CME/SAM

Fresh and Citrated Whole-Blood Specimens Can Produce Different Thromboelastography Results in Patients on Extracorporeal Membrane Oxygenation

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ABSTRACT

Objectives: *To compare thromboelastography (TEG) tracings obtained from fresh and citrated whole-blood samples in patients on extracorporeal membrane oxygenation (ECMO) or after cardiopulmonary bypass and in healthy volunteers.*

Methods: *Samples of fresh and citrated whole blood were analyzed for 25 patients and 4 healthy volunteers. Thromboelastography analysis was performed in both plain and heparinase cups.*

Results: *In 5 of 6 patients on ECMO, use of citrated samples resulted in apparent partial or complete heparin reversal. In TEG tracings from patients following cardiopulmonary bypass, there was a slight hypercoagulable appearance in the citrated sample. No differences were noted between fresh and citrated samples from healthy volunteers whose blood was spiked with heparin.*

Conclusions: *In some patients on ECMO, use of samples collected in sodium citrate tubes for TEG analysis results in significant artifacts, which could lead to heparin overdosing in these patients.*

Upon completion of this activity you will be able to:
- discuss the limitations of the use of fresh whole blood for thromboelastography (TEG).
- define the parameters measured in TEG tracings.
- recognize potential artifacts in TEG tracings in recalcified citrated patient samples.
- compare TEG tracings from citrated and fresh (noncitrated) whole-blood samples.

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Questions appear on p 274. Exam is located at www.ascp.org/ajcpcme.

The use of thromboelastography (TEG) as a comprehensive assessment of hemostasis has been shown to decrease the transfusion of blood products for liver transplant[1] and cardiovascular surgery.[2,3] With the increased awareness of the hazards of transfusion, more institutions are looking for ways to decrease blood product use and determine the best blood component for therapy.

Until recently, TEG (Haemonetics, Niles, IL) was the only test of the viscoelastic properties of blood approved by the US Food and Drug Administration. It was initially developed for use with fresh whole blood; however, the test needs to be initiated within 4 minutes of sample collection. This is not practical for many centers that perform TEG for patients in several locations or hospitals without laboratories in close proximity to critical care services.

The use of citrated whole blood has been published as an alternative to fresh (non-anticoagulated) whole blood for use in TEG. This alternative allows for more time between the blood draw and the initiation of the test. Some studies comparing TEG tracings of citrated fresh whole-blood samples have shown that citrate anticoagulant affects the parameters of the tracing, with a tendency toward a hypercoagulable effect. These studies have differed in the populations being studied (healthy volunteers vs patients) and the activator used to initiate clotting (kaolin vs celite).[4-7]

In this study, we sought to validate the use of citrated whole blood for kaolin-activated TEG performed on patients on extracorporeal membrane oxygenation (ECMO) and for patients after cardiovascular surgery.

Materials and Methods

Sample and Patient Selection

In an effort to validate the use of citrated whole blood for TEG studies in our institution, we performed tests on 3 different patient populations. Samples of fresh and citrated whole blood in 3.2% sodium citrate tubes (Becton Dickenson, Franklin Lakes, NJ) were collected nearly simultaneously by laboratory staff from 6 patients on ECMO. Samples were collected by venipuncture or from arterial catheters. The laboratory procedure for TEG collection requires a 10-mL discard for samples collected from arterial catheters and a 2- to 3-mL discard for samples collected by venipuncture. Fresh whole-blood samples were collected in a syringe and immediately transported to the laboratory, such that TEG analysis was started within 4 minutes of the sample draw. For citrated whole-blood samples, excess citrated whole blood from tubes submitted for routine coagulation monitoring was used. Before centrifuging samples for coagulation testing, a 1-mL aliquot of citrated whole blood was removed for TEG testing. For citrated samples, TEG tracings were started within 15 minutes of sample collection based on internal stability studies (data not provided) and published literature.[8] For 19 cardiovascular surgery patients, fresh whole blood was collected in syringes and 3.2% sodium citrate tubes nearly simultaneously by operating room staff immediately after protamine reversal of heparin and discontinuation of cardiopulmonary bypass. Samples were transported and handled in an identical manner as described above. Finally, whole-blood samples were collected from 4 healthy volunteers using a Becton Dickinson Vacutainer Push Button Blood Collection Set with a 21-gauge needle. After a 2- to 3-mL discard, samples were drawn into a 30-mL syringe, then pipetted into Starstedt aliquot tubes (Starstedt, Newton, NC) and spiked with incremental levels of unfractionated heparin (0.0, 0.1, 0.2, 0.3, and 0.4 U/mL) immediately after the sample draw. Samples with various levels of heparin were either kept in aliquot tubes and tested as fresh whole blood within 4 minutes of sample collection or aliquoted into 3.2% sodium citrate tubes and tested as citrated samples within 15 minutes of sample collection after thorough mixing of the citrated tubes. The study design was approved by the Mayo Clinic Institutional Review Board.

TEG Testing

The TEG parameters measured and compared for differences between fresh whole blood and citrated blood included reaction (R) time, measured in minutes (R time is a lag phase before clotting begins, which reflects clotting factor adequacy or the presence of clotting inhibitors); angle (measurement of clot kinetics/fibrinogen, determined by the angle of a line drawn tangent to the clotting curve from the initiation of clotting); and maximum amplitude (MA, measurement of platelet function/count, calculated as the maximum oscillation distance in millimeters). See **Figure 1** and **Figure 2** for a demonstration of the TEG parameters.

For all fresh whole-blood samples, 1 mL of whole blood from the collection syringe was mixed with kaolin for activation in the vial provided by the manufacturer (Haemonetics), and an aliquot (360 μL) was added to each cup (plain plastic and heparinase) within 4 minutes of sample collection. For citrated samples, 1 mL of excess 3.2% sodium citrate whole blood was mixed with kaolin for activation. Prior to putting the whole-blood sample (340 μL) in each cup (plain plastic and heparinase), 20 μL of 0.2M calcium chloride was added for recalcification per the manufacturer's recommendations. Thromboelastography analysis was performed on Haemonetics 5000 series TEG analyzers using both plain (no additive) plastic cups and heparinase cups to neutralize any heparin in the sample. Comparing TEG results between plain and heparinase cups allows clinicians to determine whether the presence of heparin (either from therapeutic administration or contamination during the sample draw) is responsible for abnormalities (especially prolonged R time) observed on the TEG tracing. Kaolin activator, calcium chloride, and plain and heparinase cups were provided as part of a kit by the instrument manufacturer (Haemonetics). Statistical significance of differences in TEG parameters between fresh and sodium citrate whole-blood samples was determined using an unpaired *t* test in GraphPad InStat, version 3.0 (GraphPad Software, San Diego, CA).

Results

Samples were collected from 6 patients on ECMO. In 3 patients, the citrated whole-blood samples showed near-normal coagulation status (R, angle, and MA values only slightly outside the normal ranges), while fresh whole-blood samples

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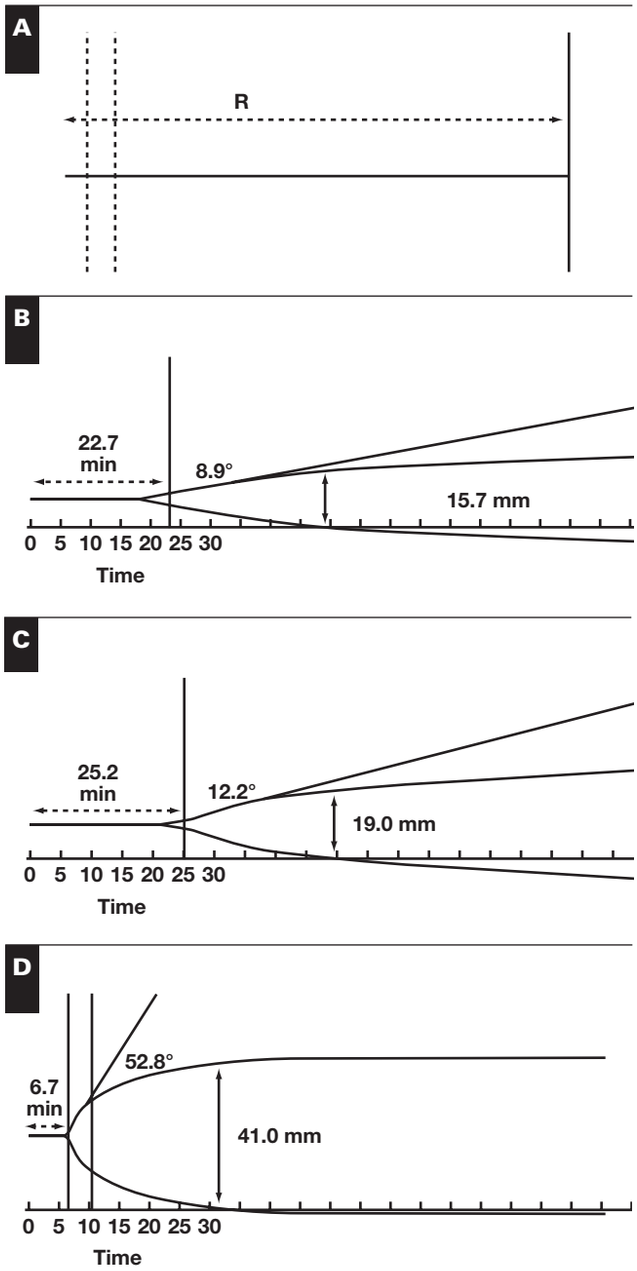

Figure 1 Comparison of thromboelastography (TEG) tracings from a patient on extracorporeal membrane oxygenation. Testing citrated whole blood in this patient showed the appearance of heparin reversal (the citrated TEG tracing resembled the heparinase tracing from the fresh sample). **A**, Kaolin, fresh whole blood. **B**, Kaolin with heparinase, fresh whole blood. **C**, Kaolin, citrated whole blood. **D**, Kaolin with heparinase, citrated whole blood. Reaction (R) time is indicated by dashed arrows. Maximum amplitude (MA) is indicated by solid arrows and angle by a line drawn tangent to the clotting curve from clot initiation. One tracing (**A**) was terminated after 84 minutes without initiation of clotting; thus, for this tracing, there is no MA or angle, and the R time is more than 84 minutes.

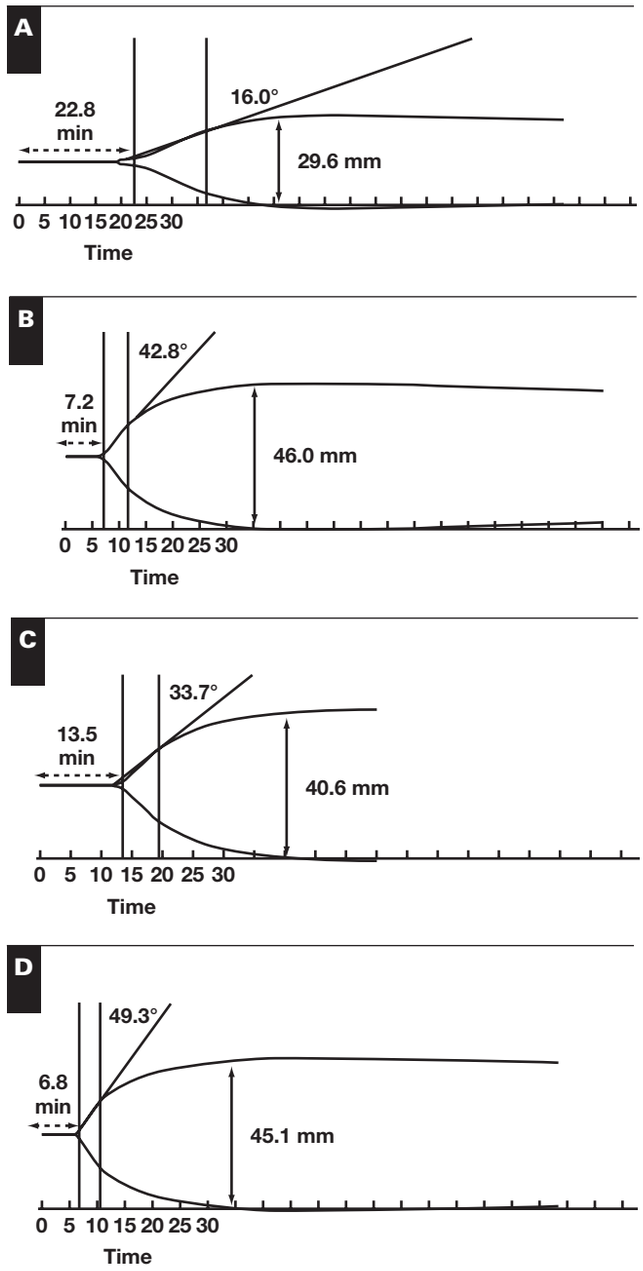

Figure 2 Comparison of thromboelastography (TEG) tracings from a patient on extracorporeal membrane oxygenation. Testing citrated whole blood in this patient showed the appearance of partial heparin reversal (the citrated kaolin TEG started to resemble the heparinase tracing from the fresh sample). **A**, Kaolin, fresh whole blood. **B**, Kaolin with heparinase, fresh whole blood. **C**, Kaolin, citrated whole blood. **D**, Kaolin with heparinase, citrated whole blood. Reaction time is indicated by dashed arrows. Maximum amplitude is indicated by solid arrows and angle by a line drawn tangent to the clotting curve from clot initiation.

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demonstrated profound coagulopathy (R times >37 minutes) attributed partially to heparin administration. For these 3 patients, the TEG tracing in citrated whole blood (Figure 1C) was nearly identical to the tracing produced after heparinase treatment of fresh whole blood (Figure 1B). For fresh whole-blood samples in these 3 patients, heparinase treatment markedly reversed the coagulopathy (prolonged R time) observed (compare Figure 1A with Figure 1B), demonstrating that heparin therapy was partly responsible for the coagulopathy observed. Thus, the most likely explanation for marked differences between fresh and citrated whole-blood TEG tracings in these patients is partial heparin reversal in the citrated tube.

In 2 patients, there was the appearance of partial reversal of heparin in the citrated sample (Figure 2). In these cases, the fresh whole-blood sample measured in the plain cup showed only moderately prolonged R values (Figure 2A), while the fresh whole-blood sample in the heparinase cup showed near-normal coagulation status (Figure 2B), indicating that heparin was responsible for the coagulopathy observed with fresh whole blood. The citrated whole-blood sample in the plain cup (Figure 2C) also showed near-normal coagulation status, again demonstrating heparin reversal in the citrated tube. In 1 patient, there was little difference between the fresh and citrated whole-blood TEG tracings (not shown).

Following these observations, samples were collected from 19 cardiovascular surgery patients who had TEG ordered for bleeding following cardiopulmonary bypass (after protamine reversal of heparin). In these patients, TEG tracings from citrated samples did not show marked differences from TEG tracings performed with fresh whole-blood samples. Consistent with earlier reports,[4,5] the R time in citrated samples was shorter than that observed in fresh whole-blood samples. Mean (SD) R time in fresh whole-blood samples was 7.1 (2.3) minutes compared with 5.0 (1.1) minutes in citrated samples ($P = .001$) **Table 1**. Differences in angle and MA were not statistically significant comparing citrated with fresh whole-blood samples for these 19 patients ($P > .05$) (Table 1).

Last, samples from 4 healthy volunteers were drawn and whole blood spiked with heparin in an effort to reproduce the findings in the patients on ECMO (who were receiving therapeutic heparin). Either in the absence of heparin or at heparin levels between 0.1 and 0.4 U/mL, neither clinically nor statistically significant differences were seen between the fresh and citrated whole-blood TEG parameters (R, angle, and MA) in either plain plastic cups or heparinase cups (data not shown). The observations of apparent heparin reversal in patients on ECMO could not be reproduced in healthy volunteers after heparin spiking or in cardiovascular surgery patients.

Discussion

At least 3 studies have found that storage of whole blood in citrate affects TEG tracings, with citrate collection or storage producing a hypercoagulable pattern, as demonstrated primarily by decreased R times compared with fresh whole blood[4,5] or decreasing R times as a function of storage time in citrate.[4,5,7] Two of these studies used celite as the activator for

Table 1
Thromboelastography Parameters, Including Reaction (R) Time, Angle, and Maximum Amplitude (MA), for 19 Cardiovascular Surgery Patients Comparing Fresh (Not Anticoagulated) With Recalcified Citrated Whole-Blood Samples

	Fresh Whole Blood			Citrated (Recalcified) Whole Blood		
Patient No.	R (min)	Angle (°)	MA (mm)	R (min)	Angle (°)	MA (mm)
1	6.2	65.1	50.8	3.5	70.4	61.4
2	6.2	60.4	55.3	4.5	69.9	63.3
3	5.9	65.4	62.0	6.0	62.1	56.1
4	6.0	69.8	74.0	4.2	74.6	68.6
5	6.9	64.2	64.0	5.3	23.6	28.5
6	8.7	54.0	55.0	5.2	64.1	57.1
7	8.6	57.4	64.0	8.2	56.1	56.5
8	10.3	50.2	65.2	5.4	66.1	62.6
9	5.5	67.8	65.9	4.8	70.2	62.6
10	7.4	54.5	57.2	5.2	60.9	52.4
11	9.0	48.3	56.7	6.2	58.5	59.1
12	11.4	41.9	46.4	5.2	54.2	44.9
13	5.8	66.4	63.8	5.3	65.7	59.4
14	5.1	70.0	70.8	4.9	66.2	63.0
15	3.9	70.6	64.7	4.2	66.9	60.6
16	7.4	45.7	50.2	5.5	50.9	47.7
17	4.3	66.9	64.3	3.8	63.7	56.8
18	12.0	47.3	57.2	4.8	69.0	66.6
19	4.5	71.7	74.5	3.6	76.3	72.1
Mean	7.1	59.9	61.2	5.0	62.6	57.9

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TEG analysis,[4,7] while 1 used kaolin.[5] In contrast, 1 study of 10 healthy volunteers found no difference in kaolin-activated TEG parameters between samples collected in citrate and fresh whole blood, although using tissue factor activation did result in a hypercoagulable pattern for citrated samples.[6] Differences between studies in number and types of subjects tested, clotting activator used, and time between citrated sample collection and testing make it difficult to generalize the extent to which use of citrated blood samples for TEG testing leads to the appearance of a hypercoagulable state (decreased R time).

Other groups have found unexpected and presumably artifactual results when testing different patient populations with TEG. Two separate groups have reported a fibrinolytic pattern in TEG tracings using heparinase cups.[9,10] In 1 of these reports, citrated samples were collected from patients on ECMO or with left ventricular assist devices.[9] In the other report, a fibrinolytic pattern was observed in the heparinase cup in TEG tracings performed in patients with severe sepsis, although it is unclear whether fresh whole blood or citrated whole blood was used.[10]

In evaluating whether citrated whole blood could be used for TEG in patients on ECMO, we found unexpected differences between TEG tracings from samples collected in sodium citrate tubes compared with fresh whole blood. In 3 patients on ECMO, use of citrated samples led to TEG tracings in plain plastic cups that were nearly identical to those obtained using fresh whole blood but tested with a heparinase cup. Thus, use of citrated samples led to apparent heparin reversal in these patients. In 2 additional patients on ECMO, use of citrated whole-blood samples for TEG resulted in apparent partial heparin reversal, while in 1 patient on ECMO, there was little difference in the TEG tracings between fresh and citrated whole blood. Because in our practice, the R time obtained by TEG is used to adjust the heparin dose for patients on ECMO, the apparent heparin reversal in the citrated samples would lead to excess heparin dosing and could pose a serious risk of patient harm.

In patients having TEG performed after cardiovascular surgery, we observed only moderate decreases in R time in citrated samples, consistent with earlier reports.[4,5] In 4 healthy volunteers whose fresh whole blood or citrated whole blood was spiked with heparin, we did not observe any significant differences between fresh and citrated samples in either plain or heparinase TEG cups. The presence of heparin alone (in healthy volunteers) is not sufficient to cause significant artifacts in TEG tracings when citrated whole-blood samples are used. It is unclear what factors in patients on ECMO lead to the appearance of heparin reversal in some citrated samples.

One limitation to our study is that we did not explore the relationship between storage time in citrated whole blood and differences between fresh and citrated whole-blood TEG tracings. All citrated whole-blood TEG was performed within 15 minutes of sample collection. Most studies have shown that with increasing time between citrated sample collection and testing, the R time decreases further compared with fresh whole blood.[4,5]

In conclusion, we observed clinically significant differences between citrated and fresh whole-blood TEG tracings for some but not all patients on ECMO. Combined with previous reports suggesting artifacts and/or clinically significant differences in TEG tracings in heparinase cups in some patient populations, it appears that use of citrated blood samples may not be appropriate for all TEG procedures performed on all patient populations. Institutions wishing to use citrated samples for TEG analysis should investigate the impact of citrated sample collection and storage time on the types of TEG testing being performed in the patient populations to be tested.

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