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The Effect of salvaged blood on Coagulation Function as measured by Thromboelastography

Gerhardt Konig*, Mark H. Yazer[^], and Jonathan H. Waters[#]

*Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[^]Department of Pathology, University of Pittsburgh, and the Institute for Transfusion Medicine, Pittsburgh, PA

[#]Departments of Anesthesiology and Bioengineering, University of Pittsburgh School of Medicine, and the McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pa

Abstract

BACKGROUND—There is concern that salvaged blood has the potential to activate the coagulation system, which might place patients at risk of thrombotic complications. The aim of this study was to determine whether transfusion of salvaged blood after total knee arthroplasty (TKA) would lead to procoagulopathic changes as measured by thromboelastography (TEG), and furthermore if washing would reduce this risk.

STUDY DESIGN AND METHODS—Twenty two patients undergoing TKA were enrolled. Control samples were venous blood samples taken before surgery. Test samples were created by mixing the control samples with postoperatively salvaged blood, either washed or unwashed. TEG profiles were measured, noting the time to initiate clotting (R), the time of clot formation (K), the angle of clot formation (α -angle), and the maximum strength of clot (MA).

RESULTS—The changes in the coagulation profile from control samples to test samples were consistent for both the washed and unwashed groups: R time decreased, MA decreased, and K and alpha-angle remained the same. However, the changes were more pronounced in the unwashed group than the washed group, with a 61% decrease in R time as compared with 14%, and a 26% drop in MA as compared with 6%.

CONCLUSION—The addition of salvaged blood to the patient's preoperative blood resulted in decreased clot strength as well as decreased time to initial clot formation. This suggests that the reinfusion of postoperatively salvaged washed or unwashed blood after TKA favors a change towards a more hypocoagulable state, and washing appears to reduce this effect.

Keywords

Autotransfusion; Blood transfusion; Coagulation; Salvaged blood; Thromboelastography; Total knee arthroplasty

INTRODUCTION

Total knee arthroplasty (TKA) is associated with significant amounts of post-operative blood loss and a high risk for requiring an allogeneic blood transfusion.¹ The transfusion of

Send correspondence and reprint requests to: Gerhardt Konig, MD, Department of Anesthesiology, Magee Womens Hospital, 300 Halket St., Suite 3510, Pittsburgh, PA 15213, Phone: (412) 641-1765, Fax: (412) 641-2432, konigg@upmc.edu.

Conflicts of Interest: None

postoperative salvaged blood to patients following TKA has been shown to reduce the need for allogeneic RBC transfusions.² However, there are concerns that the salvaged blood may contain procoagulopathic elements which might increase the risk for thrombotic complications in a patient population already at risk for deep vein thrombosis and pulmonary emboli.^{3,4}

Although such thrombotic complications can be serious, the rate of these complications after total knee arthroplasty is very low.⁵ Hence it is impractical to design a randomized controlled trial investigating the role of the reinfusion of salvaged blood with sufficient power to detect a difference in the rates of these complications in this patient population. Other authors have instead reported on changes to coagulation factor levels and traditional hematologic parameters after the reinfusion of post-operative salvaged blood.^{3,4,6} It is unclear how changes in these individual laboratory parameters correlate to differences in clinically relevant thrombotic complications, and it might be more predictive of the patient's post-operative risk of thrombosis if changes in the clotting mechanism as a whole could be analyzed.

Thromboelastography (TEG) is a tool which measures a patient's clotting mechanism *in toto*. Its output is a graphical representation of platelet function, coagulation factor and inhibitor function, fibrin polymerization, and fibrinolysis. As such, it may be among the best *in vitro* tests to use when ascertaining whether or not the transfusion of salvaged blood places the recipient at an increased risk for thrombotic complications. The aim of this study was to determine whether the transfusion of salvaged blood leads to procoagulopathic changes in the TEG profile, and to see if there was a difference in the TEG profile induced by washed vs unwashed salvaged blood.

MATERIALS AND METHODS

This was a prospective study which included 22 patients undergoing elective primary TKA. Patients with a known coagulopathy by a review of their laboratory parameters and history were excluded. Informed consent was obtained from all patients and the study was approved by the University of Pittsburgh's Institutional review board.

The patients were divided into two groups based on how the salvaged blood from their postoperative drain was processed prior to being transfused back to them. In 11 patients the blood was collected into a blood salvage system that does not wash the blood before it is readministered via a standard blood IV set (Suretrans System, Davol, Inc., Warwick, RI). In the other 11 patients the post-operative salvaged blood was washed with normal saline prior to reinfusion (Orthopat, Haemonetics, Braintree MA). The selection of the post-operative blood salvage technique was determined before the surgery began by the surgeon.

A 3 mL sample of venous blood was collected into a blood collection tube containing buffered sodium citrate (BD Vacutainer, Franklin Lakes NJ) from all patients immediately prior to surgery using standard venipuncture techniques (pre-operative sample). Another 3 mL sample was taken after surgery from the salvaged blood collection bag prior to the reinfusion of its contents (post-operative sample).

A TEG profile was measured on all of the pre-operative blood samples. A TEG profile was also measured on a blood sample created by mixing the patient's pre-operative blood with post-operative salvaged blood in a 10:1 ratio (300 μ L pre-surgical blood + 30 μ L of post-operative salvaged blood + 30 μ L of 0.2M calcium chloride) (mixed sample). This ratio was chosen as it reflects the typical amount of reinfused post-operative salvaged blood relative to the patient's total blood volume. 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

obtained from the TEG and compared between the control and the mixed samples (Figure 1). TEG profiles were run using kaolin as an activator in a Thrombelastograph Hemostasis Analyzer (Model 5000, Haemonetics, Braintree MA). Samples were run within one hour of the salvaged blood sample being collected, which was generally between 4 to 6 hours after pre-operative samples were taken.

Statistical analysis was performed using the two-tailed Student's t-test, paired for within-group testing (control vs mixed sample), and unpaired for between-group testing (washed group vs unwashed group control samples).

RESULTS

There were 11 patients in the washed group and 11 in the unwashed group. Patient demographics and preoperative hemoglobins were similar between the two groups (Table 1). There was no difference in preoperative TEG coagulation parameters between the two groups of patients (Table 2).

The TEG parameters of the pre-operative and the mixed samples are shown in Table 3. The changes in the TEG profiles between the pre-operative and the mixed samples were consistent for both the washed and unwashed groups: There were significant changes in the R time and the MA, while the K time and α -angle remained unchanged. However, the changes in the R time and MA were more pronounced in the unwashed group than the washed group, with a 61% decrease in R time and a 26% drop in the MA in the unwashed samples compared to 14% and 6%, respectively, in the washed samples.

DISCUSSION

The addition of salvaged blood to the patient's pre-operative blood resulted in significantly decreased clot strength as well as decreased time to initial clot formation. This was seen in both the washed and unwashed groups, although the effect was more pronounced in the unwashed group. This suggests that the washing process removed some of the elements responsible for these changes.

It is known that unwashed salvaged blood is different from venous blood in terms of its coagulation components. Unwashed salvaged blood has lower levels of fibrinogen and higher levels of fibrin degradation products, indicating that the clotting cascade has been activated and that fibrinolysis has been initiated.^{7,8} Unwashed salvaged blood also contains some cell breakdown products and various proteins like free hemoglobin, leukocyte enzymes, activated complement proteins, and inflammatory cytokines.⁹⁻¹³ Washing is thought to remove these proteins and cell breakdown products. However, there is a paucity of convincing evidence that proves that the differences between washed and unwashed salvaged blood lead to any differences in clinical outcomes.¹⁴ Regardless, in a surgical setting such as TKA where there is already heightened concern for postoperative deep vein thromboses (DVT) and pulmonary emboli (PE) it is prudent to examine the changes in the patient's coagulation profile caused by routine operative and post-operative interventions to determine if they might predispose the patients to these serious thrombotic complications.

With nearly all patients receiving extended courses of venous thromboembolism prophylaxis after TKA the incidence of symptomatic DVT and PE has been reduced to 0.6% and 0.3%, respectively.⁵ This means that it would be impractical to design a prospective study large enough to be powered to detect a difference in clinical outcomes related to thrombosis and salvaged blood. Other groups have looked at surrogate markers of coagulopathy using patient hematology data. De Jong and colleagues looked at the effect of transfusion of unwashed salvaged blood on patients after elective TKA and found that platelet counts were

lower and levels of prothrombin fragments were higher in the venous blood samples after transfusion of unwashed salvaged blood.³ They hypothesized that these perturbations could potentially favor thrombosis after receiving salvaged blood since it suggested platelet depletion and thrombin formation. Dalen and colleagues found that leukocyte and platelet counts were reduced after the re-infusion of unwashed salvaged blood, but not to clinically significant levels.¹⁵ Other in vitro studies looking at salvaged blood also found a reduction in the amount of platelets as compared with venous blood, and that those platelets that remain are degranulated and mostly nonfunctional.^{3,7,16–18} Hence the platelet reduction seen by De Jong and colleagues may be due to platelet consumption at the wound and not necessarily due to an initiation of coagulation by reinfusion of salvaged blood as they suggested. Regardless, clinical effects on the recipients of post-operative salvaged blood cannot be ascertained using these isolated hematologic parameters and thus the current state of the literature cannot establish causality between the re-infusion of salvaged blood and thrombotic complications.

Thromboelastography is a measurement of the patient's clotting mechanism as a whole and is potentially well suited to determine whether or not the transfusion of salvaged blood would make a patient more prone to thrombotic complications.¹⁹ After TKA patients typically receive 400–500 mL of post-operatively salvaged blood, which is about 10% of their total blood volume.²⁰ Thus the volumes of blood used to create the mixed samples in this study were designed to model a typical patient's post-operative course. The TEG results suggest that transfusion of salvaged blood, in particular transfusion of unwashed salvaged blood, leads to clot formation that is initiated sooner, but of much lower strength. We hypothesize that this is due to the transfusion of a combination of procoagulopathic and anticoagulopathic elements known to be in salvaged blood, as discussed next.

Increased levels of active tissue factor (TF) and the presence of activated coagulation products are likely responsible for the decreased time to initial clot formation, and fibrin degradation products (FDPs) are likely responsible for the decreased clot strength. Blood salvaged after orthopedic procedures contains high concentrations of tissue factor.²¹ Tissue factor is an initiator in the coagulation cascade and adding it to the patient's venous blood will serve to activate the clotting mechanism, which will manifest as a decreased R time in the TEG as seen in this study. Salvaged blood also contains high levels of FDPs and this could explain the decrease in clot strength seen.²² High levels of FDPs affect the clotting mechanism by competitively binding to fibrinogen receptors thus inhibiting platelet aggregation, as well as by direct activation of plasmin.^{16,17,23,24} Taken together, the transfusion of activated tissue factor and FDPs may be responsible for the changes to the patients' coagulation profiles seen after receiving salvaged blood. One may make the argument that the mixed sample diluted coagulation factors in the control sample, but this would have manifested as a decreased MA as well as a increased R time, not decreased as was seen in the study.

This study has several limitations. We did not measure patient TEG profiles right after surgery before they received the salvaged blood to determine the effect that fluids and the surgery itself produced, if any. However previous studies have demonstrated TEG changes favoring a hypercoagulable state, with an increased K and MA, immediately after TKA without transfusing salvaged blood.²⁵ The results in the current study indicate a change in the opposite direction, favoring a hypocoagulable state after transfusing post-operatively salvaged blood.

The cost differential between the two types of salvaged blood systems should also be taken into account. The unwashed drain collection system is completely disposable and costs about 100 USD, whereas for the washed drain collection system the disposable alone costs

more than 400 USD, which does not include the capital investment of purchasing the equipment.²⁶ Thus a cost/benefit analysis of the potentially increased risk of thrombotic complications and the costs of the washed and unwashed devices should be considered when implementing a post-operative salvage system.

From this study, it would appear that the reinfusion of post-operatively salvaged blood leads to impairment of the clotting mechanism as measured by TEG. One could theorize that the reduction in clotting function might reduce the incidence of thrombotic complications after TKA. This contention would be supported by the lack of clinical trials in orthopedic surgery demonstrating increases in thrombotic complications following the reinfusion of unwashed blood.² Besides orthopedic surgery, cardiac surgery is the other main area where postoperatively salvaged blood is used. For cardiac surgery, the reduction in clotting function may be of clinical detriment. This is also supported by clinical trials where chest tube drainage increased following the administration of unwashed blood.²⁷ While in orthopedic surgery these findings may have a beneficial effect, in cardiac surgery an intact coagulation system is imperative to prevent postoperative bleeding. The results of this study provide a better understanding of these clinical observations.

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Figure 1. Thromboelastogram (TEG) trace, which gives a graphical representation of the sum of platelet function, coagulation factors, fibrin polymerization and the fibrinolytic system. The main parameters are R: time it takes to initiate clotting; K: the time to 20mm amplitude; α -angle: Slope between R and K, an indication of the rate of fibrin polymerization once initialized; and MA: the maximum amplitude of the tracing, which represents the maximum strength of the clot.

Table 1

Demographics were similar between the two patient groups, as well as preoperative hemoglobin (preop Hgb).

	Washed (n=11)	Unwashed (n=11)	P value
Gender	91% female	82% female	n/a
Age	60+/-13	66+/-5	0.20
BMI	35+/-6	29+/-5	0.01
Preop Hgb	13.4+/-1.6	13.3+/-0.9	0.88

Table 2

The TEG coagulation parameters were similar in the preoperative venous samples from the two groups of patients. Mean $\pm$ SD (range). R: Time to initial clot formation; K: Time to 20mm amplitude; α -Angle: rate of clot formation; MA: Maximum amplitude (clot strength).

Washed group vs Unwashed group Pre-surgical (control) samples			
	Washed (n=11)	Unwashed (n=11)	P value
R	4.9 $\pm$ 1.2 (3.5–7.3)	5.1 $\pm$ 1.1 (2.0–6.1)	0.64
K	1.5 $\pm$ 0.4 (1.2–2.1)	1.5 $\pm$ 0.3 (1.1–1.9)	0.59
α-Angle	68.7 $\pm$ 4.2 (62.4–73.2)	69.2 $\pm$ 3.2 (64.8–73.9)	0.77
MA	64.8 $\pm$ 3.4 (58.5–69.7)	64.0 $\pm$ 2.6 (60.7–67.9)	0.53

Table 3

Change in TEG coagulation parameters after the patients' presurgical venous sample was mixed with either washed or unwashed salvaged blood. Data reported as mean $\pm$ SD (range). R: Time to initial clot formation; K: Time to 20mm amplitude; α -Angle: rate of clot formation; MA: Maximum amplitude (clot strength).

Mixed with washed salvaged blood (n=11)			
	Presurgical	Mixed	P value
R	4.9 $\pm$ 1.2 (3.5–7.3)	4.2 $\pm$ 1.4 (2.9–7.2)	<u>0.02</u>
K	1.5 $\pm$ 0.4 (1.2–2.1)	1.7 $\pm$ 0.6 (0.9–3.3)	0.35
α-Angle	68.7 $\pm$ 4.2 (62.4–73.2)	67.3 $\pm$ 6.6 (52.8–75.3)	0.4
MA	64.8 $\pm$ 3.4 (58.5–69.7)	60.6 $\pm$ 6.1 (46.2–68.7)	<u>0.02</u>

Mixed with unwashed salvaged blood (n=11)			
	Presurgical	Mixed	P value
R	5.1 $\pm$ 1.1 (2.0–6.1)	2.0 $\pm$ 1.0 (0.8–3.6)	<u><0.01</u>
K	1.5 $\pm$ 0.3 (1.1–1.9)	2.0 $\pm$ 0.6 (1.0–2.9)	0.06
α-Angle	69.2 $\pm$ 3.2 (64.8–73.9)	67.7 $\pm$ 5.0 (58.3–76.1)	0.53
MA	64.0 $\pm$ 2.6 (60.7–67.9)	47.2 $\pm$ 13.3 (30.2–71.2)	<u><0.01</u>