PERIODICUM BIOLOGORUM
VOL. 117, No 2, 291–296, 2015

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Does rotation thrombelastometry (ROTEM) improve early prediction of coagulopathy in breast tumor?

DINKO BAGATIN[1]
KATARINA ŠAKIĆ[1,2]
TOMICA BAGATIN[1]
DEANA ŠTURM[3]
MILAN MILOŠEVIĆ[4]

[1] Polyclinic Bagatin, Polyclynic for Maxillofacial, General and Plastic Surgery, Zagreb, Croatia

[1,2] Faculty of Medicine University of Osijek, Chair of Anesthesiology, Reanimatology and Intensive medicine, Osijek, Croatia

[3] Department of Transfusion Medicine and Coagulation in Oncology Patients, Universety Hospital for Tumors, Department of Transfusion Medicine, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia

[4] School of Medicine University of Zagreb, Zagreb, Croatia

Correspondence:
Dinko Bagatin, MD,
Polyclinic Bagatin.
Kneza Višeslava 14, 10000 Zagreb, Croatia,
Email: dinko.bagatin@poliklinikabagatin.hr

Key words: breast disease, breast cancer, benign breast disease, coagulation factors, thrombelastometry

Abbreviations:
TNM – tumor-node-metastasis classification
NST – no special type
NOS – not other specified
ROTEM – rotation thrombelastometry
TEG – Thromboelastography
CT=R – clotting time
CFT = k – clot formation time
MCF = MA – maximum clot formation
AUC – area under the curve
A – Amplitude of cloth formation after 5-30 minutes
PT – prothrombin time
APTT – activated partial thromboplastin time

Received May 12, 2015.

Abstract

***Background and Purpose**: Breast Cancer is the second most common cancer among women after skin carcinoma. Incindence in Croatia in 2012 was 2227 new cases per year with mortality 1033 women per year. One of the most pronaunced caracteristics of cancers in general are changes in coagulation factors. Except usual coagulation factors there is thrombelasometry which is dynamic method for evaluation of coagulation factors. We have been used thrombelastometry to see differences in coagulation factors for carcinomas and benign breast diseases.*

***Materials and Methods**: We included 132 patients with benign and malignant breast diseases in Institute of Thumors, Clinical Hospital Center "Sisters of Mersy", Zagreb, Croatia gathered in prospective study in 2012/2013. We compared differences in coagulation parameters with thrombelastometry and usual coagulation factors in earlier mentioned two groups of patients with Mann-Whitney U test what is graficly described with Box and Whiskers plots and correlatio coefficients are described in table with Spearman correlation coefficients.*

***Results:** A5,A10,A15,A20,A25 and A30, MCF and AUC intem are significatly higher in malignant breast disease patients. Significant trend of elevation of these values is present in both patients groups, but those are sigificantly higher in patient group with malignant tumors. While in patients group with malignant tumors almost every correlation coefficients between A5-A30, MCF and AUC intem and cogulation markers are significant, those correlations among patients with benign diseases are not significant. Those values suggests that A5-A30, MCF and AUC intem are significatly correlated with most common used coagulation markers only in patients with malignant diseases.*

***Conclusions:** There are differences in coagulation factors in patients with benign and malignant breast diseases. Trend of elevation of markers of coagulation values is present in both disease, but significantly higher values are in malignant tumor. Our results are based on small numbers and larger number of patients with precise data of coagulation parameters are still needed.*

INTRODUCTION

Breast cancer is the second most common cancer afer skin cancers and it is first cause of death from malignant tumors in women. The most common type of breast is ductal carcinoma (NST) which begins in the linining of the milk ducts. Another type of breast cancer is lobular carcinoma(NOS) which begins in the lobules of the breast. Invasive

breast cancer is breast cancer that has spread from where it began in the breast ducts or lobules to surrounding normal tissue. The incidence in Croatia is around 2300 new cases of breast carcinoma in women with around 800 deaths from these disease in 2013. in University Hospital for Tumors 600 patients had breast biopsy for suspitious breast tumors and afterward therapy after protocol for breast cancer. Surgical treatment have been based on biopsy for suspicious breast tumors and emergency patohistological analysis. Patients with carcinomas are at risk of coagulopathy which can be the first sign of malignant disease.

The hemostatic system with its procoagulant effects mediated directly by cancer cells are considered to play principal role in the development of cancer-induced hypercoagulability and major thromboembolic complications *(1).*

Recently, rotation thrombelastometry (ROTEM) is a method to evaluate the whole process of blood coagulation as a graph from the beginning of clot formation to fibrinolysis providing information related to the cumulative effects of various parameters of all stages of the coagulation and fibrinolytic processes *(2).*

The benefits of ROTEM_ technology include rapid availability of test results and enhanced reproducibility the data which are also continuous, digital, and retrievable for further calculations *(3, 4).*

The goal of the study is to prove connection between changes in coagulation factors and pathystological analysis. In the study standard methods for coagulation factors analysis and thrombelastometry (ROTEM) have been used. Thrombelastometry gives quantitive and graphic measurment from inicial thromb formation to its retraction and lysis. Coagulation factors in correlation with patohistological analysis will contribute to better understanding perioperative treatment of benign changes and malignat breast tumors and more rational thromboprofilaxis and treatment with anticoagulant and antiagregation therapy.

MATERIALS AND METHODS

Using information after prospective study2012/2013. in University Hospital for Tumors, University Hospital Center "Sisters of Mersy", Zagreb, Croatia. Our study have been included 132 patients:59 of those had a breast cancer and all the rest(73) benign breast disease. All patients had mean age 59,15 +- 11,6. This study have been done with aproval of Etical Commity of School of Medicine, University of Zagreb and University Hospital Center "Sisters of Mersy" in Zagreb.All hospitals, pathology laboatories and coagulation parameters laboratories where from University Hospital Center "Sisters of Mersy". Patohistological samples where surgicaly removed by traind specialists for oncologic breast surgery. Patohistological analysis were analysed by trained patologists who are specialised for breast surgery tumors. Mean tumor size were 20,0 +- 11,2 mm. Breast cancer staging was based on pathologic tumor-node-metastasis (TNM) classification. We also analysed therapy after surgery (Chemotherapy, radiotherapy or unknown).

From coagulation factors we analysed FVIII, PC, PT, INR PT. We also used rotation thromelastometry (ROTEM) in all patients and analysed A5, A10, A15, A20, A25, A30 intem, MCF intem and AUC intem. We analysed with differences in this coagulation factors among patients benign and malignant breast diseases.

Patients with preexisting hematological or coagulation disorders, those taking anticoagulants and those with liver or renal dysfunction were excluded from the study.

Sample collection for subsequent coagulation analysis blood samples were drawn into 4.5 ml vacutainers (Becton Dickinson) containing 3.2% trisodium citrate with a citrate/ blood ratio of 1:9. and for platelet count into Becton Dickinson EDTA tubes.

Coagulation analyses

The laboratory tests of coagulation were performed on full automated STA compact device of Diagnostica STAGO for all patients: platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT), fibrinogen, and D-Dimer. The normal ranges for these tests are: APTT (26–36 s), PT (9.4–15.4 s), Fibrinogen (200– 400 mg/dl), and D-Dimer (0.00–0.50 lg/ml).

ROTEM_ thrombelastographic analysis

Thrombelastography analysis was performed with the ROTEM_ Coagulation Analyzer (Pentapharm, Munich, Germany). Four channels were available for simultaneous measurements. Each test required 300 ll citrated whole blood. The blood was re-calcified with 20 nl 0.2 mol/l CaCl2 (star-TEM_; Pentapharm, Munich, Germany) and activation of coagulation was performed with different agents:

INTEM: Contact pathway activation of the coagulation with 20 nl of contact activator (partial thromboplastin–phospholipid from rabbit brain extract and ellagic acid, in-TEM_; Pentapharm, Munich, Germany).

EXTEM: Tissue factor pathway activation of the coagulation with 20 nl of tissue factor (TF, tissue thromboplastin from rabbit brain extract, ex-TEM_; Pentapharm, Munich,Germany).

APTEM: TF plus 20 nl of aprotinin, plasmin-antagonist (ap-TEM_; Pentapharm, Munich, Germany).

FIBTEM: TF plus inhibition of thrombocytes with 20 nl of cytochalasin (fib-TEM_; Pentapharm, Munich, Germany).

The test starts automatically after injection of the blood sample with an automated pipette and calculated graphical results are obtained by the integrated computer of the device. All ROTEM samples were analyzed within 30–90 min of blood collection.

The following ROTEM_ parameters were determined: clotting time (CT = R) represents a measure of the initiation of clot formation, clot formation time (CFT = k) represents the speed of clot formation, and maximum clot formation (MCF = MA) represents maximum clot strength.

TABLE 1

Descriptive statistics of investigated group.

		N	%
NST	NOS	24	30,5%
	NST	17	40,7%
	Without data	18	28,8%
Tumor grade	1	3	5,1%
	2	35	59,3%
	3	19	32,2%
	Without data	2	3,4%
LumTP	A	4	6,8%
	B	39	66,1%
	Without data	16	27,1%
T	1	5	8,5%
	1b	4	6,8%
	1c	4	6,8%
	2	13	22,0%
	3	2	3,4%
	Without data	31	52,5%
N	0	18	30,5%
	1	5	8,5%
	2	4	6,8%
	3	1	1,7%
	Without data	31	52,5%
M	0	15	25,4%
	1	13	22,0%
	Without data	31	52,5%
Therapy	Without data	15	25,4%
	Chemotherapy	11	18,6%
	Radiotherapy	33	55,9%
Age (years): mean ± SD	59,15 ± 11,6		
Tumor size (mm): mean ± SD	20,0 ± 11,2		

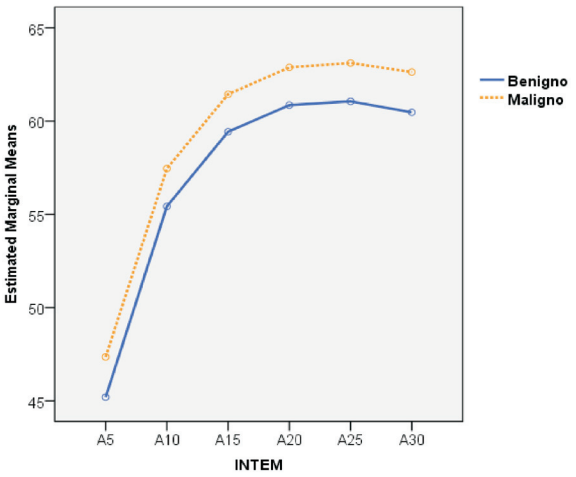

Figure 1. *Differences in A5 – A30 intem values in benign and malign breast disease.*

Statistics

Data were shown in tables in figure. Descriptive stastics were made to describe investigated patients sample Kolmogorov-Smirnov test was used to analyse data normality and due to its results appropriate non-parametric tests were used in following analysis. Differences between patients with malignant and benign breast disease were analysed with Mann-Whitney U test. Correlations between A5-A30, MCF and AUC intem and coagulation markers regarding malignant and benign breast disease were done with Spearman correlations coefficients. All P values below 0,05 were consindered significant. IBM SPSS Stastistics version 19.0.01. had been used as stastistical software (www.spss.com).

RESULTS

Majority of patients: 35 (59,3%) had tumor grade 2, 39 (66,1%) had LumTP grade B. Among those patients that have valid data from TNM clasification (N=28) 13 (46,4%) had T grade 2, 18 (64,3%) N grade 0 and 13 (46,4%) M grade 1 (metastasis in lymphatic nodules). Average tumor size was 20,0 +- 11,2 mm. There was no significan age difference between benign and malignant breast disease group (Z = -0,5; p=0,540; Mann-Whitney U test)

A5, A10,A15,A20, A25, A30, MCF and AUC intem values are significantly higher in malignant breast disease. Table 2. shows descriptive statistics and differences between benign and malignant breast disease regarding A5-A30, MCF, AUC intem and coagulation markers. Significant trend of elevation these values is present in both

TABLE 2

Differences in significant correlations between A5-A30, MCF and AUC intem and coagulation markers regarding malignant and benign breast disease: Spearman correlation.

		Malignant disease				Benign disease			
		N=59				N=70			
		FVIII	PC	PVs	INRPV	FVIII	PC	PVs	INRPV
A5 intem	Rho	0,251	0,149	–0,290	–0,261	–0,005	0,081	–0,040	–0,057
	P	0,055	0,259	0,026	0,046	0,967	0,505	0,741	0,637
A10 intem	Rho	0,256	0,235	–0,334	–0,313	0,072	0,093	–0,075	–0,093
	P	0,050	0,073	0,010	0,016	0,553	0,442	0,537	0,445
A15 intem	Rho	0,298	0,267	–0,332	–0,314	0,100	0,108	–0,090	–0,111
	P	0,022	0,041	0,010	0,016	0,412	0,373	0,458	0,361
A20 intem	Rho	0,289	0,287	–0,334	–0,304	0,146	0,139	–0,135	–0,144
	P	0,026	0,028	0,010	0,019	0,227	0,251	0,266	0,234
A25 intem	Rho	0,316	0,293	–0,330	–0,297	0,200	0,148	–0,145	–0,158
	P	0,015	0,024	0,011	0,022	0,098	0,221	0,232	0,191
A30 intem	Rho	0,314	0,280	–0,310	–0,270	0,222	0,169	–0,168	–0,185
	P	0,016	0,032	0,017	0,039	0,064	0,161	0,165	0,125
MCF intem	Rho	0,284	0,279	–0,326	–0,298	0,180	0,127	–0,128	–0,136
	P	0,029	0,033	0,012	0,022	0,135	0,294	0,289	0,260
AUC intem	Rho	0,311	0,297	–0,326	–0,289	0,219	0,135	–0,117	–0,112
	P	0,016	0,022	0,012	0,027	0,069	0,264	0,336	0,358

group (p;0,001; Friedeman test), but significantly higher values in group with malignant tumor (Figure 1).

While in patients with malignant disease almost every correlation coefficients between A5 –A30, MCF and AUC intem coagulation markers are significant,those correlations among patients with benign disease are not significant. Among patients with malignant disease positive correlations were found between A15-A30,MCF and AUC intem and FVIII and PC. Significant negative correlations were found with PV and INRPV. Different patterns of significant correlations between A5-A30, MCF and AUC intem and coagulation markers regarding malignant and benign breast disease are shown in Table 3.

DISCUSSION

Although breast cancer is one of the most common carcinoma in women there are small number of studies which analyse differences between malignant and benign breast disease with rotation thrombelastometry and correlations between thrombelastometry factors and coagulation factors which we used in our study.

After analyzing all gethered data from our prospective study we concluded that there are strong connection between A5, A10, A15, A20, A25 i A30, MCF and AUC intem with malignant disease and that it is significantly higher in group with malignant diseases than in group with benign breast diseases. Also our values suggests that A5-A30, MCF and AUC intem are significantly correlated with most common used coagulation markers only in patients with malignant disease.

The strength of our study is that we find differencies in specific coagulation parameters with thrombelastometry and standard coagulation tests in patients with malignant disease and we proved significant difference in some specific parameters what we mentioned earlier.

Many studies demonstrates thromboelastographic evidence of hypercoagulability in patients suffering from cancer with a high rate of venous thromboembolic events *(5).*Thrombotic episodes may also precede the diagnosis of cancer by months or years thus representing a potential marker for occult malignancy *(6).* Abnormal hemostasis has been reported in cancer patients, including the shortening of the activated partial thromboplastin time, elevated levels of coagulation proteins (fibrinogen, factors V, VIII, IX, and XI), thrombocytosis, elevated fibrin/fibrinogen degradation products, and an accelerated rate of fibrinogen turnover *(5).*

Hypercoagulability is difficult to detect by standard coagulation tests in cancer patients, but ROTEM *(7),* is

a sensitive method that is able to identify and measure hypercoagulability, which is not detected by routine laboratory tests *(8, 9, 7).* ROTEM, by using whole blood, measures both quantity of clotting and, most importantly quality of clotting which is not recorded by routine coagulation profile (10.4). Hypercoagulability was diagnosed readily by the presence of an accelerated clot formation, as evidenced by shortening of CFT and an increase of the clot strength, as evidenced by increasing of MCF. ROTEM_ tracings on all assays (INTEM, EXTEM, FIBTEM, APTEM) revealed a statistically significant increasing of MCF in cancer patients. While it is likely that certain cancer types are more prone to thrombosis *(11),* we could not predict which tumors are most commonly associated with thrombosis since ROTEM parameters did not differ among cancer subgroups.

In the literature, postoperative hypercoagulability, detected by TEG, has been reported in patients undergoing hepatic surgery *(12),* general abdominal procedures (13, 14), and neurosurgery *(15).* Other clinical settings associated with hypercoagulability detected by TEG include ischemic heart disease *(16),* end-stage renal failure *(17),* insertion of cutdown intravenous catheters *(18)* exposure to oral contraceptives *(19).*

Thromboelastographic analysis of hypercoagulability has been also performed in patients with malignancies in the earlier literature using first the native whole blood TEG and then the celite-activated TEG *(21, 22).* Our findings are by utilizing a newer and more powerful technique, the modified rotation thromboelastogram analyzer, ROTEM_. Hyperfibrinogenemia and thrombocytosis have been frequently reported in patients with malignant disorders *(23, 24).* We therefore sought to correlate these laboratory parameters with those of ROTEM_ and observed that MCF had a strong positive correlation with plasma fibrinogen concentration and platelet counts. MCF measures the maximum clot strength, which is dependent on platelet function and fibrinogen level. The contribution of platelet component and fibrinogen to the clot strenght has been demonstrated in adult patients without tumors *(25, 26, 27, 28).*

Since ROTEM demonstrates hemostasis as a whole dynamic process, ROTEM data gives more information on interaction between platelets and the coagulation cascade rather than the conventional coagulation screens; PT, APTT, platelet count, and fibrinogen concentrations. Identification by the ROTEM of a hypercoagulable state in patients with breast tumor may help to identify those at risk for cancer-induced thromboembolic events and the test may be more valuable if combined with scoring systems for grading deep vein leg thrombosis *(29).* Further investigations that correlate this hypercoagulability with the clinical picture are needed to determine if TEG data can be applied on therapeutic interventions in this patient population.

On the other hand, the limitation of this study are the small number of patients and we recomend to make a larger study with patients with malignant breast diseases which will confirm our data and which will help in future to diagnose breast tumors before even we found them with other diagnostic means. The thing is that coagulation disorders can be one of the first signs of malignant disease and that this can help us to discover the disease in the earlier stage and to make better prognosis in the end.

CONCLUSION

In patients with malignant breast disease almost every correlation ROTEM coefficients between A5 –A30, MCF and AUC intem coagulation markers are significant,but those correlations among patients with benign disease are not significant. Among patients with malignant disease positive correlations were found between A15-A30, MCF and AUC intem and FVIII and PC and significant negative correlations were found with PV and INR. Significant trend of elevation hypercoagulability values is present in both disease but significantly higher values in malignant breast tumor. Rotation thrombelastometry (ROTEM) may improve early prediction of coagulopathy in (benign and malign) breast tumor?

Funding/potential competing interests: No financial support and no other potential conflict of interest relevant to this article was reported.

REFERENCES

1. WINTER P C 2006 The pathogenesis of venous thromboembolism in cancer: emerging links with tumour biology. *Hematol Oncol 24:* 126–33. doi:10.1002/hon.785.**]**.
2. SPIEL A O, MAYR F B, FIRBAS C, OUEHENBERGER P, JILMA B 2005 Validation of rotation thrombelastography in a model of systemic activation of fibrinolysis and coagulation in humans. *J Thromb Haemost 4:* 411–16. doi:10.1111/j.1538-7836.2006.01715.x.**]**.
3. SCHOBERSBERGER W, MITTERMAYR M, FRIES D, INNERHOFFER P, KLINGER A *et al.* 2007 Changes in blood coagulation of arm and leg veins during a simulated long-haul flight. *Thromb Res 119:* 293–300. doi:10.1016/j.thromres. 2006. 03.003.
4. SORENSON B, JOHANSENP, CHRISTIANSEN K, WOELKE M, INGERSLEV J, *et al.* 2002 Whole blood coagulation thromboelastographic profilesemploying minimal tissue factor activation. *J Thromb Haemost 1:* 551–8. doi:10.1046/j.1538-7836.2003.00075.x.,**]**.
5. ZWICKER J I, FURIE B C, FURIE B 2007 Cancer-associated thrombosis. *Clin Rev Oncol Hematol 62(2):* 126–36. doi:10.1016/j. critrevonc.2007.01.001
6. DONATI M B 1994 Cancer and thrombosis. *Haemostasis 24:* 128–31
7. MALLETT S V, COX D J A 1992. Thromboelastography. *Br J Anaesth 69:* 307–13. doi:10.1093/bja/69.3.307
8. COHEN E, CAPRINI J P, ZUCKERMAN L, VAGHER P, ROBINSON B 1977 Evaluation of three methods used to identify accelerated coagulability. *Thromb Res 10:* 587–604. doi:10.1016/0049-3848 (77)90214-6.

9. ARCELUS J I, TRAVERSO C I, CAPRINI J A 1995 Thromboelastography for the assessment of hypercoagulability during general surgery. *Semin Thromb Hemost 2:* 21–6
10. TRAVERSO C I, CAPRINI J A, ARCELUS J I 1995.The normal thromboelastogram and its interpretation. *Semin Thromb Hemost 21(Suppl 4):* 7–13
11. LEVINE M 1997 Treatment of thrombotic disorders in cancer patients. *Haemostasis 27:* 38–43
12. HOWLAND W S, CASTRO E B, FORTNER J B, GOULD R N 1974 Hypercoagulability, thrombelastographic monitoring during extensive hepatic surgery. *Arch Surg108:* 605–8
13. BUTLER M J 1978 Thromboelastography during and after elective abdominal surgery. *Thromb Hemost 39:* 488–95
14. MAHLA E, LANG T, VICENZI M N, WERKGARTNER G, MAIER R *et al.* 2001 Thromboelastography for monitoring prolonged hypercoagulability after major abdominal surgery. *Anesth Analg 92:* 572–7. doi:10.1097/00000539-200103000-00004.
15. GOH K U C, TSIO W C 1997 Hemostatic changes during surgery for primary brain tumors. *J Neurol Neurosurg Psychiatry 63:* 334–8
16. ARTANG R, JENSEN E, PEDERSEN F, FRANDSEN N J 2000 Thrombelastography in healthy volunteers, patients with stable angina and acute chest pain. *Thromb Res 97:* 499–503. doi:10.1016/S0049-3848(99)00210-8
17. HOLLOWAY D S, VAGHER J P, CAPRINI J A, SIMON N M, MOCKROS L F 1987 Thromboelastography of blood from subjects with chronic renal failure. *Thromb Res 45:* 817–25. doi:10.1016/0049-3848 (87)90091-0
18. SPILLERT C R, MCGOVEM P J, VIDAVER R M, LAZARO E J 1981 Thrombelastographic evaluation of cut-down induced accelerated coagulation. *Thromb Hemost 46:* 567
19. FISCH I R, FREEDMAN S H 1975. Oral contraceptives, ABO blood groups, and in vitro fibrin formation. *Obstet Gynecol 46(4):* 473–9
20. PARVEZ Z, MONCADA R, FAREED J, MESSMORE H L 1983.Effect of non-ionic contrast media on the components of coagulation and complement systems. *Invest Radiol 18:* 279–84. doi:10.1097/00004424-198305000-00012.
21. CAPRINI J A, ZUCKERMAN L, COHEN A, VAGHER J P, LIPP V 1976 The identification of accelerated coagulability. *Thromb Res 9:* 167–80. doi:10.1016/0049-3848(76)90161-4. 24
22. FRANCIS J L, FRANCIS D A, GUNATHILAGAN G J 1994. Assessment of hypercoagulability in patients with cancer using the Sonoclot AnalyzerTM and Thrombelastography. *Thromb Res 74:* 335–46. doi:10.1016/0049-3848(94)90149-X
23. SUN N C, MCAFEE W M, HUM G J, WEINER J M 1979. Hemostatic abnormalities in malignancy, a prospective study in one hundred eight patients. Part I. Coagulation study. *Am J Clin Pathol 71:* 10–6. 26
24. EDWARDS R L, RICKLES F R, MORITZ T E, HENDERSON W G, ZACHARSKI L R *et al.* 1987 Abnormalities of blood coagulation tests in patients with cancer. *Am J Clin Pathol 88:* 596–602
25. KETTNER S C, PANZER O P, KOZEK S A, SEIBT F A, STOISER B *et al.* 1999 Use of abciximab-modified thrombelastography in patients undergoing cardiac surgery. *Anesth Analg 89:* 580–4. doi:10.1097/ 00000539-199909000-00007.
26. KHURANA S, MATTSON J C, WESTLEY S, O'NEILL W W, TIMMIS G C *et al.* 1997 Monitoring platelet glycoprotein IIb/IIIa-fibrin interaction with tissue factor-activated thrombelastography. *J Lab Clin Med 130:* 401–11. doi:10.1016/S0022-2143(97)90040-8.
27. NIELSEN V G, GEARY B T, BAIRD M S 2000. Evaluation of the contribution of platelets to clot strength by thrombelastography in rabbits: the role of tissue factor and cytochalasin D. *Anaesth Analg 91:* 35–9. doi:10.1097/00000539-200007000-00007
28. ZUCKERMAN L, COHEN E, VAGHER J O, WOODWART E, CAPRINI J A 1981 Comparison of thrombelastography with common coagulation tests. *Thromb Haemost 46:* 752–6
29. BJO¨RGELL O, ROBERTSON F 2002 Scoring systems for grading deep leg vein thrombosis. *Acta Radiol 43:* 299–305. doi:10.1034/j.1600-0455.2002.430311.x.