Original Article

Detection of elevated INR by thrombelastometry and thrombelastography in warfarin treated patients and healthy controls.

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Abstract

Introduction: The diagnostic potential of whole blood viscoelastic tests thrombelastography (TEG®) and thrombelastometry (ROTEM®) to detect warfarin-induced INR elevation remains elusive.

Methods: Viscoelastic tests were performed in 107 patients on warfarin and 89 healthy controls. Tests were activated by kaolin for TEG, and ellagic acid (INTEM) or tissue factor (EXTEM) for ROTEM.

Results: Viscoelastic tests revealed significant differences in clotting profiles between controls and warfarin-treated patients. Compared with healthy controls, patients treated with warfarin had prolonged EXTEM clotting and TEG reaction time ($p < 0.001$), both of which were also increased beyond the reference range. Increased INR values correlated with EXTEM CT (Spearman rho= 0.87) and TEG R-time (rho= 0.73). EXTEM CT had a sensitivity and specificity of 0.89 and 1.00, respectively, to detect elevated INR above 1.2 units, with a positive and negative predictive values (PPV and NPV) of 1.00 and 0.88, respectively. Similarly, TEG R-time had a sensitivity and specificity of 0.86 and 0.87, respectively, with a PPV of 0.89 and a NPV of 0.83. The corresponding receiver operator characteristic area under the curve was 0.99 (95% confidence interval [CI], 0.99 – 1.00) for EXTEM CT and 0.94 (95% CI, 0.91 – 0.97) for TEG R-time.

Conclusions: Tissue factor-activated viscoelastic testing (EXTEM) revealed individuals with warfarin-induced INR elevation accurately, while TEG - activated through the intrinsic pathway – still was of acceptable diagnostic value. Further studies are required to evaluate the diagnostic potential of viscoelastic tests in relation to standard laboratory tests in other mixed patient populations, where the PPV and NPV may be inferior.

Introduction

Warfarin and other vitamin-K antagonists (VKAs) are widely used for oral anticoagulation in the prevention and treatment of different thromboembolic diseases. The international normalized ratio (INR) is a normalized test based on the plasma prothrombin time and used in the laboratory control and monitoring of anticoagulation with VKAs. Today, whole blood viscoelastic tests are being increasingly used to evaluate states of hypo- and hypercoagulability (1). It has been shown that hypocoagulability detected by viscoelastic tests correlates with blood loss, transfusion need, and mortality in traumatic and obstetrical hemorrhage (1-5). However, the diagnostic value of these tests has been questioned because of an apparent low sensitivity for other bleeding conditions, including platelet disorders, von Willebrand disease and treatment with anticoagulant drugs (6,7).

For treatment with VKAs in particular, viscoelastic testing with thrombelastography (TEG) had a low sensitivity and specificity to detect elevated INR (8). Likewise, TEG had a limited sensitivity to identify deficiency of vitamin K dependent clotting factors in trauma patients (9). The correlation between TEG (activated by kaolin) and rapidTEG (activated by kaolin and tissue factor) appeared to be low (10). It remains to be established whether viscoelastic tests can be used to identify states of elevated INR in patients with oral anticoagulation.

In the present study, we analyzed whole blood coagulation profiles by viscoelastic tests in warfarin-treated individuals and healthy controls. We hypothesized that a tissue factor-activated test would be more sensitive to detect elevated INR than tests activated through the intrinsic pathway, due to the similarity with the mode of activation used in INR.

Methods and Materials

The study was approved by the regional scientific ethics committee in Stockholm and conducted in line with the second declaration of Helsinki. Patients and control subjects were included after giving written consent for participation in the study.

Patients treated with warfarin were recruited consecutively during outpatient visits at the Coagulation Unit of Karolinska University Hospital between September 2011 and April 2012. Baseline data on patient characteristics were collected from medical records.

Healthy volunteers presenting for a blood donation at the Karolinska University Hospital blood bank were included as controls between September 2011 and December 2012. Any previous blood donations were at least 4 weeks before inclusion. After giving informed consent, participating blood donors were questioned regarding current medication, and a survey was collected to indicate possible bleeding tendency.

Sample collection and laboratory analysis

Whole blood was collected by antecubital venipuncture in tubes containing 0.129 mol L^{-1} sodium citrate (BD, Becton, Dickinson and Company®, Franklin Lakes, NJ, USA) for performance of viscoelastic test and coagulation assays, and EDTA tubes (BD, Becton, Dickinson and Company®) for blood chemistry and cell counts. Hemoglobin, hematocrit, white cell and platelet count, INR based on Owren-type prothrombin time, aPTT (all Triolab AB®, Stockholm, Sweden), and plasma fibrinogen (Clauss method, Dade Behring/Siemens®, Munich, Germany) were analyzed as routine samples with the Sysmex CS 2100i® and Sysmex XE 5000/XT 2000i® system (Kobe, Japan) in the Department of Clinical Chemistry, Karolinska University Hospital Solna.

Rotational thrombelastometry was performed on a ROTEM® delta system (TEM Innovations GmbH, Munich, Germany) while thrombelastography was performed on a TEG 5000® unit

(Haemostasis System, Analytical Software Version 3, Haemoscope Corp, Niles, IL, USA). All tests were performed with fresh whole blood within 1 hour of venipuncture at 37° C, according to the manufacturer's instructions. For ROTEM, we used an assay activated through the intrinsic coagulation system by ellagic acid (INTEM reagent, TEM Innovations GmbH), or a tissue factor-activated assay for the extrinsic coagulation system (EXTEM reagent, TEM Innovations GmbH), with or without the addition of the platelet inhibitor cytochalasin D (FIBTEM reagent, TEM Innovations GmbH). Briefly, the process guided by an automated pipetting program first requires preparation of the EXTEM, FIBTEM, or INTEM reagent in a sample cuvette including 20 µL 0.2 mol L^{-1} $CaCl_2$, after which 300uL of whole blood is added, mixed, and the readout is started. TEG was activated by recalcification of 340 µL kaolin-whole blood mix with 20 µL 0.2 mol L^{-1} $CaCl_2$. Both viscoelastic testing systems automatically calculate a number of readout parameters (1). Roughly, these parameters indicate the time to initiation of coagulation, the strength of the formed clot, and clot retraction / fibrinolysis. We report the reference range for ROTEM from a multi-laboratory reference study including 6 centers and more than 140 individuals (11). To our knowledge, no such study exists for TEG, and we thus report the reference range provided by the manufacturer.

Statistical analysis

Statistical analysis was performed with R version 3.1.1 (July 2014). Non-parametric data were described as median (interquartile range, IQR), while parametric data were described as mean (standard deviation, SD). Correlation analysis was performed by Spearman and Pearson method to assess non-linear and linear associations between INR and viscoelastic test outcomes. To assess data for between-group differences, we used Welch's t-test for parametric variables, an adaptation of *Student's* t-test for independent samples assuming

unequal variances (12), or a Mann-Whitney U test for non-parametric variables. Proportions were compared with Pearson's chi-square test. Receiver operator characteristics curves were constructed with the pROC package for R (version 1.7.3, available from *http://cran.r-project.org/web/packages/pROC/*) (13). Statistical significance was defined as $p < 0.05$ for a two-sided test.

Results

We recruited a total of 200 subjects, consisting of 111 patients on warfarin treatment, and 89 healthy blood donors as controls. Two patients were excluded from analysis because of failure to obtain baseline test results, and a further two were excluded due to concomitant treatment with antiplatelet agents. The baseline characteristics of the study population are described in Table 1. Warfarin treated patients were older, had a lower platelet count, and a prolonged aPTT ($p < 0.05$ for all comparisons) (Table 1). The median INR amongst warfarin-treated patients was 2.4 (IQR 1.9 - 2.9). Patients also had increased fibrinogen levels compared to controls (mean±SD, 3.2 ± 0.6 vs. 2.7 ± 0.5 g/L, respectively; $p < 0.001$). All subjects had hemoglobin levels within the reference range.

The indication for anticoagulation encompassed primary and recurrent VTE, atrial fibrillation, as well as the presence of a mechanical aortic valve (Table 1).

Viscoelastic test results

We evaluated all subjects through viscoelastic testing by ROTEM and TEG. There were statistical significant differences in all TEG and ROTEM variables between both groups, except for EXTEM alpha angle (Table 2). Warfarin treated patients had a significantly higher EXTEM MCF than controls (mean±SD, 68.6 ± 4.6 vs. 64.4 ± 4.6 mm; $p < 0.001$), as well as an increased FIBTEM MCF (mean±SD, 16.1 ± 4.8 vs. 13.1 ± 3.5 mm; $p < 0.001$). In contrast, controls had a higher median TEG MA (57 vs. 61 mm; $p < 0.001$). Increased Clauss-method fibrinogen levels correlated with EXTEM MCF (Spearman rho 0.70, Pearson r 0.70; $p < 0.001$), but less so with TEG MA (rho 0.27, r 0.19; $p < 0.001$). Clot lysis/retraction was relatively increased in controls compared with in patients in all viscoelastic tests, but within reference limits. We noted that both EXTEM CT and TEG R-time were prolonged beyond the respective reference ranges in warfarin treated patients.

Elevated INR was linearly correlated with prolongation of EXTEM clotting time (rho 0.87, r 0.86; $p < 0.001$, Figure 1). Similarly, TEG R-time increased with higher INR values, but the correlation was weaker and non-linear (rho 0.73, r 0.47; $p < 0.001$, Figure 1). We noticed that several patients had an extremely low TEG angle and strong prolongation of R-time, while this marked hypocoagulability was not evident in the ROTEM clotting profiles. Exclusion of 12 patients with an R-time above 20 minutes (all with TEG angle < 17 °) increased the Pearson correlation coefficient to 0.72, indicating a more pronounced linear relationship. Warfarin-treated patients with an INR between 2.0 and 3.0 had EXTEM clotting times in the range of 66 to 161 sec (Figure 2).

Both INTEM clotting time and TEG R-time were linearly correlated with increased aPTT (Pearson r 0.79 and 0.61, respectively; Supplementary Figure 1).

Diagnostic value of viscoelastic tests for detection of elevated INR

We further investigated EXTEM CT and TEG R-time for their discriminative potential for elevated INR values. At an EXTEM CT cut-off of 75 sec (established in reference 11), the calculated sensitivity to detect elevated INR above 1.2 was 0.89 (95% CI, 0.81 – 0.94), with a specificity of 1.00 (95% CI, 0.94 – 1.00). The positive predictive value (PPV) was 1.00 (95% CI, 0.94 – 1.00), and the negative predictive value (NPV) was 0.88 (95% CI, 0.80 – 0.94). A receiver operating characteristic (ROC) curve for EXTEM CT had an area under the curve (AUC) of 0.99 (Figure 3), where an AUC value of 0.50 indicates random, and 1.00 perfect classification.

Dichotomizing TEG R-time at a cutoff of 8.0 minutes produced a sensitivity of 0.86 (95% CI, 0.77 – 0.92) and a specificity of 0.87 (95% CI, 0.78 – 0.93) to identify subjects with elevated INR > 1.2. The PPV was 0.89 (95% CI, 0.81 – 0.94) and the NPV 0.83 (95% CI, 0.74 – 0.90). The corresponding ROC area under the curve was 0.94 (Figure 3).

In our study, EXTEM CT reached 100% sensitivity or specificity at 56 sec and 72 sec, respectively (Table 3, upper panel). The best combination / trade-off of a high PPV and NPV was found at 64 sec. Similarly, the TEG R-time cut-off with 100% sensitivity was 4.5 min, and for 100% specificity 15.1 min. Again, an optimal PPV and NPV ratio was found at the cut-off 8.1 min (Table 3, lower panel).

For comparison, aPTT had a sensitivity of 0.69 (95% CI, 0.59 - 0.78) and a specificity of 0.96 (95% CI, 0.89 - 0.99) to detect elevated INR above 1.2. The PPV and NPV of increased aPTT was 0.95 (95% CI, 0.87 - 0.99) and 0.72 (95% CI, 0.63 - 0.80), respectively, and the ROC-AUC was 0.91 (95% CI, 0.87 - 0.95) (Supplementary Figure 3).

Discussion and Conclusion

In the present study we evaluated the diagnostic properties of viscoelastic tests amongst patients with warfarin anticoagulation and healthy controls. ROTEM activated by tissue factor had a very high sensitivity and specificity to detect elevated INR values, with almost perfect classification indicated by the ROC area under the curve. In intrinsic pathway-activated TEG, R-time also correctly classified most subjects into normal or elevated INR categories, but its diagnostic accuracy was less than EXTEM CT, with a lower sensitivity and specificity, and a lower ROC AUC.

We hypothesized that a tissue factor-activated viscoelastic test would have a better diagnostic value for the detection of increased INR compared to tests activated through the intrinsic pathway. This hypothesis was based on the similar mode of activation of the prothrombin time via the extrinsic coagulation pathway. Our study's findings evidently confirmed this hypothesis. Intrinsically activated tests rely on activation of the clotting factors XII, IX, and VIII, while tests activated through the extrinsic system provide a direct link between the tissue factor/factor VII complex and prothrombinase activation (14). It is probably therefore, in a similar manner as for aPTT, that viscoelastic tests activated through the intrinsic pathway have a lower diagnostic potential to detect coagulation deficiencies due to treatment with VKAs. Nonetheless, in our study, the aPTT in warfarin treated patients was marginally prolonged (median 44 sec, reference range 28 - 40 sec, Table 1), and this correlated with increased INTEM clotting time and TEG R-time (Supplementary Figure 1).

Both EXTEM and FIBTEM MCF were increased in warfarin treated patients as compared with controls; a finding that has been described previously (15). In contrast, patients had a significantly lower TEG MA than controls. The underlying factors accounting for the differences in clot strength between both tests require attention in future studies. Moreover, clot lysis/retraction was increased in controls, although the observed differences in our study

were relatively minor and well within the reference range for healthy individuals. Warfarin-treated individuals have pronounced fibrinolytic capacity (in a mixed population with a high atrial fibrillation/venous thromboembolism ratio) (16). Based on the limited assessment of fibrinolysis in our study, we cannot comment on the relevance of this finding, although these differences in both studies are of interest.

Traditionally, reference ranges are established through the 2.5 and 97.5 percentile of a reference population, but this approach can be questioned in situations where a reference test is available. A more dynamic approach, based on the clinical question with a specifically required positive and negative predictive value for the inclusion or exclusion of a diagnosis may be more functional. For example, the 97.5-percentile cut-off for prolongation of EXTEM CT established in a multicenter study was 75 sec (11). However, an almost perfect classification with a high PPV and NPV was achieved at a cut-off of 64 sec in our study (Table 3). Presumably, such dynamic approaches should be applied more widely to define laboratory cut-offs based on clinically relevant endpoints.

We present the first large study investigating multiple viscoelastic tests with different modes of activation in a warfarin treated population. A strength of our study is the clinical setting in which the study subjects were enrolled, as well as their consecutive inclusion. Furthermore, 25% of warfarin-treated patients had an INR above 2.9, which enabled us to include values beyond the treatment's target range in our analysis. Although it has been reported that a decreased erythrocyte volume fraction may influence the read-out of viscoelastic tests (17,18), this was probably of limited relevance in our study as all tested subjects had hemoglobin concentrations within the reference range.

Our study had a number of limitations that need to be addressed. First, all tests were conducted in a standardized manner: blood was sampled by coagulation-specialized nurses, viscoelastic tests were conducted by one trained research nurse, and the transfer time of

samples was low. Therefore, we reduced variation due to pre-analytical factors relative to a clinical testing situation, where such standardization is not possible. Second, at baseline, we were only able to include a small number of patients receiving anticoagulation for a mechanical aortic valve or atrial fibrillation, and we cannot rule out possible differences for these populations. Third, it has been previously shown that the tissue factor-activated rapidTEG® system was not sensitive for elevated INR in patients receiving warfarin (8). Unfortunately, this system was not available to us, but its diagnostic value should be compared to tissue factor-activated ROTEM in future studies. Finally, as we have demonstrated the potential of viscoelastic tests within a homogenous clinical population, care should be taken when the results of the study are being extrapolated, as the diagnostic value may prove different. We suggest that further studies are required to evaluate viscoelastic tests in relationship to standard laboratory tests in other patient populations.

In conclusion, we demonstrate the possibility of using tissue factor-activated thrombelatometry to detect elevated INR with a high positive and negative predictive value. TEG had a lower accuracy, but was still of acceptable diagnostic value. Thus, we propose that patients with warfarin anticoagulation should not be categorically precluded from analysis with viscoelastic tests, for example in the context of trauma. The study's results present an example for further population based investigations into the diagnostic value of whole blood viscoelastic tests for the evaluation of coagulation disorders.

Conflict of interest

M.H. received unrestricted grants from Baxter Medical AB and Octapharma AG for the present work. D.E.S. was supported by a scholarship from the German National Academic Foundation (Studienstiftung des deutschen Volkes).

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Tables

Table 1: Baseline characteristics of warfarin-treated patients and healthy control subjects.

	Warfarin-treated patients N = 107	**Healthy controls N = 89**	**p-value**
Age, years, median (IQR)	57 (46 – 64)	49 (37 – 56)	< 0.001
Women, *n* (%)	42 (39.3%)	37 (41.6%)	0.40
Hemoglobin, g/L, mean (SD)	141 (±15)	142 (±11)	0.99
White cell count, * 10^{12} /L, mean (SD)	6.1 (±1.8)	5.6 (±1.5)	0.09
Platelet count, *10^{9} /L, median (IQR)	235 (205 – 275)	257 (229 – 283)	0.004
Fibrinogen, g/L, mean (SD)	3.2 (±0.6)	2.7 (±0.5)	< 0.001
INR, median (IQR)	2.4 (1.9 – 2.9)	1.0 (1.0 – 1.1)	< 0.001
aPTT, sec, median (IQR)	44 (39 – 51)	33 (31 – 36)	< 0.001
Indication for anticoagulation			
First DVT or PE, *n* (%)	46 (43.0%)		
Recurrent DVT or PE, *n* (%)	46 (43.0%)		
Mechanical Aortic Valve, *n* (%)	3 (2.8%)		
Atrial Fibrillation, *n* (%)	1 (0.9%)		
Other, *n* (%)	11 (10.3%)		

INR, international normalized ratio; aPTT, activated partial thromboplastin time; DVT, deep venous thrombosis; PE, pulmonary embolism.

Table 2: Results of viscoelastic testing for patients with warfarin treatment and healthy controls, including reference ranges and statistical testing.

	Warfarin-treated patients	Healthy controls	Reference range	p-value*
ROTEM				
INTEM CT, sec, median (IQR)	176 (162 – 191)	163 (157 – 170)	137 – 246	< 0.001
INTEM alpha angle, °, median (IQR)	78 (76 – 79)	77 (75 – 78)	71 – 82	0.03
INTEM MCF, mm, mean (SD)	69.4 (± 4.1)	65.5 (± 7.0)	52 – 72	< 0.001
INTEM ML, %, median (IQR)	8 (5 –10)	10 (8 –12)	< 12	< 0.001
EXTEM CT, sec, median (IQR)	98 (83 – 120)	47 (44 – 51)	42 – 74	< 0.001
EXTEM alpha angle, °, median (IQR)	76 (72 – 79)	76 (72 – 78)	63 – 81	0.86
EXTEM MCF, mm, mean (SD)	68.6 (± 4.6)	64.4 (± 4.6)	49 – 71	< 0.001
EXTEM ML, %, median (IQR)	7 (5 – 10)	10 (8 – 13)	< 18	< 0.001
FIBTEM MCF, mm, mean (SD)	16.1 (± 4.8)	13.1 (± 3.5)	9 – 25	< 0.001
TEG				
R–time, min, median (IQR)	10.2 (8.8 – 12.5)	5.5 (4.2 – 7.0)	3 – 8	< 0.001
Angle, °, median (IQR)	55 (39 – 62)	65 (57 – 68)	55 – 78	< 0.001
MA, mm, median (IQR)	57 (50 – 62)	61 (58 – 64)	51 – 69	< 0.001
LY30, %, median (IQR)	0.4 (0.0 – 2.0)	1.9 (1.1 – 3.0)	< 15	< 0.001

* p-values are for comparisons between controls and patients. CT, clotting time; MCF, maximum clot firmness; ML, maximum lysis; R-time, reaction time; MA, maximal amplitude; LY30, lysis at 30 minutes.

Table 3: Specificity and sensitivity of tissue factor-activated ROTEM and TEG to classify into INR ≤ 1.2 and > 1.2 at various cut-offs.

Threshold EXTEM CT (sec)	**56**	**64**	**72**
Sensitivity	1.00	0.96	0.92
Specificity	0.85	0.99	1.00
PPV	0.89	0.99	1.00
NPV	1.00	0.96	0.92
Threshold TEG R-time (min)	**4.5**	**8.1**	**15.1**
Sensitivity	1.00	0.86	0.17
Specificity	0.31	0.88	1.00
PPV	0.64	0.90	1.00
NPV	1.00	0.84	0.50

The left and right outer columns indicate the calculated value at which full sensitivity or specificity is achieved, respectively. The middle column cut-off provides the best discriminative potential between reference and elevated INR above 1.2. CT, clotting time; PPV, positive predictive value; NPV, negative predictive value, R-time, reaction time.

Figure Legends

Figure 1: Scatterplot of INR (arbitrary units) against EXTEM clotting time (sec) or TEG R-time (min). Warfarin-treated cases are depicted as circles, while triangles indicate healthy control subjects.

Figure 2: Boxplot of EXTEM clotting times at different INR ranges.

Figure 3: Receiver-operator characteristics curves of EXTEM clotting time and TEG R-time to classify subjects into INR ≤ 1.2 or > 1.2.