

The use of recalcified citrated whole blood – a pragmatic approach for thromboelastography in children

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Summary

Background: Thromboelastography (TEG) is an established way of monitoring the coagulation status of children and adults requiring blood products during surgery. Serial measurements are performed using a nearside machine and blood product prescription may be titrated against changes in TEG. There may also be useful applications when the patient is remote from the TEG machine but these are limited because TEG is usually performed on fresh native whole blood within 6 min of venepuncture. Citrated whole blood can be used for TEG if transport time is more than 6 min. We wished to establish whether TEG parameters for citrated whole blood were comparable with those of native whole blood in healthy children.

Methods: Blood was obtained from 14 healthy children undergoing minor surgical procedures, at the time of intravenous cannula insertion for anaesthesia. Each sample was divided: TEG was performed on part of the sample in its fresh native state at 6 min and second portion of the sample was citrated, kept at room temperature and TEG was performed at 30 min after recalcification.

Results: There was a significant difference in TEG parameters (r , k , α , MA and LY30) for fresh native whole blood and recalcified citrated whole blood (paired t -test).

Conclusions: The normal range for fresh native whole blood TEG parameters is well established, which is routinely used in practice. There was a significant difference between TEG parameters for fresh native whole blood and citrated whole blood. We recommend that a specific normal range be established for citrated whole blood to enable it to be used in clinical practice.

Keywords: thromboelastography; fresh native whole blood; citrated whole blood; children

Introduction

Thromboelastography (TEG) monitors hemostasis as a whole dynamic process by measuring viscoelastic properties of blood. It gives significant information on clotting factor activity, interaction of clotting factors with platelets, platelet function and any clinically significant fibrinolytic process, within 20–30 min (1,2). Thromboelastography requires only a small amount of blood (0.36 ml). This can be potentially beneficial in children. Serial samples can be taken to enable the clinician to titrate blood products to maximum effect and possibly reduce exposure to blood products. The role of TEG is well established in liver and cardiac transplant surgery in both adults and children (3–5).

TEG is usually performed on fresh native whole blood within 6 min of venepuncture, ideally at 4 min (1,2). TEG analysers are usually kept in operation theatres, as a near-patient tool for monitoring hemostasis during surgery. Although TEG analysis may be valuable for ward-based patients, it is not always practical or possible to analyse blood within 6 min. With this in mind, other investigators have explored the use of citrated whole blood for TEG by using standardized technique (2,6,7). However, all these studies were performed in adults and until now, studies in children were lacking. We wished to establish whether this standardized technique produced reproducible results in the pediatric population. Hence, the present study was undertaken.

The aim of the present study was to compare TEG parameters on fresh native whole blood with TEG parameters on recalcified citrated whole blood in healthy children.

Materials and methods

After obtaining local hospital ethics committee approval, normal healthy children undergoing minor surgical procedures were recruited for the study. Written, informed consent was obtained from parents and older children. Children aged 10–16 years were recruited for the study. Children less than 1-year-old were excluded from the study because of possible sampling difficulties. Children with unexplained bleeding in the past or with a family history of bleeding disorders were also excluded. Blood

samples were obtained from the intravenous cannula at the time of anesthesia by a 'two-syringe technique' (initial 2–3 ml blood is discarded) to avoid contamination with tissue thromboplastin. Blood was analysed for full blood count, clotting studies [(prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, TT, d-dimers] and TEG. Full blood count was performed by using 'Advia 120' (Bayer, Basingstoke, UK) analyser, while clotting studies were performed by using 'Futura Advance' (Instrumentation Laboratory, Washington, UK) analyser and 'd-dimer' was measured by 'Nycocard' (Nycomed Pharma, CSO, Norway). Standardized technique was followed for citrating and subsequent recalcification of whole blood (2,6,7). Whole blood was citrated with 3.2% (0.105 M) sodium citrate (one part citrate to nine parts blood dilution). TEG was performed, by using a 5000 series TEG analyser from Haemoscope Corporation, Skokie, IL, USA. TEG was performed at a fixed temperature of 37°C on fresh native whole blood (360 µl) within 6 min of collection and on citrated whole blood at 30 min after collection. Citrated whole blood (340 µl) was first recalcified with 0.2 M calcium chloride (20 µl) before performing the TEG. TEG analytical software gave all the results for parameters in numerical values from the TEG tracings and the whole information was saved in the software.

Five TEG parameters (as demonstrated in Figure 1) were used for this study. '*r*' (*R*, reaction time) is the time from the start of tracing until the curve is 2 mm wide, it measures latency from the time blood is placed in the TEG analyser until initial fibrin formation. '*k*' (*K*, clot formation time) is the time measured from the '*r*' to the point where amplitude of the tracing reaches 20 mm, it is a measure of the speed or clot kinetics to reach a certain level of clot strength. '*α*' (angle) is the angle formed by the slope of the TEG tracing from the '*r*' to '*k*' value, it denotes the speed at which solid clot forms. '*MA*' (maximum amplitude) is the greatest amplitude on the TEG trace and is the reflection of the absolute strength of fibrin clot. '*LY30*' (clot lysis at 30 min) represents the percentage decrease in the area under curve at 30 min after maximum amplitude, it denotes per cent fibrinolysis at 30 min. '*r*', '*k*' values were measured in minutes (min) while '*MA*' in millimetres and '*LY30*' in % (1,2).

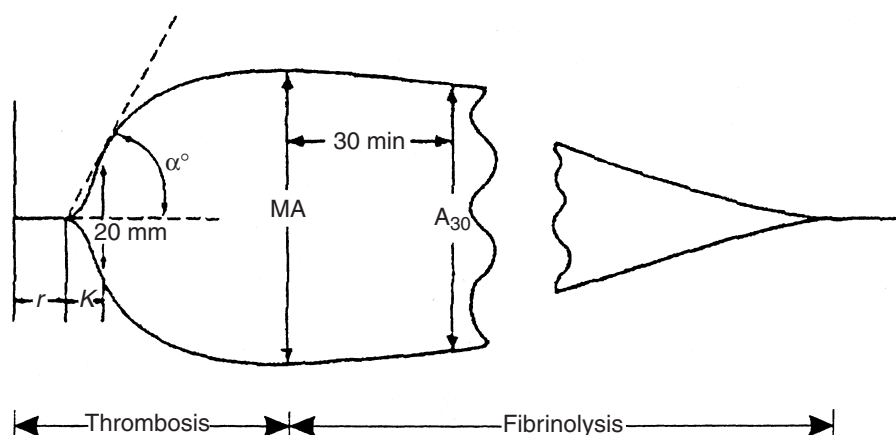


Figure 1
TEG variables.

We performed a power calculation based on the previous study in adults, which demonstrated no difference in TEG values for whole blood and citrated whole blood (6), and found that the number of subjects required for the study was seven with a correlation coefficient of 0.9. The results for the citrated whole blood were compared with fresh native whole blood by using paired *t*-test.

Results

Blood samples were obtained from 14 healthy children undergoing minor surgical procedures. The surgical procedures were orchidopexy in six, repair of hydrocele in two, repair of hernia in two, circumcision in two and excision of benign subcutaneous lump in two children. There were 13 boys and one girl. The boys were predominant as main surgical procedures were orchidopexy and repair of inguinal hernias. The median age of children was 4.9 years with the youngest being 1.6 years and

the eldest 15 years. Hematological parameters (Table 1) other than 'Thrombin time' (TT) were appropriate for the age. In seven subjects, 'TT' was slightly prolonged by 1 s above upper limit of adult normal range for our laboratory (13–18 s). However, all the subjects had normal 'fibrinogen' and 'd-dimer' levels, also there was no heparin contamination. All the subjects underwent surgery without any excessive bleeding.

TEG values were normally distributed. There was no correlation between TEG parameters and age. The sample TEG tracings for fresh native whole blood and citrated whole blood are demonstrated in Figure 2 and Figure 3, respectively. There was a significant difference between TEG values for fresh native whole blood and citrated whole blood (Table 2). The mean '*r*' for native whole blood was 17.4 min while for citrated whole blood, it was 7.7 min. The *P*-value for '*r*' was <0.001 (highly significant). The remaining mean TEG values for native whole blood were '*k*'- 7.6 min, ' α '- 31.2°, '*MA*'- 52.6 mm, 'LY30'- 0.8%, while mean TEG values for citrated whole blood were '*k*'- 3.1 min, ' α '- 55°, '*MA*'- 57.6 mm, 'LY30'- 1.9%. After comparing whole blood and citrated whole blood TEG values, the *P* value for '*k*', ' α ' and 'LY30' were <0.001 (statistically highly significant) while *P* value for '*MA*' was 0.022 (statistically significant).

Discussion

TEG is a useful measure of global hemostasis. It is a dynamic test, which gives important information about coagulation, fibrinolysis and platelet function. It may also be helpful in guiding the appropriate

Table 1
Full blood count and clotting screen

Investigations	Result*
Hb	12.1 (11.5–16) g·dl ⁻¹
WBC	8.4 (5.3–12.5) × 10 ⁹ l ⁻¹
Platelets	277 (215–369) × 10 ⁹ l ⁻¹
PT	12.3 (10–14) s
INR	1.1 (1–1.3)
APTT	35 (29.9–39.5) s
TT	19 (15–19) s
Fibrinogen	2.3 (1.6–2.9) g·l ⁻¹
d-Dimers	0.3 (0.1–1.6) mg·ml ⁻¹

*Median (range).

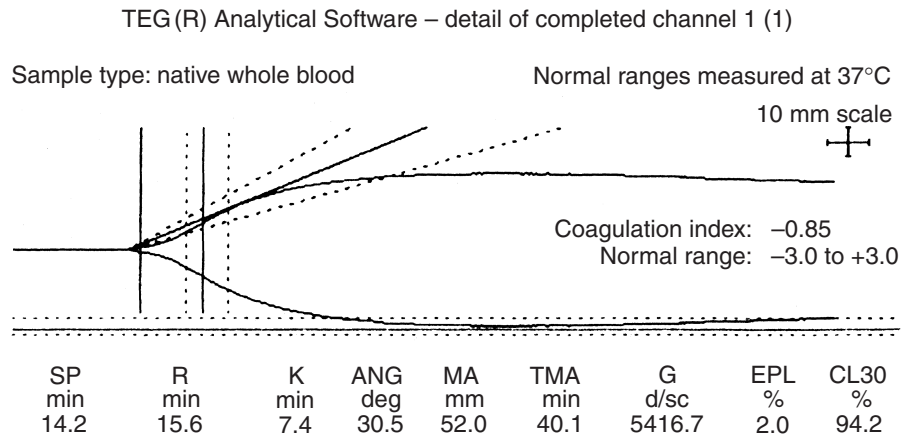


Figure 2
TEG tracing of 'fresh native whole blood'.

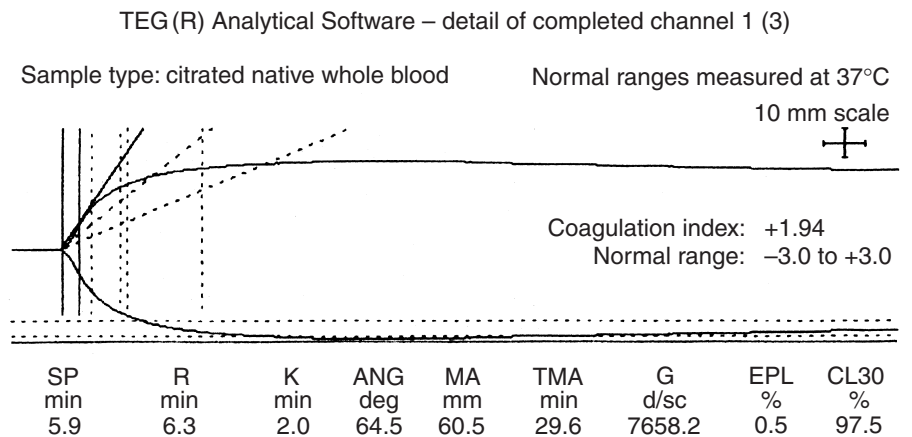


Figure 3
TEG tracing of 'recalcified citrated whole blood'.

Table 2
TEG parameters

TEG*	Native whole blood	Citrated whole blood	P-value
r (min)	17.4 ± 3.6	7.7 ± 2.6	<0.001
k (min)	7.6 ± 1.4	3.1 ± 1.4	<0.001
α (°)	31.2 ± 4.8	55 ± 10.5	<0.001
MA (mm)	52.6 ± 6.4	57.6 ± 3.7	=0.022
LY30 (%)	0.8 ± 1	1.9 ± 1.2	<0.001

*TEG values: Mean ± SD.

therapy in correcting defects in hemostasis. The specific therapy can first be applied *in vitro* to confirm its effect on the blood sample before the patient is treated (8,9). Its use is limited by the fact that fresh native whole blood must be used within 6 min of venepuncture. It is therefore useful to have a method that allows for the storage of blood while providing the results as reliable as the 'gold standard' of fresh native whole blood. Various studies in the past have suggested the use of citrated whole blood for TEG if there arose a delay in performing TEG (2,6,7). However, all the studies were conduc-

ted in adults and this is the first study in children demonstrating the usefulness of citrated whole blood samples for TEG.

The normal range for TEG parameters in healthy children has already been established by using 'celite-activated TEG' on fresh native whole blood (10). However, data related to the TEG pattern using citrated whole blood in children are lacking. This study demonstrated that, there was a significant difference between TEG values for fresh native whole blood (analysed within 6 min) and citrated whole blood (analysed after recalcification at 30 min). The 'r' and 'k' values for citrated whole blood were significantly lower than similar values for fresh native whole blood, while 'α', 'MA' and 'LY30' values were significantly higher. The normal reference range used for interpreting TEG values is based on fresh native whole blood samples. In view of this, if the citrated whole blood is used for TEG analysis, then TEG values should be interpreted with caution. Ideally, a normal reference range

should be established for citrated whole blood TEG before using this in practice.

Similar findings were also reported in adults demonstrating a significant difference between native whole blood TEG and citrated whole blood TEG parameters (7). This study also suggested analysing citrated whole blood for TEG at 1 h, as TEG parameters for citrated whole blood might be unstable up to 1 h of citrate storage, not giving reproducible results. In the current study, TEG was performed at 30 min on all citrated blood samples. As a fixed time was used in all children, comparison of TEG parameters was possible without any bias. Contrary to findings of this study, Bowbrick *et al.* have shown that in adults, there was no significant difference between native whole blood TEG parameters and citrated whole blood TEG parameters at 30 min, however there were only eight healthy adults in the study (6).

The significant difference in TEG parameters for fresh native whole blood and recalcified citrated whole blood is difficult to explain. The reduced 'r', 'k' and increased 'α', 'MA' values is partly explained by activation of coagulation and platelets in citrated blood. This was demonstrated in a previous study of citrated whole blood TEG. Although sodium citrate has been shown to be an adequate anticoagulant for measuring clotting screen in the laboratory, it is not sufficient to completely suppress thrombin generation *in vitro* (7).

This study used a computerized TEG analyser, giving TEG values directly rather than providing a trace, which requires manual analysis. This study reliably demonstrated a difference between TEG parameters for fresh native whole blood and recalcified citrated whole blood in healthy children. TEG was performed on two different blood samples in the same child, thus each child acted as its own control, eliminating source of bias. Despite all these advantages, the number of children recruited was small, so we could not establish a normal range. In this study, there was male preponderance, which might introduce a bias, as one recent study has demonstrated that blood is slightly more coagulable in adult females than adult males. However, about 50% of women in this study were on oral contraceptive pills, which is known to cause hypercoagulability (11).

The major weaknesses of TEG are (i) it cannot identify the specific factor responsible for coagulopathy; (ii) it is not validated; and (iii) it has never been

standardized. However, it still has many advantages and is routinely used in transplant surgery and in various clinical settings (3–5,12,13).

This study demonstrated that there is a difference between recalcified citrated whole blood and fresh native whole blood TEG. A further study with larger numbers is required to establish the normal range for citrated whole blood TEG parameters.

We conclude that if a delay in transport of blood sample is expected, then citrated whole blood can be used for TEG analysis. Useful trends may be recognized when serial analysis of blood samples is performed (i.e. before and after therapeutic intervention). However, a specific normal range needs to be established before it can be used for isolated analysis in practice.

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