Effects of platelet-sparing leukocyte reduction and agitation methods on in vitro measures of hemostatic function in cold-stored whole blood

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BACKGROUND: Agitation of platelet units stored at room temperature is performed routinely to maintain platelet function, and leukoreduction of blood products is the standard of care in many countries to reduce immune consequences of transfusion. The effect of agitation and leukoreduction on whole blood stored at 4°C requires investigation, as reductions in hemostatic capacity of whole blood may reduce its efficacy in treating trauma-induced coagulopathy and platelet dysfunction. We hypothesize that agitation of whole blood will not affect hemostatic function and that leukoreduction will reduce hemostatic function of whole blood.

METHODS: In this in vitro randomized controlled study, 21 units of leukoreduced and 20 nonleukoreduced whole blood units were each randomly assigned into four agitation groups. Hemostatic parameters were measured using viscoelastic assays (rotational thromboelastometry-Extrinsic Screening Test (ROTEM-EXTEM) and thromboelastography (TEG) platelet mapping), impedance aggregometry (agonists—adenosine phosphate, arachidonic acid, thrombin receptor activating peptide, and collagen), and a thrombin generation assay from these whole blood units before and after filtration and on 0, 5, 10, and 15 days of storage at 4°C.

RESULTS: Leukoreduction compared to nonleukoreduction reduced platelet concentration on Day 0. Viscoelastic measures and thrombin generation parameters revealed significant reduction in hemostatic function between the leukoreduced units and the non-leukoreduced units at a few time points. Leukoreduced units consistently demonstrated reduced platelet aggregation compared to the nonleukoreduced units. Agitation methods did not significantly affect any of the hemostatic parameters examined.

CONCLUSIONS: Leukoreduction of whole blood with a platelet-sparing filter caused a moderate but significant reduction in some measures of whole blood hemostatic function most evident early in storage. The benefits of leukoreduction should be weighed against the potential reduced hemostatic function of leukoreduced units. Agitation of whole blood is not required to maintain hemostatic function. (*J Trauma Acute Care Surg.* 2018;84: S104–S114. Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: In vitro randomized controlled trial, level 1.
KEY WORDS: Whole blood; leukoreduction; platelet-sparing filter; hemostasis monitoring.

Globally, more than five million people die from traumatic injuries each year, accounting for 9% of the total deaths worldwide.[1] Traumatic hemorrhagic shock is the leading cause of death that is preventable after injury, accounting for potentially 30,000 deaths per year in the United States.[2] Damage control resuscitation is a bundle of care that aims to reduce death from traumatic hemorrhage.[3] A central component of damage control resuscitation is hemostatic resuscitation, which is characterized by resuscitating with whole blood (WB) or its equivalent with units of red blood cells (RBCs), plasma, and platelets (PLTs) in a 1:1:1 unit ratio.[4,5]

Recent US military reports indicate potential survival benefits with warm fresh WB.[6,7] This product is typically collected on site and rapidly transfused to combat casualties at risk of traumatic hemorrhagic shock. As a result of its immediate need to be transfused, formal transfusion-transmitted disease testing cannot be performed; therefore, it is not a Food and Drug Administration (FDA)-licensed product.[6] This has been one barrier to the use of warm fresh WB in civilian trauma centers. Cold-stored (2–6°C) WB is licensed by the FDA and can be stored for up to 35 days. Due to the interest in returning to the use of WB for hemorrhagic shock, low-titer Group O WB (LTOWB) is starting to be implemented at some large civilian trauma centers and emergency medical systems in the United States and Norway.[8] These programs limit storage duration to 14 to 21 days based on concerns regarding red blood cell and PLT efficacy past 21 days of storage.[9,10] Historically, there have been hundreds of thousands of LTOWB units transfused during the Korean and Vietnam conflicts with reports indicating its safety regarding a very low risk of hemolytic reactions.[11–13]

Low-titer Group O WB use in traumatic hemorrhagic shock has several advantages over conventional component therapy

including higher hemoglobin, clotting factor, and PLT concentrations compared to reconstituted WB, and, as it is provided in one bag, its use simplifies the logistics of the resuscitation.[8,14,15] Low-titer Group O WB should also be safer than reconstituted WB, since the reconstituted WB includes PLTs stored at 22°C, which increase the risk of bacterial contamination. Low-titer Group O WB should also confer a lower risk of hemolysis compared to the current common practice of transfusing nontitered type A plasma and PLTs when transfused in an ABO incompatible manner. An additional method to further improve the safety of LTOWB is leukoreduction (LR), which can reduce febrile reactions, cytomegalovirus (CMV) transmission, and human leukocyte antigen (HLA) alloimmunization. A concern with LR using a PLT-sparing filter is that PLT number and function may be reduced, impairing its hemostatic capacity. This potential issue is compounded by the fact that PLT function is reduced over time in WB.[10] Agitation of PLT units stored at room temperature is performed routinely to maintain PLT function. While WB stored at 2°C to 6°C historically has not required agitation, the effect of agitation on a full range of functional hemostatic measures has not been examined.

The use of LTOWB for patients with life-threatening traumatic bleeding will likely expand, since the The American Association of Blood Banks (AABB) recently announced that LTOWB is now able to be used as a standard product for patients with severe bleeding.[16] With the increased implementation of LTOWB, several practical questions have arisen regarding the hemostatic consequences of LR using an FDA-approved PLT-sparing filter, and agitation of WB.[17–19] In this series of in vitro studies, the effect of LR using the FDA-approved PLT-sparing filter on hemostatic function in cold-stored WB was examined. The effect of various agitation methods on PLT function in cold-stored WB stored for 15 days was also evaluated.

METHODS

LR and Non-LR Units

The Washington University School of Medicine Institutional Review Board approved this study. At a local FDA-licensed blood center, 500 mL of WB was collected from donors and randomly placed into either an FDA-approved IMUFLEX WB-SP blood bag system with an in-line PLT-sparing LR filter (Terumo BCT, Lakewood, CO), or into a citrate-phosphate-dextrose-containing collection bag, using double blood-pack units (Fenwal, Inc, Lake Zurich, IL). Immediately after collection, the WB units were placed into a golden hour box (Pelican Biothermal, Plymouth, MA) set at 4°C and then transported to the research laboratory within 1 hour after donation for further processing and storage. Leukoreduction was performed on the units collected with the Terumo collection system approximately 1 hour after collection. Ten-milliliter aliquots from each unit were obtained by aseptic technique both before and immediately after LR to perform PLT concentration and PLT function testing. Any unit that did not meet the PLT concentration criteria of more than 110×10^9/L before or after LR was discarded. Leukoreduction with the PLT-sparing filter occurred in our research laboratory instead of the blood collection center because this filter is not used at the blood collection center and it was simpler to leukoreduce the WB units in our research laboratory.

WB Storage, Agitation Conditions, and Sampling

The units that met criteria for analysis were then randomly assigned to one of four agitation groups: (1) un-rocked (control), (2) shake up and down for 1 minute one time per day in a manner that was similar to shaking hands, (3) flat rocker, continuous mechanical horizontal agitation (PF-42; Helmer Scientific, Noblesville, IN) at approximately 70 rpm; and 4) continuous end-over-end rocking (PAS-40; Helmer Scientific) at approximately 6 rpm. All units were kept in a monitored refrigerator with an interior temperature of between 2°C and 6°C for 15 days.

Samples for testing were taken from the unit's port, using aseptic technique, on the day of collection (Day 0) and at each time point thereafter. Before sampling on Days 5, 10, and 15, all units were placed at room temperature to warm for approximately 10 minutes and gently massaged for an additional 1 to 2 minutes before removing a 10-mL aliquot for testing to ensure adequate resuspension of settled cells.

Laboratory Measurements

Platelet concentration, hematocrit, and hemoglobin were measured using a Sysmex XN-10 hematology analyzer (Anchorage, AK). Activated partial thromboplastin time, prothrombin time, and fibrinogen were measured using a STA Compact Max analyzer (Diagnostica Stago, Inc, Parsippany, NJ). Potassium and blood pH were measured using an ABL 90 Flex blood gas analyzer (Radiometer Medical, Bronshoj, Denmark).

Thromboelastography (TEG)

All samples were tested using a TEG 5000 analyzer and a PLT mapping assay kit (Haemonetics, Niles, IL), in accordance with the manufacturer's instructions. Briefly, sample aliquots (360 µL) were recalcified by the addition of calcium chloride (0.2 mol/L, 32 µL) and added to individual cups containing activator F, adenosine diphosphate (ADP), or arachidonic acid (AA). An additional 360-µL kaolin-treated aliquot was run in conjunction with the agonists. Tests were allowed to run for 1 hour at 37°C.

Impedance Aggregometry

Aggregation was determined using a multiplate impedance aggregometer (Dynabyte Medical, Munich, Germany) with WB samples following the manufacturer's instructions. Adenosine diphosphate (ADP), AA, thrombin receptor–activating peptide (TRAP), and collagen were used as agonists (Roche Diagnostics, Indianapolis, IN). The test was initiated by incubating 300 µL of WB with 300 µL of $NaCl_2/CaCl_2$ (ADP, TRAP) or 0.9% $NaCl_2$ (arachidonic acid, collagen) for 3 minutes. After incubation, 20 µL of agonist (6.5 µmol/L ADP, 0.5 mmol/L ASPI, 32 µmol/L TRAP, or 3.2 µg/mL collagen) was added. The tests were run for 6 minutes and the area under the curve (AUC) was calculated.

Rotational Thromboelastometry (ROTEM)

Clot formation was analyzed using a ROTEM delta WB analyzer (Munich, Germany) following the manufacturer's instructions. Citrated blood (300 µL) was placed into a disposable cuvette. The test was started by adding the recalcification reagent, and the extrinsic activator, Ex-tem (TEM Systems, Inc, NC). The temperature was set to 37°C. Each sample was allowed

to run for 1 hour. All ROTEM parameters were recorded. The maximum clot firmness (MCF) was reported for each sample.

Thrombin Generation

Thrombin generation was measured using a calibrated automated thrombinoscope (Diagnostica Stago) in accordance with the manufacturer's instructions. All reagents including PLT-poor plasma high and low, FluCa-kit, and thrombin calibrator were obtained from Diagnostica Stago. Platelet-poor plasma was prepared by twice centrifuging an aliquot of WB at 2500 G for 10 minutes at room temperature. The plasma was collected and spun at 10,000 G for an additional 10 minutes, transferred to a clean microfuge tube, and stored at between −70°C and −80°C. Plasma samples were thawed for exactly 10 minutes in a water bath at 37°C. A sample of thawed plasma (80 µL) was added to the PLT-poor plasma high and low reagents (20-pmol/L tissue factor and 1-pmol/L tissue factor, respectively) or thrombin calibrator (20 µL) in an Immulon 2HB transparent U-bottom 96-well plate (ThermoFischer Scientific, St. Louis, MO) and incubated for 10 minutes at 37°C. Each sample was run in triplicate. Measurements were started by the addition of Fluo-substrate buffer (40 µL) into each well and were recorded every 20 seconds for 1 hour. The endogenous thrombin potential (ETP) is reported for each sample.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics (Version 24; Armonk, New York). All data are described as median (interquartile range) unless otherwise noted. Comparison of continuous nonparametric data (LR vs non-LR) was performed with the Wilcoxon rank sum. The Kruskal-Wallis test was used to compare continuous nonparametric data between more than two groups. Analyses included comparisons between study groups (LR vs non-LR or each agitation method) at each time point, and percent change from Day 0 to Day 15 between LR and non-LR. The Wilcoxon sign rank test was used to compare absolute median values in nonparametric data from Day 0 to Day 15 in LR and non-LR and in agitation methods. A Bonferroni correction was used to adjust p values for the four time points studied in the comparisons at each time point between LR and non-LR, and agitation methods. A p value less than 0.05 was considered significant. Samples that had a PLT concentration of less than 110×10^9/L were excluded from further analyses of hemostatic function because these were determined to be filter failures according to the filter's licensing criteria for PLT units or were below this threshold before LR.

RESULTS

Effect of LR on PLT Concentration

Of 26 units that underwent LR, 5 units had a pre-LR or post-LR PLT concentration of less than 110×10^9/L and were excluded from further analysis. The initial PLT concentration at Day 0 immediately after LR (n = 21) was lower than the PLT concentration in the non-LR group (n = 20); median, 162×10^9/L (146–202) versus 231×10^9/L (196–313), respectively ($p = 0.004$). At all other time points analyzed, there was no difference in the median PLT concentration between the LR and non-LR groups. The median PLT concentration decreased

significantly from Day 0 to Day 15 in both the LR (162×10^9/L to 95×10^9/L) and non-LR groups (Day 0 = 231×10^9/L; Day 15 = 99×10^9/L), respectively ($p < 0.001$). There was no difference in the percent change from Day 0 to Day 15 between the LR and non-LR groups, −46.8% and −46.3%, respectively ($p = 0.92$; Fig. 1).

Effect of LR on Viscoelastic Parameters

On Day 0, the median ROTEM-MCF in the LR group was reduced compared to that in the non-LR group; 58 mm (55–61.5) versus 65 mm (61–67), respectively ($p = 0.003$). On Day 5, the median ROTEM-MCF in the LR group was also reduced compared to that in the non-LR group; 55 mm (53–62) versus 64 mm (58–65), respectively ($p = 0.001$). By Day 15, the median values for ROTEM-MCF in both the LR and non-LR groups were below the lower limit of the reference range of 52 mm (Fig. 2). The median ROTEM-MCF was significantly reduced from Day 0 to Day 15 in both the LR and non-LR groups, ($p < 0.001$), (Fig. 2). There was no difference in the percent reduction of ROTEM-MCF from Day 0 to Day 15 between the non-LR (−25.2%) and LR groups (−21.2%) ($p = 0.65$; Fig. 2).

There was no statistically significant difference between TEG maximal amplitude (MA), MA-ADP, and MA-AA values between the non-LR and LR units on each day tested, except for Day 0 where TEG MA was reduced in the LR group compared to the non-LR group (45 (43–55) vs 61 (56–64), respectively; ($p = 0.02$), (Fig. 2). In both LR and non-LR groups, the difference in TEG MA, MA-ADP, and MA-AA results were significantly reduced between Days 0 and 15, except for the TEG MA results in the LR group and TEG MA-ADP results in the non-LR group (Fig. 2). There was no difference in the percent reduction from Day 0 to Day 15 between LR and non-LR groups for TEG MA, (LR = −14.44% and non-LR = −13.06%, $p = 1$), TEG MA-ADP (LR = −20% and non-LR = −9.4%, $p = 0.22$),
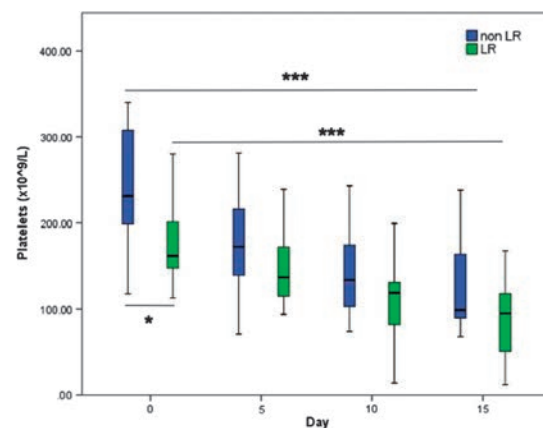
Figure 1. Platelet concentration in nonleukoreduced and leukoreduced WB units over time. This figure shows the platelet concentration of *nonleukoreduced* (*blue bars*) and *leukoreduced* (*green bars*) WB units over 15 days. Median platelet concentrations were compared at each time point between LR and non-LR groups and absolute median platelet concentrations were compared from Days 0 to 15 within LR or non-LR groups. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
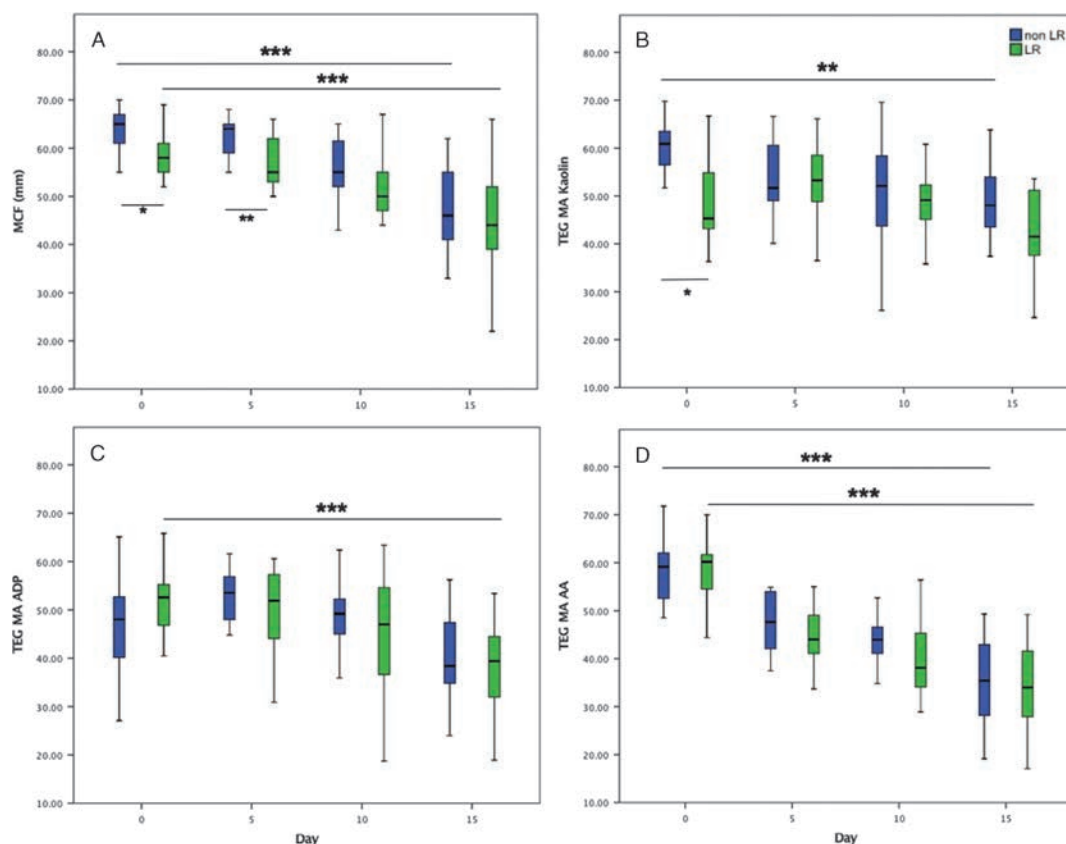
Figure 2. Viscoelastic measures in nonleukoreduced and leukoreduced WB units over time. This figure shows viscoelastic measures of *non-LR* (*blue bars*) and *LR* (*green bars*) whole blood units over 15 days. These include the *MCF* measured by ROTEM (*A*), MA measured by *TEG* (*B*), *TEG MA-ADP* (*C*), and *TEG MA-AA* (*D*). Median measurements of MCF, TEG MA, TEG MA-ADP, and TEG MA-AA were compared at each time point between LR and non-LR groups, and absolute median measurements were compared from Day 0 to Day 15 within LR or non-LR groups. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

and MA-AA (LR = −37% and non-LR = −38.7%, $p = 0.97$) from Day 0 to Day 15 (Fig. 2).

Effect of LR on PLT Aggregation

The median AUC for aggregation with all four agonists was significantly reduced in the LR compared to the non-LR units on Days 0 and 5 (Fig. 3). On Day 10, the LR units had lower aggregation with ASPI, ADP, and collagen agonists compared to the non-LR units. By Day 15 of storage, the aggregation response to all four tested agonists was low, and there were no statistically significant differences between the two groups. Platelet aggregation with all four agonists was reduced from Day 0 to Day 15 in both the non-LR and LR groups ($p < 0.001$). There was no difference in the percent reduction of aggregation with the four agonists tested from Day 0 to Day 15 between the LR and non-LR groups (Fig. 3) as follows: ADP (LR, −75.7%; non-LR, −78.2% ($p = 0.32$)), ASPI (LR, −86.6%; non-LR, −85.2% ($p = 0.53$)), TRAP (LR, −75.7%; non-LR, −85.1%; ($p = 0.1$)), and collagen (LR, −78.3%; non-LR, −82% ($p = 0.85$)).

Effect of LR on Endogenous Thrombin Generation Potential

In the low tissue factor experiments, the median ETP was reduced in LR units compared to the non-LR units on Day 0 and 10; Day 0: LR, 952 (846–1,061) and non-LR,

1,154.31 (1,035–1,285) ($p = 0.04$); Day 10: LR, 1,089 (1,031–1,150) and non-LR, 1,276.9 (1,091–1,574) ($p = 0.04$), respectively (Fig. 4). The non-LR group had a significant reduction in median ETP between Day 0 and Day 15 ($p < 0.001$). There was no difference in the percent change from Day 0 to Day 15 between non-LR and LR groups; 24.5% and 23.1%, respectively ($p = 0.6$).

In the high TF experiments, there was no difference in the median ETP values between the LR and non-LR groups at each time point measured. In both the LR and non-LR groups, there was no difference between the median ETP values on Day 0 and Day 15. There was also no difference in the percent change from Day 0 to Day 15 between non-LR and LR, −8.8% and 10.2%, respectively ($p = 0.06$) (Fig. 4).

Effect of Agitation Method on Hemostatic Parameters

For each assay, there were 21 LR and 20 non-LR units with 5 units per agitation group, with one LR group having 6 units. When all agitation methods were compared, there were no significant differences in the median PLT concentrations at each time point in either the non-LR or the LR group (Fig. 5A, B).

In LR units, within each agitation group from Day 0 to Day 15, there were significant reductions in median PLT
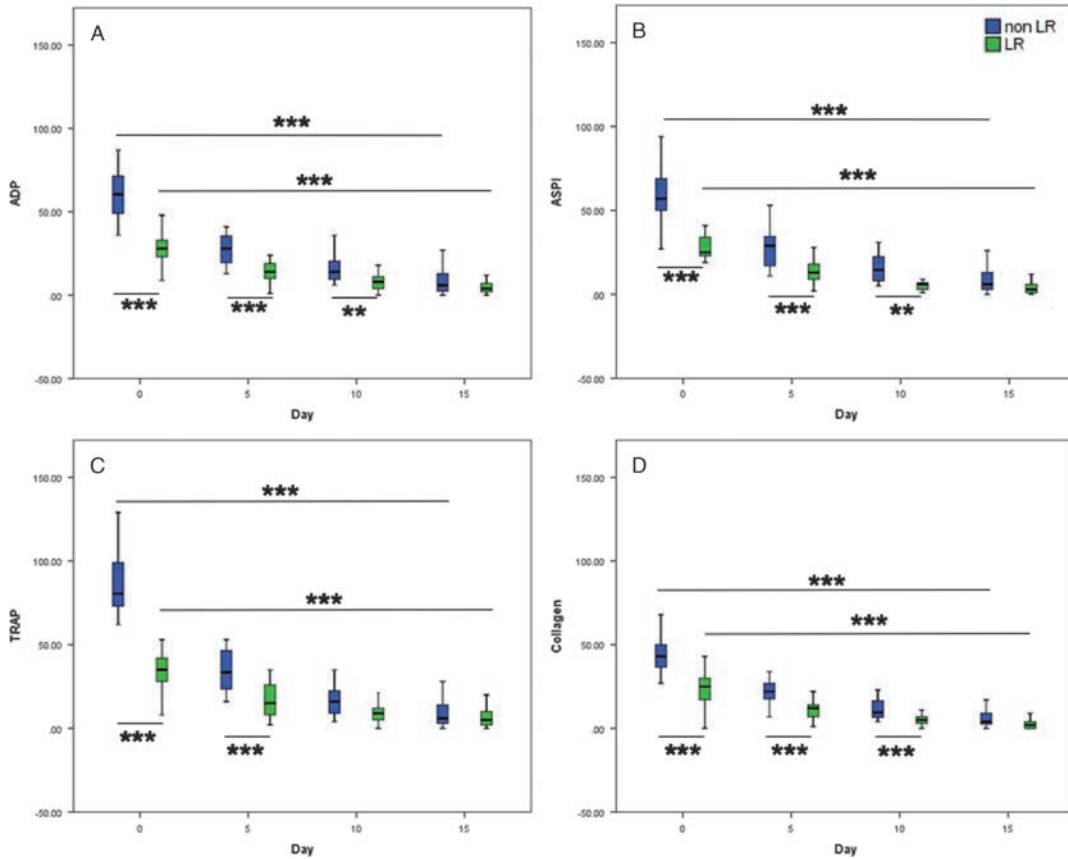
Figure 3. Impedance aggregometry measures nonleukoreduced and leukoreduced WB units over time. This figure shows impedance aggregometry measures of *non-LR* (*blue bars*) and *LR* (*green bars*) WB units over 15 days. Impedance aggregometry parameters included *ADP* (*A*) to *ASPI* (*B*), *TRAP* (*C*), and *collagen* (*D*). The median AUC aggregation responses to ADP, ASPI, TRAP, and collagen were compared for each time point between LR and non-LR units, and absolute median measurements were compared from Day 0 to Day 15 within LR and non-LR groups. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

concentration (Fig. 5A; $p < 0.05$ for all four agitation methods). Agitation method did not affect the median MCF, TEG MA, ADP-MA, and AA-MA results at each time point analyzed. However, within each agitation group from Day 0 to Day 15,

there were statistical reductions in median MCF and TEG MA-AA (Fig. 6A, D; $p < 0.05$), and in TEG MA-ADP for 360° rotator (Fig. 6C; $p < 0.05$). Agitation did not affect the median PLT aggregation AUC on Days 0 and 5 of testing, but
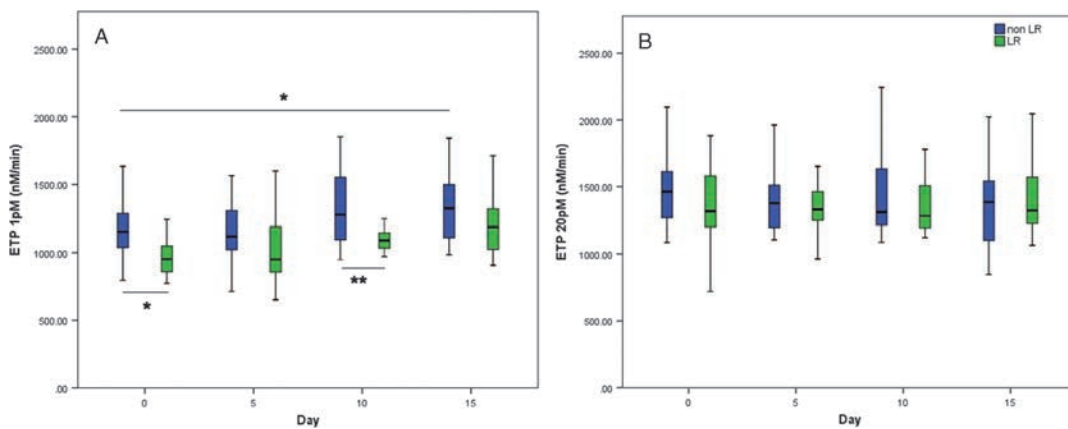

Figure 4. Thrombin generation in nonleukoreduced and leukoreduced WB units over time. This figure shows thrombin generation measures of *non-LR* (*blue bars*) and *LR* (*green bars*) WB units over 15 days. These include *ETP* dosed at 1 pmol/L or 20 pmol/L (*A, B*). Median *ETP* between non-LR and LR units were compared at each time point, and absolute median measurements were compared from Day 0 to Day 15 within LR and non-LR groups. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
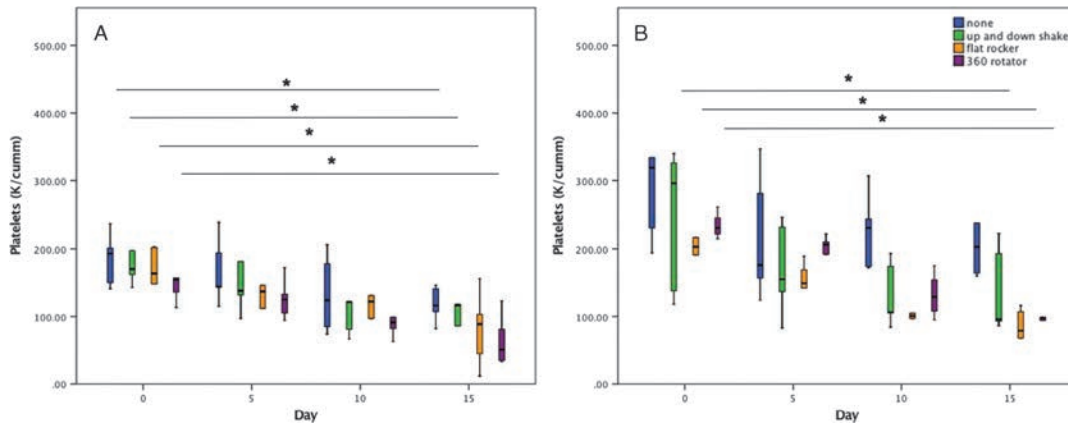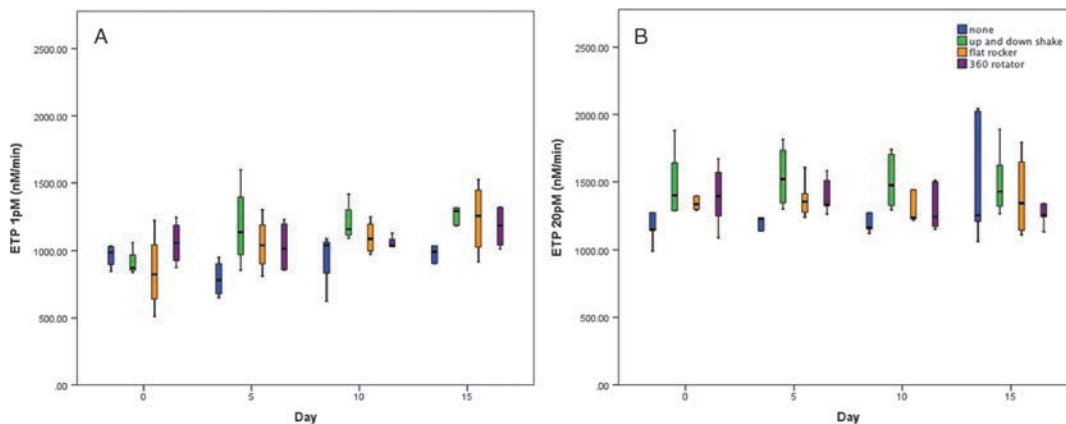
Figure 5. Platelet concentration in leukoreduced and nonleukoreduced and WB units by agitation method over time. This figure shows the platelet concentration of *LR* (*A*) and *non-LR* (*B*) WB units by differing agitation methods over 15 days. Whole blood was then randomized to four agitation methods; un-rocked (*blue bar; none*), *up and down shake* (*green bar*), continuous mechanical horizontal agitation via flat rocker (*yellow bar*), or continuous end-over-end rocking via a 360° rotator (*red*). Median platelet concentrations were compared at each time point for LR and non-LR units with no significant differences. Absolute median platelet concentrations were compared from Day 0 to Day 15 within each agitation method for LR and non-LR groups. The LR group had differences for all agitation methods, and the non-LR group had differences for up and down shake, flat rocker, and 360° rotator. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
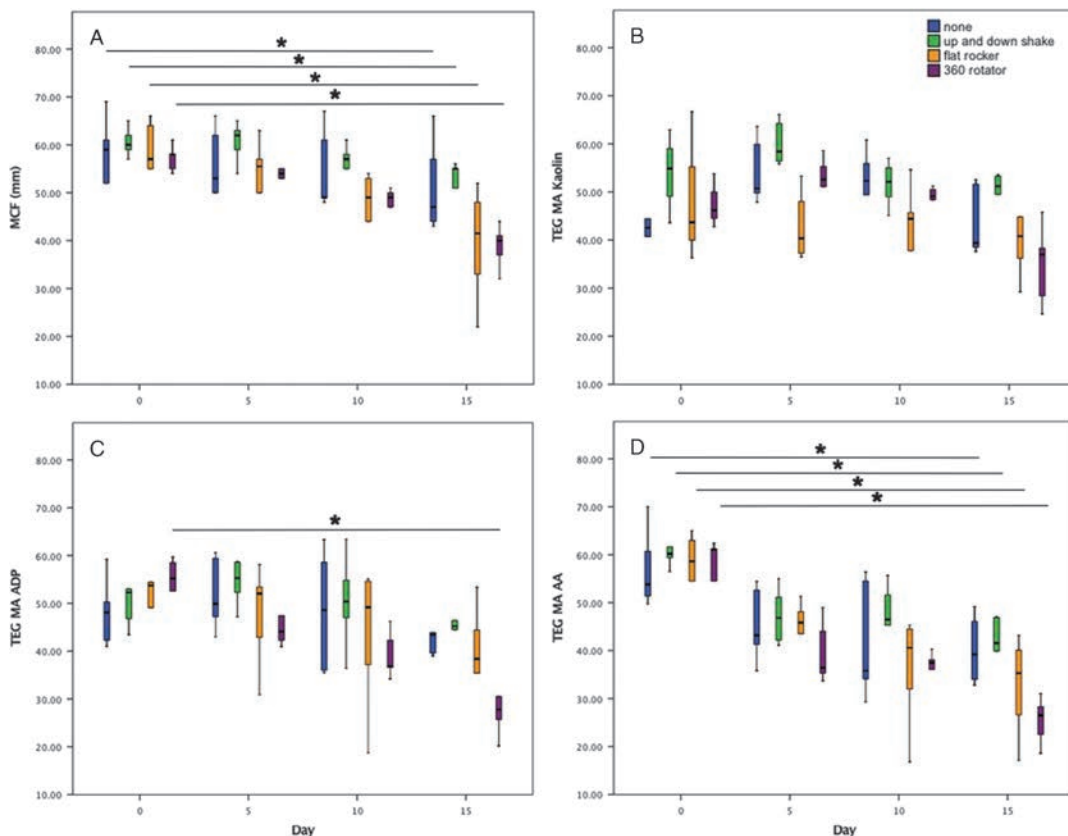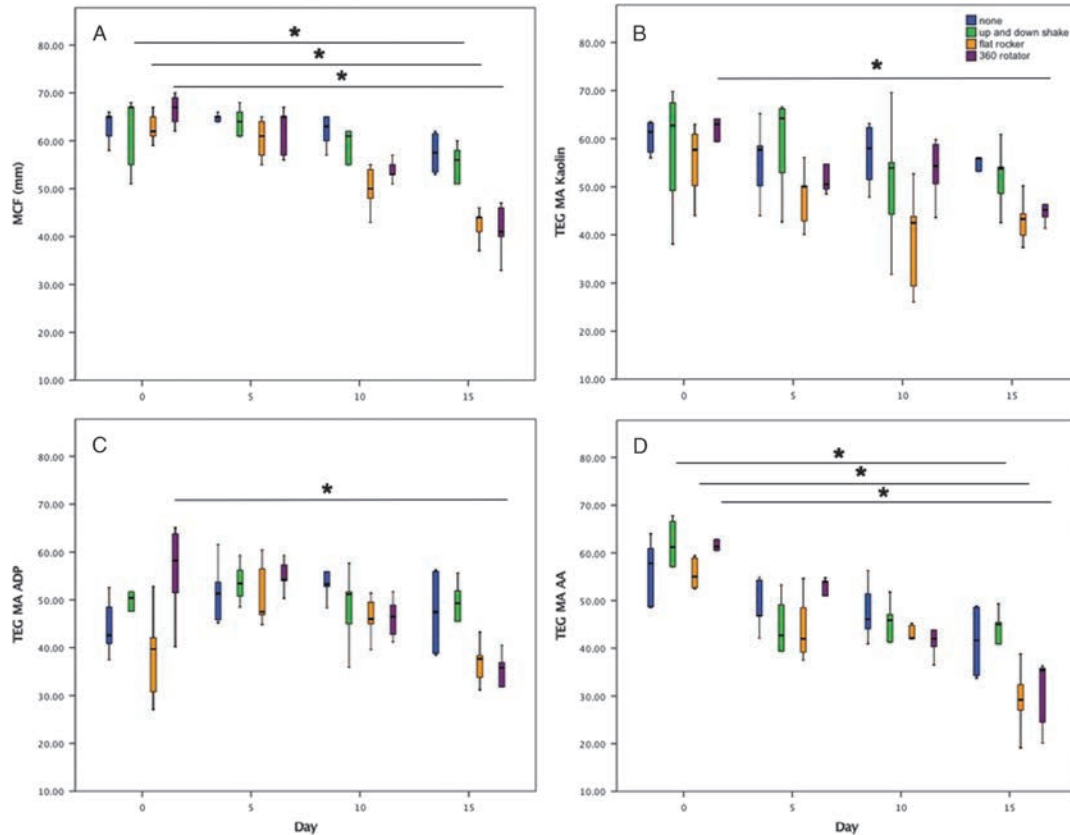

Figure 6. Viscoelastic measures in leukoreduced WB units by agitation method over time. This figure shows viscoelastic measures of LR WB units by differing agitation method over 15 days. Agitation methods included un-rocked (*blue bar; none*), *up and down shake* (*green bar*), continuous mechanical horizontal agitation via flat rocker (*yellow bar*), or continuous end-over-end rocking via a 360° rotator (*red*). The viscoelastic parameters measured include the *MCF* measured by ROTEM (*A*), *MA* measured by TEG, (*B*), *TEG MA-ADP* (*C*), and *TEG MA-AA* (*D*). Median measurements of MCF, TEG-MA, TEG MA-ADP, and TEG MA-AA were compared at each time point. No differences were noted. Median measurements of MCF, TEG-MA, TEG MA-ADP, and TEG MA-AA were compared within each agitation group from Day 0 to Day 15. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
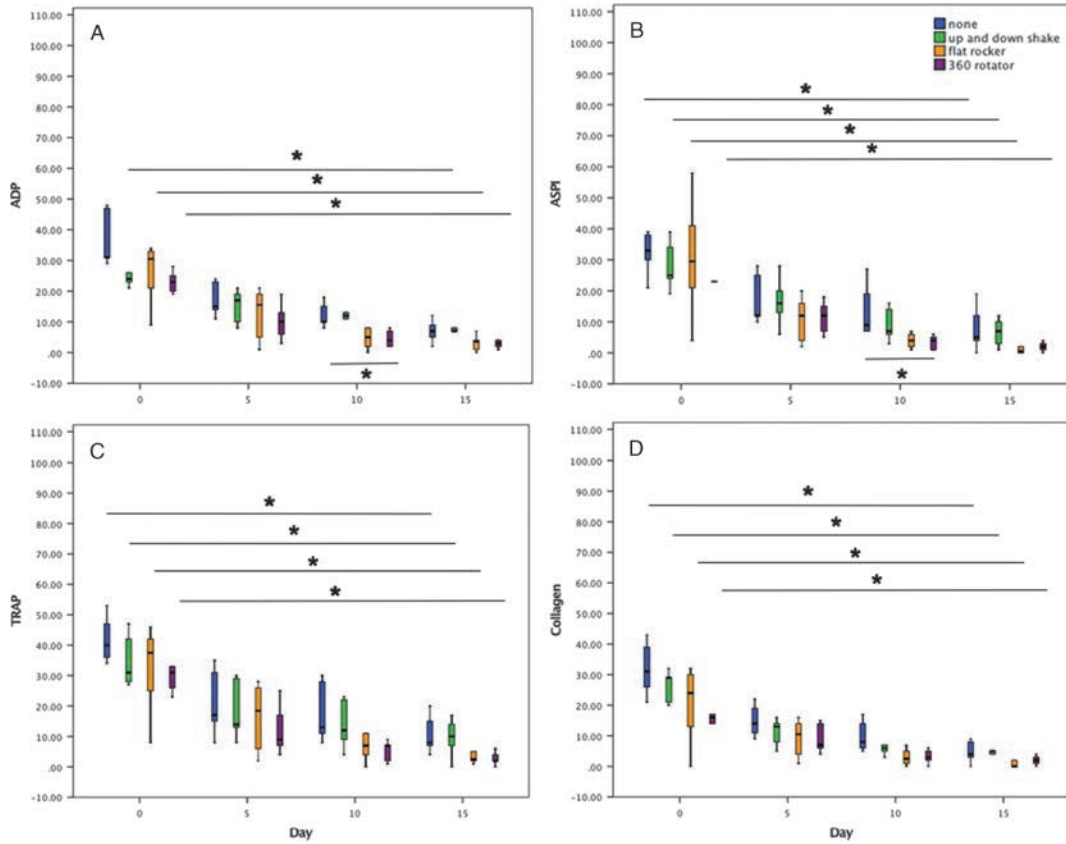
Figure 7. Impedance aggregometry measures in leukoreduced WB units by agitation method over time. This figure shows impedance aggregometry measures of LR WB units by differing agitation methods over 15 days. Agitation methods included un-rocked *(blue bar; none)*, *up and down shake* (*green bar*), continuous mechanical horizontal agitation via *flat rocker* (*yellow bar*), or continuous end-over-end rocking via a *360° rotator* (*red*). Impedance aggregometry measures included *ADP* (*A*) to *ASPI* (*B*), *TRAP* (*C*), and *collagen* (*D*). Median AUC for aggregation responses to ADP, ASPI, TRAP, and collagen were compared at each time point between agitation methods, with significant differences noted by *$p \leq 0.05$. Median AUC for aggregation responses to ADP, ASPI, TRAP, and collagen were compared within each agitation group from Day 0 to Day 15. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 8. Thrombin generation in leukoreduced WB units by agitation method over time. This figure shows thrombin generation measures of leukoreduced WB units by differing agitation method over 15 days. Agitation methods included un-rocked *(blue bar; none)*, *up and down shake* (*green bar*), continuous mechanical horizontal agitation via flat rocker (*yellow bar*), or continuous end- over-end rocking via a *360° rotator* (*red*). Thrombin generation variables included *ETP* dosed at 1 pmol/L or 20 pmol/L (*A*, *B*). Median *ETP* was compared at each time point between agitation methods, and absolute median ETP was compared within each agitation method from day 0 and day 15. No differences were noted.

Figure 9. Viscoelastic measures in nonleukoreduced WB units by agitation method over time. This figure shows viscoelastic measures of non-LR WB units by differing agitation method over 15 days. Agitation methods included un-rocked (*blue bar; none*), *up and down shake* (*green bar*), continuous mechanical horizontal agitation via *flat rocker* (*yellow bar*), or continuous end-over-end rocking via a *360° rotator* (*red*). Viscoelastic parameters measured include *MCF* measured by ROTEM (*A*), *MA* measured by TEG (*B*), *TEG MA-ADP* (*C*), and TEG MA-AA (*D*). Median measurements of MCF, TEG-MA, TEG MA-ADP, and TEG MA-AA were compared at each time point, and absolute median measurements were compared within each agitation method form Day 0 to Day 15. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

it did affect ADP and ASPI on Day 10 (Fig. 7; $p < 0.05$). However, within each agitation method, there were significant reductions in median ADP response (up and down shake, flat rocker, and 360° rotator), and ASPI, TRAP, and collagen response (Fig. 7A-D; $p < 0.05$). Agitation did not affect total thrombin generation capacity in LR groups at each time point analyzed with either high or low TF testing, and there was no difference from Day 0 to Day 15 (Fig. 8).

In non-LR units, agitation method did not affect the median MCF, TEG MA, ADP-MA, and AA-MA results at each time point analyzed (Fig. 9). However, within each agitation group from Day 0 to Day 15, there were significant reductions in median MCF and TEG MA-AA (up and down shake, flat rocker, and 360° rotator), TEG MA kaolin (360° rotator), and TEG MA-ADP (360° rotator) (Fig. 9A-D; $p < 0.05$). Method of agitation had an effect on PLT aggregation on Days 10 (ADP and ASPI) and Day 15 (ADP, ASPI, TRAP, and collagen) (Fig. 10A-D; $p < 0.05$). Within each group, the differences in median in ADP, ASPI, TRAP, and collagen was significantly reduced in up and down shake, flat rocker, and 360° rotator from Day 0 to Day 15 ($p < 0.05$). Agitation did not affect total thrombin generation capacity in non-LR units at each time

point analyzed with either high or low TF testing, and there was no difference from Day 0 to Day 15 (Fig. 11).

Effects of the Agitation and LR on Hematologic, Chemistry, and Acid Base Parameters

No significant differences occurred between Days 0 and 15 for any of the parameters measured within each agitation group. These findings are summarized in supplemental table 1 (Table, Supplemental Digital Content 1, http://links.lww.com/TA/B119).
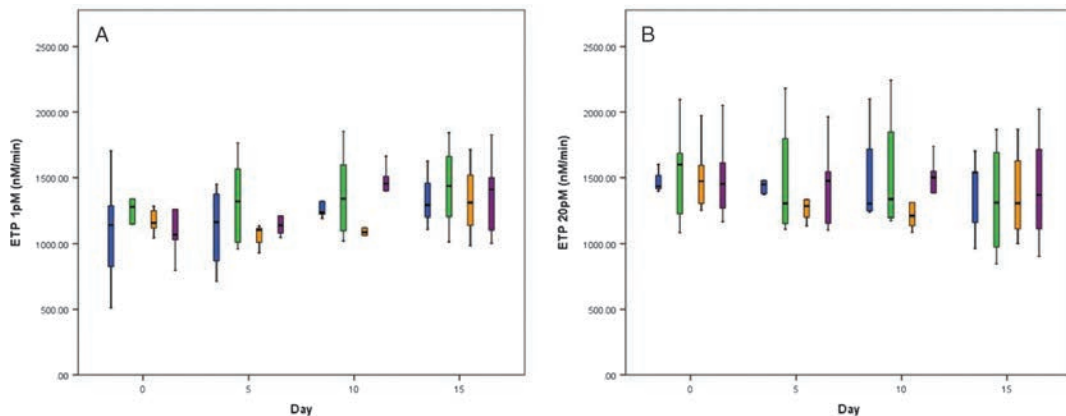
DISCUSSION

This study is a comprehensive in vitro analysis of the effect of LR and agitation on hemostatic parameters in cold-stored WB over a 15-day period. Although there was an immediate reduction in PLT concentration following LR, this difference became non-significant for the remaining duration of the storage period. In general, viscoelastic measures and thrombin generation parameters did not reveal a consistent significant reduction in hemostatic function between the LR and the non-LR units. Although the MCF was reduced in the LR units, on Days 0 and 5, the clinical
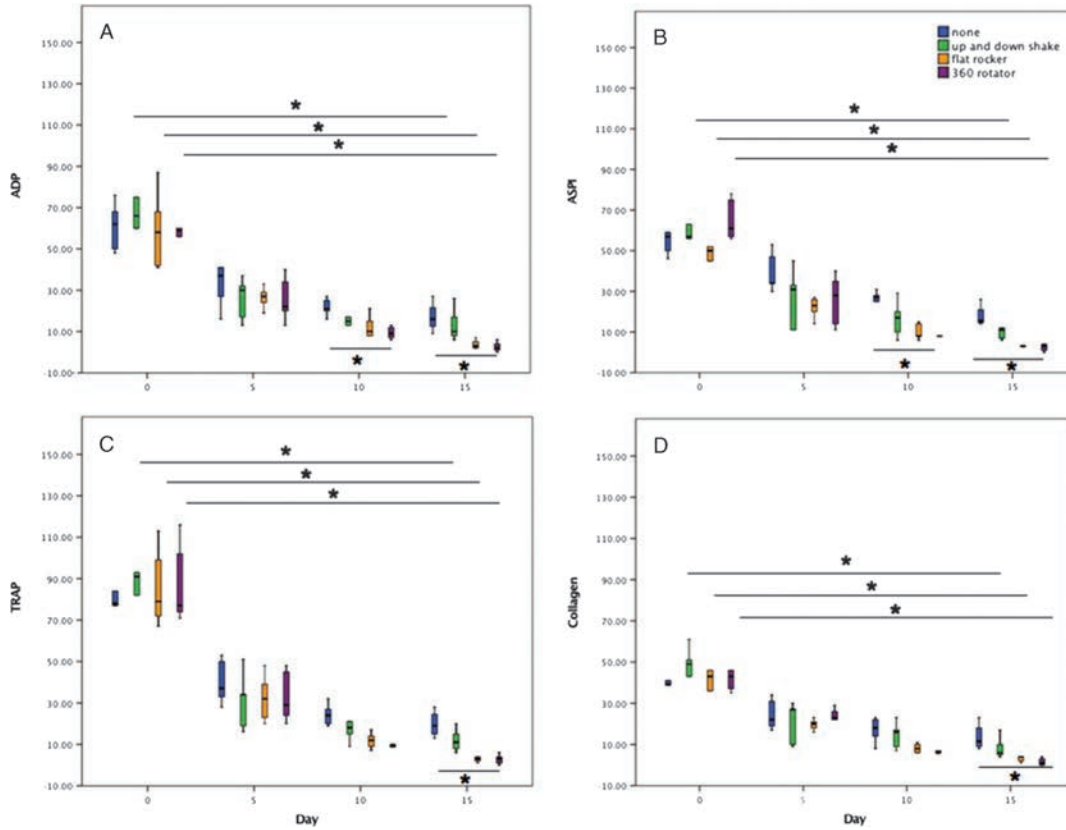
Figure 10. Impedance aggregometry measures in nonleukoreduced WB units by agitation method over time. This figure shows impedance aggregometry measures of non-LR WB units by differing agitation methods over 15 days. Agitation methods included un-rocked (*blue bar; none*), *up and down shake* (*green bar*), continuous mechanical horizontal agitation via *flat rocker* (*yellow bar*), or continuous end-over-end rocking via a *360° rotator* (*red*). Impedance aggregometry measures included *ADP* (*A*) to ASPI (*B*), TRAP (*C*), and collagen (*D*). Median AUC for aggregation responses to ADP, ASPI, TRAP, and collagen were compared at each time point between agitation methods, and absolute median measurements were compared within each agitation method from Day 0 to Day 15. Differences are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 11. Thrombin generation in nonleukoreduced WB units by agitation method over time. This figure shows thrombin generation measures of non-LR WB units by differing agitation method over 15 days. Agitation methods included un-rocked (*blue bar; none*), *up and down shake* (*green bar*), continuous mechanical horizontal agitation via *flat rocker* (*yellow bar*), or continuous end-over-end rocking via a *360° rotator* (*red*). Thrombin generation variables included *ETP* dosed at 1 pmol/L or 20 pmol/L (*A, B*). Median ETP was compared at each time point between agitation methods, and absolute median ETP measurements were compared within each agitation method from Day 0 to Day 15.

relevance of these findings are unknown. In contrast, the LR units consistently demonstrated reduced PLT aggregation parameters compared to the non-LR units, except on Day 15. Agitation methods had minimal effects on the panel of hemostatic parameters examined. The differences noted in hemoglobin, hematocrit, and fibrinogen according to agitation method do not seem to be clinically relevant.

To date, few studies have evaluated the hemostatic capacity of WB during storage. Pidcoke et al. evaluated the hemostatic capacity of non-LR WB stored for 21 days at either 4°C or 22°C. Their analysis demonstrated that measures of PLT function decreased over time at both storage temperatures, but the PLTs stored at 4°C demonstrated improved functionality compared to those stored at room temperature in all of the assays.[10] Yazer et al.[19] examined the viscoelastic effects of cold storage at 4°C on PLT function in WB collected with the same PLT-sparing filter used in this study under a variety of agitation methods. The viscoelastic test results in the current study are consistent with those in the Yazer et al. report. Another in vitro analysis, by Siletz et al., compared non-LR to LR WB and reported that LR significantly reduced WB hemostatic function. The results of the Siletz et al. study can be explained by the fact that they used a non–PLT-sparing filter, thereby eliminating PLT contribution to WB hemostatic function in LR units.

The findings of this study demonstrate that use of a PLT-sparing LR filter exerts a moderate but significant reduction in some measures of WB hemostatic function most evident early in storage. Initially, LR caused a significant reduction in the concentration of PLTs, although the effect of LR on viscoelastic parameters, and thrombin generation was minor. However, impedance aggregation was significantly lower in the LR units compared to the non-LR units. The clinical relevance of these findings is difficult to know with precision, although it is clear that LR did not improve PLT function. While there are reports indicating that changes in PLT concentration, impedance aggregation, viscoelastic measures, and thrombin generation each have been associated with increased risk of bleeding, there are limited data to indicate which of these tests is the most important in predicting the functional hemostatic potential of WB in a bleeding patient.[20–24] Each of these in vitro measures reported in this study is limited in that these do not model the contribution of endothelium or flow dynamics in hemostasis. Some consider thrombin generation to be an important in vitro global measure of hemostasis. Interestingly, thrombin generation capacity of plasma within WB remained intact during storage, even after LR. Fibrinogen concentrations also did not change from Day 0 to Day 10 in all units analyzed (Appendix Table 1). Despite the reduction of most parameters of hemostatic function tested from Days 0 to 15, in both the non-LR and LR units, the lack of difference in the percent reduction between these two groups by Day 15 gives a context to the loss of function; ultimately, storage time effects seem to take precedence over the effect of LR. From a practical perspective, programs that store WB out to 15 days will use the oldest units in inventory first to reduce waste. As a result, it will be common for WB to be transfused between 10 and 15 days of storage. At 10 days and greater, there were no differences in PLT concentration, viscoelastic measures of clot firmness, and thrombin generation. Even the aggregation differences, while statistically different, may not be clinically

meaningful at Days 10 and 15. Ultimately, the clinical relevance of each of the in vitro tests performed here need to be better understood to determine if using this WB filter has any significant adverse effects on in vivo PLT function.

Whether LR is important for trauma patients is controversial. While some benefits of LR are well documented, including reducing febrile transfusion reactions, CMV transmission, and HLA sensitization, there are no data in patients with traumatic injury that LR blood products improve outcomes.[25] Thus, it is unknown if the benefits of LR outweigh the potential reduction in hemostatic capacity in the trauma patient with severe life-threatening hemorrhage.

CONCLUSIONS

Leukoreduction of WB with PLT-sparing filter caused a reduction in some measures of hemostatic potential. This effect was most evident during the early period of storage (Days 0–10) and was gradually superseded by storage time effects. The benefits of LR in the trauma population should be weighed against the potential reduced hemostatic function of WB after LR. Clinical studies are needed to determine if maximal hemostatic function in a non-LR WB is preferable to the potential risks associated with exposure to the white blood cells within the product. In areas where universal LR is mandated, these results indicate that at the end of storage, there is no difference in hemostatic capacity of WB compared to several days earlier. In areas where universal LR is not mandated, the effect of LR on the hemostatic capacity of WB should be balanced against the modest benefits that it confers on trauma patients. Cold-stored WB does not need to be agitated, since there is no effect of agitation on hemostatic function.

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