Storage of interim platelet units for 18 to 24 hours before pooling: in vitro study

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BACKGROUND: The Atreus 3C system (CaridianBCT) automatically produces three components from whole blood (WB), a red blood cell (RBC) unit, a plasma unit, and an interim platelet (PLT) unit (IPU) that can be pooled with other IPUs to form a PLT dose for transfusion. The Atreus 3C system also includes a PLT yield indicator (PYI), which is an advanced algorithm that provides an index that is shown to correlate well with the amount of PLTs that finally end up in the IPU bag. The aim of our in vitro study was to compare the effects of holding WB overnight versus processing WB fresh (2-8 hr), both with 18- to 24-hour storage of the IPUs before pooling into a transfusable PLT dose.

STUDY DESIGN AND METHODS: WB was processed either fresh (within 8 hr after collection, Atreus F) or after overnight storage (14-24 hr, Atreus S) without agitation at 22 ± 2°C. After a subsequent resting time of 18 to 24 hours on a flat-bed shaker, five IPUs were selected for pooling with 200 mL of PAS II for in vitro quality assessments during a 7-day storage period (n = 10 in each arm). IPUs were selected for pooling using the PYI of the Atreus 3C system.

RESULTS: During storage, the glucose concentration was lower ($p < 0.05$) and the lactate concentration was higher ($p < 0.05$) in Atreus S pools, but no differences in the glucose consumption rate were noted. Adenosine triphosphate levels and hypotonic shock response reactivity were higher in Atreus S ($p < 0.05$). No significant differences in PLT counts, contents, mean PLT volume, lactate dehydrogenase, pO_2, pCO_2, extent of shape change, and CD62P between groups were detected. pH was maintained higher than 6.8 (Day 7). With exception of 2 units in the Atreus S arm, swirling remained at greater than 2 in all units at all times.

CONCLUSION: Our results suggest that PLTs prepared from fresh or overnight-stored WB and pooled after 18 to 24 hours meet necessary in vitro criteria without any relevant differences between both groups. Using the PYI, comparable yields can be obtained between WB processed within 2 to 8 hours and WB stored overnight.

Over the past few decades, manual methods for preparing platelets (PLTs) from whole blood (WB) donations based on pooled buffy coats (BCs) have been developed.[1] These techniques have been progressively replaced at several blood centers by use of a semiautomated technique, the OrbiSac system (CaridianBCT, Zaventem, Belgium),[2] a device for automating the pooling process of BCs. This technique requires several steps, affecting staff time and production flow in the blood center. Although several of those manual steps can be eliminated and combined by using the Atreus 2C+ system for BC preparation (CaridianBCT),[3] this system still requires the OrbiSac for PLT preparation. In contrast, the 3C component automatically produces three components derived from WB, a red blood cell (RBC) unit, a plasma unit, and an interim platelet unit (IPU) that can manually be pooled with other IPUs to form a transfusable PLT dose.

In addition, the Atreus 3C system includes a PLT yield indicator (PYI). Without being a cell counter, the PYI provides an estimate of the PLT content in the IPU, displayed on the Atreus screen and recorded in the Atreus system manager. This indicator may be a helpful tool to increase and/or standardize PLT content either by removal of

ABBREVIATIONS: Atreus F = platelets obtained from whole blood processed within 2 to 8 hours; Atreus S = platelets obtained from overnight-stored whole blood; BC = buffy coat; ESC = extent of shape change; HSR = hypotonic shock response; IPU = interim platelet unit; PYI = platelet yield indicator; WB = whole blood.

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low-yield IPUs from inclusion in the pooling process or by offering the possibility to combine low- and high-yield IPUs in a more consistent manner (reducing variability in the final pool) or by both.

WB units can be either kept overnight at room temperature before initial separation into components or separated within 2 to 8 hours. The effect of either WB separation within 8 hours or holding WB overnight before preparation of PLTs on in vitro PLT quality has recently been described for the 2C+ protocol.[3] However, the potential effect of storing IPUs overnight in the provisional IPU bags has not been previously studied. Extension of the IPU storage interval for the Atreus 3C system could solve logistical problems associated with PLT preparation. For this reason, in vitro effects on the extension of the IPU storage interval were evaluated.

The aim of our in vitro study was to compare the effects on PLTs after holding WB for either less than 8 or 14 to 24 hours before processing to IPUs, followed by a further 18 to 24 hours storage of the IPUs to PLT units. The quality of the RBCs and plasma prepared from the Atreus 3C system are not evaluated in this study.

MATERIALS AND METHODS

Blood collection, preparation of blood components, and storage of IPUs

The Atreus collection and processing set is similar to a conventional blood bag system (containing both CPD and SAGM), except that it also includes an additional separation bag ("conical bag"). The three-component procedure will automatically produce an RBC unit, a plasma unit, and an IPU that can be manually pooled with other IPUs to form a transfusable PLT dose. After the 3C procedure, the RBC unit is transferred through an inline leukoreduction filter to an RBC storage bag containing 100 mL of SAGM. By the Atreus centrifugal separation process, consistently leukoreduced plasma is produced in the system with no further need of filtration.

The Atreus device includes a "platelet yield index" (PYI) feature. This is an advanced algorithm that, based on the reflective signal of one of the photocells and the position of the hydraulic system in the device, provides an index that is shown to correlate well with the amount of PLTs that finally end up in the IPU bag (data on file, CaridianBCT). Although it is not a PLT counter, this PYI allows selecting the IPUs with the higher PLT content for pooling.

A total of approximately 450 mL of WB was collected and within 30 minutes placed on cooling plates (Sebra, Tucson, AZ) to allow its temperature to adjust to 20 to 24°C. The blood donors met standard donation criteria and gave their written informed consent in accordance with our institution's guidelines. In this study, outlined in Fig. 1, we evaluated the in vitro quality of PLTs during a 7-day storage period. PLTs were obtained by pooling 18- to 24-hour-old IPUs prepared either from fresh blood (<8 hr, Atreus F) or from overnight-stored blood (14-24 hr, Atreus S). Storage of the IPUs during this 18 to 24 hours occurred on a horizontal shaker in the IPU bag, which is a standard PVC-DEHP bag with the normal relatively low gas permeability for O_2 and CO_2. Five IPUs and 200 mL of PAS II (SSP, Macopharma, Mouvaux, Belgium) were pooled and leukoreduced using the Atreus pooling set into a leukoreduced PLT concentrate for in vitro quality assessments. The storage bag, included in the pooling set, is made of PVC plastic with a citrate-based plasticizer (BTHC) and intended to contain up to 5×10^{11} PLTs in 400 mL of plasma. Both arms consisted of 10 pools selected arbitrarily (n = 10 in each arm).

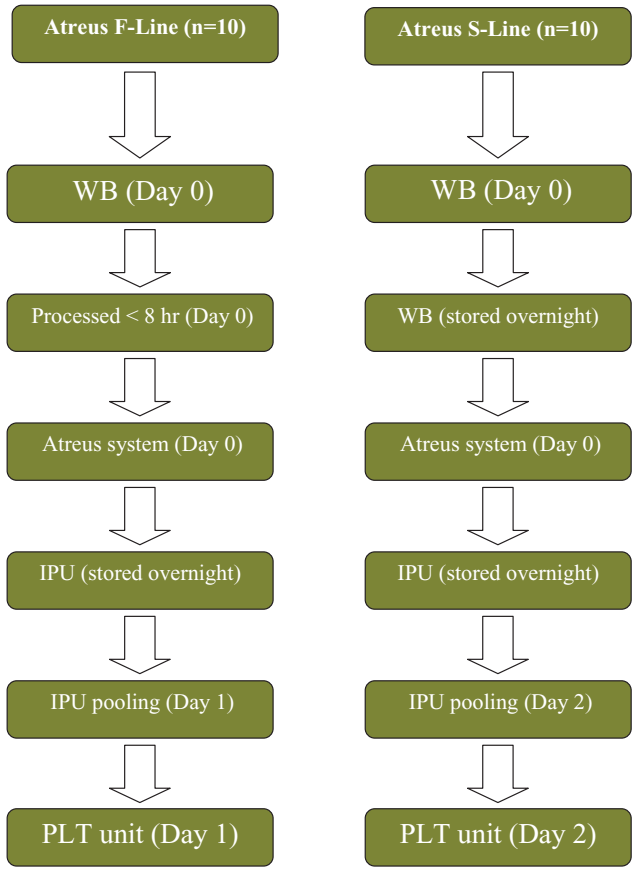

Fig. 1. Study design.

IPU selection:

For Atreus F, more units of blood (n = 87) were processed than needed for the pools (n = 50 IPUs for 10 pools), and the IPUs with the highest PYI were selected for inclusion into the pools, except when aggregates were visible in the IPU at the time of selection of the IPUs. For Atreus S, a lower PYI was selected compared with the PYI selected for Atreus F. Air and foam were removed from the con-

tainers and PLT units were stored in approximately 60% PAS II PLT additive solution and 40% plasma on a flat-bed agitator (60 cycles/min, Model LPR-3, Melco, Glendale, CA) in a temperature-controlled chamber at 22 ± 2°C. The samples (10 mL) were drawn on Days 1 or 2 (PLT preparation day), 3, 5, and 7. To avoid disturbing pO_2 and pCO_2, sampling was not done using a sample pouch, but by direct insertion of a needle in the bag through a sampling site coupler, followed by slow aspiration and immediate analysis on the blood gas analyzer.

Analysis of metabolic and cellular variables

Cellular and metabolic in vitro variables were evaluated in a PLT storage study, including measurements of PLT counts (10^9/L and 10^9/unit) and mean PLT volume using hematology equipment (CA 620 Cellguard, Boule Medical, Stockholm, Sweden). The volume (mL) was calculated by weighing the contents of the storage bag on a scale (Mettler PB 2000, Mettler-Toledo, Albstadt, Switzerland) and the results, in grams, were divided by 1.01 (1.01 g/mL is the density of the storage medium composed of approx. 70% PAS II and 30% plasma). By use of routine blood gas equipment (ABL 800, Radiometer, Copenhagen, Denmark), we also measured glucose (mmol/L), lactate (mmol/L), pH, pO_2, and pCO_2 (kPa at 37°C). Bicarbonate (mmol/L) was calculated based on other measured variables.

According to Bertolini and Murphy,[4] the assessment of swirling was scored as 0, 1, and 2. The white blood cell (WBC) count on Day 1 was determined with a Nageotte chamber and a microscope (Zeiss standard, Zeiss, Chester, VA).[5] Hypotonic shock response (HSR) reactivity and extent of shape change (ESC) measurements were performed using a dedicated microprocessor-based instrument (SPA 2000, Chronolog, Havertown, PA) with the modifications of these tests described by Vanden-Broeke and colleagues.[6] The total adenosine triphosphate (ATP) concentration (μmol/10^{11} PLTs) was determined with a luminometer (Orion Microplate luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin.[7] Extracellular lactate dehydrogenase (LDH) activity, a marker for disintegration of PLTs, was measured with a spectrophotometric method (Kit 063K6003, Sigma Aldrich, St Louis, MO; spectrophotometer DMS 100, Varian Techtron, Springvale, Australia).[8] The LDH activity is presented as extracellular percentage of total activity.

CD62P analysis by flow cytometry

PLT concentrate samples, fixed by adding an equal volume of 1% paraformaldehyde–phosphate-buffered saline (PBS; pH 7.2-7.4), at 22°C for 10 minutes, were then stained for 20 minutes at the same temperature in the dark by incubating with 20 μL of fluorochrome-labeled monoclonal antibodies (MoAbs) per approximately 20×10^6 PLTs. Phycoerythrin-conjugated (immunoglobulin G [IgG]1 isotype) CD62P (P-selectin/GMP-140/PADGEM; Clone CLB Thromb/6) MoAbs and control specimens (IgG1 isotype) were used for single-color staining purchased from Immunotech (Beckman Coulter, Marseilles, France). After incubation with fluorochrome-conjugated antibodies, the samples were washed twice by adding 2.0 mL of filtered PBS-ethylenediaminetetraacetic acid (0.33%, pH 6.9) with 0.1% Na-azide, and centrifuged at $2760 \times g$ (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) for 10 minutes at +18°C. A total of 100,000 PLTs were acquired on a flow cytometer (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ) equipped with 15-mW argon ion lasers and its accompanying software (CellQuest Pro, Becton Dickinson). Daily controls of optics and fluorescence intensity were performed using standardized beads (Calibrite, Becton Dickinson). The flow cytometer settings were optimized for the acquisition of PLTs by logarithmic signal amplification in all four detectors (forward and side scatter channels and fluorescence channels FL1 and FL2). For analysis, the gate was set around intact PLT population as defined by forward and side scatter characteristics. The percentage of positive PLTs of total PLT expressing activation markers CD62P above that of background (negative control) as well as the mean fluorescence intensity (MFI) were recorded.

Bacterial cultures

Bacterial cultures were performed on Day 7 with the routine methods of the bacteriologic laboratory at Karolinska University Hospital, Huddinge, including aerobic and anaerobic cultures performed on Columbia blood agar plates, with 48 hours to final report.

Statistical analyses

The mean values and standard deviations (SDs; n = 10) are usually given. Repeated measurement analysis of variance was performed including post hoc test and Fisher's least significant differences. Results of Fisher's least significant differences are presented in the result section. Two different groups (Atreus F and Atreus S) were studied over time (days). "Days" was the repeated factor and "Group" was a between factor. The p value represents the significance of the differences from baseline (Day 1 and 2) over time. The p value for the interaction term between days and group is reported. The difference was considered significant at p values of less than 0.05. The analyses were carried out using computer software (Statistica, Version 6.0, 1984-2007 StatSoft, Inc., SPSS, Chicago, IL).

TABLE 1. Comparison of PLTs, obtained either from fresh (<8 hr) or overnight-stored WB (14-24 hr) processed on the Atreus 3C system (CaridianBCT) and pooled after a subsequent resting time of 18 to 24 hours*

WB processing line	Volume (mL)	PLTs ($\times 10^9$/L)	PLTs ($\times 10^9$/unit)	WBCs ($\times 10^6$/units)
Atreus F (WB)	364 ± 4	942 ± 81	342 ± 29	<0.2
Atreus S (WB)	362 ± 5	940 ± 90	341 ± 33	<0.2

* Sampling was performed on Day 1 (Atreus F) and Day 2 (Atreus S), respectively. Results are expressed as mean ± SD. n = 10.

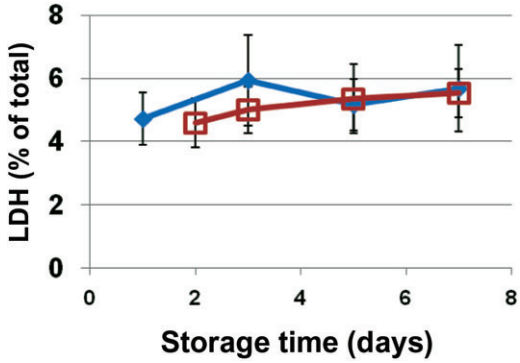

Fig. 2. LDH activity (% of total) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, ◆) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, ⊟). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

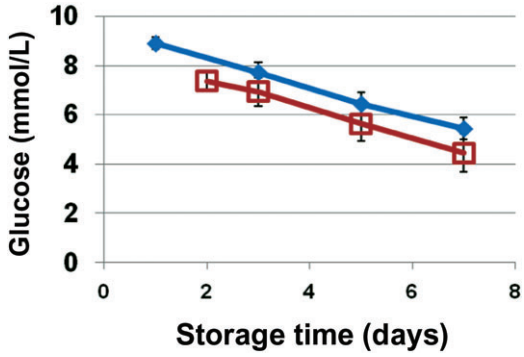

Fig. 3. Glucose concentration (mmol/L) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, ◆) and WB stored overnight and pooled after 18 to 24 hours (stored, ⊟). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. $p < 0.05$.

RESULTS

Cellular assays

The cellular content data on Day 1 (Atreus F) and Day 2 (Atreus S) are given in Table 1. PLT concentration and pool yield showed no significant difference between groups. No significant difference in mean PLT volume between groups was detected throughout storage (data not shown). LDH activity (% of total) remained stable at low levels without any significant difference between groups (Fig. 2).

Metabolic assays

Although the glucose concentration (Fig. 3) at the start of the storage was significantly lower ($p < 0.05$) and lactate concentration was significantly higher ($p < 0.05$) in the Atreus S arm (Fig. 4), the glucose consumption rate (Fig. 5) was the same (0.58 ± 0.05 µmol/10^9 PLTs/day in Atreus F vs. 0.57 ± 0.04 µmol/10^9 PLTs/day in Atreus S; p = NS). pH (Fig. 6) was slightly higher in the Atreus F group ($p < 0.05$). No significant difference in carbon dioxide (pCO_2) and the oxygen tension (pO_2) was detected (Fig. 7 versus Fig. 8). ATP levels (Fig. 9) were higher in the Atreus S arm ($p < 0.05$, Fig. 2).

HSR, ESC, and swirling

HSR (Fig. 10) was higher in the Atreus S arm ($p < 0.05$). No significant difference in the ESC levels (Fig. 11) between

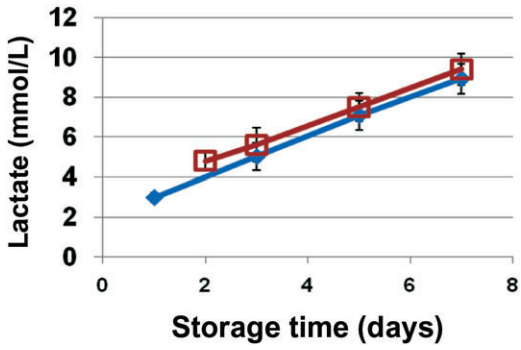

Fig. 4. Lactate concentration (mmol/L) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, ◆) and WB stored overnight and PLT pooling after 18-24 hours (stored, ⊟). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. $p < 0.05$.

groups was detected (Fig. 2). With exception of two Atreus S units (score = 1) on Days 5 and 7, swirling remained at the highest levels (score = 2) in all units at all times.

CD62P analysis

The percentage of PLTs expressing the activation markers CD62P (Fig. 12) as well as the MFI (data not shown)

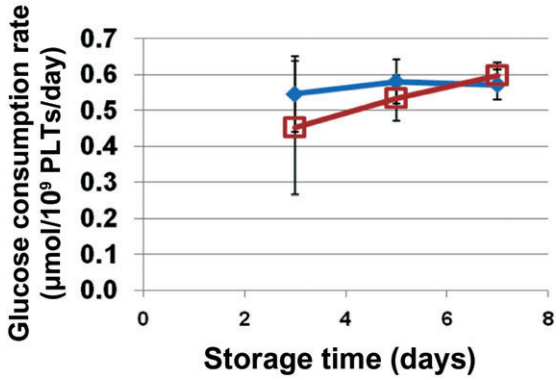

Fig. 5. Glucose consumption rate in PLTs obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and pooled after 18 to 24 hours (stored, —□—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

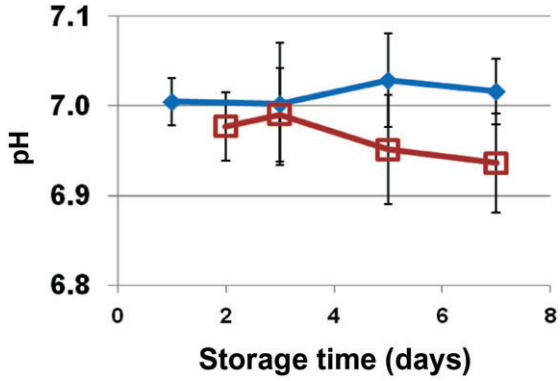

Fig. 6. pH (at 37°C) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —□—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. $p < 0.05$.

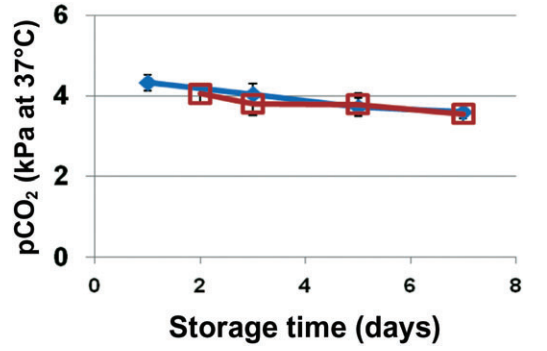

Fig. 7. Carbon dioxide (kPa at 37°C) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —□—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

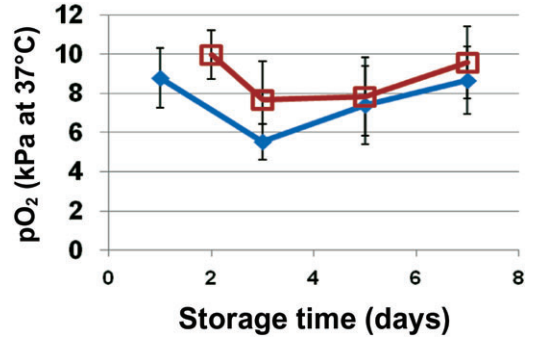

Fig. 8. Oxygen tension (kPa at 37°C) during 7 days of storage in PLT units obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —□—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

increased during storage in all units. No significant difference in the percentage and/or MFI of PLTs expressing the activation marker CD62P between groups was detected. All bacterial cultures were negative.

DISCUSSION

In this study, in vitro quality was evaluated, comparing PLTs prepared from either fresh or overnight-stored WB processed on the Atreus 3C system and pooled at 18 to 24 hours after processing. The Atreus 3C system offers the possibility to eliminate several manual steps and standardize production in a single integrated process.

In this study, PLTs obtained from the Atreus 3C system showed a satisfactory PLT content (Table 1) according to current European standards (>240×10^9/unit). Recent published data indicate that PLT yield may be significantly improved when WB is stored overnight before processing.[3,9,10] In contrast, this study suggests that by using the PYI feature, PLT yield from WB processed within 2 to 8 hours can be improved to the same level as that for WB stored overnight. Of course, the strategy used in this study implies that—for fresh blood—a number of IPUs are excluded from being used for composing pools. In case the need of PLT pools dictates the use of all WB units for production of PLT pools, this might not be an option (and use of overnight processing could be envisioned). However, for the many centers that now only use a portion of their WB units for final pool production, this might be a great opportunity, since the PYI guides the operation into which IPUs to select for pool optimization, a feature not

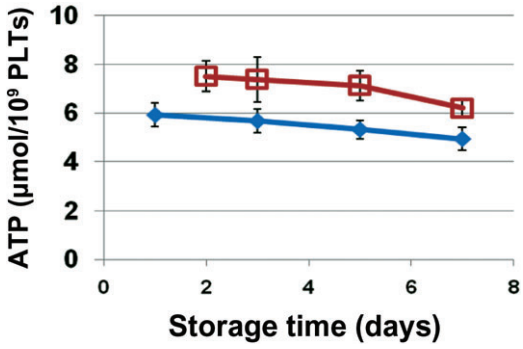

Fig. 9. ATP (µmol/10^{11} PLTs) during 7 days of storage in PLTs obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —⊟—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. $p < 0.05$.

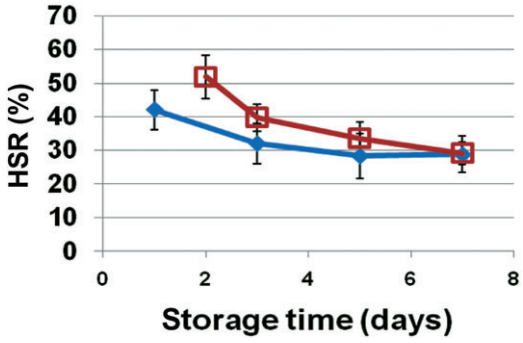

Fig. 10. HSR (%) during 7 days of storage in PLTs obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —⊟—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. $p < 0.05$.

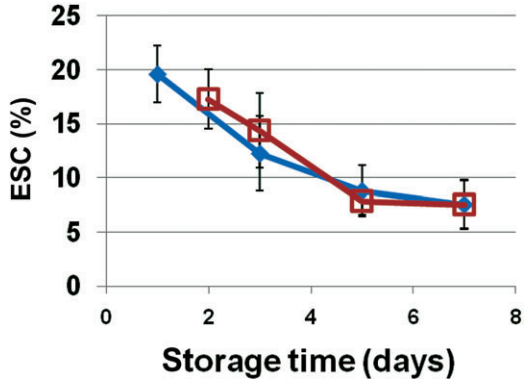

Fig. 11. ESC (%) during 7 days of storage in PLTs obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —⊟—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

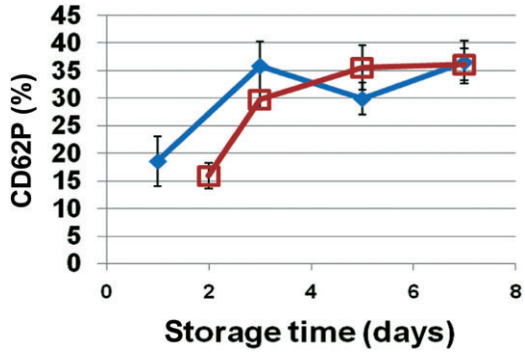

Fig. 12. Changes in PLT expression of CD62P (%) during 7 days storage of PLTs obtained from WB processed within 2 to 8 hours and PLT pooling after 18 to 24 hours (fresh, —◆—) and WB stored overnight and PLT pooling after 18 to 24 hours (stored, —⊟—). Values given as mean ± SD, n = 10. The p value represents the interaction term between days and group during storage. Not significant.

available in conventional BC or PLT-rich plasma operation. This is a significant improvement in the preparation technique of PLTs from WB.

In general, we found that variables used as markers of PLT function, metabolism, and activation gave quite similar results between groups. The differences observed initially and during storage in glucose-lactate concentration as well as pH are most likely related to the storage of WB overnight in Atreus S units. The storage overnight will result in additional consumption of glucose and production of lactate by RBCs and WBCs in WB. This explanation is further supported by similar glucose consumption rate in both groups indicating similar metabolism during storage.

We observed significantly lower ATP levels and HSR in the fresh units. Regarding HSR, this difference is leveled out during storage. Even though all ATP levels were seen to decrease gradually throughout the storage period, they all exceeded the concentrations (>4.0 µmol/10^{11} PLTs) associated with acceptable in vivo viability.[11] Differences in response to HSR can sometimes be explained by differences in the PAS-to-plasma dilution required for the HSR test.[6] In contrast to earlier data, suggesting that overnight storage of WB improves the response to HSR compared to less than 8-hour-processed WB,[3] the differences in HSR levels shown in this study may not primarily be explained by the technique of measurement, since the PLT concentrations were similar in both groups. Therefore, the reason for the differences in HSR and ATP in this study seems to be unknown. However, the response to HSR may be improved by using storage solutions with inclusion of

certain components such as potassium and magnesium with proven favorable effects on PLTs.[12,13]

To summarize, these results suggest that PLTs prepared from either fresh or overnight-stored WB and pooled after a subsequent resting time of 18 to 24 hours meet necessary in vitro criteria without any relevant differences between PLT units in the two groups. By using the PYI feature, PLT yield from WB processed within 2 to 8 hours can be improved to the same level as that of WB stored overnight. PYI as a predictor of the PLT yield in the final unit certainly will have a potential to be a helpful tool in improving and standardizing the PLT preparation process. Our study also suggests that it will be possible to store IPUs prepared after WB overnight hold for additional 18 to 24 hours before pooling, without negative effects on PLT quality. However, there is still little insight into the impact on the hemostatic behavior after PLT transfusion. Therefore, these data may be supplemented with in vivo studies of recovery and survival.

CONFLICT OF INTEREST

PS, AS, and HSG performed this study. The study was independently performed without any other interest than scientific knowledge. GvW and CV are CaridianBCT employees and supplied equipment and technical advice.

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