Platelet concentrates from fresh or overnight-stored blood, an international study

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BACKGROUND: Whole blood and also buffy coats (BCs) can be held for a few hours or overnight before processing into blood components or platelet concentrates (PCs). Individual studies have reported a range of outcomes regarding in vitro variables for PCs prepared from fresh and stored whole blood. In this multicenter study, effects of storage of whole blood or BCs on the in vitro quality of PCs were studied.

STUDY DESIGN AND METHODS: The leukoreduced BC PCs were prepared from fresh BCs (2-8 hr after collection; fresh/fresh), from BCs at 20 to 24 hours after collection (fresh/stored), or from BCs prepared from whole blood stored for 20 to 24 hours (stored/fresh). PCs were stored on a flat-bed shaker at 20 to 24°C for 7 days. PCs were tested on Days 0 (only fresh/fresh), 1, 5, and 7 for in vitro quality. There were six participating centers that tested all three conditions with n = 6 per condition.

RESULTS: In comparison to fresh/stored and stored/fresh PCs, fresh/fresh PCs exhibited a lower platelet (PLT) count (Day 1—$220 \times 10^9 \pm 70 \times 10^9$ vs. $324 \times 10^9 \pm 50 \times 10^9$ and $368 \times 10^9 \pm 56 \times 10^9$ PLTs/PC), lactate, pCO_2, and hypotonic shock response (HSR; Days 5 and 7; Day 7—50 ± 13% vs. 57 ± 12 and 63 ± 11%) and a higher pH, glucose, pO_2, and CD62P expression (than stored/fresh PCs only; Day 7—33 ± 10% vs. 28 ± 12 and 24 ± 11%; $p < 0.05$). No differences were observed for volume, swirling effect, white blood cell count, annexin V binding, or aggregation between these conditions.

CONCLUSION: Based on PLT count, HSR, and PLT activation, PCs are best prepared after 20 to 24 hours hold of the whole blood or BCs.

Platelet (PLT) concentrates (PCs) can be prepared by immediate processing of fresh whole blood. For this approach the advantages are that red blood cells (RBCs) can be refrigerated within 8 hours after collection, which leads to optimal 2,3-diphosphoglycerate acid (2,3-DPG) and adenosine triphosphate (ATP) levels, and plasma can be frozen rapidly, leading to higher Factor (F)VIII levels, since it is known that storage of whole blood or nonfrozen plasma results in FVIII loss up to 1% per hour.[1-3] In a number of countries, it is mandatory to process the whole blood on the day of collection due to concerns over bacterial contamination if blood is stored at ambient temperature for longer periods. Alternatively, PCs can be prepared from buffy coats (BCs) separated from whole blood within a few hours of collection and then stored at ambient temperature overnight. A third option is to store whole blood overnight at room temperature and then process it to PLTs. This is the normal procedure in many European countries and Canada, but is not yet permitted in the United States. Studies have shown that overnight storage of whole blood can reduce the risk of bacterial contamination since bacteria will be phagocytosed by white blood cells (WBCs).[4-6] Furthermore, the advent of recombinant FVIII has reduced demand for plasma-derived FVIII concentrates meaning the FVIII content of plasma is less critical. For RBCs, it is

ABBREVIATIONS: BC(s) = buffy coat(s); HSR = hypotonic shock response; PC(s) = platelet concentrate(s).

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TABLE 1. Whole blood/BC storage methods studied

Whole blood processing*	PC preparation*	Short description	Number
2-8 hr	2-8 hr	fresh/fresh	35
2-8 hr	20-24 hr	fresh/stored	36
20-24 hr	20-24 hr	stored/fresh	36

* After blood collection.

known that in the body 2,3-DPG will be replenished in a few hours after transfusion.[7] For all these reasons the benefits of immediate processing are less than in the past and there are strong logistic and operational advantages of using overnight storage to allow blood processing during normal business hours.[8]

For PCs there is no consensus between studies on the comparative in vitro quality of PCs prepared from fresh or stored blood. A lower PLT yield, a higher degree of PLT activation, and higher pH for PCs from fresh whole blood compared to PCs from stored whole blood or BCs has been described.[9,10] These differences are hypothesized to be due to PLT aggregates in the fresh PCs. However, in other studies no differences between these different processing approaches were observed.[2,11]

To investigate the effect of overnight storage of either whole blood or BCs on the preparation of PCs by the BC method, a standardized multicenter study was performed. Three preparation methods were compared (Table 1): PCs prepared from fresh whole blood on the day of collection (fresh/fresh), PCs prepared from BCs from fresh whole blood that are subsequently stored up to 20 to 24 hours after collection at room temperature (fresh/stored), and PCs prepared from whole blood stored for 20 to 24 hours at room temperature (stored/fresh).

MATERIALS AND METHODS

Study design

The evaluation of the use of fresh versus overnight stored whole blood or BCs for preparation of PCs was studied in an unpaired study design. The study consisted of three conditions, PCs prepared from fresh whole blood (fresh/fresh), PCs prepared from overnight-stored BCs from fresh whole blood (fresh/stored), and PCs prepared from overnight stored whole blood (stored/fresh; Table 1).

The six participating centers were as per author affiliations. All centers tested all three conditions with six PCs per condition, except for one center where n = 5 for the fresh/fresh PCs. All PCs were prepared using four to six BCs and 100% plasma and all were leukoreduced according to the centers' standard methods. Sampling was undertaken on Days 0 (only fresh/fresh), 1, 5, and 7. Day 0 was the day of blood collection.

The primary endpoint was PLT count in the PC. The secondary endpoints were volume, swirling effect, WBC count, pH, glucose, lactate, pO_2, pCO_2, PLT surface CD62P expression, annexin V binding (performed by four centers), PLT aggregation using adenosine 5′-diphosphate (ADP) or collagen (performed by three centers) and hypotonic shock response (HSR). Whole blood was collected, stored, and processed to PCs according each centers standard operating procedures (Table 2), except for the storage times to meet the criteria of each study condition.

In vitro assays

All in vitro assays were undertaken according to the established and validated method used in the individual centres. In general the methods were as follows: PLT count was determined using an automated cell analyser (Sysmex, Toa, Japan; Advia; Bayer, Leverkusen, Germany or Cellguard, Boule Medical, Stockholm, Sweden). WBC count was determined using a bead-based flow cytometric method (LeucoCOUNT on the FACSCalibur or FACS CANTO-II, BD Biosciences, San Jose, CA). pH was measured with a blood gas analyzer (Rapidlab, Siemens Medical Solutions Diagnostics BV, Breda, the Netherlands; Radiometer, Copenhagen, Denmark or Gem Premier 3000, Instrumentation Laboratory, Bedford, MA) and sampling using a sampling bag or syringe and needle, or pH was measured using a general pH meter (Mettler Toledo Ltd; Leicester, UK). The pO_2, pCO_2, glucose and lactate were measured using a blood gas analyzer (together with pH), or glucose and lactate were measured using dry chemistry on a chemistry analyzer (Vitros DT6011, Axis-Shield, Kimbolton, United Kingdom). PLT surface CD62P expression and annexin V binding were measured using flow cytometry (FACSCalibur, FACS CANTI-II or EPICS XL, Beckman Coulter, Miami, FL) and fluorescein isothiocyanate- or phycoerythrin-labeled monoclonals. PLT aggregation using ADP or collagen and HSR were performed using an aggregometer (Chrono-log Corp., Havertown, PA).

Statistical analysis

Data for the three study conditions were compared using an ordinary analysis of variance with Kruskal-Wallis as posttest, calculated by using computer software (Instat, GraphPad, San Diego, CA, 2005). A p value of less than 0.05 was considered to be significant.

RESULTS

General PC variables

The PCs were made at six different centers and showed mean volumes of 336 ± 38, 333 ± 41, and 336 ± 38 mL on

TABLE 2. Conditions of PC preparation per center

Center	BCs per PC	Pooling system	Cooling whole blood	Minimal rest BC*
1	5	Fresenius T3961†	CompoCool†	1 hr
2	4	Fresenius T3961†	CompoCool†	2 hr
3	4	Fenwal Optipure PLT‡	CompoCool†	2 hr
4	6	Orbisac§	Cooling plates Sebra‖	1 hr
5	4	Pall ELX¶	No active cooling	4 hr
6	5	Terumo BC pooling kit**	CompoCool†	1 hr

* Between whole blood separation and pooling of BCs (BCs) for PLT production.
† Fresenius Hemocare, Emmer Compascuum, the Netherlands.
‡ Fenwal, Mont Saint Guibert, Belgium.
§ Gambro BCT, Zaventem, Belgium.
‖ Sebra, Tucson, AZ.
¶ Pall, Portsmouth, England.
** Terumo, Tokyo, Japan.

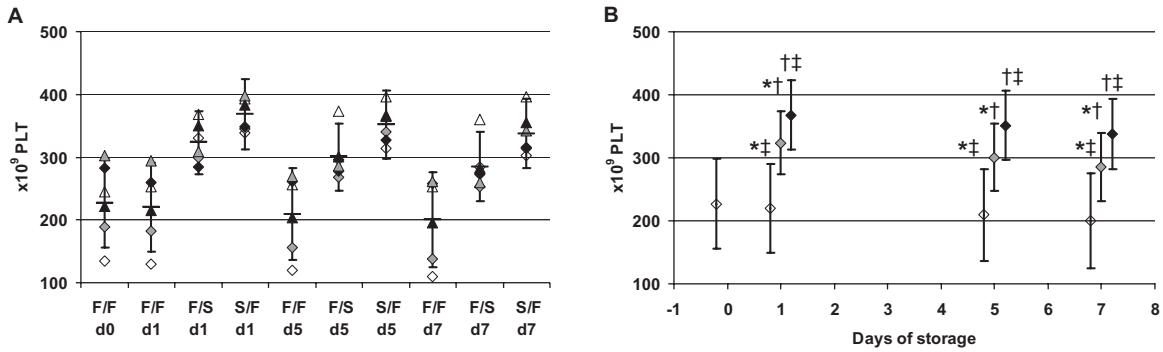

Fig. 1. PLT count per PC per center (A) with results (mean) of Centers 1 (◇), 2 (◈), 3 (◆), 4 (△), 5 (◬), and 6 (▲); overall mean ± SD (—); and PLT count in total (B) with results (mean ± SD) of fresh/fresh (◇), fresh/stored (◈) and stored/fresh PCs(◆). F/F = fresh/fresh; F/S = fresh/stored; S/F = stored fresh; d = day of storage. *p < 0.05 between fresh/fresh and fresh/stored PCs; †p < 0.05 between fresh/stored and stored/fresh PCs; ‡p < 0.05 between fresh/fresh and stored/fresh PCs.

day of preparation for fresh/fresh, fresh/stored, and stored/fresh PCs, respectively (not significant). For all PCs the swirling effect was observed throughout the storage period with no significant differences among the three study conditions. All PCs showed WBC values well below the current European acceptance level of 1.0×10^6 per PC, other than two PCs from one center with 1.1×10^6 and 1.4×10^6 WBC for one fresh/stored PC or one stored/fresh PC, respectively.

PLT counts showed high variability among the participating centers, especially for the fresh/fresh PCs that showed a much higher standard deviation than PCs of the other two conditions (Fig. 1). Overall, it can be seen that fresh/fresh PCs had the lowest PLT counts and stored/fresh PCs had the highest PLT counts. The PLT counts were below 60×10^9 PLTs/donor unit, that is, the lower limit according to European guidelines, for 25 of 35 fresh/fresh PCs, while this was the case for only 6 of 36 fresh/stored PCs and for 3 of 36 stored/fresh PCs. The PLT counts were below 55×10^9 PLTs/donor unit, that is, the lower limit according to US and Canadian guidelines, for 22 (fresh/fresh), three (fresh/stored), and two PCs (stored/fresh), respectively. On average the PLT contents of fresh/fresh PCs were 32 and 40% lower than in PCs prepared after overnight storage of BCs or whole blood, respectively.

Metabolism

The $pH_{22°C}$ remained well above 6.8, glucose remained present, and lactate remained below 20 mmol/L during storage for 7 days for all three study conditions (Table 3). The $pH_{22°C}$ and glucose values of fresh/fresh PCs were significantly higher than those of the other two conditions, whereas for lactate the opposite was observed (Fig. 2). The pO_2 was highest for fresh/fresh PCs and remained quite stable during storage. The pCO_2, which decreased 1 day after preparation, was lowest for fresh/fresh PCs.

PLT activation

The PLT surface CD62P expression, which increased during storage, was significantly higher for fresh/fresh PCs

TABLE 3. In vitro quality variables of PCs under various conditions (Day 7, mean ± SD)

Variable	F/F	F/S	S/F	$p < 0.05$
PLTs ($\times 10^9$/PC)	201 ± 75	285 ± 55	338 ± 55	F/F-F/S, S/F; F/S-S/F
$pH_{22°C}$	7.40 ± 0.15	7.30 ± 0.15	7.28 ± 0.14	F/F-F/S, S/F
Glucose (mmol/L)	15.9 ± 1.6	13.5 ± 1.6	13.6 ± 1.7	F/F-F/S, S/F
Lactate (mmol/L)	12.6 ± 2.7	15.8 ± 2.6	15.1 ± 2.2	F/F-F/S, S/F
PO_2 (kPa)	16.9 ± 4.9	13.2 ± 4.3	11.6 ± 5.4	F/F-F/S, S/F
PCO_2 (kPa)	3.0 ± 0.9	3.5 ± 0.8	3.9 ± 1.1	F/F-F/S, S/F
CD62p expression (%)	33.3 ± 9.9	28.0 ± 11.6	24.1 ± 11.0	F/F-S/F
Annexin V binding (%)	30.4 ± 17.4	26.7 ± 18.9	18.4 ± 14.2	
Aggregation using ADP (%)	38.9 ± 16.5	33.8 ± 11.4	40.0 ± 22.2	
Aggregation using collagen (%)	38.0 ± 20.2	45.6 ± 16.5	50.2 ± 30.2	
HSR (%)	49.9 ± 13.4	56.8 ± 12.2	62.9 ± 11.1	F/F-S/F

F/F = fresh/fresh; F/S = fresh/stored; S/F = stored/fresh.

than for stored/fresh PCs throughout storage for 7 days. On Day 7 two centers showed CD62P expression levels higher than 40% for all three study conditions. Annexin V binding showed large differences among the centers with values less than 5% at Day 7 for one center and values higher than 50% for another center. Given this wide range the overall differences in this variable were not significantly different.

PLT in vitro functionality

PLT aggregation using ADP or collagen decreased during storage. On Day 1 there was a significantly higher aggregation using ADP for stored/fresh PCs than fresh/fresh PCs, but this difference disappeared by Day 5 of storage. HSR was well maintained during storage for all three study conditions, with the best response being for stored/fresh PCs.

DISCUSSION

In this multicenter study, the effects of overnight hold of whole blood or BCs at ambient temperature on PCs was compared to PCs prepared without delay. It was found that fresh/fresh PCs showed a lower PLT count, lactate, pCO_2, and HSR compared to fresh/stored and stored/fresh PCs ($p < 0.05$). In addition, a higher pH, glucose, pO_2, and CD62P expression were observed for fresh/fresh PCs compared to fresh/stored and stored/fresh PCs ($p < 0.05$). No significant differences were found for volume, swirling effect, WBC count, annexin V binding, and aggregation using ADP or collagen.

Although fresh/fresh PCs showed worse results for CD62P expression and HSR, the reduced PLT count is probably the only clinically relevant difference observed between the three study conditions. The lower PLT count for fresh/fresh PCs may be explained by the relatively short rest period of the whole blood and BCs such that PLTs are still activated and form aggregates, which are removed during centrifuging or filtration of the PC. Fresh/stored PCs were also prepared from fresh processed whole blood; however, further processing of the separated BCs to PCs was the following day and resulted in higher PLT counts than fresh/fresh, but lower than stored/fresh PCs.

For fresh/fresh PCs a PLT count below the lower limit of acceptance ($<60 \times 10^9$ per donor unit according to European guidelines and $<55 \times 10^9$ per donor unit according to US and Canadian guidelines) was observed in the majority of units. This lack of conformance with these specifications may lead to greater rejection rates of components as substandard, an increased usage of PLT donations, and greater patient exposure to multiple donors, with the risk of an increased frequency of adverse reactions.[12-14]

Differences in PLT count as seen among the centers may be explained by differences in the number of BCs pooled to make one PC, by differences in the details of processing (e.g., filters, centrifugation conditions, or PLT-rich plasma harvesting from spun BCs).[9,15] Furthermore, it has been described that cell counters can vary for up to 20 or 30% in PLT counts.[16,17] However, since all participating centers tested all three methods for PLT production so that despite differences between centers, the comparison of the three methods is valid.

The metabolism variables pH, glucose, and lactate showed best results for fresh/fresh PCs, compared to the other two conditions. This might be explained by the lower amount of PLT in the storage bag, resulting in a lower total metabolism in the bag.[18] However, since the differences are already seen on Day 1, it is more probable that glucose is consumed and lactate is formed by the RBCs and WBCs in the whole blood and the BC during overnight storage resulting in worse starting conditions for fresh/stored and stored/fresh PCs compared to fresh/fresh PCs.

PLT activation seemed to be lowest for stored/fresh PCs, due to the longer resting periods for whole blood and BCs compared to fresh/fresh and fresh/stored PCs. This supports our hypothesis that aggregates are formed in fresh PCs, which then disaggregate with increased resting times. For annexin V no significant differences were observed between methods although there was a trend

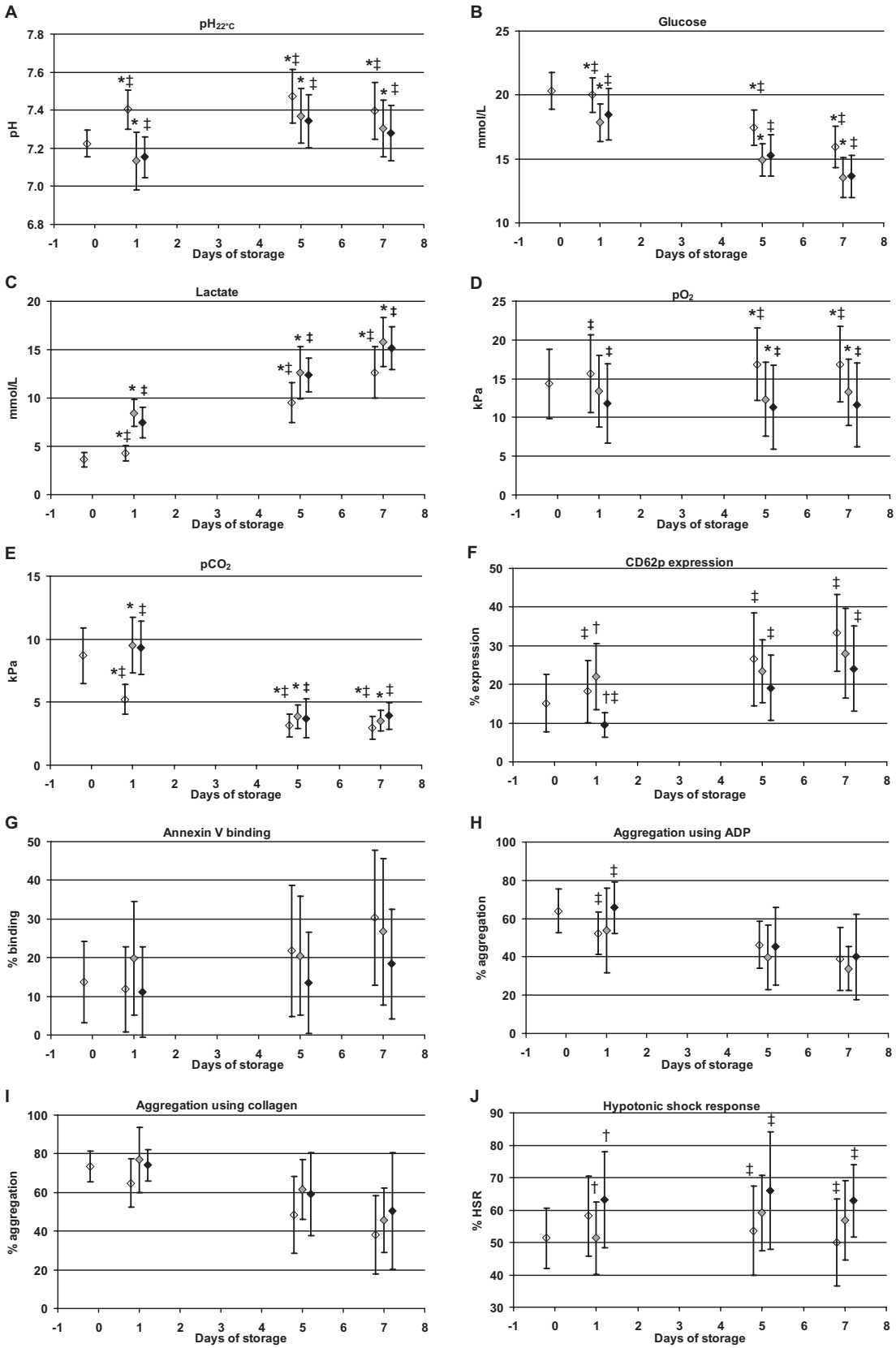

Fig. 2. pH (A), glucose level (B), lactate level (C), pO_2 (D), pCO_2 (E), CD62P expression (F), annexin V binding (G), aggregation using ADP (H), or collagen (I) and HSR (J) of PCs that were fresh/fresh (◇), fresh/stored (◈), or stored/fresh (◆). *$p < 0.05$ between fresh/fresh and fresh/stored PCs; †$p < 0.05$ between fresh/stored and stored/fresh PCs; ‡$p < 0.05$ between fresh/fresh and stored/fresh PCs.

toward higher values in the fresh/fresh group. A high annexin V binding for PCs is unexpected, since this variable is usually regarded as an indication of apoptosis (expected at longer storage times) resulting in PS exposure.[19] The swirl and metabolic variables indicate good conditions for all PCs, so that for this no increase for annexin V need to be expected for fresh/fresh PCs. Since CD62P and HSR did differ for the three conditions, it can be hypothesized that at longer storage annexin V binding will also differ between the conditions.

For aggregation by ADP or collagen no differences were observed among the three conditions after 5 days of storage. For these tests there was also a high degree of variability between centers, which may be partly due to differences in testing methods and thus differences in PLT quality of the three conditions may not be detectable when assessing aggregation.

For PC preparation the stored/fresh method produces PCs with the highest PLT content and lowest PLT activation. When using this method of production, plasma and RBCs are also be made from the whole blood stored at room temperature, which is allowed in Europe and Canada but not in the United States. While this results in a lower FVIII concentration than blood processed on the day of collection, it has been shown that plasma derived from overnight stored whole blood meets current European guidelines for FVIII content,[2,3,20,21] and results in minor differences for other coagulation factors.[22] Studies have shown that ambient storage of whole blood results in a more rapid depletion of 2,3-DPG during subsequent storage of RBCs, but ATP levels are increased or remained unchanged.[2,23,24] Since 2,3-DPG is almost totally depleted in RBCs after 3 weeks and most RBCs are stored for more than 2 weeks before transfusion,[25] and it is known that in the body 2,3-DPG will be rejuvenated a few hours after transfusion,[7] the lower 2,3-DPG level would not appear to be a reason to prevent the use of RBCs from overnight-stored whole blood.

In conclusion, PCs can be best prepared from overnight stored whole blood or overnight-stored BCs (fresh/stored or stored/fresh). The PLT content of fresh/fresh PCs does not comply with European, US, or Canadian guidelines for PLT content. The PLT content and HSR of stored/fresh PCs is highest, with the lowest level of PLT activation as evidenced by CD62P expression. Besides the differences in qualitative PLT aspects, efficient production center operational issues like working during business hours only, fewer discards of PCs due to low PLT counts, and reduced donor exposure might be important reasons to prepare and use stored/fresh PCs.

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CONFLICT OF INTEREST

None of the authors have affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this article.

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