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Sensitivity of routine coagulation assays to direct oral anticoagulants: patient samples versus commercial drug-specific calibrators

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Summary

Most studies on the sensitivities of coagulation assays to direct oral anticoagulants (DOACs) are based on normal plasma spiked with anticoagulant in the laboratory. Recent studies have shown that reagent sensitivity varies significantly depending on whether spiked or patient samples are used. The aim of this study was to compare the sensitivities of routine coagulation assays in patient samples and commercial drug specific calibrators using commonly used activated partial thromboplastin time (APTT) and prothrombin time (PT) reagents (i.e., Actin FS and Neoplastine CI Plus for APTT and PT, respectively) in Australian laboratories. Samples collected at Pathology North Hunter (PN-H) for dabigatran ($n = 39$), rivaroxaban, ($n = 56$) or apixaban levels ($n = 22$) between February 2013 and November 2015 were analysed and compared to two different commercial drug specific calibrators from different manufacturers for each DOAC. Our results show that dabigatran (Hyphen and Technoclone) and rivaroxaban (Stago) calibrators tend to overestimate the APTT but are similar to patient samples for PT. A cut-off DOAC level of 50 ng/mL based on results from patient samples within the laboratory can be used as the lower limit which will result in prolongation of APTT for dabigatran (sensitivity 96%, $n = 25$) and PT for rivaroxaban (sensitivity 97%, $n = 29$), respectively. Individual laboratories should be familiar with the sensitivity of their coagulation reagents to different DOACs including differences between patient samples versus different commercial drug specific calibrators.

Key words: Direct oral anticoagulants; DOACs; activated partial thromboplastin time; prothrombin time; patient samples; calibrators.

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INTRODUCTION

Direct oral anticoagulants (DOACs) have the advantage of more predictable pharmacokinetics and pharmacodynamics compared to vitamin K antagonists such as warfarin and therefore do not require routine laboratory monitoring. However in some situations, such as bleeding, recurrent or progressive thrombosis, emergency surgery, renal failure or liver failure, laboratory monitoring may be required. This includes baseline coagulation assays and functional anticoagulant levels. Previous studies have shown that the sensitivities of coagulation assays to the DOACs are reagent and method dependent.[1–7] Most of these early studies have been based on spiked normal pooled plasma. Very few studies were performed using patient samples.[8–12] Regarding the use of routine coagulation assays in the laboratory assessment of DOACs, most guidelines recommend the use of activated partial thromboplastin time (APTT) for dabigatran and prothrombin time (PT) for rivaroxaban for urgent screening of DOACs when the drug being assessed is known.[13] Previous guidelines such as the 2012 British Committee for Standards in Haematology (BCSH) have suggested that a normal APTT for dabigatran and PT for rivaroxaban excludes a therapeutic intensity of the drug.[14] This has been called into question by several later studies performed using patient samples which showed coagulation tests within the normal range even at therapeutic levels of DOAC. In addition, the use of calibrators (spiked samples) tended to overestimate the sensitivity of routine coagulation tests to dabigatran and rivaroxaban.[10,15–19] In Australia, current guidelines by the Australasian Society of Thrombosis and Haemostasis (ASTH) recommend using specific quantitative assays, i.e., dilute thrombin time and drug specific anti-factor Xa chromogenic assay to assess dabigatran and rivaroxaban levels, respectively.[20] Since these assays may not be available in many regional and remote laboratories and/or after hours, ASTH recognises that routine coagulation assays such as thrombin time (TT), APTT and PT can be utilised as screening tests to provide qualitative information about the presence of DOACs. However, caution should be given in adopting these recommendations if commercial drug-specific calibrators are used to determine the relative sensitivity of routine coagulation to DOACs, as there are differences in spiked compared to patient samples. Previous studies have comprehensively evaluated the effect of DOACs on haemostasis tests in spiked or patient samples but none have compared the sensitivity of both the APTT and PT to different DOACs in patient samples versus different drug-specific commercial calibrators.[11,12,16] A recent study evaluated the use of a single drug-specific commercial calibrator for determining PT or APTT reagent sensitivity to dabigatran and rivaroxaban compared to patient

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samples but not for apixaban or using different drug-specific commercial calibrators.[18]

Actin FS and Neoplastine CI Plus are the most common APTT and PT reagents, respectively, used in Australian laboratories, with a third of laboratories using these reagents. In a survey of laboratories participating in the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPA QAP) Haemostasis Program, Actin FS was the most commonly used APTT reagent in 194 of 708 laboratories (27%) among 15 other reagents. Neoplastine CI Plus was the second most common PT reagent used after SM Thrombrel S in 240 out of 730 laboratories (33%) among nine other PT reagents.[21]

Our first aim was to use a common APTT and PT reagent in Australia (Actin FS and Neoplastine CI Plus, respectively) to compare the sensitivities of routine coagulation assays to DOACs in patient samples versus different commercial drug-specific calibrators. If routine coagulation studies can be used as a rough guide to determine whether significant DOAC is present, it would also be useful to know what approximate level of DOAC would cause prolongation of the APTT or PT using these reagents. The median trough level for dabigatran, rivaroxaban and apixaban has been estimated to be around 90 ng/mL [95% confidence interval (CI) 31–225], 32 ng/mL [interquartile range (IQR) 19–60] and 63 ng/mL (95%CI 22–177), respectively.[22–24] Corresponding median peak levels were 184 ng/mL (95% CI 64–443), 244 ng/mL (IQR 175–360) and 132 ng/mL (95%CI 59–302), respectively. Given the lower level sensitivity of the quantitating assays (approximately 40 ng/mL for dilute thrombin time; 25 ng/mL for chromogenic anti Xa assays),[25–29] a level of 50 ng/mL may be a reasonable target to suggest a limited haemostasis related effect at that concentration/time point. Some studies have reported so called 'peak' level ranges for some DOACs of below 50 ng/mL,[13,22,30,31] although it would be expected that this will be a low occurrence rate, and potentially representative of 'non-compliance' or maybe even 'DOAC resistance' in occasional patients. Therefore, the second aim of this study was to determine the distribution of samples with routine coagulation assays above the normal range at a specified cut-off drug level of 50 ng/mL using different DOACs in patient samples.

MATERIALS AND METHODS

Patient samples

For this analysis, patient samples collected at Pathology North Hunter (PN-H) between February 2012 and November 2015 requesting for dabigatran ($n = 65$), rivaroxaban ($n = 112$), or apixaban levels ($n = 31$) were analysed retrospectively. The data analysed consisted of results from coagulation assays performed using Neoplastine CI Plus (Diagnostica Stago, France) and Actin FS (Siemens, Germany) which are the primary reagents used in our laboratory for PT and APTT testing, respectively. The testing was done by mechanical clot detection method on a STA-R Evolution instrument (Diagnostica Stago). Dabigatran levels were tested by an in-house dilute thrombin time akin to the commercially available Hemoclot assay from Hyphen Biomed. Rivaroxaban and apixaban levels were performed using Liquid anti-Xa chromogenic assay (STA liquid anti-Xa; Diagnostica Stago). Limits of detection for dilute thrombin time and liquid anti-Xa chromogenic assays are 40 ng/mL[26] and 25 ng/mL (package insert from manufacturer), respectively. Levels below this were reported as 'zero'. Patient samples where neither APTT nor PT were requested (dabigatran $n = 9$; rivaroxaban $n = 41$; apixaban $n = 8$) were excluded. Patient samples where only one of the PT or APTT results were available which made up the minority were included in analysis for the individual tests. Further samples were excluded if patients were on other oral anticoagulants ($n = 3$), had liver disease ($n = 7$), multiorgan failure ($n = 1$) or if coagulation tests were abnormal when levels of DOACs were undetectable ($n = 18$). After exclusions, the number of remaining samples available for the final analysis were dabigatran ($n = 39$), rivaroxaban ($n = 56$), and apixaban ($n = 22$).

Commercial drug-specific calibrators

PT and APTT were performed on commercial drug-specific calibrators in which DOAC levels were predetermined by the manufacturer using liquid chromatography mass spectrometry (LC/MS-MS). Two calibrators sourced from different manufacturers were used for each DOAC. For rivaroxaban and apixaban, the calibrators were sourced from Diagnostica Stago (France) and Technoclone (Austria), while for Dabigatran the calibrators were sourced from Hyphen Biomed (France) and Technoclone (Austria).

Data analysis

Data were analysed using Microsoft Excel (Microsoft, USA). Linear regression lines and correlation coefficient values, R^2 were derived using the statistical software package included in Microsoft Excel. To compare the sensitivity of APTT and PT in patient samples with commercial drug-specific calibrators, APTT and PT were plotted against DOAC level in separate graphs for each DOAC.

To further evaluate the differences between patient samples and commercial drug-specific calibrators, we used difference plots (Bland–Altman plots) obtained from the Analyse-it extension statistical software package for Excel. For this analysis, a derived PT or APTT for the corresponding DOAC level in patient samples was obtained from the drug-specific calibrator curve. The difference or percentage difference between the derived APTT/PT from the calibrator curve and the APTT/PT for patient samples was then plotted against the mean APTT/PT (mean of APTT/PT from patient samples and derived APTT/PT from calibrator curve). Mean and mean percentage differences were indicated by the 'plus' sign if the derived clotting time was more prolonged and 'minus' sign if the clotting time was shorter for commercial drug-specific calibrators compared to patient samples. A two-tailed paired t test was used to determine if the difference between mean APTT/PT for patient samples compared with mean derived APTT/PT for commercial drug-specific calibrators were significant ($p < 0.05$).

Ethics

This study was approved by Hunter New England (NSW) Human Research Ethics Committee.

RESULTS

Sensitivity of APTT and PT to DOACs in patient samples compared with commercial drug-specific calibrators

The DOAC levels used for commercial drug-specific calibrators from various manufacturers and their corresponding APTT/PT values are summarised in Table 1. Difference results from the Bland–Altman analysis comparing sensitivity of APTT and PT to DOACs in patient samples versus drug-specific commercial calibrators are shown in Table 2. For DOAC levels in patient samples, a level below the limit of detection in our laboratory as described in methods was reported as 'zero'.

Dabigatran

Dabigatran levels in patient samples ranged from 0 to 667 ng/mL and the corresponding APTT and PT ranges were 28–104 s and 13–33 s, respectively. Correlation between APTT clotting times and dabigatran levels in commercial drug-specific calibrators ($R^2 = 1$ for Hyphen and $R^2 = 0.9959$ for Technoclone) was higher compared to patient samples ($R^2 = 0.7717$). Drug-specific calibrators overestimated the sensitivity of the APTT to dabigatran compared to patient