

# Dabigatran Monitoring Was Influenced by Thrombin Time Reagent With Different Thrombin Concentrations

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## Abstract

To describe the effect of dabigatran on thrombin time (TT) reagents at different concentrations of thrombin. Pooled normal plasma enriched with dabigatran was dissolved in dimethylsulfoxide (DMSO) at concentrations of 0, 20, 50, 100, 200, 300, and 500 ng/mL. Samples with each concentration were evaluated using a semiautomatic coagulation analyzer to assess the effect of dabigatran on internal normalized ratio (INR), thromboplastin time (APTT), and TT, which were purchased from Instrument Laboratory (IL), Sysmex (SYS), and Stago (STA), respectively. Regarding INR, no reagent showed good sensitivity to increasing concentration of dabigatran, despite all reagents showing good linear response curves ( $P = .012$ ). Regarding APTT, all reagents had low sensitivity to increasing dabigatran concentration, but SYS-APTT showed a better linear response curve ( $P = .001$ ). Regarding TT, all reagents had a good linear response to the concentration of dabigatran; however, SYS-TT was very sensitive at low concentrations of dabigatran (0-100 ng/mL), while IL (TT-5 mL) and STA-TT were sensitive at medium concentrations of dabigatran (0-300 ng/mL), and IL (TT-2 mL) was less sensitive for a wide concentration of dabigatran (0-500 ng/mL;  $P = .007$ ). Internal normalized ratio and APTT showed low sensitivity and SYS-TT showed high sensitivity to concentrations of dabigatran that were unsuitable to monitor. Both IL (TT-5 mL) and STA-TT were useful at medium concentrations of dabigatran by semiautomatic coagulation analyzer, which calculated results using the end point method of coagulation. Instrument Laboratory (TT-2 mL), which contains a higher concentration of thrombin, had better sensitivity to the concentration of dabigatran than APTT and was suitable for routine monitoring by an automatic analyzer.

## Keywords

anticoagulants, bleeding, thrombosis

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## Introduction

Dabigatran etexilate is an oral direct anticoagulant and a direct thrombin inhibitor.<sup>1,2</sup> Dabigatran is frequently used to prevent nonvalvular atrial fibrillation, thromboprophylaxis in knee and hip replacement surgery, and for the treatment as well as secondary prevention of venous thromboembolic disease.<sup>2</sup> Dabigatran etexilate has a wide therapeutic window and can be pharmacokinetically assessed without any kind of monitoring.<sup>3</sup> However, there are several clinical situations where determination of the level of anticoagulation may be of value, such as patients with massive hemorrhage or thrombosis, or excessive anticoagulation treatment.<sup>2</sup> In addition, reasonable monitoring (thromboplastin time [APTT]/thrombin time [TT]/ecarin clotting time [ECT]) can ensure blood concentrations of dabigatran within the normal range before operation, especially of patient with renal impairment.<sup>4</sup>

There are many coagulation screening assays to monitor dabigatran, such as APTT, prothrombin time (PT), TT, diluted thrombin time (dTT), and ECT. Ecarin clotting time and dTT are sensitive to the blood concentration of dabigatran; however, they are not routine laboratory screening assays.<sup>2,5</sup> Dager et al<sup>6</sup> found that APTT and internal normalized ratio (INR) showed a

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**Table 1.** Results of Thromboplastin Time (APTT), Thrombin Time (TT), Internal Normalized Ratio (INR) for Different Concentrations of Dabigatran.<sup>a</sup>

Dabigatran (ng/mL)	APTT-IL (s)	APTT-SYS (s)	APTT-STA (s)	INR-IL	INR-SYS	INR-STA	TT-IL-2 mL (s)	TT-SYS (s)	TT-STA (s)	TT-IL-5 mL (s)
0	39.3 (1.0)	30.4 (1.7)	34.7 (0.9)	1.02 (0.01)	1.01 (0.01)	1.03 (0.01)	8.9 (0.4)	20.9 (1.5)	19.4 (0.2)	17.4 (0.5)
20	62.0 (1.5)	33.5 (1.9)	43.6 (1.6)	1.11 (0.02)	1.08 (0.02)	1.06 (0.01)	31.9 (1.2)	76.2 (1.4)	57.3 (2.6)	51.2 (2.1)
50	72.0 (2.7)	42.1 (1.8)	54.3 (2.1)	1.17 (0.01)	1.14 (0.01)	1.36 (0.01)	86.5 (1.7)	189.5 (2.2)	128.2 (1.8)	105.2 (1.8)
100	84.1 (2.9)	51.5 (3.0)	64.2 (1.7)	1.26 (0.02)	1.23 (0.02)	1.63 (0.01)	149.2 (2.5)	331.8 (2.3)	200.3 (2.9)	174.1 (3.0)
200	105.9 (2.3)	58.6 (1.1)	78.5 (1.2)	1.47 (0.02)	1.41 (0.01)	2.01 (0.01)	202.8 (2.0)	>400	262.5 (1.9)	220.1 (2.2)
300	119.7 (2.7)	65.4 (2.2)	80.5 (2.3)	1.62 (0.01)	1.55 (0.02)	2.31 (0.01)	261.7 (2.7)	>400	334.4 (2.8)	305.6 (2.5)
500	128.0 (2.4)	80.2 (2.1)	101.8 (2.5)	2.39 (0.02)	1.79 (0.02)	2.78 (0.02)	349.1 (2.4)	>400	>400	>400
		P = .001			P = .012			P = .007		

Abbreviations: IL, Instrument Laboratory; STA, Stago; SYS, Sysmex.

<sup>a</sup>>400 seconds was the upper limit.

linear response to dabigatran concentration without enough sensitivity, and that TT was extremely sensitive to the changes in dabigatran concentration, but they did not have enough data to analyze TT because of the limitations of the detection range in automatic coagulant analyzers. The basic reaction principle for PT, APTT, and TT is blood coagulation. However, analyzers have different methods to generate data, for example, analyzers from SYS and Instrument Laboratory (IL) obtain the results via immunoturbidimetry, whereas the analyzer from STA uses coagulation as the end point (Golden Standard). Due to these method differences and the variation of detection levels among analyzers, the correlations between dabigatran's plasma levels and their impact on clotting assays remain unclear. The semiautomatic coagulation analyzer (STA-R Evolution) is quite different. In contrast to automatic coagulation analyzers, STA-R, which obtains results using the end point method of coagulation, can detect the result over a long range of time, allowing the selection of better reagents for monitoring dabigatran.

The half-life of blood dabigatran in adults is reportedly 14 to 17 hours; in healthy males receiving a single 150 mg dose, the peak blood concentration is 90 to 130 ng/mL, which is maintained for 1 to 4 hours.<sup>7</sup> The RELY Trial indicated that the blood concentration of dabigatran in 80% of patients was 22.9 to 238.3 ng/mL. We prepared several samples with concentrations of dabigatran between 0 and 500 ng/mL, and we aimed to analyze the results of APTT, TT, and INR using a semiautomatic coagulate analyzer. We then aimed to further elucidate the relationship between the thrombin concentration of different TT reagents and blood concentrations of dabigatran.

## Materials and Methods

### Materials

Dabigatran etexilate (Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany) was bought from a pharmacy. Pooled normal plasma was provided by Stago (Lot No: 00539, Diagnostica Stago, Asnières sur Seine, France). Dimethylsulfoxide was obtained from Sagan Corporation (Shanghai, China). The core reagents (PT, APTT, TT) were obtained from the 3

companies: SYS from Japan, STA from France and IL from United States. Importantly, the TT reagent from IL has 2 concentrations of thrombin (IL-5 mL: 3.0 UNIH/ mL and IL-2 mL: 7.5 UNIH/mL). The thrombin concentration of SYS-TT was 1.15 UNIH/mL and the thrombin concentration of STA was 1.5 UNIH/mL. The STA-R was provided by STA.

### Methods

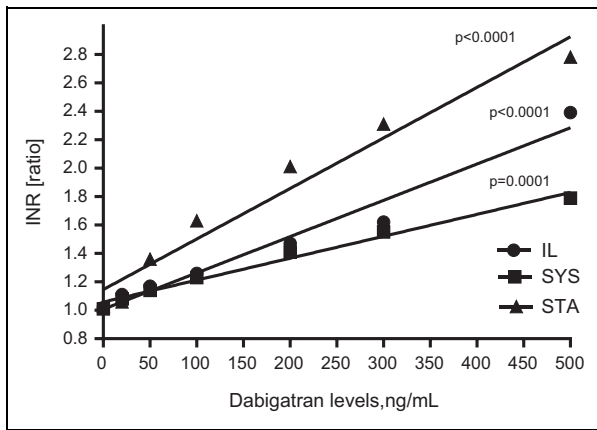
**Samples with different concentrations of Dabigatran.** Pooled normal plasma was dissolved in 1-mL distilled water at room temperature for 30 minutes. A capsule of dabigatran etexilate with the capsule shell removed was dissolved in 2.2 mL of dimethylsulfoxide to form the stock solution (50 mg/mL). Prepared mixed plasma was diluted to final concentrations of 0, 20, 50, 100, 200, 300, and 500 ng/mL.

**Samples measurement.** According to the instructions of the reagents (PT: SYS, Lot No-546810; STA, Lot No-111649; IL, Lot No-0020003050. APTT: SYS, Lot No-557126B; STA, Lot No-112290; IL, Lot No-0020006800. TT: SYS, Lot No-504485; STA, Lot No-111597; IL, Lot No-0009758515) and the operating procedure of STA-R, all of the samples with different drug concentrations were analyzed to detect the time of blood coagulation, with each sample being measured 3 times and the actual values reflect the mean.

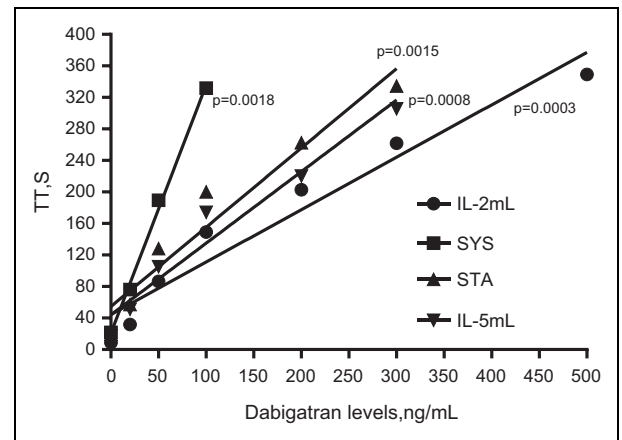
**Statistical analyses.** The relationships between the results of reagents from different companies and dabigatran concentrations were expressed by the Pearson correlation analysis. The coagulation assays were compared using the Friedman test, with  $P < .05$  considered to be significant. Data analysis was performed using SPSS.

### Results

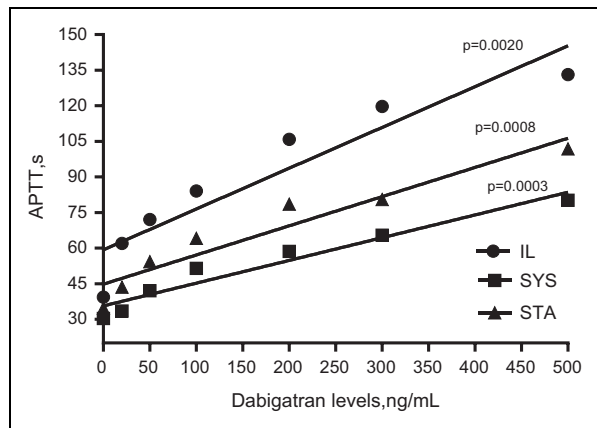
The reagents PT, APTT and TT, selected from 3 different companies and the STA-R Evolution, were employed to assay the relationship between coagulation test items and the blood



**Figure 1.** Internal normalized ratio detected by reagents of 3 companies with increasing concentrations of dabigatran. Correlation (slope): IL, 0.9690 (0.0026); SYS, 0.9853 (0.0015); and STA, 0.9579 (0.0036). IL indicates Instrument Laboratory; STA, Stago; SYS, Sysmex.



**Figure 3.** Results of thrombin time (TT) detected by reagents of 3 companies with increasing concentrations of dabigatran. Correlation (slope): IL-2 mL, 0.9438 (0.67); SYS, 0.9964 (3.15); STA, 0.9368 (1.0); and IL-5 mL, 0.9530 (0.91). APTT indicates thromboplastin time; IL, Instrument Laboratory; STA, Stago; SYS, Sysmex.



**Figure 2.** Results of APTT detected by reagents of 3 companies with increasing concentrations of dabigatran. Correlation (slope): IL, 0.8732 (0.17); SYS, 0.9414 (0.10); and STA, 0.9138 (0.12). APTT indicates thromboplastin time; IL, Instrument Laboratory; STA, Stago; SYS, Sysmex.

concentration of dabigatran. We evaluated the related data from Table 1.

### Internal Normalized Ratio

The results of INR show that the STA-PT was more sensitive to the changes in the blood concentration of dabigatran than SYS-PT and IL-PT (Figure 1). All reagents had a good linear response curve. The distribution of INR results from different companies with increasing concentration of dabigatran was significantly different ( $P = .012$ ).

### Thromboplastin Time

Figure 2 shows that the STA-APTT reagent had the best sensitivity and correlation with changes in the blood concentration of dabigatran. The IL reagent sensitivity was medium and SYS

reagent sensitivity was lower, but its correlation was better than that of IL reagent. The distribution of APTT results from different companies with increasing concentration of dabigatran was significantly different ( $P = .001$ ).

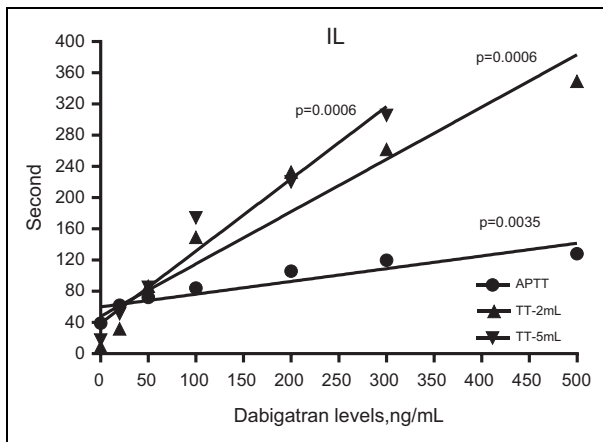
### Thrombin Time

Instrument Laboratory-TT reagents include TT-5 mL and TT-2 mL, representing different thrombin concentrations. Table 1 shows that TT-5 mL and STA-TT could detect concentrations of dabigatran within 300 ng/mL, whereas TT-2 mL could detect concentrations of dabigatran within 500 ng/mL, and SYS-TT could detect concentrations of dabigatran within 100 ng/mL. Figure 3 shows that SYS-TT is most sensitive to changes in the blood concentration of dabigatran, with TT-5 mL and STA-TT being medium sensitive and then TT-2 mL being least sensitive. Notably, the 4 reagents showed good linear correlation. The distribution of TT results from different companies with increasing concentration of dabigatran was significantly different ( $P = .007$ ).

In a side-by-side comparison of the 3 IL detection methods (Figure 4), APTT sensitivity was low and TT-5 mL was very sensitive; when the blood concentration exceeded 300 ng/mL, the detection value was greater than 400 seconds. Additionally, IL (TT-2 mL) could detect the blood concentration within 500 ng/mL with medium sensitivity. All parameters showed good linear correlation.

### Discussion

The standard oral dosage of dabigatran etexilate is 110 mg or 150 mg, twice per day. The blood concentration of dabigatran is stable without routine coagulation monitoring.<sup>8</sup> However, for some patients with renal insufficiency, as well as for elderly patients and those at risk of stroke, 110 mg dabigatran twice daily can lead to an overdose. To prevent damage to renal



**Figure 4.** Results of TT-2 mL, TT-5 mL, and APTT detected by reagents from IL with increasing concentrations of dabigatran. Correlation (slope): IL-2 mL, 0.9222 (0.67); IL-5 mL, 0.9597 (0.93); and APTT, 0.8427 (0.16). APTT indicates thromboplastin time; IL, Instrument Laboratory; TT, thrombin time.

function and increased risk of bleeding, some hospitals of European countries have chosen to use 75-mg dabigatran twice daily.<sup>9</sup> Therefore, monitoring of the blood concentration of dabigatran is critical for some patients. Although ECT and dTT are suitable for the monitoring of dabigatran, they are not recognized by the United States Food and Drug Administration; thus, they are not widely used for clinical monitoring.<sup>5</sup>

William et al<sup>6</sup> stated that although INR and APTT showed a good linear relationship with the blood concentration of dabigatran, their sensitivity was poor, and it was unclear whether these parameters could be used for monitoring. William et al randomly sent prepared blood samples to laboratories in South America and the United Kingdom for testing and statistical analysis. Considering the inconsistent results by different analyzers, some interference caused by delivering samples, and the narrow range of blood concentration of dabigatran, we have made some improvements to make clear relationship between reagents and dabigatran. First, we chose reagents from 3 companies popularly used in clinical thrombosis and hemostasis laboratories. Second, we applied the semiautomatic coagulator STA-R as the detection analyzer. The STA-R can detect the result over a long range of time at higher concentrations of dabigatran and using the end point method of coagulation potentially eliminating some detection interference. Finally, we prepared samples with a wide range of blood concentrations of dabigatran and analyzed them within the suitable time and temperature.

Internal normalized ratio was less sensitive to blood concentrations. Stago-INR increased by 0.1 to 0.3 per 50 ng/mL and was less sensitive to low blood concentrations. If the blood concentration of dabigatran had no large shift, it was very hard to recognize changes of the results. Van et al<sup>10</sup> analyzed the reason behind INR producing high results in point-of-care (POC) equipment. Both POC-INR and direct INR

measurements were not helpful for dabigatran monitoring, whereas APTT was appropriate. In a comparison among the 3 APTT reagents, STA-APTT had the highest sensitivity and showed a good linear relationship with the blood concentration of dabigatran ( $P = .001$ ). Dubé et al<sup>11</sup> compared the sensitivity and specificity of 5 different APTT reagents with TT and dTT reagents. Although APTT showed good sensitivity, its specificity was very poor for low blood concentrations. So APTT is not a good choice in monitoring the blood concentration of dabigatran.

The semiautomatic coagulation analyzer was better in assays, resulting in high TT results. Sysmex-TT was highly sensitive: when the blood concentration of dabigatran exceeded 100 ng/mL, the detection value was >400 seconds. The SYS-TT reagent, which contained thrombin at approximately 1.15 UNIH/mL, suffered the greatest interference by dabigatran, which was at the lowest concentration among 4 TT reagents. The sensitivity and correlation measurements of IL (TT-5 mL) and STA-TT were similar. When the blood concentration reached 500 ng/mL, the detection values were >400 seconds. However, the thrombin concentrations of IL (TT-5 mL) and STA-TT were 3.0 UNIH/mL and 1.5 UNIH/mL, respectively. As shown in Table 1, the results of IL (TT-5 mL) were lower than those of STA-TT in low blood concentrations of dabigatran, which were determined by residual thrombin concentration. When the thrombin concentration increased, the difference of TT results between them was not obvious, and the detection values exceeded 400 seconds when the concentration reached 500 ng/mL. In comparison, IL (TT-5 mL) with a high thrombin concentration was optimal for monitoring. With the increase of blood drug concentration of dabigatran, IL (TT-2 mL) retained a high correlation, and the sensitivity was much higher than APTT.

According to the automatic coagulation analyzer, Avecilla et al<sup>12</sup> prepared dTT reagent, which contains 0.75 UNIH/mL of thrombin, to reduce sensitivity to dabigatran. Compared to APTT and TT, dTT showed monitoring advantages. The RELY Trial indicated that the blood concentration of 10% to 90% dabigatran was 22.9 to 238.3 ng/mL. The upper limit of TT detection of SYS and STA analyzers is approximately 200 seconds, whereas that of the IL analyzer is 300 seconds. Instrument Laboratory-2 mL contained a thrombin concentration of 7.5 UNIH/mL, the residual thrombin would have a reaction with prothrombin after combining with dabigatran, but the TT results had a good sensitivity and correlation with the concentration of dabigatran. Using various coagulation reagents for detection on a semiautomatic coagulation analyzer and using the end point method of coagulation to interpret the results, this study facilitated analysis of the differences of various reagents. The end point method of coagulation remains the golden standard. The semiautomatic coagulation analyzer is suitable for anticoagulant monitoring when an automatic coagulation analyzer cannot meet the requirements for anticoagulant monitoring.

Importantly, it is difficult to obtain samples of patients with different blood concentrations of dabigatran; thus, this was an

in vitro test, and it is not clear whether dabigatran monitoring and potential dose adjustments have any impact on clinical outcome. However, it is meaningful to monitor the blood concentration of dabigatran in consideration of thrombin concentration from different TT reagents.

As some actual situations in the laboratory, and we did not consider the problem of lot-to-lot variability, we obtained reagent with one lot number to measure each blood concentration of dabigatran, however, we tested each blood concentration of dabigatran for 3 times, the results of our study could reflect potential outliers.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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