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ORIGINAL ARTICLE

Measurement of rivaroxaban and apixaban in serum samples of patients

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ABSTRACT

Background The determination of rivaroxaban and apixaban from serum samples of patients may be beneficial in specific clinical situations when additional blood sampling for plasma and thus the determination of factor Xa activity is not feasible or results are not plausible.

Materials and methods The primary aim of this study was to compare the concentrations of rivaroxaban and apixaban in serum with those measured in plasma. Secondary aims were the performance of three different chromogenic methods and concentrations in patients on treatment with rivaroxaban 10 mg od (n = 124) or 20 mg od (n = 94) or apixaban 5 mg bid (n = 52) measured at different time.

Results Concentrations of rivaroxaban and apixaban in serum were about 20–25% higher compared with plasma samples with a high correlation (r = 0·79775–0·94662) using all assays (all $P < 0·0001$). The intraclass correlation coefficients were about 0·90 for rivaroxaban and 0·55 for apixaban. Mean rivaroxaban concentrations were higher at 2 and 3 h compared with 1 and 12 h after administration measured from plasma and serum samples (all P-values < 0·05) and were not different between 1 vs. 12 h (plasma and serum).

Conclusions The results indicate that rivaroxaban and apixaban concentrations can be determined specifically from serum samples.

Keywords Apixaban, chromogenic substrate methods, direct oral anticoagulants, plasma, rivaroxaban, serum.

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Introduction

The direct oral factor Xa inhibitors rivaroxaban and apixaban prevent venous thromboembolism following elective primary total hip and knee replacement surgery [1,2], recurrent venous thromboembolism in patients with acute venous thrombosis or pulmonary embolism [3,4] and cerebral and noncerebral embolism in patients with atrial fibrillation [5,6]. Rivaroxaban reduces recurrence of acute coronary syndromes in combination with acetylsalicylic acid and a thienopyridine [7]. These anticoagulants are given at fixed daily doses without need for laboratory-guided dose adjustments. If clinically indicated, current practice is to use factor Xa-specific chromogenic substrate assays to determine the drugs. For determination of rivaroxaban [8–13], and apixaban [14, 15] activities, blood needs to be anticoagulated by sodium citrate during withdrawal to avoid clotting *in vitro*. However, determination of rivaroxaban or apixaban concentrations in blood may be required even if such pretest conditions are not met, for example, if serum is the only available test material. Therefore, the determination of drugs from serum may be desirable in specific clinical situations such as in renal failure, concomitant administration of inhibitors of *P*-glycoprotein-I [16–18], very elderly, bleeding or thrombotic complication while on anticoagulation, before operations, before initiation of thrombolytic therapy or for control of adherence to therapy [19–21], or if plasma activity determinations do not appear to be plausible.

Unlike plasma samples, in particular those containing citrate, serum samples are taken from all patients in acute and nonacute clinical situations. Upon adequate handling, clinical chemical analyses are performed on automated clinical chemistry analysers. Further to citrate addition, blood for the determination of

coagulation parameters needs to be collected into plastic or siliconised glass tubes to inhibit mechanical coagulation activation *in vitro*. Potential pitfalls of blood drawing for coagulation parameters are incomplete filling of the tube resulting in wrong ratios of anticoagulant to blood leading to incorrect coagulation results and the activation of blood clotting during and after blood sampling due to incorrect handling. In addition, correct handling also includes centrifugation at given temperatures, analysis within a given time frame and specific procedures for freezing and thawing of samples [22].

The concentrations of drugs are predominantly determined in serum samples from patients using liquid chromatography techniques [14,23–25]. Drug levels or other clinical chemical parameters may be requested by clinicians after blood collection has been completed. Therefore, serum samples are often stored routinely for several days. This becomes important for patients with drug overdose or intoxication or for forensic purposes [26,27]. Rivaroxaban and apixaban bind directly to activated and nonactivated factor X, and they do not require cofactors. To determine the activities of both inhibitors in plasma – so far representing the only method of determination which is being accessible to clinical use – factor Xa is added in excess, and the residual factor Xa activity releases a chromophor in relation to the anticoagulant concentration. In a preliminary investigation, we reported on a photometric method to quantify the concentrations of new oral anticoagulants in serum samples of patients during treatment [28]. The aims of the study are the following: (i) a comparison of serum rivaroxaban and apixaban concentrations with those measured in plasma using several chromogenic methods, (ii) to compare the performance of three different chromogenic methods and (iii) to compare rivaroxaban and apixaban concentrations in patients measured at different time. Samples were obtained from patients on treatment with rivaroxaban 10 and 20 mg od and apixaban 5 mg bid.

Methods

Patients

Plasma and serum samples were obtained from patients before and after 4–6 days of treatment with rivaroxaban 10 mg od 12 h after intake of medication for prevention of thromboembolic complications after primary elective total knee (TKR) and hip replacement (THR) (n = 144). In a second group of patients (n = 74), plasma and serum samples were collected at 1, 2, 3 or 12 h after intake of rivaroxaban 20 mg od. They were treated to prevent systemic embolism in nonvalvular atrial fibrillation or recurrent events following venous thromboembolism. In a third group of patients with atrial fibrillation (n = 52) who received apixaban 5 mg bid, blood samples were collected at 2 h after administration. The studies were approved by the local ethics committee, and participants gave written informed consent prior to blood sampling.

Preparation of samples

Blood was collected into plastic tubes containing 3·8% sodium citrate (1/9, v/v, citrate/blood) to obtain platelet-poor plasma and into plastic tubes containing kaolin to obtain serum during the same venipuncture. Latter samples remained for 30 min at room temperature to allow serum formation in kaolin-containing tubes. They were centrifuged for 30 min at 1800 *g* and 4 °C, and several aliquots of the supernatant were [29] frozen with liquid nitrogen and kept at −72 °C until analysed.

Origin and quality of anticoagulants

Apixaban was kindly supplied by Bristol-Meyers-Squibb (Plainsboro,NJ, USA). Rivaroxaban was purified from commercially available Xarelto® tablets, and its purity was characterised by analytical methods as described [30].

Analysis of serum and plasma samples

Three chromogenic substrate assays were used for the determination of drug concentrations from serum [31] and plasma samples. The chromogenic assay in serum was performed as described for plasma. Following the addition of the chromogenic substrate, factor Xa was incubated in excess with the serum sample for 2 min (Coamatic and HemosIL assays) or 10 min (S2222 chromogenic substrate assay). The S2222 chromogenic substrate assay was performed as described using human factor Xa [71 nkat/mL, both reagents from Haemochrom, Essen, Germany, lower limit of detection (LOD) for plasma and serum about 25 ng/mL] [32]. Coamatic (Haemochrom, Essen, Germany, LOD for plasma and serum about 9 ng/mL) and HemosIL (Instrumentation Laboratory GmbH, Kirchheim, Germany, LOD for plasma and serum about 27 ng/mL, limit of quantification about three times higher for all methods in plasma and serum) chromogenic substrate assays were performed as described by the manufacturers. All assays were run on the microtiter plate system Multiskan FC connected to the software program SkanIt 3.1 (Thermo Fisher Scientific, Langenselbold, Germany). Standard curves were computed using serial concentrations of rivaroxaban and apixaban ranging from 0 to 500 ng/mL added to normal human plasma or serum pooled from 20 volunteers.

Statistical analysis

All statistical calculations have been performed with SAS software, release 9.3 (SAS Institute Inc., Cary, NC, USA). Quantitative variables are given as mean and standard deviation (SD). Furthermore, Pearson's correlation coefficient has been assessed to estimate the strength of correlation.

To compare mean values and error variances of plasma and serum concentrations, the t-test for two paired samples and the test of Maloney and Rastogi have been performed, respectively.

Table 1 Concentration of rivaroxaban (ng/mL) in controls and patients on treatment with 10 and 20 mg od 12 h after medication, and *P*-values for comparisons between plasma and serum. Assays and doses and correlations between plasma vs. serum

		Plasma		Serum		Plasma vs. serum	Plasma	Serum	Plasma	Serum	Plasma vs. serum Pearson correlation and intraclass correlation (ICC)		Plasma vs. serum Maloney Rastogi
							Control vs. treatment		10 vs. 20 mg				
	n	Mean [ng/mL]	SD [ng/mL]	Mean [ng/mL]	SD [ng/mL]	*P*-value *t*-test	*P*-value two-sample *t*-test	*P*-value two-sample *t*-test	*P*-value two-sample *t*-test	*P*-value two-sample *t*-test	*r*	ICC	*P*-value
Control													
Coamatic	137	9·8	8·1	4·5	9·6	< 0·0001					0·1465		0·0941
HemosIL	137	10·8	11·3	4·9	11·5	< 0·0001					0·4370		0·2346
S2222	137	14·3	11·5	17·2	16·7	0·0271					0·2504		< 0·0001
10 mg od													
Coamatic	120	52·9	38·8	63·6	49·5	< 0·0001	< 0·0001	< 0·0001			0·9434	0·8887	< 0·0001
HemosIL	120	56	40·2	64·9	54·9	0·0003	< 0·0001	< 0·0001			0·8796	0·8560	0·0005
S2222	121	67·8	41	82·6	40·4	< 0·0001	< 0·0001	< 0·0001			0·8074	0·7584	0·4930
20 mg od													
Coamatic	99	182·7	119·2	226·7	156·1	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·9466	0·8549	< 0·0001
HemosIL	99	185·6	119·2	197·5	145·2	0·0109	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·9113	0·8922	0·0004
S2222	99	233·8	113·1	270·1	138·3	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·9129	0·8596	< 0·0001

Table 2 Inter-assay comparisons for patients on treatment with rivaroxaban 10 and 20 mg od

	Plasma				Serum			
	t-test for paired samples *P*-value	Maloney Rastogi *P*-value	Pearson correlation (*r*) Intraclass correlation (ICC) *r*	ICC	*t*-test for paired samples *P*-value	Maloney Rastogi *P*-value	Pearson correlation (*r*) Intraclass correlation (ICC) *r*	ICC
Control								
Coamatic-HemosIL	0·3431	< 0·0001	0·2913		0·7291	0·0706	0·8632	
Coamatic-S2222	0·0002	< 0·0001	0·0626		< 0·0001	< 0·0001	0·0574	
HemosIL-S2222	0·0043	0·8369	0·2496		< 0·0001	0·0013	0·0687	
10 mg od								
Coamatic-HemosIL	0·0043	0·1801	0·9573	0·9582	0·3270	0·0096	0·9492	0·9470
Coamatic-S2222	< 0·0001	0·1764	0·8932	0·8385	< 0·0001	< 0·0001	0·8749	0·8337
HemosIL-S2222	< 0·0001	0·6136	0·8786	0·8466	< 0·0001	0·0085	0·8377	0·8203
20 mg od								
Coamatic-HemosIL	0·1215	0·5290	0·9482	0·9466	< 0·0001	0·0773	0·9263	0·9073
Coamatic-S2222	< 0·0001	0·3334	0·9111	0·7874	< 0·0001	0·0003	0·9653	0·9200
HemosIL-S2222	< 0·0001	0·2876	0·8721	0·7672	< 0·0001	0·3363	0·9532	0·8379

The latter test indicates whether serum and plasma measurements differ regarding their precision. It is based on the correlation between the sum and the difference of the two measurements and thus is equivalent to Bland–Altman analysis which is based on the correlation between measurements' mean and difference. Finally, the intraclass correlation coefficient was used to determine the strength of agreement between two measurement methods.

Mean values of two independent groups (e.g. control vs. treatment) have been compared using the two-sample *t*-test.

To evaluate changes over time, ANOVA for repeated measurements has been performed using the SAS procedure PROC MIXED. In the case of a statistically significant test result, Scheffe tests have been performed for pairwise comparisons. Statistical significance was assumed for test results with $P < 0{\cdot}05$.

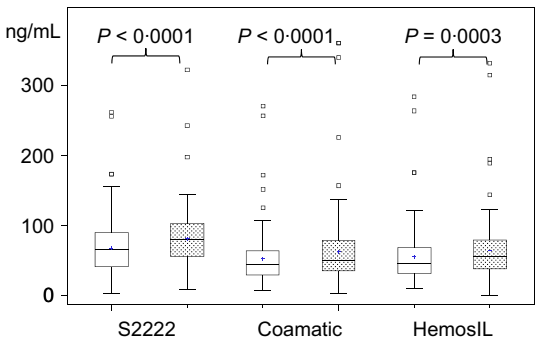

Figure 1 Boxplots of plasma (open boxes) and serum (stippled boxes) concentrations of rivaroxaban at 12 h after administration of 10 mg rivaroxaban od for 4–6 days in patients with elective hip or knee replacement surgery.

Results

Rivaroxaban and apixaban standard curves in plasma and serum

The serial dilutions of rivaroxaban and apixaban added to serum and plasma of six volunteers gave correlation coefficients [optical density (405 nm) vs. concentration] for rivaroxaban of $r = 0{\cdot}993$ with the S2222 assay, $r = 0{\cdot}998$ for the Coamatic assay and $r = 0{\cdot}9925$ for the HemosIL assay and for apixaban of $r = 0{\cdot}9993$ for the S2222 assay, $r = 0{\cdot}9980$ for the Coamatic assay and $r = 0{\cdot}9961$ for the HemosIL assay (all $P < 0{\cdot}0001$).

Control values of patients not on treatment with an anticoagulant

The mean concentrations of *rivaroxaban* ($n = 137$, Table 1) in serum samples of controls were higher compared with those

Table 3 Concentrations in plasma and serum samples at different time points in patients on treatment with rivaroxaban 20 mg od using three chromogenic substrate assays

					Plasma			Serum		
			Hour	*N*	Mean	SD	*P*-value *t*-test	Mean	SD	*P*-value *t*-test
S2222			1	14	174·2	71·7	0·0323	201·1	105·9	0·0015
			2	32	300	100·3		362	116·6	
			3	13	324·5	93·3		413·2	90·9	
			12	35	175·8	87·0		187·6	77·5	
	Hour		Hour				*P*-value Scheffe test			*P*-value Scheffe test
	1	vs.	2				0·0763			0·0228
	1		3				0·1779			0·0348
	1		12				0·8970			0·9992
	2		3				0·9999			0·9548
	2		12				0·1997			0·0118
	3		12				0·3607			0·0220
HemosIL			1	14	126·9	97·2	0·0004	99·5	85·9	0·0003
			2	30	269·9	105·2		296·4	133·2	
			3	13	297·3	85·1		357·3	99·2	
			12	31	110·3	57·4		114·1	71	
	1	vs.	2				0·0113			0·0027
	1		3				0·0305			0·0092
	1		12				0·9860			0·9222
	2		3				0·9960			0·9687
	2		12				0·0024			0·0032
	3		12				0·0094			0·0092
Coamatic			1	14	120·5	93·3	0·001	137·5	110·5	0·0002
			2	30	257·5	97·7		344·9	136·3	
			3	13	298·2	110·2		380·2	75·3	
			12	29	109·2	51·4		134·2	76·3	
	1	vs.	2				0·0169			0·0045
	1		3				0·0418			0·0085
	1		12				0·9934			0·9965
	2		3				0·9872			0·9110
	2		12				0·0068			0·0015
	3		12				0·0136			0·0043

in plasma samples. The S2222 assay showed higher values compared with the other assays, which themselves were not different from each other (Table 1). The correlation of plasma and serum values was rather low ($r = 0{\cdot}437$) but statistically significant with the HemosIL assay, and values did not correlate for the other assays. The precision was higher for the

Coamatic assay compared with the other assays using plasma and serum samples (Maloney-Rastogi test, Table 2). For *apixaban*, the mean, SD and variance values of plasma and serum samples of controls did not differ (Table 4), and values of plasma and serum samples did not correlate (Table 4).

Patients on treatment with rivaroxaban 10 mg od

Concentrations of rivaroxaban in plasma and serum were significantly higher during treatment (n = 121) compared with controls using all chromogenic assay (Table 1). Mean and SD values are shown in Table 1 and as Boxplots in Fig. 1 for plasma and serum samples for all assays. Despite the higher mean and variance values for serum samples, there was a strong correlation between plasma and serum values (Table 1). All chromogenic assays measured 15–20% higher concentrations in serum samples compared with plasma samples (P-values Table 1). S2222 assay determined about 20% higher values in serum and plasma compared with the other two chromogenic assays (P-values Table 2). The precision of the assays did not differ in plasma samples and was lower for the Coamatic assay in serum samples compared with HemosIL and S2222 assays and was lower for the HemosIL compared with the S2222 assay (Maloney-Rastogi test, P-values Table 2). The correlation of the concentrations of rivaroxaban in plasma and serum samples between assays ranged from r = 0·8377–0·9482 (Table 2). The ICC supported the results of the other statistical examinations.

Patients on treatment with rivaroxaban 20 mg od

Plasma and serum values were strongly correlated in all assays (Table 1). Mean concentrations and variances of rivaroxaban in plasma samples were lower compared with serum samples using all chromogenic assays (Table 1). The interassay comparisons showed no differences between plasma values and variances determined by the Coamatic and HemosIL assays (Table 2). The other intercomparisons of plasma and serum concentrations showed differences, but variances were not different except a lower variance of results of serum concentrations for Coamatic compared with S2222 assay (Table 2). The correlation between assays was strong for plasma and serum samples ranging from 0·8721 to 0·9653 (Table 2).

The concentrations of rivaroxaban in plasma and serum were different across all time points with all assays (t-test, Table 3). Detailed analysis using Scheffe test showed that in plasma, the concentrations between the different time points were not different using the S2222 chromogenic substrate assay. In contrast, values in serum samples were higher at 2 and 3 h after intake compared with 1 and 12 h (Table 3). Using the HemosIL and Coamatic chromogenic assays, concentrations were higher at 2 and 3 h compared with 1 and 12 h in plasma as well as in serum samples (Table 3). The concentrations of rivaroxaban were higher in serum samples compared with plasma samples with all chromogenic assays (Table 3). Values in plasma and serum samples were higher using the S2222 compared with the results using the Coamatic and HemosIL chromogenic assays (Table 3, Fig. 2a–c).

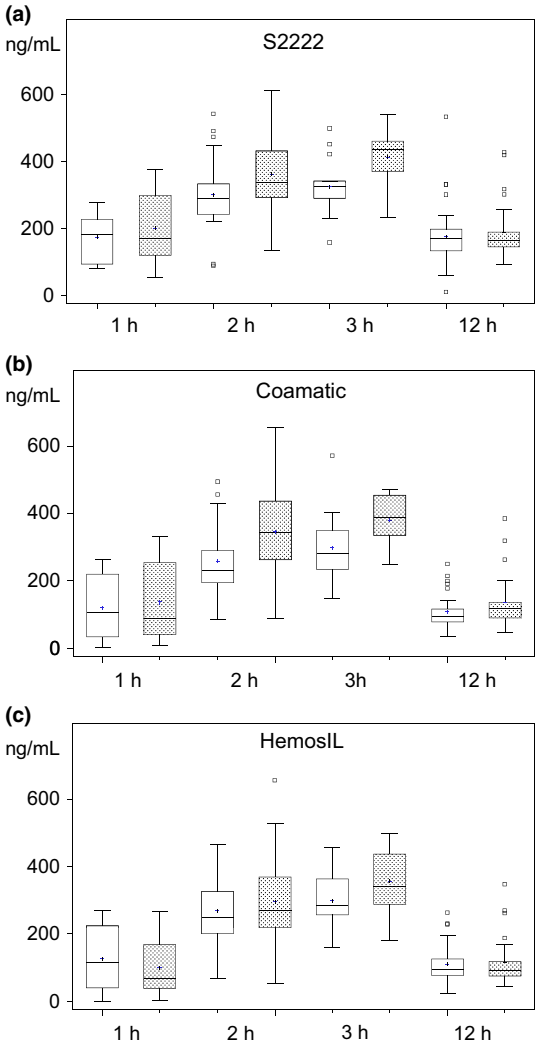

Figure 2 (a–c) Boxplots (S2222 chromogenic substrate method, (a) Coamatic chromogenic substrate assay, (b) and HemosIL chromogenic substrate assay, (c) of plasma (open boxes) and serum (stippled boxes) concentrations of rivaroxaban at 1, 2, 3 and 12 h following administration of 20 mg rivaroxaban od at steady state levels. Samples were taken from patients at different outpatient visits at indicated time intervals after intake of medication. *P*-values are given in Table 3.

Table 4 Concentration of apixaban (ng/mL) in controls and patients on treatment, and *P*-values for comparisons between plasma and serum. Assays and correlations between plasma vs. serum

		Plasma		Serum		Plasma vs. serum	Plasma Control vs. treatment	Serum Control vs. treatment	Plasma vs. serum Malony Rastogi	Plasma vs. serum Pearson correlation and ICC	
	n	Mean ng/mL	SD	Mean ng/mL	SD	*P*-value *t*-test for paired samples	*P* value two–sample t-test		*P*-value	*r*	ICC
Control											
Coamatic	72	16·7	17·7	15·3	15·1	0·6146			0·1893	0·0346	
HemosIL	72	24·9	19·7	19·9	19·3	0·1358			0·8754	−0·0412	
S2222	48	16·8	23·2	24·4	18·1	0·0808			0·0957	−0·0107	
Apixaban											
Coamatic	43	199·1	66	282·1	144·5	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·9399	0·5347
HemosIL	43	208·1	77	314·3	148·4	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·9301	0·5151
S2222	52	211·4	68·4	284·7	130·4	< 0·0001	< 0·0001	< 0·0001	< 0·0001	0·7977	0·5147

The values in plasma and serum 12 h after administration of rivaroxaban 10 mg od were lower compared with 20 mg od using all chromogenic substrate assays (Table 1).

Patients on treatment with apixaban 5 mg bid

In controls, the mean values and variances of the concentrations of apixaban were not significantly different in plasma and serum samples using all assays (Table 4). Correlations were strong for plasma and serum values with all assays (Table 4).

During treatment with apixaban, plasma concentrations were higher compared with control values (Table 4). Values are given as Boxplots for plasma and serum concentration as determined with all chromogenic assays (Fig. 3). Correlations of plasma and serum concentrations were strong using all assays (Table 4). Concentrations and variances were lower in plasma compared with serum samples using all assays (Table 4). The ICC values were lower somewhat than the Pearson's correlation coefficients due to the higher mean concentrations of apixaban in serum compared with plasma.

The inter-assay comparisons of the mean values (*t*-test) and of the variances (Maloney-Rastogi test) of plasma and serum concentrations were rather strong using plasma and serum samples (Table 5). Mean values of the concentrations of apixaban were lower with the Coamatic assay for both plasma and serum samples compared with the other assays (Table 5). Precision was higher for Coamatic compared with the other assays using plasma samples. Using serum samples, however, S2222 assay was more precise than Coamatic or HemosIL assay (Table 5). The ICC confirmed the high interassay correlations for plasma and serum samples.

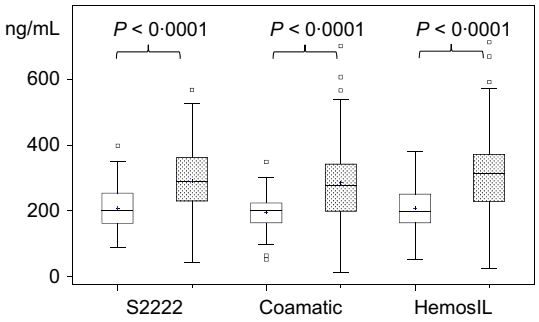

Figure 3 Boxplots of plasma (open boxes) and serum (stippled boxes) concentrations at 12 h following administration of 5 mg apixaban bid at months 3–6 in patients with atrial fibrillation or prevention of recurrent venous thromboembolism. Concentrations were measured using different chromogenic substrate assays.

Discussion

The present data indicate that rivaroxaban and apixaban can be determined from serum samples of patients on treatment and that values correlate well with those determined from plasma samples. The concentrations following intake of 20 mg rivaroxaban, which were obtained from different patients at different days, showed that they were significantly higher at 2

Table 5 Inter-assay comparisons for patients on treatment with apixaban

		Plasma				Serum			
		t-test for paired samples	Maloney Rostogi	Pearson correlation and ICC		*t*-test for paired samples	Maloney Rostogi	Pearson correlation and ICC	
	n	*P*-value	*P*-value	*r*	*P*-value	*P*-value	*P*-value	*r*	
Control									
Coamatic-HemosIL	72	< 0·0001	0·2783	0·5860		0·0114	0·0087	0·6433	
Coamatic-S2222	48	0·9773	0·0623	−0·0499		0·0002	< 0·0001	0·0456	
HemosIL-S2222	48	0·0155	0·6602	−0·1217		0·1427	0·1142	−0·1609	
Apixaban									
Coamatic-HemosIL	53	0·0012	< 0·0001	0·9663	0·9377	< 0·0001	0·3720	0·9632	0·9473
Coamatic-S2222	53	0·0250	0·0005	0·8316	0·8167	0·6186	0·0046	0·9196	0·9032
HemosIL-S2222	53	0·8477	0·0032	0·8353	0·8429	0·0045	0·0005	0·9257	0·8906

and 3 h compared with 1 and 12 h after application in plasma as well as serum samples. Serum samples are preferred for determination of drugs because of the absence of many proteins which may interfere with the determination such as fibrinogen and other coagulation factors [26,27,33]. Oral direct factor Xa inhibitors require only factor Xa to act on an enzyme which is added in excess to serum samples in the present assay. An enzyme-specific substrate releases a chromophor quantitatively from the substrate in competition with the factor Xa inhibitor [34,35]. Therefore, the oral direct factor Xa inhibitors can be determined in serum samples, and the adoption of plasma samples is not prerequisite.

In real life studies, the concentration of rivaroxaban varied widely in small groups of patients using plasma samples and a chromogenic substrate assay [36] and coagulation based assays [37]. However, using high-performance liquid chromatography mass-spectrometry as detection method, concentrations were significantly different 2, 12 and 24 h after intake of 10 mg rivaroxaban od [38]. In our study after intake of 20 mg rivaroxaban od we also found significant different concentrations in plasma as well as in serum samples of treated patients between 1 vs. 2 or 3 and 1 or 2 vs. 12 hours using chromogenic assays. Despite some differences between assays in our study, all three chromogenic substrate tests confirm the feasibility to determine rivaroxaban also from serum samples following administration of 10 or 20 mg rivaroxaban od.

The effect of apixaban spiked at increasing concentrations in pooled citrated normal human platelet-poor plasma substantially prolonged coagulation times of clotting assays and varied between methods used [39–41]. The variability of the results of chromogenic methods was reduced using methods adapted to a specific coagulation platform based on data of an international collaborative study [42]. Pharmacokinetic studies in healthy volunteers resulted in peak levels at steady state conditions using high-performance liquid chromatography from serum samples [40,43] as we found in our patients using chromogenic substrate assays. Our data from the chromogenic substrate assay correlated well between serum and plasma samples. However, plasma values were significantly lower than serum values with all assays. The values of the interassay and intraclass correlation coefficients were high with rivaroxaban for the three chromogenic assays support the validity of this determination from serum samples despite minor systematic differences between the three tests. These values were lower for the comparison of apixaban in plasma and serum samples using all chromogenic assays.

The values for rivaroxaban and apixaban determined in serum samples were about 20–25% higher compared with plasma samples.

The concentrations of rivaroxaban or apixaban may require determination in specific clinical situation such as deterioration of renal function, in the elderly or oldest population, before surgery or in acute clinical situations, during recurrent events or during bleeding complications or to check adherence to therapy [19,20]. Such determinations may be ordered also with some time delay when plasma samples are not at all or not any more available (too long storage period). Serum samples may be an alternative to measure rivaroxaban and apixaban in such situations.

Limitations of the present investigations are that the determination of rivaroxaban and apixaban in serum samples remains to be validated in clinical studies. However, even the validation of the plasma assays for both anticoagulants is still missing in relation to the incidences of thrombotic or bleeding

events. A strength of this study is that the determination of rivaroxaban and apixaban in serum samples involves commercially available methods which can be adapted to current coagulation platforms following the description for plasma samples.

In conclusion, the determination of rivaroxaban and apixaban from serum samples of patients offers an additional method to determine their concentrations in specific clinical situations. They can be performed with all chromogenic assays, but some differences may exist between them indicating requirement of standardization of every chromogenic assay. An adaptation of the chromogenic assays to current coagulation platforms seems feasible and may even improve the results, but yet remains to be tested.

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Conflicts of interest

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