Correspondence

Cord Blood Processing by Using a Standard Manual Technique and Automated Closed System "Sepax" (Kit CS-530)

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To the Editors:

SEVERAL TECHNIQUES for umbilical cord blood (UCB) volume reduction ("processing") have been developed, but some of their disadvantages, such as long duration of procedure and insufficient reliability, are usually in evidence (1–5). Recently, a new cell separator adapted to low-volume cell suspensions has been developed (Sepax, Biosafe S.A., Eysins/Nyon, Switzerland). This system consists of a computer-controlled automated cytapheresis procedure ensuring a very effective volume reduction and low amounts of red blood cells (RBC) in the final products in a functionally closed system. In addition, the final product is collected directly into the freezing bags and can be frozen immediately after dimethylsulfoxide (DMSO) addition. We compared the efficacy of this device on UCB samples volume reduction with a manual system based on blood bags and using hydroxyethyl starch (HES) ($n = 19$ for each technique).

UCB samples were obtained after vaginal delivery of full-term newborns. They were collected (with informed consent of mothers) in sterile bags (cord blood bags, 150-ml CPD Vacuvam Macopharma, Tourcoing, France) containing anticoagulant and delivered to the Cell Therapy Unit of the Bordeaux Blood Center (Etablissement Français du Sang Aquitaine-Limousin, Site de Bordeaux). Only samples unsuitable for allogeneic transplantation were used for our experiments.

To perform the standard blood bag centrifugation technique of UCB volume reduction (PALL Medical Stem Cell Processing System), 6% (wt/vol) HES (Gri-fols Laboratory, Barcelona, Spain) was added to the anticoagulated UCB sample in a 1:5 ratio. The blood bag was then centrifuged (Sigma GK 15; Sigma Osterode am Hartz, Germany) for 15 min at 4°C at $50 \times g$. The plasma with white blood cell (WBC) fraction was transferred to a processing bag (Stem Cell Processing System kit, Pall Medical, Portsmouth, England) using a press. The WBC were then concentrated by a second centrifugation ($400 \times g$ for 10 min at 4°C) and the WBC-poor plasma was transferred to a transfer bag. After determining the volume, WBC-poor plasma was added to obtain a concentrate of a volume <30 ml before adding DMSO.

The automated separation was performed using the Sepax cell centrifugation device (Sepax S.A., Eysins-s/Nyon, Switzerland) for processing blood volumes from 20 to 200 ml in a one-step procedure with the CS-530 cell separation kit. Technical details concerning this device were described in ref. 6. Briefly, the bag containing UCB with 20% of HES was connected to the separation kit with a sterile docking system (Terumo TSCD SC-201). When UCB was transferred from the bag to the separation chamber, the centrifugation speed was automatically increased from 3,500 rpm ($360 \times g$) to 6,500 rpm ($1,250 \times g$) and maintained for 4 min before it was automatically decreased to 4,500 rpm ($600 \times g$). The extraction and collection phase were effected at this centrifugation speed, and UCB was separated into plasma, buffy coat, and RBC. The WBC concentrate was then collected by several synchronized automatic actions (see ref. 6 for details). At the end of procedure, the bags were

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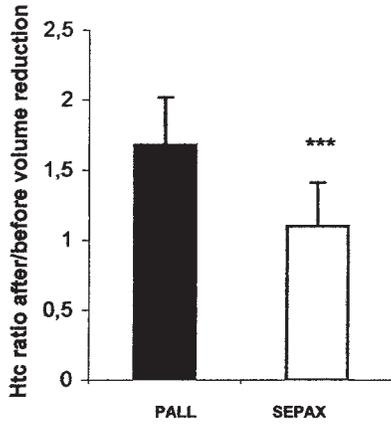
FIG. 1. Ratios of hematocrit values after/before reduction. *** = $p < 0.001$, *t*-test.

sealed and weighed for determining the volume of the collected WBC suspension before adding DMSO.

A DMSO-dextran solution (2.1 ml) was added to the volume-reduced UCB while being shaken at $+4°C$. The original UCB bag was then placed in a "protection bag," frozen in a metallic container using a controlled-rate procedure (Thermogenesis Bio Archive™-System 1G 36 26, Hemo System, Marseille, France), and stored in liquid nitrogen (7). The samples were thawed at $37°C$, washed twice (dextran-sorbitol, 1:1 solution), and analyzed.

Cell count and differential cell count was performed by using automatic cell counter (Argos 3diff ABX, Monpellier, France). CD34$^+$ cells were enumerated in the UCB, and WBC concentrates were analyzed by flow cytometry (8). Three-color fluorescence (flow cytometer FACSCalibur, Beckton Dickinson) was used to count vi-
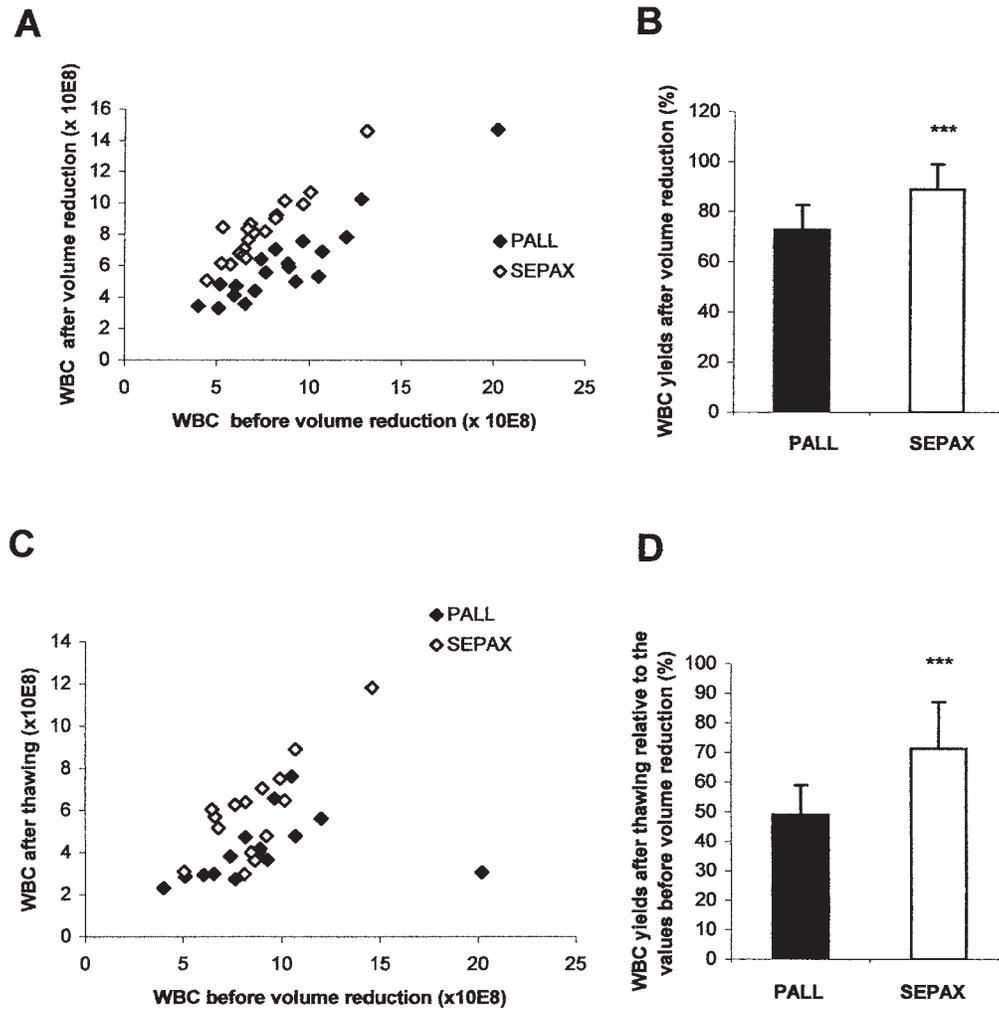
FIG. 2. Individual UCB WBC values before and after volume reduction (**A**) and mean WBC yields after volume reduction (**B**). The same parameters after freezing and thawing of volume-reduced UCB samples relative to the values before volume reduction (**C,D**). *** = $p < 0.001$, *t*-test.
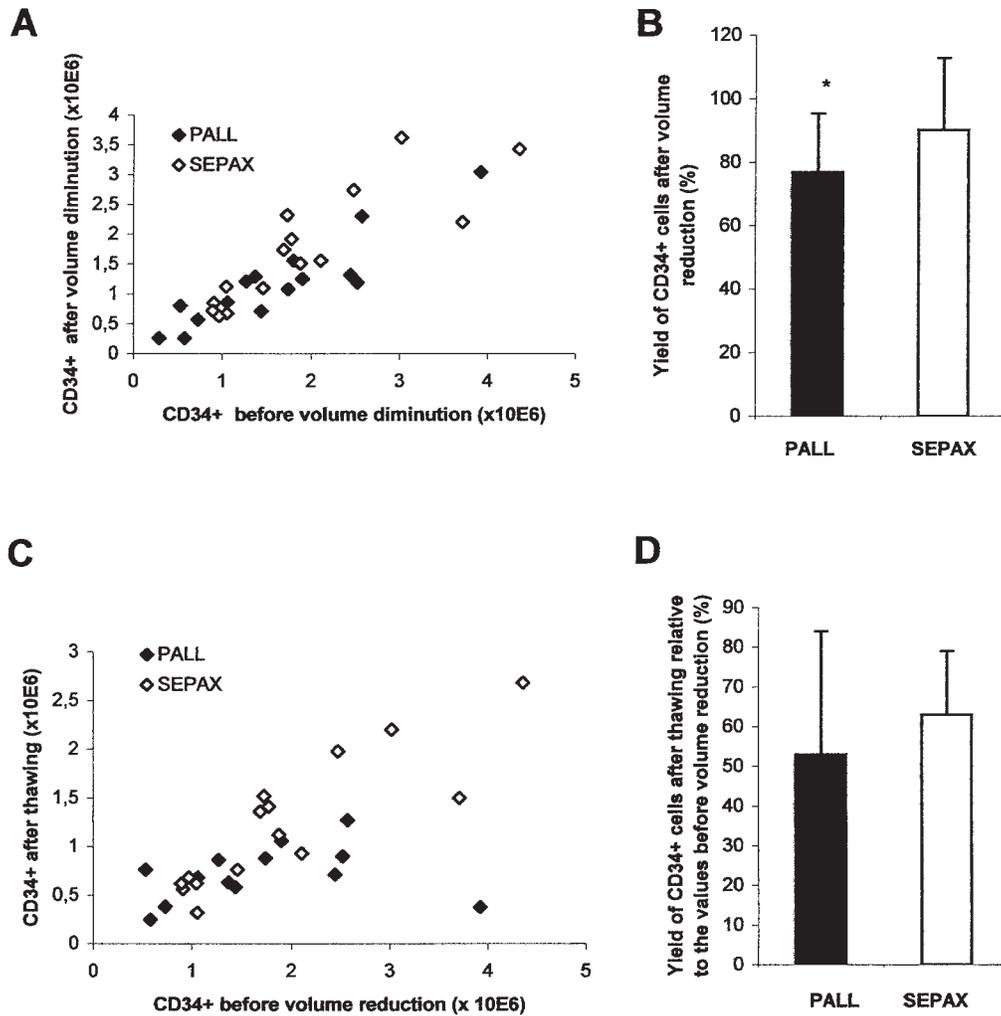
FIG. 3. Individual UCB CD34$^+$ cell numbers before and after volume reduction (**A**) and mean CD34$^+$ cell yields after volume reduction (**B**). The same parameters after freezing and thawing of volume-reduced UCB samples relative to the values before volume reduction (**C,D**). * = $p < 0.05$, t-test.

able CD45$^+$/CD34$^+$ cells directly by using microbeads (ProCOUNT, Becton Dickinson commercial kit). The yields of WBC and of CD34$^+$ cells were calculated by dividing total number of concerned cells after a processing step with the total number of the corresponding cell type prior to that step.

Our comparative test of Sepax separator and a manual technique (Pall) confirmed the advantage of first one with respect to the duration of manipulation (only 20 min against 90 min), as it was already stressed (6).

The volumes after processing using Pall system were heterogeneous, varying from 17 to 27 ml (mean = 22.5 ± 2.7 ml), whereas Sepax enabled very homogenous volumes after processing (22.5–24.5 ml; mean 23.7 ± 0.52 ml) whatever the initial volume of UCB sample (not shown). By contrast, the hematocrits in the

UCB samples after volume reduction were more homogenous in samples processed by using Pall technique (33–49%; mean = 56.8 ± 7.4%) than Sepax (23–47%; mean = 36.8 ± 9%) (not shown). The mean ratio of hematocrit values after/before volume reduction was significantly lower in the series of UCB samples processed by using the Sepax separator (Fig. 1), demonstrating that the automated technique was more efficient in RBC depletion. Indeed, we found a better RBC reduction in samples processed on Sepax by using the kit CS-530 (73.1 ± 10.8%; not shown) in our experiments than by manual technique and Sepax in previous study (47.5 ± 9.1%) (6).

The automated technique was also more efficient in the WBC recovery with respect to the initial values (Fig. 2A,B) ($p < 0.001$; t-test). The yield of CD34$^+$ cells was

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TABLE 1. MORPHOLOGY OF CELL CONTENT IN CORD BLOOD SAMPLES BEFORE AND AFTER VOLUME REDUCTION

Device	% of lymphocyte-like cells			% of monocytes			% of granulocytes		
	Before volume reduction	After volume reduction	Ratio after/before	Before volume reduction	After volume reduction	Ratio after/before	Before volume reduction	After volume reduction	Ratio after/before
PALL (n = 16)	38.6 ± 6.4	34.0***[a] ± 5.8	0.88 ± 0.07	13.4 ± 3.1	15.9*[a] ± 6.2	1.19 ± 0.35	48.6 ± 6.6	50.0 ± 8.2	1.04 ± 0.11
SEPAX (n = 15)	42.7 ± 8.4	40.2***[a] ± 8.	0.94*[b] ± 0.07	20.2 ± 9.7	15.9*[a] ± 5.9	0.91*[b] ± 0.36	37.1 ± 12.9	43.9***[a] ± 11.6	1.25*[b] ± 0.32

Student t-test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
[a]Relative to the values before volume diminution.
[b]SEPAX ratios relative to PALL.

also better ($p < 0.05$) by using the automatic separator Sepax than manual technique Pall (Fig. 3A,B). Thus, unlike Zingsem et al. (6), we demonstrated a better recovery of WBC ($90.0 \pm 9.1\%$) and CD34$^+$ cells ($90.2 \pm 22\%$) by using the Sepax system than by using a manual technique. Our values obtained by Sepax are also higher than corresponding values for WBC and CD34$^+$ cell recovery ($78.6 \pm 24.9\%$ and $83.6 \pm 32.5\%$, respectively) recently published (6). Two factors potentially could explain these differences: (1) the higher volumes of UCB samples in our study (103.1 ± 4.0 vs. 75.5 ± 10 in (6)) and (2) the separation kit (we used CS-530 and Zingsem et al. used CS-510). However, due to the absence of functional differences between two kits (only the final collection bag is different) this better efficiency of Sepax seems to be related primarily to the bigger volume of UCB samples in our study. If confirmed, this implicates an improvement of the separation program to adapt the dynamic parameters to the volume of each individual UCB sample.

The better yield of WBC in UCB samples by Sepax volume-reduced is maintained after freezing/thawing of UCB (Fig. 2C,D). However, the better yield of CD34$^+$ cells using Sepax (Fig. 3A,B) was abrogated when the samples are frozen and thawed (Fig. 3C,D). We think that the reason for this relatively lower efficiency of CD34$^+$ cell cryopreservation could be a suboptimal homogenization of DMSO-dextran with the cell suspension in the small bicompartmental bags of Sepax kit. Conversely, in the manual Pall technique, a larger volume of processing bags permits better homogenization of DMSO-dextran with the cell suspension before transferring the final suspension into small bags for cryopreservation. In addition, the better relative maintenance of lymphoid-like cells (the CD34$^+$ cell population reside in this fraction) is accompanied by a diminution in monocyte concentration and an increase in the concentration of granulocytes in the samples processed by Sepax (Table 1). This should be improved in the future evolution of the Sepax device since the depletion in granulocytes would improve considerably the properties of UCB cell suspension.

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