Interoperator and Intraoperator Variability of Whole Blood Coagulation Assays: A Comparison of Thromboelastography and Rotational Thromboelastometry

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Objectives: **Near-patient viscoelastic tests have proved useful in decreasing blood and blood product use in cardiac surgery. Two different analyzers are available, TEG and ROTEM. Many different individuals operate these devices, which raises concern that this factor may significantly affect results. The present study sought to objectively assess variability in results between operators.**

Design: **Prospective study.**

Setting: **Regional cardiac center.**

Participants: **Adult patients undergoing elective cardiac surgery.**

Interventions: **Thirty-six mL of blood were taken from each of 21 patients. TEG kaolin and functional fibrinogen (FF) analyses and the equivalent ROTEM INTEM S and FIBTEM S analyses were performed. Six operators performed one of each test per patient to assess interoperator variability. One further operator performed 6 of each test per patient to assess intraoperator variability.**

Measurements and Main Results: **All routine measurement parameters were noted and the coefficient of variation (CV) calculated, analyzing comparable parameters. All interoperator CVs were significantly lower for ROTEM analyses compared with TEG. CV for INTEM S CT/ kaolin r time was 4.7 versus 16.3 and MCF/MA was 2.6 versus 4.3 ($p < 0.01$). Similarly, FIBTEM S MCF/ FF MA was 8.3 versus 12.2. All intraoperator CVs were significantly lower for ROTEM analyses compared with TEG ($p < 0.01$). CV for INTEM S CT/ kaolin r time was 3.1 versus 9.8 and MCF/ MA was 1.6 versus 4. Similarly, FIBTEM S MCF/ MA was 6.9 versus 12.1.**

Conclusions: **This series of results suggested ROTEM analyses are more reproducible than TEG and, consequently, that ROTEM may be better suited for use in a multiuser environment.**

KEY WORDS: thromboelastography, thromboelastometry, near-patient testing, interindividual variation

THROMBOELASTOGRAPHY first was described as a global test of blood coagulation by Hartert.[1] The method allows rapid assessment of several aspects of coagulation: Initiation of clot, propagation kinetics, clot firmness, and fibrinolysis. The results produced provide a qualitative rather than quantitative assessment of the coagulation process. Whole blood coagulation analyses using thromboelastographic/thromboelastometric techniques are increasingly popular, especially for point-of-care management of acute perioperative bleeding;[2–11] given the increased awareness of the complications associated with red cell and blood product transfusion, there has been increasing interest in such devices as a means to decrease blood use.

Two principal manufacturers, Haemonetics (Braintree, MA) and TEM International GmbH (Munich, Germany) have developed this technology and have produced thromboelastographic instruments for use in the perioperative point-of-care setting. The system developed by Haemonetics is the thrombelastograph (TEG), and that developed by TEM International is the ROTEM. Both systems produce a trace that is a graphic representation of clot strength over a period of time. TEG analysis is termed "thromboelastography", and ROTEM analysis is termed "thromboelastometry." Although the nomenclature of identical parts of the trace varies between the 2 systems,[12] the primary hardware difference concerns the movement of the cup and pin. The TEG cup rotates while the pin is suspended freely in the cup by a torsion wire, whereas the ROTEM pin rotates while the cup is stationary; each rotates through an arc of 4.75° every 6 seconds. ROTEM, the more recently developed system, is an adaptation of the original TEG technique; the stabilization of the pin using a ball bearing in the mechanical axis is claimed to improve performance in terms of greater resilience to vibration and interference.

Use of these devices does not require a specialized coagulation laboratory or experienced laboratory personnel; therefore, both systems are used extensively to assess hemostasis in the operating room, intensive care unit, or emergency room. Accordingly, many different individuals may operate these devices, including anesthesiologists, intensivists, nursing staff, and perfusionists, who often are inexperienced in laboratory techniques. This is potentially a major factor influencing the results of point-of-care tests. Point-of-care testing produces rapid results; however, this should not be at the expense of accuracy and precision.[13] Although the use of standardized reagents and computer-assisted pipetting improve experimental reproducibility, TEG and ROTEM still require some individual pipetting steps. Therefore, interoperator variability may produce clinically relevant discrepancies in the results produced. At present there are little data available concerning the equivalency or superiority between the 2 widely-used devices specifically comparing operator-dependent variability, although there have been some limited assessments of intraoperator variability.[14]

The aim of this study was to assess interoperator variability using TEG and ROTEM under identical conditions and to

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determine whether clinically relevant differences in inter-operator variability of the 2 devices could be detected.

METHODS

After institutional review board approval and written informed consent, 21 adult patients scheduled for elective cardiac surgery with no pre-existing coagulation abnormalities demonstrable on routine preoperative coagulation tests were included in the study. Although antiplatelet medication should have no effect on results from either system, patients on dual-antiplatelet medication were excluded; those on aspirin had had it stopped 1 week before surgery in line with routine practice in the authors' institution. Thirty-six mL of blood was taken from the central venous pressure (CVP) catheter placed after induction of anesthesia. Three sampling time points were chosen to ensure testing reflected a variety of normal and potentially abnormal coagulation patterns. In 7 of the patients, blood was taken after induction of anesthesia but before surgery to assess patients with normal coagulation. In a further 7 patients, blood was taken after protamine reversal of heparin post-cardiopulmonary bypass to assess patients who were hemodiluted and possibly coagulopathic. In a further 7 patients, the blood was taken on the first postoperative day while in the intensive care unit to assess patients in their postoperative phase who may demonstrate an acute-phase reaction to surgery and consequent hypercoagulable coagulation pattern. For each patient, after discarding the initial 4 mL of blood aspirated from the CVP, the 36 mL of blood was collected into 12 citrated Vacutainer tubes (BD Biosciences, Plymouth, Devon, UK) containing 3.2% (0.105 mmol/L) sodium citrate. The samples were analyzed using the TEG and ROTEM devices as per manufacturers' instructions and, in keeping with the study protocol, no earlier than 30 minutes and no later than 90 minutes after sampling. Samples were analyzed at 37°C and samples were prewarmed for 5 minutes before analysis.

Thromboelastographic measurements were performed simultaneously using 6 two-channel TEG (Model 5000 Haemonetics, Braintree, MA) and 3 four-channel ROTEM (Model Delta, TEM International GmbH, Munich, Germany) devices. All channels used had passed electronic and liquid quality controls immediately before the test run. Intrinsically activated and platelet-inhibited thromboelastographic assays were performed; on the TEG analyzer, kaolin-activated thromboelastography and the functional fibrinogen level assay (FF) were run. On the ROTEM device, the equivalent tests (the intrinsically-activated INTEM S and the platelet-inhibited FIBTEM S assays) were performed using single-use reagents. Detailed descriptions of these assays previously have been documented.[12,15,16] Parameters reflecting clot initiation, propagation, and strength were assessed using both systems. Clot initiation was defined by the reaction time for TEG or the coagulation time for ROTEM, clot propagation by the alpha angle and clot strength by the maximum amplitude (MA) of the trace for TEG and firmness parameters (A5, A10, A20, or MCF) for ROTEM. A typical trace from each system is demonstrated in Fig 1. Platelet-inhibited thromboelastography gives the functional fibrinogen level on the TEG expressed as the MA and the maximum clot firmness of the fibrin clot (MCF) on the ROTEM. All measurements were allowed to run at least until a stable maximum amplitude and maximum clot firmness were reached.

Seven anesthesiologists voluntarily participated as operators in the study. One of the 7 operators had established experience in performing TEG assays and clinical and research experience performing ROTEM assays and was defined as an expert user (operator A). The other 6 users had performed TEG and ROTEM analyses on a minimum of 10 occasions in clinical practice (operators B, C, D, E, F, and G). All had undergone training with the same manufacturers' representatives within 1 month before the start of the study, irrespective of previous levels of experience.

All operators (A-G) were given 1 citrated tube of blood each and performed 4 assays—kaolin and functional fibrinogen (FF) on TEG and INTEM S and FIBTEM S (single-use reagents)[17] on ROTEM. Fig 2 shows the sequence for each patient sample (n = 21). Results from these measurements were used to quantify the variability as assessed by the coefficient of variation (CV)—the SD/ mean. As overall variability includes both inter- and intraoperator variability, a second step evaluated intraoperator variability (method imprecision). For this, operator A was given 6 citrated tubes of blood and performed a total of 6 sets of the measurements previously described (Kaolin and FF on TEG and INTEM S and FIBTEM S on ROTEM) on each sample, allowing quantification of the imprecision of the assays by calculating the intraoperator coefficient of variation for the expert user (CV_A).

The measurements were stored automatically in the database of each device and the following parameters were extracted for further statistical analysis: Reaction time (r, min), k time (min), alpha angle (αTEG, degrees), maximum amplitude (MA, mm), and functional fibrinogen level (as MA) from the TEG. The equivalent parameters were extracted from the ROTEM for analysis: Clotting time (CT, seconds), clot formation time (CFT, seconds), alpha angle (αROTEM, degrees), and maximum clot firmness (MCF, mm) for both INTEM S and FIBTEM S.

Statistical analyses were performed using Statistica software v.9 (Statsoft Inc., Tulsa, OK). CV was calculated for each assay measurement in every series. Because each patient's sample was analyzed 12 times in total (6 times by operator A and 6 times by operators B-G), a total of 12 results were generated for each parameter of each assay performed on each patient. The CV was calculated for each parameter of each assay on each patient using the 6 results from operator A for intraoperator variability and 7 results from operators A-G (using the first of the 6 results from operator A) for interoperator variability. Because there were 21 patients in total, there were 21 CVs generated for each parameter of each assay for both intra- and interoperator variability. These 21 CVs for the corresponding parameters for each assay obtained from TEG and ROTEM analyses were compared using a paired *t* test. Sample size analysis was performed with regard to the parameter reflecting maximum clot strength in the intrinsically activated thromboelastographic assays (MA in TEG and MCF in ROTEM), because the variability was anticipated to be lowest for this parameter. Expected difference of means for interoperator variability was taken as 2% and expected standard deviation was taken as 3%. Using a desired power of 0.8 and a p value of <0.05, a sample size of at least 20 was required to detect significant differences in the CV for interoperator variability between TEG and ROTEM analyses. Therefore, 21 patients were included in the study.

RESULTS

Twenty-one patients' samples were analyzed; 7 samples were from patients pre-bypass, 7 were from patients post-bypass and post-protamine, and 7 were from patients on their first postoperative day. All analyses were run in accordance with protocol; only where any obvious technical procedural error occurred was the analysis stopped and immediately repeated. Of the 1008 analyses performed, this occurred on fewer than 20 occasions.

The results of all analyses comparing CVs for equivalent parameters for each system performed by operators A-G (assessing interoperator variation) are presented in Table 1. The results of all analyses performed by operator A (showing intraoperator variation and precision) are presented in Table 2.

For operators A-G all mean INTEM S CVs were significantly lower than the kaolin equivalent ($p < 0.01$). All mean FIBTEM S CVs were significantly lower than the FF equivalent ($p < 0.03$). For operator A, all mean INTEM S CVs were significantly lower than the kaolin equivalent ($p < 0.01$).