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Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study

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Dear Editor,

Guidance regarding dabigatran monitoring has been published [1,2], but the specific assays are not all targeted to low levels of dabigatran encountered in the preoperative setting. For example, Hemoclot® Thrombin Inhibitor (HTI) and the ecarin chromogenic assay (ECA) have a limit of detection and quantification (LOD and LOQ) between 30 and 50 ng/ml [3–5]. In our recent study, a modified HTI (HTI LOW) and ECA (STA®-ECA II) adapted for low dabigatran concentrations showed better performances than conventional HTI to assess plasma dabigatran concentrations below 50 ng/ml [6], but these assays are not widely available. The activated partial thromboplastin time should not be used to assess dabigatran in plasma as it has only a modest correlation with dabigatran levels and is affected by factor deficiencies, lupus anticoagulant or elevated FVIII in inflammatory conditions [7].

Some recent publications have suggested that thrombin time (TT) could be useful to assess the presence of dabigatran to guide the periprocedural management [8,9]. However, TT is affected by many variables [10] and there is a lack of standardization between laboratories [11].

Therefore, we decided to analyse TT following validation methods published by *Marlar et al.* and *Chandler* [12,13] in plasma samples containing dabigatran. We assessed the TT of different spiked dabigatran concentrations with intra- and inter-assay precision, its stability in time (at 2, 4 and 24 hours) and at different temperatures (room temperature and 4 °C), its limit of detection (LOD) and quantification (LOQ) for dabigatran, the linearity of the results, and finally, the sensitivity of TT to different spiked concentrations of unfractionated heparin (UFH) (Leo Pharma, Ballerup, Denmark) and enoxaparin (Clexane®, Sanofi-Aventis, Diegem, Belgium) compared to HTI. Afterwards, we analysed TT in 24 plasma samples from dabigatran treated patients expected to be in the low ranges.

The stability of TT was compared using the Wilcoxon signed-rank test. We used Medcalc software version 6.0 for Windows® and GraphPad Prism® version 6.0 for Mac OSx®.

The study was approved by the Medical Ethical Committee of the CHU Dinant Godinne UcL Namur (BU3920096633). We prepared normal pool plasma (NPP) and platelet-poor-plasma (PPP) samples and stored them as described previously [1]. Dabigatran was purchased from Alsachim® (Strasbourg, France) and spiked at increasing concentrations (0, 10, 20, 30, 40, 50, 100 and 200 ng/ml) in NPP as described previously [1]. These concentrations were designed to cover preprocedural ranges (from 0 to 50 ng/ml) [14,15] and trough therapeutic ranges (from 50 to 200 ng/ml). The limits of the trough therapeutic range represent the range in which ischemic stroke/systemic embolic events and major bleeding events are lowest [16]. Two thrombin reagents were tested (a bovine thrombin: HemosIL® TT, Instrumentation Laboratory, Lexington, KY, USA, and a human thrombin: STA®-Thrombin, Diagnostica Stago, Asnière, France) on two coagulometers (STA-R® Evolution and ACL TOP®700). We prepared 6 different concentrations of bovine thrombin (3.33, 3.75, 3.90, 4.05, 4.28 and 5.00 NIH/ml) and 4 different concentrations of human thrombin (1.36, 1.50, 1.76 and 2.00 NIH/ml). We defined the optimal thrombin concentration (OTC) for three arbitrarily selected conditions, as the minimal concentration that gave a reproducible TT:

- less than 120 seconds at 50 ng/ml;
- less than 300 seconds at 200 ng/ml;
- and higher than the minimum TT measurable with the coagulometer at 0 ng/ml of dabigatran (e.g. > 8 seconds for ACL TOP®).

We set T_{MAX} at 300 seconds (instead of the recommended 120 seconds on STA-R® and 100 seconds on ACL TOP®) to allow measurement of dabigatran concentration up to 200 ng/ml. This was technically not possible to achieve for the combination STA®-Thrombin on ACL TOP®.

As shown, in Fig. 1. the OTCs were 1.76 NIH/ml for STA-R® with STA®-Thrombin, 4.28 NIH/ml for STA-R® with HemosIL® TT, 3.75 NIH/ml for ACL TOP® with HemosIL® TT and we found no OTC for ACL TOP® with STA®-Thrombin (T_{MAX} = 100 seconds). The type of coagulometer (different mechanism of clot detection, i.e. mechanical (STA-R®) versus optical (ACL TOP®) clot detection) and, especially, the thrombin origin were two important variables. We decided afterwards to use a homogeneous system for the validation methods and chose two OTCs for each coagulometer (1.50 and 1.76 NIH/ml of STA®-Thrombin for STA-R®; 3.75 and 3.90 NIH/ml of HemosIL® TT for ACL TOP®). For STA®-Thrombin, the concentration recommended by the manufacturer is 1.50 NIH/ml. At this thrombin concentration, TT was sometimes > 120 seconds for dabigatran concentrations of 50 ng/ml, so that it failed to respect *one* of the OTC's arbitrary conditions, but for routine facilities we tested it further. For HemosIL® TT, the manufacturer's recommendations (1.9, 3.0 and 7.5 NIH/ml) were never optimal following *our* arbitrary definitions (preliminary tests not shown).

Abbreviations: HTI, Hemoclot Thombin Inhibitors®; ECA, Ecarin chromogenic assay; LOD, Limit of detection; LOQ, Limit of quantification; TT, Thrombin time; UFH, Unfractionated heparin; NPP, Normal pool plasma; PPP, Platelet-poor-plasma; NIH/ml, National Institutes of Health (NIH) units per ml; OTC, Optimal thrombin concentration; T_{MAX}, Limit of measurement of thrombin time; CV%, Coefficient of variation expressed in percentage; SD, Standard deviation.

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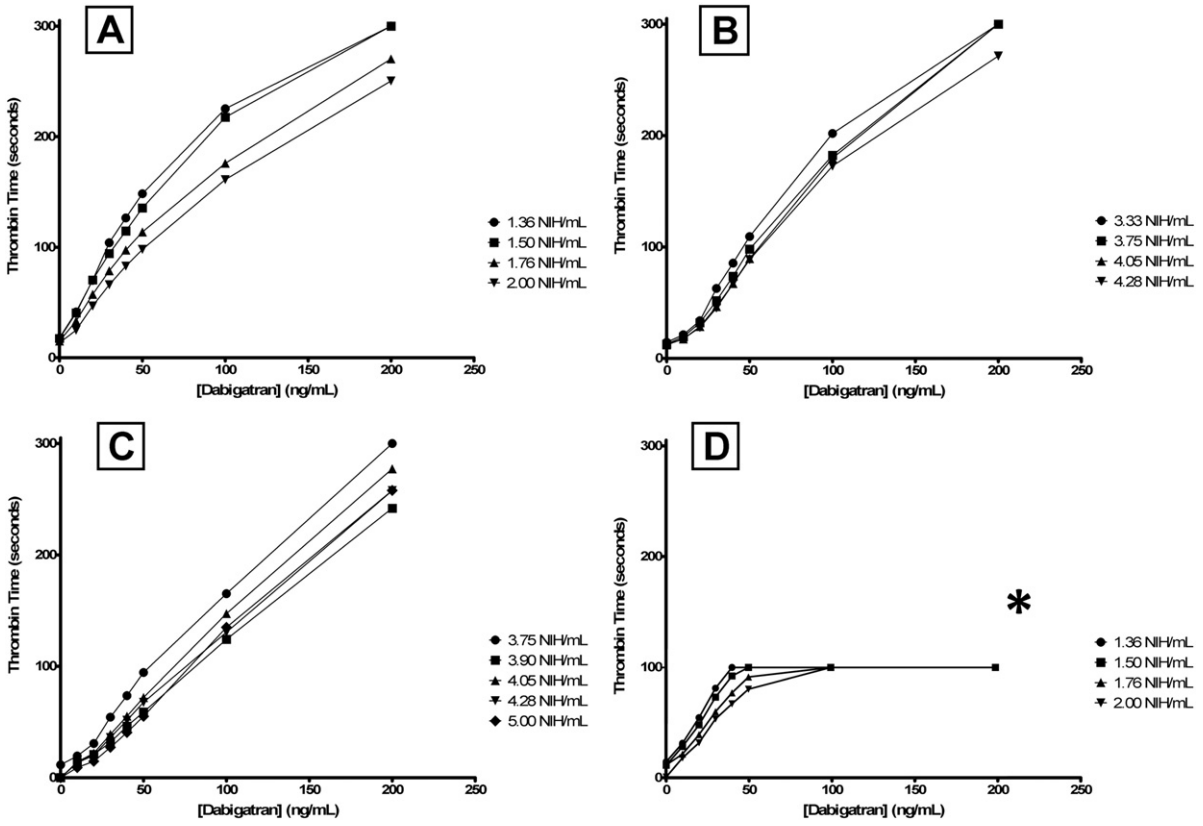

Fig. 1. Optimization of thrombin time on different coagulometers and with different reagents. A) STA-R Evolution®, STA®-Thrombin; B) STA-R Evolution®, HemosIL® TT; C) ACL TOP®700, HemosIL® TT; D) ACL TOP®700, STA®-Thrombin. Thrombin times < 8 seconds were placed at zero in images C and D. Thrombin time > 300 seconds (A-C) or 100 seconds (D)* were set at 300 and 100 seconds, respectively.

Our repeatability experiments showed an acceptable variability (below 10% for intra-assays and below 12% for inter-assays) [12]. ACL TOP® with HemosIL® TT showed higher CV% than STA-R® with STA® Thrombin. This may be explained by the mechanism of clot detection. Mechanical clot detection (STA-R®) may be preferable to optical clot detection (ACL TOP®) due to its increased sensitivity [17] and greater precision [18].

Stability experiments were tested for one OTC on each coagulometer. A slight increase of TT appeared after 2 hours of storage at room temperature. For 1.50 NIH/ml on STA-R®, the stability tests showed a statistically significant increase in TT for all dabigatran concentrations when plasma samples were stored at room temperature for 4 or 24 hours. For plasma samples stored at 4 °C, we observed statistically and clinically significant increases in TT for samples with dabigatran concentrations > 30 ng/ml that were stored for 24 hours.

For 3.75 NIH/ml on ACL TOP®, only plasma samples stored at room temperature showed a statistically and clinically significant increase in TT for dabigatran concentrations > 30 ng/ml and stored for 24 hours.

The stability for the different dabigatran concentrations at room temperature and 4 °C is shown in Fig. 2. This time-related increase in TT may be due to platelet and soluble coagulation factor activation [19]. These findings should be validated on samples from patients treated with dabigatran. We suggest to measure TT as soon as possible (within 2 hours of sampling) in plasma containing dabigatran. If this is not possible, samples should be rapidly stored at 4 °C.

We tested TT and HTI in the presence of UFH and enoxaparin both in NPP at 0, 0.15, 0.30, 0.60, 0.90, 1.20, 1.50 and 2.00 UI/ml. Thrombin time was above the reference range at a concentration of 0.15 UI/ml of UFH and 0.30 UI/ml of enoxaparin whereas the HTI gave a false positive result at 0.60 UI/ml of UFH and 1.20 UI/ml of enoxaparin on STA-R®. The aim of this comparison was to show the differences in sensitivity of TT to two different heparins and to underline the limit of HTI in patients bridged with heparin. A commercially available heparinase can be added to the blood sample to detect the presence of heparin [20].

Limit of detection and LOQ were lower than 0.9 and 3.0 ng/ml respectively for STA®-Thrombin on STA-R®, and lower than 2.2 and 7.2 ng/ml for HemosIL® TT on ACL TOP®. As stated in previous publications [1,9], TT is a reliable test to exclude clinically relevant dabigatran presence in the blood.

Following the linear regressions presented in Table 1, TT measured with STA®-Thrombin concentration proposed by the manufacturer (1.5 NIH/ml) can also detect dabigatran concentrations < 50 ng/ml (R-square = 0.97). In addition, optimised HemosIL® TT on ACL TOP® is a reliable qualitative test for dabigatran concentrations up to 200 ng/ml (R-squares = 0.98), which is *not* the case for STA®-Thrombin on STA-R® (R-squares = 0.78 and 0.75 for 1.5 NIH/ml and 1.76 NIH/ml respectively). Therefore, we do not support the suggestion of *Chin et al.* [21] to use TT to assess trough dabigatran concentrations and adjust treatment doses. Furthermore, other studies are needed to validate the improvement in outcomes with dose adjustment related to plasma levels and patient characteristics [16,22].

Table 1
Linearity of thrombin time and dabigatran concentrations following optimized thrombin concentrations.

	1.50 NIH/ml STA®-Thrombin STA-R® Evolution	1.76 NIH/ml STA®-Thrombin STA-R® Evolution	3.75 NIH/ml HemosIL® TT ACL TOP®700	3.90 NIH/ml HemosIL® TT ACL TOP®700
For dabigatran concentrations: 0, 30, 50, 100 and 200 ng/mL				
R-square	0.78	0.75	0.98	0.98
For dabigatran concentrations: 0, 30 and 50 ng/ml				
R-square	0.97	0.99	0.98	0.99