Measurement of apixaban concentrations in real-world clinical and laboratory settings: the first Polish experience

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Introduction  Rivaroxaban and apixaban, activated factor X inhibitors, are direct oral anticoagulants (DOACs), which are used in stroke prevention in atrial fibrillation, treatment of acute venous thromboembolism (VTE), and prevention of recurrent VTE.

No need for routine laboratory monitoring is the advantage of DOACs over vitamin K antagonists. Therapeutic ranges have not been established for DOACs, but the "on-therapy range" has been defined as the interval between the 5th percentile trough concentration and the 95th percentile peak concentration. DOACs affect prothrombin time (PT), activated partial thromboplastin time (aPTT), and the dilute Russell viper venom time (dRVVT) used in the detection of lupus anticoagulant (LA), together with some other coagulation tests. The impact of DOACs on coagulation assays depends on the method, reagents and analyzer, and on the drug itself and its concentrations, which is relatively rapidly changing due to the half-life of around 12 hours. In vitro studies showed that PT and aPTT are generally less sensitive to apixaban than to rivaroxaban. It has been suggested that PT and aPTT may be considered as the screening tests for DOACs, but evidence to support this hypothesis is inconsistent, particularly in patients receiving apixaban.

In Poland, apixaban is used in fewer than 10% of patients on DOACs, largely due to lack of reimbursement. Recently, the first Polish data on its use in patients with high bleeding risk have been published. At our institution as in the only center in Poland, the measurement of apixaban on a 24/7 basis was started in September 2016 and is readily available for inpatients and outpatients. We present here our first experience with the determination of apixaban concentrations and their effects on PT-, aPTT-, and dRVVT-based tests.

Materials and method  We prospectively recruited the first 42 patients from John Paul II Hospital in Kraków, Poland, who received apixaban, including 10 individuals in whom the samples were drawn twice (a total of 52 samples). These patients were compared with 37 age- and sex-matched patients on rivaroxaban. Indications to measure the drug levels were doubts regarding compliance, bleeding complications, or assessment prior to invasive procedures.

The PT (Thromborel S; reference range, 10.4–13.0 s), aPTT (Pathromtin SL; reference range, 25.9–36.6 s), and dRVVT assays using LA1 Screening Reagent for screening tests (LA1) and LA2 Confirmation Reagent for confirmatory tests (LA2) were performed on the BCS-XP automated analyzer (Siemens Healthcare, Marburg, Germany). Positive LA was defined as the LA1-to-LA2 ratio exceeding 1.2. Apixaban and rivaroxaban concentrations were measured using the chromogenic Biophen DiXal test (Hyphen, BioMed, Neuville-Sur-Oise, France) with specific calibrators.

We adopted on-therapy ranges based on a summary of product characteristics for apixaban, 2.5 mg twice daily; peak, 30–153 ng/ml; trough, 11–90 ng/ml and suggested by Dousфils et al for apixaban, 5 mg twice daily; peak, 59–302 ng/ml; trough, 22–177 ng/ml for rivaroxaban, 20 mg once daily; peak, 189–419 ng/ml; trough, 6–87 ng/ml.

Statistical analysis  Continuous data were expressed as median (interquartile range [IQR]). Spearman’s rank correlation analyses were used to estimate associations between variables. The Mann–Whitney test was used to compare differences between variables. The χ² test was used to compare categorical variables. A P value of less than 0.05 was considered significant.
**Results** VTE was the main indication for the use of rivaroxaban or apixaban (100% and 83%, respectively). There were no intergroup differences in estimated glomerular filtration rate (98 ml/min/1.73 m² [IQR, 92–107] vs 94 ml/min/1.73 m² [IQR, 66–90]; P = 0.14). The standard dose of rivaroxaban (20 mg once daily) was administered in 33 patients (89%), while apixaban (5 mg twice daily) was administered in 20 patients (48%), and the remaining patients received apixaban at a dose of 2.5 mg twice daily largely due to high bleeding risk.

The median (IQR) apixaban concentration in samples taken 2 to 12 hours after the last dose (n = 35 [67%]) was 110 (85–199) ng/ml, while that of rivaroxaban taken 2 to 24 hours (all patients) was 169 (87–232) ng/ml. For apixaban, the median (IQR) time from the receipt of the sample in the laboratory to the release of the results was 129 (89–173) minutes. Both rivaroxaban and apixaban were inversely correlated with the time since the last dose ($r = −0.64$, $P < 0.001$ and $r = −0.66$, $P < 0.001$, respectively).
We found stronger associations of PT and aPTT with rivaroxaban \((r = 0.62, P < 0.001\) and \(r = 0.43, P = 0.01\), respectively) than with apixaban \((r = 0.28, P = 0.04\) and \(r = 0.35, P = 0.01\), respectively). Prolonged PT \((> 13.0 \text{ s})\) was observed less commonly in patients receiving apixaban than rivaroxaban \((9\,[17\%]\) and 22\,[59\%], respectively, \(P < 0.001)\). The corresponding values for prolonged aPTT \((> 36.6 \text{ s})\) were 9\,[17\%] and 5\,[14\%], respectively \(P = 0.85\). Only one 25-year-old woman with the greatest prolongation of aPTT while on apixaban was later diagnosed with antiphospholipid syndrome. Prolonged PT and concomitantly aPTT were found in 4 subjects \((11\%)\) with rivaroxaban concentrations of 203 ng/ml, 268 ng/ml, 290 ng/ml, and 303 ng/ml and in 4 cases \((8\%\) with apixaban concentrations of 163 ng/ml, 199 ng/ml, 209 ng/ml, and 522 ng/ml.

The distribution of PT and aPTT in patients with VTE treated with apixaban or rivaroxaban against the on-therapy ranges is shown in FIGURE 1A–1F. Whereas all concentrations for rivaroxaban were within the on-therapy range, 2 patients \((7\%)\) and 5 patients \((20\%)\) for apixaban administered at a dose of 2.5 mg and 5 mg twice daily, respectively, exceeded their on-therapy ranges.

Positive LA screening tests were less common among the 35 tested patients on apixaban versus 35 patients receiving rivaroxaban \((51\%\) and 89\%, respectively, \(P = 0.01)\), while the LA1-to-LA2 ratio exceeding 1.2 was found in 3 patients \((9\%)\) and 20 patients \((57\%)\), respectively \(P = 0.001)\).

**Discussion** This study is the first to show apixaban measurements on the 24/7 basis in a Polish hospital, with about 2-hour time till the final result. We confirmed that the PT assay displays a lower sensitivity to apixaban than to rivaroxaban. As expected, apixaban and rivaroxaban had little impact on aPTT in our patients, with normal values even at high rivaroxaban and apixaban concentrations \((356 \text{ ng/ml} \text{ and 477 ng/ml, respectively})\). We also found an inverse correlation between time since the last dose and drug concentrations.

Significantly prolonged aPTT while on rivaroxaban and apixaban may be caused by the presence of LA, as illustrated by 2 outlying points in FIGURE 1B. LA testing could be challenging on DOACs, especially while dRVVT-based assays are used. Interestingly, the dRVVT confirmatory test correctly most positive screen results in patients on apixaban, but only in one-third of those taking rivaroxaban, which is in line with the results of Antovic et al. The risk of false-positive LA in DOAC-treated patients can be reduced without interrupting treatment by taking blood samples before the next dose, when the lowest concentrations of the drug can be expected.

Study limitations included a small sample size and use of a single reagent for each test, although using more sensitive reagents (e.g., neoplastic CI+ or APTT-A [both Diagnostica Stago]), might lead to more prolonged PT or aPTT on both anticoagulants.

In conclusion, apixaban concentrations associated with the time since the last dose can and should be used in everyday laboratory practice, since PT and aPTT values are insensitive to detect even high concentrations of apixaban. Our study confirms that to assess anticoagulant effects of DOAC-specific coagulation tests should be available for tertiary centers, especially in order to reliably interpret LA testing. If a patient is treated with DOACs, blood collection for coagulation tests just before the next dose should be recommended.

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