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LETTER TO THE EDITOR

Are the DOAC plasma level thresholds appropriate for clinical decision-making? A reappraisal using thrombin generation testing

Dear Editors,

The direct oral anticoagulants (DOACs) are now used for several thromboembolic diseases with indications being dependent of the drugs and of the approvals by regulatory bodies.[1] Routine monitoring of their anticoagulant activity is generally not necessary, but the measurement of DOAC plasma levels can be required in some situations such as bleedings or thromboembolic events while on anticoagulation.[1] In emergency situations such as acute ischemic strokes, the decision to perform thrombolysis in a DOAC patient is complicated and may pose an additional risk of bleeding.[2] In these situations, there is a need to exclude residual anticoagulant activity prior to initiation of thrombolysis or to avoid unnecessary administration of reversal agent.[1] A safe-for-treatment threshold of 30 ng/mL has been suggested for apixaban, dabigatran, and rivaroxaban covering both perioperative situations and thrombolysis. The threshold of 50 ng/mL has been proposed to permit surgery with moderate bleeding risk.[3] In the absence of reversal agents, some groups of expert have proposed other cutoffs for safe thrombolysis, that is, 100 ng/mL for rivaroxaban, 50 ng/mL for dabigatran, and 10 ng/mL for apixaban.[4] However, these are subjective, based on expert's opinions, and do not reflect the different pharmacodynamic profile of DOACs.[5]

Specific laboratory tests, that is, anti-IIa or anti-FXa assays calibrated for each DOAC, are more sensitive than routine tests and report the results in ng/mL. This unit corresponds to the one used for the definition of the abovementioned thresholds, making these tests quite relevant in routine for clinical decision-making.[2] On the other hand, global tests such as the thrombin generation assay (TGA) have been described as promising to assess the pharmacodynamic profile of anticoagulants which may better reflect the true anticoagulant activity than measuring the ponderal concentration.[6] Given the known thrombin generation (TG) profile of DOAC, the ponderal thresholds proposed in the literature may provide highly different anticoagulant activities in a particular patient. The aim of this study was to assess the impact of DOACs on TGA at the different concentrations used as thresholds proposed for urgent clinical decision-making.

The study protocol is in accordance with the Declaration of Helsinki, and the recruitment of the volunteers has been approved by the Ethical Committee of the CHU UCL Namur, Yvoir, Belgium (approval number: B03920096633). Apixaban, betrixaban, edoxaban, rivaroxaban, and dabigatran were spiked in normal pooled plasma (NPP) at five relevant plasma concentrations (0 [buffer], 10, 30, 50, and 100 ng/mL).[3,4] Previous reports showed that results obtained in spiked samples correlate well with those in real-life samples, although some interindividual variability may not be excluded.[6] The NPP was obtained by mixing the platelet-poor plasma (PPP) from 52 healthy volunteers. The exclusion criteria and the protocol for venipuncture were the same as previously described.[7] The TG profile was assessed by the calibrated automated thrombogram (CAT) using the Thrombinoscope software version 5.0 (Thrombinoscope bv, Maastricht, the Netherlands) with the PPP Reagent® as triggering reagent. The ratio for the lag time (LT) and the time to peak (Ttpeak) and the percentage of inhibition of the peak height (Peak), the endogenous thrombin potential (ETP), and the mean velocity rate index (mVRI) were calculated relative to the NPP buffer condition. Friedman and Dunn's multiple comparison tests have been used to assess statistical differences between different thresholds of DOACs for each parameter. TGA has been performed in triplicate and assessed by 3 independent runs. The mean of these 3×3 experiments has been calculated for each parameter at each concentration of DOAC. The threshold for significance has been set at 0.05. All data were processed using GraphPad Prism software 8.0 (San Diego, California, USA).

The ratio of the LT and the Ttpeak and the percentage of inhibition of the Peak, ETP, and mVRI for each DOAC are summarized in Table 1. The comparison of parameters and thresholds is summarized in Table S1. All DOACs demonstrated a concentration-dependent prolongation of the LT and the Ttpeak (Figure 1). Factor Xa inhibitors demonstrated a concentration-dependent reduction in the Peak, ETP, and mVRI, whereas dabigatran had little/no impact on these parameters (Figure 1 and Table 1). The most influenced parameters were the mVRI and Peak for FXa inhibitions, while for dabigatran, these were the LT and Ttpeak. Depending on the parameters and the thresholds, significant differences were observed between DOACs and principally between FXa inhibitors and dabigatran (Table S1). At the lowest threshold investigated (ie, 10 ng/mL), only apixaban did not show a difference in TG parameters compared with condition in the absence of anticoagulant. In summary, the TG profile of dabigatran significantly differs from all FXa inhibitors, and within FXa inhibitors, betrixaban appears to be the most potent TG inhibitor, followed by rivaroxaban, while apixaban and edoxaban possess a similar TG inhibitory activity. Figure 1 showed that 10, 30, 50, or 100 ng/mL of dabigatran are significantly different from

TABLE 1 Summary of CAT parameters for DOACs at 5 relevant plasma concentrations

	[ ] ng/mL	0	10	30	50	100	p value†
Lag time (ratio)	Apixaban	1.00	**1.09**	1.27	1.27	1.51	<0.05
	Betrixaban	1.00	1.43	1.97	2.15	2.66	<0.05
	Edoxaban	1.00	1.24	1.59	1.72	1.94	<0.05
	Rivaroxaban	1.00	1.20	1.40	1.56	**1.79**	<0.05
	Dabigatran	1.00	1.50	1.51	**1.63**	2.22	<0.05
	P value‡	/	<0.05	<0.05	<0.05	<0.05	/
Ttpeak (ratio)	Apixaban	1.00	**1.10**	1.21	1.22	1.24	<0.05
	Betrixaban	1.00	1.60	2.50	2.81	3.28	<0.05
	Edoxaban	1.00	1.27	1.69	2.02	2.40	<0.05
	Rivaroxaban	1.00	1.22	1.84	2.27	**2.70**	<0.05
	Dabigatran	1.00	1.18	1.20	**1.23**	1.46	<0.05
	P value‡	/	<0.05	<0.05	<0.05	<0.05	/
Peak (inhibition %)	Apixaban	100.0	**83.4**	56.0	41.1	27.7	<0.05
	Betrixaban	100.0	58.7	32.1	25.0	14.9	<0.05
	Edoxaban	100.0	79.6	57.4	45.2	34.2	<0.05
	Rivaroxaban	100.0	64.8	39.8	31.0	**21.8**	<0.05
	Dabigatran	100.0	103.7	102.7	**107.0**	103.9	<0.05
	P value‡	/	<0.05	<0.05	<0.05	<0.05	/
ETP (inhibition %)	Apixaban	100.0	**97.1**	91.5	83.9	70.9	<0.05
	Betrixaban	100.0	91.7	74.0	64.4	44.2	<0.05
	Edoxaban	100.0	96.9	93.7	87.5	75.9	<0.05
	Rivaroxaban	100.0	92.1	80.6	74.8	**59.4**	<0.05
	Dabigatran	100.0	102.0	101.7	**106.2**	96.0	<0.05
	P value‡	/	<0.05	<0.05	<0.05	<0.05	/
mVRI (inhibition %)	Apixaban	100.0	**75.8**	47.8	34.8	26.9	<0.05
	Betrixaban	100.0	33.7	11.4	7.5	4.0	<0.05
	Edoxaban	100.0	62.7	31.6	20.0	12.2	<0.05
	Rivaroxaban	100.0	51.2	18.0	11.1	**6.5**	<0.05
	Dabigatran	100.0	111.4	106.6	**115.2**	121.1	<0.05
	P value‡	/	<0.05	<0.05	<0.05	<0.05	/

Abbreviations: ETP, endogenous thrombin potential; Lag time, time required to reach 10 nM of thrombin; mVRI, mean velocity rate index; Peak, peak height of thrombin; Ttpeak, time to reach the thrombin peak.

The table reports data for the lag time and the Ttpeak (ratio) and for the Peak, the ETP, and the mVRI (percentage of inhibition) for DOACs at five concentration thresholds. Significant differences were observed between DOACs‡ and between thresholds†. Boxes corresponding to the different thrombolysis thresholds, that is, 10 ng/mL for apixaban, 50 ng/mL for dabigatran, and 100 ng/mL for rivaroxaban, are marked in bold to facilitate the comparison for a particular TGA parameter.

the same amount of apixaban, betrixaban, edoxaban, or rivaroxaban (Table S1). The less pronounced effect observed with apixaban and edoxaban compared to rivaroxaban and betrixaban might be due to a different inhibition constant (Ki) on FXa.[5] The differences between FXa inhibitors and dabigatran are certainly related to the coagulation protein targeted.[5,8] Table 1 showed that the effects of FXa inhibitors were more pronounced on the Peak and mVRI than on other parameters while dabigatran had major effects on the LT and Ttpeak, also demonstrating the importance of the choice of the parameter to be analyzed according to the DOAC to reflect the intrinsic anticoagulant activity.[6,7]

Recommendations for the perioperative management are discrepant. Experts are still debating on the best approach, that is, laboratory versus pharmacokinetic. In the perioperative setting, the residual plasma concentrations of DOACs vary with the elective or urgent nature of the invasive procedure. However, the risk is mainly driven by the criticality of the intervention (ie, low versus high risk procedures) and the residual DOAC concentrations.[9] However, our results showed that TG profiles from DOACs significantly differ and the decision to interrupt anticoagulation before surgery should be based on residual activity profile rather than DOAC levels.

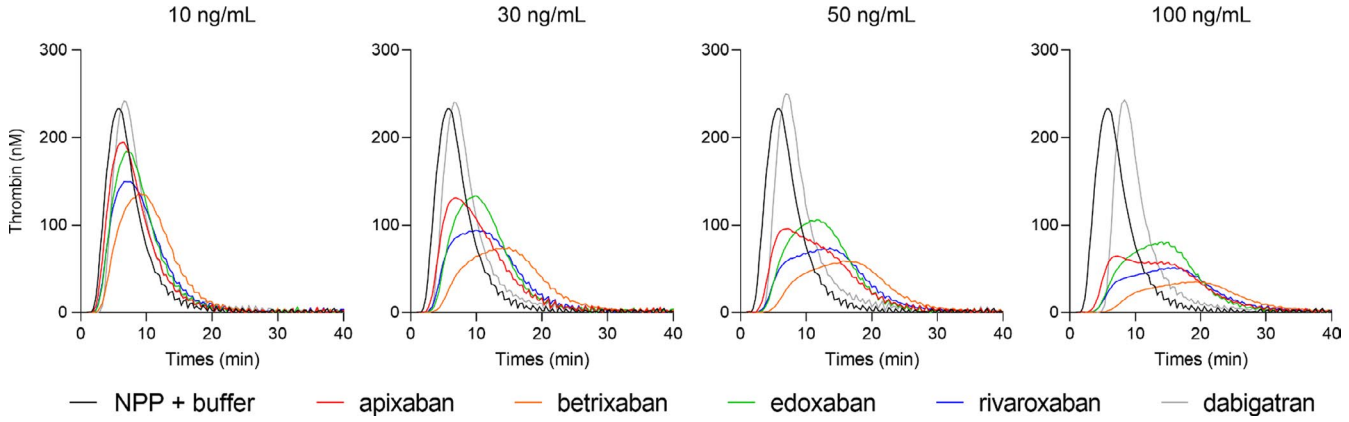

FIGURE 1 Impact of DOACs at 4 relevant plasma concentrations on the calibrated automated thrombogram using PPP Reagent® [Colour figure can be viewed at wileyonlinelibrary.com]

Recommendations on thrombolysis procedures in DOAC patients are heterogeneous in the literature.[2-4,10] According to the American Heart Association/American Stroke Association (AHA/ASA), thrombolysis can be performed in patients on DOAC if the drug was last taken since at least 48 hours and creatinine clearance ≥ 50 mL/min.[10] The French Vascular Neurology Society further mentions that thresholds ≤ 30 ng/mL, or ≤ 50 ng/mL if the bleeding risk is moderate, should be reached before performing thrombolysis.[2] Other cutoffs have been suggested, that is, 10 ng/mL for apixaban, 50 ng/mL for dabigatran, and 100 ng/mL for rivaroxaban, before intravenous (IV) thrombolysis, but still with a lack of clinical validation.[4]

Knowing the different pharmacodynamic and pharmacokinetic profiles of these drugs, both the kinetic and the ponderal approaches have limitations. While the former is highly dependent on the elimination rate of the individual patient, and therefore on the residual activity after a determined time frame,[6] the latter is limited by the assumptions that similar concentrations of DOACs will provide similar anticoagulant effects. The TG data obtained in this study revealed that the threshold of 50 ng/mL of dabigatran gave a LT ratio clinically equivalent to 100 ng/mL of rivaroxaban but was significantly different than 10 ng/mL of apixaban, demonstrating that these thresholds are certainly not equivalent when considering the residual anticoagulant effect. This is also confirmed with other TG parameters. For the Ttpeak, the ratio of apixaban and dabigatran was lower than that rivaroxaban. For the Peak, mVRI, and ETP, dabigatran had no impact and again significant differences were observed between 10 ng/mL of apixaban and 100 ng/mL of rivaroxaban. These results show that thresholds mentioned in the literature are not equivalent in terms of residual anticoagulant activity as observed by these well-controlled TG experiments. A similar observation is made by comparing the thresholds of 30 and 50 ng/mL of DOACs (Table 1).

Therefore, further research is needed to define the optimal approach for the perioperative management or the management of thrombolysis in DOAC patients. More investigations are needed to confirm these in vitro data and establish the suitability of global coagulation tests such as TGA, thromboelastography, or sonorheometry for the management of thrombolysis, urgent surgery, or administration of reversal agents. For stroke management, the ideal laboratory test should have a low turnaround time to provide results within a short time frame (ie, within 30 maximum). Thus, to date, even if automated devices are available to assess TG,[11] the actual time required to provide an interpreted result might be too long, and therefore, this technique deserves further improvements (ie, reduction in centrifugation times, possibility to extract partial results during the analytical process). However, this study has the advantage of addressing the question whether the thresholds currently proposed for safe thrombolysis and urgent procedures are appropriate given that they provide highly different anticoagulant profiles. Due to the different targets and inhibition constant (Ki) between DOACs, the use of specific TGA parameters could be more accurate in assessing the residual anticoagulant activity in plasma. Such observations will be useful to redefine safe and appropriate procedure for thrombolysis and urgent procedures.

KEYWORDS

dabigatran, direct oral anticoagulants, factor Xa inhibitors, thrombin generation, thrombolysis thresholds

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

J. Evrard and J. Douxfils designed the study. J. Evrard interpreted the data. J. Evrard and J. Douxfils wrote the first draft. V. Maloteau, M. Hardy, S. Lessire, J.-M. Dogné, and F. Mullier supplied additional information. All authors contributed to critical review and manuscript revision.

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