Efficient granulocyte collection method using high concentrations of medium molecular weight hydroxyethyl starch

Running title: HES130/0.4 and granulocyte collection

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Conflicts of Interest

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Key Points:

1. This study highlights an efficient approach for granulocyte collection using medium molecular weight hydroxyethyl starch (HES130/0.4), which has a better safety profile than the high molecular weight hydroxyethyl starch.

2. High concentrations of medium molecular weight hydroxyethyl starch in the separation chamber are required for efficient granulocyte collection.

Abstract

BACKGROUND: Granulocyte transfusion therapy is a rational therapeutic option for patients with prolonged severe neutropenia. Although high molecular weight hydroxyethyl starch (hHES) facilitates the separation of red blood cells during granulocyte collection, renal dysfunction has been noted as a potential side effect. HES130/0.4 (Voluven®) is a medium molecular weight HES (mHES) with superior safety profiles than hHES. Although HES130/0.4 is reportedly effective in the collection of granulocytes, we lack studies comparing the efficiency of granulocyte collection using HES130/0.4 and hHES.

STUDY DESIGN AND METHODS: We retrospectively collected the data from 60 consecutive apheresis procedures performed on 40 healthy donors at the Okayama University Hospital between July 2013 and December 2021. All procedures were performed using the Spectra Optia system. Based on the HES130/0.4 concentration in the separation chamber, granulocyte collection methods using HES130/0.4 were classified into m0.46, m0.44, m0.37, and m0.8 groups. We used HES130/0.4 and hHES groups to compare the various sample collection methods.

RESULTS: The median granulocytes collection efficiency (CE) was approximately 24.0% and 28.1% in the m0.8 and hHES groups, respectively, which were significantly

higher than that in the m0.46, m0.44, and m0.37 groups. One month following granulocyte collection with HES130/0.4, no significant changes were observed in serum creatinine levels compared to that before the donation.

CONCLUSION: Therefore, we propose a granulocyte collecting approach employing HES130/0.4, which is comparable to the use of hHES in terms of the granulocyte CE. A high concentration of HES130/0.4 in the separation chamber was considered to be crucial for granulocyte collection.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment strategy for hematological malignancies and congenital bone marrow disorders. Infection is one of the major complications following HSCT despite advances in supportive care over the past decade, such as the advent of novel anti-infective drugs [1].

Granulocyte transfusion therapy (GTx) is a viable therapeutic option for patients with prolonged severe neutropenia, including those who have undergone HSCT. At least 10^{10} granulocytes from a healthy donor must be infused into the patient for the successful outcome of GTx treatment [2]. Granulocyte apheresis has been able to provide a stable yield owing to the development of the continuous blood-flow separator [3], use of hydroxyethyl starch (HES) to enhance the separation of red blood cells (RBC) [4], and administration of granulocyte colony-stimulating factor (G-CSF) and dexamethasone to the donor [5,6].

HES is used as a selective sedimentation agent to induce RBC rouleaux formation since the specific gravities of granulocytes and red blood cells are relatively similar [4]. High molecular weight HES (hHES) is more effective for granulocyte collection than low molecular weight HES [7–9]. However, several emergency medicine studies revealed that hHES is associated with an increased risk of renal injury or malfunction, hemorrhage, and mortality [10]. The development of hematological abnormalities and diffused tissue storage

has been reported in patients with renal failure who had excessive HES exposure [11]. Recent reports demonstrated that sufficient granulocytes could be harvested via apheresis using a medium molecular weight HES (mHES) (HES130/0.4: Voluven®; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) [12–14]. HES130/0.4 is a third-generation HES characterized by an average molecular weight of 130,000 Dalton and a molar substitution of 0.4. HES130/0.4 does not accumulate in the body and is eliminated within 24 h following ten days of continuous administration [15]. Moreover, in cardiac surgery, a meta-analysis revealed no significant difference in the incidence of acute kidney damage and renal replacement therapy between patients administered with HES130/0.4 and human albumin [16]. Another meta-analysis study suggested that tetrastarch containing HES130/0.4 was superior to pentastarch and hetastarch containing hHES in terms of blood loss or transfusion requirements [17]. However, we lack reports on the methods of granulocyte collection using HES130/0.4 and comparing the granulocyte collection efficiency (CE) using HES130/0.4 and hHES.

Our institution's policy is to perform apheresis with donor safety as the primary concern. For granulocyte collection, our institution formerly employed hHES (HES400/0.7: HES40®; NIPRO, Osaka, Japan); however, due to adverse outcomes of patients already reported so far, we have been using HES130/0.4 since 2014 and developing improved

procedures. In particular, we focused on the previous report[18] that showed HES130/0.4 has a fast erythrocyte sedimentation rate at high concentrations ex vivo and succeeded in increasing the CE by increasing the HES130/0.4 concentration in the separation chamber. Here, we presented granulocyte collection methods and proposed a novel approach based on HES130/0.4. We further compared its efficiency with that of HES400/0.7 and determined the number of granulocytes that can be collected in a short time.

Materials and Methods

Donor characteristics

This retrospective analysis was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board at Okayama University Hospital. Our cohort comprised 40 healthy donors who underwent 60 consecutive apheresis procedures for granulocyte collections at our institute between July 2013 and December 2021.

The eligibility criteria for GTx donors were as follows: (1) family members within the third degree of kinship of the recipient, (2) whose ABO blood type was a match or minor-mismatch to the recipient, (3) aged between 18–65, and (4) with no viral infections (human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and human T-cell leukemia virus type 1) established by laboratory testing at the time of apheresis. Informed consent was obtained from all donors prior to the procedure.

Granulocyte mobilization

GTx donors underwent granulocyte mobilization with granulocyte colony-stimulating factor (G-CSF; Kyowa Kirin, Tokyo, Japan) plus dexamethasone (Nichi-Iko Pharmaceutical, Toyama, Japan). Donors were administered 300 μg of G-CSF subcutaneously at 18 h and 8 mg of dexamethasone orally 6 h before granulocyte collection. Granulocyte collections were performed twice per episode from a single donor.

In repeated collections, there were at least seven-day time intervals between each collection. The same mobilization method was used as in the first collection.

Apheresis procedure for collecting granulocyte

Granulocyte harvesting was performed using the two-needle approach using the Spectra Optia system (Terumo BCT, Lakewood, CO, USA) and the polymorphonuclear collection program (PMN) protocol. The processed blood volume was measured until all the HES and anticoagulant (AC) were exhausted. The packing factor, which indicates the centrifugation forces, and the collection flow rate were set to their default values. The collection preference (CP) value was adjusted for the optimal interface positioning by real-time monitoring of hematocrit concentrations in the apheresis product. When the erythrocyte and granulocyte layers are in proximity, setting a deeper CP value increases the number of granulocytes that can be collected and the number of RBCs in the apheresis product. To minimize the burden of blood loss to the donor, our institutional standard is to adjust the CP to a hematocrit not exceeding 20% in the apheresis product. The initial CP setting was optimized at the first implementation of each protocol. To prevent hypocalcemia, 8.5% calcium gluconate hydrate (Nichi-Iko Pharmaceutical, Toyama, Japan) was administered via continuous intravenous infusion (12 mL/h).

The other settings such as AC ratio and the combination of HES and AC are outlined in

Table 1 and as follows: (1) hHES: 6 % HES400/0.7 400 mL plus anticoagulant citrate dextrose solution A (ACD-A containing 3% citric acid; Terumo, Tokyo, Japan) 500 mL and AC ratio set 8.5:1, the initial CP value set to 60, and initial blood flow rate set to 60 mL/min and increased gradually up to 75 mL/min, (2) m0.46: 6%HES130/0.4 500mL plus ACD-A 500 mL and AC ratio set 6.5:1, the initial CP value set to 27, and initial blood flow rate set to 40 mL/min and increased gradually up to 70 mL/min, (3) m0.44: 6%HES130/0.4 500mL plus 46.7% sodium citrate hydrate 30 mL, AC ratio set 12:1, the initial CP value set to 35, and initial blood flow rate set to default and increased gradually up to 40 mL/min, (4) m0.37: 6%HES130/0.4 500mL plus 10% sodium citrate hydrate (Citramin "FUSO" for Transfusion®; Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) 175 mL and AC ratio set 13:1, the initial CP value set to 27, and initial blood flow rate set to 40 mL/min and increased gradually up to 60 mL/min, or (5) m0.8: 6%HES130/0.4 500mL plus 10% sodium citrate hydrate 80 mL, AC ratio set 6.5:1, the initial CP value set to 40, and blood flow rate set to 40 mL/min.

Granulocyte irradiation (15 Gy) was performed immediately after harvesting. One month following granulocyte donation, every donor in the m0.8 group was offered a follow-up visit, whereas donors in the other groups were provided a follow-up appointment at the discretion of the attending physician. Notably, the HES concentration in the chamber was

estimated using the HES product volume and concentration used, anticoagulant volume, and AC ratio. For example, in HES (0.8%) group, it was calculated as follows: HES concentration in chamber (%) = (6% × 500 mL / 580 mL) × (1/6.5) = 0.8 %.

CE analysis

CE was calculated based on the average of the pre- and post-apheresis peripheral blood counts (CE1) or pre-apheresis granulocyte count (CE2) [19]. We only evaluated CE2 (%), which was determined using the pre-apheresis absolute peripheral blood granulocyte counts as follows: CE (%) = {Total number of granulocytes collected × 10^{-4}} / {peripheral granulocyte counts × blood volume processed (L)}.

Statistical analysis

Values are presented as mean ± standard error unless otherwise specified. We used a one-way analysis of variance to compare more than two groups. Pearson's correlation coefficient was utilized to assess correlation intensity, and the strength of the correlation was determined by the absolute *r* value in each evaluation, as previously described [20]. Statistical significance was set at $p < 0.05$, and all tests were two-tailed. We used GraphPad Prism 6 software (GraphPad, Inc., La Jolla, CA, USA) for each analysis.

Results

Our study population comprised 40 donors who underwent 60 granulocyte harvest

procedures using the Spectra Optia system between July 2013 and December 2021 (Table 2). Our analysis revealed that 20 donors underwent granulocyte apheresis twice, with no one receiving it more than two times. The donors who underwent second granulocyte apheresis were subjected to the same apheresis method as the first. Eight apheresis procedures were performed on four donors in the hHES group, eight procedures were performed on seven donors in the m0.46 group, 15 procedures were performed on ten donors in the m0.44, and the m0.8 groups and 14 procedures were performed on nine donors in the m0.37 group. No significant difference was observed between the five apheresis methods in terms of donor age, sex, body weight, pre-apheresis hematocrit, and the number of donors who underwent two apheresis procedures. However, there was a slight disparity across the five apheresis method groups for pre-apheresis white blood cell (WBC) count, absolute neutrophil count (ANC), and platelet (PLT) count (Table 2).

First, we evaluated the apheresis procedures. The mean processing time for granulocyte apheresis for the hHES group was 114.8 ± 4.5 min, 114.1 ± 3.9 minutes for m0.46 group, 176.0 ± 3.0 min for m0.44 group, 145.3 ± 5.0 min for m0.37 group, and 109.7 ± 3.1 min for m0.8 group (Figure 1A). That was the longest for the m0.44 group, followed by the m0.37 group, which was significantly longer than the other three groups. Mean processed blood volume (PBV) and product volume were 6721 ± 19.8 mL and 555.8 ± 1.9 mL for

204 hHES group, 5607 ± 62.1 mL, and 481.9 ± 5.1 mL for m0.46 group, 6651 ± 39.2 mL, and
205 525.7 ± 3.3 mL for m0.44 group, 7276 ± 55.6 mL, and 564.6 ± 17.8 mL for m0.37 group,
206 and 3346 ± 27.6 mL and 262.1 ± 2.6 mL for m0.8 group, respectively (Figure 1B,C). Data
207 analysis revealed that mean PBV and product volume in the m0.8 group were
208 significantly lower than in the other groups. Of note, adjustment of the collection
209 preference from the initial settings was not necessary for 2 of 8 procedures in the hHES
210 and m0.46 groups, 4 of 15 procedures in the m0.44 group, 3 of 15 procedures in m0.37
211 group, and 10 of 15 procedures in the m0.8 group.
212 Next, we evaluated the apheresis products. The mean total granulocyte count per
213 apheresis product was 7.7 ± 0.67 × 10^{10} for hHES group, 1.5 ± 0.14 × 10^{10} for m0.46
214 group, 2.7 ± 0.39 × 10^{10} for m0.44 group, 3.2 ± 0.78 × 10^{10} for m0.37 group, and 2.8 ±
215 0.29 × 10^{10} for m0.8 group (Figure 2A). Results indicated that the mean total granulocyte
216 count in the hHES group was significantly higher than in the other groups. The target of
217 1.0 × 10^{10} granulocytes was achieved in 8 of 8 procedures in the hHES group, 7 of 8
218 procedures in the m0.46 group, 13 of 15 procedures in the m0.44 group, 14 of 15
219 procedures in the m0.37 group, and 15 of 15 procedures in the m0.8 group. Mean
220 granulocyte CE was 28.1 ± 2.1% for hHES group, 8.0 ± 0.8% for mHES (0.46%) group,
221 15.0 ± 2.0% for m0.44 group, 11.1 ± 2.2% for m0.37 group, and 24.0 ± 1.9% for m0.8

group (Figure. 2B). Data analysis revealed that mean granulocyte CE of the hHES and m0.8 groups were comparable and significantly higher than the other groups. The proportions of monocytes and lymphocytes in leukocyte containing apheresis products were 3.8 ± 0.8% and 7.6 ± 2.5% for hHES group, 14.1 ± 1.4% and 17.1 ± 2.1% for m0.46 group, 5.1 ± 1.3% and 12.8 ± 2.5 % for m0.44 group, 12.6 ± 1.6% and 18.3 ± 2.9% for m0.37 group, and 2.2% ± 0.9% and 7.8 ± 1.1% for m0.8 group, respectively (Figure 2C, D). Monocyte and lymphocyte proportions in the m0.8 group were comparable to those in the hHES group. Hematocrit in the apheresis product was 4.7 ± 0.36% for hHES group, 19.5 ± 0.7% for m0.46 group, 23.9 ± 0.7% for m0.44 group, 20.8 ± 1.1% for m0.37 group, and 12.6 ± 1.2% for m0.8 group (Figure 2E). Hematocrit of the m0.8 group was higher than that of the hHES group but significantly lower than the other three groups using HES130/0.4. No difference was observed in the percentage of platelet count contamination in apheresis products between the five groups (Figure 2F). In contrast, the absolute number of platelets in the product was $17.3 \pm 1.3 \times 10^{10}$ for hHES group, 14.1 ± 1.3×10^{10} for m0.46 group, $12.3 \pm 0.9 \times 10^{10}$ for m0.44 group, $15.9 \pm 0.8 \times 10^{10}$ for m0.37 group, and $7.5 \pm 0.5 \times 10^{10}$ for m0.8 group (Figure 2G) and was significantly lower in m0.8 group compared with the other groups.

Next, we assessed the correlation between the granulocyte CE and donor's hematocrit

among apheresis performed with HES130/0.4. There was a non-significant but positive correlation trend in the m0.46 (r = 0.67, p = 0.07), m0.44 (r = 0.41, p = 0.13), and m0.37 groups (r = 0.45, p = 0.09). In addition, when these three groups were combined, which had lower HES130/0.4 concentrations in the chamber than the m0.8 group, there was a weak positive correlation between granulocytes CE and hematocrit (r = 0.37, p = 0.02) (Figure 3A). However, in the m0.8 group, there was no correlation between granulocyte CE and hematocrit (r = 0.10, p = 0.71) (Figure 3B).

Finally, we assessed adverse events in donors. Citrate reactions during granulocyte apheresis were recorded in 1 of 8 procedures in each of the hHES and m0.46 groups, none in the m0.44 and m0.8 groups, and 3 of 14 procedures in m0.37 group. No further adverse events during the apheresis procedure were recorded. At the follow-up one month after granulocytes donation, no significant changes were observed in serum creatinine level compared to that before the donation in HES130/0.4 groups with adequate records (Figure 4). The serum creatinine levels were available for analysis in the one donor in m0.37, three in m0.44, and 10 in m0.8 groups.

Discussion

This is the first report comparing hHES with HES130/0.4 in granulocyte collection performed using the Spectra Optia system in a single-center experiment. Our findings demonstrated that granulocyte apheresis with m0.8 had the same collection efficiency as with hHES ~~but with less contamination~~. In addition, every procedure in the m0.8 group could provide the desired granulocyte counts of 1×10^{10}. Notably, the collection by m0.8 has a faster processing time and fewer PBV to achieve successful apheresis compared to the previous studies using HES130/0.4[12,13]. Maintaining a high HES130/0.4 concentration in the separation chamber might have resulted in the efficient collection of granulocytes.

Firstly, we observed that the m0.8 group performed granulocyte apheresis in a shorter time and had less PBV than other groups. The PBV depended on the AC ratio and total volume of HES and anticoagulant. This was attributed to the AC ratio set to 6.5:1 and the rapid consumption of mHES containing anticoagulant solution which was the lowest volume among mHES groups. Generally, a large quantity of PBV, such as 7–10 L, is required to collect an adequate dose of granulocytes [21]. Citrate anticoagulant administration during longer procedures results in citrate accumulation [22]. Common

274 complications associated with citrate anticoagulant administration include hypocalcemia.
275 For donor safety, it might be crucial to minimize the processing time and PBV. In contrast,
276 the granulocyte CE in the m0.8 group was similar to the hHES group and was not inferior
277 to other reports with HES130/0.4[12,13]. Although there was no significant difference, the
278 CE tended to be highest with the m0.44 method, with slowest flow rates of 40 mL/min
279 among m0.46, m0.44, and m0.37. Fixing the flow rate to 40 mL/min in the m0.8 method
280 may have also contributed to the stabilization of CE. From all m0.8 group donors, more
281 than 1×10^{10} granulocytes was collected. These results indicated that granulocyte
282 apheresis using the m0.8 method could rapidly collect a significant quantity of
283 granulocytes, thereby reducing the physical burden on the donor. Notably, the m0.8 group
284 had fewer preference adjustments based on real-time monitoring of hematocrits in the
285 apheresis product than the other groups. Therefore, the m0.8 use seemed to be an easy-
286 to-operate method with stable yields.
287 Next, we demonstrated that a high HES130/0.4 concentration in the separation chamber
288 of spectra Optia enabled stable granulocyte yields regardless of donor hematocrit values.
289 As previously reported [13], in granulocyte apheresis using the approach of low HES130/0.4
290 concentration in the separation chamber (Figure 3A), a low donor hematocrit negatively
291 affected granulocyte CE. Erythrocyte sedimentation rate had been shown to be

292 proportional to HES concentration [18]. This should have been considered in the centrifugal
293 environment, where a high plasma-to-blood ratio (i.e., low hematocrit) could adversely
294 affect the erythrocyte sedimentation rate at low HES concentrations. Moreover, these
295 methods increased erythrocyte, monocyte, and lymphocyte contamination rates compared
296 to the m0.8 group (Figure 2). RBCs, granulocytes, and lymphocytes are distributed by
297 size, with erythrocytes and granulocytes in close proximity [23]. Therefore, a high
298 concentration of HES130/0.4 may effectively segregate granulocytes along the
299 centrifugal gradient.
300 Notably, we confirmed granulocyte apheresis using hHES, which is an established
301 technique. Granulocyte apheresis with hHES collected the highest number of
302 granulocytes among the methods tested. However, recent investigations have raised
303 safety concerns for HESs in patients with sepsis [24–26]. In contrast, studies using
304 HES130/0.4 in patients with penetrating trauma [27], sepsis [28], and surgery [16,17,29] revealed
305 that its safety profile was comparable to saline or human albumin in terms of mortality,
306 acute kidney injury, and hemorrhage. Although the actual volume of HES infused into
307 granulocyte donors is smaller than that given to the patients included in these studies,
308 there is little evidence of HES effects on healthy donors. Hence, we used HES130/0.4,
309 which is considered safer than hHES. Despite the modest number of cases, no adverse

effects such as renal damage or bleeding were noted following the use of HES130/0.4 in our study. HES130/4.0 might be safer than other HES for healthy donors, although further studies are needed to confirm its safety because the pathophysiology of these patients differs from healthy donors.

The study may have some potential limitations. First, this is a retrospective study with a limited sample size for each group. However, the results reported and the numerous procedures we had undertaken are beneficial for future considerations regarding the improvement of the granulocytes apheresis method. Second, this trial was limited to the apheresis method and did not examine the clinical impacts of the number of infused granulocytes on the recipients. While a recent study suggested that a higher number of infused granulocytes would be more effective for clinical outcome [2], others have disputed this claim [30], and there is still no consensus regarding the optimal granulocyte dose. For this reason, our institution accepted the AABB criteria and established the target value of 1.0×10^{10} for granulocyte collection.

However, some researchers suggest that infusing more granulocytes is preferable. One expert recommends a dose of 6–8 × 10^{10} per granulocyte transfusion given daily, with a minimal dose of 4 × 10^{10} [31]. With the m0.8 method, a median of 2.8 × 10^{10} granulocytes was collected with a median PBV volume of 3346 mL. As the blood volume of an adult

is approximately 5000 mL, a yield of approximately 5×10^{10} can be expected if 6692 mL of blood—twice the volume used here—is processed using the m0.8 method. This would require 1000 mL of HES130/0.4; however, to the best of our knowledge, there are no data on this dosing in healthy donors. In Japan, HES130/0.4 is approved for use up to 50 mg/kg/day for patients, and as 1000 mL is considerably less than this, it could be safely used for healthy donors. Therefore, an attempt to collect more granulocytes using the m0.8 method with an increased volume of HES130/0.4 is worth considering and a subject for future research.

In conclusion, we proposed the method of granulocyte apheresis using HES130/0.4, which could collect a sufficient quantity of granulocytes in a short time, with granulocyte CE comparable to high molecular weight HES, and with simple procedures requiring minimal or no adjustments, while avoiding the donor safety concerns associated with hHES. Granulocyte collection using the m0.8 method can be an important alternative in situations where hHES cannot be used or when its safety is a concern.

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Authorship Contributions

T.K. and K.F.: Designed the study; Y.S., T.U., M.K, M.M., S.I., K.W., H.F., N.A., H.N., and K.M: Contributed to the data collection; T.K.: Analyzed the data; T.K., K.F., and N.F.: Wrote the paper; F.O. and Y. M.: Supervised the studies and edited the paper.

References

1. Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H, et al. Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: An analysis of the Japan Transplant Outcome Registry. Bone Marrow Transplant. 2013 Apr;48(4):529–36.

2. Price TH, Boeckh M, Harrison RW, McCullough J, Ness PM, Strauss RG, et al. Efficacy of transfusion with granulocytes from G-CSF/dexamethasone–treated donors in neutropenic patients with infection. Blood. 2015 Oct 29;126(18):2153–61.

3. Freireich EJ. Leukocyte transfusion and the development of the continuous-flow blood cell separator. Transfus Med Rev. 2011 Oct;25(4):344–50.

4. Janes AW, Mishler JM, Lowes B. Serial infusion effects of hydroxyethyl starch on ESR, blood typing and crossmatching and serum amylase levels. Vox Sang. 1977 Mar;32(3):131–4.

5. Stroncek DF, Yau YY, Oblitas J, Leitman SF. Administration of G-CSF plus dexamethasone producesgreater granulocyte concentrate yields while causing nomore donor toxicity than G-CSF alone. Transfusion (Paris). 2001 Aug;41(8):1037–44.

6. Strauss RG, Klein HG, Leitman SF, Price TH, Lichtiger B, Martinez F, et al. Preparation of granulocyte concentrates by apheresis: Collection modalities in the USA. Vox Sang. 2011 May;100(4):426–33.

7. Lee J, Leitman S, Klein H. A controlled comparison of the efficacy of hetastarch and pentastarch in granulocyte collections by centrifugal leukapheresis. Blood. 1995 Dec 15;86(12):4662–6.

8. Ikemoto J, Yoshihara S, Fujioka T, Ohtsuka Y, Fujita N, Kokubunji A, et al. Impact of the mobilization regimen and the harvesting technique on the granulocyte yield in healthy donors for granulocyte transfusion therapy. Transfusion (Paris). 2012 Dec;52(12):2646–52.

9. Bux J, Cassens U, Dielschneider T, Duchscherer M, Edel E, Eichler H, et al. Tolerance of granulocyte donors towards granulocyte colony-stimulating factor stimulation and of patients towards granulocyte transfusions: Results of a multicentre study. Vox Sang.

388 2003 Nov;85(4):322–5.

389 10. Li B, Zhao H, Zhang J, Yan Q, Li T, Liu L. Resuscitation fluids in septic shock: A
390 network meta-analysis of randomized controlled trials. Shock. 2020 Jun;53(6):679–85.

391 11. Auwerda JJA, Leebeek FWG, Wilson JHP, van Diggelen OP, Lam KH, Sonneveld P.
392 Acquired lysosomal storage caused by frequent plasmapheresis procedures with
393 hydroxyethyl starch. Transfusion (Paris). 2006 Oct;46(10):1705–11.

394 12. Nanya M, Yurugi K, Kato I, Hiramatsu H, Kawabata H, Kondo T, et al. Successful
395 granulocyte apheresis using medium molecular weight hydroxyethyl starch. Int J
396 Hematol. 2019 Dec;110(6):729–35.

397 13. Henzan T, Yamauchi T, Yamanaka I, Sakoda T, Semba Y, Hayashi M, et al. Granulocyte
398 collection by polymorphonuclear cell-targeting apheresis with medium-molecular-
399 weight hydroxyethyl starch. Int J Hematol. 2021 Dec;114(6):691–700.

400 14. Mandal S, Naim F, Kumar R, Gupta S, Gupta VR, Kathuria I. A pilot study on impact of
401 use of medium molecular weight hydroxyethyl starch in granulocyte apheresis using
402 Spectra Optia. Transfus Apher Sci. 2022 Mar;61(5):103436.

403 15. Waitzinger J, Bepperling F, Pabst G, Opitz J. Hydroxyethyl starch (HES) [130/0.4], a
404 new HES specification: pharmacokinetics and safety after multiple infusions of 10%
405 solution in healthy volunteers. Drugs R D. 2003;4(3):149–57.

406 16. Wei L, Li D, Sun L. The comparison of albumin and 6% hydroxyethyl starches (130/0.4)
407 in cardiac surgery: A meta-analysis of randomized controlled clinical trials. BMC Surg.
408 2021 Dec;21(1):342.

409 17. Jacob M, Fellahi JL, Chappell D, Kurz A. The impact of hydroxyethyl starches in cardiac
410 surgery: A meta-analysis. Crit Care. 2014 Dec;18(6):656.

411 18. Liu FC, Liao CH, Chang YW, Liou JT, Day YJ. Hydroxyethyl starch interferes with
412 human blood ex vivo coagulation, platelet function and sedimentation. Acta
413 Anaesthesiol Taiwan. 2009 Jun;47(2):71–8.

414 19. Cancelas JA, Scott EP, Bill JR. Continuous CD34+ cell collection by a new device is safe
415 and more efficient than by a standard collection procedure: results of a two-center,
416 crossover, randomized trial. Transfusion (Paris). 2016 Nov;56(11):2824–32.

20. Evans JD. Straightforward statistics for the behavioral sciences. Pacific Grove : Brooks/Cole Pub. Co.; 1996.

21. Klein K, Castillo B. Historical perspectives, current status, and ethical issues in granulocyte transfusion. Ann Clin Lab Sci. 2017;47(4):7.

22. Bolan CD, Cecco SA, Wesley RA, Horne M, Yau YY, Remaley AT, et al. Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. Transfusion (Paris). 2002 Jul;42(7):935–46.

23. Bøyum A, Løvhaug D, Tresland L, Nordlie EM. Separation of leucocytes: Improved cell purity by fine adjustments of gradient medium density and osmolality. Scand J Immunol. 1991 Dec;34(6):697–712.

24. Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med. 2008 Jan 10;358(2):125–39.

25. Perner A, Haase N, Guttormsen AB, Tenhunen J, Klemenzson G, Åneman A, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med. 2012 Jul 12;367(2):124–34.

26. Qureshi SH, Rizvi SI, Patel NN, Murphy GJ. Meta-analysis of colloids *versus* crystalloids in critically ill, trauma and surgical patients. Br J Surg. 2015 Dec 15;103(1):14–26.

27. James MFM, Michell WL, Joubert IA, Nicol AJ, Navsaria PH, Gillespie RS. Resuscitation with hydroxyethyl starch improves renal function and lactate clearance in penetrating trauma in a randomized controlled study: The FIRST trial (Fluids in Resuscitation of Severe Trauma). Br J Anaesth. 2011 Nov;107(5):693–702.

28. Guidet B, Martinet O, Boulain T, Philippart F, Poussel JF, Maizel J, et al. Assessment of hemodynamic efficacy and safety of 6% hydroxyethylstarch 130/0.4 vs. 0.9% NaCl fluid replacement in patients with severe sepsis: The CRYSTMAS study. Crit Care. 2012 Jun;16(3):R94.

29. Futier E, Garot M, Godet T, Biais M, Verzilli D, Ouattara A, et al. Effect of hydroxyethyl starch vs saline for volume replacement therapy on death or postoperative complications among high-risk patients undergoing major abdominal surgery: The FLASH randomized clinical trial. JAMA. 2020 Jan 21;323(3):225–36.

30. Lee JM, Choi SJ, Kim HS, Yang M, Kim Y, Lee JW, et al. Analysis of hematologic parameters of donors, patients, and granulocyte concentrates to predict successful granulocyte transfusion. BLOOD Res. 2019 Mar 31;54(1):52–6.

31. Strauss RG. Commentary on white blood cell transfusions for control of infections in neutropenic patients by Vallejos C. (Transfusion 1975; 15(1):28–33). Transfusion (Paris). 2022 Apr;62(4):731–9.

Figure Legends

Figure 1. Outcomes of granulocyte apheresis procedures. Processing time (A), processed blood volume (PBV) (B), and product volume (C) for granulocyte apheresis are depicted. Data are expressed as the mean ± SE of the mean and are presented in Supplemental Table 1. *p < 0.05, **p < 0.01 ***p < 0.001, ****p < 0.0001.

Figure 2. Analysis of granulocyte apheresis products. Granulocyte counts in apheresis products are presented in terms of their absolute number (A), collection efficiency (CE) (B), populations of monocytic (Mn) (C), and lymphocytic (Ly) leukocytes (D), hematocrit (Ht) (E), and the absolute number of platelets (Plt) (F). Data are expressed as the mean ± SE of the mean and are presented in Supplemental Table 1. *p < 0.05, **p < 0.01 ***p < 0.001, ****p < 0.0001.

Figure 3. Correlation between granulocyte CE values and donor peripheral blood hematocrit before initiating apheresis using HES130/0.4. A positive correlation was observed between the granulocyte CE and donor hematocrit in low concentration HES groups [m0.46, m0.44, and m0.37] in the apheresis chamber (A) but not in m0.8 (B). The line of best fit and 95% confidence intervals are depicted in (A).

Figure 4. **HES did not affect renal function.** Serum creatinine levels were measured prior to granulocyte apheresis and during follow-up visits one month later.

Table 1. Summary of the apheresis settings

	HES	Anti-coagulant	AC ratio	CP	Blood flow rate	Ceoncentration in chamber (%)	
						HES	CA
(1) hHES	6%HES400/0.7 400 mL	ACD-A 500 mL	8.5:1	60	60 mL/min (max 75 mL/min)	0.31	0.20
(2) m0.46	6%HES130/0.4 500 mL	ACD-A 500 mL	6.5:1	27	40 mL/min (max 70 mL/min)	0.46	0.23
(3) m0.44	6%HES130/0.4 500 mL	46.7% sodium citrate hydrate 30 mL	13:1	35	Default (max 40 mL/min)	0.44	0.20
(4) m0.37	6%HES130/0.4 500 mL	10% sodium citrate hydrate 175 mL	12:1	27	40 mL/min (max 70 mL/min)	0.37	0.22
(5) m0.8	6%HES130/0.4 500 mL	10% sodium citrate hydrate 80 mL	6.5:1	40	40 mL/min	0.80	0.21

HES: hydroxyethyl starch, CA: citric acid, AC: anticoagulant; CP: collection preference; ACD-A: anticoagulant citrate dextrose solution A

Table 2. Granulocyte donor characteristics

	hHES	m0.46	m0.44	m0.37	m0.8	*p*-value
Median age (years, range)*	26 (24–60)	42 (35–66)	48 (25–57)	41 (23–50)	49.5 (21–59)	0.93
Sex (male/female) †	3 / 1	4 / 3	5 / 5	5 / 4	5 / 5	0.86
Body weight (kg, range)*	60.6 (52.0–87.0)	63.0 (53.0–70.6)	60.8 (49.0–92.5)	58.9 (40.7–76.7)	57.2(46.8–115.7)	0.88
Baseline WBC (/μL) ‡	43,680 ± 2929	36,933 ± 3098	29,269 ± 2283	41,167 ± 2311	37,387 ± 2976	<0.01
Baseline ANC (/μL) ‡	40,956 ± 2727	33,732 ± 2936	27,598 ± 2203	38,097 ± 2319	35,653 ± 3059	0.01
Baseline Ht (%) ‡	43.4 ± 1.40	43.2 ± 1.54	42.3 ± 0.91	41.5 ± 1.13	42.0 ± 1.62	0.27
Baseline Plt ($\times 10^4$/μL) ‡	25.5 ± 1.36	27.2 ± 1.30	24.3 ± 1.67	27.1 ± 1.28	26.2 ± 2.20	0.04
Number of the previous collections†						0.65
0	4	7	10	9	10	
1	4	1	5	5	5	

WBC: white blood cell; ANC: absolute neutrophil count; Ht: hematocrit; Plt: platelet.

The symbols denote median with range (*), number (†), and mean with SEM (‡). The p-value was determined using one-way ANOVA.