Automated preparation of whole blood–derived platelets suspended in two different platelet additive solutions and stored for 7 days

Joan Cid, Laura Magnano, Patricia Molina, Maribel Diaz-Ricart, Nuria Martínez, Rosa M. Maymó, Lluís Puig, Miguel Lozano, Ginés Escolar, and Ana M. Galán

BACKGROUND: The Atreus system (Terumo BCT) automates the preparation of blood components from whole blood donations. Intermediate platelet (PLT) products can be pooled manually or with the OrbiSac (Terumo BCT) and suspended in different PLT additive solutions (PASs) to obtain PLT concentrates (PCs). The aim of our study was to compare the in vitro PLT quality of PCs obtained with either the Atreus 2C+ and the OrbiSac or the Atreus 3C and suspended in PAS-II or PAS-IIIM during storage for up to 7 days.

STUDY DESIGN AND METHODS: We prepared eight PCs from buffy coats obtained with Atreus 2C+, pooled with the OrbiSac, and suspended in PAS-II and eight PCs from interim PLT units obtained with the Atreus 3C and suspended either in PAS-II or in PAS-IIIM. We measured volume, PLT content, and mean PLT component and performed metabolic assays (pH, glucose, lactate, pO_2, and pCO_2) and flow cytometry analyses (GPIb, GPIIbIIIa, GPIV, CD62P, CD63, von Willebrand factor [vWF], fibrinogen, Factor V, and annexin V).

RESULTS: PCs prepared with the Atreus 3C showed lower volume and higher PLT concentration when compared with PCs prepared with the Atreus 2C+ and the OrbiSac ($p < 0.05$). Glucose consumption rate and the expression of CD62P, CD63, and vWF were lower in PCs suspended in PAS-IIIM when compared with PCs suspended in PAS-II ($p < 0.05$).

CONCLUSION: PCs prepared with the Atreus 3C and suspended in PAS-IIIM preserve satisfactorily the in vitro PLT quality during 7-day storage. PLT activation during a 7-day storage period was lower when the storage solution was PAS-IIIM in comparison with PAS-II.

Automation of blood component preparation from whole blood (WB) donations is progressively replacing manual methods.[1] Today, the almost complete automation of blood component preparation from WB bags is achieved with the Atreus system (Terumo BCT, Zaventem, Belgium).

The Atreus whole blood processing system is a self-contained, automated manufacturing system that generates the blood components from WB units. This innovative system replaces many labor-intensive manual process steps, such as balancing, centrifugation, expression, sealing, volume determination, and information management.[1]

The Atreus system can be configured in three different protocols. First, the 2C protocol was designed to obtain 1 unit of red blood cells (RBCs), 1 unit of plasma, and 1 unit

ABBREVIATIONS: BC(s) = buffy coat(s); IPU(s) = interim platelet unit(s); MPC(s) = mean PLT component(s); PAS(s) = platelet additive solution(s); PC(s) = platelet concentrate(s); PerCP = peridinin chlorophyll protein; PYI = platelet yield indicator; WB = whole blood.

From the Department of Hemotherapy and Hemostasis, CDB, IDIBAPS, Hospital Clínic, University de Barcelona; and the Banc de Sang i Teixits, Barcelona, Spain.

Address reprint requests to: Joan Cid, MD, PhD, Servei d'Hemoteràpia i Hemostàsia, Hospital Clínic, C/. Villarroel, 170, 08036 Barcelona, Spain; e-mail: jcid@clinic.ub.es.

This work has been partially supported by research grant from Terumo BCT as well as by grants FIS (CP04-00112, PS09/00664) (Fondo de Investigaciones de la Seguridad Social), German Jose Carreras Leukemia Foundation (R 07/41v), SAF2009-10365, SAF2011-28214 (Ministerio de Ciencia y Tecnología), and RD06/ 0009/1003 (Red HERACLES, Instituto de Salud Carlos III).

Received for publication February 27, 2013; revision received April 30, 2013, and accepted April 30, 2013.

doi: 10.1111/trf.12283

TRANSFUSION **;**:**-**.

of residual white blood cells (WBCs).[2,3] Second, the 2C+ protocol was designed to obtain 1 unit of RBCs, one buffy coat (BC), and 1 unit of plasma.[4] The obtained BCs can be pooled manually[5] or automatically with either the OrbiSac (Terumo BCT)[6] or the TACSI (Terumo BCT)[7,8] to obtain a pooled platelet (PLT) concentrate (PC). Finally, the most recent 3C protocol was developed to obtain 1 unit of RBCs, 1 unit of plasma, 1 interim PLT unit (IPU), and 1 unit of residual WBCs. The Atreus 3C system also 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 in the pools either by removal of 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. The IPUs can be manually pooled with other IPUs to form a transfusable PLT dose, without the need for a second centrifugation step.[9]

During recent decades, there has been great interest in developing and using PLT additive solution (PAS) for the storage of PLTs, because PAS can improve storage conditions of PLTs. At present, such PASs are in use for transfusion purposes in several countries, mostly in Europe. PAS is generally used as a substitute for plasma to 1) reduce the amount of plasma transfused with PLTs and to recover plasma for other purposes, 2) avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload, 3) make some photochemical treatments possible for the inactivation of pathogens in PLTs, and 4) improve storage conditions. The use of PAS offers the possibility of including substances in the storage solution that have specific effects on PLTs and that are not present in plasma or in the anticoagulant.[10] PAS developed in 1980s are of historical interest;[11] newer PASs, such as PAS-II (contains NaCl, citrate, and acetate) and PAS-III (contains the previous components and also phosphate), are widely used for preparation and storage of PLTs, mostly in Europe.[12] The more recent PAS-IIIM, which contains the previous components and also potassium, and magnesium, will probably replace previous PASs.[13]

The aims of our study were: 1) to analyze the in vitro PLT quality of WB-derived PLTs obtained with the Atreus 2C+, pooled with the OrbiSac, and suspended in PAS-II during storage for up to 7 days; 2) to analyze the in vitro WB-derived PLTs obtained with the Atreus 3C, pooled manually and suspended in PAS-II or PAS-IIIM during storage for up to 7 days; and 3) to compare the previous in vitro quality results.

MATERIALS AND METHODS

Study design (Fig. 1)

This study, with three arms, is outlined in Fig. 1 and evaluated in vitro quality of WB-derived PLTs during a 7-day storage period, prepared from either the Atreus 2C+ and the OrbiSac and stored in PAS-II or the Atreus 3C and stored in PAS-II or PAS-IIIM.

We collected 450 mL of blood (±10%) in Atreus WB bags from volunteer donors meeting European guidelines criteria for blood donation (Day 0).[14] The WB bags were cooled to 22 ± 2°C after collection, employing butane-1,4-diol plates until fractionation on the morning of the day after the blood donation (Day +1).

The Atreus equipment includes an automatic centrifuge, a hydraulic expressor, valve/sealers, and a bar code reader integrated in the same equipment. The Atreus WB set for the preparation of blood components is similar to a conventional blood bag system (containing both CPD and saline-adenine-glucose-mannitol), except that it also includes an additional separation bag ("round bag"). After the Atreus processing, RBC units are transferred through a leukoreduction filter to the appropriate bag for storage. By the Atreus centrifugal separation process, leukoreduced plasma is produced in the system with no further need of filtration.

The BCs obtained from the Atreus 2C+ system were held undisturbed for a minimum of 4 hours and a maximum of 8 hours at 22 ± 2°C until they were processed in the afternoon of the day after the blood donation (Day +1) with the OrbiSac system. We pooled five ABO-identical BCs and 300 mL of PAS-II (SSP, MacoPharma, Lille, France) with a sterile connecting device (TSCD, Terumo BCT) in an OrbiSac standard BC set. This set consisted of a number of lines to dock the BCs and PAS, a round bag that served as the pooling and separation bag, a filter (LRP6, Pall, East Hills, NY), and a PLT storage bag. The bag system was placed into the OrbiSac device following the manufacturer's instructions. The system first performed a pooling procedure and it continued on automatically by centrifugation, inline filtration, transfer of the PLT-rich supernatant into the storage bag, and sealing.

The IPUs obtained from the Atreus 3C system were left to rest at least 1 hour, followed by a minimum of 2

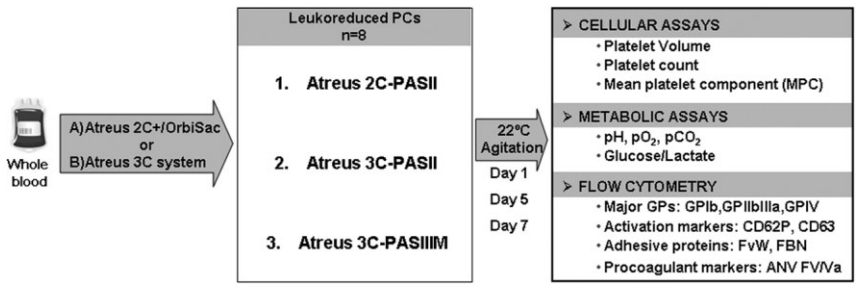

Fig. 1. Study design.

hours and a maximum of 8 hours on a flatbed shaker at 22 ± 2°C until they were processed in the afternoon of the day after the blood donation (Day +1). The IPU bag is a standard polyvinylchloride (PVC)-DEHP bag with the normal relatively low gas permeability for O_2 and CO_2. We manually pooled 4 ABO-identical IPUs obtained with Atreus 3C and 200 mL of PAS-II (SSP, MacoPharma) or 200 mL of PAS-IIIM (SSP+, MacoPharma) using the Atreus pooling set into a leukoreduced PC for in vitro quality assessments. We selected the IPUs to pool according to the results of the PYI. The storage bag, included in the pooling set, is made of PVC plastic with a citrate-based plasticizer (BTHC).

Sampling and determinations

We calculated the volume of the PCs according to the net weight and density (1.014 g/mL). We sampled PCs on Days 1, 5, and 7. We determined the PLT content of the PCs and the mean PLT component (MPC) in tubes containing EDTA (1.6 mg EDTA/mL; Sarstedt & Co., Nümbrecht, Germany) with a hematology analyzer (Advia 2120 hematology system, Siemens Healthcare Diagnostics, Deerfield, IL) and expressed the results as PLTs $\times 10^6$/L and g/dL, respectively. We performed pH, pO_2, and pCO_2 measurements using a blood gas analyzer (Rapidlab 860, Bayer, Deerfield, IL). We determined levels of glucose and lactate, expressed as mg/dL, using an analyzer (ADVIA 2400, Siemens Healthcare Diagnostics). We determined glucose concentration in PLT samples collected in tubes anticoagulated with lithium heparin (Vacuette Premium, Greiner Bio One, Frickenhausen, Germany) and we determined lactate concentration in PLT samples collected in BD Vacutainer tubes (BD368920 plastic fluoride/oxalate glucose preservation, Becton Dickinson, San Jose, CA). Glucose consumption rate was calculated using glucose consumption between Day 1 and 7 corrected by PLT concentration in the PCs.

Flow cytometry analysis

We analyzed PLTs by dual-flow cytometry using combinations of antibodies or markers conjugated with fluorescein isothiocyanate (FITC), phycoerythrin, or peridinin chlorophyll protein (PerCP). Monoclonal antibodies (MoAbs) used were commercially available (Immunotech, Marseille, France; except where expressly indicated). Presence of major glycoprotein GPIIbIIIa was detected with an anti-CD41a-PerCP (Clone P2) from BD Biosciences (San Jose, CA), GPIb was detected with an anti CD42b-FITC (Clone SZ2), and GPIV with an anti-CD36-FITC (clone FA6.152). Activation markers were detected using antibodies against P-selectin with anti-CD62P-FITC (Clone CLBThromb/6) and the 53-kDa lysosomal membrane protein with anti-CD63-FITC (Clone CLBGran/12). The binding of adhesive proteins, fibrinogen, and von Willebrand factor (vWF) was detected with the corresponding antibodies (Dako A/S, Glostrup, Denmark, for fibrinogen and AbD Serotec, Kidlington, UK, for vWF). Detection of procoagulant activity was measured using coagulation Factor (F)V with a MoAb that recognizes the light chain of both FV and FVa (Clone V237 from American Diagnostica, Stamford, CT), and the exposure of anionic phospholipids on the outer leaflet of the PLT membrane was quantified using annexin V (BD Biosciences). Nonspecific membrane immunofluorescence was determined by using an immunoglobulin G1 (Clone 679.1Mc7) as a negative control.

Immunolabeling of PLTs with MoAbs was performed using dual-color analysis as previously described.[15] Briefly, after collection 2.5-µL aliquots of PCs were added to polypropylene tubes preloaded with 50 µL of phosphate-buffered saline (PBS). Samples were first incubated with saturating concentrations of anti-CD41a-PerCP in the dark, without stirring, for 15 minutes at room temperature, followed by the addition of labeled conjugated MoAbs and an additional incubation for 15 minutes. Samples were then diluted with 1 mL of PBS and analyzed immediately with a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA) as previously described.[16]

Bacterial cultures

We performed bacterial cultures on Day 7 with the routine methods of the bacteriologic laboratory at Hospital Clínic, including aerobic and aerobic cultures performed on BD BACTED Standard 10 (Becton Dickinson), with 5 days to final report.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). One-way analysis of variance test for independent experiments was applied when multiple comparisons were required, followed by Bonferroni's test if significance was detected. The level of statistical significance was established at a p value of less than 0.05. Statistical analysis was carried out with computer software (SPSS Software, release 18.0, IBM Corp., Armonk, NY).

RESULTS

We analyzed eight PCs prepared in each of the three arms of the study. All bacterial cultures were negative.

Cellular assays (Table 1)

The volume of PCs prepared with the Atreus 3C system was 21% lower when compared with the volume of PCs

TABLE 1. Cellular assays*

Cellular assay	Day 1	Day 5	Day 7
PLT bag volume (mL)			
2C+ PAS-II	444 ± 12	436 ± 13	421 ± 12
3C PAS-II	352 ± 12†	342 ± 12†	326 ± 13†
3C PAS-IIIM	360 ± 12†	350 ± 12†	335 ± 13†
PLT count $\times 10^9$/L			
2C+ PAS-II	740 ± 106	809 ± 74	760 ± 99
3C PAS-II	1014 ± 270†	1123 ± 274†	1040 ± 275†
3C PAS-IIIM	1116 ± 266†	1154 ± 264†	1089 ± 209†
PLT count $\times 10^9$/unit			
2C+ PAS-II	330 ± 44	353 ± 27	319 ± 39
3C PAS-II	356 ± 92	383 ± 94	337 ± 86
3C PAS-IIIM	398 ± 82	403 ± 93	363 ± 74
MPC (g/dL)			
2C+ PAS-II	23.4 ± 1.1	22.1 ± 0.8‡	21.8 ± 0.9‡
3C PAS-II	24.5 ± 1.8	22.9 ± 0.4‡§	22.4 ± 1.1‡
3C PAS-IIIM	24.7 ± 1.8	23.6 ± 1.6§	22.5 ± 1.6‡

* Results are expressed as mean ± SD (n = 8).
† $p < 0.01$ vs. 2C+ PAS-II.
‡ $p < 0.05$ vs. Day 1.
§ $p < 0.05$ vs. 2C+ PAS-II.

TABLE 2. Metabolic assays*

Metabolic assay	Day 1	Day 5	Day 7
pH (37°C)			
2C+ PAS-II	7.05 ± 0.06	7.10 ± 0.04	7.08 ± 0.03
3C PAS-II	6.98 ± 0.07	7.04 ± 0.16	7.06 ± 0.12
3C PAS-IIIM	7.10 ± 0.05†	7.19 ± 0.06‡	7.19 ± 0.06‡
Glucose (mg/dL)			
2C+ PAS-II	164 ± 6	127 ± 8	107 ± 10
3C PAS-II	164 ± 9	112 ± 17§	89 ± 20§
3C PAS-IIIM	161 ± 14	119 ± 19	102 ± 20
Lactate (mg/dL)			
2C+ PAS-II	28 ± 2	66 ± 6	94 ± 8
3C PAS-II	32 ± 5	90 ± 23§	123 ± 31§
3C PAS-IIIM	32 ± 5	78 ± 20	102 ± 24
pO_2			
2C+ PAS-II	89.94 ± 33.6	93.6 ± 23.5	84.5 ± 27.5
3C PAS-II	72.1 ± 39.8	82.5 ± 44.4	58.1 ± 38.7
3C PAS-IIIM	69.6 ± 31.5	67.5 ± 43.0	69.2 ± 40.0
pCO_2			
2C+ PAS-II	39.2 ± 16.1	26.4 ± 2.5	25.4 ± 2.3
3C PAS-II	33.4 ± 3.3	27.2 ± 6.2	24.8 ± 5.8
3C PAS-IIIM	28.7 ± 2.6	24.3 ± 5.0	23.0 ± 3.3

* Results are expressed as mean ± SD (n = 8).
† $p < 0.05$ vs. 3C PAS-II.
‡ $p < 0.01$ vs. 2C+ PAS-II.
§ $p < 0.05$ vs. the other two groups.

prepared from the Atreus 2C+ and the OrbiSac ($p < 0.01$). In contrast, the PLT concentration was 37% higher when the PCs were prepared with the Atreus 3C in comparison with the Atreus 2C+ and the OrbiSac ($p < 0.01$). The resulting PLT yield was 9% higher in PCs prepared with the Atreus 3C when compared with PCs prepared from the Atreus 2C+ and the OrbiSac, although the difference was not significant.

The MPC decreased throughout the PLT storage in the three groups of the study, thereby indicating degranulation of PLTs. PLTs suspended in PAS-II showed lower MPC on Day 5 and on Day 7 when compared with MPC values on Day 1 ($p < 0.05$). PLTs suspended in PAS-IIIM showed lower MPC on Day 7 when compared with MPC values on Day 1 ($p < 0.05$). On Day 5, MPC was higher in PCs obtained from the Atreus 3C when compared with PCs obtained from the Atreus 2C+ ($p < 0.05$).

Metabolic assays (Table 2)

The pH was higher during 7-day storage in PCs prepared with the Atreus 3C and stored in PAS-IIIM when compared with the other two groups ($p < 0.05$). The glucose concentration was lower and lactate concentration higher in PCs prepared with the Atreus 3C and stored in PAS-II when compared with those prepared with the Atreus 2C+ and stored in PAS-II, and the difference was significant on Days 5 and 7. However, the glucose consumption rate in both groups using PAS-II was similar to each other (0.129 ± 0.012 and 0.126 ± 0.028 mg/10^9 PLTs/day), but it was significantly lower in PAS-IIIM (0.089 ± 0.016 mg/10^9 PLTs/day; $p < 0.01$). The rest of metabolic assays showed no significant difference among the three arms of the study.

Flow cytometry studies (Fig. 2)

The expression of major glycoproteins (GPIb, GPIIbIIIa, and GPIV) on PLT membrane showed no significant difference among the three arms of the study.

The expression of activation markers (CD62P and CD63) remained stable up to 7-day storage and it was lower in PCs prepared with the Atreus 3C and stored in PAS-IIIM when compared with the other two arms of the study, and the difference was significant at 5-day and at 7-day storage ($p < 0.05$).

In the case of adhesive proteins, the expression of vWF was lower in PCs prepared with the Atreus 3C and stored either in PAS-II or in PAS-IIIM when compared with PCs prepared with the Atreus 2C and the OrbiSac and stored in PAS-II, and the difference was significant at 5-day storage ($p < 0.05$), and the expression of fibrinogen showed no significant difference among the three arms of the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout

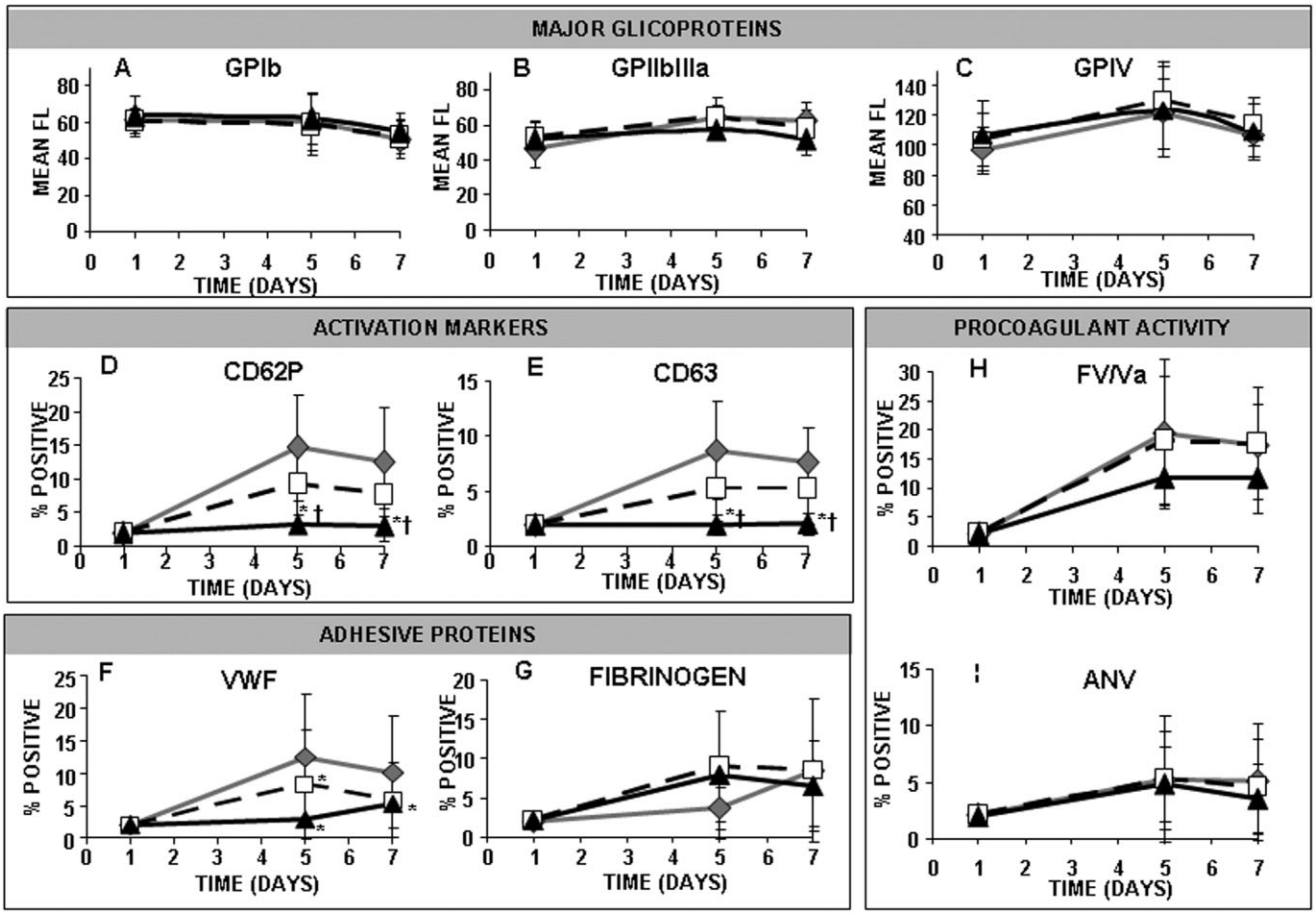

Fig. 2. Results of flow cytometry studies to detect changes in the presence of major glycoproteins, activation markers, adhesive proteins, and procoagulant activity during 7 days of storage. Graphs show modifications on PLT membrane expression during 7 days storage of: (A) major glycoproteins GPIb, (B) GPIIbIIIa, and (C) GPIV; expression of activation dependent antigens expressed as percentage of positive PLTs for (D) P-selectin (CD62) and (E) LIMP (CD63), membrane-bound adhesive proteins are (F) for vWF and (G) fibrinogen. Finally, procoagulant activity was measured by exposure of (H) FV/Va on PLT membrane and (I) annexin V (ANV) binding as a measurement of exposure of anionic phospholipids along 7 days' storage. Results expressed as mean ± SD; *p < 0.05 versus 2C+ PAS-II; †p < 0.05 versus 3C PAS-II. (—◆—) 2C+ PAS-II; (– □ –) 3C PAS-II; (—▲—) 3C PAS-IIIM.

the storage in the three arms of the study, and the difference was not significant.

DISCUSSION

Our study showed that PCs prepared with the Atreus 3C and suspended in PAS-IIIM preserve the in vitro PLT quality during 7-day storage satisfactorily. Those PCs had also the advantage of a smaller total volume when compared with PCs prepared with the Atreus 2C+ and the OrbiSac. Moreover, direct and indirect signs of PLT activation markers were lower when the storage solution was PAS-IIIM in comparison with PAS-II.

To our knowledge, this is the first study to compare PLT in vitro quality variables of PCs obtained with two different configurations of the Atreus system and stored in two different PASs. There were previous validation studies performed in a controlled environment focused on analyzing PLT characteristics of PCs prepared from BCs obtained from Atreus 2C+ and either pooled manually and suspended in plasma[5] or pooled with OrbiSac and suspended in PAS-II (T-Sol, Fenwal Europe sprl, Mont Saint Guibert, Belgium).[6] Other authors focused on analyzing RBCs, plasma, and BCs obtained with the Atreus 2C+[4] or PCs prepared from IPUs obtained with the Atreus 3C and stored in PAS-II (SSP, MacoPharma).[9] Finally, in a routine environment, other authors focused on operational values and in vitro quality of blood components obtained with the Atreus 3C.[17]

Taking together all the previous studies, it seems that automation of PC preparation from WB bags helps in standardization and reduction of variability in the final pool.[1] In this sense, Cid and colleagues published a study in 2007, where authors compared the results of pooled PCs

prepared with the OrbiSac system with pooled PCs prepared manually.[18] Authors showed that automation of the pooling process increased not only the PLT content but also the consistency of the final product: the coefficients of variation for the volume and the PLT content decreased from 30% to 20% and 17% to 11%, respectively.[1,18] Interestingly, the present study, as reported previously,[9] showed that the use of PYI in the Atreus 3C configuration helped to improve the PLT content of the final pooled PC when compared with pooled PCs prepared with the Atreus 2C+ and the OrbiSac, because the content of PLTs in the final pooled PC was the same pooling 4 IPUs obtained from the Atreus 3C than pooling 5 BCs obtained from the Atreus 2C+. Because the introduction of automation in blood component preparation is changing the classic way of preparing PLTs, and we are reporting here changes in PLT volume, PLT concentration, metabolic changes, and lower expression of vWF, it should be desirable to perform future studies to compare the clinical outcome of PLT transfusions prepared by these two automated methods, as others did in the past comparing PLTs prepared either manually or semiautomatically with OrbiSac.[19]

Our study showed a slightly stronger decrease in glucose concentration and increase in lactate concentration in PCs prepared with the Atreus 3C when compared with PCs prepared with the Atreus 2C+ and the OrbiSac, and both variables were significant on Day 5 and Day 7 ($p < 0.05$). However, the glucose consumption rate was similar for both systems. This observation could be related to the fact that PCs prepared with the Atreus 3C resulted in a lower volume and higher PLT concentration when compared with PCs prepared with the Atreus 2C+ and the OrbiSac. Interestingly, when we compared the two groups of PLTs prepared with the Atreus 3C, the presence of PAS-IIIM resulted in a decrease of glucose consumption rate when glucose consumption was corrected by PLT concentration in the PCs. Thus, in all PCs prepared with both systems and both PASs, there was still plenty of glucose at the end of the storage.

Regarding PAS used in our study, PCs suspended in PAS-IIIM showed higher pH, lower expression of GPIbIIIa, lower expression of activation markers on PLT membrane (CD62P and CD63), and lower expression of vWF when compared with PCs suspended in PAS-II and prepared either with the Atreus 2C+ and OrbiSac or with the Atreus 3C ($p < 0.05$). We believe that these results can be explained in part because PAS-IIIM contains phosphate, potassium, and magnesium, in addition to NaCl, citrate, and acetate, present in PAS-II, and it is known that the combination of these three new components in PAS-IIIM is associated with complex effects and interdependence.[10] For example, previous studies have shown that the presence of magnesium in PAS significantly inhibited exposure of P-selectin, decreased binding of fibrinogen to ADP-activated PLTs, and significantly decreased agonist-induced PLT aggregation.[20,21] Additional studies showed that magnesium present in PAS reduced PLT activation and metabolic rate.[22,23]

In our study, we also measured MPC as an indicator of PLT activation. MPC is a new PLT variable reported by modern ADVIA cell blood count analyzers that measure the mean refractive index of the PLTs.[24,25] The previous hematology analyzer measures the intensity of light scattered by PLTs at two different angles (2-3° and 5-15°) and from the paired values computes the PLT volume and the PLT component concentration on a cell-by-cell basis. These values are then averaged to provide the MPC expressed in g/dL. MPC is linearly related to PLT density and is reduced when PLTs degranulate, thus indicating that PLTs have undergone activation.[26] Several studies have recently demonstrated that MPC values are inversely correlated with PLT membrane activation and subsequent P-selectin expression.[27] In fact, the MPC variable has been used as an indicator of PLT activation in different conditions and physiologic states,[28-31] as well as stored PCs.[25,32,33] In the present study, we observed that MPC decreased during 7-day PLT storage indicating PLT activation in the three arms of the study. However, higher MPC indicating lower PLT activation was seen in PCs prepared with the Atreus 3C and suspended in PAS-IIIM when compared with the other two arms of the study. Therefore, using MPC as a variable of PLT activation, PCs prepared with the Atreus 3C and suspended in PAS-IIIM showed lower PLT activation when compared with the other two arms of the study.

In conclusion, our study showed that PCs prepared with the Atreus 3C and suspended in PAS-IIIM had similar or slightly improved in vitro quality during a 7-day storage period compared to PCs prepared with the Atreus 2C+ and the OrbiSac and suspended in PAS-II. Moreover, those PCs had the advantage of a smaller total volume. Finally, PLT activation during a 7-day storage period was lower when the storage solution was PAS-IIIM in comparison with PAS-II.

ACKNOWLEDGMENT

AMG belongs to the Researcher's stabilization program of "Instituto de Salud Carlos III" from the Spanish government and "Direcció d'Estratègia i Coordinació del Departament de Salut" from the Generalitat de Catalunya.

CONFLICT OF INTEREST

GE received lecture fees and research grants from Terumo BCT. The rest of authors have no conflicts to disclose.

REFERENCES

1. Cid J. Automation in blood component preparation methods. In: Blajchman M, Cid J, Lozano M, editors. Blood

component preparation. From benchtop to bedside. 1st ed. Bethesda (MD): American Association of Blood Banks Press; 2011. p. 271-86.
2. Sawyer S, Whitley P, Cuartas I, Wellington M, Beddard R, Vasallo R, Rugg N, Cancelas J, Graminske S, Friedman KD, Foster S, Felt T, McAteer M. In vitro evaluation of leukoreduced RBC units obtained after automated separation of whole blood by the Atreus system. Transfusion 2008;48 Suppl 2:170-1.
3. Rugg N, Cancelas J, Pratt P, Worsham N, AuBuchon JP, Dumont LJ, Herschel L, Dumont D, Baker S, Waters S, McAteer M, Foster S, Felt T. In vivo evaluation of leukoreduced RBCs obtained after automated separation of whole blood units by the novel Atreus system. Transfusion 2008;48 Suppl 2:171.
4. Thomas S, Beard M, Garwood M, Callaert M, Waeg G, Cardigan R. Blood components produced from whole blood using the Atreus processing system. Transfusion 2008;48: 2515-24.
5. Thomas S, Beard M, Garwood M, Callaert M, Cardigan R. Platelet concentrates produced from whole blood using the Atreus processing system. Vox Sang 2009;97:93-101.
6. Sandgren P, Callaert M, Shanwell A, Gulliksson H. Storage of platelet concentrates from pooled buffy coats made of fresh and overnight-stored whole blood processed on the novel Atreus 2C+ system: in vitro study. Transfusion 2008; 48:688-96.
7. Cid J, Lozano M, Ramiro L, Escolar G, Puig L, Pla RP. Comparison of two semi-automated devices for preparing leukoreduced pooled buffy-coat derived pooled platelet concentrates in additive solution. Vox Sang 2008;95 Suppl: 318.
8. Sandgren P, Hild M, Sjodin A, Gulliksson H. Storage of buffy-coat-derived platelets in additive solutions: in vitro effects on platelets prepared by the novel TACSI system and stored in plastic containers with different gas permeability. Vox Sang 2010;99:341-7.
9. Sandgren P, van Waeg G, Verheggen C, Sjodin A, Gulliksson H. Storage of interim platelet units for 18 to 24 hours before pooling: in vitro study. Transfusion 2011;51:1213-9.
10. Gulliksson H. Containers and additive solutions for platelets. In: Blajchman M, Cid J, Lozano M, editors. Blood component preparation. From benchtop to bedside. 1st ed. Bethesda (MD): American Association of Blood Banks Press; 2011. p. 135-55.
11. Gulliksson H. Additive solutions for the storage of platelets for transfusion. Transfus Med 2000;10:257-64.
12. Gulliksson H. Storage of platelets in additive solutions: the effect of citrate and acetate in in vitro studies. Transfusion 1993;33:301-3.
13. Gulliksson H, AuBuchon JP, Cardigan R, Van der Meer PF, Murphy S, Prowse C, Richter E, Ringwald J, Smacchia C, Slichter S, de Wildt-Eggen J. Storage of platelets in additive solutions: a multicentre study of the in vitro effects of potassium and magnesium. Vox Sang 2003;85:199-205.
14. European Committee on Blood Transfusion. Guide to the preparation, use and quality assurance of blood components. 16th ed. Strasbourg: EDQM; 2011.
15. Perez-Pujol S, Lozano M, Perea D, Mazzara R, Ordinas A, Escolar G. Effect of holding buffy coats 4 or 18 hours before preparing pooled filtered PLT concentrates in plasma. Transfusion 2004;44:202-9.
16. Galan AM, Lozano M, Molina P, Navalon F, Marschner S, Goodrich R, Escolar G. Impact of pathogen reduction technology and storage in platelet additive solutions on platelet function. Transfusion 2011;51:808-15.
17. Jurado M, Algora M, Garcia-Sanchez F, Vico S, Rodriguez E, Perez S, Barbolla L. Automated processing of whole blood units: operational value and in vitro quality of final blood components. Blood Transfus 2012;10:63-71.
18. Cid J, Claparols M, Pinacho A, Hernandez JM, Ortiz P, Puig LS, Pla RP. Comparison of blood component preparation methods from whole blood bags based on buffy coat extraction. Transfus Apher Sci 2007;36:243-7.
19. Cid J, Ramiro L, Escoda L, Llorente A. Efficacy of transfusion of platelet concentrates obtained by manual pooling or by semiautomated pooling of buffy-coats: a retrospective analysis of count increment, corrected count increment and transfusion interval. Vox Sang 2009;96:29-33.
20. Gawaz M, Ott I, Reininger AJ, Neumann FJ. Effects of magnesium on platelet aggregation and adhesion. Magnesium modulates surface expression of glycoproteins on platelets in vitro and ex vivo. Thromb Haemost 1994;72:912-8.
21. Sheu JR, Hsiao G, Shen MY, Fong TH, Chen YW, Lin CH, Chou DS. Mechanisms involved in the antiplatelet activity of magnesium in human platelets. Br J Haematol 2002;119: 1033-41.
22. de Wildt-Eggen J, Schrijver JG, Bins M, Gulliksson H. Storage of platelets in additive solutions: effects of magnesium and/or potassium. Transfusion 2002;42:76-80.
23. Gulliksson H, AuBuchon JP, Vesterinen M, Sandgren P, Larsson S, Pickard CA, Herschel I, Roger J, Tracy JE, Langweiler M. Storage of platelets in additive solutions: a pilot in vitro study of the effects of potassium and magnesium. Vox Sang 2002;82:131-6.
24. Brummitt DR, Barker HF. The determination of a reference range for new platelet parameters produced by the Bayer ADVIA120 full blood count analyser. Clin Lab Haematol 2000;22:103-7.
25. Lim YA, Hyun BH. Evaluation of platelet parameters on the ADVIA 120 as the quality indicator for stored platelets. Clin Lab Haematol 2002;24:377-84.
26. Díaz-Ricart M, Brunso L, Pino M, Navalon F, Jou JM, Heras M, White JG, Escolar G. Preanalytical treatment of EDTA-anticoagulated blood to ensure stabilization of the mean platelet volume and component measured with the ADVIA counters. Thromb Res 2010;126:e30-e35.
27. Ahnadi CE, Sabrinah CE, Lepine M, Okrongly D, Pujol-Moix N, Hernandez A, Boughrassa F, Grant AM. Assessment of platelet activation in several different

anticoagulants by the Advia 120 Hematology System, fluorescence flow cytometry, and electron microscopy. Thromb Haemost 2003;90:940-8.
28. Brummitt DR, Barker HF, Pujol-Moix N. A new platelet parameter, the mean platelet component, can demonstrate abnormal platelet function and structure in myelodysplasia. Clin Lab Haematol 2003;25:59-62.
29. Boos CJ, Beevers GD, Lip GY. Assessment of platelet activation indices using the ADVIATM 120 amongst "high-risk" patients with hypertension. Ann Med 2007;39:72-8.
30. Chung I, Choudhury A, Lip GY. Platelet activation in acute, decompensated congestive heart failure. Thromb Res 2007; 120:709-13.
31. Chung I, Choudhury A, Patel J, Lip GY. Soluble CD40L, platelet surface CD40L and total platelet CD40L in congestive heart failure: relationship to platelet volume, mass and granularity. J Intern Med 2008;263:313-21.
32. Park IJ, Lim YA, Cho SR. Usefulness of delta value of platelet parameters on ADVIA 120 for the functional reactivity of stored platelets. J Clin Lab Anal 2010;24:38-43.
33. Beard MJ, Jeewa Z, Bashir S, Cardigan R, Thomas S. Comparison of platelet activation in platelet concentrates measured by flow cytometry or ADVIA 2120. Vox Sang 2011;101:122-30.