ml paraformaldehyde (2% solution, pH 7.4) and the probe was scanned immediately in a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with a 15-mW argon ion laser. By electronic gating, 30,000 CD41-positive single platelets were analyzed per sample using CellQuest software (Becton Dickinson, Heidelberg, Germany). Electronic gating was performed to exclude large CD41-positive events, which are typical for platelet-leukocyte and platelet-platelet aggregates. To validate the gating procedure, in each sample an additional staining with fluorescein isothiocyanate (FITC)-labeled CD45 (ALB12, FITC-labeled, 5 µg/ml) was performed.

Specific antibodies and in vitro stimulation

Fluorescein-tagged antibodies against the following antigens were used (all Immunotech, Hamburg, Germany): CD42b (SZ2, 7.5 µg/ml), CD49b (GI9, 4 µg/ml), CD62P (CLB-thromb/6, 5 µg/ml), CD63 (CLB-gran12, 5 µg/ml), CD29 (Gi9, 10 µg/ml). In addition, an antibody against a neoepitope appearing after activation of the GP IIb/IIIa receptor (PAC-1, clone SP-2, Becton Dickinson, Heidelberg, Germany, 10 µg/ml) was used. For measurement of platelet reactivity, some samples were additionally stimulated with adenosine diphosphate (ADP) (CD62P: 8 µM, CD63: 18 µM) for 10 min at 37 C immediately before incubation with the antibodies. The reaction was stopped immediately after antibody staining by adding paraformaldehyde.

Mepacrine staining

Dense bodies were quantified by mepacrine staining (5 µM, Sigma, Deisenhofen, Germany) for 30 min at 37 C in the dark. For identification of platelets, the tube additionally contained the phycoerythrin-tagged CD41 antibody (P2, 5 µg/ml, Immunotech, Hamburg, Germany). At the end of the incubation time, platelets were fixed with 2 ml paraformaldehyde (2% solution, pH 7.4) and immediately scanned in the flow cytometer. For measurement of the degranulation reaction of dense bodies, the sample was additionally incubated with the hexapeptide thrombin receptor-activating peptide (TRAP, 20 µM) for 10 min at 37 C immediately before mepacrine staining.

Microparticles

The CD41-positive microparticles were distinguished from intact normal platelets on the basis of their size as described by Abrams et al. [1]. Double staining technique with CD41 enabled an exclusion of endothelial or red blood cell fragments from the analysis. To quantify and discriminate between platelets and microparticles, the limit was set at the lower border of the forward scatter profile of a normal platelet population, which is between the first and second quartile of a four-decade logarithmic forward scatter scale. The number of the microparticles was expressed as the percentage of particles below this limit to the total number of CD41-positive fluorescent particles counted (i.e., platelets plus microparticles).

Quality controls

The instrument settings were gauged daily by use of standard beads (Quantum 24, FCSC, Fishers, Ind., USA). This enables standardized results independent from a possible long-term instrument drift and makes it possible to express the fluorescence intensities in absolute values [standard units: molecules of equivalent soluble fluorochrome (MESF)] except for the mepacrine staining [arbitrary units (AU)]. All data were collected using four-decade logarithmic amplification. To calibrate size measurement of microparticles, a daily calibration was made by running fluorescent-labeled beads of known size (Flow-Check, Coulter, Fullerton, Calif., USA) and adjusting the gain such that the 1.0-µm beads fall at the beginning of the second decade of a four-decade log forward-angle light scatter scale.

Statistical analyses

All analyses were performed with SPSS 10.0 for Windows software (SPSS, Chicago, Ill., USA). All results are expressed as mean values (±SD). Comparisons between groups were done using the Mann-Whitney test and the Kruskal-Wallis test. A $p<0.05$ was considered statistically significant. All significant differences are given as two-sided values.

Results

A total of 40 analyses following chemotherapy (CT), 39 following total body irradiation (TBI, 8–12 Gy), 39 following ATG in parallel with cyclophosphamide (ATG/Cy), and 45 following transplantations were performed.

Conditioning therapy caused a rapid decrease of the platelet count (Fig. 2). Platelet transfusions were required in 4 (10%) patients during chemotherapy, in 9

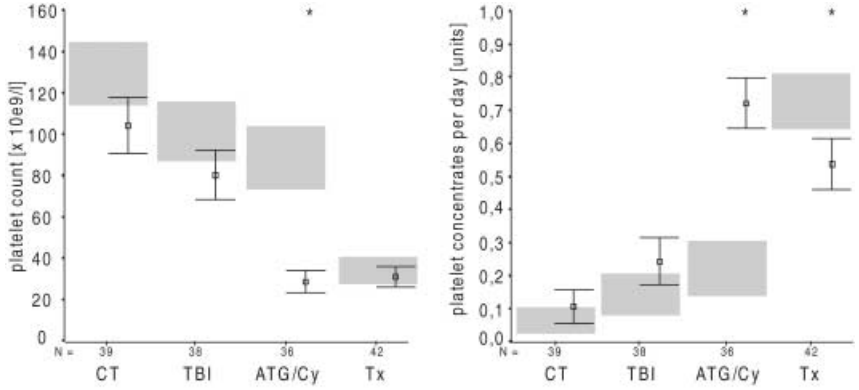

Fig. 2 Platelet count and consumption of platelet concentrates per day. The figures give the values before (*grey bar*) and after particular treatment blocks. All bars represent the 95% confidence interval; the dots are the mean values. *Significant change ($p<0.05$) with respect to the value before start of each treatment block. *CT* chemotherapy, *TBI* total body irradiation, *ATG-F/Cy* therapy with ATG and cyclophosphamide, *Tx* transplantation

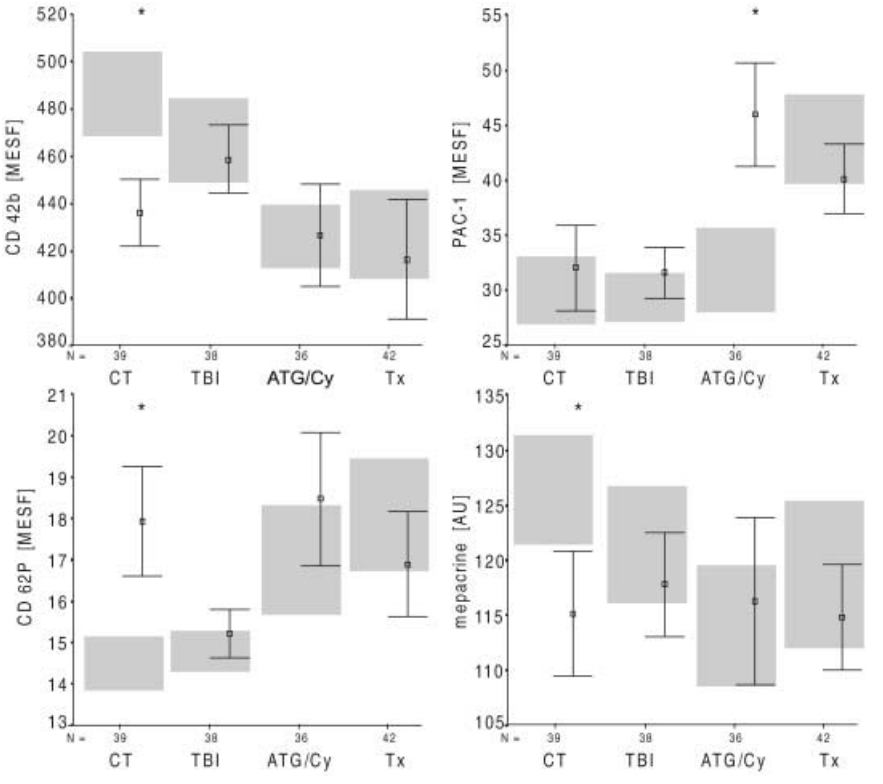

Fig. 3 Platelet surface receptors and granules of all patients under study. The figures give the mean fluorescence values before (*grey bar*) and after the treatment block. All bars represent the 95% confidence interval; the dots are the mean values. *Significant change ($p<0.05$) with respect to the value before start of each treatment block. *AU* arbitrary fluorescence units, *MESF* molecules of equivalent soluble fluorochrome, *CT* chemotherapy, *TBI* total body irradiation, *ATG-F/Cy* therapy with ATG and cyclophosphamide, *Tx* transplantation

(23.1%) patients during TBI, in 26 (66.7%) patients during application of ATG/Cy, and in 25 (55.6%) on the transplantation day. A marked rise in the need for platelet transfusions as well as an increase in body temperature during the treatment period was observed in patients receiving ATG/Cy (Fig. 2), whereas during the other conditioning blocks no patient had a body temperature beyond 38.0 C. Comparisons between patients who did or did not receive platelet transfusions revealed no difference in the flow cytometric parameters (Table 2). The various chemotherapeutic agents were similar in their effects on platelets (Table 3).

During chemotherapy a decrease of the surface integrin CD42b (part of the GP Ib/V/IX complex) as well as mepacrine staining and an increase of CD62p (P-selectin) surface expression was observed (Fig. 3). The integrin CD41 (GP IIb) was comparable in all groups (Table 4). In vitro stimulation of platelets with ADP led to an augmented surface expression of CD62P. In addition, the percentage of circulating microparticles rose to 143% when compared to the level before chemotherapy (Fig. 4).

During application of ATG/Cy surface expression of PAC-1 antigen, a neoepitope appearing after activation of the GP IIb/IIIa receptor, increased. However, significant signs of platelet degranulation were not detectable and the level of circulating microparticles did not change. TBI or the process of transplantation itself (transfusion of hematopoietic stem cell preparations) had no influence on flow cytometric parameters of platelets.

Table 2 Impact of platelet transfusions on the flow cytometric results. The data show no significant differences among patients with or without platelet concentrates during the distinctive treatment blocks. The numbers give the mean (±SD) fluorescence intensities at the end of each treatment block in absolute values (molecules of equivalent soluble fluorochrome) except for the mepacrine staining (arbitrary units). *TBI* total body irradiation (4–12 Gy), *ATG-F/Cy* therapy with ATG and cyclophosphamide, *PC* platelet concentrate

	Chemotherapy		TBI		ATG/Cy		Transplantation	
n	No PC 36	PC 4	No PC 30	PC 9	No PC 13	PC 26	No PC 20	PC 25
Platelet surface receptors								
CD49b (GP Ia)	37.3±12.7	38.8±4.2	37.4±11.4	41.7±10.0	37.2±11.0	36.5±11.4	39.3±6.8	39.0±10.8
CD41 (GP IIb)	131.9±44.2	128.0±23.6	137.4±49.3	133.3±36.0	131.7±56.1	131.1±25.9	135.3±50.4	153.0±200.5
PAC-1	32.4±25.2	26.8±5.9	31.9±14.1	36.8±17.2	42.3±20.3	41.5±30.9	39.3±21.5	40.1±22.2
CD42b (GP Ib)	436.1±94.7	450.5±26.5	464.1±96.7	396.6±39.3	489.9±200.6	395.2±82.1	441.8±112.2	399.4±202.7
Platelet granules								
CD62P	17.9±8.9	18.3±4.1	14.8±3.4	13.3±4.2	19.1±7.9	16.4±6.1	19.1±12.9	15.5±4.2
ADP	78.7±83.2	62.0±19.8	58.5±24.2	56.7±16.2	64.1±32.8	61.4±15.6	58.0±44.4	61.2±14.8
CD63 (GP 53)	25.0±6.0	27.0±8.2	23.9±5.8	22.3±4.9	27.4±9.6	23.9±5.9	24.6±4.4	23.1±4.9
ADP	44.3±17.6	42.5±7.0	43.2±16.9	41.9±10.7	45.0±19.1	45.1±8.7	38.9±14.9	33.4±7.3
Mepacrine	118.8±34.4	100.0±47.5	122.1±25.9	126.8±38.4	119.2±45.6	114.3±48.1	125.2±34.3	121.6±28.8
TRAP	102.3±36.9	90.5±56.1	117.8±22.7	110.1±42.9	117.8±39.6	110.7±27.0	117.9±32.7	114.6±31.0
Micaroparticles								
Microparticles	4.0±4.4	3.9±1.1	2.4±1.6	2.0±2.1	2.2±2.2	2.3±2.9	2.5±3.1	2.3±1.6

Table 3 Impact of three different chemotherapeutic regimens on the flow cytometric results. The data show no significant differences among patients receiving the three different chemotherapeutic regimens. The numbers give the mean (±SD) fluorescence intensities at the end of each treatment block in absolute values (molecules of equivalent soluble fluorochrome) except for the mepacrine staining (arbitrary units). For the dosage of the chemotherapeutic regimens see Table 1

n	FLAMSA 16	Fludarabine 16	Busulfan 6
Platelet surface receptors			
CD49b (GP Ia)	38.1±12.6	37.7±15.2	35.0±9.8
CD41 (GP IIb)	130.7±27.1	128.3±43.3	125.0±42.8
PAC-1	28.8±12.9	30.6±18.4	27.0±51.9
CD42b (GP Ib)	438.5±109.4	423.9±64.1	423.0±83.1
Platelet granules			
CD62P	15.1±3.6	17.3±9.1	14.2±14.6
ADP	52.6±14.2	104.0±126.7	73.8±45.0
CD63 (GP 53)	24.9±6.9	25.8±6.3	25.8±6.7
ADP	45.3±8.1	47.5±22.3	44.2±12.0
Mepacrine	108.0±30.5	111.7±44.8	115.5±28.5
TRAP	109.4±33.5	101.2±47.7	113.8±20.7
Microparticles			
Microparticles	3.3±2.2	4.3±3.8	3.5±1.1

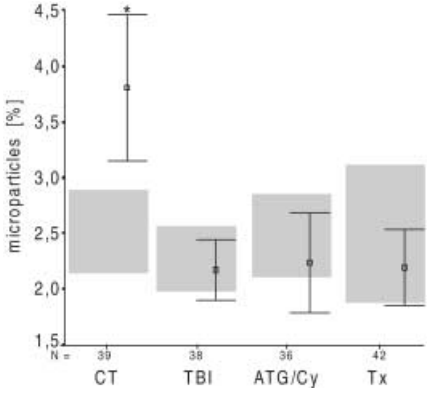

Fig. 4 Circulating microparticles of all patients under study. The figures give the percentage of circulating microparticles before (*grey bar*) and after the treatment block. All bars represent the 95% confidence interval; the dots are the mean values. *Significant change ($p<0.05$) with respect to the value before start of each treatment block. *CT* chemotherapy, *TBI* total body irradiation, *ATG-F/Cy* therapy with ATG and cyclophosphamide, *Tx* transplantation

Discussion

In this study, flow cytometric parameters of circulating platelets were evaluated in patients during conditioning therapy for HSCT. Using whole blood flow cytometry for a minimal manipulation of platelets, the major findings of this study can be summarized as follows:

1. Transfused platelets did not influence our results.
2. Chemotherapy strongly activated and degranulated platelets.
3. Treatment with ATG/Cy lead to an activation of GP IIb/IIIa, but significant degranulation was not detectable.
4. Following chemotherapy an elevated level of circulating microparticles was observed.
5. TBI and the transplantation itself had no influence on platelets.

The conditioning regimen irreversibly damages hematopoiesis of the recipient and causes a rapid fall in the

Table 4 Impact of the conditioning blocks on the flow cytometric results. *Significant change ($p<0.05$) with respect to the value before start of the treatment phase. The numbers are the mean values (SD). Fluorescence intensities are given in absolute values (standard units: molecules of equivalent soluble fluorochrome) except for the mepacrine staining (arbitrary units). *TBI* total body irradiation (4–12 Gy), *ATG-F/Cy* therapy with ATG and cyclophosphamide

	Chemotherapy		TBI		ATG/Cy		Transplantation	
	Before	After	Before	After	Before	After	Before	After
n	40		39		39		45	
Temperature (°C)	36.7±0.6	37.0±0.9	36.9±0.7	37.2±0.9	37.3±0.9	38.4*±1.2	38.2±1.2	37.5*±1.2
Platelet surface receptors								
CD49b (GP Ia)	39.9±14.3	38.2±12.2	39.1±11.2	38.4±10.9	36.4±12.0	41.1±11.4	41.6±10.9	39.2±9.4
CD41 (GP IIb)	141.5±48.1	132.4±41.4	143.1±56.1	137.4±47.9	134.0±47.8	126.8±36.4	126.53±38.9	143.0±149.5
Platelet granules								
CD62P	14.5±4.2	17.9*±8.3	14.8±3.0	15.2±3.7	17.0±8.4	18.5±9.7	18.1±9.0	16.9±8.4
ADP	53.2±21.5	73.4*±77.6	55.1±21.8	55.2±22.3	68.3±78.8	48.9±23.2	51.2±23.6	45.6±30.2
CD63 (GP 53)	26.4±7.4	25.1±6.1	25.7±6.1	23.3±5.5	22.7±6.7	25.8±9.1	25.6±8.5	23.4±4.8
ADP	48.7±21.5	42.4±17.0	41.4±12.8	40.0±16.0	39.1±18.6	39.3±14.7	40.1±15.4	35.6±11.0
Mepacrine	126.5±31.3	115.2*±35.5	122.7±32.6	116.9±29.6	114.1±34.4	116.3±46.1	118.7±44.6	110.4±31.7
TRAP	114.5±29.1	110.6±37.8	120.5±35.1	115.6±27.9	113.3±37.5	111.1±31.8	112.0±32.5	109.2±31.1

platelet count making platelet transfusions an essential supportive measure in the course of HSCT. For substitution, irradiated platelets from apheresed single donors were used. It is well-known that both apheresis and storage of platelet concentrates activate and degranulate platelets, which may by seen by an increased surface expression of CD62P on these platelets [16]. Interestingly, we found no differences between those patients who received platelet transfusions and those who did not. However, it has been described that CD62P-positive transfused platelets are rapidly removed from circulation by degradation [21] and adhesion to leukocytes [22] within a few minutes. As all concentrates were administered routinely in the afternoon and our measurements were done in the morning, the time lag and the rapid sequestration especially of the preactivated portion of transfused platelets might explain the missing impact on our results. In contrast, however, chemotherapy and ATG may cause a continuous influence on platelets due to their long half-life, which is reflected in the detected platelet changes.

We found a decrease of the surface expression of GP Ib/V/IX and a degranulation of alpha-granules and dense bodies as a result of conditioning chemotherapy, indicating a strong and irreversible in vivo activation of platelets. A direct effect of chemotherapeutic agents on platelets has been described for cisplatin [34], but not for the chemotherapeutics used in this study. In our view, an indirect platelet activation by endothelial cell damage is more probable. Chemotherapeutic agents may change the endothelial production of nitric oxide [35] and secondarily stimulate platelets [3]. In addition, conventional and high-dose chemotherapy may induce an activation of plasmatic coagulation causing increased plasma levels of thrombin-antithrombin complexes, prothrombin fragments 1+2, D-dimers, and low levels of antithrombin, protein C, and protein S [25, 26, 36]. As a consequence, the elevated thrombin levels may activate and degranulate the platelets.

Application of high-dose ATG commonly causes a systemic inflammatory response syndrome (SIRS) and a disseminated intravascular coagulation [28]. Indeed, we found an increased consumption of platelet concentrates and an elevated body temperature in these patients. Surprisingly, however, therapy with ATG only activated the fibrinogen receptor GP IIb/IIIa, which could be seen by the high surface expression of PAC-1 neoepitopes. A degranulation reaction or an internalization of the GP Ib/V/IX [13, 24] was not detectable, indicating a much weaker impact on platelets than that affected by chemotherapy. As the first chemotherapeutic phase activated and degranulated platelets, some activated platelets may have survived and become refractory to a second activation hit. However, similar results have also been obtained in sepsis [6], where significant platelet degranulation was only found in severe septic shock accompanied by multiorgan failure, a situation which did not occur in our study. In septic patients without organ dysfunction, only an activation of GP IIb/IIIa may be observed [6]. As the cytokine release during sepsis may be similar to that during ATG therapy [28], the findings in sepsis may be comparable with our results. It may be speculated that in sepsis and SIRS activating and inhibiting influences on platelets are balanced, thus preventing irreversible platelet activation. Indeed, enhanced synthesis of NO and prostacyclin, both highly effective platelet inhibitors, has been described in sepsis [37]. On the other hand, the intensive platelet sequestration during sepsis and ATG therapy might preferentially remove activated platelets from the circulation of affected patients. This might also explain the only mild signs of platelet activation under these clinical conditions.

The present study demonstrates a pronounced number of circulating microparticles in the blood of patients receiving chemotherapy. Microparticles are membrane vesicles shed from the outer platelet membrane [15]. They are observed in various clinical situations associated with platelet activation as acute myocardial infarction, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, heparin-induced thrombocytopenia, and other thrombotic disorders [5]. However, the importance of circulating microparticles in the pathogenesis of these diseases is still being debated. Microparticles may also be detected in stored platelet concentrates and contribute to the hemostatic effect of transfused platelets. They might be highly thrombogenic [39] as they provide a large catalytic surface for the assembly of procoagulant enzymes [8, 33] and activate other platelets [2].

Indeed, a thrombophilic influence of intravenous chemotherapy has been demonstrated [17, 18, 27], possibly due to the stimulation of the plasmatic coagulation system, a decrease of coagulation inhibitors [31], and/or the activation of the endothelium and platelets [3]. Indwelling central venous catheters, commonly used in HSCT patients, pose an additional risk factor for axillary/subclavian vein thrombosis in cancer patients [10]. We presume that these patients also have an additional risk of developing thromboses due to microparticles and activated platelets.

A strong correlation between hepatic veno-occlusive disease, which represents the most dangerous thrombotic complication following HSCT, and a myeloablative therapy with busulfan or cyclophosphamide has been described [4, 29]. We found no differences with regard to microparticles and platelet activation between nonmyeloablative (FLAMSA, fludarabine) and myeloablative chemotherapy regimens (busulfan). Therefore, an impact of microparticle generation by the conditioning therapy on the pathogenesis of VOD is unlikely.

In this study we were able to demonstrate an activating effect of the conditioning regimen on platelets in patients receiving HSCT. Chemotherapy, in particular, led to a strong and irreversible platelet activation and a generation of microparticles. Our findings underline the impact of platelets on the pathogenesis of hemostatic complications during HSCT.

Acknowledgements This work is part of the doctoral thesis of Birgit Höhnberg.

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