CPC047

Prothrombin time assay for measuring rivaroxaban plasma concentrations using calibrators and controls: results of a multicentre field trial

Meyer Michel Samama[1,2], Genevieve Contant[3], Theodore E Spiro[4], Elisabeth Perzborn[5], Léna Le Flem[2], Céline Guinet[2], Yves Gourmelin[3], Jean Luc Martinoli[3], for the Rivaroxaban Prothrombin Time Field Trial Laboratories

[1]Hôtel-Dieu University Hospital, Paris, France; [2]Biomnis Laboratories R&D, Ivry-sur-Seine, France; [3]Diagnostica Stago SA, Gennevilliers, France; [4]Bayer HealthCare Pharmaceuticals Inc., Montville, NJ, USA; [5]Bayer HealthCare Pharmaceuticals, Wuppertal, Germany

Introduction

- **Rivaroxaban – an oral, direct Factor Xa inhibitor[1] – is currently used in clinical practice for the prevention and treatment of thromboembolic disorders**
- **Routine coagulation monitoring is not required,[2] but a quantitative determination of rivaroxaban exposure might be useful in certain clinical circumstances (e.g. prior to urgent surgery)**
- **Because of its mode of action, rivaroxaban prolongs the prothrombin time (PT), but the results vary depending on the assay reagents; the international normalized ratio (INR) correction used for monitoring the vitamin K antagonists cannot be used for rivaroxaban[3,4]**

Objective

- To evaluate the suitability of the PT assay for the measurement of rivaroxaban plasma concentrations (ng/ml) using rivaroxaban calibrators and controls, and to assess the inter- and intra-laboratory precision of the measurements

Methods

- Participating laboratories in Europe and North America were provided with sets of rivaroxaban calibrators (0, 41, 219 and 430 ng/ml) and pooled human plasma controls containing 19, 160 and 643 ng/ml of rivaroxaban. The concentrations of rivaroxaban in the pooled human plasma controls were unknown to the participating laboratories
- Evaluations were carried out over 10 days by each laboratory using its own local PT reagent (Table 1) as well as a centrally provided PT reagent, STA® Neoplastine® CI Plus (Diagnostica Stago, Gennevilliers, France)
- Day-to-day precision and accuracy were evaluated by producing a calibration curve each day and by testing in duplicate the three pooled human plasma controls
- The control plasma samples were diluted with calibrator containing 0 ng/ml rivaroxaban when the values were greater than the calibration range

Results

- Local PT reagents:
  - A large inter-laboratory variation was seen when results were expressed in seconds; the coefficient of variation (CV) was 13.6–29.7%. Less variation was found when the results were expressed as rivaroxaban concentrations (ng/ml; CV 3.9–15.5%; undiluted samples), although over-estimation was observed (Figure 1; Table 2)
  - The intra-laboratory CV was 2.7–34.1% (for 19 ng/ml), 1.1–7.9% (160 ng/ml) and 1.1–9.6% (643 ng/ml)

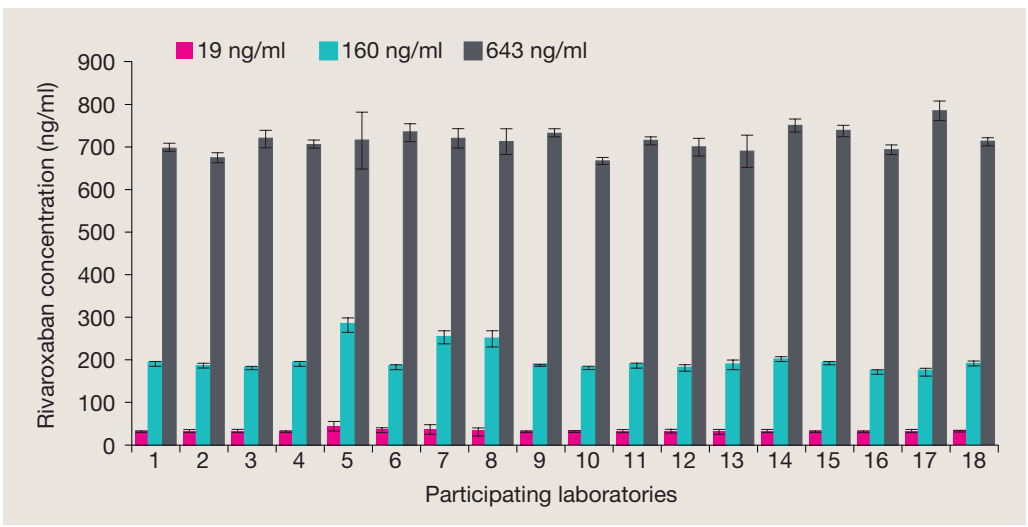

Figure 1. Rivaroxaban concentrations in control plasma samples reported from individual laboratories using local prothrombin time reagents. Results are presented as median values ± standard deviation (N=18).

Table 1. Local PT reagents and instruments used by the participating laboratories

Reagents			Central PT reagent	Local PT reagents					Total Local PT reagents
				Diagnostica Stago		Siemens	Instrumentation Laboratory		
			STA® Neoplastine® CI Plus	STA® Neoplastine® CI Plus	STA® Neoplastine® CI	Thromborel® S	Innovin®	Recombi PlasTin® 2G	
Routine instrument	Diagnostica Stago	STA-R	7	3	1	1		1	6
		STA Compact®	3	1			1	1	3
	Heinrich Amelung	KC 10	2	1			1		2
	Instrumentation Laboratory	ACL 1000	1		1				1
		ACL TOP®	2					2	2
	Siemens	BCS	4	1		2	1		4
Total			19	6	2	3	3	4	18

PT, prothrombin time.

- Central PT reagent:
  - There was less inter-laboratory variation when the central PT reagent was used (CV 2.0–7.5%; undiluted samples; expressed as rivaroxaban concentrations) compared with local PT reagents; however, measured rivaroxaban concentrations were higher than the actual values (Figure 2; Table 2), as with local PT reagents
  - The intra-laboratory CV was 4.5–19.3% (for 19 ng/ml), 1.2–8.3% (160 ng/ml), and 0.9–5.0% (643 ng/ml)
- The CV of the calibrators was 4.4–6.5% for the central PT reagent compared with 12.5–27.2% for local PT reagents (Table 2)

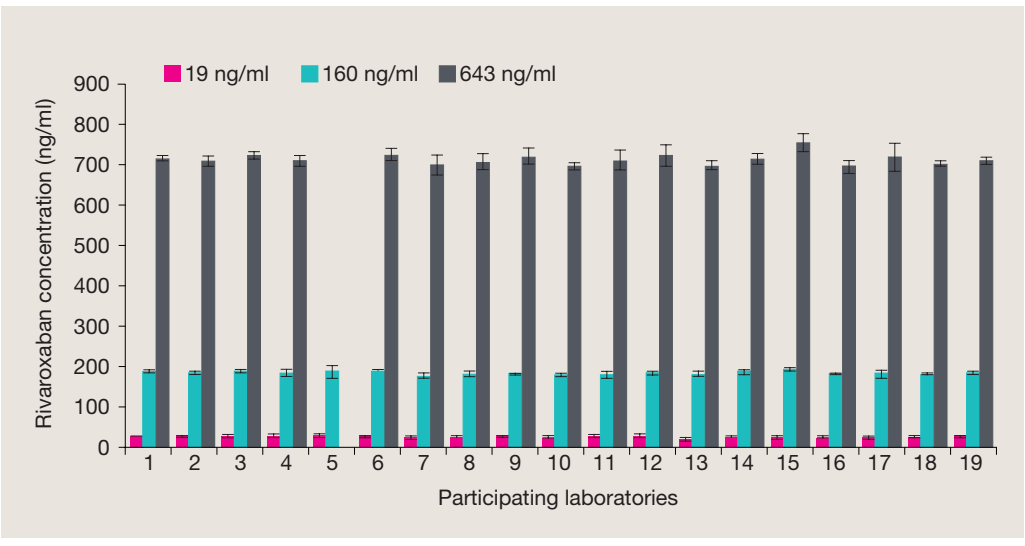

Figure 2. Rivaroxaban concentrations in control plasma samples reported from individual laboratories using the centrally provided prothrombin time reagent (STA® Neoplastine® CI Plus). Results are presented as median values ± standard deviation (N=19).

Table 2. Inter-laboratory variations: comparison of results obtained with local PT reagents versus central PT reagent

	Actual rivaroxaban concentration	Local PT reagents		Central PT reagent (STA® Neoplastine® CI Plus)	
		Mean±SD (N=18)	CV, %	Mean±SD (N=19)	CV, %
Control samples, time (seconds)	19 ng/ml	12.6±1.7	13.6	13.8±0.8	5.9
	160 ng/ml	20.2±4.0	19.9	23.7±1.0	4.2
	643 ng/ml	44.7±13.3	29.7	57.2±2.5	4.4
	643 ng/ml*	23.9±5.9	24.9	28.5±1.8	6.4
Control samples, rivaroxaban concentration (ng/ml)	19 ng/ml	31±4	11.9	29±2	7.5
	160 ng/ml	197±31	15.5	186±4	2.2
	643 ng/ml	715±28	3.9	712±14	2.0
	643 ng/ml*	263±13	5.1	261±15	5.9
Calibrators, time (seconds)	0 ng/ml	11.0±1.38	12.5	11.8±0.77	6.5
	41 ng/ml	13.0±1.80	13.9	14.3±0.84	5.8
	219 ng/ml	22.1±5.09	23.0	26.5±1.20	4.6
	430 ng/ml	31.3±8.52	27.2	38.8±1.70	4.4

*Control samples were diluted twofold with calibrator (containing 0 ng/ml rivaroxaban).
CV, coefficient of variation; PT, prothrombin time; SD, standard deviation.

Conclusions

- **The results of this field trial suggest that it is feasible to measure rivaroxaban plasma concentrations (expressed in ng/ml) using the PT combined with rivaroxaban calibrators and controls, in contrast to the conventional INR, which cannot be used**
- **Owing to the variability of the measurements observed (in particular at low rivaroxaban plasma concentrations), more specific and sensitive methods (i.e. anti-Factor Xa chromogenic assays [please see poster CPC049]) are a better alternative when more precise measurements of rivaroxaban exposure are required**
- **Further validation of this method is required in clinical settings**

References

1. Perzborn E *et al. J Thromb Haemost* 2005;3:514–521.
2. Bayer Pharma AG. Xarelto® (rivaroxaban) Summary of Product Characteristics. 2011. Available at: http://www.xarelto.com/html/downloads/Xarelto_Summary_of_Product_Characteristics_Dec2011.pdf. Accessed 2 March 2012.
3. Samama MM *et al. Thromb Haemost* 2010;103:815–825.
4. Lindhoff-Last E *et al. Ther Drug Monit* 2010;32:673–679.

Disclosure of conflict of interest
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Participants
DM Adcock, Englewood, CO, USA; JP Cambus, Toulouse, France; I Elalamy, Paris, France; G Gerotziafas, Paris, France; I Gouin-Thibault, Ivry-sur-Seine, France; J Harenberg, Mannheim, Germany; MH Horellou, Paris, France; M Jacquemin, Leuven, Belgium; K Jochmans, Brussels, Belgium; C le Courvoisier-Flaujac, Paris, France; C Legnani, Bologna, Italy; E Lindhoff-Last, Frankfurt, Germany; JL Martinoli, Gennevilliers, France; TL Ortel, Durham, NC, USA; G Palareti, Bologna, Italy; D Peetz, Mainz, Germany; MM Samama, Ivry-sur-Seine, France; A Schade, Cleveland, OH, USA; S Testa, Cremona, Italy; A Tripodi, Milan, Italy; AMHP van den Besselaar, Leiden, The Netherlands; J Weitz, Hamilton, Ontario, Canada; W Wijns, Brussels, Belgium.

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