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Letter to the Editor

Comparison between specific and nonspecific assay in the evaluation of the anticoagulant effect of the Direct Oral Anticoagulants: Our experience in a cardiovascular hospital

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To the Editor

The new generation of antithrombotic agents, known as Direct Oral Anticoagulants (DOACs), act on a single factor within the coagulation cascade. Although the monitoring of therapy is not necessary for DOACs, measuring the anticoagulant effect is useful in many clinical situations, especially pre-operative evaluation [1]. Specific tests, which quantify the drug indirectly, and nonspecific tests, which are altered by DOACs, are used to evaluate the anticoagulant effect of DOACs. The aim of this study is to assess the responsiveness of the specific and non-specific assays used at IRCCS Policlinico San Donato to low DOAC concentrations and to compare the responsiveness between calibrators and patients with nonspecific assays.

All tests were performed using completely automated assays on STA-R Max® instruments (Diagnostica Stago, Asnières-sur-Seine, France). We used the ecarin chromogenic assay (ECA) with a detection limit of 15 ng/mL as a specific test for dabigatran and the Liquid anti-Xa chromogenic assay (Liquid anti-Xa) with detection limits of 25 ng/mL and 23 ng/mL for rivaroxaban and apixaban respectively. We used activated partial thromboplastin time (aPTT) as non-specific test for dabigatran, with normal range 24–35 s, and pro-thrombin time (PT) for rivaroxaban and apixaban, with normal range 10.7–16.1 s.

To compare responsiveness between specific and non-specific assays we used calibrators supplied by Stago. For each level of DOACs, we performed 5 repetitions with the specific and non-specific assay, calculating media, standard deviation, minimum and maximum values. For comparison of responsiveness between calibrators and patients with nonspecific assays we used the plasma of 40 patients attending the IRCCS Policlinico San Donato, of whom 12 were being treated with dabigatran, 11 with rivaroxaban, and 17 with apixaban. To study the correlation between the normality of non-specific tests and the concentration of drugs, we calculated the drug concentration corresponding to the upper limit of normality of the non-specific test using the linear regression obtained using the plasma of the 40 patients included in the study. The study protocol was approved by the local Ethics Committee and patients gave their written informed consent to the examination protocol, conducted in accordance with the Declaration of Helsinki, as revised in 2013.

For the comparison of responsiveness between specific and non-specific tests we used the following statistical regressions: second-degree polynomial for ECA, Liquid anti-Xa and aPTT, and linear regression for PT. For these regressions we used the drug concentrations of the calibrators determined by the manufacturer with liquid chromatography-mass spectrometry: 0, 49, 99, 171, 257 ng/mL for dabigatran; 0, 97, 248, 493 ng/mL for rivaroxaban; 0, 91, 233, 455 ng/mL for apixaban. For the comparison of responsiveness between calibrators and patient samples using the non-specific assays we used the following statistical regressions: second degree polynomial for aPTT using calibrators, linear regression for aPTT using patient samples, and linear regression for PT using both calibrators and patient samples. For these regressions we used the drug concentrations determined in calibrators and patient samples with specific tests. For all statistical regressions we calculated the correlation coefficient value (R^2) and a p-value < 0.05 was considered statistically significant. For calibrator has been accepted the mathematical model more easily with an $R^2 > 0.98$. Responsiveness was evaluated by comparing the values of the 2 Clotting Time (2CT), defined as the final concentration in DOACs needed to double the measured parameter [2], obtained from the statistical

Table 1
Comparison of responsiveness using 2CT.

Dabigatran		Rivaroxaban		Apixaban	
Comparison between functional specific and non-specific assay (using calibrators at known concentrations of the drug)					
ECA (STA-ECA II) 2CT ng/mL	aPTT (STA-Cephascreen)	Liquid anti-Xa (STA-Liquid anti-Xa) 2CT ng/mL	PT (STA-Neoplastin CI plus)	Liquid anti-Xa (STA-Liquid anti-Xa) 2CT ng/mL	PT (STA-Neoplastin CI plus)
130	189	162	352	166	1164
Comparison between calibrators and patients' plasma using non-specific assay (using calibrators and plasma: drug concentrations determined with a specific assay)					
Non-specific assay: aPTT		Non-specific assay: PT		Non-specific assay: PT	
Calibrators 2CT ng/mL	Patients	Calibrators 2CT ng/mL	Patients	Calibrators 2CT ng/mL	Patients
143	228	313	423	1106	780

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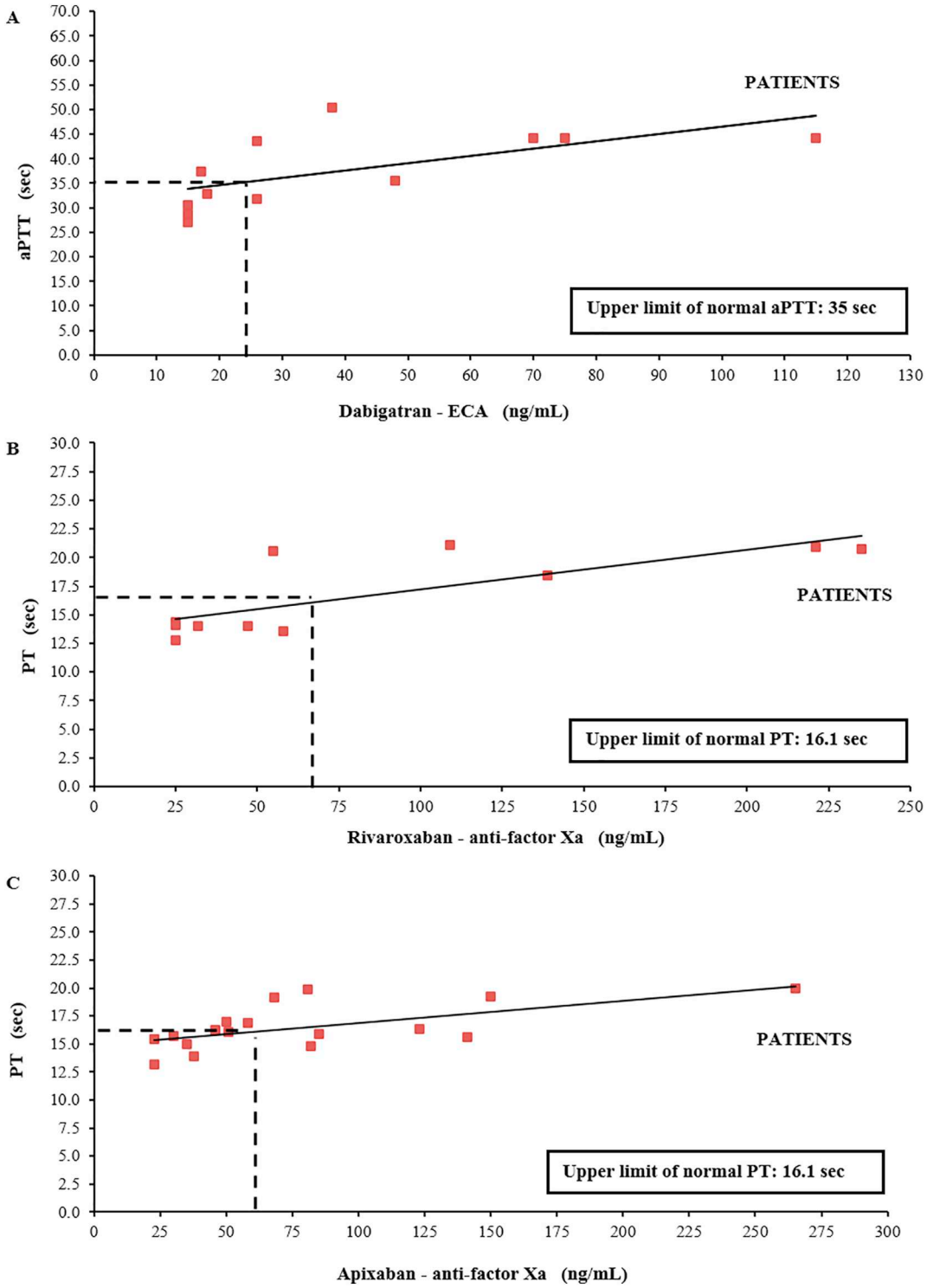

Fig. 1. Interpolation of the upper limit of test of normality of nonspecific assays with the regression line obtained using the plasma of 40 patients, to locate the respective concentration of DOAC. Y axis: nonspecific test used; X axis: concentration of DOAC dose in plasmas of patients using the respective method. (A) dabigatran; (B) rivaroxaban; (C) apixaban.

regressions of the specific and non-specific assays. We considered the test with the lowest 2CT more responsive. For the calculation of 2CT we used concentrations of DOACs corresponding to the limits of detection of the specific tests.

Table 1 compares specific and non-specific functional test responsiveness through the values of 2CT. In agreement with the literature [2,3], we obtained lower 2CT values from the specific assays than from non-specific methods for all three DOACs, identifying greater responsiveness to low concentrations by using the specific assays. The British Committee for Standards in Haematology [4] and the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis [5] recommend, in the absence of specific assays, that non-specific test responsivity be determined using drug-specific calibrators. Table 1 contrasts the 2CT obtained using non-specific assays of calibrators and patients' plasma. In agreement with the study by Lim et al.

[6], the reagent used in our laboratory for aPTT in the presence of dabigatran has shown an overestimation of responsivity when using the calibrators, compared to results from patients. As regards the inhibitors of factor Xa, the evaluation of responsivity of PT in the presence of rivaroxaban, would be overestimated using the calibrators, as confirmed in the literature [7]. Conversely, for apixaban, the lowest values of 2CT obtained using PT on patient samples revealed a possible underestimation of responsivity when using the calibrators. This data also correlates with the results obtained in the work of Lim et al. [6]. Another important parameter in evaluating the possible use of non-specific tests in managing patients anticoagulated with DOACs is the relationships between the normal test result and the concentration of the drug. The drug concentration corresponding to the upper limit of aPTT (Fig. 1A) identifies a dabigatran concentration of 22 ng/mL. The PT upper limit of normality corresponds to a rivaroxaban concentration of 68 ng/mL (Fig. 1B) and an apixaban concentration of 60 ng/mL (Fig. 1C). These results are in agreement with the literature for dabigatran, rivaroxaban and apixaban [3,8,9].

The data obtained in this study show the superiority of ECA and Liquid anti-Xa assays over the non-specific tests, aPTT and PT, for which responsivity should be evaluated not only with calibrators but also with patient samples. Moreover, the poor concordance demonstrated between non-specific test results and DOAC concentration in patients' plasma suggests that a normal test result is not correlated with the absence of the drug.

This work suggests that for a true evaluation of anticoagulated patients with DOACs specific methods should be preferred. In the absence of these functional specific tests, each laboratory should assess the responsiveness of non-specific assays using not only the calibrators, but also patient samples.

Conflict of interest

The author reports no conflicts of interest related to this work.

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