SAJS
Review

ROTEM analysis: A significant advance in the field of rotational thrombelastography

R. C. FRANZ, M.B. CH.B., M.MED. (CHIR.), M.D.

Emeritus Professor of Surgery, University of Pretoria, and Director, Fibrinolysis Laboratory, Department of Surgery, University of Pretoria

Summary

At the turn of the century, a significant advance in the rapidly expanding field of rotational thrombelastography (ROTEG), known at present as thrombelastometry or ROTEM analysis, was developed at the Ludwig-Maximillian University in Munich. The measuring unit is operated by a laptop computer. There are four temperature-controlled measuring channels in which the global assays not only detect coagulation factor defects but also platelet function, abnormal fibrinogen polymerisation, anticoagulant defects and hyperfibrinolysis.

ROTEM has steadily increased in popularity, particularly because of its well-established application as a diagnostic aid at the point of care in the critical care situation. It also provides a useful guide in the choice of an appropriate therapeutic option in the bleeding patient, reducing costs by avoiding administration of costly component therapy such as fresh-frozen plasma, cryoprecipitate, platelet concentrates or antifibrinolytic agents.

As well as being useful in monitoring anticoagulant therapy and the use of pharmacological agents, ROTEM is a valuable research tool in the field of hypercoagulability, fibrinolysis, factor XIII activity and the ultimate phases of the blood coagulation process.

Research in the fibrinolysis laboratory from 1968 has shown that the original Hellige thrombelastograph, which was developed by Hartert of Heidelberg during World War II,[1] was an easily operated mechanical optical system for identifying the key areas of haemostatic failure.

However, at the turn of the century a significant advance in the rapidly expanding field of rotational thrombelastography (ROTEG) (known at present as rotational thrombelastometry or ROTEM®Analysis (Pentapharm GmbH)) was developed at the Ludwig-Maximillian University in Munich.[2,3] In contrast to clotting tests, which only detect the clotting time, ROTEM detects the changes in all the phases of coagulation and fibrinolysis.[2-5]

Like the exemplary Hellige instrument, ROTEM also measures the firmness or the modulus of elasticity of the coagulum. It appears that a fibrin clot is analogous to the concept of a Maxwell body, which implies that it has elastic as well as viscous properties.[6]

ROTEM measures the formation, stabilisation and eventual lysis of the clot, including platelet function and fibrin polymerisation (including factor XIII activity), important parameters that are not detected in standard clotting assays. However, a limitation of Hellige thrombelastography as well as ROTEM is the inability to identify platelet adhesion and Von Willebrand factor or aspirin-like defects during primary haemostasis. Whereas in classic thrombelastography only a single test is performed to provide a global test for haemostatic integrity, ROTEM analysis is an attempt to gain selected information on the main coagulation pathways and the effect of anticoagulants, as well as hyperfibrinolysis which experience has shown is clearly under-diagnosed in the critical care situation.[5]

The ROTEM approach[2-5]

In contrast to the Hellige thrombelastograph where the cuvette is rotated, in the case of ROTEM the pin is rotated with a stationary cuvette. As long as the blood sample remains fluid this movement remains unimpeded. However, as soon as the blood clots, the rotation is restricted by the developing fibrin fibres. The rotation of the pin (which is therefore inversely proportional to the fibrin build-up and clot firmness) is calculated by a laptop computer and presented as a direct visual readout in the form of a ROTEM curve as well as its numerical parameters.

Based on rotation thrombelastography, ROTEM has overcome several limitations of the Hellige instrument. The ROTEM system comprises a detection system, electronic pipette, software and disposable plastic cuvettes. The measuring unit is operated by a laptop computer. There are four temperature-controlled measuring channels in which whole blood or plasma samples may be evaluated. The global assays not only detect coagulation factor defects but also defective platelet function, abnormal fibrinogen polymerisation, anticoagulant defects and hyperfibrinolysis.[2-5]

Another advantage of ROTEM over the Hellige instrument is its rugged construction. Whereas the classic thrombelastograph is sensitive to mechanical disruption, the Israelis have shown that the ROTEM may be transported over rough terrain right up to the battle front without interference with the overall accuracy of the instrument (personal communication).

The following measurements are evaluated on the TEM tracing (the Hellige nomenclature is shown in parentheses).

Clotting time (CT). Time taken from the start of the measurement until initiation of clotting (Hellige: r-time).

Clot formation time (CFT). Time taken from the

initiation of clotting until a firmness of 20 mm is detected (Hellige: k-time).

Maximum clot firmness (MCF). Stabilisation of the clot by polymerised fibrin, thrombocytes as well as FXIII (Hellige: Ma or maximum amplitude).

Maximum lysis (ML). Reduction of clot firmness after MCF. Lysis at a fixed time in minutes (e.g. CL30, CL60).

The objectives of ROTEM analysis[2,3]

The various assays not only exclude a surgically correctable cause but can also differentiate between abnormal platelet function, defective fibrin polymerisation, coagulation factor defects and aggressive fibrinolysis.

The various tests are briefly outlined in Table I.

Over the years experience has shown that certain components of ROTEM may be used as guidelines for blood component therapy or pharmacological manipulation in the critical care situation. The CFT is used as a dosage guide for fresh-frozen plasma, the MCF to monitor platelet infusion or fibrinogen requirements, heparinase (Heptem) to calculate protamine dosage, and the addition of aprotinin to assess the need for antifibrinolytic therapy. This differential diagnostic and therapeutic algorithm has now been developed and elegantly refined by Vorweg and his colleagues at the University Clinic in Cologne.[3]

From classic thrombelastography to ROTEM

The Pretoria experience with the use of the classic Hellige thrombelastograph in the first 3 000 patients with suspected haemostatic defects was published in 1981.[7] The text, which included notes on the evolution of thrombelastography, the basic principles and description of the apparatus, the interpretation of the thrombelastogram using native whole blood, citrated whole blood and citrated plasma, the principles of normal haemostasis, and the thrombelastographic pattern in various haemorrhagic states, hypercoagulability, disseminated intravascular coagulation and fibrinolysis, was briefly summarised as follows:

'The thrombelastograph as designed by Hartert has now been in clinical use for over thirty years. Within thirty minutes it provides a global account of clot formation and fibrinolysis or the time and degree of the interaction between the activators and inhibitors of both systems.

'On the basis of our own experience it seems justifiable to assert that although its sensitivity to technical variables requires stringent laboratory control, the thrombelastogram offers a simple and reliable method of identifying the key areas of haemostatic incompetence or assessing the efficacy of a therapeutic programme.

'Thrombelastographic hypercoagulability, which has deservedly been given prominence in the recent literature, may add an exciting dimension to the diagnostic spectrum of an elegantly conceived instrument.'[7]

Despite an inexplicable decline in interest during the 1970s, there has clearly been an upsurge in the use of the Hellige instrument (especially in the USA), with a significant impetus at the turn of the century when the use of the Hellige as well as the ROTEM systems provided a major advance in cases of haemostatic failure, notably during liver transplantation, cardiac surgery, major abdominal surgery and seriously traumatised patients.[8-10] Within minutes ROTEM analysis offers a targeted therapeutic approach based on specialised differential diagnostic information. In effect, the diagnostic information provided by the ROTEM method has provided well-defined advantages over the clotting tests because all the phases of clot formation and stabilisation as well as fibrinolysis, platelet function and factor XIII activity are evaluated.[2-5]

Hypercoagulability

The role of classic thrombelastography as an adjunctive test in thrombophilia screening has enjoyed considerable interest. It has been shown that although a hypercoagulable thrombelastogram was demonstrated in 45% of patients with a previous thrombosis, the thrombelastograph and standard thrombophilia testing did not identify the same set of individuals.[11] However, Traverso *et al.* have found that the maximal amplitude of the thrombelastogram was predictive of deep-vein thrombosis development, with a sensitivity of 72% and a specificity of 69%.[12]

As other studies have also shown the maximum amplitude to be the most sensitive thrombelastographic parameter in patients with a thrombophilic trait, this raises the question whether factors concerning the initial phases of venous thrombogenesis at the endothelial interface should be included in the thrombophilia screening profile.

As a diagnostic tool the thrombelastogram also showed significant differences between the sexes, with a highly

TABLE I. THE METHODS AND OBJECTIVES OF ROTEM ANALYSIS

Test	Method	Objective
Extem	**Activation of clot formation with tissue factor**	**Assessment of factors VII, X, V, II, I, platelets and fibrinolysis**
Intem	**Activation of clot formation via the contact phase**	**Assessment of factors XII, XI, IX, VII, X, V, II, I, platelets and fibrinolysis**
Fibtem	**Activation as in Extem with addition of a platelet-blocking substance**	**Fibrinogen function**
Aptem	**Activation as in Extem with addition of an antifibrinolytic (aprotinin)**	**Recognition of hyper-fibrinolysis**
Heptem	**Activation as with Intem with the addition of heparinase**	**Detection of heparin and heparin-related substances**

significant trend of increasing whole-blood coagulability from men through non-pregnant to pregnant women (p<0.0001).[13]

Experimental work

Our own experience has shown that both the classic Hellige thrombelastogram and the ROTEM system are valuable research tools, as shown in the following examples.

The effect of platelets on tissue plasminogen activator (tPA) activity[14]

In view of the documented differential sensitivity of platelet-rich (arterial) and erythrocyte-rich (venous) thrombi to thrombolytic therapy, a study was designed to assess the effect of circulating platelets on tissue plasminogen activator (tPA)-induced fibrinolysis.

Ten microlitres of tPA (0.5 mg Actilyse per ml) were added to a Hellige thrombelastograph cuvette containing platelet-free and platelet-rich plasma samples (prepared by differential centrifugation). The fibrinolytic activity was derived from the thrombelastographic lysis time (TLT) and the fibrin build-up from the maximal amplitude (Ma).

The quantification of fibrinolytic activity and the effect of platelets on tPA-induced fibrinolysis are shown in Fig. 1.

These results showed that the Hellige thrombelastogram provides a simple and reproducible method of determining the lytic activity in a fibrin clot. Platelet-rich fibrin appeared to be significantly more resistant to exogenous tPA-induced fibrinolysis than platelet-poor fibrin, a differential sensitivity that may be attributed to the high concentration of plasmin inhibitors in platelets, notably factor XIII, α_2-plasmin inhibitor and plasminogen activator inhibitor (PAI) (Fig. 2). These results may have fundamental therapeutic implications in cases of fibrinolytic haemorrhage as well as in the field of thrombolytic therapy where individual cases of resistance to thrombolysis could pose a major problem.[14]

Use of the rotational thrombelastograph (ROTEG) in the quantification of the 'lytic state' during thrombolytic therapy: A comparative analysis in the subhuman primate[15]

The primary aim of this study was to assess the diagnostic efficacy of ROTEG in the quantification of plasma fibrinolytic activity (PFA) or the intensity of the 'lytic state' during thrombolytic therapy in the Chacma baboon.

tPA was administered via the lesser saphenous vein at the following dosage regimens: 0.125, 0.25, 0.5 and 1 mg/kg body weight, given as a constant infusion over 2 hours. Thirty minutes after commencement of the infusion (after achieving a 'steady state'), free-flowing superior vena caval blood samples were taken via a transbasilic venous catheter for the following determinations: PFA as determined by ROTEG, tPA antigen, plasminogen and fibrinogen plasma levels, prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT) and lytic zones on fibrin plates.

Analysis of variance showed a highly significant fibrinolytic response (ROTEG) to incremental rtPA dosage (p<0.0001) (Fig. 3). A similar pattern was found between rtPA dosage and the tPA-antigen blood levels (p=0.0015) (Fig. 4). In contrast to these findings, the effect of the various dosage regimens on the other haemostatic parameters (PT, PTT,

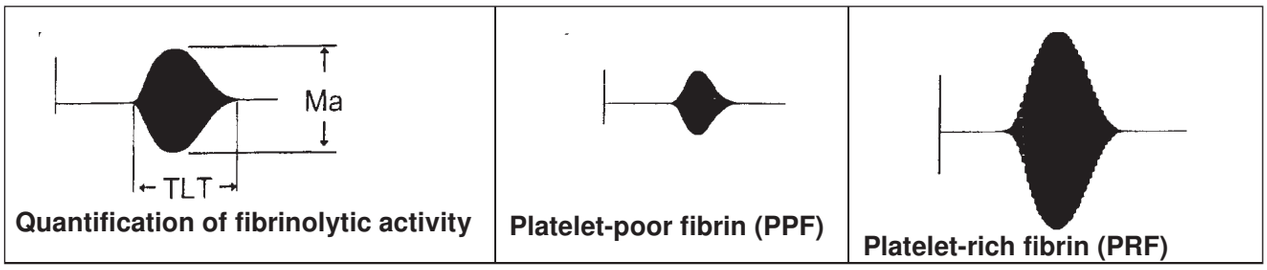

Fig. 1. Hellige thrombelastographic patterns during tPA-induced fibrinolysis. Note the increased rate and degree of fibrin build-up (Ma) and longer lysis time (decreased fibrinolytic activity) in the platelet-rich sample.

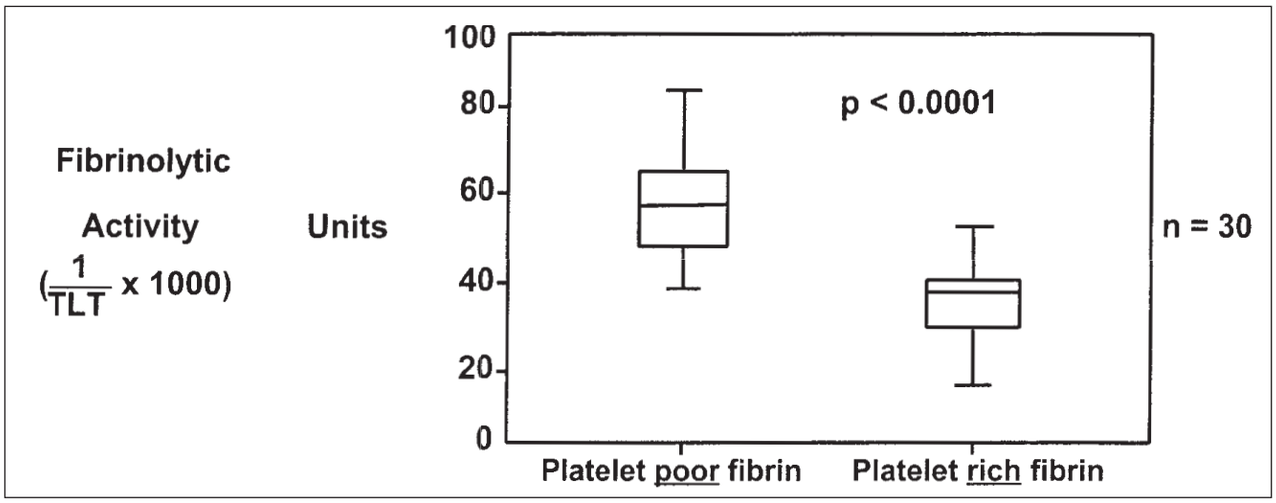

Fig. 2. Box-and-whisker plots showing the effect of platelets on tPA-induced fibrinolysis.

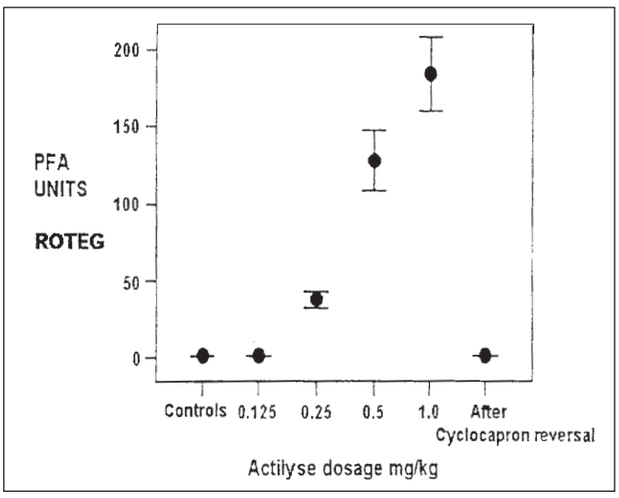

Fig. 3. Effect of tPA dosage on plasma fibrinolytic activity (ROTEG method).

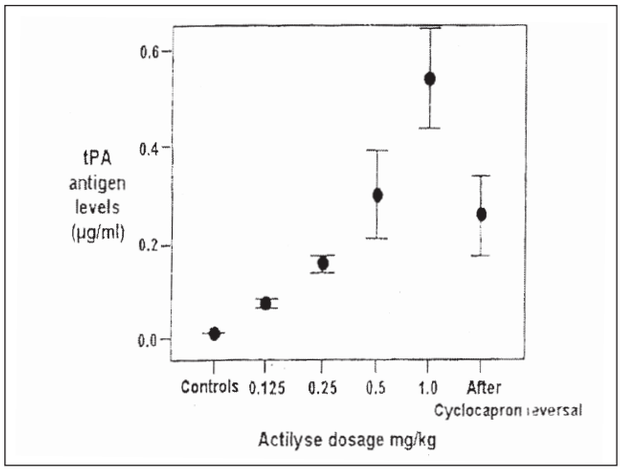

Fig. 4. Effect of tPA dosage on tPA antigen levels.

TT and lytic zones on fibrin plates) showed unpredictable results.

ROTEM has also been found to be a valuable research tool to assess the intensity of the lytic state.[16,17] It was also able to identify a Pretoria family with fibrinogen dysfunction.[18]

Streptokinase versus tPA-induced fibrinolysis – a comparative analysis[19]

A study was designed to assess the differences in individual fibrinolytic response to two commonly used thrombolytic agents with a known differential sensitivity to antistreptococcal antibodies.

Ten international units of streptokinase (Kabikanase) and 10 µl of tPA (0.5 mg Actilyse per ml) were added to different platelet-free citrated plasma samples in a rotational thrombelastograph cuvette. The fibrinolytic activity (FA) was derived from the thrombelastographic lysis time (TLT) in minutes:

$$(FA = \frac{1}{TLT} \times 1\,000).$$

The results are briefly summarised in Fig. 5.

These results showed that the variances of the lytic activity induced by tPA versus streptokinase (SK) (excluding no lysis

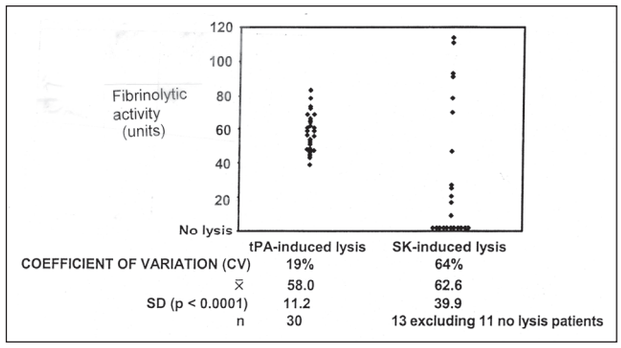

Fig. 5. Effect of streptokinase and tPA on thrombelastographic fibrinolysis.

patients) differ significantly (p<0.0001, Bartlett's test of equal variances). In view of the wide scatter (elevated coefficient of variation) in the lytic activity induced by SK therapy, tissue plasminogen activator therapy appears to offer a significantly more predictable response.

Factor XIII assays

Although it has been well documented that platelet-rich (arterial) thrombi are significantly more resistant to thrombolysis than erythrocyte-rich (venous) thrombi, the exact mechanism has not been clearly defined. The main physiological function of factor XIII (FXIII) is to cross-link fibrin and to protect it from the fibrinolytic enzyme, plasmin. The latter effect is achieved mainly by covalently linking α_2-antiplasmin, the most potent physiological inhibitor of plasmin, to fibrin.

The enzyme FXIIIa is the final component of the blood clotting cascade. In 2001 Schroeder *et al.* demonstrated the sensitivity and specificity of ROTEG to demonstrate the influence of FXIII on the final stages of fibrinogenesis.[20] Work in our own laboratory has shown that FXIII-enriched fibrin exhibits a dose-dependent resistance to plasminogen-activator-induced fibrinolysis.[21]

The effect of FXIII and platelets on SK-induced fibrinolysis: A rotational thrombelastographic study[21]

In view of the large quantity of FXIII in platelets, a study was designed to assess the global effect of FXIII and platelets on SK-induced fibrinolysis. Recombinant FXIII (Fibrogammin; Behringwerke A.G., Marburg) was added to SK-enriched (10 IU/ml), platelet-rich and platelet-free plasma samples in a standard ROTEG cuvette to a final concentration of 0, 4, 8 and 12 IU FXIII per ml. The lytic activity in units was derived from the TEG lysis time (TLT) (Fig. 6).

From these findings it would seem that ROTEG provides a simple and reproducible method to determine the lytic activity in a fibrin clot. FXIII-enriched fibrin exhibits a dose-dependent resistance to exogenous plasminogen activator-induced fibrinolysis (Fig. 7). Furthermore, platelets (which are a rich source of FXIII antiplasmin) appear to supplement this inhibitory function. These results may have fundamental therapeutic implications in cases of fibrinolytic haemorrhage, as well as in the field of thrombolytic therapy where individual cases of resistance to thrombolysis could pose a major problem.[21]

These experiments have shown ROTEG to be a simple

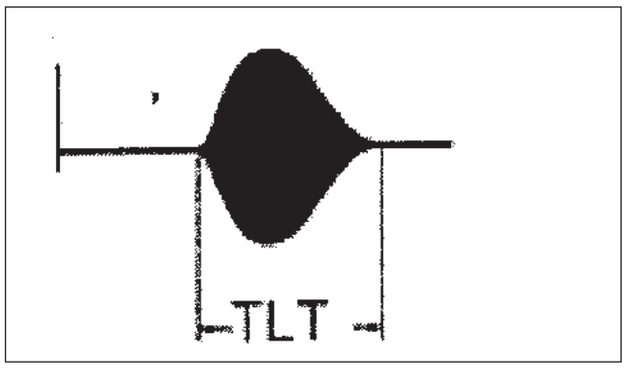

Fig. 6. TEG lysis.

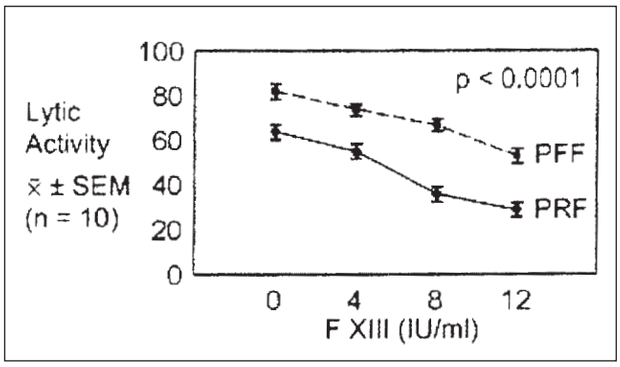

Fig. 7. Effect of FXIII on SK-induced lysis in platelet-rich (PRF) and platelet-free fibrin (PFF).

though effective method for the investigation of FXIII function in plasma (information that is not readily available in the standard laboratory tests).

Conclusion

ROTEM has steadily increased in popularity, especially because of its well-established application as a diagnostic aid at the point of care in the critical care situation. This remarkable innovation also provides a useful guide in the choice of an appropriate therapeutic option in the bleeding patient, thereby reducing costs by avoiding the unmethodical administration of costly component therapy such as fresh-frozen plasma, cryoprecipitate, platelet concentrates or antifibrinolytic agents.[22]

ROTEM has also been shown to be a useful research tool in the field of hypercoagulability, fibrinolysis, FXIII activity, the ultimate phases of the blood coagulation process, as well as monitoring of anticoagulant therapy and the use of pharmacological agents.

REFERENCES

1. Hartert H. Blutgerinnungstudien mit der Thrombelastographie, einem neuen Untersuchungsverfahren. *Klin Wochenschrift* 1948; 26: 577-583.
2. Calatzis AN, Fritzsche P, Kling M, *et al*. A new technique for fast and specific coagulation monitoring. *Surg Res* 1996; 28(1): 89.
3. Vorweg M, Hartman B, Knüttgen D, Jahn MC, Doehn M. Management of fulminant fibrinolysis during abdominal aortic surgery. *J Cardiothorac Vasc Anesth* 2001; 15: 764-767.
4. Calatzis A, Schramm W, Spannagl M. Management of bleeding in surgery and intensive care. In: Scharrer I, Schramm W, eds. *31st Hemophilia Symposium Hamburg 2000*. Berlin: Springer Verlag, 2002.
5. Lang T, Bauters E, Braun SL, *et al*. Normal range. Multi-centre investigation on reference ranges for ROTEM® thromboelastometry. *Blood Coagul Fibrinolysis* 2005; 16: 301-310.
6. Scott-Blair GW, Burnett J. On the rates of coagulation and subsequent softening of bovine and human blood and of thrombinfibrinogen. *Biorheology* 1968; 5: 163.
7. Franz RC, Coetzee WJC. The thrombelastographic diagnosis of haemostatic defects. *Surgery Annual* 1981; 13: 75-106.
8. Kang Y, Marquez D, Lewis J, *et al.* Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. *Anesth Analg* 1985; 64: 888-896.
9. Mittermayr M, Margeiter J, Velik-Salchner C, *et al.* Heparin/protamin monitoring. Effects of protamine and heparin can be detected and easily differentiated by modified thrombelastography (ROTEM®). An *in vitro* study. *Br J Anaesth* 2005; 95: 310-316.
10. Cammerer U, Dietrich W, Rampf T, Braun SL, Richter JA. Predictivity in cardiac surgery. The predictive value of modified computerized thromboelastography and platelet function analysis for postoperative blood loss in routine cardiac surgery. *Anesth Analg* 2003; 96: 51-57.
11. O'Donnell J, Riddel A, Owen D, *et al*. Role of the thrombelastograph as an adjunctive test in thrombophilia screening. *Blood Coagul Fibrinolysis* 2004; 15: 207-211.
12. Traverso C, Arcelus J, Gomez E, Luna D, Lopez-Cantareno M, Garcia J. Prospective assessment of the risk of deep vein thrombosis in elective abdominal surgery. Predictive role of thrombelastography. *Thrombotic and Haemorrhagic Disorders* 1993; 71: 9-15.
13. Gorton HJ, Warren ER, Simpson NAB, Lyons GR, Columb MO. Thromboelastography identifies sex-related differences in coagulation. *Anesth Analg* 2000; 91: 1279-1281.
14. Franz RC. The effect of platelets on tissue plasminogen activator (tPA) activity. Paper presented at the Congress of the Surgical Research Society of Southern Africa, Cape Town, 28 July 2000.
15. Franz RC, Joubert JEH, Briedenhann S, Meintjes CJ. The use of the rotational thrombelastograph (ROTEG) in the quantification of the 'lytic state' during thrombolytic therapy. A comparative analysis in the subhuman primate. *S Afr J Surg* 2004; 42(2): 56 (Abstract).
16. Du Plessis AA, Van Zyl E, Joubert E, Briedenhann S, Van der Merwe CA, Franz RC. The thrombin clot lysis time (TCLT). A single test to assess the intensity of the lytic state during thrombolytic therapy. *S Afr J Surg* 1996; 34(1): 50 (Abstract).
17. Snyders E, Van Zyl E, Joubert E, Briedenhann S, Van der Merwe CA, Franz RC. The effect of regional thrombolytic therapy on the local and systemic lytic states. A comparative analysis in Chacma baboons. *S Afr J Surg* 1996; 34(1): 50 (Abstract).
18. Franz RC, Matsuda M. Fibrinogen Pretoria. A clinical, biochemical and electronmicroscopical study. *S Afr J Surg* 1999; 37(4): 116 (Abstract).
19. Van Niekerk H. Streptokinase versus tPA-induced fibrinolysis – a comparative analysis. MMed Dissertation, University of Pretoria, 2003.
20. Schroeder V, Chatterjee T, Kohler HP. Influence of blood coagulation factor XIII and FXIII Val34Leu on plasma clot formation measured by thrombelastogrpay. *Thromb Res* 2001; 104: 467-474.
21. Franz RC, Meintjes CJ, Joubert JEH, Briedenhann S, Becker P. The effect of factor XIII and platelets on SK-induced fibrinolysis: A thrombelastographic study. Paper presented at the XVIII Congress of the International Society of Thrombosis and Haemostasis, Paris, 6 -12 July 2001. Abstract: CD 3203 (ISSN 0340-6245).
22. Anderson L, Quasim I, Soutar R, Steven M, Macfie A, Korte W. Cost savings. An audit of red cell and blood product use after the institution of thromboelastometry in a cardiac intensive care unit. *Transfus Med* 2006; 16: 31-39.