A New Method for Determining Concentrations of Direct Oral Anticoagulants

Bloemen, Saartje; Zwaveling, Suzanne; Douxfils, Jonathan; Peters, T.; Wagenvoord, Rob; Hemker, Hendrick Coenraad; Mullier, François; de Laat, Bas; Roest, Mark; Kremers, Romy

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PB 1199 | A New Method for Determining Concentrations of Direct Oral Anticoagulants

S. Bloemen[1,2], S. Zwaveling[1,2], J. Douxfils[3], T. Peters[1,2], R. Wagenvoord[1,2], H.C. Hemker[1,2], F. Mullier[3,4], B. de Laat[1,2], M. Roest[1,2], R. Kremers[1,2]

[1]Synapse Research Institute, CARIM, Maastricht University, Maastricht, The Netherlands, [2]CARIM, Maastricht University, Department of Biochemistry, Maastricht, The Netherlands, [3]Namur Research Institute for Life Sciences, University of Namur, Department of Pharmacy, Namur, Belgium, [4]Université Catholique de Louvain, CHU UCL Namur, Hematology Laboratory, Namur Thrombosis and Hemostasis Center, Yvoir, Belgium

Background: Direct oral anticoagulants (DOACs) are commonly provided without monitoring. However, reliable assays to measure DOAC levels and activity in emergency situations are needed. We developed a test based on the inhibition of $\alpha_2$macroglobulin-thrombin (α_2M-IIa) by dabigatran (DAB) and of α_2M-factor Xa (α_2M-Xa) by rivaroxaban (RIV), making it possible to evaluate both DOAC classes in combination with thrombin generation (TG).

Aims: To quantify DOAC levels and activity in plasma.

Methods: Consenting patients using RIV (n=50) and DAB (n=28) were included. TG was performed in platelet poor plasma (5 pM tissue factor), with idarucizumab in calibrator wells for DAB samples. The new DOAC assays measured the effect of diluted plasma samples on Z-Gly-Gly-Arg-AMC conversion by α_2M-Xa or α_2M-IIa. The slopes of these curves were compared to a reference curve with known DOAC concentrations. DOAC levels were also estimated by 'classical' assays. Spearman correlation coefficients were determined.

Results: A concentration of 30 nM α_2M-Xa or α_2M-IIa was optimal to measure 50-500 nM RIV/DAB and 90 nM α_2M-Xa or 70 nM α_2M-IIa, respectively were optimal for 500-1000 nM RIV/DAB (Fig.1).

The intra- and inter-assay CV were below 2.5 % and 5% (n=2). Both the RIV and DAB assay correlated with TG parameters. The RIV assay correlated with ‚classical' assays and had a very good correlation with Biophen DiXaI. The DAB assay did not correlate with hemoclot thrombin inhibitor (probably since DAB concentrations were too low), but showed variable correlation with the other assays (Table1).

Conclusions: The new DOAC assays show good correlations with other assays that were confirmed to accurately assess DOAC levels (particularly the RIV assay with the Biophen DiXaI, which had the best correlation with mass spectrometry). Our assay can simultaneously evaluate DOAC concentrations as well as the DOACs effect on thrombin generation, providing an overview of the anticoagulation status of a patient in relation to circulating DOAC levels.

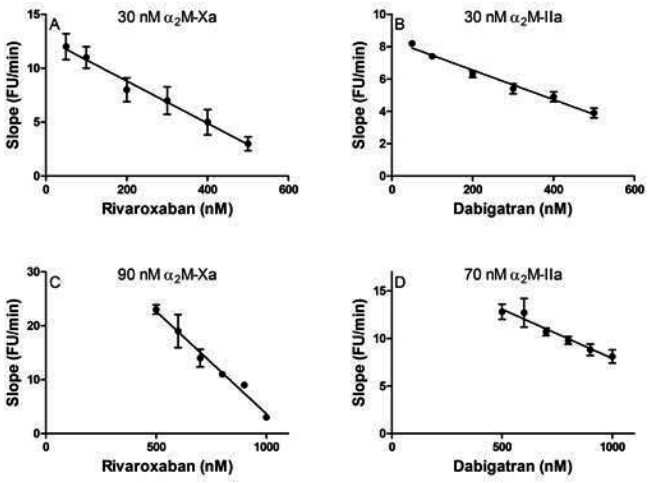

FIGURE 1 Dose-response of several DOAC concentrations at chosen α2M-Xa and α2M-IIa concentrations

TABLE 1 Correlation (Spearman) of rivaroxaban or dabigatran concentration (as determined by the new assay) with TG and other calibrated assays

	Assay	Correlation coefficient	p-value		Assay	Correlation coefficient	p-value
Rivaroxaban	Calibrated prothrombin time (ng/ml)	0.468	0.002	Dabigatran	Activated partial thromboplastin time (s)	0.581	0.002
	Dilute Russel viper venom time (s)	0.760	0.000		Dilute Russel viper venom time (s)	0.649	0.001
	Biophen DiXaI (ng/ml)	0.915	0.000		Hemoclot thrombin inhibitor (ng/ml)	0.391	0.097
	TG endogenous thrombin potential(nM.min)	-0.525	0.000		Ecarin chromogenic assay (ng/ml)	0.591	0.001
	TG Peak (nM)	-0.550	0.000		TG endogenous thrombin potential(nM.min)	-0.323	0.100
	TG lag time (min)	0.671	0.000		TG Peak (nM)	-0.354	0.201
	TG time-to-peak (min)	0.702	0.000		TG lag time (min)	0.423	0.028
					TG time-to-peak (min)	0.339	0.084