Measurement of Anticoagulation in Patients on Dabigatran, Rivaroxaban and Apixaban Therapy by Novel Automated Thrombelastography

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DOI: 10.1055/a-1692-1415

Please cite this article as: Artang R, Dias J, Walsh M et al. Measurement of Anticoagulation in Patients on Dabigatran, Rivaroxaban and Apixaban Therapy by Novel Automated Thrombelastography. TH Open 2021. doi: 10.1055/a-1692-1415

Conflict of Interest: JDD and JH were employees of Haemonetics Corporation at the time of the study. This study was supported by Haemonetics Corporation (Boston, Massachusetts).

Trial registration: NCT02798328, ClinicalTrials.gov (http://www.clinicaltrials.gov/), Prospective observational multi-center study

Abstract:
Background: Direct-acting oral anticoagulants (DOACs) do not require monitoring. Measurement of DOAC effect would be useful in the event of bleeding, trauma, and thromboembolism while on anticoagulation. We evaluated the effectiveness of the investigational DOAC assays on the TEG®6s Hemostasis Analyzer to assess the anticoagulant effect of DOACs in patients treated for atrial fibrillation or DVT.

Methods: Patients on treatment for a minimum of 7 days with standard doses of dabigatran, rivaroxaban and apixaban were included. DOAC plasma concentrations and TEG®6s R-time were measured and correlated. The sensitivity, specificity and negative predictive value (NPV) of R-time to detect DOAC concentrations of ≥ 30, ≥ 50 and ≥ 100 ng/mL were calculated.

Results: 189 Subjects were included, (n=50) on apixaban, (n= 62) on rivaroxaban, (n=53) on dabigatran and (n=24) on no DOAC were studied. Using the direct thrombin inhibitor (DTI) channel, R-time demonstrated strong linear correlation with dabigatran levels (r = 0.93, p < 0.0001). Using the anti-factor Xa (AFXa) channel, R-time demonstrated strong non-linear correlation with rivaroxaban and apixaban levels (rs = 0.92 and 0.84 respectively, p < 0.0001 for both). R-time revealed strong sensitivity and NPV in detecting low DOAC levels for the predefined concentrations.

Conclusion: R-time measured by TEG®6s DOAC Specific cartridge has a strong correlation with concentrations of the most commonly used DOACs, with high sensitivity and NPV for detecting lower drug levels that are considered clinically relevant for patients in need of antidote, or prior to urgent surgery. Further studies to determine the relation of R-time to clinical outcomes are warranted.
Measurement of Anticoagulation in Patients on Dabigatran, Rivaroxaban and Apixaban Therapy by Novel Automated Thrombelastography

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Abstract

Background: Direct-acting oral anticoagulants (DOACs) do not require monitoring. Measurement of DOAC effect would be useful in the event of bleeding, trauma, and thromboembolism while on anticoagulation. We evaluated the effectiveness of the investigational DOAC assays on the TEG®6s Hemostasis Analyzer to assess the anticoagulant effect of DOACs in patients treated for atrial fibrillation or DVT.

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or prior to urgent surgery. Further studies to determine the relation of R-time to clinical outcomes are warranted.

Introduction

Laboratory assessment of the anticoagulant effect of the DOACs remains a challenge a decade after this class of anticoagulants entered the market. While routine monitoring of these agents is not warranted, the ability to detect anticoagulation may be an important clinical tool in certain situations, such as overdose, bleeding, and urgent surgery [1].

The Thrombelastograph® (TEG®)6s Analyzer is a novel site-of-care test for global evaluation of hemostasis. The TEG®6s applies resonance-frequency viscoelasticity measurements and premixed disposable multichannel microfluidic cartridges [2]. The DOAC Specific cartridge for the TEG®6s system is an experimental prototype that was proven more sensitive and specific than the conventional Global Hemostasis cartridge in identifying the anticoagulant effect from DOAC therapy comparing DOAC treated patients to healthy volunteers without anticoagulation [3]. A study on healthy volunteers revealed a significant correlation between the TEG® Reaction time (R-time) and blood DOAC levels using this cartridge [4]. In a recent study, the normal reference ranges for the DOAC Specific cartridge, and its effectiveness in detecting and classifying the DOAC treatment, were established using blood samples from 160 healthy subjects and 190 patients on treatment for atrial fibrillation and venous thromboembolism [5].

The clinically relevant DOAC concentration cutoffs, based on current available literature, are 30 ng/mL for urgent invasive procedures with high bleeding risk, 50 ng/mL for antidote administration [6, 7], and 100 ng/mL for thrombolysis in stroke [8]. The purpose of this present
study was to demonstrate the correlation between the R-time and the DOAC plasma concentrations, as well as to assess sensitivity, specificity and negative predictive value (NPV) of the R-time for the aforementioned clinically useful DOAC concentrations in the same cohort of patients.

**Materials and methods**

This study was conducted at the following five clinical sites in the United States from August 2016 until September 2017: Essentia Institute of Rural Health, Duluth, MN; Memorial Hospital of South Bend, South Bend, IN; Spartanburg Regional Medical Center, Spartanburg, SC; Inova Heart and Vascular Institute, Falls Church, VA; and Inova Cardiology Ambulatory Research Center, Manassas, VA. In accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, Institutional Review Boards at each participating site conferred ethical approval. All participants were 18 years or older and gave informed consent prior to the enrollment.

For the on-DOAC group, patients were included if they were 18 years or older, and on a DOAC at doses recommended by the manufacturers for treatment of atrial fibrillation, venous thromboembolism or thromboembolism prophylaxis for minimum of 7 days uninterrupted. The exclusion criteria were, genetic bleeding disorders, known or subsequently discovered inherited defects of coagulation (e.g. hemophilia or Von Willebrand disease), DOAC dosage outside of manufacturer’s recommended range (e.g. study subject with renal impairment and supratherapeutic dose), Heparin or LMWH administered within 7 days prior to blood draw, on any other type on FDA approved or experimental anticoagulant, bruising, wounds or scarring in the area of venipuncture. Treatment with aspirin was not an exclusion criteria.
Blood samples were collected at a random time point at patient presentation to outpatient clinic or during inpatient presentation. A non-DOAC control group of patients was included in the analysis for comparison. Subjects were included if they were 18 years or older. The exclusion criteria for the non-DOAC group were, medical evidence of atrial fibrillation, deep vein thrombosis or pulmonary embolism requiring anticoagulation, genetic Bleeding disorders, known or subsequently discovered inherited defects of coagulation (e.g. hemophilia or Von Willebrand disease), on any medication containing heparin or LMWH within 7 days, on a DOAC or other anticoagulant, on any medications known to affect coagulation status, on strict vegan diet, bruising, wounds or scarring in the area of venipuncture.

**Blood Sampling and Analysis**

Up to ~20 mL of blood was drawn standard venipuncture from each subject. No restrictions were made on use of tourniquet. The first 3-6 mL Non-Additive tube was discarded at each draw. The second tube of 4.5 mL was collected in a standard 3.2% sodium citrate tube (BD Vacutainer, Franklin Lakes, NJ, USA), and was used for the TEG®6s analysis. The third tube of 4.5 mL was collected in a standard 3.2% sodium citrate tube and then centrifuged at 2000g for 10 minutes. The plasma samples were stored at ~80° Celsius for the measurement of DOAC concentrations. DOAC concentrations were measured by High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) assay at Quest Diagnostics (Wood Dale, IL, USA) [9].

**Thrombelastography**

The Thrombelastograph® 6s (TEG®6s, Haemonetics Corp, Boston, MA, USA) technique has previously been described in detail [2, 3]. The TEG®6s system measures the clot viscoelasticity using resonance-frequency. The blood sample suspended in a micro ring structure within the
cartridge is exposed to a fixed vibration frequency. With LED illumination, a detector measures vertical motion of the blood film. The frequency leading to resonance is identified and then converted to the TEG system readout. The DOAC Specific cartridge contained kaolin in channel 1 (the citrated kaolin [CK] channel), ecarin in channel 2 (the direct thrombin inhibitor [DTI] channel), factor Xa in channel 3 (the antifactor-Xa [AFXa] channel), and kaolin with abciximab in channel 4 (the functional fibrinogen channel). Based on an unpublished pilot studies on patients treated with warfarin, the R-time was not altered beyond the established reference range for both DTI and AFXa channels with INRs ranging between 1.2 and 3.5. Heparin increased the R-time in the AFXa channel in a dose dependent fashion but caused no change of R-time in the DTI channel. Experience with low molecular weight heparin is pending and is considered as necessary part of the product development but we anticipate similar response to the R-time on the AFXa and DTI channels as heparin given its effect on factor Xa inhibition. The DOAC specific cartridge is an experimental prototype that is currently not commercially available and is under investigation for use in patients who are treated with DOACs. The current protocol for the TEG®6s system is to allow the samples to stabilize for 10 minutes prior to analysis unless the analysis is needed urgently for medical emergency. In the present study all the R-time analyses were performed between 10 minutes and 2 hours after the phlebotomy.

Statistical Analysis

The importance of measuring the R-time in patients treated with DOACs has been previously demonstrated (3, 4). We determined the correlation between R and DOAC concentrations. Pearson’s correlation coefficient $r$ was calculated for linear association between R-times and DOAC concentration. Spearman’s rank correlation $r_s$ was calculated if the association of R-times and DOAC concentrations were non-linear. Correlation coefficient values of $> 0.8$ were
considered strong. *P* values of less than 0.05 were considered significant. If the association pattern did not fit a linear model, the best fit nonlinear model was selected. Goodness of Fit expressed by $R^2$ was calculated for both linear and non-linear regressions. Concentrations of rivaroxaban, apixaban, and dabigatran were classified in 3 categories: <30 vs. $\geq$ 30, <50 vs. $\geq$ 50, <100 vs. $\geq$ 100. Logistic regression models compared R-times to the binary concentrations of ($\geq$ 30, $\geq$ 50, $\geq$ 100) to identify initial cut-points to initiate the detailed analysis of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood (LR+) and negative likelihood ratios (LR−). Analysis of sensitivity, specificity, PPV, NPV, LR+, and LR− began at the R-time cut-point identified by the logistic regression and continued for the next 6 R-times, consecutively. The best model fit was identified as the model with the highest LR+ and lowest LR− levels. [10]. The rational for the sample size was previously described [5].

The correlations and graphs were generated using Prism software version 9 (GraphPad SoftwInc., La Jolla, CA). The sensitivity data were analyzed using IBM SPSS statistics (Version 23) predictive analytics software (IBM Corp. Armonk NY, Released 2015).

**Results**

189 subjects were included in the study. 165 of those subjects were in the on-DOAC group and 24 subjects were included on the non-DOAC group. The clinical characteristics for each DOAC and non DOAC groups are outlined in Table 1. Among the subjects on DOAC, 50 were on apixaban, 53 on rivaroxaban, and 62 on dabigatran. The mean age of DOAC cohort was 71± 10.2 years and 42% were female. Among subjects not anticoagulated, the mean age was 48 ± 11.6 years and 67% were female. Using the DTI channel, R-time demonstrated a strong linear correlation with dabigatran levels ($r = 0.93$, $p < 0.0001$, $R^2 = 0.86$ for goodness of Fit). Using the AFXa channel, R-time demonstrated strong non-linear correlation with rivaroxaban and
apixaban levels ($r_s = 0.92$ for rivaroxaban, $r_s = 0.84$ for apixaban, $p < 0.0001$ and $R^2 = 0.86$ for goodness of Fit for both) (Fig 1).

The DOAC levels among the non-DOAC group were 0. Among the 50 subjects on Apixaban, 1 subject had a level of 29 ng/mL or less, 1 subject between 30 and 49 ng/mL and 6 subjects between 50 and 99 ng/ml. Among the 53 subjects on rivaroxaban, 4 subjects had a level of 29 ng/mL or less, 7 subjects between 30 and 49 ng/mL and 17 subjects between 50 and 99 ng/ml. Among the 62 subjects on dabigatran, 2 subjects had a level of 29 ng/mL or less, 9 subjects between 30 and 49 ng/mL and 17 subjects between 50 and 99 ng/ml. The sensitivity, specificity, LR+, LR− and NPVs for the R-time to detect DOAC concentrations of ≥ 30, ≥ 50 and ≥ 100 ng/mL for the entire cohort of on-DOAC and non-DOAC subjects are presented in Table 2. R-time revealed strong sensitivity and NPV for the predefined DOAC concentrations. Due to only single acquired concentration between 30 and 49 ng/mL in the apixaban group, no sensitivity and specificity calculations were performed in that range.

Discussion

To date, this is the largest study correlating the DOAC concentrations to TEG-R value using the DOAC specific cartridge with TEG®6s device. In this study a strong correlation between R-time and DOAC concentration in 165 patients on DOAC was demonstrated. The R-time also revealed high sensitivity and NPV for the low doses of the 3 most commonly used DOAC agents.

This technology may be useful in clinical situations requiring rapid assessment of the coagulation status in patients who present with acute bleeding while on DOAC treatment. Other
clinical situations where such test may prove to be useful are patients on DOAC needing urgent surgery, and prior to DC-cardioversion, or radiofrequency ablation for patients with atrial fibrillation when there is uncertainty about the hemostatic function or adequacy of anticoagulation. The R-time assessment test duration was under 6 minutes.

The association observed between R-time, and the concentration of the FXa inhibitors rivaroxaban and apixaban, was best fitted in a nonlinear pattern as increasing concentrations of the FXa inhibitor concentration would not cause further increase of the R-time displaying a plateau in the curve. We hypothesize that this observation may be related to the fixed concentration of Factor Xa used in the FXa channel chamber. Alternative explanation may be the fixed amount of factor Xa in the whole blood sample that is present in the chamber, thus limited number of FXa binding sites for the FXa inhibitor molecules. It is therefore plausible that at higher concentrations of FXa inhibitor (>150-200 ng/mL) a saturation point may be achieved where higher levels of the in vivo FXa inhibitor concentration would no longer impact the effect of FXa in converting prothrombin to thrombin and thereby fibrin generation. Since the R-time reflects the beginning of fibrin-platelet clot generation, the mechanism described above may explain the plateau in R-time with further increase of the FXa inhibitor concentration in the present study. This hypothesis requires further investigation with escalating FXa dose in in-vitro samples analyzed. The correlation of Dabigatran concentrations and R-time was linear in the concentrations observed in this study.

The routine monitoring of patients on chronic DOAC treatment, without thrombotic or bleeding events, is not recommended by the guidelines [11, 12]. The challenge for the clinicians is how to respond to a measured level that falls at the upper or lower end of a very wide range reported in patients on chronic DOAC treatment [13]. Secondary analysis of the randomized
DOAC trials has revealed association of higher drug concentration with bleeding and lower concentrations with thrombotic events. There is so far, no evidence that adjustment of the DOAC dose, beyond what was demonstrated in the original DOAC trials and stated in the prescription information, would improve the clinical outcome. Patients’ propensity for thrombosis or bleeding may differ significantly based on patient characteristics including but not limited to age and renal function. It is therefore possible that the same concentration of DOAC in plasma can result in adequate protection in some patients, but thrombosis or bleeding in others [14]. The global coagulation assay parameters, such as the R-time in Thrombelastography and Lag-time in Thrombin Generation assay have a statistically significant correlation with DOAC concentration levels but do not offer an exact prediction of the DOAC concentration [4, 15]. The latter observation is further confirmed as seen by the scatter in figure 1 in the present study. Our observation reveals that the strength of the TEG®6s technique beyond its short turnaround time may be its high sensitivity and NPV for the predefined DOAC concentrations in a qualitative binary pattern (≥ 50 or 100 ng/mL of drug present, versus no clinically significant amount present) rather than quantitative (ng/mL) assessment of DOAC concentrations. For concentrations of 30 ng/mL or lower and for potential clinical application of this technology in general, these observations will need to be verified with much larger cohorts and more robust and consistent high sensitivity and NPVs across different predefined low concentrations. The present study was not designed to answer the question of clinical outcomes correlation with R-times but may be considered as hypothesis generating research. Establishing such correlation is therefore warranted using the results from large clinical studies, associating DOAC concentrations and different coagulation parameters, including the global assays with the clinical outcomes or conducting new studies where the global assays are included.
The limitation of the present study includes a lack of adequate data on edoxaban, the other currently FDA approved factor Xa inhibitor. Given only 4 subjects included edoxaban group no meaningful analysis could be performed on the data which was omitted from this manuscript. The thresholds achieved in the present study on rivaroxaban and apixaban can therefore not be used or deduced for edoxaban. The study design did not include hour by hour monitoring of the DOAC level, but rather approaching the patients consecutively as they presented to the clinic or the hospital, regardless of the time of DOAC intake. The sensitivity and specificity calculations on apixaban for levels between 30 and 49 ng/mL were therefore less certain. A larger number of observations at DOAC levels less than 50 ng/mL would have further strengthen the sensitivity and specificity of this technique in lower concentrations, where it considered clinically relevant. The study would furthermore have demonstrated much higher specificity, NPV and LR+ if the non DOAC cohort was larger by design providing a larger true negative value.

In conclusion, R-time measured by TEG®6s DOAC Specific cartridge has a strong correlation with concentrations of the most commonly used DOACs. This technique has high sensitivity and negative predictive value for detecting lower drug levels that are considered clinically relevant for potential use in patients in need of antidote, or prior to urgent surgery. Further larger studies with focus on lower concentrations, and the relation of R-time to clinical outcomes is warranted. This novel assay has promise in the personalization of antithrombotic therapy in patients with cardiovascular and thrombotic diseases.

What is known about this topic?

- Prior studies on the thrombolastograph TEG®6s have shown favorable correlation between direct oral anticoagulants (DOACs) concentrations and TEG R-time in small number of healthy volunteers.
What does this paper add?

- We studied the correlation of TEG®6s R-time to DOAC plasma concentrations in 165 patients on chronic DOAC treatment.
- TEG®6s R-time demonstrated highly significant correlation with dabigatran, rivaroxaban, and apixaban blood concentrations.
- The R-time demonstrated high sensitivity and negative predictive value to detect DOAC concentrations of ≥30, ≥ 50 and ≥ 100 ng/mL.

Acknowledgements

We would like to thanks Rebecca Starr for her assistance with the language of the manuscript.

Author Contributions

RA and JH conceived and designed the study. MW, KB, BCT, MA, JDN, and PAG contributed to data collection and interpretation. All authors revised the article for important intellectual content and approved the final version for submission.

Disclosure of Conflict of Interests

JDD and JH were employees of Haemonetics Corporation at the time of the study. This study was supported by Haemonetics Corporation (Boston, Massachusetts). The other authors have no other relevant financial interest in the products or companies described in this article.

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**Fig 1.** The scatter diagram of DOAC concentrations against R-time. $R_{\text{DTI}}$ indicates using the DTI channel; $R_{\text{FXa}}$ indicates using the AFXa channel.

**Table 1.** Subjects clinical characteristics

|                          | Apixaban | Dabigatran | Rivaroxaban | Non-DOAC |
|--------------------------|----------|------------|-------------|----------|
| **Mean Age, years (SD)** | n=50     | n=62       | n=53        | n=24     |
|                          | 72 (10.3)| 70.9 (9.1) | 70.8 (11.9) | 48 (11.6)|
| **Gender, n (%)**        |          |            |             |          |
| Female                   | 25 (50)  | 24 (39)    | 25 (47)     | 16 (67)  |
| Male                     | 25 (50)  | 38 (61)    | 28 (53)     | 8 (33)   |
| **Ethnicity, n (%)**     |          |            |             |          |
| Caucasian                | 44 (88)  | 59 (95)    | 49 (92)     | 21 (88)  |
| Other                    | 6 (12)   | 3 (5)      | 4 (8)       | 3 (12)   |
| **DOAC indication, n (%)**|         |            |             |          |
| Atrial Fibrillation or Flutter | 43 (86)  | 62 (100)   | 45 (85)     |          |
| DVT/PE Treatment         | 4 (8)    | 0          | 6 (11)      |          |
| DVT Prophylactic         | 3 (6)    | 0          | 2 (4)       |          |
| **Comorbidities, n (%)** |          |            |             |          |
| Hyperlipidemia           | 27 (54%) | 37 (60%)   | 24 (45%)    | 5 (21%)  |
| Hypertension             | 38 (76%) | 43 (69%)   | 33 (62%)    | 10 (42%) |
| Heart Failure            | 14 (28%) | 7 (11%)    | 6 (11%)     |          |
| Diabetes                 | 12 (24%) | 16 (26%)   | 8 (15%)     |          |
| Hypothyroidism           | 4 (8%)   | 8 (13%)    | 6 (11%)     | 1 (4%)   |
| Coronary artery disease  | 3 (6%)   | 6 (10%)    | 10 (19%)    |          |
| **Antiplatelet Therapy ConMed, n (%)** | | | | |
| Aspirin                  | 9 (18%)  | 17 (27%)   | 10 (19%)    | 2 (8%)   |
| Clopidogrel              | 4 (8%)   | 1 (2%)     | 1 (2%)      |          |
| Prasugrel                |          | 1 (2%)     |            |          |

**Table 2.** Sensitivity, specificity and negative predictive value of R-time to detect DOAC concentrations above thresholds mentioned

| DOAC            | Threshold (ng/mL) | R-time (min) | Sensitivity | Specificity | LR+ | LR- | NPV |
|-----------------|-------------------|--------------|-------------|-------------|-----|-----|-----|
| Apixaban        | $\geq$           |              | %           | %           |     |     |     |
| Dabigatran      | $\geq$           |              | %           | %           |     |     |     |
| Rivaroxaban     | $\geq$           |              | %           | %           |     |     |     |
| Non-DOAC        | $\geq$           |              | %           | %           |     |     |     |
|                | 30   | 50   | 100  | 82 (82-100) | 83 (70-95) | 92 (82-100) | 94 (88-100) | 100 (81-100) | 82 (72-93) | 100 (81-100) |
|----------------|------|------|------|-------------|-----------|-------------|-------------|--------------|-------------|--------------|
| **Dabigatran** |      |      |      |             |           |             |             |              |             |              |
| 30             | 2.6  | 100  | 13   | 0           | 100       |             |             |              |             |              |
| 50             | 3.1  | 94    | 83   | 0.07        | 91 (81-100)|             |             |              |             |              |
| 100            | 3.4  | 100  | 82   | 5.6         | 0         | 100         |             |              |             |              |
| **Rivaroxaban**|      |      |      |             |           |             |             |              |             |              |
| 30             | 1.7  | 98    | 86   | 6.9         | 96 (88-100)|             |             |              |             |              |
| 50             | 2.1  | 95    | 80   | 4.8         | 93 (84-100)|             |             |              |             |              |
| 100            | 2.6  | 96 (78.9-99.9) | 85 (72.4-93.3) | 5.1 | 0.05 | 98 (93-100) |           |              |             |              |
| **Apixaban**   |      |      |      |             |           |             |             |              |             |              |
| 30             | *    |      |      |             |           |             |             |              |             |              |
| 50             | 1.7  | 100  | 96   | 25          | 0         | 100         |             |              |             |              |
| 100            | 2.2  | 98 (93-100) | 81 (67-95) | 5 | 0.03 | 96 (89-100) |           |              |             |              |

The reference range of R-time for the FXa channel is 0.6–1.5 min, and for DTI channel is 1.6–2.5 min.

AFXa, antifactor-Xa; AUC, area under the curve; DOAC, direct oral anticoagulant; DTI, direct thrombin inhibitor; R, reaction time. LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value.

a Indicates using the DTI channel.
b Indicates using the AFXa channel.

* Due to only single acquired concentration between 30 and 49 ng/mL, no calculations were performed.
**Fig 1**

**Dabigatran**

![Graph A](image)

$r = 0.93, p < 0.0001$

$Y = 0.01^*X + 2.2$

**Rivaroxaban**

![Graph B](image)

$r_s = 0.92, p < 0.0001$

$Y = 0.9 + 3.4^*(1-exp(-0.01^*X))$

**Apixaban**

![Graph C](image)
