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Stationary Versus Agitated Storage of Whole Blood During Acute Normovolemic Hemodilution

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Abstract

Background—Acute normovolemic hemodilution is an intraoperative technique to reduce the number of red blood cells lost in shed blood during surgery. Standard guidelines for storage of platelets recommend constant gentle agitation to maintain gas exchange for the metabolically active platelets. The collected whole blood (WB) for acute normovolemic hemodilution remains stationary for as long as 8 hours before reinfusion. We hypothesized that gentle agitation of WB throughout storage would improve the coagulation properties of the WB at the time of reinfusion.

Methods—WB was collected from 10 volunteer donors and control samples taken. The units were split into two storage groups: agitated (rocked), and stationary (unrocked). Cell counts and fibrinogen levels, as well as thromboelastography (TEG®) measurements, including TEG® PlateletMapping® assays, were performed on the control sample and the test samples after 8 hours of rocked or unrocked storage at room temperature.

Results—Nine units of WB from 9 different healthy volunteers were tested. There were no significant differences in hematocrit, hemoglobin, red blood cells counts, platelet counts, or fibrinogen levels between the control samples and the rocked and unrocked WB samples. WB coagulation as measured by TEG® was preserved during the 8-hour storage period in both the rocked and unrocked samples. There were no significant differences between the control, rocked, and unrocked samples in time to initiate clotting, time of clot formation, rate of clot formation, or maximum strength of clot values. There were also no significant differences in the fibrin contribution to clot strength between the control, rocked, and unrocked samples, and no significant difference between the platelet activation from adenosine diphosphate or arachidonic acid among any of the three groups.

Conclusion—Given the small sample size, there is no statistical evidence on which to reject the null hypothesis of there being no difference in the changes from the baseline between coagulation function as measured by TEG® between WB that is either agitated or kept stationary for 8 hours. These findings need to be confirmed in a larger study.

Introduction

Acute normovolemic hemodilution (ANH) is an intraoperative technique that can reduce the need for allogeneic red blood cell transfusion.^{1–3} Reinfusion of the platelet (PLT)- and coagulation factor-containing whole blood at the end of the surgical procedure may also help restore any coagulation dysfunction due to blood loss during the procedure, reducing the need for postoperative PLT transfusions.⁴

Standard guidelines for storage of PLTs recommend constant gentle agitation to maintain gas exchange, since the PLTs are metabolically active.⁵ However, AABB (formerly the American Association of Blood Banks) standards for whole blood that is phlebotomized for ANH do not discuss agitation.⁶ Currently, most practitioners do not place the ANH whole blood on a rocker to maintain agitation during its *ex vivo* storage. Because the current practice of not rocking whole blood collected for ANH differs from how PLTs would normally be stored in the blood bank, this pilot study was designed to determine if

coagulation function was better maintained during rocked storage compared to stationary storage of ANH whole blood over the maximum 8-hour storage period.

Thromboelastography® (TEG®) measures the clotting mechanism as a whole and may be among the best clinically relevant *in vitro* tests to use when ascertaining coagulation function in whole blood. The aim of this study was to determine if there was a difference in coagulation function as measured by TEG® between rocked versus stationary storage of whole blood at ambient temperature for 8 hours.

Methods

Blood collection and study design

This study was given a no human subjects designation by the University of Pittsburgh's IRB and thus donor consent was not required. To determine the optimum method of storing whole blood during ANH, 10 units of whole blood, collected according to standard protocols, were randomly selected within 4–6 hours of donation from among the units collected by the local Food and Drug Administration-licensed blood center. The blood was collected into phlebotomy bags containing citrate phosphate dextrose. Each whole blood unit was divided evenly into two daughter bags. Samples were taken from each bag and served as the control samples. A complete blood count (CBC) and TEG® analysis (standard and TEG® PlateletMapping®) were performed on the control samples. The bags were then stored for eight hours at ambient temperature (21°C), with one bag being constantly agitated by rocking ("rocked" sample), and the other bag stationary ("unrocked" sample). The rocked sample was placed on a tilting platform and rocked at an angle of $\pm 17^\circ$ from the horizontal at a rate of 20 cycles/min. After eight hours a CBC and TEG® analysis were performed on the rocked and unrocked test samples.

Whole blood clotting potential as measured by TEG®

The standard TEG® output is a graphical representation of the clotting mechanism. Through analysis of this graph the elements of coagulation can be quantified and compared, including PLT function, coagulation factor and inhibitor function, fibrin cross-linking, and fibrinolysis. In this study the standard TEG® analysis was performed per the manufacturer's instructions, using a 1 ml sample of blood transferred to a vial containing kaolin and mixed by inversion. Then, 340 μ l of the kaolin activated blood was transferred to a TEG® cup containing 20 μ l of 0.2 M CaCl_2 to reverse the effects of the citrate anticoagulant. The TEG® was performed using the TEG® 5000 instrument (Haemoscope Corporation, Braintree, MA). The time to initiate clotting (R), the time of clot formation (K), the rate of clot formation (α -angle), and the maximum strength of clot (MA) were measured (noted in the equation below as $\text{MA}_{\text{Thrombin}}$).

Fibrin and platelet receptor function as measured by TEG®

$\text{MA}_{\text{Thrombin}}$ is the maximum uninhibited clot strength in the presence of an artificial activator. We also performed the TEG® PlateletMapping® assays that show the contribution of fibrin and PLT receptors to clot strength. First $\text{MA}_{\text{Fibrin}}$ is measured, which is the contribution to $\text{MA}_{\text{Thrombin}}$ by fibrin alone. Then the MA's from adenosine diphosphate (ADP) activation (MA_{ADP}) and from thromboxane A2 activation (MA_{AA} , for arachidonic acid (AA) pathway) are measured.

For this assay heparin is first added to the samples of whole blood to eliminate PLT activation by thrombin. Three hundred-sixty μ l of this heparinized blood is transferred to a TEG® cup containing 10 μ l of Activator F (Reptilase and Factor XIII) which promotes fibrin cross-linking, yielding $\text{MA}_{\text{Fibrin}}$. The process is repeated, with 360 μ l of heparinized

blood transferred to a TEG® cup containing 10 µl of Activator F, but then either 10 µl of ADP, or 10 µl of AA are also added, yielding MA_{ADP}, and MA_{AA}, respectively. The percentage of PLT activation due to ADP or AA is then calculated as follows:

$$(MA_{ADP \text{ or } AA} - MA_{Fibrin}) / (MA_{Thrombin} - MA_{Fibrin}) \times 100.$$

Statistics

Data are descriptively summarized as mean ± standard deviation. The changes from controls (baseline) were compared between the rocked and unrocked groups. D'Agostino and Pearson omnibus test was used to check the normality assumption in the dataset. The only groups in which both pairs of datasets were normal were %ADP activation and PLT count. Unpaired t-tests were used to compare these two sets. The remaining sets were compared using the Wilcoxon- Mann-Whitney rank-sum tests. The 95% confidence intervals were estimated for the median and mean differences between two groups by using the Hodges-Lehmann approach and the unpaired t-test, respectively. Two-sided p-values less than 0.05 were considered statistically significant.

Results

Basic whole blood analysis

Although 10 units of whole blood were collected, one donor had been taking aspirin as evidenced by greatly reduced PLT activity using the AA agonist. Thus this donor's unit was not included in the overall analysis. In the 9 whole blood units that were included in the analysis, there were no significant differences in hematocrit, hemoglobin, red blood cell counts, PLT counts, or fibrinogen levels between the control samples and the rocked and unrocked whole blood samples (Table 1). When the lack of variation in fibrinogen was recognized after testing of the first five units it was not measured in the last four.

Whole blood clotting potential measured by TEG®

Overall, whole blood coagulation as measured by TEG® was preserved during the 8-hour storage period in both the rocked and unrocked samples (Figure 1). There were no differences between the control, rocked, and unrocked samples in R, K, α-angle, or MA (p values all higher than 0.05, range 0.17 to 0.29).

Fibrin and platelet receptor function as measured by TEG®

Table 2 shows the results of the TEG® PlateletMapping® assays. There was no difference in the fibrin contribution to clot strength, or in the platelet activation from ADP or AA, among any of the three groups. PLT activation due to AA did trend lower in the rocked group, but this did not reach statistical significance.

Discussion

No significant changes were seen among the whole blood samples when received, and after 8 hours of either agitated or stationary storage. Clot initiation, amplification, propagation, and termination, as well as cell count and fibrinogen levels all remained steady.

TEG® and TEG® PlateletMapping® assays have been increasingly used to assess whole blood clotting potential and PLT function, respectively.⁷⁻⁹ In a porcine model of hypothermia and hemorrhage, the TEG® was shown to be a superior assay in differentiating clotting abnormalities compared to conventional assays such as prothrombin time, activated partial thromboplastin time and activated clotting time.¹⁰ Earlier studies on ANH investigated the changes in coagulation profiles after the infusion of various crystalloid or

colloid fluids after whole blood phlebotomy.¹¹ To our knowledge, however, no studies have been published that addressed the question of whether or not the whole blood collected for ANH should be kept under gentle agitation, as are the recommendations for the storage of PLTs.⁵

Our values for the standard TEG® assay and the TEG® PlateletMapping® assay are similar to that of other healthy blood donors.⁹ Previous studies of stored whole blood found a reduction in PLT function after 72hrs of stationary storage at room temperature.¹² When refrigerated, coagulation changes as measured by TEG® are not noticed until 11 days of storage.¹³ Our study expanded on these previous observations by demonstrating that at room temperature, the storage of whole blood under stationary or agitated conditions for 8 hours did not reduce its coagulation potential.

Normally, PLT concentrates are agitated during storage in the blood bank because these cells are aerobically active during storage, and rocking facilitates gas exchange. In fact, several investigations on PLT concentrates found that an interruption in agitation for less than 24 hours, while not inducing permanent platelet damage, did cause increases in lactic acid production and decreased oxygen utilization.^{14–16} However, these studies only assessed changes in metabolic markers and not perturbations in PLT function or coagulation as a whole. Other studies have shown that viable PLT concentrates can be prepared from whole blood or buffy coats that had been stored overnight, which is a longer period than is permitted for the storage of ANH whole blood.^{17–19} We have demonstrated that PLTs in whole blood remain functional after 8 hours of stationary or agitated storage, is consistent with these previous findings, and supports the current stationary storage practice of ANH whole blood.

This study has several limitations. PLT aggregometry, and not TEG®, is the gold standard for assessing PLT function. However this test is typically only performed in specialized coagulation laboratories, is not available around the clock, and has a turnaround time which makes it impractical for clinical decision making based on samples taken during a surgical case. We did not have access to aggregometry testing when we concluded the 8-hour storage time, and we used the point-of-care TEG® device. The results of the TEG® have been shown to closely correlate with that from PLT aggregometry.²⁰ A further limitation was our sample size, and therefore the study was not powered to detect the observed small differences among the three groups with 80% certainty for all of the tests. More importantly, however, was that the variation among the three groups for all of the underpowered tests was small and not clinically significant. Also, as the whole blood for this study came from volunteer donors, we did not actually transfuse the whole blood back to surgical patients after being stored for 8 hours, and thus we could not measure clinical outcomes such as bleeding or the need for perioperative PLT transfusion. Perhaps rocking or not rocking the whole blood induced subtle changes in the PLTs that could either enhance or diminish their activity in a manner that was not reflected in the CBC or TEG® results. Another limitation of the study is that we used donor whole blood to replicate actual patient blood. The whole blood collected for ANH from a surgical patient would contain anesthetic drugs and perhaps other medications that are not present in donor blood. The effects of these medications on coagulation function, if any, would not have been captured in this study. Furthermore, although unlikely to have induced any significant changes, the whole blood in this study was between four and six hours old when we began our experiments; blood drawn for ANH would not have had this time lapse before the commencement of agitated or stationary storage.

Our results suggest that there is no difference in coagulation properties of whole blood as measured by TEG®, including TEG® PlateletMapping®, after either stationary or agitated

storage of whole blood for 8 hours at room temperature. Given the small sample size, there is no statistical evidence on which to reject the null hypothesis of there being no difference in the changes from the baseline between coagulation function as measured by TEG® between whole blood that is either agitated or kept stationary for 8 hours. These findings need to be confirmed in a larger study. The present study may provide some preliminary evidence that whole blood does not need to be agitated in order to maintain coagulation function during ANH, although these *in vitro* results should be validated clinically in the perioperative setting.

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Figure 1. Results from the whole blood coagulation experiments using the thromboelastography (TEG®). Data are presented as mean $\pm$ SD. Coagulation was preserved during the 8-hour storage period in both the rocked and unrocked samples. N=9 for all groups.

Table 1

Basic analysis of the whole blood when received (control), and after 8 hours of either stationary (unrocked) or agitated (rocked) storage. Values are reported as mean $\pm$ SD. n=9 for all measurements except fibrinogen which is n=5.

	Control	Unrocked	Rocked	Unrocked (difference)	Rocked (difference)	Difference-in- difference between groups (95% CI) ^a	p
Hematocrit (%)	35 $\pm$ 3	36 $\pm$ 3	39 $\pm$ 9	0.9 $\pm$ 1.1	4.7 $\pm$ 10.5	-0.10 (-1.20, 1.90)	0.80
Hemoglobin (g/dl)	11.9 $\pm$ 1.0	12.3 $\pm$ 1.0	13.4 $\pm$ 2.9	0.31 $\pm$ 0.36	1.43 $\pm$ 3.22	-0.10 (-0.50, 0.70)	0.73
Red Blood Cell count (10 ⁶ /mm ⁶)	3.8 $\pm$ 0.4	3.9 $\pm$ 0.4	4.3 $\pm$ 0.7	0.07 $\pm$ 0.14	0.50 $\pm$ 0.98	0.10 (0.00, 0.31)	0.19
Platelet count (10 ³ /mm ⁶)	239 $\pm$ 51	246 $\pm$ 59	223 $\pm$ 86	6.5 $\pm$ 20.8	-16.0 $\pm$ 58.9	-22.48 (-68.84, 23.89) ^b	0.30
Fibrinogen (mg/dl)	274 $\pm$ 44	267 $\pm$ 33	270 $\pm$ 34	-6.8 $\pm$ 12.6	-3.6 $\pm$ 11.8	4.00 (-17.00, 26.50)	0.42

^aHodges-Lehmann exact approach for median difference

^bUnpaired t-test for mean difference

Table 2

The results of the thromboelastography (TEG®)platelet function assays of the whole blood when received (control), and after 8 hours of either stationary (unrocked) or agitated (rocked) storage. Values are reported as mean $\pm$ SD(n=9).

	Control	Unrocked	Rocked	Unrocked (difference)	Rocked (difference)	Difference- in-difference between groups (95% CI) ^a	p
MA _{Thrombin} (mm)	64.3 $\pm$ 3.9	64.6 $\pm$ 4.9	61.1 $\pm$ 9.5	0.36 $\pm$ 2.49	-3.19 $\pm$ 6.93	-1.80 (-6.80, 1.60)	0.30
MA _{Fibrin} (mm)	10.2 $\pm$ 11.7	8.3 $\pm$ 7.5	7.4 $\pm$ 7.8	-1.82 $\pm$ 6.03	-2.76 $\pm$ 5.08	-1.00 (-3.70, 0.60)	0.34
% ADP activation (%)	71 $\pm$ 13	61 $\pm$ 25	56 $\pm$ 29	-9.7 $\pm$ 20.1	-15.1 $\pm$ 27.6	-5.08 (-29.18, 19.02) ^b	0.66
% AA activation (%)	82 $\pm$ 9	79 $\pm$ 15	69 $\pm$ 12	-3.3 $\pm$ 10.8	-13.1 $\pm$ 15.8	-7.77 (-22.14, 3.95)	0.19
TEG® R	6.3 $\pm$ 0.8	6.2 $\pm$ 0.8	6.8 $\pm$ 1.2	-0.02 $\pm$ 0.83	0.51 $\pm$ 1.00	0.40 (-0.50, 1.30)	0.16
TEG® K	1.7 $\pm$ 0.4	1.7 $\pm$ 0.4	2.2 $\pm$ 1.3	0.01 $\pm$ 0.31	0.58 $\pm$ 1.23	0.20 (-0.20, 0.90)	0.34
TEG® α -angle	66.5 $\pm$ 5.4	66.5 $\pm$ 5.0	61.0 $\pm$ 12.4	-0.04 $\pm$ 3.14	-5.52 $\pm$ 11.05	-2.00 (-8.60, 1.80)	0.26

^aHodges-Lehmann exact approach for median difference

^bUnpaired t-test for mean difference