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LETTER TO THE EDITOR

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Reduction of the turn-around time for the measurement of rivaroxaban and apixaban: Assessment of the performance of a rapid centrifugation method

To the editor,

Phase III trials and registries[1] have highlighted the safety and efficacy of fixed drug regimen for direct oral anticoagulants (DOACs) without routine drug monitoring. However, measuring plasma DOACs level can be useful in several circumstances such as (i) detecting drug overdose, (ii) assessing the contribution of DOACs to serious bleeding, (iii) planning the timing of urgent invasive procedure, (iv) determining the suitability for thrombolytic therapy in acute ischemic strokes and (v) measuring the effect of antidote administration. Besides, guidance of the International Society on Thrombosis and Hemostasis warrants antidote administration for urgent procedure at high risk of bleeding when drug concentration exceeds 30 ng/mL, whereas for serious bleeding, antidote administration should be considered if drug concentration exceeds 50 ng/mL.[2] Routine assays like prothrombin time (PT) and activated partial thromboplastin time (aPTT) are not recommended for estimating DOAC plasma levels or assessing the intensity of anticoagulation at on-therapy doses due to lack of sensitivity, specificity, inter-reagent/instrument variation and lack of comparability with specific assays.[3] Currently, specific tests assessing DOAC plasma levels are recommended but their turn-around time (TAT) is still too long in many centres, reducing their usefulness in emergent situations which require accurate, rapid and 24/7 available specific tests. The rapidity is expressed by the TAT, defined as the time from registration of the blood sample in the laboratory to first result communicated, including centrifugation steps and the analysis process. The analysis process may also involve the reconstitution of the reagents when lyophilized, preparation of the calibration curve when the test is not yet calibrated on the analyser and validation of the control plasma, in addition to the sample's analysis and result's validation. Currently, some technical means are available to reduce the TAT of specific assays.[4]

One option is to reduce the duration of the centrifugation process. However, the preanalytical step may impair sample quality and modify the results of coagulation assays.[5,6] Therefore, we first aimed to study the impact of 2 centrifugation protocols (ie the standard protocol named "protocol A," vs a shortened centrifugation protocol named "protocol B") on the accuracy of screening and specific coagulation assays. Secondly, we aimed to measure the TAT of a chromogenic anti-Xa assay qualified for rivaroxaban or apixaban measurements in a real-life setting.

The study was approved by the Ethics Committee of the CHU UCL Namur (Yvoir, Belgium) (number: B039201630144). Written informed consent was obtained from each patient.

Sixty-four plasma samples from patients treated with apixaban and 27 plasma samples from patients treated with rivaroxaban were included in the study between January and April 2017. Patients were either on-therapy (n = 26 apixaban, 13 rivaroxaban) or stopped their treatment before surgery/invasive procedure (n = 38 apixaban, 14 rivaroxaban (delay since last dose median: median (interquartile range (IQR)), apixaban: 72 hours (55.5-89.5 hours), rivaroxaban: 79.5 hours (50-100 hours)).

Platelet-poor plasma (PPP) was obtained from the supernatant fraction after centrifugation of the blood samples for 15′ at 1500 g at room temperature (protocol A) or for 3′ at 4400 g at room temperature (protocol B). For each patient, the plasmas collected by protocol A or B were analysed simultaneously. All the analyses were performed on fresh samples on a STA-R MAX coagulometer (Diagnostica Stago®, Asnière-sur-Seine, France). Activated partial thromboplastin time (aPTT, STA®-C.K.Prest, Diagnostica Stago), PT (RecombiPlasTin 2G®, Werfen®, Netherlands), thrombin time (TT, STA®-Thrombin, Diagnostica Stago®) and fibrinogen using the Clauss method (Fib, STA®-Thrombin, Diagnostica Stago®) were performed on all samples. Plasma concentrations of rivaroxaban and apixaban were estimated using calibrated STA®-Liquid anti-Xa assay (STA-LAX). Four levels (0, 100, 250 and 500 ng/mL) of rivaroxaban and apixaban calibrators (Diagnostica Stago) were used according to the recommendations of the manufacturer. It should be noted that, in order to avoid reagents standing at room temperature (18-25°C) for 30 minutes before use, reagents of the STA-LAX were prepared each week and maintained in their original capped vials with STA®-Reducer for maximum 7 days on the STA-R MAX analyser. The 2 levels of rivaroxaban and apixaban controls (Diagnostica Stago) were also prepared each week and maintained in their original capped vials for a maximum 7 days at 2-8°C (according to the manufacturer's recommendations). As some laboratories do not have the possibility to prepare reagents and controls each week, we tested the impact of the reduction of the stabilization period of controls from 30 to 15 minutes or 5 minutes, on the 2 levels of controls and on 10 different days.

The TAT of STA-LAX was calculated for the standard centrifugation protocol for all the experiments. During the period preceding the study (November-December 2016), the median TAT for apixaban and rivaroxaban measurement was 246 minutes (n = 29). Recorded measurement of the TAT was only possible for the standard centrifugation protocol since the samples can only be registered once

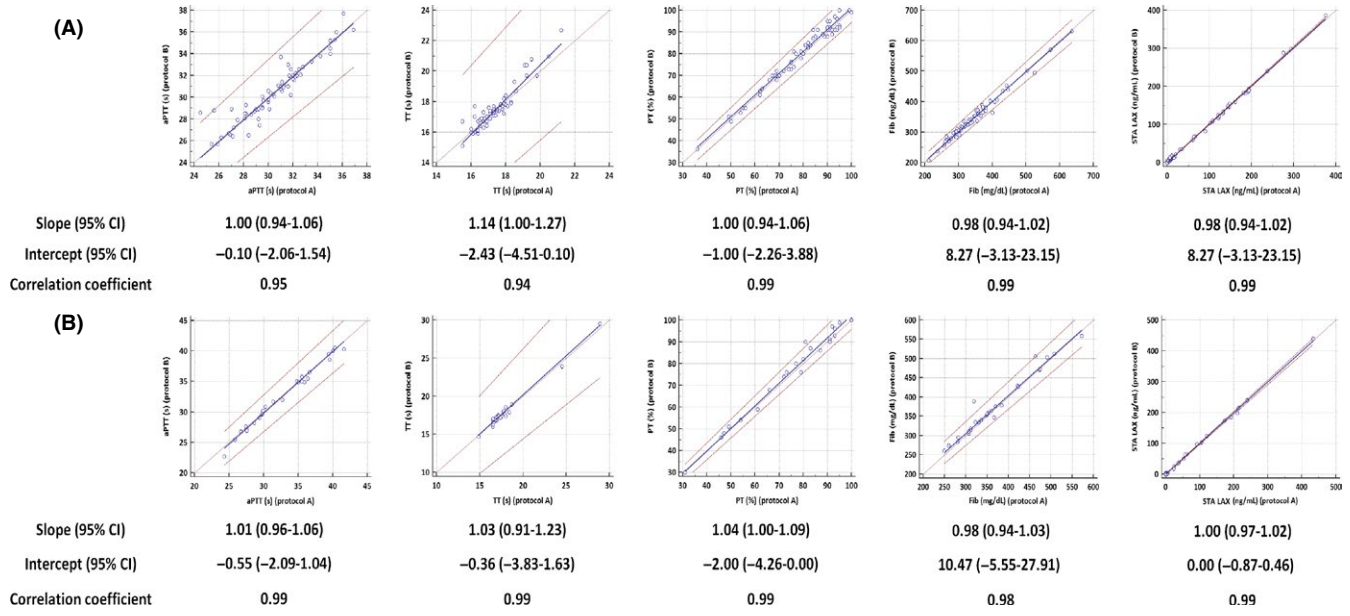

FIGURE 1 Comparison of protocol A with protocol B by Passing-Bablok regression analysis for aPTT, TT, PT, Fib, and STA LAX in plasma samples from patients treated with apixaban (A) and rivaroxaban (B) 95% CI = confidence interval at 95%. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Laboratory test results (aPTT, TT, PT, Fib and STA LAX) of plasma samples from patients treated with apixaban (n = 64) or rivaroxaban (n = 27)

	Protocol A[a]		Protocol B[b]			
	Median	IQR	Median	IQR	*P* value	Difference: mean B vs mean A (%)
Apixaban N = 64						
aPTT (s)	30.3	28.3-32.0	30.3	28.5-32.0	.68	0
TT (s)	17.2	16.7-17.9	17.3	16.7-17.8	.29	− 0.57
PT (%)	82.0	71.5-91.0	84.0	72.0-91.0	**.003**	−1.12
Fib (mg/dL)	331.0	293.7-370.2	333.5	298.5-370.0	.1	−0.41
STA LAX (ng/mL)	13.5	2.7-122.2	16.0	3 .7-122.0	.052	−1.33
Rivaroxaban N = 27						
aPTT (s)	31.4	28.3-35.9	31.7	28.0-35.5	**.025**	0.92
TT (s)	17.1	16.7-17.8	17.2	16.9-17.6	>.2	−0.56
PT (%)	78.0	54.0-91.0	78.0	54.0-90.3	**<.05**	−1.65
Fib (mg/dL)	350.0	311.8-421.0	357.0	317.8-427.0	**.003**	−2.08
STA LAX (ng/mL)	36.0	1.0-105.0	35.0	2.7-103.0	>.2	0

For PT, aPTT, Fib and TT, CV_A were equal or lower than 5.5% (data not shown). For rivaroxaban, CV_A was at 2.6% for a mean concentration of 313 ng/mL and at 4.0% for a mean concentration of 76 ng/mL. For apixaban, CV_A was at 2.3% for a mean concentration of 281 ng/mL and at 3.6% for a mean concentration of 73 ng/mL. The CV_I for PT, aPTT, Fib and TT was obtained in the literature.[11,12]
RCV (%) for aPTT, TT, PT and Fib are 3.60, 8.32, 34.79 and 20.11, respectively. Differences between protocols A and B were always below the corresponding RCV.
Statistically significant *P* values are written in bold.
aPTT, activated partial thromboplastin time; Fib, fibrinogen; IQR, interquartile range; PT, prothrombin time ; STA LAX, STA®-Liquid Anti-Xa assay; TT, thrombin time.
[a]Protocol A = Standard centrifugation (15 min).
[b]Protocol B = Shortened centrifugation (3 min).

in the Laboratory Information Management System. However, for each sample, procedure B allows a gain of 12 minutes compared to the standard procedure.

The biological results obtained by the 2 centrifugation protocols were compared by a standard t test in case of normal distribution (apixaban group) or by Wilcoxon test if the distribution failed

the normality and/or n < 30 (rivaroxaban group). GraphPad Prism 6.0e (GraphPad Software, La Jolla CA, USA, www.graphpad.com) and MedCalc 12.7.0 (MedCalc Software, Ostend, Belgium) were used to perform statistical analysis. A *P* value <.05 was considered statistically significant. The reference change value (RCV) which includes analytical variation (CV_A) and intraindividual biological variation (CV_I) was also employed to assess the clinical relevance of a statistical difference. Because DOACs are xenobiotics, it is difficult to determine the "biological" variation.[7] Therefore, we used the Passing-Bablok regression analysis to determine if both protocols may be interchangeable. Interchangeability is defined by the intercept and slope of each comparison that are not significantly different from 0 and 1, respectively. Figure 1A,B showed that protocols A and B may be used interchangeably for apixaban and rivaroxaban, respectively.

For apixaban, we found no difference between protocols A and B for aPTT, TT, Fib and STA-LAX. Concerning the PT, we found a statistically significant difference which was however not clinically relevant (Table 1). The median TAT was 61.0′ (min: 30.0′, max: 208.8′). The use of Protocol B (rapid centrifugation) allows reaching a TAT lower than 60 minutes in 12 additional samples.

For rivaroxaban, we found no difference between protocols A and B for TT and STA-LAX, while we observed statistically significant differences for aPTT, PT and Fib. Again, these differences were lower than the RCV, and thus not clinically relevant (Table 1). The median TAT was 67.6′ (min: 43.3′, max: 177.4′). The use of Protocol B allows reaching a TAT lower than 60 minutes in 7 additional samples.

In conclusion, the present study demonstrates that a rapid centrifugation protocol has no impact on the accuracy of rivaroxaban and apixaban plasma concentration assessments using the STA-LAX. High-acceleration centrifugation conditions are frequently used in laboratory automation systems to reduce the TAT of clinical chemistry samples, but also for PT, aPTT, fibrinogen and TT.[8]

A recent study in patients with acute stroke showed a median TAT of 34 minutes when specific assays are routinely implemented. However, details on the preanalytical and analytical processed used to achieve this TAT were not given.[9] The same team has also recently shown that determining rivaroxaban levels within a short TAT allowed thrombolysis for one-third of patients taking rivaroxaban who would otherwise be ineligible for this procedure.[10]

Our real-life study confirms the possibility of reducing the TAT around 30 minutes for specific rivaroxaban and apixaban assays. This was confirmed by TAT measurements (median: 32.8 minutes, IQR: 22.4-40.7 minutes) in 20 urgent requests (suspected or confirmed stroke, major bleedings, polytrauma, major surgeries or invasive procedures) collected after the study completion.

However, a wide variation of the TAT was observed during the study period and may be explained by the following reasons: the lack of awareness of an emergent request and of the possibility to use ready-to-use reagents/controls, and also by a delay between result delivery (ie result available in the lab) and technical validation (ie result available for the physician).

By reducing the period of stabilization of the controls (most manufacturers mention 30 minutes) to 15 or 5 minutes, we showed that the control results were always in the acceptable range (apixaban level 1: 56-94 ng/mL, level 2: 231-321 ng/mL), rivaroxaban level 1 (62-100 ng/mL), level 2: 251-345 ng/mL) after 15 or 30 minutes of reconstitution. After 5 minutes, there was only one outlier for rivaroxaban (control high: 379 ng/mL) and apixaban (control high: 332 ng/mL).

In conclusion, a rapid centrifugation protocol is feasible to reduce the TAT of the laboratory assessment of apixaban and rivaroxaban plasma concentrations below 60 minutes without impacting the accuracy of the results. Other option such as reducing the period of stabilization of the reagents and controls, and preparing and storing reagents/controls every week following manufacturer's recommendations may reduce the TAT to around 30 minutes. However, this requires implementation of specific laboratory management procedure and continued training of all the staff members.

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REFERENCES

1. Beyer-Westendorf J, Gelbricht V, Forster K, et al. Peri-interventional management of novel oral anticoagulants in daily care: results from the prospective Dresden NOAC registry. *Eur Heart J*. 2014;35:1888-1896.
2. Levy JH, Ageno W, Chan NC, Crowther M, Verhamme P, Weitz JI. Subcommittee on Control of A. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. *J Thromb Haemost*. 2016;14:623-627.
3. Testa S, Legnani C, Tripodi A, et al. Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study. *J Thromb Haemost*. 2016;14:2194-2201.
4. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. *J Thromb Haemost*. 2018;16:209-219.
5. Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favaloro EJ. Quality standards for sample collection in coagulation testing. *Semin Thromb Hemost*. 2012;38:565-575.
6. Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thromb J*. 2016;14:49.
7. Pauwels S, Vermeersch P, Desmet K, Vanstapel F. Practice-oriented quality specifications for therapeutic drug monitoring. *Clin Chem*. 2014;60:559-560.
8. Suchsland J, Friedrich N, Grotevendt A, et al. Optimizing centrifugation of coagulation samples in laboratory automation. *Clin Chem Lab Med*. 2014;52:1187-1191.
9. Seiffge DJ, Traenka C, Polymeris A, et al. Feasibility of rapid measurement of Rivaroxaban plasma levels in patients with acute stroke. *J Thromb Thrombolysis*. 2017;43:112-116.
10. Seiffge DJ, Traenka C, Polymeris AA, et al. Intravenous thrombolysis in patients with stroke taking rivaroxaban using drug specific plasma levels: experience with a standard operation procedure in clinical practice. *J Stroke*. 2017;19:347-355.
11. de Maat MP, van Schie M, Kluft C, Leebeek FW, Meijer P. Biological variation of hemostasis variables in thrombosis and bleeding: consequences for performance specifications. *Clin Chem*. 2016;62:1639-1646.
12. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. *Semin Thromb Hemost*. 2008;34:635-641.