

Research Article

A Study of Serial Monitoring of DOAC Levels: A Lesson of What Test to Order and When to Order it

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Abstract

The new direct oral anticoagulants (DOACs) are an attractive choice for prevention of stroke in atrial fibrillation, prophylaxis of hip/knee replacement and treatment of venous thromboembolism (VTE). DOACs have gained popularity over warfarin as their therapeutic range can be achieved rapidly, have fewer drug-drug interactions and have fewer food restrictions. But more importantly, DOACs do not require routine monitoring. Nonetheless, there is great uncertainty and lack of consensus about how to monitor patients on DOACs that are at risk of bleeding due to renal disease, trauma, or being underweight, or on the other hand, may be subtherapeutic due to malabsorption, obesity or lack of compliance. Herein we present our experience using several methods to monitor the direct FXa inhibitors, rivaroxaban and apixaban, in a serial study of compliance. We studied the sensitivity of prothrombin time (PT), partial thromboplastin time (PTT), dilute Russell viper venom (DRVVT) and two anti-Xa chromogenic assays to measure rivaroxaban and apixaban levels in patients at serial time points for up to 12 weeks. We determined that DRVVT is a good assay to screen for the presence of DOACs but not reliable to monitor the drug levels whereas the chromogenic anti-Xa assays should be used to monitor drug level. We observed a broad range of drug levels based on the time gap from last dose and thus we recommend that blood samples be drawn at peak intervals.

Introduction

The new oral direct FXa inhibitors are growing in popularity over warfarin due to their rapid anticoagulant effect, broad therapeutic range, and their anticoagulant efficacy is as good, or better, than warfarin, and more importantly, they are safer than warfarin

as clinical trials have shown a reduced incidence of intracranial hemorrhage [1,2]. DOACs, have a rapid onset of action, short half-life, predictable pharmacokinetics and pharmacodynamics, and less drug interference. The peak drug level for rivaroxaban occurs 2-4 hours following ingestion and for apixaban takes 3-4 hours. The half-life of rivaroxaban is 5-9 hours and for apixaban is 8-15 hours (average 12 hr.) [3,4]. Apixaban and rivaroxaban are substrates for P-glycoprotein (P-gp) transport and are metabolized by CYP3A4 [5]. Thus P-gp and CYP3A4 inducers, such as rifampin, carbamazepine, phenytoin, and St. John's wort, can increase drug clearance whereas strong P-gp and CYP3A4 inhibitors such as ketoconazole, itraconazole, ritonavir, clarithromycin can decrease drug clearance [6]. Clearance of FXa inhibitors is mainly by renal and, to a lesser extent, intestinal excretion. Thus, patients with liver disease and kidney disease should be evaluated before anticoagulation and monitored at least annually during anticoagulation [7]. Although DOACs do not require routine monitoring, there are certain conditions that may lead to subtherapeutic or supratherapeutic levels. Malabsorption, obesity, and bariatric surgery may be associated with subtherapeutic levels [8], whereas renal disease, liver disease, and being underweight may lead to supratherapeutic levels and increased risk of bleeding. In addition, drug monitoring or screening may be needed in patients with trauma or in patients that require emergent surgery. This study shows the sensitivity of routine coagulation tests (PT, PTT, DRVVT) and two anti-Xa chromogenic assays to measure rivaroxaban and apixaban levels in patients at serial time points for up to 12 weeks.

Materials and Methods

Samples were received from 7 patients on rivaroxaban 20 mg once daily and 9 patients on apixaban (8 patients on 5 mg twice

daily and one patient on 2.5 mg twice a day) enrolled in the AiCure study, a randomized 12-week study in adults diagnosed with ischemic stroke who were anticoagulated with DOACs. The AiCure study was designed to determine the efficacy of a mobile application to monitor and improve compliance with taking DOACs and the results of improved compliance with this intervention are published elsewhere [9]. All patients were enrolled in the AiCure study after they had been receiving anticoagulation treatment for at least a month. Blood samples were obtained at baseline, week 4, week 8 and week 12. Informed consent was obtained before entering the study. The study was approved by the Montefiore Medical Center institutional review board. PT, PTT, and DRVVT were performed with the Dade Innovin, Dade Actin PTT FSL and HemosIL DRRVT (Siemens, Erlangen, Germany) as per manufacturer recommendations. Chromogenic anti-Xa was performed at Montefiore Medical Center with the BIOPHEN DiXaI kits and calibrators (Hyphen biomed, Neuville-Sur-Oise, France) for rivaroxaban and apixaban as per manufacturer recommendations. These assays were performed in the BCS XP coagulation analyzers. To determine accuracy of the Hyphen biomed chromogenic assays, we sent out a subset of samples blindly to University of Washington Coagulation lab for testing using the STAGO liquid Xa assay in the STAR Evolution analyzer for a correlation study. Graphs and statistics (linearity equations and R^2 correlation) were performed in Microsoft excel.

Results

We observed a strong correlation between the Biophen and Stago chromogenic anti-Xa assays ($R^2 > 0.9$) (Figure 1). Thus, all the results presented from this point on were obtained with the Biophen chromogenic anti-Xa assay.

We then assessed if routine coagulation assays such as prothrombin time (PT) and partial thromboplastin time (PTT) are sensitive enough to detect therapeutic levels of rivaroxaban and apixaban. Approximately 50% of samples from patients on rivaroxaban had PT values within the normal range (Figure 2). The sensitivity of the PT assay to detect apixaban was even worse with more than 66% of patients' samples with results in the normal range. Although we observed a positive linear correlation

between prolongation of PT with increasing rivaroxaban levels, no positive correlation was observed between PT and apixaban levels. This indicates that PT may be prolonged in patients on rivaroxaban with plasma levels greater than 200 ng/mL, whereas patients on apixaban cannot be reliably screened with the PT assay. Similar results were observed with the PTT assay (Figure 2). A positive linear correlation of PTT prolongation with increasing rivaroxaban levels was observed, with PTT results above the normal range with all samples that had rivaroxaban levels greater than 200 ng/mL. The majority of the samples from patients on apixaban had PTT results within normal limits.

Russel viper venom directly activates coagulation factor X (FX), thus the DRVVT assay has been proposed as a good assay to screen FX inhibitors. Indeed, we observed that all the samples from patients on rivaroxaban had DRVVT times above the upper limit of the normal range, and only two samples (6%) from patients on apixaban had DRVVT results within normal limits (Figure 2). Again, we observed a positive linear correlation of DRVVT prolongation with increasing rivaroxaban levels whereas correlation of DRVVT with increasing apixaban levels was linear but weak.

We then study if the broad range of drug plasma levels, measured with the Biophen anti-Xa chromogenic assay, was due to the time lapse from last dose. Rivaroxaban and apixaban have similar kinetics; they are rapid acting drugs with peak time of 2-4 hours for rivaroxaban and 3-4 hours for apixaban. It is a general assumption that the best time to monitor drug levels is at peak, however, guidance of how to monitor DOACs is scarce. We observed a positive correlation of highest drug levels at peak time for both rivaroxaban and apixaban, however, there is a broad range of levels even at peak time (Figure 3). For instance, at rivaroxaban's peak time, there are two patient populations: patients with levels higher than 200 ng/mL (high responders) vs. patients with levels less than 200 ng/mL (low responders). A similar pattern is observed with patients on apixaban at a cut off of 150 ng/mL, although the pattern is not as clear. This indicates there are a significant number of factors accounting for the broad therapeutic range which includes issues with absorption, metabolism, and excretion as previously reported

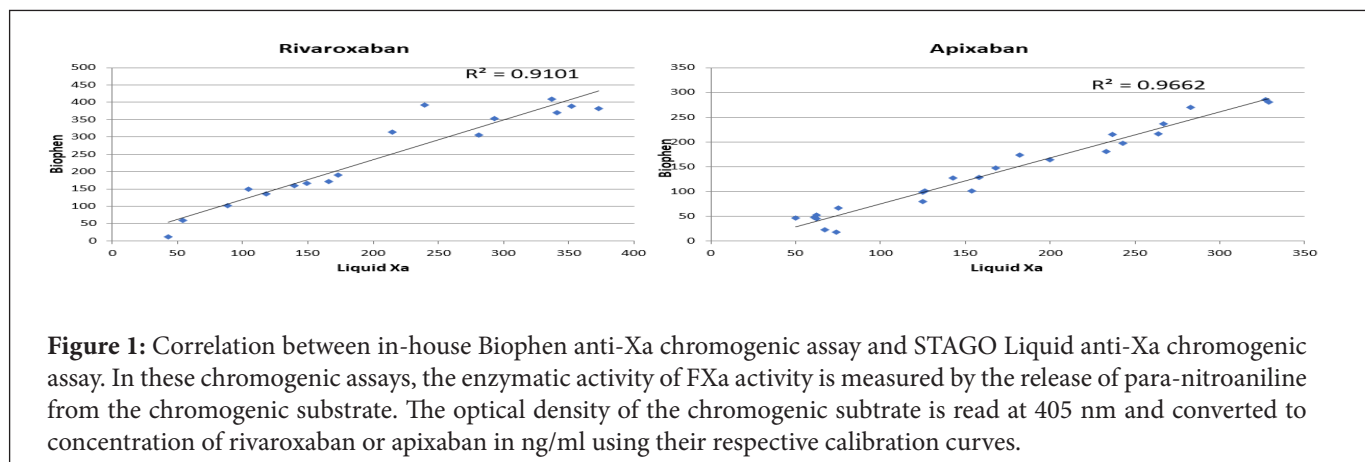
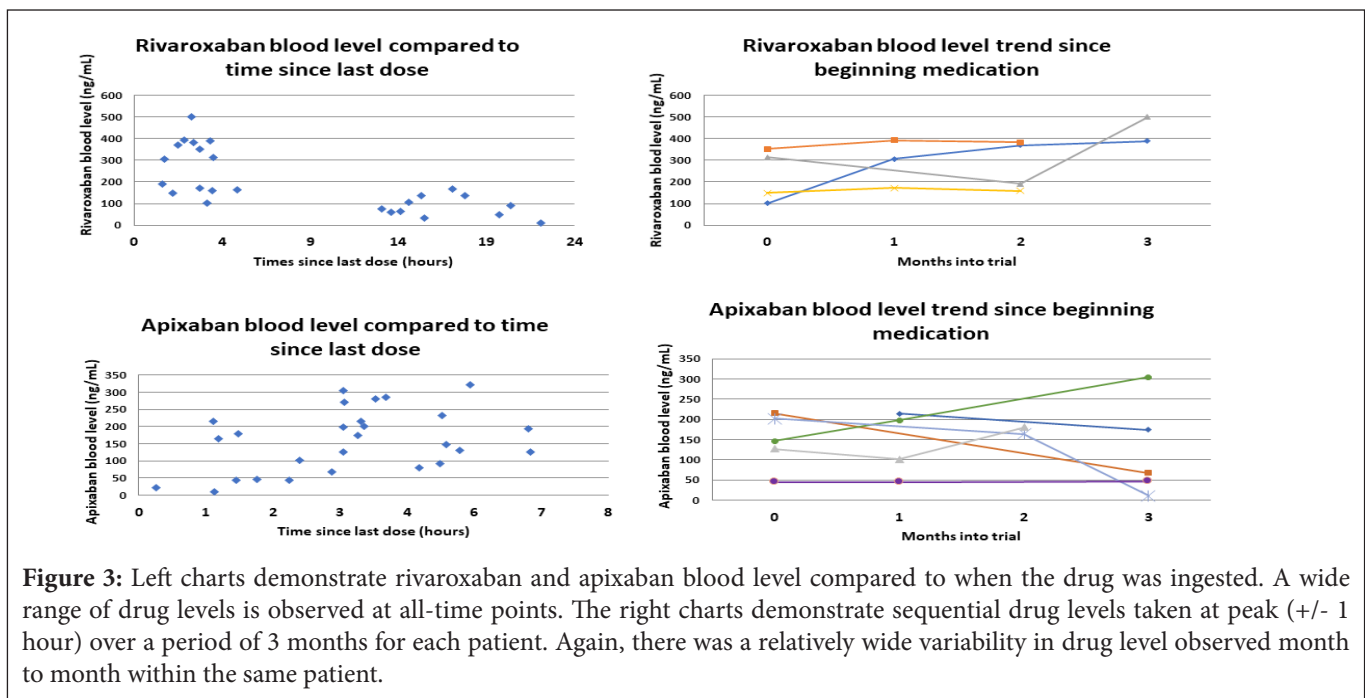
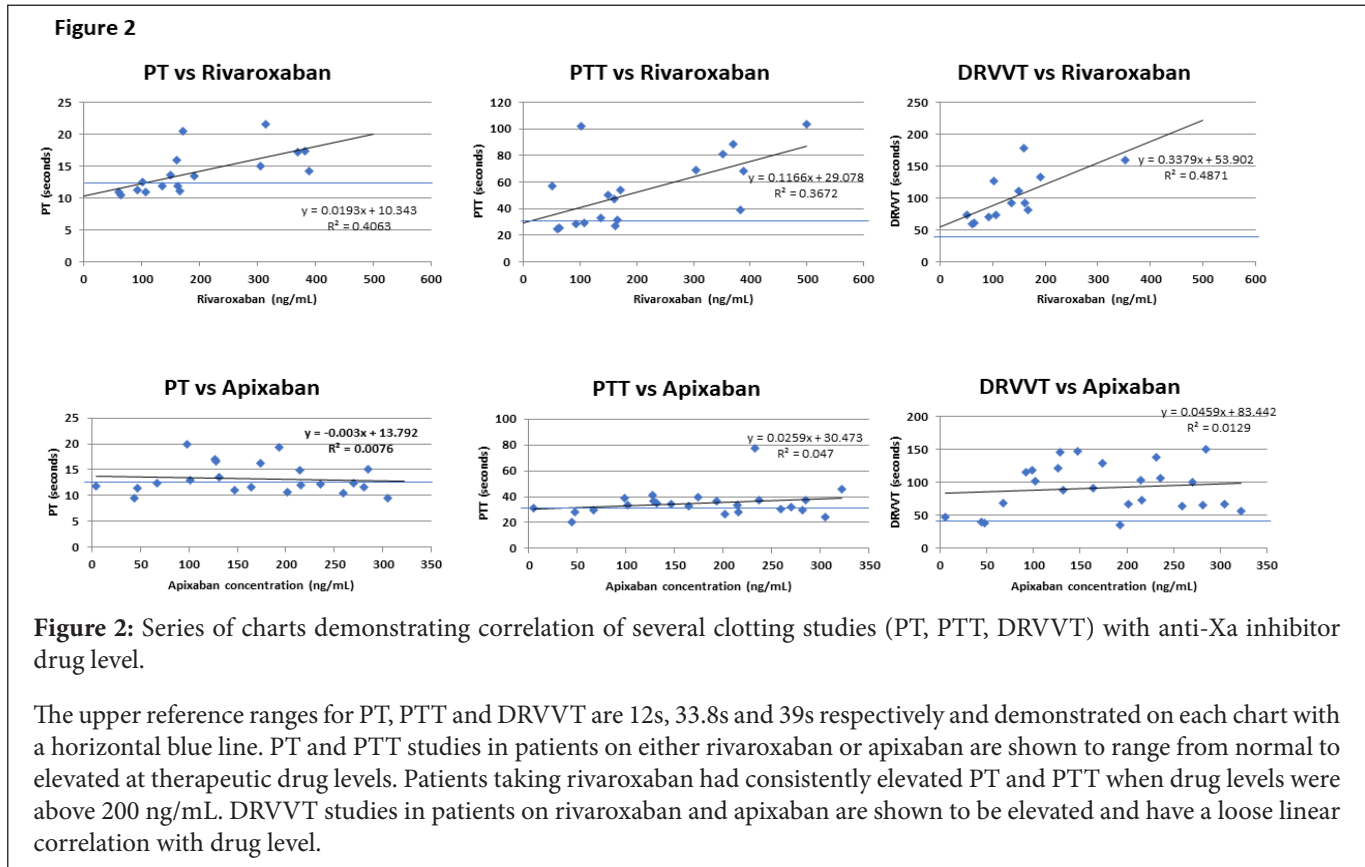


Figure 1: Correlation between in-house Biophen anti-Xa chromogenic assay and STAGO Liquid anti-Xa chromogenic assay. In these chromogenic assays, the enzymatic activity of FXa activity is measured by the release of para-nitroaniline from the chromogenic substrate. The optical density of the chromogenic substrate is read at 405 nm and converted to concentration of rivaroxaban or apixaban in ng/ml using their respective calibration curves.

[5, 10-12]. To determine if the broad range of drug levels is solely due to kinetics or if it is patient-dependent, we tracked the sequential drug levels for each patient taken at peak (+/- 1 hour). Indeed, we observed that 3 patients on rivaroxaban had average levels above 200 ng/mL and 1 patient had levels always below 200 ng/mL (Figure 3). The individual patient pattern of

serial drug levels for apixaban was not as clear. One patient had consistent drug levels around 50 ng/mL and this was the only patient on a regimen of 2.5 mg twice a day, whereas the majority of the patients on apixaban 5 mg twice a day had drug levels above 100 ng/mL. One patient had a drug level below 20 ng/mL that likely represents a skipped dose prior to testing.



Conclusion

Routine coagulation assays (PT and PTT) are fairly insensitive in the detection of DOACs. Sensitivity of PT/PTT is better for rivaroxaban levels greater than 200 ng/mL, but these routine coagulation tests lack sensitivity and linearity for apixaban. On the other hand, DRVVT was fairly sensitive and had a linear correlation for both DOACs (more sensitive for rivaroxaban than apixaban). We recommend the DRVVT assay as a useful screening assay for the presence of DOACs in emergency situations, however careful attention to possible interferences should be given. DRVVT, like PTT, is sensitive to lupus anticoagulants and, depending on the reagents used, may also be sensitive to heparin [13]. Direct thrombin inhibitors will interfere with all coagulation assays based on clot formation. It is important to note that the sensitivity of routine coagulation tests and DRVVT assays greatly depends on the specific reagents and methodologies used and given the high number of commercially available kits, we recommend independent validation studies at each institute or selecting kits and methods that have been validated elsewhere.

To estimate the plasma drug level, we recommend the chromogenic anti-Xa assay with appropriate calibrators. To obtain reliable, representative plasma drug levels it is important to draw the sample at peak time with careful documentation of the time between drug administration and time of blood collection. Correlation with creatinine clearance may also be useful while monitoring DOAC levels as the kidney is the main metabolizer of these drugs, but utility of this was not evaluated for in this study. There are no current guidelines regarding dose adjustment for patients who are subtherapeutic or supratherapeutic, and there is no evidence that adjusting the dose alters the outcome. Thus, there is an urgent need to develop prospective clinical trials to determine if dose adjustment is beneficial. Nonetheless, there are legitimate circumstances in which patients can be subtherapeutic or supratherapeutic, which may require a change in the anticoagulation regimen. We observed a large intra-patient variability of Xa inhibitor levels at peak which is consistent with previous studies [14]. Therefore, we advise no decision should be based on a single measure of plasma drug level but rather serial measures at peak (timing from last dose should be closely monitored). Of course, the exception to this recommendation are cases of life threatening bleeds for which there are appropriate guidelines, which include screening for drug presence in patient plasma and reversing the drug effect [5]. Andexanet alfa is an antidote for FXa inhibitors recently approved by FDA [15]. Prothrombin complex concentrates are commonly recommended for reversal of FXa inhibitors during bleeding episodes but institutions are encouraged to establish their own DOAC reversal protocols and guidelines.

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