Recovery of white blood cells and platelets from leukoreduction system chambers of Trima Accel and COBE Spectra plateletpheresis devices

Recently, Dietz and coworkers[1] described a new source of viable peripheral blood mononuclear cells (PBMNCs) that they recovered from leukoreduction system chambers (LRSCs) of Trima Accel apheresis devices (Gambro BCT, Lakewood, CO) after routine donor plateletpheresis procedures. These white blood cells (WBCs), which are usually discarded, functioned excellently in conventional in vitro assays and therefore represent a potential and novel source for research-grade cellular products.

In the following report, we describe our results comparing the yields of WBCs recovered from LRSCs in Trima Accel (software version 5.1, Gambro BCT) and COBE Spectra (software version 7.0, Gambro BCT) plateletpheresis devices. After a storage period of 2 hours at room temperature, we drained the contents of the LRSCs into 20-mL conical tubes. We counted WBCs, platelets (PLTs), and red blood cells with a blood cell counter (ADVIA 120, Bayer HealthCare Diagnostics Division, Tarrytown, NY) and quantified CD14+ monocytes by flow cytometry (FACSCalibur, BD, San Jose, CA), as previously described.[2] WBC and PLT yields were significantly different for the plateletpheresis devices (Table 1). Lymphocyte yields were greater and more consistent than monocyte yields. The minimum and maximum monocyte yields differed by 10-fold. WBC yields from Trima Accel LRSCs were 30 times greater than those from COBE Spectra LRSCs. Dietz and coworkers recovered 1.88×10^9 PBMNCs from Trima Accel LRSCs. We recovered a mean of 66.5 percent fewer PBMNCs (0.69×10^9 PBMNCs) from Trima Accel LRSCs.

These different results may reflect actual differences in recoveries between the two plateletpheresis devices or, possibly, different instrument settings during plateletpheresis collections. For our study, we used an anticoagulant ratio of 11:1, whereas the ratio was 13:1 in the study by Dietz and coworkers.[1] Also, the draw management (6 vs. 3), return management (4 vs. 1), and maximal draw flow (medium vs. fast) were different in our study compared with that by Dietz and colleagues. Thus, direct comparison of the cell yields of both studies is limited. The wide range of CD14+ monocytes recovered in LRSCs could impair the consistency of the results of monocyte-derived dendritic cells (DCs). Typically, monocyte yields from the Trima Accel LRSCs consisted of only approximately 10 percent of the cell yield of a leukapheresis unit.[2] With current culture techniques, the

TABLE 1. Comparison of Trima Accel and COBE Spectra LRSC cell yields

Measure*	Median†	Range	Mean ± SD
Donation time (min)	(1) 54	38-91	57 ± 15
	(2) 68	43-90	67 ± 18‡
Separation volume (L)	(1) 3.20	1.95-4.19	3.21 ± 0.68
	(2) 4.56	3.10-6.44	4.61 ± 1.25§¶
Product volume (mL)	(1) 8.50	5.8-9.5	8.0 ± 1.1
	(2) 9.35	8.8-10.0	9.3 ± 0.4‡§
Preapheresis WBC count ($\times 10^9$/L)	(1) 5.6	3.4-7.5	5.52 ± 1.01
	(2) 5.9	4.5-7.3	6.01 ± 0.89¶
Postapheresis WBC count ($\times 10^9$/L)	(1) 6.4	3.7-10.4	6.40 ± 1.44
	(2) 6.6	4.6-8.3	6.60 ± 1.17¶
PLT yield ($\times 10^{11}$)‖	(1) 5.65	2.29-6.67	4.82 ± 1.47
	(2) 5.15	2.37-7.58	5.18 ± 2.06‡
WBC concentration ($\times 10^9$/L)	(1) 112.1	65.2-205.2	122.6 ± 38.8
	(2) 3.09	0.85-8.86	3.37 ± 2.31‡§
WBC yield ($\times 10^9$)	(1) 0.93	0.54-1.76	0.98 ± 0.33
	(2) 0.03	0.008-0.084	0.032 ± 0.022§¶
CD14+ product (%)	(1) 17.6	1.25-25.3	16.7 ± 5.6
	(2) 10.3	2.15-25.1	11.5 ± 6.2**¶
CD14+ yield ($\times 10^8$)	(1) 1.60	0.22-3.16	1.60 ± 0.74
	(2) 0.03	0.008-0.12	0.037 ± 0.036§‡
Lymphocytes (%)	(1) 56.3	40.7-64.2	54.8 ± 6.79
	(2) 53.3	30.3-73.6	54.1 ± 14.2¶
Lymphocyte yield ($\times 10^8$)	(1) 5.33	2.50-8.38	5.33 ± 1.72
	(2) 0.14	0.023-0.62	0.186 ± 0.168‡§
PBMNCs (%)	(1) 72.9	42.0-83.6	71.5 ± 8.5
	(2) 68.9	35.5-82.9	65.5 ± 15.7‡
PBMNC yield ($\times 10^8$)	(1) 7.14	3.47-11.0	6.93 ± 2.16
	(2) 0.16	0.03-0.70	0.22 ± 0.19§‡
PMCs†† (%)	(1) 9.0	1.7-14.5	8.85 ± 3.11
	(2) 18.6	4.2-47.7	19.87 ± 13.79¶**
PMC†† yield ($\times 10^8$)	(1) 0.80	0.28-1.53	0.83 ± 0.34
	(2) 0.03	0.172-0.168	0.052 ± 0.046‡§
PLT concentration ($\times 10^{12}$/L)	(1) 2.33	1.40-4.90	2.51 ± 0.97
	(2) 0.53	0.32-0.63	0.50 ± 0.10‡§
PLT yield ($\times 10^{10}$)	(1) 1.74	0.86-4.21	2.04 ± 0.92
	(2) 0.48	0.28-0.58	0.47 ± 0.09‡§
Hematocrit (%)	(1) 58.0	2.7-64.8	52.1 ± 17.7
	(2) 6.60	4.5-26.5	8.7 ± 6.4§‡
RBC ($\times 10^{12}$/L)	(1) 5.99	0.30-7.31	5.53 ± 1.90
	(2) 0.68	0.39-2.87	0.90 ± 0.71§‡

* Mean ± 1 SD.
† (1) Trima Accel (n = 27); (2) COBE Spectra (n = 10).
‡ U test.
§ $p < 0.01$.
¶ t Test.
‖ Product.
** $p < 0.05$.
††PMCs = polymorphic cells (granulocytes).

production of a therapeutic DC vaccination series requires more than 10^9 monocytes. WBC elutriation to enrich monocytes also requires a minimum of 10^9 monocytes as starting population for DC cell culture.[3] Currently, WBC yields from Trima Accel LRSCs are insufficient for the production of a whole therapeutic DC vaccine series. With improved ex vivo monocyte expansion protocols, the required minimum cell yield for DC vaccines could decrease. Given the mean loss of 10^9 WBCs per plateletpheresis collection by the Trima Accel, a 24-apheresis-procedures-per-year donor could lose more than 2×10^{10} WBCs. In comparison, the same number of plateletpheresis procedures with the COBE Spectra LRSCs would remove only 3.2×10^7 WBCs per donation or a total of 7.7×10^8 WBCs for 24 collections per year. Strauss[4] reported high losses of lymphocytes per PLT donation with previous plateletpheresis methods. This observation of immediate and long-term decreases in donors' lymphocyte counts lead to limits on the frequency of plateletpheresis. The number of WBCs retained by Trima Accel LRSCs after plateletpheresis is considerably less than the calculated WBC loss of these earlier studies. Only 10.8 percent of the PLT donors of our study (4/37) had decreased WBC counts immediately after plateletpheresis. For 89 percent of donors there was an increase in the postdonation WBC count (Table 1). The difference between predonation and postdonation WBC counts was slightly higher ($p = 0.32$) for Trima Accel procedures. A possible reason for that observation could be WBC recruitment, which may be greater during increased WBC loss.[2] Rock and coworkers found only minimal changes of donor WBC counts after repeated plateletpheresis.[5] Reasons for the variable number of WBCs retained in the Trima Accel LRSCs should be subject to further investigation to get a better standardization of this new WBC product.

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Reply

We recently described apheresis LRS chambers as an efficient source of research-grade PBMNCs.[1] Strasser and colleagues[2] could not replicate our data and challenged their reproducibility. They are correct that the type and settings of the apheresis machine can influence the number of recovered cells. The machine-borne differences are expected as the COBE Spectra uses a two-stage channel for blood separation whereas the Trima Accel uses a single-stage channel. We did not use the COBE Spectra and cannot comment on the findings of Strasser and colleagues.

The reported differences between the results of Strasser and colleagues and ours with the Trima Accel are most likely associated with differences in the volume of processed blood. Strasser and colleagues processed a mean of 3.21 L during 54-minute platelet (PLT) collections.[2] In contrast, we routinely process a mean of 4.25 L in 80 minutes in plateletpheresis collections (not including triple-PLT-product collections, which were not performed with LRS chambers in our report). Thus, the 66.5 percent difference in recovered PBMNCs between Strasser and colleagues and us can largely be attributed to the fact that Strasser and colleagues processed only 77.5 percent of our typical volume. It is noteworthy that we process the volume of 4.2 L because the Trima Accel LRS chamber is considered full at 4.7 L of blood processed for a donor with a body mass index (BMI) of less than 30 and 4.1 L for a donor with a BMI of more than 30; the machine automatically clears the LRS chamber at these volumes. Thus, the settings we used maximized the number of cells in the LRS chamber.

Other discrepancies between the results of Strasser and colleagues and us may be attributed to different collection procedures including different draw management, return management, or maximal draw flow settings. Additional factors influencing LRS chamber cell content—not mentioned by Strasser and colleagues—include the BMI of donors and concurrent plasma collection; we did not

collect the concurrent units of plasma on the donors whose LRS chambers were used in our study.

Both our data and the data of another recent independent report[3] indicate that monocyte recovery in the LRS chamber does not cover a "wide range," in contrast to the suggestion by Strasser and colleagues. We routinely use the LRS chambers as a source of normal PBMNCs and have found them a consistent and reliable cell source for monocyte selection for dendritic cell culture. Discussion regarding the best source of monocytes for therapeutic use goes beyond monocyte yields and includes ease of selection, cost, required dose, efficiency of culture, and other variables beyond the scope of our article. We agree with Strasser and colleagues that LRS chambers would not provide adequate cell doses for the manufacture of therapeutic products at present. For therapeutic products, a MNC collection should be performed on the patient and/or donor, not a PLT collection.

The consistency of LRS chambers as a source of research-grade cells has proven so reliable that we have eliminated the preparation of buffy coats and replaced them with LRS chambers (approx. 300 in the first 5 months of 2007) for scientists at the Mayo Clinic.

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Leukoreduction of transfused blood and postoperative infection: the "confounder variable" hypothesis

Blumberg and coworkers[1] illustrate how the intention-to-treat principle makes it difficult to asses the randomized controlled trials (RCTs) investigating leukoreduced blood transfusion to decrease postoperative infections. We report on an additional difficulty that, despite being less known, may affect the very substance of these RCTs.

Previously, we reported that in RCTs designed to study the effect of allogeneic white blood cells on postoperative infections, patients transfused with filtered, leukoreduced RBCs may have received blood units that were stored for a shorter duration compared to blood units transfused to controls.[2] This effect could result from the common practice of selecting younger RBC units for laboratory or bedside filtration, whereas units approaching their expiration date are selected for routine transfusion. In the minority of trials where relatively fresh RBCs were used independently of the randomization arm, heavily transfused patients were likely to have received older RBCs (off-protocol), and these were the specific patients who experienced more postoperative complications (Refs. 12, 14, and 15 in Blumberg et al.[1]). Therefore, it is quite possible that leukoreduction was a confounder for fresher blood in many of the RCTs reviewed by Blumberg and coworkers.[1]

The "confounder variable" hypothesis points to a biologically plausible mechanism for the variety of deleterious effects that have been ascribed to transfusion of nonleukoreduced blood. Upon storage, RBCs undergo a series of biochemical and biomechanical changes that impair oxygen delivery to tissues, which may contribute to infection and organ failure (reviewed by Tinmouth et al.[3]). Indeed, longer storage of transfused RBCs has been associated with a higher risk of adverse outcomes in the postoperative period and in critically ill patients, including surgery-related and nosocomial infection, sepsis, multiorgan failure, and increased length of stay in the intensive care unit.[3] Although stored RBCs recover their normal function several hours after transfusion, the first few hours after tissue contamination by bacteria are critical for wound infection to be established.[4]

Therefore, besides other difficulties in interpreting the results from the RCTs cited by Blumberg and coworkers[1] the question of whether or not leukoreduction prevents the variety of deleterious effects ascribed to allogeneic blood transfusion cannot be answered until the storage time of transfused RBCs is taken into account.

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The intention-to-treat principle in clinical trials and meta-analyses of leukoreduced blood transfusions in surgical patients

We read with much interest the article by Blumberg and colleagues[1] on the different techniques used in meta-analyses of by-filtration leukoreduced (FLR) blood transfusions. Several meta-analyses have been published, but their contradictory conclusions made it difficult to reach consensus. Blumberg and coworkers nicely illustrate the different approaches that have been used and that explain the different conclusions. Sadly, instead of using this knowledge to aim for consensus, they continued with further polarizing the discussion. They accuse others of including invalid data, while in their presented results they invalidly exclude 204 patients (175 transfused with buffy coat–depleted blood, who happen to have an infection rate lower than in the FLR group). They made a good start, on page 574, when they compared intention-to-treat with as-treated analyses and concluded: “Each approach has strengths and weaknesses.”

The as-treated (scientific) analysis focuses on the patients who received transfusions, to prevent dilution of a potential effect. Therefore, it is the best method to investigate if the transfusion of FLR blood products can reduce postoperative infections. It will answer the questions on which is the best product to transfuse and inform us about the efficacy of the treatment. In other words, it supplies us with answers on the product (patient) level.

In contrast, the intention-to-treat analysis takes the whole population into account, including differences in infection rates in the subpopulation that did not receive transfusions due to differences in transfusion triggers used. Therefore, this is the best method to investigate if changing the standard procedure to transfusing FLR blood products will reduce postoperative infections. It will answer the questions on which is the best policy and inform us about the effectiveness of a policy change. In other words, it supplies us with answers on the policy (population) level.

How to analyze and what now to report? When reporting a transfusion study it would be best to include both the intention-to-treat and the as-treated results because “Each approach has strengths and weaknesses.” For meta-analyses, this is more difficult. If a meta-analysis is performed to clarify etiologic issues, the analyses could best focus on the “scientific” as-treated data. To convince society to accept a policy change, however, it is best when a meta-analysis shows benefit using an intention-to-treat approach.

I draw attention to two other issues within the transfusion setting where there is a discrepancy between the patient level and the population level. The first is matching for RBC antigens like c, E, and K. Some patients will absolutely benefit from this policy; none will be harmed. For the majority of transfused patients, however, no clinical benefit will be shown. Still this policy is introduced and/or used in specific subgroups of patients. The second issue is the use of a type-and-screen policy to reduce the number of cross-matches. For the majority of patients, no adverse clinical effect will be shown, but some patients will definitely have adverse effects from this policy; still, it is introduced and/or used in specific subgroups of patients.

In these two situations with conflicting results, no general decision was forced. The policies were introduced for only specific subgroups of patients, where the clinical benefits were optimal and/or the adverse clinical effects minimal. In the issue of FLR blood transfusions in surgical patients, would it not be better to stop trying to force a general decision and use our energy to identify subgroups of patients where we should introduce and/or use FLR blood products, especially because from the included studies only one study does inform us on patients undergoing other than cardiac or colorectal surgery?

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