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ORIGINAL RESEARCH

TRANSFUSION

Granulocyte concentrate splitting does not affect phenotype and function

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Abstract

Background: More granulocyte concentrates (GCs) could be produced for more patients from the same donor if apheresis bags were split and stored for longer periods of time. Hence, we tested the hypothesis that splitting and extension of storage of GCs do not impair granulocyte function or viability.

Study Design and Methods: Granulocyte apheresis concentrates were produced using modified fluid gelatin as a separation enhancer, split into two portions, and stored for 24 and 48 h. Granulocyte function, represented by cell migration, reactive oxygen species (ROS) production, and neutrophil extracellular trap formation (NETosis), was measured by live-cell imaging. ROS production, adhesive surface protein expression, and viability were measured by flow cytometry.

Results: Splitting had no effect on any of the tested parameters. After 24 h of storage, live-cell imaging showed no significant difference in migration, time to maximum ROS production, time to half-maximum NETosis, viability, or CD11b expression, but ROS production induced by phorbol 12-myristate 13-acetate (PMA) decreased from an initial median fluorescence intensity of 1775–590 artificial units. After 48 h, PMA-induced ROS production, viability, and migration declined, as reflected by decreases in median total distance (119 vs. 63.5 μm) and median Euclidean distance (30.75 vs. 14.3 μm).

Conclusion: Splitting GC products has no effect on granulocyte viability or function, but extended storage >24 h does compromise granulocyte function. The findings confirm that GCs should be transfused within 24 h of collection. Longer storage cannot be recommended.

KEYWORDS

distribution, granulocyte concentrates, granulocytes, modified fluid gelatin, neutrophils, separation, storage, transfusion

Abbreviations: 7-AAD, 7-amino-actinomycin D; APC, allophycocyanin; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DHR, dihydrorhodamine; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; fMLP, N-formyl-Met-Leu-Phe; GC, granulocyte concentrate; G-CSF, granulocyte colony stimulating factor; HES, hydroxethyl starch; MFG, modified fluid gelatin; NETosis, neutrophil extracellular trap formation; PE, phycoerythrin; PMA, phorbol 12-myristate 13-acetate; QC, qality control; ROS, reactive oxygen species.

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1 | INTRODUCTION

Granulocyte concentrate (GC) transfusion is a potentially lifesaving option for patients with a low number of functional neutrophils.[1] The production of GC from apheresis requires pretreatment of the donors with glucocorticoids and/or G-CSF as well as apheresis with sedimentation enhancers like hydroxyethyl starch (HES) or modified fluid gelatin (MFG).[2] Alternatively, granulocytes can be obtained from buffy coats from whole blood donations.[3] The latter does not require pretreatment and may result in superior granulocyte function with respect to Candida killing despite elaborated post donation procedures.[4] In fact, HES may impair granulocyte function in a dose-dependent way.[5] Regardless of the production method, GC are laborious to manufacture, and their short shelf life limits their availability even further.

In a recent study, only nine out of 34 patients could be provided with GC, reflecting potential shortages in the GC supply.[6] Theoretically, it might be possible to increase supply by simply splitting GC products and extending their shelf-life beyond the present maximum storage period of 24 h, if sufficient granulocyte doses were obtained.

While there is some agreement that at least 1×10^{10} granulocytes are required for therapeutic efficacy,[7] optimal doses would contain 4×10^{10} granulocytes for adult patients.[8] Absolute values are not applicable for pediatric transfusions, hence, these are usually expressed as cells per kilogram bodyweight. Optimal efficacy was recently found for $1.5–3.0 \times 10^{8}$ cells/kg, implicating underdosage for lower doses and side effects by cytokines and others outweighing benefits for higher doses.[9] Under-dosage, however, does not exclude efficacy completely.[10]

Currently, studies on the storage stability of granulocytes are limited to GCs produced with HES. The available data show that after 24 h of storage, more than 90% of cells remain viable, cell morphology is unchanged, and functional parameters, such as reactive oxygen species (ROS) formation and chemotaxis, are intact.[11,12] However, as HES has an unfavorable risk profile, authorities have acted to limit its use in GC production as well as other applications.[13,14]

Therefore, MFG is now used as an alternative sedimentation enhancer for GC production.[15] Compared to HES, MFG produces comparable cell yields, and the granulocyte function of MFG-derived GCs is at least equal to that of HES-derived products.[2,5] However, the storage of GCs collected using MFG-assisted apheresis has not yet been investigated.

Moreover, there is no published data available on whether splitting and extended storage of GCs impair granulocyte quality. To answer these questions, we tested cellular parameters of granulocyte viability and function immediately after splitting and after 24 and 48 h of storage.

2 | MATERIALS AND METHODS

2.1 | Granulocyte concentrate production

GCs were acquired from 11 healthy donors as approved by the local ethics committee (17-565-101). Apheresis was performed as previously described.[2] Briefly, donor granulocytes were mobilized with granulocyte colony stimulating factor (G-CSF) and dexamethasone. MFG (Gelafundin, Braun) admixed with citrate at a ratio of 1–12 was used for sedimentation enhancement. A peripheral blood volume of 7 L was generally processed.

Each granulocyte apheresis donation bag was split into two empty GC bags (GC1, GC2) of the same gas permeable plastic (Accessory Platelet Storage Set, Terumo BCT) as the original one and one quality control (QC) sample. Bag volumes were reduced by welding to ensure an equal surface-to-volume ratio as the primary apheresis bag.

The GCs were analyzed immediately after apheresis by flow cytometry or live-cell imaging. A similar analysis was performed on QC, GC1, and GC2 samples within 120–150 min after splitting. While the flow cytometry was performed on whole cell suspensions, live-cell imaging was performed on isolated neutrophils. Samples from the apheresis bag, GC1, GC2, and QC were investigated separately at 0 h. Samples after 24 and 48 h storage at room temperature without agitation were investigated by QC only.

2.2 | Isolation of granulocytes for live-cell imaging

Cell suspension samples (700 μl) were diluted with Dulbecco's phosphate buffered saline (PBS, 700 μl) without calcium and magnesium (Sigma-Aldrich). Dual density gradient isolation of neutrophils was achieved using Leuko Spin and Lympho Spin Medium (both pluriSelect Life Science). Granulocytes were adjusted to a concentration of 20×10^{3} cells/μl.

2.3 | Preparation of chemotaxis gels and substrates

Collagen I gels were prepared as previously described.[16] The gels were incubated at 37°C and 5% CO_2 (HeraCell 150i CO_2 Incubator, Thermo Fisher Scientific).

Dihydrorhodamine 123 (DHR123, Sigma-Aldrich) was used to detect ROS by fluorescence after oxidation to rhodamine123, whereas DNA in NETs was stained

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with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich).[5,17,18] DAPI (5 mg/ml) were diluted with Dulbecco's Phosphate Buffered Saline with $MgCl_2$ and $CaCl_2$ (PBS Mg/Ca, Sigma-Aldrich) and RPMI-1640 (RPMI, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich). DHR (1 mM) was diluted with RPMI to a final concentration of 1 μM. Isolated granulocytes (150 μl, final concentration 3×10^3 cells/μl) were added to a separate gel and combined with DAPI (3 μl, final concentration 0.5 μg/ml), Bovine Collagen Solution Type 1 (150 μl, Advanced BioMatrix, final concentration 1.5 mg/ml) and DHR (5 μl, final concentration 10 μM).

The granulocyte/collagen mix (6 μl each) was put into one of the three central canals of a μ-Slide (μ-Slide Chemotaxis, ibidi GmbH). Each slide consists of three central canals with two adjacent reservoirs, allowing for parallel chemotaxis assays.[19] After loading, the μ-Slides were incubated for 30 min at 37°C and 5% CO_2 to harden the collagen gel.

2.4 | Preparation of chemotaxis reservoirs

N-formyl-Met-Leu-Phe (1 mM fMLP, Sigma-Aldrich) was diluted with PBS and RPMI supplemented with 10% FBS. fMLP (final conc. 10 nM), RPMI, and 10% FBS were added to the three reservoirs left of the central canals of the μ-Slide, and RPMI and 10% FBS were added to the three on the right to create separate chemotactic gradients.

2.5 | 3D live cell imaging

Every 30 s for 270 min in total, one phase contrast image and two fluorescence microscopy images (one for DAPI and one for DHR/rhodamine123) were taken of all canals with a camera and microscope (Leica DFC9000 and Leica DMi8) using Leica Application Suite X 3.0.4.16529 software (Leica Microsystems GmbH), yielding 540 sets of images for each canal. The following parameters of migration were assessed for each cell using Imaris 8.3.4 software (Bitplane AG): total distance, Euclidean distance, displacement X, and straightness. Displacement X represents forward migration along the x-axis of the μ-Slide. As the chemotactic gradient was established along the x-axis, displacement X represents chemotaxis towards fMLP. Straightness, which represents a granulocyte's tendency to travel in a straight line towards a target, was calculated as Euclidean distance divided by total distance (accumulated distance).[5]

2.6 | Flow cytometry

ROS analysis comprised a separate control test, an fMLP and human tumor necrosis factor alpha test (TNF-a, PeproTech Inc), and a phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) test for each sample, respectively.[20] fMLP was used because of its capability to induce direct, albeit minor, ROS production in granulocytes.[21,22] The stimulating effect of fMLP on granulocytes was amplified by using TNF-α for priming.[21,23] Samples were combined with DHR and 1 mM 5-(and-6)-carboxy SNARF-1 (Thermo Fisher Scientific). First, TNF-α was added and all compounds were incubated at 37°C for 10 min. Afterwards, fMLP or PMA (positive control) were added, and the samples were incubated at 37°C for 20 min, followed by the addition of propidium iodide (Serva Electrophoresis GmbH). Granulocytes were assessed by flow cytometry using a FACSCalibur flow cytometer (BD Biosciences) with CellquestPro Version 5.2 software (Becton-Dickinson Bioscience) and FlowJo 10.0.7 (FlowJo, LLC) for subsequent analysis.

Viability testing by flow cytometry was performed using fluorescein isothiocyanate (FITC), Annexin V (BioLegend), and 7-amino-actinomycin D (7-AAD, BioLegend). Control tests were performed by the fluorochrome minus one (FMO) method. Each sample combined with PBS was centrifuged at 425 g for 3 min at 4°C. The supernatant was then discarded, and Annexin V Binding Buffer (BioLegend) was added. Annexin V and 7-AAD were added accordingly. All samples were then incubated for 15 min at room temperature, and an Annexin V Binding Buffer was added.

Surface protein analysis was performed using phycoerythrin (PE) fluorochrome-conjugated anti-human CD11b antibodies, FITC fluorochrome-conjugated anti-human CD62L antibodies, and allophycocyanin (APC) fluorochrome-conjugated anti-human CD66b antibodies (all BioLegend).[5,21] Each sample was diluted with PBS Mg/Ca, centrifuged (425 g, 3 min, 4°C) and stained. All samples were incubated at 4°C for 15 min, washed, and resuspended with PBS Mg/Ca prior to flow cytometry.

2.7 | Statistical analysis

SPSS Statistics 25 (IBM) was used for statistical analysis. The normality of the data distribution was tested using the Shapiro–Wilk test. Further analyses were done by one-way analysis of variance (ANOVA), median tests, Kruskal-Wallis tests, and Bonferroni post hoc corrections for multiple comparisons.

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3 | RESULTS

3.1 | Granulocyte concentrate production

All donors were male, and the average age was 28 years. Mean white blood cell count of GC bags was 200/nl of which 83.3%, or 167/nl were neutrophil granulocytes.

The time course of neutrophil migration (summary in Table 1) and activation with ROS production, and final NETosis blast was detected by live cell imaging as described (Videos S1 and S2).

Functional microscopy was also performed for 4.5 h, but the observation period was not divided into 30-minute intervals. Rhodamine123 was evaluated as T_{max}ROS and NETosis was evaluated as Et_{50}NET as described previously.[5,24,25] The results are summarized in Table 2.

Flow cytometry was used to quantify ROS production intensity, viability, and changes in surface protein expression. Results obtained before and after 24 and 48 h of storage are displayed in Table 2.

3.2 | Splitting of GCs

3D live cell imaging—Migration: Because granulocyte migration started vividly but decreased during the 4.5 h observation period (270 min), analysis of the following parameters was restricted to 30 min intervals for the first 90 min after apheresis: Euclidean distance, displacement X, straightness (Table 1) and total distance (Table 1, Figure 1). Splitting itself at 0 h had no statistically significant (all *p*-values >.05) effect on the migration parameters total distance, Euclidean distance, and displacement X. Example given, the median total distances traveled by granulocytes in the granulocyte apheresis donation bag, GC1, GC2, and QC groups were 160, 121, 106, and 119 μm, respectively, in the first 30 min of testing, and they were 70.4, 85.8, 79.5 and 67.1 μm, and 27.1, 27.6, 35.8 and 37.7 μm for 31–60 min and 61–90 min, respectively (Table S1)

3D live cell imaging—functional microscopy: Splitting had no effect on the timing of ROS production or the timing of NETosis. T_{max}ROS was an average of 64.5 ± 12.8 min in all samples (granulocyte apheresis donation bag, GC1, GC2, and QC), and no significant differences were found within this group (Table S2). Et_{50}NET for the same samples was 120 ± 9.77 min. Similarly, no significant differences were found (Table S2). The time between the two reactions was an average of 54.7 ± 8.57 min

3.3 | Flow cytometry

Splitting granulocyte apheresis donations in two did not result in any significant differences in PMA and

TABLE 1 Granulocyte migration parameters determined immediately following granulocyte apheresis (in all bags 0 h) and after 0, 24, and 48 h of storage in QC bags

Parameter	Observation period [min]	All bags 0 h	QC 0 h	QC 24 h	QC 48 h
Euclidean distance [μm] *	1–30	34.3 (22.5)	30.8 (26.1)	40.6 (20.0)	14.3 (12.0)‡
	31–60	10.3 (19.1)	9.35 (13.3)	8.05 (11.5)	5.32 (6.8)‡
	61–90	5.01 (15.3)	10.75 (6.79)	2.63 (6.0)	2.46 (4.8)‡
Total distance [μm] *	1–30	123 (40.7)	119 (39.2)	114 (37.6)	63.5 (36.5)
	31–60	73.6 (56.0)	67.1 (52.0)	44.7 (376.7)	34.0 (25.1)
	61–90	29.0 (50.5)	37.7 (40.3)	23.8 (21.0)	20.7 (21.9)
Displacement X [μm] *	1–30	6.44 (18.3)	6.43 (23.5)	11.6 (15.5)	1.55 (7.9)
	31–60	2.06 (13.5)	2.63 (9.63)	2.17 (6.9)	0.01 (4.6)§
	61–90	0.69 (10.6)	0.78 (3.97)	0.24 (2.5)	0.03 (2.5)
Straightness†	1–30	0.29 (0.11)	0.27 (0.11)	0.31 (0.09)	0.23 (0.07)§
	31–60	0.19 (0.07)	0.178 (0.05)	0.17 (0.06)	0.13 (0.03)‡
	61–90	0.17 (0.06)	0.168 (0.05)	0.14 (0.05)	0.12 (0.04)§

Note: Standard deviations in parenthesis, data expressed as *median or †mean values; ‡ $0.001 < p \leq .05$ for comparisons with QC 0 h samples; § $p \leq .001$ for comparisons with QC 0 h samples. Displacement X: positive values correspond to forward movement towards the chemoattractant fMLP. Straightness: valid values lie between 1 and 0, where 1 represents precise target-specific migration and 0 represents unspecific movement. The displayed values reflect measurements taken following storage. All 0 h.

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fMLP/TNF-α-induced ROS production. In addition, no significant differences in the observed fractions of viable cells, apoptotic cells, and necrotic cells were observed. Splitting did not result in any significant differences in surface proteins, measured as average fluorescence intensities of CD11b and CD66b and median fluorescence intensities of CD62L (all in Table S2).

3.4 | Storage of GCs

3D live cell imaging—Migration: In extended storage samples (48 h), the total migration distance was nearly halved compared to that of fresh samples (Figure 1). During the first 30 min of the microscopic observation period, the total distance decreased to a median of

TABLE 2 Parameters of granulocyte function and viability determined immediately following granulocyte apheresis (in all bags 0 h) and after 0, 24, and 48 h of storage in QC bags

	Parameter	All bags 0 h	QC 0 h	QC 24 h	QC 48 h
Microscopy	T_{max}ROS [min] * §	64.5 (12.8)	58.1 (36.1)	70.4 (29.3)	61.4 (31.2)
	Et_{50}NET [min] * §	114 (23.3)	113 (38.2)	126 (45.0)	103 (36.2)
Flow cytometry	ROS induced by PMA [afu] † ‡	1705 (1101)	1775 (1120)	590 (645)‖	588 (813)
	ROS induced by fMLP + TNF-α [afu] † ‡	0.02 (7.43)	0.15 (4.44)	0.70 (1.21)‖	0.98 (3.13)
	$Annexin^{neg}$, $7\text{-}AAD^{neg}$ [%] † ‡	87.6 (8.0)	86.9 (6.47)	78.1 (15.4)	81.6 (14.4)‖
	$Annexin^{pos}$, $7\text{-}AAD^{neg}$ [%] † ‡	10.6 (8.2)	11.4 (6.66)	0.42 (14.4)	0.79 (3.44)‖
	$Annexin^{pos}$, $7\text{-}AAD^{pos}$ [%] † ‡	0.57 (1.19)	0.59 (1.05)	9.84 (12.1)¶	17.4 (15.1)¶
	CD11b [afu] † ‡	1140 (673)	869 (699)	1280 (977)	1230 (725)
	CD62L [afu] † ‡	131 (135)	123 (108)	111 (38.6)	98.8 (269)
	CD66b [afu] † §	614 (360)	594 (338)	794 (447)	728 (379)

Note: * Areas of high fluorochrome intensity were measured by fluorescence microscopy; † Fluorochrome intensity was measured by flow cytometry. Data with standard deviation in parenthesis and ‡ median values or § mean values; ‖ $.01 \le p < .05$ for comparisons with QC 0 h samples; ¶ $p < .001$ for comparisons with QC 0 h samples; afu = arbitrary fluorescence units; $Annexin^{neg}$ and $7\text{-}AAD^{neg}$ represent vital cells, $Annexin^{pos}$ and $7\text{-}AAD^{neg}$ mark apoptotic cells and $Annexin^{pos}$ and $7\text{-}AAD^{pos}$ represent necrotic cells.

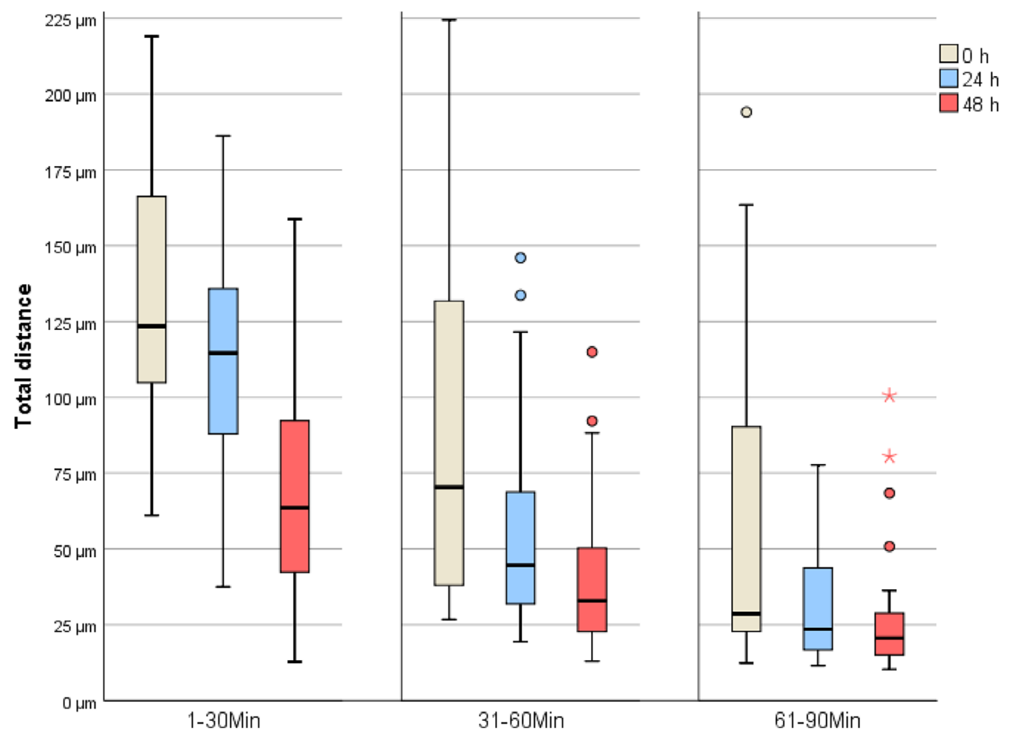

FIGURE 1 Total distance of granulocyte migration in the first 90 minutes after apheresis in samples stored for 0, 24, and 48 h*, as observed by microscopy. *The original granulocyte apheresis donations as well as the two split donations (GC1, GC2) and quality control (QC) samples obtained from them were analyzed before storage (0 h), while only QC samples were evaluated after 24 and 48 h of storage. [Color figure can be viewed at wileyonlinelibrary.com]

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63.5 μm (interquartile range [IQR] = 52.4 μm) in 48 h samples, compared to neutrophil migration of 119 μm (IQR = 35.8 μm) in all non-stored samples. In contrast, 24 h granulocytes still covered a distance of 114 μm (IQR = 55.9 μm). Euclidean distance, displacement X, and straightness were also impaired after 48 h of storage (Table 1). Interestingly, 24-h-old granulocytes exhibited a tendency toward more target-specific migration compared to fresh granulocytes (0 h). Statistically significant differences were found between the 0 versus 48 h and 24 versus 48 h samples. *p*-values for differences in cell migration parameters between the three storage time groups are shown in Table S3.

3D live cell imaging—Functional microscopy: Granulocytes in 24 and 48 h storage samples did not show significant differences in ROS production or NETosis compared to 0 h samples or each other (Table 2).

Flow cytometry: PMA-induced ROS production (fluorochrome intensity) decreased from 1775 arbitrary fluorescence units (afu) at 0 h (IQR = 1858 afu) to 590 afu (IQR = 1430 afu) at 24 h and 588 afu at 48 h. Conversely, fMLP/TNF-α-induced ROS production (rhodamine123 intensity), which was initially 0.15 afu (IQR = 1.23 afu), increased to 0.70 afu (IQR = 1.42 afu) at 24 h of storage and to 0.98 afu (IQR = 3.31) afu at 48 h, albeit insignificantly. The relative number of viable neutrophil cells measured by flow cytometry decreased from a median 86.9% (IQR = 7.46%) at 0 h to 81.6% (IQR = 22.1%) at 48 h. After 48 h of storage, the median percentage of apoptotic cells had decreased significantly from baseline ($p = .019$). A significant difference (increase) in necrotic cells was observed for 0 versus 24 h ($p < .001$) and 0 versus 48 h ($p < .001$), but not for 24 versus 48 h. Although there was a slight increase in CD11b and CD66b and a small decrease in CD62L fluorescence intensity, there were no significant differences in surface proteins from baseline to 24 or 48 h of storage.

4 | DISCUSSION

4.1 | Splitting of GCs

Cell migration capacities remained unchanged after separation of the primary granulocyte apheresis donation. Fluorescence microscopic values for ROS production and NETosis were consistent with these findings, as there was no significant difference in T_{max}ROS or Et_{50}NET between granulocyte apheresis donation bags and split samples GC1 and GC2. Furthermore, flow cytometry studies of fMLP/TNF-α and PMA-induced ROS production revealed no significant differences between granulocyte activities before and after separation. Viability measurements confirmed these findings, as no changes in the fractions of vital, apoptotic cells or necrotic cells were observed. Likewise, surface protein expression experiments did not reveal any significant differences in activity before and after splitting.

In light of these findings, we conclude that the process of separating granulocyte apheresis donations into two GC products each did not alter granulocyte migration, function, or viability.

Splitting of GCs is, independently of its feasibility, limited to those with sufficient yield for the intended patients. Pediatric transfusions for low body weight patients are therefore easier to serve with split GCs than those for adult patients. Granulocyte apheresis, in contrast to whole blood donations, varies widely between both donors and donations. Not all granulocyte apheresis may yield sufficient cell numbers for splitting. Finally, MFG as a sedimentation agent is more demanding than high molecular weight HES and might decrease the percentage of apheresis procedures suited for splitting.

4.2 | Prolonged storage of GCs

After 48 h storage, there was a significant decrease in the tested parameters of migration, that is, total migration distance, Euclidean distance, straightness, and displacement along the *x* axis towards the chemoattractant. This decrease is plausible in view of the limited lifespan of intravascular granulocytes of about 5 days and the stress apheresis and preparation exert on granulocytes.[26] Extracorporeal storage of neutrophils might further decrease their lifespan, for example, by glucose depletion in the cell suspension medium over time, pH changes, accumulation of toxic metabolites, or cytokines.

Interestingly, analysis of Euclidean distance showed that 24 h granulocytes migrated slightly farther (median range of 40.6 μm, IQR = 26.0 μm) than fresh granulocytes of the control group (30.8 μm, IQR = 21.9 μm), but the difference was not statistically significant (Table 1). A similar observation was made for straightness: the data shows that cell migration was less target-specific at 48 h than at 0 or 24 h. The straightness of granulocytes depends on a variety of factors, such as the surrounding environment.[27] Displacement X (movement along the x axis towards the chemoattractant fMLP) was used as a marker of chemotaxis functionality.[28,29] Following 48 h after donation, granulocytes lost nearly all ability to migrate along a chemotactic gradient. Taking the total distance traveled and Euclidean distance into account, this could point towards severe implications for immune defense.

The data from this study also suggest that granulocytes are weakened immediately after apheresis, as granulocytes displayed the tendency towards improved target-directed migration and chemotaxis following 24 h of storage.

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The results of the migration experiments were partially confirmed by those of the fluorescence microscopic T_{max}ROS and Et_{50}NET analyses. After 24 h storage, T_{max}ROS was delayed by more than 10 min, from an initial 59.3 ± 12.9 min to 70.4 ± 29.3 min. NETosis, a more important factor in extending the life span of granulocytes, took place at an Et_{50}NET of 126 ± 45 min at 24 h compared to a DAPI Et_{50}NET of 113 ± 30.1 min at 0 h. In addition to the small delays in both reactions, the time between DHR T_{max}ROS and DAPI Et_{50}NET increased slightly from 54.4 ± 39.3 min at 0 h to 55.9 ± 40.3 min at 24 h. Conversely, the reactions occurred earlier after 48 h storage.

The microscopic data suggests that 24 h storage was associated with enhanced chemotaxis of granulocytes and an extended half-life of neutrophils, although the difference was not statistically significant. The reason for this may be that apheresis caused an initial weakening of granulocytes at 0 h, from which they recovered after 24 h of storage. In addition, donor granulocytes were mobilized with G-CSF and dexamethasone, of which G-CSF is known to improve chemotaxis and increase half-life.[30–33]

Flow cytometric analysis showed that after 24 h storage, PMA-induced ROS production decreased, while fMLP/TNF-α-induced ROS production increased. ROS production is essential for a number of important mechanisms, such as chemotaxis, intracellular signaling, and NETosis.[23,34–38]

Although the number of vital cells in 48 h samples had decreased as expected, the bulk of necrotic cells were derived from earlier apoptotic cells. This equates to a shift in the apoptotic to necrotic cell ratio from the time when apoptotic cells dominated in the earlier samples. The majority of tested granulocytes (81.6%, IQR 22.1%) were vital after 48 h of storage, reflecting a life span that could have been extended due to G-CSF exposure.[39] An extended half-life, on the contrary, may also lead to excessive immune reactions.[21,23,40–42]

The findings of the surface protein experiments also support the notion that granulocytes are weakened immediately after apheresis. On the whole, however, the various tendencies revealed here were statistically insignificant.

Granulocyte function seemed unchanged after 24 h of storage. However, the importance of the observed decrease in PMA-induced ROS production remains to be seen. Extending the storage of GCs to 48 h had a negative impact on neutrophils. The exact time at which neutrophils shifted from being adequate to impaired in the interval between 24 and 48 h storage remains unclear. Therefore, we conclude that the storage of GCs for more than 24 h cannot be recommended.

Study limitations: Additional tests showed that the process of isolating granulocytes from GC samples by density gradient separation and subsequently preparing them for microscopic analysis also modulates neutrophil migration.[43]

5 | CONCLUSION

Splitting granulocyte apheresis donations had no effect on granulocyte function or viability. One day of storage (24 h) did not impair the chemotaxis, migrational abilities, time course of ROS production, time course of NETosis, or viability of the granulocytes. After 48 h of storage, however, these parameters of granulocyte function and viability were impaired. Therefore, storing GCs for more than 24 h cannot be recommended.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted.

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