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The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an *in vitro* study

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Millions of patients are involved worldwide in the treatment/prevention of thromboembolic diseases with vitamin K antagonists (VKAs), heparins or congeners. In the next few years, many of these patients will presumably be switched to the new antithrombotics, such as rivaroxaban. Apparently, this drug does not require laboratory monitoring for dose adjustment, because of its predictable pharmacokinetics/pharmacodynamics [1]. However, the measurement of its pharmacologic effect might be of value in selected patients, such as those with renal failure [2]. Furthermore, laboratory monitoring might help in checking patient compliance or in revealing over-anticoagulation [2]. Because of its simplicity and availability, the prothrombin time (PT) could be a good candidate. A recent study [3] investigated its suitability using normal plasma spiked with increased concentrations of rivaroxaban, chosen to mimic plasma levels (up to 1.00 μg mL^{-1}) in excess of those that would be obtained after administration of oral doses of 10 mg or more once-daily or twice-daily [1]. The study showed that the PT determined with different thromboplastins was suitable for reflecting the concentration effect of rivaroxaban, but important differences in between-thromboplastin responsiveness were observed [3]. Although this variability does not necessarily prevent the PT from being used in clinical laboratories, it does prevent comparison of results across thromboplastins, and therefore sharing of experience with this drug, which will start to accumulate as soon as rivaroxaban is licensed for the treatment/prevention of thrombosis in the most common cardiovascular diseases. Normalization of results across thromboplastins could also pave the way to the establishment of 'universal' therapeutic intervals for rivaroxaban if they are ultimately needed in selected patients. We reasoned that the standardization model used for VKAs could also be applied for rivaroxaban. In particular, we hypothesized that an international sensitivity index (ISI), valid for rivaroxaban (ISIrivaroxaban), could be determined by testing for PT with working and standard thromboplastin plasmas spiked with increasing concentrations of the drug. The ISIrivaroxaban could then be used as a substitute for the ISIvka in the equation of the International Normalized Ratio (INRvka) [$(PTpatient/PTnormal)^{ISIvka}$] to obtain the INRrivaroxaban [$(PTpatient/PTnormal)^{ISIrivaroxaban}$].

To test this hypothesis, a pooled normal plasma (PNP) (Cryopep, Saint-Jean-de-Vedas, France) was used to prepare 10 plasmas spiked with increasing amounts of rivaroxaban (Bayer-Schering-Pharma, Wuppertal, Germany). The drug was dissolved in 100% dimethylsulfoxide (DMSO) to obtain a 1 mM stock solution, which was then diluted in 5% DMSO

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and used as a working solution to prepare plasmas with rivaroxaban concentrations ranging from 0 to 1.0 μg mL^{-1}. These plasmas were characterized for their anti-factor Xa activity in a previous study [3]. Seven of the above plasmas (from 0 to 1.0 μg mL^{-1}) were used as calibration plasmas to derive the ISIrivaroxaban. The remaining three, with low rivaroxaban concentration (plasma A, 0.1 μg mL^{-1}), intermediate rivaroxaban concentration (plasma B, 0.3 μg mL^{-1}) and high rivaroxaban concentration (plasma C, 0.7 μg mL^{-1}) were used as test plasmas to validate the efficacy of the ISIrivaroxaban in result normalization.

We used the following thromboplastins: Thromborel-S (human placenta; Siemens, Marburg, Germany); Innovin (human recombinant tissue factor [hrTF]; Siemens); Recombiplastin (hrTF; Instrumentation Laboratory, Orangeburg, NY, USA); Triniclot (rabbit; Tcoag, NY, USA); Neoplastin (rabbit; Stago, Asnieres, France); and Neoplastin-Plus (rabbit; Stago). Recombiplastin and Neoplastin-Plus were considered as the arbitrary standards against which to derive the ISIrivaroxaban.

PT testing for rivaroxaban-spiked plasmas was performed with all thromboplastins according to the manufacturers' specifications by means of the Sta-R coagulometer (Stago). Six repeated PT measurements for each of the rivaroxaban-spiked plasmas were performed on different occasions.

The ISIvka for each thromboplastin was the one declared by the manufacturer. The ISIrivaroxaban was determined by a modification of the World Health Organization (WHO) protocol for ISIvka [4]. Briefly, log-transformed PTs (working vs. arbitrary standard thromboplastin) were plotted, and the slope of the orthogonal regression line was calculated, together with its coefficient of variation (CV). Data points at a perpendicular distance of > 3 standard deviations from the line were omitted from the final calculation. The ISIrivaroxaban was assigned as the product of the slope and the ISIrivaroxaban of the arbitrary standard (set at 1.00).

PT values for each of the three rivaroxaban-spiked test plasmas and thromboplastins were divided by the mean normal PT obtained with the PNP (called the PT ratio). To obtain the valid INR for VKA or rivaroxaban (INRvka or INRrivaroxaban), PT ratios were raised to a power equal to the corresponding thromboplastin ISIvka or ISIrivaroxaban. Finally, the overall (all thromboplastins) mean values and their CVs were calculated within each set of PT ratio, INRvka or INRrivaroxaban. The between-thromboplastin variability according to the result expression was eventually evaluated by means of the corresponding CV.

PT values increased with increasing dose of rivaroxaban, and the linearity of the relationship was excellent for all thromboplastins (not shown). The ISIrivaroxabans for all working thromboplastins relative to the arbitrary standard and their CVs are reported in Table 1. The responsiveness to rivaroxaban (when Recombiplastin was used as the arbitrary standard) was highest for Neoplastin-Plus (ISIrivaroxaban = 1.014) and lowest for Innovin (ISIrivaroxaban = 1.712). The rivaroxaban responsiveness of each thromboplastin did not depend on the species, as shown by the fact that Innovin (human recombinant) and Thromborel-S (human placenta) had different ISIrivaroxaban values (1.712 vs. 1.382). Furthermore, Innovin and Recombiplastin had different ISIrivaroxaban values (1.712 vs. 1.00), despite the fact that both are from hrTF. Although measurable, the ISIrivaroxaban values observed for the three rabbit thromboplastins (Neoplastin, Neoplastin-Plus and Triniclot) were less pronounced (range, 1.014–1.224). In general, the rivaroxaban and VKA responsiveness are not allied, as shown by the lack of association between the ISIrivaroxaban and ISIvka (Table 1). The precision of the calibration was excellent for all thromboplastins, as shown by the very low CVs (range, 1.0–1.8%). The results did not change when Neoplastin-Plus was used as an arbitrary standard.

Table 1 International sensitivity index (ISI) calibration

		ISIrivaroxaban (CV% of the slope)	
Thromboplastin	ISIvka	Recombiplastin as arbitrary standard	Neoplastin-Plus as arbitrary standard
Recombiplastin	1.00	1.000	0.987 (1.0)
Thromborel S	1.07	1.382 (1.8)	1.361 (1.8)
Neoplastin-Plus	1.17	1.014 (1.0)	1.00
Neoplastin	1.75	1.196 (1.0)	1.177 (0.8)
Triniclot	1.22	1.224 (1.6)	1.208 (1.6)
Innovin	0.93	1.712 (1.4)	1.691 (1.6)

CV, coefficient of variation of the slope. ISIvka and ISIrivaroxaban refer to the ISIs valid for vitamin K antagonists and rivaroxaban, respectively.

PT ratios, INRvka and INRrivaroxaban for the three test plasmas tested with different thromboplastins are reported in Table 2. Between-thromboplastin variability (expressed as CV) was relatively high when results for plasmas A, B and C were expressed as PT ratio (5.5%, 12.1% and 18.1%, respectively) or as INRvka (10.4%, 24.6% and 39.0%, respectively), but decreased considerably when results were expressed as INRrivaroxaban (2.1%, 3.3% and 1.9%). The overall between-thromboplastin variability (including the three test plasmas) was 14.0%, 29.6% and 2.1% when results were expressed as PT ratio, INRvka or INRrivaroxaban, respectively (Table 2). The degree of improvement of the between-thromboplastin variability with results expressed as INRrivaroxaban did not change when Neoplastin-Plus was used as the arbitrary standard (not shown).

This feasibility study shows that the proposed model of PT standardization is suitable, as demonstrated by the following observations: (i) ISIrivaroxaban can be determined with excellent precision with CV of the slope ≤ 3% as recommended by WHO guidelines [4]; (ii) it is relatively simple (few PT measurements for rivaroxaban-spiked plasmas); and (iii) it is effective in minimizing the between-thromboplastin variability. The study also provides evidence that the INRvka, if inappropriately used for reporting PT results in patients on rivaroxaban, may cause clinicians to make misleading interpretations

Table 2 Validation of the efficacy of ISIrivaroxaban in results normalization

	PT ratio			INRvka			INRrivaroxaban		
	A	B	C	A	B	C	A	B	C
Mean	1.27	1.76	2.67	1.34	2.01	3.39	1.33	1.96	3.23
CV%	5.5	12.1	18.1	10.4	24.6	39.0	2.1	3.3	1.9
Overall CV	14.0			29.6			2.1		

CV, between-thromboplastin coefficient of variation; INR, International Normalized Ratio; PT, prothrombin time. Mean PT values were obtained with different thromboplastins and different ways of expressing the results. PT ratio, INRvka and INRrivaroxaban refer to the ratio of the clotting time (test plasma-to-normal plasma), and the INRs valid for vitamin K antagonists and rivaroxaban, respectively. A, B and C refer to the test plasmas with 0.1 μg mL^{-1}, 0.3 μg mL^{-1} and 0.7 μg mL^{-1} rivaroxaban.

of the levels of the circulating drug, as shown by the fact that the thromboplastins that are very VKA-responsive (ISIvka close to 1) are not necessarily equally rivaroxaban-responsive (Table 1). The discrepancy (ISIvka vs. ISIrivaroxaban) is probably a result of the different thromboplastin compositions [5].

According to the ISTH guidelines [6], the INR can be determined not only by raising the PT ratio to a power corresponding to the ISI of the working thromboplastin, but also by use of a simpler model, whereby coumarin plasmas certified for their INRs in terms of an international standard can be tested locally with the working thromboplastins. Then, local PTs can be plotted against the certified INR, and the resulting best-fit regression line be used to extrapolate the patient's INR. We used our set of data to validate this alternative model for deriving the INRrivaroxban for the three test plasmas, and the results (not shown) demonstrate that this simplified standardization model is also feasible.

Some limitations of this work should be recognized. First, none of the WHO standards was used to determine the ISIrivaroxaban, because they were not available at the time of the study. We elected to use as an arbitrary standard one of the thromboplastins included in the study (either Recombiplastin or Neoplastin-Plus). This choice was driven by two reasons. Both thromboplastins represent the two WHO routes of calibration (human and rabbit), and both displayed the highest responsiveness to rivaroxaban, as observed in the previous study [3]. It should, however, be realized that the choice of any other standard would have not infringed the principle of the calibration, and would have had an effect only by changing the relative responsiveness of the working thromboplastins, leaving unaltered results and conclusions on the feasibility of the INRrivaroxaban calibration. In fact, substituting Recombiplastin with Neoplastin-Plus did not affect the results and conclusions of this study. Second, our results stem from a single-center calibration study. A working party should be appointed within the frame of activities of the SSC-ISTH to carry out a multicenter study to confirm/extend the results of this feasibility study. Additional thromboplastins, coagulometers and WHO standards could be included. Third, we included rivaroxaban-spiked normal plasmas, which, although representative of the real situation, do not necessarily mirror what occurs in vivo when rivaroxaban is administered to the patients. Plasmas from patients treated with rivaroxaban (not yet available) will, in the future, provide the opportunity to perform appropriate ISIrivaroxaban calibrations.

In conclusion, the results of this study are consistent with the hypothesis that the model of ISI/INR calibration, once used for VKA and later applied to liver disease [7] and disseminated intravascular coagulation [8], is also feasible for rivaroxaban and possibly for other new direct FXa inhibitors.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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