Diagnostic Accuracy of a Novel Chromogenic Direct Thrombin Inhibitor Assay: Clinical Experiences for Dabigatran Monitoring

Sven Poli1 Florian Härtig1 Charlotte Spencer1 Matthias Ebner3 Ingvild Birschmann4 Joachim Kuhn4 Susanne Faix2 Ulf Ziemann1 Hans-Ulrich Häring2,5,6 Rainer Lehmann2,5,6 Andreas Peter2,5,6 Sebastian Hörber2

1 Department of Neurology and Stroke, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
2 Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, Department of Internal Medicine, University of Tübingen, Germany
3 Department of Internal Medicine and Cardiology, Charité University Medicine Berlin – Campus Virchow Klinikum, Berlin, Germany
4 Institute for Laboratory and Transfusion Medicine, Heart and Diabetes Center, Bad Oeynhausen, Ruhr University, Bochum, Germany
5 Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany
6 German Center for Diabetes Research (DZD), München-Neuherberg, Germany

Address for correspondence Prof. Dr. Andreas Peter, MD, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, Department of Internal Medicine, University of Tübingen, Otfried-Müller-Str.10, 72076 Tübingen, Germany (e-mail: Andreas.Peter@med.uni-tuebingen.de).


Abstract

Background Direct oral anticoagulants (DOACs) are increasingly replacing vitamin K antagonists (VKA) for clinical indications requiring long-term oral anticoagulation. In contrast to VKA, treatment with DOAC including dabigatran—the only direct thrombin inhibitor amongst them—does not require therapeutic drug monitoring. However, in case of treatment complications (e.g., major haemorrhage) and conditions requiring urgent surgery or thrombolytic therapy, information about actual DOAC plasma levels is needed to guide treatment decisions. Due to short reagent stability, limited accuracy at low dabigatran levels and high heparin sensitivity, the applicability of the widely used Hemoclot thrombin inhibitor (HTI) coagulation assay is limited in the emergency setting.

Methods Dabigatran concentrations of 288 citrated plasma samples taken from 48 dabigatran-treated patients with drug concentrations of up to 300 ng/mL were measured with the chromogenic anti-IIa Biophen direct thrombin inhibitor (BDTI) assay and results compared with HTI using ultra performance liquid chromatography—tandem mass spectrometry as the reference method for measuring dabigatran plasma concentrations.

Results BDTI results showed a very strong correlation with dabigatran concentrations (r = 0.965, p < 0.0001) as well as a low intra- and inter-assay variation of <5%. Compared with HTI, BDTI provides an improved on-board reagent stability of 72 hours, rapid turnaround times comparable to routine coagulation assays, high accuracy at low drug levels and reduced heparin sensitivity.

Conclusion The BDTI is an ideal coagulation assay for the around-the-clock determination of dabigatran plasma levels in clinical routine including emergency situations.

Keywords ► oral anticoagulants ► clinical trials ► dabigatran ► NOAC ► DOAC
Introduction

Direct oral anticoagulants (DOAC), like dabigatran, have proven both efficacy and safety in the prevention of ischemic stroke in patients with non-valvular atrial fibrillation\(^1\) as well as treatment and prevention of venous thrombosis and thromboembolism.\(^1\)-\(^3\) Consequently, DOAC are recommended by international guidelines and thus increasingly replacing vitamin K antagonists (VKA) for these indications.\(^4\)-\(^7\) Due to low but still relevant rates of haemorrhagic complications and ischemic events during DOAC therapy, the management of emergency situations requiring reversal of anticoagulation, urgent surgical intervention or thrombolysis in patients treated with DOAC is gaining relevance.\(^8\)-\(^10\)

Although safety and efficacy of fixed dosing regimens have been established by phase III trials and confirmed by registries\(^5\),\(^11\)-\(^13\) and therapeutic drug monitoring is deemed unnecessary for DOAC treatment guidance,\(^14\) fast and reliable determination of DOAC plasma concentrations may be of vital importance in emergency situations.\(^15\) With the arrival of the highly effective but also costly idarucizumab and the development of further DOAC-specific reversal agents, 24/7 availability of suitable coagulation tests to exclude clinically relevant levels of dabigatran and other DOAC may avoid unnecessary expenses.

Standard coagulation assays like activated partial thromboplastin time (aPTT) or prothrombin time (PT) do not have sufficient sensitivity for DOAC and show strong reagent-specific variations in their ability to detect DOAC.\(^1\),\(^16\)-\(^18\) Apixaban, edoxaban and rivaroxaban act as direct inhibitors of activated coagulation factor X, and chromogenic anti-Xa assays—originally developed for monitoring of low-molecular-weight heparins (LMWH)—can be applied for the determination of anti-Xa activity. Commercial calibrators may be used for the respective compound of interest. The high stability and short test duration of chromogenic anti-Xa assays enables routine diagnostic laboratories to rapidly provide reliable results 24/7.\(^19\)

The determination of dabigatran plasma concentrations is usually performed using a calibrated, diluted thrombin time assay like the European Conformity (CE) labelled Hemoclot thrombin inhibitor (HTI) assay (Hyphen BioMed, Neuville-sur-Oise, France) or a calibrated Ecarin clotting time (ECT). HTI is distinguished by an adequate correlation to gold standard ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS),\(^20\) but has some major disadvantages regarding the emergency setting: first, once reconstituted, the lyophilized reagents have short stability phase of only 8 hours. Time-consuming preparation of reagents for each sample delays the turnaround time in the laboratory in emergency situations. Second, diagnostic accuracy of HTI is limited at low dabigatran levels. Third, HTI is sensitive to heparin, which excludes heparinized patients from reliable analysis.\(^21\) ECT-based dabigatran assays circumvent these disadvantages of HTI, but are not available with CE-labelled quality controlled diagnostic reagents.\(^17\),\(^18\),\(^22\),\(^23\)

Recently, the novel Biophen direct thrombin inhibitor (BDTI; Hyphen BioMed) assay was made available for the determination of dabigatran concentrations. It is a chromogenic, kinetic anti-\(\text{IIa}\) method for quantitative determination of direct thrombin inhibitors in plasma and is suitable for CE-marked automated measurements. According to the manufacturer, it overcomes the disadvantages of HTI for emergency situations due to improved reagent stability and lower heparin sensitivity.

In the present study, we aim to evaluate this novel assay with real-life patient samples under routine conditions in a defined clinical cohort and validate the results by comparison against the gold standard method UPLC-MS/MS.

Materials and Methods

Methods

Study Design

This assay validation study was conducted in two parts.

First, in a method comparison study, we retrospectively analysed citrated plasma samples taken from dabigatran-treated patients during two previous prospective observational trials with blinded endpoint assessment (Clinical Trial Registration Information unique identifiers: NCT02371044 and NCT02371070). Institutional review board approval was obtained for both trials from the ethics committee at the University Hospital Tübingen (protocol number 259/2013B01).

Second, in a prospective observational trial with blinded endpoint assessment, we analysed fresh citrated plasma samples taken from dabigatran-treated patients under routine conditions (NCT02825394). Institutional review board approval was obtained from the ethics committee at the University Hospital Tübingen (protocol number 270/2015B01).

All studies were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants prior to enrolment.

Setting and Eligibility Criteria

All three clinical studies were conducted at the Department of Neurology & Stroke and the Department of Cardiology of the University Hospital Tübingen, a tertiary care facility.

Between August 2013 and January 2016, we enrolled and collected blood samples from patients either receiving the first dose of dabigatran or being on treatment with dabigatran. Subjects who had received VKA or other DOAC than dabigatran less than 14 days, LMWH less than 24 hours or unfractionated heparin (UFH) less than 12 hours prior to enrolment were excluded to rule out interference with measurements. Patients with either abnormal coagulation values at baseline (international normalized ratio [INR] > 1.2 or aPTT > 37 seconds) or history of coagulopathy were also excluded. Concomitant use of antiplatelet drugs was permitted.

Sample Collection

Six blood samples were collected from each subject via a venous catheter or by direct venipuncture at six different time points to cover a wide spectrum of dabigatran concentrations: before drug intake, and 30 minutes, 1, 2, 8 and 15...
12 hours (trough level) after drug intake. Samples were collected in sodium citrate tubes (Sarstedt, Nümbrecht, Germany) and instantly centrifuged to acquire plasma for analysis. Further plasma aliquots were immediately frozen and stored at $-80^\circ$C.

**Laboratory Analysis**

Anti-IIa activity was tested using BDTI and HTI reagents on a Sysmex CS5100 (Siemens Healthcare Diagnostics, Eschborn, Germany).

BDTI measurements were performed with all collected citrated plasma samples, in the first part of the study from frozen ($-80^\circ$C) and re-thawed samples. In the second part of the study, after having established around-the-clock availability, BDTI measurements were performed under routine conditions (i.e. individually at any time of collection, 24/7) with fresh citrated plasma.

HTI measurements were only performed during the first part of the study with frozen ($-80^\circ$C) and re-thawed citrated plasma.

Using the gold standard for measurements of DOAC concentrations, UPLC-MS/MS was performed for all collected samples using frozen ($-80^\circ$C) and re-thawed citrated plasma as recently described. In addition to these specific coagulation assays, routine coagulation parameters, clinical chemistry parameters, and full blood counts were determined at baseline for each study patient (see methods part in online supplements).

BDTI, HTI, UPLC-MS/MS and standard laboratory assays were conducted and interpreted by technicians blinded to patient details and treatment. Furthermore, technicians who conducted and interpreted BDTI were blinded to HTI and UPLC-MS/MS results, and vice versa. All tests were performed according to respective manufacturers’ instructions by thoroughly trained investigators and technicians.

**Calibration and linearity of BDTI and HTI:** For initial calibration of BDTI and HTI, human plasma samples with known dabigatran concentrations were used as provided by the manufacturer.

To determine linearity of BDTI and HTI, six fresh control samples of patients receiving dabigatran were diluted as indicated (1:2, 1:4, 1:10 and 1:100). BDTI and HTI results were correlated with calculated dabigatran concentrations and a linearity curve was determined by linear regression analysis. Linearity of the curve was considered acceptable at a linear correlation coefficient $r^2 > 0.98$.

**Determination of Reagent Stability and Limit of Detection:** Reagent stability was assessed during the second phase of our study, where BDTI was used and plasma-based quality controls were performed in clinical routine. According to the manufacturer, the detection threshold of BDTI assay is specified as 6.3 ng/mL. Limit of detection (LOD) of BDTI at our centre was determined by adding 3 SDs to the mean of samples without dabigatran verified by UPLC-MS/MS.

**Heparin sensitivity check:** To evaluate the influence of heparin or LMWH on BDTI results and HTI, six fresh plasma samples of six patients on dabigatran therapy were spiked with 0, 1, 2 and 4 IU/mL of UFH or with 0, 1 and 2 IU/mL of LMWH. BDTI and HTI results were compared with dabigatran concentrations determined by UPLC-MS/MS out of those six samples. According to statements of the BDTI manufacturer, UFH up to 4 IU/mL and LMWH up to 2 IU/mL should not interfere with BDTI results due to heparin neutralizing substances.

**Statistical Analysis**

Passing and Bablok linear regression analyses were performed for comparison of assay systems and the sample types. Correlation strength was graded as proposed by Evans: less than 0.20: very weak; 0.20 to 0.39: weak; 0.40 to 0.59: moderate; 0.60 to 0.79: strong; and greater than 0.80: very strong. The Bland–Altman approach was used for analyses of the differences between the two methods as recommended by Hollis for method comparison studies using the EVAPAK 3.1.2 software package.

### Results

**Linearity:** BDTI showed very strong linear correlation ($r = 0.9894$) and the recovery was between 91 and 120% (see Supplementary Table S1 [online only]). Analytic performance and stability of BDTI (dilution experiments)–Intra- and inter-assay coefficient of variance: Intra-assay coefficient of variation (CV; $n = 20$) was 2.79% at 133.4 ng/mL and 2.81% at 346.2 ng/mL, and inter-assay CV ($n = 15$) was 5.06% at 115.71 ng/mL and 4.72% at 322.05 ng/mL (see Supplementary Table S2 [online only]). This is in accordance with the CVs for high and low dabigatran concentrations, which are provided as CV < 5% by the manufacturer.

**Evaluation of BDTI and HTI during the clinical trial:** A total of 288 samples from 48 patients receiving either the first dose of dabigatran ($n = 26$) or being on treatment with dabigatran ($n = 22$) were enrolled in this study. Patients’ characteristics and baseline laboratory results are shown in Table 1.

In the first part of the study, a method comparison of BDTI and HTI versus UPLC-MS/MS was performed in 138 frozen and re-thawed citrated plasma samples collected from 23 dabigatran-treated patients between August 2013 and February 2015. BDTI results showed very strong correlation with dabigatran plasma concentrations determined by UPLC-MS/MS ($r = 0.908$; $r = 0.787$; $r = 0.727$; $r = 0.772$; see Fig. 1A). The same is true for plasma concentrations less than 60 ng/mL (UPLC-MS/MS = 1.147 BDTI; $r = 0.889$ vs. UPLC-MS/MS = 0.780 HTI – 7.195; $r = 0.727$; see Fig. 1B). Bland–Altman plots exhibit high agreement for samples measured with BDTI (bias = 26.41; +1.96 SD = 210.7 and –1.96 SD = –146.5; see Fig. 1C) compared with HTI (bias = 87.19; +1.96 SD = 254.8 and –1.96 SD = –80.44; see Fig. 1C).

In the second part of the study, 150 fresh citrated plasma samples taken from 25 dabigatran-treated patients were analysed between September 2015 and January 2016 under routine conditions. Again, a very strong correlation of BDTI with UPLC-MS/MS was found for dabigatran concentrations...
from 0 to 300 ng/mL (UPLC-MS/MS = 1.048 BDTI; \( r = 0.965 \))
and from 0 to 60 ng/mL (UPLC-MS/MS = 1.385 BDTI; \( r = 0.848 \)); see > Supplementary Tables S3 and S4 [online only]).

**Discussion**

In the present study, we evaluated—for the first time—the novel chromogenic BDTI assay for determination of plasma dabigatran concentrations in real-life plasma samples under routine conditions. We compared the BDTI results to those of the widely used coagulometric HTI as well as to UPLC-MS/MS, the gold standard reference method for dabigatran concentration measurements.

We were able to demonstrate that BDTI is an accurate method for reliable around-the-clock determination of dabigatran plasma levels. It shows strong correlation with the gold standard UPLC-MS/MS over the relevant range of possible concentrations, particularly including the lower range. Furthermore, it shows excellent precision, linearity and is not sensitive to artificial contamination with heparin. Compared with the widely used HTI assay, it features improved on-board stability, which makes it suitable for application in clinical routine around the clock.

**Measurement of Low Dabigatran Concentrations**

Routine measurement of dabigatran concentration is not required by clinical standards. However, in case of treatment complications (e.g. major haemorrhage) and conditions requiring urgent surgery or thrombolytic therapy, information about actual DOAC plasma levels is needed to guide time-critical treatment decisions. So far, no prospectively validated data exist for specific concentration thresholds that increase bleeding risk. It can only be estimated based on retrospective analyses, pharmacodynamic considerations, and expert opinions.

For emergency surgery, dabigatran concentrations of less than 30 ng/mL have been considered to be safe. Following these recommendations, the BDTI assay is an appropriate method to determine low dabigatran concentrations. Compared with the LOD of HTI—stated as 50 ng/mL by the manufacturer—the BDTI assay shows a clearly improved ability to detect low levels of dabigatran in plasma samples. Despite this improvement over HTI, BDTI may not completely rule out the presence of low but, nevertheless, potentially clinically significant dabigatran concentrations, thus limiting its validity prior to delicate procedures such as neuraxial anaesthesia. Hence, development should focus on increasing accuracy of the assay in the low concentration range.

**Strengths and Limitations**

To evaluate the BDTI assay for clinical applicability, we analysed samples from dabigatran-treated patients; spiked

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Mean ± SD</th>
<th>Reference range</th>
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<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 ± 0.2</td>
<td>0.6–1.1</td>
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<tr>
<td>GFR MDRD (ml/min × 1.73 m²)</td>
<td>80 ± 24</td>
<td>&gt;60</td>
</tr>
<tr>
<td>GFR CKD-EPI (ml/min × 1.73 m²)</td>
<td>75 ± 16</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>39 ± 5</td>
<td>42–52</td>
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<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.0 ± 1.7</td>
<td>14.0–18.0</td>
</tr>
<tr>
<td>Platelet count (10³/μL)</td>
<td>218 ± 67</td>
<td>150–450</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>27 ± 5</td>
<td>&lt;40</td>
</tr>
<tr>
<td>anti-Xa activity (IU/μL)</td>
<td>&lt;0.1 ± 0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Dabigatran dose°C</td>
<td>110 mg BID: 19 (39.48%)</td>
<td>150 mg BID: 29 (60.42%)</td>
</tr>
<tr>
<td>Female sex°C</td>
<td>26 (54.17%)</td>
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<tr>
<td>Age (y)</td>
<td>72 ± 13.72</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78.7 ± 20.67</td>
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<tr>
<td>Body mass index (BMI; kg/m²)</td>
<td>27.18 ± 5.05</td>
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</tr>
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</table>

**Indication for dabigatran therapy**

- Atrial fibrillation°C: 38 (79.17%)
- Patent foramen ovale (PFO): 9 (18.75%)
- Embolic stroke of undetermined source (ESUS): 1 (2.08%)
- Concomitant antiplatelet therapy (last dose < 7 d): 1 (2.08%)
- Acetylsalicylic acid°C: 18 (37.50%)
- Clopidogrel°C: 1 (2.08%)

**Detection threshold:** We determined the LOD of BDTI to be 5.89 ng/mL, which matches the manufacturer’s specifications of 6.3 ng/mL.

**Heparin sensitivity:** In accordance with the manufacturer’s statement, we did not detect an influence of UFH up to 4 IU/mL and of LWMH up to 2 IU/mL on BDTI results in dabigatran-containing plasma samples of patients. In contrast, results of the HTI assay were significantly influenced by spiking with UFH > 1 IU/mL, as described earlier (see > Supplementary Tables S3 and S4 [online only]).

**Abbreviations:** ALT, alanine transaminase; aPTT, activated partial thromboplastin time; AST, aspartate transaminase; CHE, cholinesterase; CKD-EPI, chronic kidney disease epidemiology collaboration; GFR, glomerular filtration rate; GGT, gamma-glutamyltransferase; INR, international normalized ratio; MDRD, modification of diet in renal disease; PT, prothrombin time.

°CNumber (%).

°Mean ± standard deviation.

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plasma samples were avoided. For all patients, baseline characteristics and laboratory assessments were available. In addition, detailed information on medication and comorbidities was known for each patient. Thus, real-life conditions can be assumed for method evaluation. Furthermore, comparisons of HTI and BDTI results with actual dabigatran concentrations determined by the gold standard method, ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), as conducted in this study, represent the most reliable benchmark for assay validation.

Supratherapeutic dabigatran levels as seen in intoxicated patients have not been observed during this study; likewise, no patients with renal failure or impaired liver function were included. Consequently, our analyses do not allow conclusions about BDTI performance at (very) high dabigatran concentrations.

![Fig. 1](image-url) Measurement of dabigatran concentrations in plasma samples in series. One hundred and thirty-eight plasma samples were taken at eight different time points and analysed with the Biophen direct thrombin inhibitor (BDTI) and the Hemoclot thrombin inhibitor (HTI) assay on the Sysmex CSS100 from Siemens Healthineers. Results were compared with the gold standard method for measurements of direct oral anticoagulants, ultra-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS). Shown are Passing and Bablok linear regression analyses for the BDTI and HTI assay with ranges for dabigatran concentrations from 0 to 200 ng/mL (A) and from 0 to 60 ng/mL (B), respectively. A cut-off value was additionally drawn at 30 ng/mL dabigatran concentration. Below this value, emergency surgery is considered to be safe.29,32,33 (C) Bland–Altman plots show the percentage difference and mean values of BDTI and HTI assay compared with LC-MS/MS.
plasma concentrations (above 300 ng/mL). However, treatment decisions made at (very) high dabigatran levels (e.g., reversal therapy in major haemorrhage) do not require perfectly accurate determination of plasma concentrations. In contrast, in the lower concentration spectrum covered by our study, BDTI performance was excellent, which is essential for treatment guidance in emergency situations requiring urgent surgery or thrombolysis in ischemic stroke.

**Conclusion**

In conclusion, the novel chromogenic anti-IIa BDTI assay overcomes the disadvantages of the HTI assay in terms of reagent stability, heparin sensitivity and the ability to detect low concentrations of dabigatran in real-life plasma samples. It is suitable for use on CE-marked automated systems with high accuracy, specificity and reproducibility, thus making the assay very suitable for routine clinical use, especially for day and night emergency situations in which rapid determination of dabigatran levels in plasma is pivotal to guide treatment.

**What is known about this topic?**

- The increasing use of direct oral anticoagulants made them an important consideration in emergency situations. However, the ability to timely measure the plasma concentrations of especially the direct thrombin inhibitor dabigatran to guide clinical decisions in emergency situations is still limited.

**What does this paper add?**

- The Biophen direct thrombin inhibitor assay allows the determination of dabigatran concentrations in turnaround times comparable to global coagulation tests under routine conditions on a 24/7 basis. We believe that the data are of great importance to improve laboratory diagnostic and clinical management of patients on dabigatran therapy.
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