From laboratory to clinical practice: Dabigatran effects on thrombin generation and coagulation in patient samples

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ABSTRACT

INTRODUCTION

Dabigatran (Dabi) is not routinely monitored. However, in emergency cases quantitative assessment is required and laboratories must provide suitable tests at all hours. Little is known about Dabi effects on thrombin generation.

MATERIALS AND METHODS

Patient samples (n=241) were analyzed for functional Dabi concentrations (Dabi-TT) using a combination of the Hemoclot Thrombin Inhibitors assay (HTI®) and, for samples with low levels, undiluted thrombin time (TT). Results were compared to prothrombin time (PT) and activated partial thromboplastin time (APTT). In 49 samples Dabi effects were further investigated with Calibrated Automated Thrombogram (CAT®) for thrombin generation and with Russell's viper venom time (RVVT), prothrombinase-induced clotting time (PiCT®), chromogenic Anti-IIa® and ecarin clotting assay (ECA®). Fibrinogen and D dimer were assessed to reflect the coagulation status of the patient. A subset of these samples (n=21) were also analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

RESULTS

Dabi-TT correlated with RVVT (R^2 = 0.49), PiCT® (R^2= 0.73), ECA® (R^2 = 0.89), Anti-IIa® (R^2 =0.90) and LC-MS/MS (R^2 =0.81). APTT correlated curvi-linearly with Dabi-TT (R^2 =0.71), but was normal in many cases (18/70) despite Dabi-TT > 40 ng/mL. There was no association between Dabi-TT and fibrinogen or D dimer levels. Increasing Dabi concentrations prolonged lag time (R^2 =0.54) and, surprisingly, elevated the ETP and Peak of CAT® ($p < 0.001$).

CONCLUSIONS

Thrombin-specific tests measure Dabi accurately, whereas coagulation time based assays depend more on other factors. The enhanced thrombin generation in Dabi-treated patients may predict clinically relevant hypercoagulability and warrants further investigation.

Keywords:

Anticoagulants, Blood Coagulation Tests, Dabigatran Etexilate, Drug Monitoring, Thrombin Generation

Abbreviations:

Dabi, dabigatran; DTI, direct thrombin inhibitor; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; ECA, ecarin clotting assay; HTI: Hemoclot Thrombin Inhibitors; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PiCT, prothombinase-induced clotting time; FXa, activated coagulation factor X; RVV-V, Russel's viper venom V; RVVT, Russel's viper venom time; IIa, thrombin, Anti-IIa, anti-thrombin activity; PPP, platelet-poor plasma; Dabi-TT, functional dabigatran concentration; CAT, Calibrated Automated Thrombogram; ETP, endogenous thrombin potential

INTRODUCTION

Dabigatran etexilate (Pradaxa®, Boehringer Ingelheim; Dabi) is a direct thrombin inhibitor (DTI) anticoagulant. Due to what is considered predictable pharmacokinetics, routine laboratory monitoring is claimed to be unnecessary [1,2]. Nevertheless, under special circumstances, e.g. renal or hepatic dysfunction, acute bleeding complications or thrombosis, and emergency surgery, assessment of anticoagulation becomes necessary [3,4]. Coagulation laboratories must provide readily available (all hours) and practical tests for measurements of Dabi anticoagulation. In the absence of routine methodologies for assessments under clinically stable situations, it becomes challenging to evaluate anticoagulation during medical emergencies.

The screening tests prothrombin time (PT) and activated partial thromboplastin time (APTT) are of limited value [5-9]. To better assess Dabi effects, more sensitive methods must be used. Thrombin time (TT) is linear, highly sensitive, and can be calibrated with Dabi to depict its effects [10]. Chromogenic ecarin clotting assay (ECA®) uses the snake venom ecarin to generate meizothrombin, [11-13] with less interferences with, e.g., lupus anticoagulant and warfarin than clot-based assays [14]. TT calibrated with Dabi, Hemoclot® Thrombin Inhibitors (HTI®), and ECA® correlate well with actual Dabi concentrations in plasma as measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), whereas correlations with APTT are modest and with PT non-existent [8,9].

Prothrombinase-induced clotting time (PiCT®) uses activated coagulation factor X (FXa), phospholipids and Russel's viper venom V (RVV-V) for activation, leading to

prothrombinase complex activation and thrombin formation. Modified PiCT® has been used to measure both DTIs and FXa inhibitors [15]. Russell's viper venom time (RVVT) with a FX-specific activator is sensitive to FXa inhibitors, but might also be useful to assess DTIs [16]. The chromogenic anti-thrombin (IIa) activity (Anti-IIa®) assay detects DTIs. In this assay, excess thrombin is added to the sample and the amount of residual thrombin is measured. Again, interference is less pronounced than in clot-based assays [17].

Since most coagulation assays measure the time to fibrin formation (i.e., the initiation phase of coagulation), Dabi's many biological effects remain underestimated. Thrombin generation assays measuring the full spectrum of thrombin formation seem justified and potentially more informative [18]. DTIs have been reported to decrease thrombin formation, but the effects vary with different DTIs and are somewhat controversial [19].

Previously, we have studied the suitability and variability of coagulation assays using *in vitro* spiked plasma samples shipped to several European laboratories [7], and compared methods in anonymized patient samples [8,9]. The relationships between Dabi plasma concentrations by LC-MS/MS and the risks of suffering thromboembolic or major bleeding events in the RE-LY study were recently published, yielding an excellent basis for the interpretation of Dabi concentration data [20]. As patients referred for laboratory testing likely vary in their coagulation status, it is important to evaluate Dabi also in real life patient samples (i.e., beyond the selected trial patients with standardized sampling). Here, we aimed to assess how well different clotting assays detect Dabi in actual patient samples using indirect measurements by Dabi-calibrated TT (Dabi-TT) as a reference (where Dabi-TT is undiluted

thrombin time for dabigatran < 40 ng/mL, and HTI for dabigatran ≥ 40 ng/dL). The performance of the functional analysis was confirmed by gold standard measurements by LC-MS/MS in a subset of the samples. Our additional aim was to gain more insight into the impact of Dabi on thrombin generation.

MATERIALS AND METHODS

Study samples

Patient samples sent to the Meilahti hospital coagulation laboratory (HUSLAB Laboratory Services, Helsinki University Central Hospital, Finland) for Dabi concentration analysis were collected during a 5 year period (between 2008 and 2013). A total of 241 random plasma samples (from 85 patients) were accumulated. The specific clinical situation was not recorded, but at that time, Dabi was indicated for postoperative thromboprophylaxis after elective orthopedic surgery (150 or 220 mg once daily) and in patients with atrial fibrillation (either 150 or 110 mg twice daily). The Dabi concentration analysis was freely available in our hospital district with the recommendation to order the test only under special circumstances, such as major bleeding complications, thrombosis or emergency surgery and to order simultaneously PT and APTT. Blood samples were collected into sodium citrate anticoagulant (3.2%; 109 mM) tubes according to the local sampling protocol as part of hospital routine, centrifuged (at 2500 g for 15 min) and the platelet-poor plasma (PPP) was separated within 2 hours and stored at -80 °C before analysis.

Original Dabi concentration analysis supplemented with screening tests

Dabi concentrations were assessed using diluted, Dabi-calibrated TT with HTI® (Aniara) in the 241 stored samples, the analytical range being 40-1000 ng/mL (85-2120 nmol/L) with intra-assay and inter-assay CVs of 7 % and 10 %, respectively. The screening tests TT (BC Thrombin Reagent®, Siemens Healthcare Diagnostics), APTT (Actin FSL®, Siemens Healthcare Diagnostics) and PT (Nycotest PT®, Axis-Shield; Owren-type assay) were performed in parallel according to our routine hospital protocol. TT had a local reference range of 17-25 s and an analytical range of 12-140 s and APTT 23-33 s and 18-180 s, respectively. PT (standard human plasma, Siemens Healthcare Diagnostics) had a reference range of 19-24 s and an analytical range of 16-180 s. The analyses were performed using the BCS® XP automatic analyzer (Siemens Healthcare Diagnostics).

Since very low Dabi concentrations could not be measured using HTI® (with the the lower detection limit of 40 ng/mL), we needed to estimate undiluted TT values as Dabi concentrations: TT < 60 s was set to correspond to 0 ng/mL of Dabi; TT 60-100 s to 10 ng/mL; TT 100-120 s to 20 ng/mL and TT 120-140 s to 30 ng/mL. The decision of these arbitrary categories was based on the literature and our data; TT has been shown to be linear with Dabi [10] and here, all the samples with measurable quantities of Dabi using HTI®, had an undiluted TT > 60 s. TT values < 60 s were chosen to represent 0 ng/mL instead of the upper local reference range for TT (25 s), since TT is not specific for Dabi effects and the prolongation at TT values ≥ 60 s most likely reflect Dabi effects. We then combined the TT data (< 40 ng/mL) with HTI® data (≥ 40 ng/mL) to obtain functional estimates of Dabi concentrations (Dabi-TT) covering the entire concentration range.

Further analysis of Dabi with thrombin-specific assays

We further analyzed the stored patient samples and assessed them with a large panel of clotting assays and thrombin generation as described below. We included only samples with comprehensive results from all methods, i.e., a total of 49 samples (35 patients).

The chromogenic Anti-IIa® (Direct Thombin Inhibitor assay®, Siemens Healthcare Diagnostics), the chromogenic ECA® (Haemosys® ECA-T, JenAffin), the RVVT (DVVTest 10®, Sekisui Diagnostics) and the PiCT® (Pefakit® PiCT, Pentapharm) were evaluated regarding their potential to quantify Dabi. All analyses were performed using BCS® XP.

In the Anti-IIa® assay, 25 µL of sample was mixed with 50 µL of substrate and the reaction initiated with 250 µL of thrombin reagent. In the ECA®, 25 µL plasma sample was diluted with 100 µL ECA® prothrombin buffer and mixed with 25 µL ECA® substrate, incubated (37 ºC, 1 min), and the reaction was started with 50 µL of the ECA® ecarin reagent. Both the Anti-IIa® and the ECA® assays were calibrated for Dabi using calibrators from Aniara to achieve plasma Dabi concentrations of 0, 30, 250 and 510 ng/mL.

The RVVT, which is part of a screening panel in lupus anticoagulant testing, had the local upper normal reference value of 41 s. The PiCT® assay was performed using the 2-step protocol: 50 µL of sample was mixed with 50 µL of activator (containing RVV-V, FXa and phospholipids). At 180 s incubation 50 µL of 25 mM $CaCl_2$ was added and the clotting time measured. The PiCT® reagents induce antithrombin inhibition of FXa. The manufacturer reports a reference range of 19-31 s for PiCT®.

Thrombin generation was measured using Calibrated Automated Thrombogram® (CAT®, Diagnostica Stago) with the Stago PPP reagent (tissue factor 5 pM and phospholipids 4 µM) without corn-trypsin inhibitor. The lag time of the initiation of thrombin generation, and the endogenous thrombin potential (ETP), peak, time to peak, and time to the end of thrombin generation (start of tail) were measured according to the manufacturer's instructions. For comparison, ten plasma samples from three healthy volunteers were analyzed in parallel with the patient samples. These healthy controls had averages of 2.1 min (range 1.6-2.7 min), 997 nM/min (range 682-1248 nM/min) and 166 nM (range 90-254 nM) for lag time, ETP and peak, respectively. The inter-assay variability of the thrombin generation runs was studied with ten samples of standardized plasma (Octaplas®, Octapharm) and CVs of 7 %, 24 % and 17 % were obtained for lag time, ETP and peak, respectively, in agreement with previously reported CV values [21].

D-dimer (Tina Quant D Dimer®, Roche Diagnostics) and fibrinogen (with two different reagents, Multifibren U® and Dade Thrombin®, both Siemens Healthcare Diagnostics) levels were obtained according to manufacturer’s recommendations. These fibrinogen reagents were chosen because of the previously reported interference of Dabi [22]. Our local normal range for D-dimer was < 0.5 mg/L and for the fibrinogen method Multifibren U® 1.7-4.0 g/L.

Comparison of Dabi-TT with mass spectrometry

The Dabi-TT measurement was validated in a subgroup of 21 unselected samples at the Karolinska University Hospital, Stockholm, Sweden, by measuring free Dabi concentrations using LC-MS/MS, which quantifies Dabi accurately down to 1 ng/mL [8,9]. The samples were prepared by protein precipitation with acetonitrile containing internal standard dabigatran–d3 (Toronto Research Chemicals). After centrifugation, the supernatant was diluted with an equal amount of mobile phase A (10 mM ammonium formate pH 4.5). The analytes were detected using a mass spectrometer operating in positive electrospray ionization mode, utilizing selected reaction monitoring with ion transitions 472→289 m/z for Dabi and 475→292 m/z for the internal standard. The method was linear over the range 1.1–412 ng/mL.

Statistical analysis

Linear or non-linear (for APTT) regression analysis was used to assess correlations between the measurements. We compared the means of different sample groups with Mann-Whitney´s U test. Microsoft Excel®, and IBM SPSS statistics® programs (version 21) were used for the statistical analysis.

RESULTS

Dabi-TT compared with other clotting assays – all samples

Dabi-TT values varied markedly in the 241 patient samples, averaging 71 ng/mL, with a median of only 10 ng/mL, and a range of 0-1000 ng/mL (SD 118). The undiluted TT was, as expected, sensitive to Dabi and yielded values within the measurement range at Dabi-TT

values below 40 ng/mL. 153/241 (63 %) samples gave TT values < 140 s (corresponding to HTI values < 40 ng/mL), and 88/241 (37 %) were prolonged above an undiluted TT value of 140 s. There was one outlier: TT 46 s with a HTI of 160 ng/mL (APTT being normal, 29 s).

In a subset of 21 samples, Dabi concentrations in plasma were assessed using LC-MS/MS with a mean of 73 ng/mL, (median 78, range 2-150 ng/mL). The Dabi-TT values correlated well with LC-MS/MS values, but were, on average, 10 ng/mL lower (R^2 = 0.81, bias 13.7 %; Fig 1A). The Anti-IIa® and ECA® values correlated better than Dabi-TT with LC-MS/MS, but had larger overall bias than Dabi-TT at these rather low Dabi concentrations (R^2 = 0.96 and 0.90; bias 18.4 % and 14.5 %, respectively; Fig 1B-C).

The APTT averaged 35 s with a median of 33 s (range 20-77 s). The APTT was prolonged with increasing Dabi-TT in a curvilinear manner (linear R^2 = 0.68, quadratic R^2 = 0.71; Fig 2A). One sample with an unmeasurable APTT value above 180 s and a Dabi-TT of 46 ng/mL, was excluded from the correlation analysis as an outlier. The APTT was normal (within the reference range of 23-33 s) in 18/70 (26 %) samples having Dabi-TT values of 40-160 ng/mL (Fig 2B) and 137/241 (56 %) of all samples. Even at a Dabi-TT of 160 ng/mL, one sample had normal APTT (29 s). When Dabi-TT exceeded 160 ng/mL (n=30), the APTT was always prolonged.

The PT results averaged 22 s with a median of 22 s (range 17-50 s). The PT (Owren type) results correlated poorly with Dabi-TT (R^2 = 0.13). The PT was abnormal (> 24 s) in only

48/241 (20 %) of the samples. Also, in samples with Dabi-TT of 40 ng/mL or above, the PT was abnormal in only 26/99 (26 %).

Dabi-TT compared with other clotting assays – subgroup with a full set of data

In the patient subgroup with a full methodological set of data (n=49), Dabi-TT, APTT and PT gave similar results as observed in the entire material (Table 1). The Anti-IIa® and the ECA® correlated very well with the Dabi-TT method, with R^2 values of 0.90 and 0.89, respectively (Fig 3A-B). As expected, Anti-IIa® and ECA® results were almost identical (R^2 = 0.99, bias = 3.6 %).

The PiCT® method showed prolonged readings with increasing Dabi-TT (R^2 = 0.73; Fig 3C). The PiCT® values were prolonged in 33/49 samples (67 %), and also correlated with the APTT values (R^2 = 0.76). When Dabi-TT was above 40 ng/mL, the PiCT® was prolonged in 48/49 (98 %) samples.

The RVVT was also prolonged with increasing Dabi-TT (R^2 = 0.49). The RVVT was normal (< 41 s) in only 9/49 samples (18 %), which all had low Dabi-TT values (< 40 ng/mL). The correlation appeared to be linear, as opposed to that for APTT (Fig 3D). When Dabi-TT exceeded 40 ng/mL, the RVVT was always prolonged. However, RVVT and PiCT® results correlated only modestly (R^2 = 0.59).

Thrombin generation using CAT®

In 49 patient samples, the lag time was prolonged as expected with increasing Dabi-TT values (R2 = 0.54; Fig 4A). Lag time values with Dabi-TT below 40 ng/mL were

significantly shorter than with Dabi-TT above 40 ng/mL ($p < 0.001$). Time to peak resembled the lag time. Surprisingly, ETP and peak values increased with Dabi-TT values above 40 ng/mL, and up to 225 ng/mL ($p < 0.001$). However, in one sample, at a high Dabi level of 335 ng/mL, ETP and peak values were relatively low, 2757 nM/min and 272 nM, respectively (Fig 4B-C). Despite the prolonged lag time and time to peak, Dabi did not affect the total width of the thrombin generation curve (from start of tail to lag time). Accordingly, the lag time, ETP and peak were all significantly elevated in samples with prolonged PiCT® (over 31 s; $p < 0.001$).

In addition to these 49 samples, CAT® did not provide valid results in 23 other samples: thrombin generation curves were produced, but the calibrator control (using alpha-2-macroglobulin-thrombin complex) failed. Thus, these samples could not be included in the analysis. These samples had significantly higher Dabi-TT values (259 ng/mL, on average) than the other 49 (42 ng/mL; $p < 0.001$).

Fibrinogen and D-dimer

In the 49 sample subset neither fibrinogen nor D-dimer levels correlated with Dabi-TT. D-dimer levels were fairly high with a median of 0.6 mg/L (range 0.0-4.0 mg/L). Fibrinogen levels were also somewhat elevated and the two methods gave similar results: median of 4.3 g/L for both and ranges of 2.4-10.6 g/L for Multifibren U® and 2.2-9.0 g/L for the Dade thrombin® reagents. Generally, the reagents provided similar results at all Dabi-TT levels ($R^2=0.98$; $p < 0.001$). Fibrinogen levels did not associate with the ETP or peak. In samples

where fibrinogen exceeded 4.0 g/L, the results were similar to those with fibrinogen within the normal range (Mann-Whitney U test for difference: p = 0.43 for ETP, p = 0.12 for peak).

DISCUSSION

With increasing usage of Dabi (Pradaxa®), laboratories will face the challenge of providing methods to accurately measure its effects. Even if routine measurements are considered unnecessary, measurements will be valuable for the adjustment of Dabi dosages in certain patient groups, as data from the RE-LY trial point out [20]. Rapid and reliable measures of anticoagulation will often be needed in connection with serious bleeds or risky interventions.

We have shown that Dabi analyses in spiked samples yield highly variable results [7]. In clinical patient samples, the challenge is even greater. Methods assessing thrombin activity might offer good overall knowledge on Dabi effects. Here, the specific and commercially available assays HTI®, ECA® and Anti-IIa® were shown to measure Dabi with acceptable precision, as confirmed by LC-MS/MS. To assess Dabi effects on coagulation more broadly, we performed coagulation time assessments with different activators (APTT, RVVT, PiCT®) and the CAT® assay. To our knowledge, such a wide comparison of different methods using patient samples has not been performed previously. There is little or no data on the effects of Dabi on the RVVT. Dabi prolonged the coagulation time with the APTT in an unpredictable manner, whereas the RVVT and PiCT® assays showed linear relationships. CAT® provided paradoxically elevated ETP and peak values with increasing Dabi concentrations, whereas the lag time was prolonged, suggesting that *in vivo* the consequences of inhibiting thrombin are not straightforward.

The ECA® and Anti-IIa® assays provided results which correlated very well with Dabi-TT and, in a subset of samples, also with LC-MS/MS. It has been previously reported that the HTI® assay can measure Dabi concentrations in plasma accurately above 50 ng/mL, but less reliably than the ECA® at lower concentrations [8,9]. As the Anti-IIa® measures thrombin directly it is potentially less susceptible to interference. The ECA® and Anti-IIa® had a wide measurement range and agreed well with LC-MS/MS. They seemed to measure levels below 40 ng/mL of Dabi more accurately than HTI®, but this difference might not be clinically relevant. Anti-IIa® was the functional assay that correlated best with LC-MS/MS.

Dabi significantly affects thrombin generation in patient samples. In this study, the lag time and time to peak were prolonged with increasing Dabi concentrations, as reported previously [23,24]. However, the ETP and peak values were very high in comparison with normal samples and also paradoxically enhanced with increasing concentrations of Dabi. Previous reports on this have been inconsistent. Dabi has been shown to exert hypercoagulable effects at therapeutic concentrations [25-27], but some contradictory reports exist [19,23,24, 28]. DTIs have been reported to inhibit thrombomodulin-mediated activation of protein C, and fibrinolysis at high concentrations of thrombomodulin [25,29,30]. Thus, Dabi might be less effective than anticipated in thrombogenic situations. States with enhanced thrombin generation, such as the anti-phospholipid syndrome, cancer, and ongoing thrombosis may compromise the efficacy of Dabi [31-33].

There are limitations when using the CAT® assay, due to confounding preanalytical and analytical factors. Since the patient samples were collected as part of our hospital protocol,

only a single centrifugation step was performed before CAT® analysis. Single as opposed to double centrifugation might elevate the ETP and peak values, but not at the high tissue factor concentration of 5 pM [34]. These values increased with higher Dabi concentrations while being low in our controls. Interferences in the method were likely, as the CAT® assay often failed at high Dabi concentrations. Dabi may affect the alpha-2-macroglobulin-thrombin complex, required in the calibration of the assay [18,26,27]. This has been speculated to erroneously elevate ETP and peak values in the CAT® assay at thrombin inhibitor concentrations up to 200 nM (94 ng/ml) [35]. Yet, in a recent study using spiked plasma samples, prothrombin fragments F1+2 were also elevated [25]. In our study, ETP and peak values were elevated with increasing Dabi concentration (Fig 4B-C).

The RVVT and PiCT® assays correlated fairly well with Dabi concentrations above 40 ng/mL; the RVVT was consistently prolonged as was the PiCT®, with only a single exception (Fig 3). As the RVVT is generally available for lupus anticoagulant screening it could be adopted also as an indicator test for Dabi. Conversely, it is important to exclude the presence of Dabi in the RVVT-based diagnosis of lupus anticoagulant. Both the PiCT® and the RVVT can be used to measure FXa inhibitors as well [15,16]. With these assays, laboratories might be able to use a single test to exclude pronounced effects of thrombin and FXa inhibitors. However, we must learn more about potentially interfering and complicated preanalytical and clinical factors when using these assays for this purpose.

The major limitation of the present study is the lack of clinical data on the patient samples and the timing in relation to last drug intake. However, the aim of the study is merely to document the performances of various laboratory tests. Clinical outcomes require much

larger materials for interpretation, such as that provided for Dabi measurements in plasma [20]. Dabi concentration measurements were requested according to clinical need, and the many samples with low levels imply exclusion of important Dabi concentrations before an intervention or continued monitoring after the cease of a bleed.

The majority of patients had high levels of fibrinogen and D-dimer, and as expected, Dabi levels did not correlate with those. The two different fibrinogen measurement methods gave similar results at all Dabi levels. In previous spiking experiments some fibrinogen assays have underestimated the fibrinogen level in the presence of Dabi at concentrations exceeding 200 ng/mL (22). In our patient samples, only 3/49 contained more than 200 ng/mL of Dabi, thus precluding firm conclusions. Dabi interferes with many clot-based assays [36]. These findings reflect the challenges facing clinicians and laboratorians alike; the Dabi concentration alone will not suffice for the interpretation of coagulation.

CONCLUSIONS

Several laboratory methods may be used to assess the anticoagulant effects of Dabi in patients. Using screening tests supplemented with additional specific coagulation-based tests, Dabi effects can be accurately assessed. Measurements by TT (undiluted and diluted as in the Hemoclot Thrombin Inhibitors® assay), ECA® and Anti-IIa® can be calibrated to quantify Dabi. However, Dabi concentrations alone are not comprehensive, as thrombin generation can be paradoxically high and the APTT normal, reflecting enhanced coagulation activity even in patients with rather high levels of Dabi. PiCT® and RVVT measurements cover a broader spectrum of coagulation and could be applied in addition to Dabi quantification to

depict the coagulation status of the patients´ plasma. The CAT® had limited technical performance and abilities to quantify Dabi in plasma, but may provide new insight into the effects of thrombin inhibition *in vivo*.

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FIGURE CAPTIONS:

Figure 1: Functional dabigatran concentrations (Dabi-TT) with modified thrombin time were well correlated (R^2=0.81) with gold standard liquid chromatography-tandem mass spectrometry (LC-MS/MS; A). Anti-IIa® correlated with LC-MS/MS even better (R^2=0.96; B). ECA® also correlated well (R^2=0.90; C). ECA®, Ecarin clotting assay.

Figure 2: Increasing functional dabigatran concentrations (Dabi-TT) were associated with the APTT in a curvilinear manner (A). However, APTT values could be normal (below 33 s) even with therapeutic dabigatran concentrations up to 160 ng/mL (B).

Figure 3: Functional dabigatran concentrations (Dabi-TT) correlated with Anti-IIa® (A; R^2=0.90), ECA® (B, R2=0.89), PiCT® (C; R^2 =0.73) and RVVT (D; R^2 =0.49). Anti-IIa® and ECA® results were virtually the same (R^2=0.99). ECA®, Ecarin clotting assay; PiCT®, prothrombinase induced clotting time; RVVT, Russel's viper venom time.

Figure 4: Increasing functional dabigatran concentrations (Dabi-TT) prolonged the lag time (A; R^2 = 0.54), but the ETP (B) and the peak were paradoxically increased with increasing Dabi-TT (C). Maximum values of lag time, ETP and peak obtained in the controls are shown. Dabi-TT; dabigatran concentration measured by dabigatran-calibrated thrombin time,

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Table 1: Coagulation and thrombin generation in samples from 49 patients using dabigatran

	Clotting assays					Chromogenic assays		Thrombin generation (CAT®)		
	Dabi-TT (ng/mL)	**APTT (s)**	**PT (s)**	**PiCT® (s)**	**RVVT (s)**	**Anti-IIa® (ng/mL)**	**ECA® (ng/mL)**	**Lag time (min)**	**ETP (nM/min)**	**Peak (nM)**
Mean	63	33	23	48	66	68	66	8.7	2912	629
Median	42	30	22	45	59	40	40	6.7	1856	429
SD	75	8	4	21	29	78	77	5.9	2127	466
Range	0-335	21-59	18-41	22-113	31-181	0-450	0-440	2.0-24.0	836-7945	165-2023
Normal range	NA	23-33	19-24	19-31	< 41	NA	NA	1.6-2.7	682-1248	90-254
Number of abnormal	NA	20 (40%)	9 (18%)	33 (67%)	40 (82%)	NA	NA	44 (90%)	40 (82%)	39 (80%)

CAT®, Calibrated Automated Thrombogram; Dabi-TT, functional dabigatran concentration using modified thrombin time; APTT, activated partial thromboplastin time; PT, prothrombin time; PiCT®, prothrombinase induced clotting time; RVVT, Russel's viper venom time; Anti-IIa®, anti-thrombin activity; ECA®, ecarin clotting assay; ETP, endogenous thrombin potential; SD, standard deviation; NA, not applicable.

Figure 1

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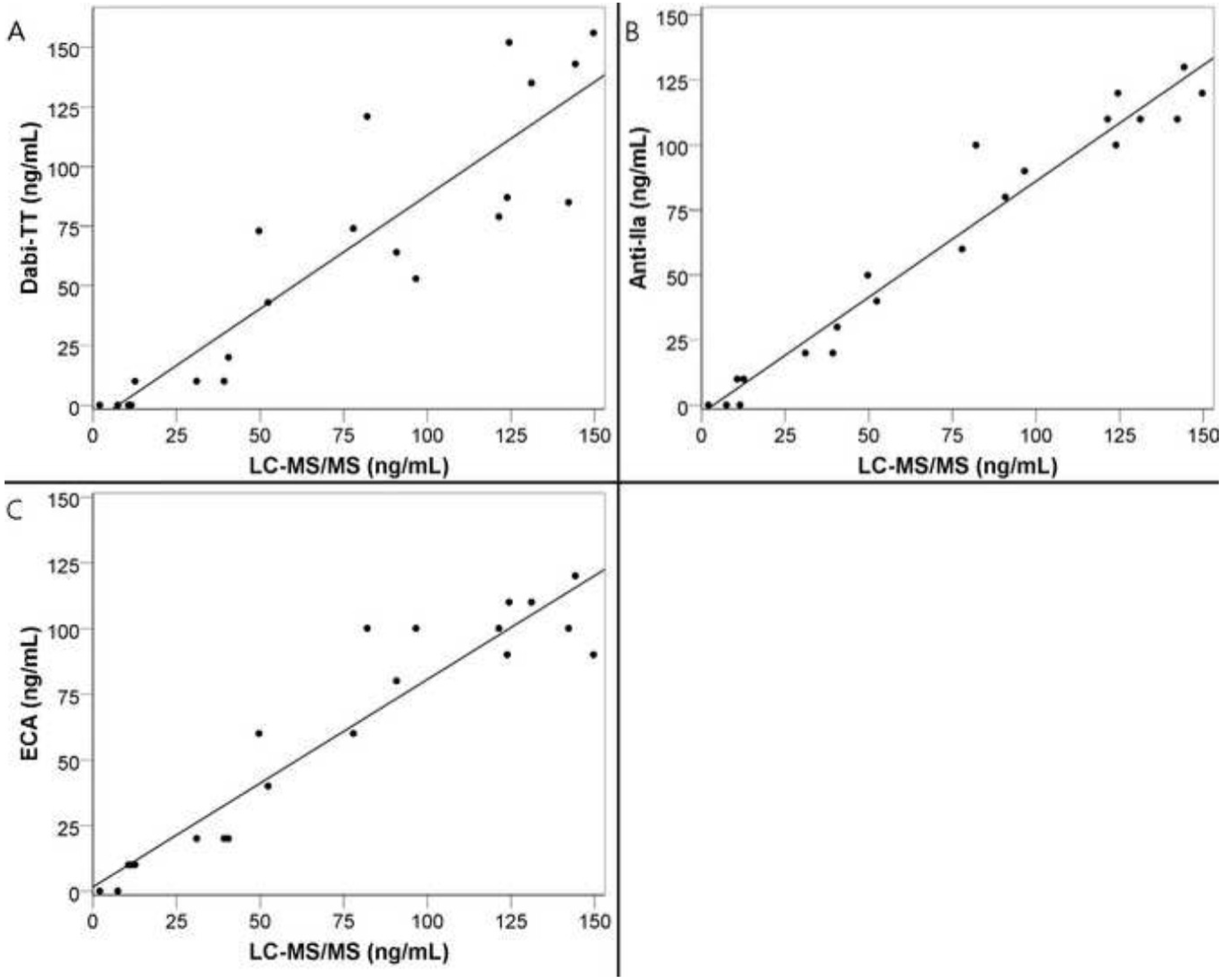

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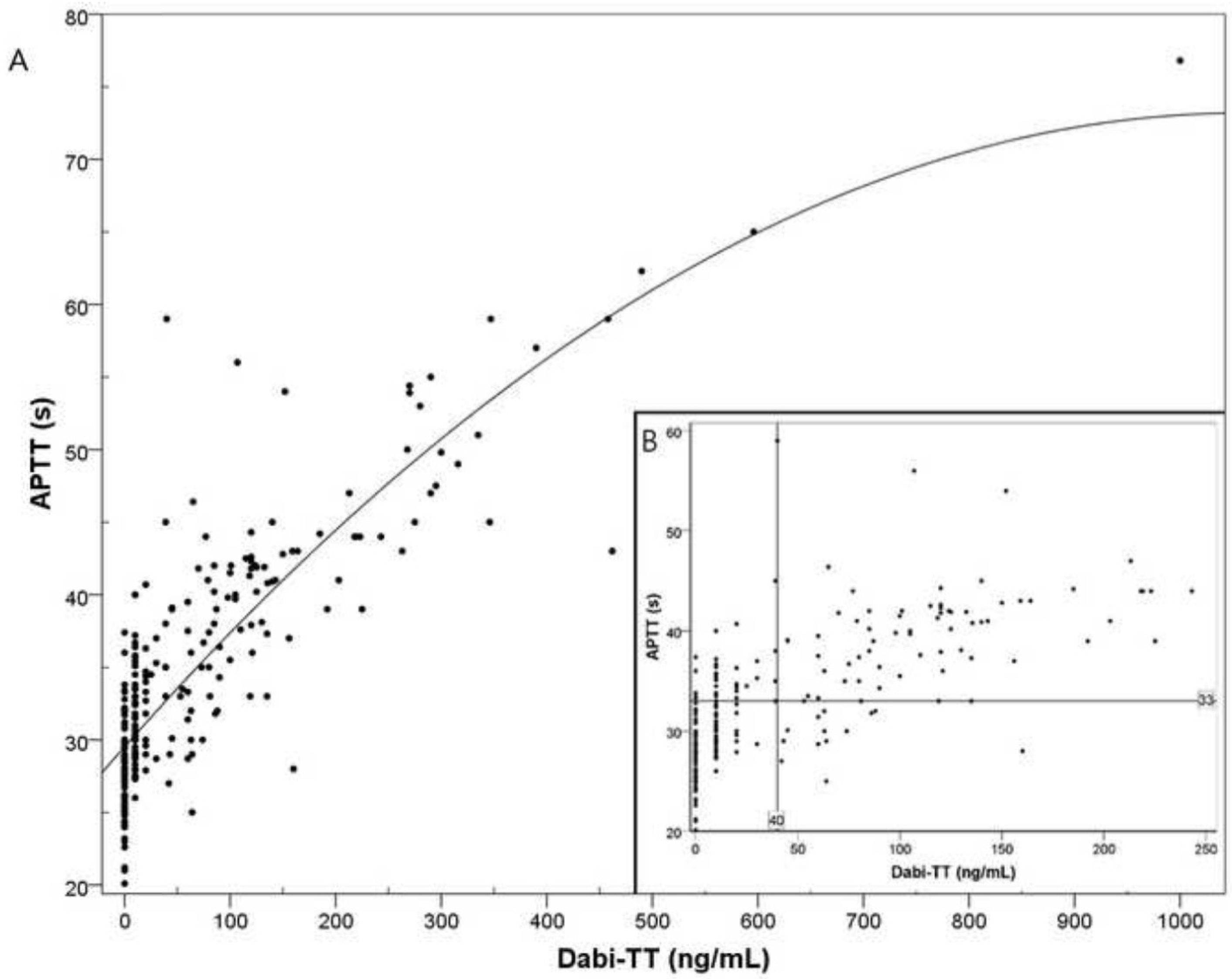

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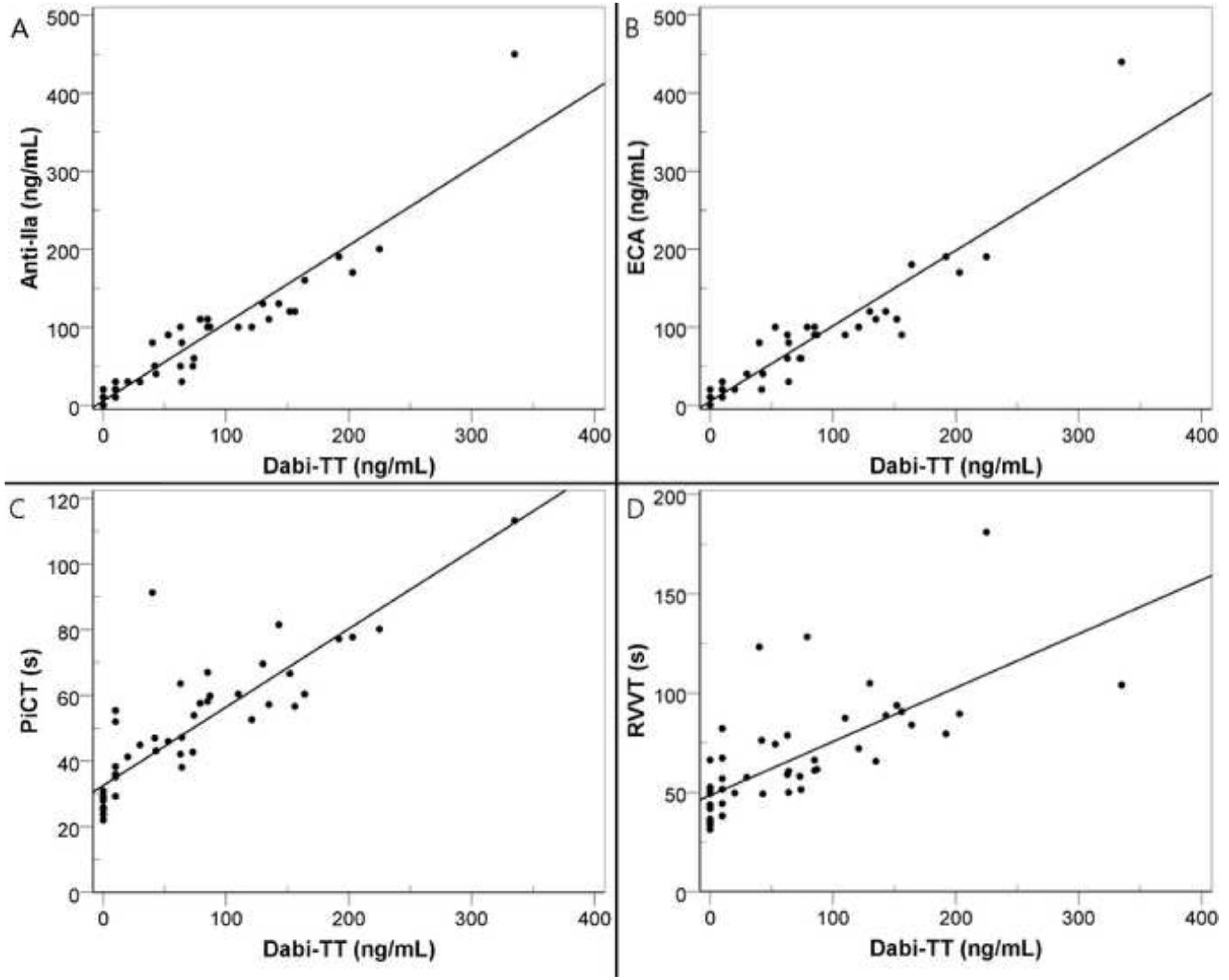

Figure 4

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