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Full Length Article

Clotting test results correlate better with DOAC concentrations when expressed as a "Correction Ratio"; results before/after extraction with the DOAC Stop reagent

T. Exner[a,*], J. Favresse[b], S. Lessire[c], J. Douxfils[d], F. Mullier[b]

[a] Haematex Research, Sydney, Australia
[b] Université Catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), Namur Institute for Life Sciences (NARILIS), Haematology Laboratory, Yvoir, Belgium
[c] Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), Namur Institute for Life Sciences (NARILIS), Department of Anesthesiology, Belgium
[d] Université de Namur, NTHC/NARILIS, Department of Pharmacy, Belgium

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ABSTRACT

Introduction: Clotting test results are currently not useful for estimating direct oral anti-coagulant (DOAC) concentrations because baseline results vary. DOAC Stop is a DOAC extracting agent with no effect on clotting factors. We investigated if aPTT (activated partial thromboplastin time) and dRVVT (dilute Russells viper venom time) results might correlate better with DOAC concentrations if results after DOAC extraction were used to estimate a "before/after" value (Correction Ratio).

Materials and methods: We used activated partial thromboplastin time (aPTT, PTT-LA) and dilute Russells viper venom time clotting test (dRVVT) results previously recorded on DOAC patient plasmas (25 dabigatran, 15 apixaban, 19 rivaroxaban) without known thrombotic risk factors before and after DOAC extraction. DOAC concentrations had been determined by standard chromogenic assays.

Results: Correlations between aPTT and dabigatran, apixaban, and rivaroxaban concentrations were initially poor (0.64, 0.15 and 0.39 respectively). However, they improved significantly to 0.94, 0.89 and 0.80 when the ratios of initial aPTT to the aPTT obtained after DOAC extraction were plotted against DOAC concentration. Still better correlations (0.99, 0.97, 0.95) and much higher sensitivities to the DOACs were obtained when dRVVT (LA Confirm) tests were used following this procedure on the same samples.

Conclusions: The correlations of aPTT and dRVVT tests with DOAC concentrations were significantly improved by using the ratio of result "before" to those "after" DOAC extraction. The results indicate that dRVVT (especially LA Confirm) and similar tests might be useful for determining DOAC concentrations more reliably and with better sensitivity than currently possible with clotting tests.

1. Introduction

DOACs affect traditional clotting tests such as the activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT) and dilute Russell's viper venom time (dRVVT) tests to a variable degree depending on their mode of action, concentration and underlying variations in test plasmas [1]. The latter may be due to normal range distribution of clotting factors and endogenous inhibitors, lupus inhibitors or other interfering agents. Recently, an activated carbon-based product (DOAC Stop) appeared to be useful to specifically extract DOACs from test plasmas [2–4]. Accordingly, it has been shown that this extraction may allow the generation of more reliable results regarding factor assays [5] and thrombophilia screening tests (including lupus anticoagulants, LAC) [4] by overcoming the initial presence of DOACs.

The aim of this retrospective study was to estimate correlations between DOAC concentration and aPTT or dRVVT results on a panel of uncomplicated DOAC plasmas from an earlier study. Then, to determine if the ratio of initial clotting time (ie. in presence of DOAC) to clotting time after DOAC extraction (Correction Ratio) might provide more

Abbreviations: DOACs, direct oral anti-coagulants; aPTT, activated partial thromboplastin time; TT, thrombin time; PT, prothrombin time; dRVVT, dilute Russells viper venom time; PNP, pooled normal plasma; LAC, lupus anticoagulant; DOAC Stop, DS

* Corresponding author at: Haematex Research, 9/17 King Rd, Hornsby, Sydney, NSW 2077, Australia.
E-mail address: exner@optushome.com.au (T. Exner).

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reliable estimation of DOAC concentration as compared to the initial test result alone.

2. Material and methods

Results from 59 plasma from patients without abnormalities in factor V (Leiden), G20210A prothrombin mutation, lupus anticoagulant tests or factor deficiencies and taking DOACs (15 apixaban, 25 dabigatran and 19 rivaroxaban) obtained in an earlier study were used [4]. A pooled normal plasma (PNP) was also included for a total of 60 test samples.

Concentrations of dabigatran were previously estimated using the ecarin chromogenic assay (STA-ECA-II, Diagnostica Stago) and concentrations of apixaban and rivaroxaban with the corresponding procedure using an anti-Xa chromogenic assay (STA-liquid anti-Xa, Diagnostica Stago) on a STA-R MAX analyzer (Diagnostica Stago). The dilute Russell's viper venom time (dRVVT) screen and confirm (STA-Staclot dRVV Screen and Confirm, Diagnostica Stago) and the aPTT sensitive to lupus anticoagulants (PTT-LA, Diagnostica Stago) were assayed on the KC10 coagulometer (Amelung GmbH, Lemgo, Germany).

The plasma of each patient had been then treated with DOAC Stop™ (Haematex Research, Hornsby, Australia) and the same clotting tests repeated [4].

The clotting time results as well as clotting time ratios (Before/After DOAC Stop, B/A) were graphed against concentration of each DOAC and lines of best fit, along with coefficients of determination (R^2) were calculated using a regular Microsoft Office 365 XL program. Linear regression was preferred throughout except where alternative curve fitting gave a better correlation. Only graphed results with the aPTT and dRVVT (LA Confirm) tests are shown for brevity. We also compared the sensitivities of the aPTT and the 2 dRVVT tests by interpolating the B/A values at 100 ng/ml of each DOAC onto the trend lines.

3. Results

3.1. Initial aPTT results

Fig. 1 shows the scatterplot of aPTT results versus DOAC concentration with linear trend lines for apixaban, dabigatran and rivaroxaban. One apixaban plasma, 3 dabigatran plasmas and 2 rivaroxaban plasmas were excluded because of unusually prolonged APTT results.

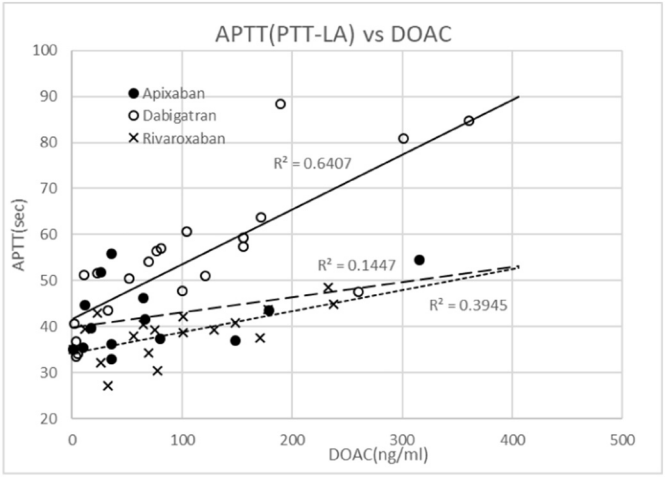

Fig. 1. Showing aPTT results (*sec*) obtained with PTT-LA reagent on plasmas from patients being treated with Dabigatran (○, solid line), Apixaban (●, long-dashed line) and Rivaroxaban (X, short-dashed line) plotted against concentration of each DOAC (ng/ml). Linear trendlines and R^2 values were assigned by the graphing program.

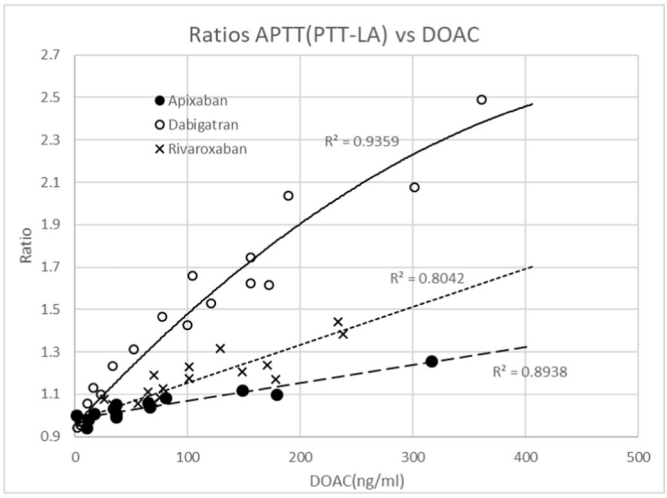

Fig. 2. This shows ratios of aPTT results before DOAC extraction to those after this plasma treatment plotted against DOAC concentration (ng/ml) for the various DOACs as identified previously.

The coefficients of correlation were quite low: 0.64 0.14 and 0.39 for dabigatran, apixaban and rivaroxaban respectively.

3.2. aPTT correction ratios

The scatterplot of aPTT ratio (before/after DOAC extraction) versus DOAC concentration with trend lines for apixaban, dabigatran and rivaroxaban is shown in Fig. 2.

For the dabigatran results the trendline was based on a power regression because it gave a better fit than linear regression. It is apparent that the correlations were much improved (0.94, 0.80, and 0.89 for dabigatran, apixaban, and rivaroxaban, respectively) from those obtained using the initial clotting times. Results for 4 dabigatran cases were incomplete (not available).

3.3. Initial dRVVT results

Similar clotting time vs DOAC concentrations scatterplots were prepared for dRVVT (LA Confirm) results.

As apparent from Fig. 3, this dRVVT test was more sensitive to all DOACs than the aPTT reagent used here (PTT-LA, similar to results reported previously [4,6–8]). Also, the correlations between DOAC

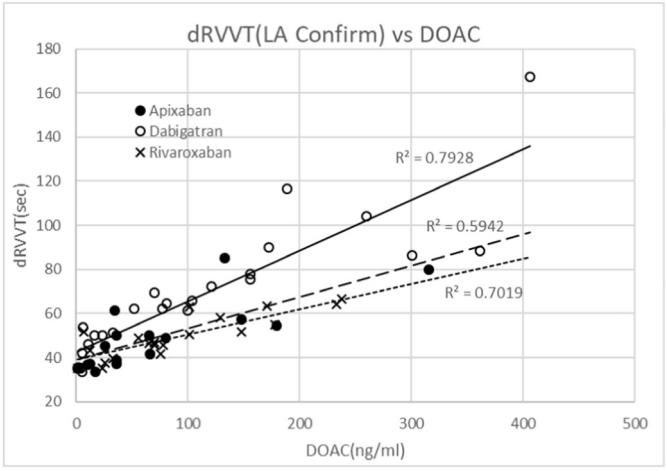

Fig. 3. This scatterplot shows dRVVT results obtained with LA Confirm reagent on plasmas from patients being treated with Dabigatran (○), Apixaban (●) and Rivaroxaban (X) plotted against concentration of each DOAC (ng/ml).

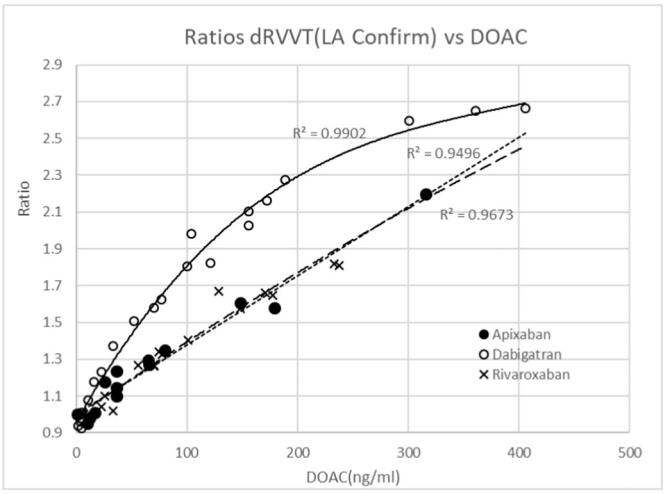

Fig. 4. This shows ratios of dRVVT (LA Confirm) results before removal of DOACs to those after this plasma treatment plotted against DOAC concentrations (ng/ml) for the various DOACs as identified previously. The curve fitting for Dabigatran and Rivaroxaban was power regression.

concentration and these dRVVT results were better than those obtained with the aPTT; 0.79, 0.59 and 0.70 respectively for dabigatran, apixaban and rivaroxaban. This is perhaps not surprising since fewer variable clotting factors affect dRVVT results.

3.4. dRVVT corrected ratios

As shown in Fig. 4, the dRVVT (LA Confirm) correlations were greatly improved with the use of before/after ratios for every DOAC (0.99, 0.97, and 0.95 for dabigatran, apixaban and rivaroxaban respectively).

Two of the apixaban results with abnormal dRVVT (LA Confirm) after DOAC Stop treatment were excluded as outliers (possibly misidentified). There were 3 missing (incomplete) results on dabigatran plasmas and one outlier ratio on a rivaroxaban plasma was also excluded (pending further investigation). Results for dRVVT (LA Screen) tests were also analysed but are only shown in Table 1 for brevity.

A summary of the correlation coefficients for both raw dRVVT and aPTT-LA tests and ratios of such tests to results after DOAC Stop on all dabigatran, apixaban and rivaroxaban plasmas is shown in Table 1.

This table also shows test result ratios interpolated onto each line of best fit for 100 ng/ml of each DOAC as an indicator of sensitivity. The higher values confirm that the dRVVT methods had better sensitivity for DOACs than the aPTT (PTT-LA) used here. It is apparent that the test with the low phospholipid dRVVT (LA Screen) reagent was slightly more sensitive to dabigatran and rivaroxaban than the high phospholipid dRVVT (LA Confirm) but provided worse correlations.

4. Discussion

These results suggest that significantly improved correlations between some clotting tests and DOAC concentrations might be achieved by using the underlying aPTT or dRVVT clotting time results obtainable after DOAC extraction in combination with the initial result expressed as a “correction ratio”.

The number of results with each DOAC was small because these plasmas had been used simply as control groups in an earlier study which proved the validity of thrombophilia testing after DOAC extraction [4]. Patient samples had been collected at random times after taking the oral agents. Some DOAC concentrations were quite low and the STA-liquid anti Xa method lacks discrimination below 20 ng/ml. A few outliers were not included in the analysis because if they had been,

Table 1
Summary of correlation and sensitivity results.

Test used and DOAC in plasma	Correlation (R^2) of result or ratio with DOAC concentration		Sensitivity
	Result (n)	Ratio Before/After DS (n)	Ratio at 100 ng/ml
aPTT (PTT-LA)			
Dabigatran	0.64 (20)	0.94 (16)	1.45
Apixaban	0.14 (13)	0.89 (13)	1.09
Rivaroxaban	0.39 (17)	0.80 (17)	1.15
dRVVT (LA Confirm)			
Dabigatran	0.79 (21)	0.99 (19)	1.80
Apixaban	0.59 (13)	0.97 (13)	1.40
Rivaroxaban	0.70 (17)	0.95 (16)	1.42
dRVVT (LA Screen)			
Dabigatran	0.86 (23)	0.90 (23)	1.90
Apixaban	0.60 (15)	0.95 (12)	1.35
Rivaroxaban	0.63 (18)	0.84 (17)	1.90

This shows results derived from aPTT (PTT-LA), dRVVT (LA Confirm) and dRVVT (LA Screen) tests on plasmas containing known concentrations of dabigatran, apixaban or rivaroxaban and no other known clotting defect. Correlation (R^2) values were determined from scatterplots of clotting time or Before/After ratio versus DOAC concentration. Number of points available are shown in brackets. Also showing the relative sensitivity of the Before/After test ratios for each DOAC interpolated from DOAC concentration at 100 ng/ml.

the correlations with raw clotting time results would have been close to zero. Thus, the significance of our observations is debatable and further larger studies are needed.

However, the principle developed here may probably be applied with advantage to uncomplicated test plasmas depleted of DOACs by any of the in vitro techniques currently being developed or the therapeutic DOAC antagonists such as Andexanet alfa and Idarucizumab. These are new developments and pose interesting questions regarding efficiency of removal and side effects in plasmas with additional abnormalities.

We previously showed that dRVVT tests were very sensitive to DOACs [6,7] and that they might be used for their approximate quantitation. However, as is well known from other tests, responses to anticoagulants are dependent on variables in the underlying plasma including factor levels and other inhibitors, (especially LAC if low phospholipid reagents are used [8,9]). This was a problem also with an improved high phospholipid dRVVT reagent (DOAC-Test) with better sensitivity to DOACs. We previously attempted to overcome underlying variations by mixing test plasmas 1:1 with PNP but correlations of DOAC Test clotting times with DOAC concentrations remained inadequate [10]. Also, the dilution from the mixing step reduced the sensitivity of the test for DOACs.

Ratios of clotting times (particularly from PT tests) relative to those from a normal plasma have been used for many years to simplify result interpretation. In the International Normalized Ratio (INR) system a sensitivity index is applied to express results from various reagents on a common scale. Calibration curves may serve a similar purpose. We propose the term “Correction Ratio” (B/A) for the initial clotting time (before DOAC extraction, B) divided by the clotting time after specific extraction of a DOAC (A).

This correction ratio method may offer a simple alternative to current chromogenic assays methods for testing for DOACs in otherwise normal plasmas. It could use existing aPTT or dRVVT reagents and might provide a more functional method for DOACs wherein their anticoagulant effects are assessed in conjunction with other clotting factors. All that is needed for its use is a calibration curve showing the dose-response for each individual DOAC in any dRVVT or aPTT test and then the clotting time ratio B/A (before/after) may be interpolated onto the line of best fit. The aPTT reagent used here (PTT-LA) was designed

for detecting LAC and alternative aPTT reagents, providing better correlation and higher sensitivity to DOACs may be more appropriate for future work along these lines. It is not clear why results with the LAC-sensitive dRVVT reagent appeared less useful in this proposed system. The DOAC extracting agent does not affect LAC [2] but perhaps the current procedure removes some procoagulant phospholipid from inadequately centrifuged samples, thereby inducing a variable into the correction ratio.

This new approach is different to current chromogenic assays for DOACs and could be applied generally to both anti-thrombin and anti FXa anticoagulants. Whereas chromogenic assays need kits and calibrators for individual DOACs, this extraction method might provide a low cost estimate of DOAC concentration simply from the correction ratio of two quick clotting tests. It would be necessary to use clotting reagents adequately sensitive to DOACs and to have appropriate calibration curves. The extraction method also allows labs to obtain a sample freed of DOACs for subsequent testing [2–5]. Additional studies are clearly required before this correction ratio method might be applied more widely. It is not yet clear how it might work with plasmas having additional clotting defects and how it might work with various aPTT, dRVVT or PT testing systems. It is also important to know if test plasmas might contain other coagulation inhibitors which might be removable by DOAC Stop [11].

Acknowledgements

Francois Mullier and Jonathan Douxfils designed the original study. Sarah Lessire and Julien Favresse carried out the tests. Thomas Exner analysed the results and wrote the paper while employed by Haematex Research. Test results used here were drawn from an earlier project [4] which had investigated the potential use of the DOAC-extracting agent to allow thrombophilia tests to be carried out despite the initial presence of DOACs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.04.028.

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