Sensitivity of routine coagulation assays to Direct Oral Anticoagulants: Patient samples versus commercial drug-specific calibrators

Ming Sheng Lim1, Kent Chapman1, Priscilla Swanepoel2, Anoop K Enjeti1,2
1. Haematology Department, Calvary Mater Hospital, Edith St, Waratah, NSW 2296, Australia
2. Pathology North Hunter, NSW Pathology

AIM/BACKGROUND

Most studies on the sensitivities of coagulation assays to DOACS (Direct Oral Anticoagulants) 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.

Method

Samples collected at Pathology North Hunter (PN-H) for dabigatran (n=39), rivaroxaban, (n=56) or apixaban levels (n=22) between February 2013-November 2015 were analysed and compared to 2 different commercial drug specific calibrators from different manufacturers for each DOAC.

Results

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 50ng/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.

Discussion

Recommendations on the use of PT and APTT alone as an indicator of DOAC levels based on commercial drug-specific calibrators should be interpreted with caution as routine coagulation assays are based on patient samples. For example, a normal APTT in patient samples for dabigatran and rivaroxaban may correspond to a higher level of DOAC than suggested by studies based on drug-specific commercial calibrators as the APTT is less sensitive in patient samples. Whether these differences translate clinically into significant differences in bleeding however is unknown. Using Actin FS and Neoplastine CI Plus as reagents in patient samples, a specified cut-off drug concentration of 50ng/ml could be used as an estimate of the lower limit above which the APTT for dabigatran and the PT for rivaroxaban would be prolonged in most samples. In general, a DOAC concentration of <50ng/ml would suggest a limited haemostasis related effect at that concentration/time point. Thus a normal APTT for dabigatran and PT for rivaroxaban can be used as a rough guide as to whether significant drug is present using a sensitive reagent such as Actin FS for DOAC and Neoplastine CI Plus for PT. This would be especially useful for laboratories where routine or afterhours drug levels are not available. However for accurate estimation, the dilute thrombin time for dabigatran and drug specific anti-Factor Xa assay for rivaroxaban and apixaban should still be used.

Conclusions

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.

Fig 1 Sensitivity of APTT Actin FS and PT Neoplastine CI Plus to DOACS in patient samples vs commercial drug-specific calibrators (A) Dabigatran: APTT in Patient samples versus Technoclone or Hyphen Biomed calibrators (B) Dabigatran: PT in Patient samples versus Technoclone or Hyphen Biomed calibrators (C) Rivaroxaban: APTT in patient samples versus Stago or Technoclone calibrators (D) Rivaroxaban: PT in patient samples versus Stago or Technoclone calibrators. (E) Apixaban: APTT in patient samples versus Stago or Technoclone calibrators (F) Apixaban: PT in patient samples versus Stago or Technoclone calibrators. Shown also are the equations and correlation coefficients (R²) for the line of best fit. DOAC levels below the limits of detection (<40ng/ml for dabigatran, <25ng/ml for rivaroxaban and apixaban) in our laboratory were reported as ‘zero’.

Fig 2 Distribution of samples with prolonged coagulation tests above and below a cut-off drug level of 50ng/L for (A) APTT (B) PT Boxed area indicates the normal range for the specific coagulation assay i.e. 24-36 secs for APTT and 12-16 secs for PT. n= number of samples within or above the normal range for the APTT or PT.