


Effect of Dabigatran on Clotting Time in the Clotpro Ecarin Clotting Assay: A Prospective, Single-Arm, Open-Label Study

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Abstract

Routine coagulation tests do not enable rapid, accurate determination of direct oral anticoagulant (DOAC) therapy. The ecarin clotting assay (ECA), performed on the ClotPro viscoelastic testing device, may enable sensitive and specific detection of dabigatran. We assessed the association between trough plasma dabigatran concentration and clotting time (CT) in the ClotPro ECA, in patients with non-valvular atrial fibrillation (NVAf). Each patient provided a single venous blood sample, ~1 hour before dabigatran dosing. The study included 118 patients, of whom 64 were receiving dabigatran 110 mg twice daily and 54 were receiving 150 mg twice daily. ECA CT was moderately correlated with trough plasma dabigatran concentration ($r = 0.80$, $p < 0.001$). Slight trends toward increased plasma dabigatran concentration and prolonged ECA CT were apparent with 150 mg versus the 110 mg dose (differences not statistically significant). Individuals with creatinine clearance below 50 mL/minute had significantly higher plasma dabigatran concentrations and significantly prolonged ECA CT versus those with creatinine clearance ≥ 50 mL/minute. In conclusion, this preliminary study has demonstrated that CT in the ClotPro ECA reflects the plasma concentration of dabigatran in patients with NVAf. The ECA could potentially be used to assess the impact of dabigatran on a patient's coagulation status.

Keywords

ClotPro, dabigatran, Ecarin clotting assay (ECA), non-valvular atrial fibrillation (NVAf)

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Introduction

Anticoagulation therapy is prescribed to large numbers of individuals with cardiovascular disease.^{1,2} Vitamin K antagonists (VKAs, e.g. warfarin) were previously considered as the standard of care, but these drugs have a narrow therapeutic window and may increase patients' risk of hemorrhagic events.³⁻⁵ Therefore, regular INR testing is needed in patients receiving VKAs to ensure appropriate dosing.

Over the last 10 years, direct oral anticoagulants (DOACs) such as dabigatran have increasingly been advocated in place of VKAs. Data from clinical trials and observational studies show that DOACs are at least as effective as VKAs, and that they may improve safety (e.g. reduced risk of major bleeding).⁶⁻¹⁰ DOACs are administered at fixed doses without a need for their effects to be monitored, and they have relatively few dietary interactions.¹¹ Consequently, DOACs are likely to be

perceived by patients as more convenient than VKAs and to be associated with higher patient satisfaction rates.¹²

In certain circumstances (e.g. trauma, emergency surgery), it may be necessary for the effects of DOAC therapy to be

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reversed.^{13,14} If the drug(s) an individual is taking or the time of last dose (hence the plasma drug level) are unknown, it may be difficult to determine the patient's need for DOAC reversal or the optimal agent(s) for achieving reversal. Coagulation tests can be performed, and DOACs have been shown to affect the results of standard laboratory tests as well as viscoelastic tests.¹⁵⁻¹⁸ However, most coagulation tests were not designed to evaluate the effects of DOACs, meaning that abnormal results may be caused by a range of factors. The ecarin clotting time (ECT), a test based on direct prothrombin activation, provides high sensitivity and specificity for the detection of dabigatran, but it is not performed routinely and, as with other laboratory tests, the turnaround time may be prolonged.¹⁹

ClotPro (Dynabyte, Germany) is a recently developed viscoelastic testing system that uses a different measurement method from ROTEM or TEG.^{20,21} This device was designed to provide rapid evaluation of whole-blood coagulation, with 6 testing channels allowing multiple assays to be performed simultaneously. The original method for viscoelastic testing, developed by Hartert, involved transferring a blood sample into a cylindrical cup and suspending a cylindrical pin into it.²² The cup was rotated to the right and left by a small angle (4.75°) and the ability of the blood to transfer the movement of the cup to the pin was recorded. The format of the ClotPro device is similar: a blood sample is contained within a cup that is rotated using an elastic element and a stationary pin is immersed in the sample. Clot firmness is measured through high-sensitivity capacitive detection of forces acting on the pin, using robust twin-bearing technology. A broad range of ClotPro assays are available, and this may be valuable in numerous clinical settings: for example, the possibility of using ClotPro to evaluate the coagulation status of patients with COVID-19 is being explored.²³

The ClotPro ecarin clotting assay (ECA) was developed to enable similar assessment as ECT, in a test with a short turnaround time that can be performed at the point of care. We undertook a study to assess the association between trough plasma dabigatran concentration and clotting time (CT) in the ClotPro ECA, in patients with non-valvular atrial fibrillation (NVAF).

Patients and Methods

This prospective, single-arm, open-label study was performed at Sarawak Heart Centre over a 7-month period (19 December 2018 to 19 July 2019). The study was approved by the medical research ethics committee at the Malaysian Ministry of Health and registered in the Malaysian National Medical Research Register (NMRR-16-1867-32865). It was performed in accordance with Good Clinical Practice and the Declaration of Helsinki. All patients provided signed informed consent before participating in any of the study procedures.

Patients aged ≥ 18 years with a diagnosis of NVAF and receiving treatment with dabigatran (110 or 150 mg twice daily; at least 3 doses administered) were eligible for the study. Further requirements for inclusion were accurate measurement

of ECA CT, and laboratory assessment of trough plasma dabigatran concentration. Any patients receiving dabigatran for indications other than NVAF (e.g. valvular atrial fibrillation; venous thromboembolism) were excluded.

Each patient provided a single venous blood sample, collected by venipuncture approximately 1 hour before dabigatran dosing. A total of 6 mL was collected into 2 sodium citrated vacutainers. The ECA was performed on a ClotPro device using the whole blood. All materials for the ECA were obtained from Dynabyte GmbH, Germany, and this analysis was conducted within 3 hours of blood collection.

For measurement of the dabigatran concentration, 2 mL plasma was obtained from the whole blood by centrifuging at 4°C and 3000 revolutions per minute for 10 minutes. The plasma samples were stored at -80°C until analysis. The dabigatran concentration was determined by liquid chromatography tandem mass spectrometry (LC-MS). The Agilent 1290 Infinity Liquid Chromatography system was coupled with the Agilent 6490 Triple Quadrupole LC-MS system (Agilent Technologies, USA), and a Poroshell 120EC-C18, $2.7\ \mu\text{m}$ ($2.1 \times 50\ \text{mm}$) column was used with a UHPLC Guard 3PK column, $2\ \mu\text{m}$ (Agilent Technologies, USA). An internal standard (apixaban-D3) and dabigatran were eluted under gradient conditions using a flow rate of 0.35 mL/min. The mobile phase consisted of ultra-pure water containing 0.1% of formic acid and acetonitrile containing 0.1% formic acid. Dabigatran was extracted from plasma using the protein precipitation method. The lowest limit of quantification (LLOQ) was 3 ng/mL; all measurements below this limit were recorded as zero. The intra-day and inter-day accuracy (%RE) and precision (%CV) of the method were assessed over 3 days, using quality control (QC), highest limit of quantification (HLOQ) and LLOQ samples. Guidance from the US Food and Drug Administration (FDA) states that accuracy and precision between runs should be within 15%.²⁴ Accordingly, measurements of %RE and %CV were less than $\pm 15\%$ for the QC, HLOQ and LLOQ samples. All calibration standards were linear ($R > 0.990$) over the concentration range of 3 ng/mL to 1000 ng/mL.

Statistics

The study hypothesis was that there is a linear relationship between CT in the ECA and trough plasma concentration of dabigatran, characterized by a Pearson correlation coefficient (r) ≥ 0.3 . Based on sample size formula for Pearson's correlation test, the minimum sample size required to reject the null hypothesis ($r < 0.3$), with $\alpha < 0.05$ and 80% power, is 84 patients.^{25,26} To allow for a dropout rate of 15%, we aimed to recruit 100 patients.

Results were analyzed for all patients, and with subgroup analysis by dabigatran dose (110 or 150 mg bid) and creatinine clearance (< 50 or ≥ 50 mL/min). The t-test was used to assess whether the degree of correlation between ECA CT and plasma dabigatran concentration reached statistical significance (significance level 0.05). The statistical significance of differences between subgroups was also determined (dabigatran dose,

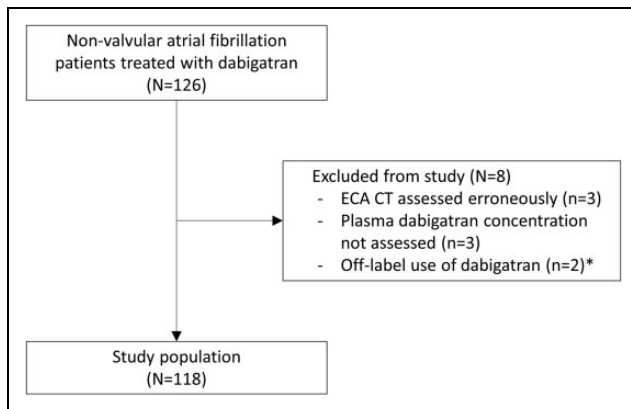


Figure 1. Patient disposition. *for apical thrombus.

Table 1. Patient Demographics and Baseline Characteristics.

Characteristic	N = 118
Age (Mean \pm SD)	69.7 \pm 9.7
Gender [male, n (%)]	70 (59.3)
Race; n (%)	
Malay	23 (19.5)
Chinese	58 (49.2)
Non-Malay Bumiputera	37 (31.4)
Cardiovascular risk factors; n (%)	
Hypertension	101 (85.6)
Congestive heart failure	23 (19.5)
Diabetes mellitus	46 (39.0)
Family history of cardiovascular disease	13 (11.0)
Smoking history	33 (27.9)
History of acute coronary syndrome	25 (21.2)
Prior venous thrombosis; n (%)	1 (0.8)
Prior stroke; n (%)	35 (29.7)
Prior hemorrhage; n (%)	12 (10.1)
Intracranial hemorrhage	2 (1.7)
Gastrointestinal bleed	5 (4.2)
Others	5 (4.2)
CHA ₂ DS ₂ -VASc score (Mean \pm SD)	3.86 \pm 1.40
HAS-BLED score (Mean \pm SD)	1.28 \pm 0.74

CHA₂DS₂-VASc, congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, sex category; HAS-BLED, hypertension, abnormal renal or liver function, stroke, history of or predisposition to bleeding, labile international normalized ratio, elderly age (>65 years), and drugs or alcohol; SD, standard deviation.

t-test; creatinine clearance, Mann-Whitney U test; significance level 0.05).

Results

A total of 126 NVAf patients receiving dabigatran treatment attended the study center during the study period. Eight of these patients were excluded, resulting in a study population of 118 patients (Figure 1). Sixty-four study participants were being treated with dabigatran 110 mg twice daily, and the remaining 54 were receiving the 150 mg dose. The mean age of the study population was 69.7 years, and 59.3% of the study

participants were male (Table 1). Most of the patients (85.6%) had hypertension; 29.7% had prior stroke, and 10.1% had prior hemorrhage. The mean congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, sex category (CHA₂DS₂-VASc) score was 3.86.

In the whole study population, the mean trough plasma dabigatran concentration (\pm standard deviation [SD]) was 59.8 \pm 50.9 ng/mL. This corresponded to a mean ECA CT of 361 \pm 210 seconds. As illustrated in Figure 2, ECA CT was moderately correlated with trough plasma dabigatran concentration ($r = 0.80$, $p < 0.001$).

Slight trends toward increased trough plasma dabigatran concentration and prolonged ECA CT were apparent with the 150 mg dose of dabigatran compared with the 110 mg dose (Figure 3). However, the differences were modest and not statistically significant. Creatinine clearance was measured in 73 of the study participants. Individuals with creatinine clearance below 50 mL/minute had a significantly higher mean trough plasma dabigatran concentration than those with creatinine clearance of 50 mL/minute or higher (70.2 vs 43.9 ng/mL, $p = 0.025$; Figure 3). Accordingly, ECA CT was significantly prolonged among patients with creatinine clearance below 50 mL/minute (mean 408.2 vs 277.8 seconds, $p = 0.008$).

Discussion

This study demonstrates that ClotPro ECA CT is sensitive to the changes in plasma concentration of dabigatran that occur in patients with NVAf. These findings suggest that the ECA could potentially be used to assess the coagulation status of patients receiving dabigatran therapy. Application of the ECA to direct thrombin inhibitors other than dabigatran is also possible, although studies are needed for confirmation. The importance of the current findings is underlined by the fact that “traditional” viscoelastic coagulation monitoring assays were not designed to measure the effects of dabigatran or other DOACs.²⁷ The range of factors that can affect the results of “traditional” assays limits their use in measuring the effects of dabigatran on coagulation status. Quantitative laboratory tests enable DOAC levels to be determined accurately, but these are more time-consuming than viscoelastic tests, and multiple assessments would be needed to identify an unknown drug.^{28,29}

As with other viscoelastic coagulation monitoring devices, ClotPro assays can be performed at the point of care, minimizing the delay before results are available. The “active tip” format of the ClotPro device facilitates an uncomplicated testing procedure, with no requirement for reagent handling by the device user.^{20,21} As well as increasing the speed of testing, this format also improves accuracy compared with manually configured assays. The current generation of TEG and ROTEM devices (TEG 6s and ROTEM sigma) have similarly increased the degree of automation with viscoelastic testing. The TEG 6s device may also provide an option for assessing DOAC anticoagulation. Studies have been performed with a developmental TEG 6s cartridge, where one channel with ecarin enables

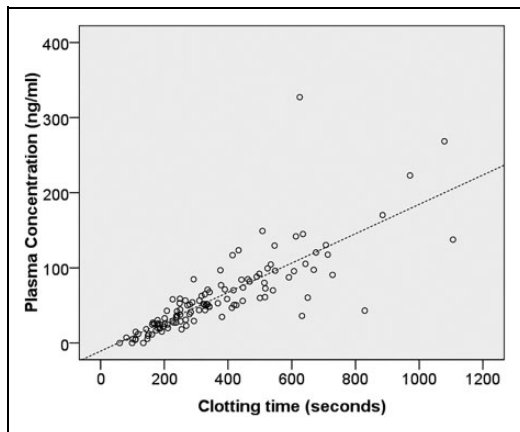


Figure 2. Correlation between clotting time in the Ecarin clotting assay (ECA CT) and trough plasma concentration of dabigatran. Pooled data from both doses of dabigatran (110 and 150 mg twice daily) are presented.

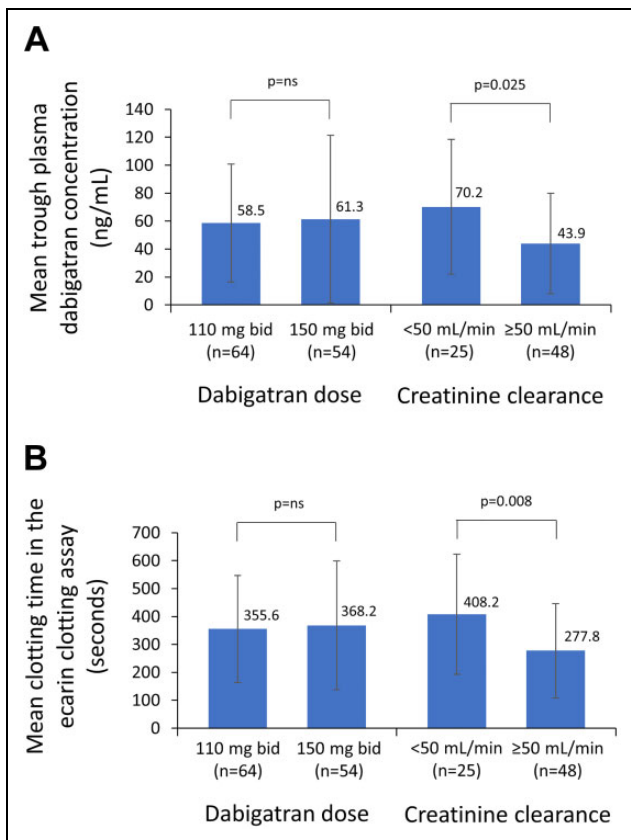


Figure 3. Stratification of patients by dabigatran dose and creatinine clearance: effects on trough plasma dabigatran concentration (A) and clotting time in the Ecarin clotting assay (B). Error bars represent standard deviation. bid, twice daily; ns, not significant.

detection of dabigatran and another channel allows detection of FXa inhibitors.^{18,30} TEG 6 s and ROTEM sigma each have 4 channels. With ClotPro, there are 6 channels and therefore a larger number of tests can be performed simultaneously. This could be clinically beneficial, for example when a patient is

first admitted to hospital a more comprehensive initial assessment may be performed. Similar measurement of “standard” viscoelastic parameters, relating to the speed and strength of clot formation following extrinsic or intrinsic activation, is possible using any of TEG 6s, ROTEM sigma or ClotPro. Studies to compare the performance of these devices may be informative and, for detection and identification of DOACs, comparison of ClotPro versus TEG 6s would be of particular interest.

Limitations of the current study include the lack of a control group, lack of measurement of sensitivity or specificity, the availability of only 1 ECA reading per patient (meaning that reproducibility of ECA CT readings was not assessed), and the lack of comparison with an alternative viscoelastic testing device. In addition, results from ClotPro assays other than ECA were not collected or analyzed. Study strengths include the real-world setting, meaning that the range of dabigatran concentrations was clinically relevant and that the results are applicable to routine clinical practice. Another strength was the inclusion in our study of laboratory-based measurement of plasma dabigatran concentrations. This enabled definitive assessment of the accuracy of the ECA data.

Conclusion

This preliminary study has demonstrated that CT in the ClotPro ECA reflects the plasma concentration of dabigatran in patients with NVAF. The ECA assay could potentially be used for real-time assessment of the impact of dabigatran on a patient’s coagulation status. Further investigations of ClotPro in the detection of dabigatran and other DOACs, including comparison with other viscoelastic testing devices, are awaited with interest.

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