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Is hydroxyethyl starch necessary for sedimentation of bone marrow?

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ABSTRACT

Hydroxyethyl starch (HES) is used to separate hematopoietic progenitor cells after bone marrow (BM) collection from red blood cells. The aims were to study alternatives for HAES-steril (200 kDa; not available anymore) and to optimize the sedimentation process. Using WBC-enriched product (10×10^9 WBC/L), instead of BM, sedimentation at 10% hematocrit using final 0.6 or 0.39% Voluven (130 kDa) or without HES appeared to be good alternatives for 0.6% HAES-steril. MNC recovery >80% and RBC depletion >90% was reached. Optimal sedimentation was reached using 110–140 mL volume. Centrifugation appeared not suitable for sedimentation. Additional testing with BM might be necessary to confirm these results.

1. Introduction

Hematopoietic progenitor cells (HPC) from patients or donors can be collected either after stimulation using growth factors (e.g. G-CSF) by apheresis or by bone marrow (BM) collection. Although apheresis is the most common method for adult patients [1,2], for some indications the transplant centers prefer BM [3].

Hydroxyethyl starch (HES) can be used to separate the HPC after BM collection from RBC. Sedimentation can be done by gravity or automated machine processing [4–9].

HES with molecular weights ≥200 kDa will become less available on the market because of its adverse effects when used as plasma volume expander. Adverse effects of HES when used as plasma expander are impairment of coagulation, renal function impairment, inhibitory effect on platelet function, deterioration of rheological parameters, and accumulation in tissues [8–10]. Although the volume effect is prolonged for higher molecular weight HES compared to lower molecular weight HES, adverse effects of HES are more pronounced for higher molecular weight HES [8–10]. However, no adverse effects are described for HES when used for sedimentation of BM, probably because of the low volumes of HES that are transfused.

For HPC from BM, RBC depletion is needed especially in case of AB0 incompatibility, to avoid AB0 induced transfusion reactions. Another reason for RBC depletion is volume reduction to reduce cryogenic storage space or in the case of allogeneic transplantation in the pediatric setting, irrespective of the blood group [11–13].

The standard procedure in our processing facility is as follows: BM is filtrated and diluted to a concentration of 10×10^9 WBC/L and divided over 400-mL bags with 140–200 mL per bag, after which 9:1 (v/v) HAES-steril 6% (final 0.6%) is added. After sedimentation for 60–90 min the plasma and buffy coat are pressed into a satellite bag (Table 1).

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Table 1
Values for BM processing (n = 11).

	Mean ± SD	Range
Volume, mL	1386 ± 373	458–1891
WBC, $\times 10^9$/L	17.6 ± 7.9	9.7–36.7
Hematocrit, %	31.4 ± 6.7	21.5–41.7
Dilution factor	0.6 ± 0.2	0.3–1.0
Volume after dilution, mL	2432 ± 1087	948–4509
WBC after dilution, $\times 10^9$/L[a]	9.8 ± 0.9	7.3–11.1
Hematocrit after dilution, %[a]	19.9 ± 7.9	7.7–34.3
Number of bags processed	14 ± 6	6–24
Volume per bag, mL[b]	168 ± 13	146–188
MNC recovery, %	88 ± 15	67–119
RBC depletion, %	95 ± 2	93–99

[a] Calculated from values before dilution and dilution factor.
[b] Calculated from total volume and number of bags processed.

The objective of this study was to investigate whether sedimentation of BM using 0.6% HAES-steril (200 kDa/0.5; standard) can be substituted by sedimentation using 0.6% or 0.39% Voluven (130 kDa/0.4) or by sedimentation without HES. The second objective was to optimize the sedimentation process of BM in relation to the volume of the product before sedimentation and sedimentation by centrifugation instead of by gravity.

2. Materials and methods

2.1. WBC-enriched product

Because BM derived HPC products are not readily available for research purposes, a WBC-enriched product from buffy coats and plasma, with WBC, RBC and platelet counts in the same range as real BM, but absence of $CD34^+$ cells or immature cells, was used in this study. The preparation of a WBC-enriched product was as follows: Five buffy coats and one plasma unit, derived from overnight stored whole blood, were pooled and centrifuged using a soft spin (930 *g* for 4.5 min, brake 3). The platelet rich plasma was used to form a therapeutic platelet concentrate. The sediment was stored overnight at RT on a flat bed shaker. Next day, a 2 mL sample was taken and WBC count was determined. The sediment was diluted using plasma until a WBC concentration of about 10×10^9/L and a hematocrit of 7–11% in a total volume of about 600 mL were obtained resulting in the WBC-enriched product. If necessary the plasma was pressed in opposite direction over a WBC reduction filter (Compostop, Fresenius Hemocare; Emmer Compascuum, The Netherlands) that was used for the preparation of the platelet concentrate, to increase the number of WBC.

2.2. The influence of using HAES-steril, Voluven or no HES

The WBC-enriched product was equally divided over four 400-mL transfer bags (about 140 mL per bag; R4R2074; Fenwal, Inc. Lake Zurich, USA). After sampling and weighing the product its volume was calculated and from this the amount of HES necessary to result in either 0.6% or 0.39% HES or without HES. For the 140 mL WBC-enriched product 15.6 mL or 9.7 mL HES was added with continuous gentle mixing, to reach a final 0.6% or 0.39% HES respectively. This resulted in four bags, containing (1) WBC-enriched product and 0.6% HAES-steril (standard), (2) WBC-enriched product and 0.6% Voluven, (3) WBC-enriched product and 0.39% Voluven, and (4) WBC-enriched product without HES. The products with 0.6% HAES-steril and 0.39% Voluven have the same molarity for the HES (3 μM). All bags were allowed to sediment by gravity for 90–105 minutes and were placed into a manual press. The supernatant including the buffy coat was pressed into an empty 400 mL transfer bag. This bag was disconnected from the bag containing the sediment that was discarded. After weighing, the bag containing the supernatant was sampled (1 mL sample).

2.3. The influence of the volume of the product before sedimentation

Four WBC-enriched products were pooled, mixed and split into 15 portions, three of 110 mL, three of 140 mL, three of 170 mL, three of 200 mL and three of 230 mL. To each bag HES or no HES was added resulting in the variations 0.6% HAES-steril, 0.39% Voluven or without HES for each volume. These bags were allowed to sediment by gravity and the supernatant was collected as described above.

2.4. Sedimentation by centrifugation or gravity

Two WBC-enriched products were pooled, mixed and split into three portions of 350 mL. To each bag HES or no HES was added resulting in the variations 0.6% HAES-steril, 0.39% Voluven or without HES. Subsequently, each bag was split in a bag containing 185 mL WBC-enriched product with additional HES or without HES and a bag containing 140 mL WBC-enriched product with additional HES or without HES. The bags with 185 mL WBC-enriched product and HES or without HES were centrifuged at 250 *g* for 10 min at RT and brake at 2. Volume and forces were optimized in pilot experiments (not shown). The bags containing 140 mL WBC-enriched product and HES or without HES were allowed to sediment by gravity and the supernatant of all bags was collected as described above.

2.5. Tests

Samples were taken of the WBC-enriched product before adding HES and after sedimentation from the supernatant. WBC counts (including differentiation), RBC counts and hematocrit were determined using the Sysmex XT 1800i (Sysmex, TOA, Japan). If the cell count was out of the linear range, samples were diluted using Cell Pack (Sysmex). The percentages of MNC recovery and RBC depletion were calculated. The MNC consists of monocytes and lymphocytes. A visual inspection of the distinction between sediment and supernatant after sedimentation for 90–105 minutes was performed.