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Douxfils, Jonathan; Chatelain, Bernard; Hjemdahl, Paul; Devalet, Bérangère; Sennesael, Anne-Laure; Wallemacq, Pierre; Rönquist-Nii, Yuko; Pohanka, Anton; Dogné, Jean Michel; Mullier, François

Published in:
Thrombosis Research

DOI:
10.1016/j.thromres.2015.02.020

Publication date:
2015

Document Version
Early version, also known as pre-print

Link to publication
Citation for published version (HARVARD):
https://doi.org/10.1016/j.thromres.2015.02.020
Regular Article

Does the Russell Viper Venom time test provide a rapid estimation of the intensity of oral anticoagulation? A cohort study

Jonathan Douxfils a,⁎, Bernard Chatelain b, Paul Hjemdahl c, Bérangère Devalet b, Anne-Laure Sennesael b, Pierre Wallemacq d, Yuko Rönquist-Ni k c, Anton Pohanka c, Jean-Michel Dogné a, François Mullier a,b

a Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), University of Namur, Belgium
b Haematology Laboratory, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), CHU Dinant Godinne UcL Namur, Université Catholique de Louvain, Belgium
c Department of Clinical Pharmacology, Karolinska University Hospital and Clinical Pharmacology Unit, Department of Medicine Solna, Karolinska Institute, SE-171 76 Stockholm, Sweden.
d Laboratory of Clinical Chemistry, Saint-Luc University Hospital, Université catholique de Louvain, Brussels, Belgium

ABSTRACT

Background: Dilute Russell Viper Venom Time (DRVV-T) might be useful in urgent settings for screening patients on Non-VKA Oral Anticoagulants (NOACs).

Aim: To compare the accuracy of DRVV-T with gold standard assays for the assessment of pharmacodynamics of dabigatran, rivaroxaban and vitamin K antagonist (VKA) in plasma samples from patients.

Methods: Sixty rivaroxaban, 48 dabigatran and 50 VKA samples from patients were included. DRVV-T was performed in all groups using STA®-Staclot®DRVV-Screen and -Con. For NOACs, PT and aPTT were performed using different reagents while plasma drug concentrations were measured by liquid mass-spectrometry (LC-MS/MS). For VKA, INR was performed using RecombiPlasTin 2G®.

Results: For NOACs, correlations between calibrated STA®-Staclot®DRVV-Con and LC-MS/MS (rs = 0.88 and 0.97 for rivaroxaban and dabigatran, respectively) were higher than the ones obtained with STA®-Staclot®DRVV-Screen (rs = 0.87 and 0.91), PT (rs = 0.83 to 0.86) or aPTT (rs = 0.84 to 0.89). Bland Altman analyses showed that calibrated DRVV-T methods tend to overestimate plasma concentrations of NOACs. ROC curves revealed that cut-off to exclude supra-therapeutic levels at Ctrough (i.e. 200 ng/mL) are different for dabigatran and rivaroxaban. Neither STA®-Staclot®DRVV-Screen nor -Con correlated sufficiently with the intensity of NOAC therapy (rs = 0.35 and 0.52).

Conclusions: STA®-Staclot®DRVV-Con provides a rapid estimation of the intensity of anticoagulation with rivaroxaban or dabigatran without specific calibrators. At Ctrough, thresholds for rivaroxaban and dabigatran can be used to identify supra-therapeutic plasma level. However, this test cannot differentiate the nature of the NOACs. The development of a point-of-care device optimising this method would be of particular interest in emergency situations.

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Introduction

Non-VKA oral anticoagulants (NOACs) do not require close monitoring or frequent dose adjustments as needed for vitamin-K antagonists (VKAs). However, searching for the optimal response at the individual level may still be useful in some situations (e.g. renal or hepatic impairment; drug-drug interactions, genetic variants) with these agents [1,2]. In addition, there is a definite need for knowledge about the intensity of NOAC-induced anticoagulation in acute situations such as major bleeds or before invasive procedures [3].

Currently, activated partial thromboplastin time (aPTT), Hemoclot Thrombin Inhibitor® (HTI), ecarin clotting time (ECT) and ecarin chromogenic assay (ECA) have been proposed for measurements of dabigatran [4,5] whereas prothrombin time (PT) or anti-Xa chromogenic assays have been suggested for rivaroxaban [6]. Recent studies concluded that the aPTT [7] and PT [8] tests do not correlate well with dabigatran and rivaroxaban concentrations, respectively. Therefore, more specific coagulation assays are recommended but these assays are not widely available and require specific calibrators and controls that increase the turnaround time in emergency situations [9]. Consequently, there is a need for a general test, easily implementable, able to screen the relative intensity of anticoagulation of all NOACs that could also provide the nature of the treatment in unconscious patients [10].
Rivaroxaban 20 mg treatment in non-valvular AF (n = 34; among these, 30 and 4 were treated or VKA only, since at least 2 weeks, without any bridging with other anticoagulants). To be included in the study, patients must have been on rivaroxaban, dabigatran etexilate or VKA were treated for stroke prevention in non-valvular AF. The majority (n = 47) of VKA samples were from patients treated with acenocoumarol. There were 3 patients treated with warfarin, and rivaroxaban measured by validated LC-MS/MS methods. We also examined if DRVV-T could differentiate between anticoagulant therapies and if thresholds that reflect supratherapeutic levels of NOACs in plasma could be found for the DRVV-T.

Materials and methods

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Centre Hospitalier Universitaire (CHU) Dinant Godinne Ucl. Namur in Yvoir, Belgium, and the Ethical Review Board in Stockholm, Sweden. Hospitalized patients and ambulatory patients have been recruited at the CHU Dinant Godinne Ucl. Namur and at the Karolinska University Hospital. Written informed consent was obtained from each donor.

Normal pool plasma

Twenty-seven healthy individuals were included. The exclusion criteria were thrombotic and/or hemorrhagic events, pregnancy, and use of antiplatelet and/or anticoagulant medication and/or drugs potentially affecting platelet and/or coagulation factor functions during two weeks prior to sampling. Blood was taken by antecubital venipuncture and collected into 0.109 M sodium citrate (9:1 v/v) tubes (Venosafe®, Terumo, Belgium) using a 21-gauge needle (Terumo, Belgium). Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double centrifugation for 15 minutes at 1500 g at room temperature. Immediately after centrifugation, PPPs from the 27 donors were mixed to obtain the normal pooled plasma (NPP) which was frozen at −80 °C without delay. Frozen NPP aliquots were thawed and heated to 37 °C for 5 minutes just before experiments.

Clinical samples

Sixty rivaroxaban, 48 dabigatran and 50 VKA plasma samples from real-life patients were included in the study for retrospective analysis. The majority (n = 47) of VKA samples were from patients treated with acenocoumarol. There were 3 patients treated with warfarin, phenprocoumon or fluindione. Exclusion criteria were concomitant treatment with linezolid (for rivaroxaban samples). To be included in the study, patients must have been on rivaroxaban, dabigatran etexilate or VKA only, since at least 2 weeks, without any bridging with other anticoagulant(s). Patients on rivaroxaban were treated for stroke prevention in non-valvar AF (n = 34; among these, 30 and 4 were treated with rivaroxaban 20 mg od and 15 mg od, respectively) or for the prevention of recurrent DVT and PE following an acute DVT (n = 26; among these, 24 were treated with rivaroxaban 20 mg od, one with 15 mg od and one with 15 mg bid, respectively). All patients on dabigatran etexilate or VKA were treated for stroke prevention in non-valvar AF. Blood was taken by venipuncture and PPP was obtained and stored as described above for the healthy volunteers. When scheduled, plasma samples from patients treated with NOACs were collected just before the next pill intake (at Cmax, i.e. 24 hours for rivaroxaban od and 12 hours for rivaroxaban and dabigatran etexilate bid) and 3 hours after the pill intake to achieve measurements at Cmax. For other samples (n = 12 and 21 for rivaroxaban and dabigatran, respectively), blood was collected randomly and no information regarding the delay was available. For VKA samples, blood was taken in a random fashion.

Home-made calibrators of rivaroxaban and dabigatran

Powder of rivaroxaban for analyses in Belgium (coagulation assays and LC-MS/MS measurement) was a generous gift of Bayer A.G. (Leverkusen, Germany). We used linezolid (Sigma-Aldrich, Diegem, Belgium) as internal standard due to structural similarities with rivaroxaban. Rivaroxaban for coagulation analysis was prepared from a stock solution (1 mg/mL) in 100% DMSO and diluted in PBS without Ca2+ and Mg2+.

Dabigatran for coagulation testing in Belgium was a generous gift from Boehringer-Ingelheim GmbH (Ingelheim am Rhein, Germany). Dabigatran for LC-MS/MS analyses in Stockholm was purchased from Alschim (Strasbourg, France) and dabigatran-d3 from Toronto Research Chemicals (Ontario, Canada). Dabigatran for coagulation analysis was prepared from a stock solution (10 mM) in DMSO plus HCl 3% and diluted in PBS without Ca2+ and Mg2+.

Working solutions of 1000, 500, 250, 100 and 50 ng/mL of rivaroxaban and dabigatran were prepared in normal pooled plasma (NPP). The DMSO concentration in plasma was ≤0.05% (v/v) which does not influence the coagulation. “Home-made calibrators” were always within 20% of the expected values with the LC-MS/MS method.

Coagulation assays

Dilute Russell’s Viper Venom time

The commercially available DRVV-T systems include a screening reagent with low phospholipid concentration and a confirmatory product with high phospholipid concentration. We used the DRVV-T system from Diagnostica Stago® (STA®-Staclot®DRVV-Screen; NPP baseline clotting time: 39.8 seconds; standard deviation: 0.4 seconds; and STA®-Staclot®DRVV-Confirm; NPP baseline clotting time: 35.9 seconds; standard deviation: 0.3 seconds). The same batch was used for each reagent. Briefly, 100 μL of plasma sample was incubated during 240 seconds at 37 °C. Thereafter, 100 μL of STA®-Staclot®DRVV-Screen or -Confirm was added, starting the reaction on a STA-R Evolution® coagulometer. Results are given in seconds, as ratios (versus NPP), and, for rivaroxaban and dabigatran, in ng/mL. For results express in ng/mL, calibration was performed with homemade calibrators.

Prothrombin Time, International Normalized Ratio (INR) and activated partial thromboplastin time

Prothrombin Time was determined in all patients treated with rivaroxaban with Triniclot PT Excel S® (TrinityBiotech, Bray, Ireland; NPP baseline clotting time: 15.6 seconds; standard deviation: 0.2 seconds) and RecombiPlasTin 2G® (Instrumentation Laboratory, Lexington, USA; NPP baseline clotting time: 10.6 seconds; standard deviation: 0.1 seconds) while activated partial thromboplastin time was determined in all patients on dabigatran with STA®-C.K. Prest® (Diagnostica Stago, Asnière, France; NPP baseline clotting time: 30.3 seconds; standard deviation: 0.2 seconds) and SynthasIL® (Instrumentation Laboratory; NPP baseline clotting time: 31.4 seconds; standard deviation: 0.2 seconds). Results are given in seconds, as ratios (versus NPP), and, for rivaroxaban and dabigatran, in ng/mL. For results express in ng/mL, calibration was performed with homemade calibrators for VKA samples, the INR was determined using RecombiPlasTin 2G®, TriniClot PT Excel S®, STA®-C.K. Prest® and SynthasIL® were performed on a STA-R Evolution® coagulometer and RecombiPlasTin 2G® was performed on an ACL-TOP® analyser.

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**Liquid chromatography coupled with tandem mass spectrometry**

LC-MS/MS measurements were performed in dabigatran and rivaroxaban samples as previously [7,8]. The detection limits (LOD) are 1 and 5 ng/mL for dabigatran and rivaroxaban, respectively.

**Rivaroxaban**

This method has been already described previously and represents an adaptation of the procedure described by Rohde et al. [14]. Briefly, sample preparation consisted of mixing 500 μL of methanol containing the internal standard (linezolid) and 200 μL of plasma sample. The mix was gently shaken and centrifuged. Separation of the analytes was achieved on an HPLC Kinetex column (Phenomenex® C18, 2.6 μm, 3.0 mm x 150 mm), using a gradient run with mobile phase A (10 mM ammonium formate) and mobile phase B (methanol). The analytes were detected using a Waters® Quattro Micro mass spectrometer operating in positive electrospray ionisation (ESI) mode utilising multiple reaction monitoring (MRM). No interfering peaks were observed in 15 blank plasmas. The calibration curve for rivaroxaban in plasma was linear over the range 5–500 ng/mL, and the lower limit of detection (LOD) was estimated to 3 ng/mL. Validation experiments with three levels of control samples (15, 60 and 125 ng/mL) on three different occasions (15 determinations per concentration) showed an inter-assay precision between 5.79% and 8.11% and an inter-assay accuracy within the confidence range (±15%).

**Dabigatran**

This method has been already described and represents a simplification of the procedure described by Stangier et al. [16]. Briefly, sample preparation consisted of mixing 500 μL of methanol containing 150 μL acetonitrile containing dabigatran-d3 as an internal standard. After centrifugation, the sample was diluted with an equal amount of mobile phase A (see below), after which the sample was gently shaken and re-centrifuged. Separation of the analytes was achieved on an Acquity UPLC BEH column (Shield RP18, 1.7 μm, 2.1 x 50 mm), using a gradient run with mobile phase A (10 mM ammonium formate pH 4.5) and mobile phase B (acetonitrile). The analytes were detected using a Waters Quattro Premier XE mass spectrometer operating in positive ESI mode utilising selected reaction monitoring (SRM). No interfering peaks were observed in 18 blank plasmas. The calibration curve for dabigatran in plasma was linear over the range 1–400 ng/mL and LLOD was estimated to <0.5 ng/mL. Validation experiments with three levels of control samples (8.1, 202 and 393 ng/ml) on three different occasions (8 determinations per concentration), showed an inter-assay precision between 6.00% and 9.25% and an inter-assay accuracy between 172 – 426 ng/mL for rivaroxaban samples as previously [7,8]. The detection limits (LOD) are 1 and 5 ng/mL for dabigatran and rivaroxaban, respectively.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego California, USA, www.graphpad.com) and MedCalc (MedCalc Software bvba, Ostend, Belgium, www.medcalc.org) for Windows. Results for coagulation tests expressed in ng/mL were compared to the respective LC-MS/MS method by Spearman correlation analysis and by linear regression. Bland-Altman analyses were also provided. Sensitivity was defined as the concentration in rivaroxaban or dabigatran required to double the clotting time of the NPP. ROC curve analyses were also performed to find the best cut-off to exclude supratherapeutic NOAC concentrations with STA®, Staclor®DRVV-Screen and –Confirm. The calibration of STA®, Staclor®DRVV-Screen and –Confirm as well as the correlation between STA®-Staclor®DRVV assays performed in patients and the true concentration provided by LC-MS/MS analyses is described by a first order equation. The mathematical model is defined as follow: Y = Y0 + (Plateau – Y0)*(1-e(-K*x)) where Y0 is the value when x is zero; plateau is the Y value at infinite x and K is the rate constant.

The limit of detection (LOD) and the limit of quantitation (LOQ) of STA®-Staclor®DRVV assays were calculated as follow: LOD = [(3*standard deviation of Y0)/slope] and LOQ = [(10*standard deviation of Y0)/slope]. As both STA®-Staclor®DRVV-Screen and –Confirm calibrations were not defined by a linear correlation on the entire concentration range (from 0 to 1000 ng/mL), we used the three first points of the calibration curves (0, 50 and 100 ng/mL) to generate a linear response (R² > 0.98). The corresponding slope and Y0 were extracted from this linear model.

**Results**

**Plasma concentrations**

Plasma concentrations ranged from 6 to 426 ng/mL for rivaroxaban and from 0 to 413 ng/mL for dabigatran as determined by LC-MS/MS. Among the 60 rivaroxaban and the 48 dabigatran samples, 12 (18.6%) and 6 (12.5%), respectively, were above 200 ng/mL.

Among patients on rivaroxaban and dabigatran etexilate, the interval between the last intake of the drug and the blood sampling was available for 60 and 35 samples, respectively (Table 1). Among the 50 plasma samples of patients treated with VKA, the INR-values ranged from 1.4 to 9.4. Six samples were above an INR of 5.0 and 4 samples were < 2.0.

**Correlations between plasma drug concentrations/effects and STA®-Staclor®DRVV-Screen**

The STA®-Staclor®DRVV-Screen yielded a curvilinear concentration-dependent prolongation of clotting time (Fig. 1 – rivaroxaban (A)). The LOD and LOQ were 14 and 46 ng/mL, respectively. The first order equation gave r²-values of 0.82 when results were expressed in seconds or as ratios (Fig. 1 – rivaroxaban (A and B)). The Spearman correlation,

**Table 1**

Mean plasma drug concentrations according to the delay since the drug administration in patients treated with rivaroxaban and dabigatran etexilate. Two hours and 3 hours represent the expected Cmax of the drugs while 12 hours (dabigatran etexilate) and 24 hours (rivaroxaban) represent the Cmin. Only samples with the exact delay between last administration and drug sampling have been included (n = 46 and 27 for rivaroxaban and dabigatran etexilate, respectively).

<table>
<thead>
<tr>
<th></th>
<th>RIVAROXABAN</th>
<th></th>
<th>DABIGATRAN ETEXILATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h (n = 17)</td>
<td>3 h (n = 15)</td>
<td>24 h (n = 14)</td>
</tr>
<tr>
<td>Mean in ng/mL</td>
<td>196</td>
<td>179</td>
<td>58</td>
</tr>
<tr>
<td>Standard Deviation (SD) in ng/mL</td>
<td>110</td>
<td>109</td>
<td>68</td>
</tr>
<tr>
<td>Coefficient Variation (CV)</td>
<td>56%</td>
<td>61%</td>
<td>116%</td>
</tr>
<tr>
<td>Min-Max range in ng/mL</td>
<td>17 - 426</td>
<td>35 - 386</td>
<td>3 - 245</td>
</tr>
<tr>
<td></td>
<td>2 h (n = 8)</td>
<td>3 h (n = 9)</td>
<td>12 h (n = 10)</td>
</tr>
<tr>
<td>Mean in ng/mL</td>
<td>168</td>
<td>164</td>
<td>86</td>
</tr>
<tr>
<td>Standard Deviation (SD) in ng/mL</td>
<td>111</td>
<td>89</td>
<td>37</td>
</tr>
<tr>
<td>Coefficient Variation (CV)</td>
<td>66%</td>
<td>54%</td>
<td>43%</td>
</tr>
<tr>
<td>Min-Max range in ng/mL</td>
<td>53 - 386</td>
<td>55 - 363</td>
<td>51 - 172</td>
</tr>
</tbody>
</table>

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RIVAROXABAN

A

$y = 0.9692x + 25.76$

$\text{Calibrated DRVV-Screen (nM/L)}$

$n=60$

$r^2 = 0.82$

LC-MS/MS (ng/mL)

B

$y = 1.283x + 5.68$

$\text{Calibrated DRVV-Screen (nM/L)}$

$n=48$

$r^2 = 0.81$

LC-MS/MS (ng/mL)

D

$50\text{th upper percent limit of agreement}: 99 \text{ ng/mL}$

$5\text{th lower percent limit of agreement}: -173 \text{ ng/mL}$

DABIGATRAN

A

$y = 0.9692x + 25.76$

$\text{Calibrated DRVV-Screen (nM/L)}$

$n=60$

$r^2 = 0.82$

LC-MS/MS (ng/mL)

B

$y = 1.283x + 5.68$

$\text{Calibrated DRVV-Screen (nM/L)}$

$n=48$

$r^2 = 0.81$

LC-MS/MS (ng/mL)

D

$50\text{th upper percent limit of agreement}: 99 \text{ ng/mL}$

$5\text{th lower percent limit of agreement}: -173 \text{ ng/mL}$

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Vitamin K antagonists

The STA®-Staclot® DRVV-Screen yielded a concentration-dependent prolongation of clotting time (Fig. 1 – dabigatran (A)). The LOD and LOQ were 6 and 20 ng/mL, respectively. The first order equation gave r²-values of 0.77 and 0.78 when results were expressed in seconds or as ratios, respectively (Fig. 1 – dabigatran (A and B)). The Spearman correlation, the linear regression and the Bland-Altman analyses for results expressed in ng/mL are provided in Fig. 1 – dabigatran (C & D) and in Table 2.

Dabigatran

The STA®-Staclot® DRVV-Screen yielded a concentration-dependent prolongation of clotting time (Supplementary Material – Fig. 1). The mean prolongation of clotting time was 23.39 (95% CI: 19.07 – 27.71) seconds for dabigatran (C & D). The Spearman correlation, the linear regression and the Bland-Altman analyses for results expressed in ng/mL are provided in Fig. 2 – dabigatran (A and B)). The Spearman correlation, the linear regression and the Bland-Altman analyses for results expressed in ng/mL are provided in Fig. 2 – dabigatran (C & D) and in Table 2.

Vitamin K antagonists

The STA®-Staclot® DRVV-Screen reflected the prolongation of clotting time poorly (Supplementary Material – Fig. 1). The Spearman correlation coefficients between INR-values and STA®-Staclot® DRVV-Screen in seconds or ratio were 0.35 (95% CI: 0.27 – 0.70; p = 0.0011; r² for the linear regression 0.28) and 0.52 (95% CI: 0.27 – 0.70; p = 0.0001; r² for the linear regression 0.28), respectively.

ROC curve analyses

ROC curves analyses yielded different cut-offs for the threshold at 200 ng/mL. Sensitivities, specificities, positive and negative likelihood ratios, and positive and negative predictive values are summarised in Table 3. ROC curves are provided in Supplementary Material - Fig. 2.

Correlations between plasma drug concentrations and PT or aPTT

Table 2 mentions Spearman correlations, Bland-Altman analyses and the slopes of the linear regressions performed for rivaroxaban and dabigatran samples with calibrated PT and aPTT. Supplementary Material - Figs. 3 and 4 show results for the linear correlations between PT (rivaroxaban) and aPTT (dabigatran), plasma concentrations measured by LC-MS/MS and the corresponding Bland-Altman analyses.

Clinical outcomes

This study was not intended to investigate the efficacy and safety of rivaroxaban, dabigatran etexilate and VKAs. However, clinical data were obtained from patients in this cohort (Table 4). This includes recurrence of thrombus in the right atrium (n = 1: dabigatran etexilate), superficial venous thrombosis (SVT) (n = 1: rivaroxaban and n = 2: dabigatran etexilate), recurrence of stroke (n = 2: rivaroxaban and n = 1: VKA) or pulmonary embolism (n = 1: rivaroxaban and n = 3: VKA), embolism (n = 1: VKA), hematoma (n = 1: dabigatran etexilate and n = 2: VKA), gynaecological bleedings (n = 3: rivaroxaban) and
RIVAROXABAN

A

\[ y = 0.8764x + 30.72 \]

B

Calibrated DRVV-Confirm (ng/mL)

C

LC-MS/MS (ng/mL)

D

Difference between LC-MS/MS and calibrated DRVV measurement

Average of LC-MS/MS and calibrated DRVV measurement

DABIGATRAN

A

\[ y = 1.343x + 2.66 \]

B

Calibrated DRVV-Confirm (ng/mL)

C

LC-MS/MS (ng/mL)

D

Difference between LC-MS/MS and calibrated DRVV measurement

Average of LC-MS/MS and calibrated DRVV measurement

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gingival bleedings (n = 1: rivaroxaban). The plasma drug concentrations by LC-MS/MS at the different blood sampling times (for rivaroxaban and dabigatran), the INR (for VKA) and the results of the STA®-Staclot®DRVV-Confirm expressed in ratio (for rivaroxaban, dabigatran etexilate and VKA) for these outcomes are detailed in Table 4. All analyses were performed after the clinical outcome occurred.

Discussion

Both STA®-Staclot®DRVV-Screen and -Confirm correlated well with dabigatran and rivaroxaban plasma concentrations (Table 2). STA®-Staclot®DRVV-Confirm always provides better r²-values than did PT and aPTT for rivaroxaban and dabigatran samples (Table 2). In addition, the present results confirm previous observations of inter-reagent variability not only in terms of sensitivity but also regarding the inter-individual response when testing for PT with rivaroxaban (Table 2). STA®-Staclot®DRVV-Screen and -Confirm showed higher LOD and LOQ than the Biophen Duxal® for rivaroxaban and lower LOD and LOQ values than the ones obtained previously with the Hemoclot Thrombin Inhibitor® (HTI) for dabigatran [7,8].

In terms of sensitivity, STA®-Staclot®DRVV-Screen performed better than PT and aPTT. The sensitivity of STA®-Staclot®DRVV-Confirm was higher than the one of aPTT for dabigatran, while for rivaroxaban, depending on the PT reagent; the sensitivity was either similar (Triniclot PT Excel S®) or better (RecombiPlasTin 2G®).

Bland-Altman analyses show that STA®-Staclot®DRVV-Screen and -Confirm also perform slightly better than PT and aPTT, mainly due to closer 5th-95th limits of agreement (Table 2). However, the mean deviation from the reference measurement is large (i.e. 40 ng/mL, which represents a mean deviation of ±30% compared to the mean concentration of all dabigatran samples) for both DRVVT-T assays. This precludes their recommendation for the accurate estimation of plasma drug concentrations. Nevertheless, the present LC-MS/MS assay for dabigatran measured free (unconjugated) dabigatran, which is ±20% lower than the total concentration in plasma [16,18]. This can contribute but does not explain entirely the discrepancy for dabigatran plasma concentrations measured by this technique and the estimations of DRVVT-T assays. Moreover, the different cut-offs in the regulatory documents are established with the dTT (i.e. HTI) [19] and we previously found that the LC-MS/MS method used in this study correlates very well with this test [7]. An adapted methodology of this test even allowed an accurate assessment of plasma concentrations < 50 ng/mL [20].

A limitation of our study is that we only tested STA®-Staclot®DRVV-Confirm on a STA-R Evolution® coagulometer. However, compared to routine coagulation assays, i.e. PT or aPTT, DRVVT-T should be less affected by inter-reagent variability since there are fewer sources of Russell’s venom compared to the sources of thromboplastins (for PT) and activators of the contact pathway (for aPTT). However, the composition of phospholipids may differ between the different DRVVT reagents commercially available and could affect the sensitivity towards dabigatran and rivaroxaban [12]. Furthermore, the availability of DRVVT is not guaranteed in all laboratories 24/7 and its cost may also be a limitation since to date, the majority of DRVVT reagents are not liquid stable. This latter concern could lead to a waste of reagent, an increased turn-around time and finally a more expensive analysis.

The current Pradaxa® EU-SmpPC states that it can be assumed that measures of anti-coagulant activity reflect dabigatran levels and can provide guidance for the assessment of bleeding risk, and that exceeding the 90th percentile of dabigatran trough levels (i.e. 200 ng/mL in NVAF) is associated with an increased risk of bleeding [19]. This is in line with a recent investigation made by the BMJ revealing that if the plasma levels of dabigatran were measured and the dose was adjusted accordingly, major bleeds could be reduced by 30-40% compared with well controlled warfarin [21]. The French group “Groupe d’Intérêt en Hémostase Périopératoire (GIHP)” proposed a similar approach for rivaroxaban than the one stated for dabigatran and mention a cut-off at 200 ng/mL at C_rough, (i.e. 24 hours after the previous dose) as associated with an increased bleeding risk [22]. Therefore, a global test, easily implementable and widely performed, able to identify the anticoagulant and to screen the relative intensity of anticoagulation of all NOACs in urgent settings, is required [10].

Previous studies stated that the DRVVT-T is influenced by rivaroxaban [13,23,24] and that it could be useful to assess pharmacodynamics of NOACs [12,13,25]. This test might therefore be used for testing a wide range of NOACs rather than using different tests for each agent [12].

Is the Dilute Russell Viper Venom Time a useful test to complement routine coagulation assays facing a bleeding emergency?

Based on the present results we recommend the use of STA®-Staclot®DRVV-Confirm which assessed the relative intensity of anticoagulation due to rivaroxaban and dabigatran more accurately than PT or aPTT. STA®-Staclot®DRVV-Screen and –Confirm showed similar properties regarding the Spearman correlation, the Bland-Altman analysis or the LOD and LOQ while STA®-Staclot®DRVV-Confirm was a bit more sensitive. On the other hand, due to a high level of phospholipids, STA®-Staclot®DRVV-Confirm is less influenced by APS antibodies. This is the main reason why STA®-Staclot®DRVV-Confirm should be preferred to the screening test but this also results into the decreased sensitivity towards rivaroxaban and dabigatran (Figs. 1 & 2).

At the threshold concentration of 200 ng/mL, data obtained are significantly different for rivaroxaban and dabigatran either with STA®-Staclot®DRVV-Screen or –Confirm (Table 3). As a consequence, no single specific cut-off for both agents can be proposed and thus, different interpretations are needed for rivaroxaban and dabigatran treated patients. Importantly, it is preferable to express the results in ratio since it reduces the inter-lot variability.

The main advantage of STA®-Staclot®DRVV-Confirm is that it provides rapid estimation of the intensity of anticoagulation without requiring specific calibrants like more specific coagulation assays. More explicitly, at C_rough, above-mentioned thresholds (Table 3) for rivaroxaban and dabigatran can be used to identify supra-therapeutic plasma levels while the results of the thrombin time (TT), PT and aPTT may help to differentiate between therapies [10]. Concerning the VKA-treated patients, both STA®-Staclot®DRVV-Screen and –Confirm did not correlate with INR. One possible hypothesis is that, as DRVVT-T is not sensitive towards factor VII while the INR well, it is possible that a part of the variability is due to this difference in the principle of the tests. This has also been underlined with the Fix-HT, a modified PT which is sensitive only to reduction of FII and FX [26]. The authors of this previous study found that while the Fix-HT correlogram correlated well with PT (INR), it fluctuated less than the INR in anticoagulated patients reflecting its insensitivity to FVII.

Clinical outcomes

For rivaroxaban, 2 recurrences of stroke were associated with plasma concentrations below the 5th percentile observed in AF population at

Fig. 2. Correlation between STA®-Staclot®DRVV-Confirm and LC-MS/MS. Results for rivaroxaban and dabigatran are presented on the left and on the right of the figure, respectively. Fig. 2A, B and 2C show results expressed in seconds, as ratio and in ng/mL, respectively. Fig. 2D presents the results of the Bland-Altman analysis. For the Bland Altman analysis, the difference is calculated as follow: [difference (A–B) vs. average] where A is the result of LC-MS/MS and B the result of STA®-Staclot®DRVV-Confirm. For rivaroxaban results below 69.7 seconds or below a ratio of 1.9 could exclude plasma drug concentration above 200 ng/mL, as provided by the ROC analysis. For dabigatran, the same cut-off results in 98.8 seconds and a ratio of 2.7, as provided by the ROC analysis.

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C_{\text{trough}} (i.e. 12 ng/mL) [27]; one pulmonary embolism was associated with a plasma level at C_{\text{max}} below the 5th percentile of the population studied in DVT treatment (i.e. 189 ng/mL) and one gingival bleeding was seen in a patient with a plasma drug concentration above the 95th percentile of the ROCKET-AF study (i.e. 137 ng/mL) [27]. For the 3 gynaecological bleedings and the SVT, rivaroxaban plasma concentrations were within the 5th – 95th percentile range for both C_{\text{trough}} and C_{\text{max}}. Among patients on dabigatran etexilate, one SVT and one recurrence of thrombus in the right atrium were associated with a concentration below the 25th percentile of plasma concentrations observed in RE-LY at C_{\text{trough}} (i.e. 61 ng/mL) and at C_{\text{max}} (i.e. 117 ng/mL), respectively [19]. The other outcomes were not associated with plasma concentrations outside concentration ranges obtained in large phase-III trials. Several case reports showed an association between extreme plasma drug concentrations and efficacy/safety outcomes [28,29] but large cohort studies are required to confirm this hypothesis. For VKA treated patients, failure of treatment occurred in 3 patients within the therapeutic range while one recurrence of stroke was observed in one patient with subtherapeutic INR. Supratherapeutic INR was observed in 2 patients experiencing massive hematoma.

Table 4

<table>
<thead>
<tr>
<th>Clinical event</th>
<th>Delay since the drug intake</th>
<th>2 hours</th>
<th>3 hours</th>
<th>24 hours (+/- 1 hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hours</td>
<td>3 hours</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ng/mL</td>
<td>ng/mL</td>
<td>ng/mL</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>NA</td>
<td>183</td>
<td>213</td>
<td>247</td>
</tr>
<tr>
<td>Recurrence of stroke (1)</td>
<td>2.0</td>
<td>1.9</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Recurrence of stroke (2)</td>
<td>NA</td>
<td>206</td>
<td>35</td>
<td>238</td>
</tr>
<tr>
<td>Gynaecological bleeding</td>
<td>NA</td>
<td>192</td>
<td>190</td>
<td>206</td>
</tr>
<tr>
<td>Gynaecological bleeding</td>
<td>NA</td>
<td>213</td>
<td>36</td>
<td>238</td>
</tr>
<tr>
<td>Dabigatran etexilate</td>
<td>107</td>
<td>136</td>
<td>75</td>
<td>1.8</td>
</tr>
<tr>
<td>Recurrence of thrombus in the right atrium</td>
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<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Superficial venous thrombosis (1)</td>
<td>143</td>
<td>154</td>
<td>51</td>
<td>1.7</td>
</tr>
<tr>
<td>Superficial venous thrombosis (2)</td>
<td>386</td>
<td>363</td>
<td>172</td>
<td>2.6</td>
</tr>
<tr>
<td>Hematoma</td>
<td>152</td>
<td>NA</td>
<td>106</td>
<td>1.9</td>
</tr>
<tr>
<td>VITAMIN K ANTAGONISTS</td>
<td>INR</td>
<td>DRVV-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>2.5</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>2.2</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1.9</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence of stroke</td>
<td>1.4</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematoma</td>
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<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematoma</td>
<td>5.4</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embolism*</td>
<td>2.0</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA: Not available

† DRVV-T: results are those obtained with STAK®-Staclot®DRVV-Screen expressed in ratio.

‡ The same patient complained a superficial venous thrombosis and a gingival bleeding at the consultation.

* Localisation not specified

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Perspectives

Our proposed thresholds for STA®-Staclot®DRVV-Confirm should be confirmed in large, preferably multicentric prospective studies comparing different DRVV-T kits with clinical outcomes. These preliminary results should also serve as hypotheses to develop a point-of-care device that would be of particular interest in emergency situations.

Conclusions

STA®, Staclot®DRVV-Confirm may provide clinicians and biologists with rapid information on the intensity of anticoagulation with NOACs. However, it cannot directly inform the physician on the nature of the drug but the use of TT, alone or complement with PT and aPTT, may help in differentiating therapies. DRVV-T using the confirm reagent is more informative than PT and aPTT to identify supra-therapeutic levels and may provide clinicians and biologists with rapid information on the intensity of anticoagulation with NOACs. However, it cannot directly inform the physician on the nature of the drug but the use of TT, alone or complement with PT and aPTT, may help in differentiating therapies.

Addendum

Jonathan Douxfils, Bernard Chatelain, François Mullier and Jean-Michel Dogné were the main investigators and were responsible for the conception of the study. Jonathan Douxfils wrote the first draft of the manuscript and the final version.

Bernard Chatelain, Paul Hjemplo, Bérangère Devault, †Meyer-Michel Samama, François Mullier and Jean-Michel Dogné were responsible for the review of the manuscript.

Jonathan Douxfils, François Mullier and Bernard Chatelain were responsible of the coagulation assays. Some of the coagulation assays were performed by Mrs. Justine Baudar, Mr. Philippe Devel and Mr. Sébastien Walbrecq.

Paul Hjemplo, Pierre Wallemacq, Yuko Rönquist-Nii and Anton Pohanka were responsible of LC-MS/MS measures of dabigatran samples and Dr. Arnaud Capron was responsible of LC-MS/MS measures of rivaroxaban samples.

Conflict of interest statement

The authors have no relevant conflicts of interest to disclose.

Acknowledgements

The authors would like to thank †Prof. Meyer Michel Samama for reviewing the manuscript and providing technical expertise. We also would like to thank Dr. Arnaud Capron, Mrs. Justine Baudar, Ms. Noémie Despas, Mr. Philippe Devel and Mr. Sébastien Walbrecq for their contributions to this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2015.02.020.

References


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