Detection of Dabigatran Interference in an Activated Protein C Resistance (APCR) EQA

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Introduction

Protein C is a vitamin K-dependent glycoprotein and natural anticoagulant synthesised in the liver and circulated in the plasma. It is activated when thrombin binds to thrombomodulin. It then inactivates factors Va and VIIIa, demonstrating antithrombotic activity[1].

Resistance to activated protein C describes an impaired anticoagulant response to activated protein C, resulting in a haemostatic disorder that can lead to deep vein thrombosis and pulmonary embolism. It can be diagnosed through blood tests. Multiple methods are currently available for the laboratory testing of Activated Protein C Resistance (APCR). Pathology laboratories may encounter difficulties obtaining true haemostasis results for patients on direct oral anticoagulants (DOACs), which can interfere with these assays[2].

The Royal College of Pathologists of Australasia Quality Assurance Programs, RCPAQAP, provides an external quality assessment of APCR testing. Participating laboratories are supplied with lyophilised plasma samples to test using Activated Partial Thromboplastin Time (APTT) and Russell Viper Venom Test (RVVT) -based APCR assays.

Participants enrolled in the APCR program by RCPAQAP were provided with duplicate samples containing Dabigatran over a 7-year interval. We reviewed the results, methods, and detection of dabigatran interference from the same sample tested seven years apart.

Method

Participants were provided with lyophilised plasma samples that were prepared in-house. The samples contained 195 ng/mL of Dabigatran and had a normal APCR Ratio. The results from sample APC-16-08a, issued in 2016, were compared with those from sample HA-APC-23-04, issued in 2023. The medians of the APCR ratio from both activated partial thromboplastin time (APTT) and Russell viper venom test (RVVT) based assays were analysed. We examined trends and changes that favour the use of APTT, RVVT, or other more sensitive assays for detecting dabigatran interference over the seven-year period.

Results and Discussion

Most participants used FV-deficient plasma to differentiate between FV-driven APCR. The results obtained in 2016 and 2023 showed no significant variation in the all-methods medians of APTT and RVVT-based APCR methods. Interestingly, all participants performing the Staclot method reported a higher result on average in 2023 (Figure 2).

In 2016, 21 participants submitted results using APTT + FV methods and 12 using RVVT + FV methods. Of these, 86% recorded normal APCR with APTT + FV methods and 92% using RVVT + FV methods.

Of the non-FV-based methods, all 5 participants reported the expected normal APCR with the RVVT method for APC-16-08a, and all 8 participants using the Staclot method also reported a negative result (normal APCR) in 2016.

In 2023, all participants using APTT + FV returned a negative result for APCR along with a quantitative value. It was observed that 12 out of the 23 participants using RVVT + FV returned a quantitative result for HA-APC-23-04, of which 11 reported the expected normal interpretation for APCR.

Five out of six participants using the Staclot method in 2023 reported a quantitative value for APCR. Two participants tested using APCR–RVVT, and both reported the expected normal interpretation.

None of the participating laboratories detected Dabigatran in the 2016 sample. In 2023, 17 participants (40%) detected the presence of Dabigatran in conjunction with prolonged APTT. Of these, 35% used activated charcoal to neutralise the DOAC before performing the APCR tests and obtaining the expected APCR negative result. 14 of the 17 participants who reported DOAC interference in 2023 used Pefakit APC-R Factor V Leiden (Table 1). Five of them treated the sample with DOAC Stop and one with another form of activated charcoal before obtaining a normal APCR ratio ranging from 5.1 to 8.1.

As shown in Table 1, 11 out of the 17 participants did not use any DOAC neutraliser; only two of which provided a normal numerical value and interpretation. The remaining nine participants (eight of whom used Pefakit) were restricted by the presence of DOAC and hence could not give a valid interpretation. This suggests that Pefakit may not yield reliable APCR data when dabigatran is present, but using a DOAC neutraliser can eliminate this interference and lead to reliable results and interpretations[3].

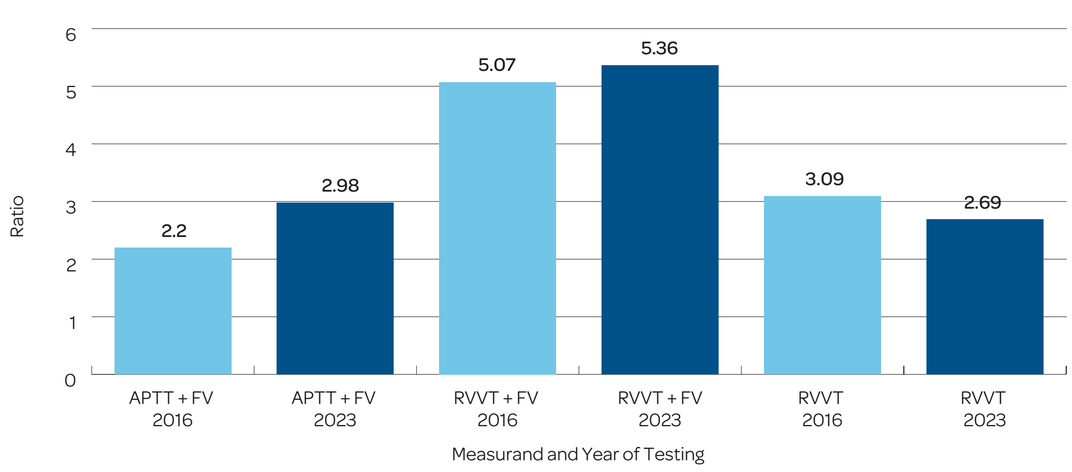

Figure 1 Comparison of 2016 and 2023 APCR survey data

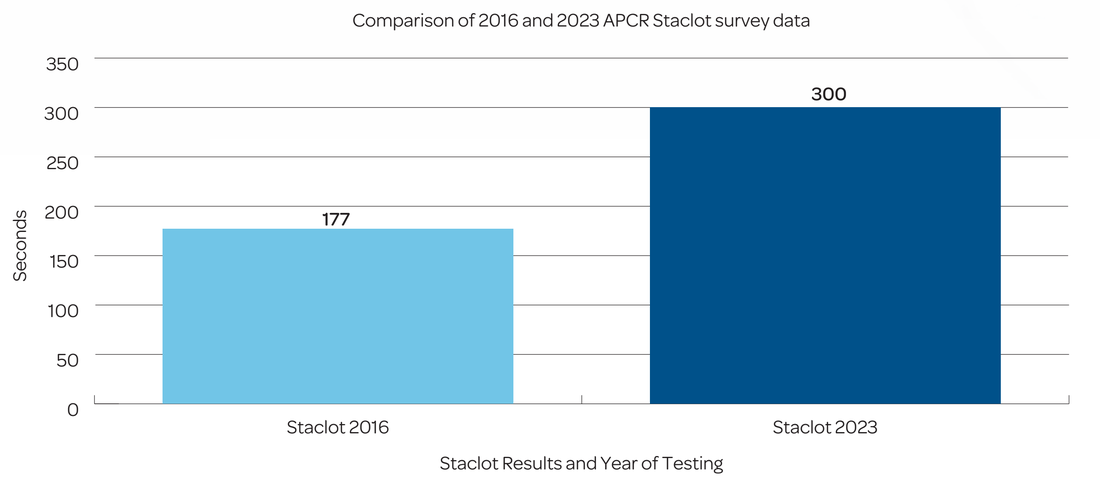

Figure 2 Comparison of 2016 and 2023 Staclot survey data

Table 1 Data from participating laboratories that detected DOAC interference in 2023 indicating improved sensitivity of the Staclot and the Pefakit reagent groups

Lab No.	Quant Value	Inter-pretation	Method	Analytical Principle	Measurement system	Reagent	DOAC Stop
1	5.73	Normal	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	Yes
2	5.61	Normal	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	Yes
3	5.11	Normal	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	Yes
4	5.98	Normal	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	Yes
5	6.59	Normal	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	Yes
6	8.11	Normal	RVVT + FV deficient	Optical clot detection	Siemens Atellica COAG 360	Pefakit APC-R Factor V Leiden	Activated Charcoal
7	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	No
8	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Werfen ACL TOP	Pefakit APC-R Factor V Leiden	No
9	10.98	Normal	RVVT + FV deficient	Optical clot detection	Sysmex CS 5100	Pefakit APC-R Factor V Leiden	No
10	Not Reported	Not Reported	Staclot	Mechanical clot detection	Stago STA-R Max	Stago Staclot APC-R	No
11	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Sysmex CS 5100	Pefakit APC-R Factor V Leiden	No
12	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Sysmex CS 5100	Pefakit APC-R Factor V Leiden	No
13	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Sysmex CN 6000	Pefakit APC-R Factor V Leiden	No
14	>300	Not Reported	Staclot	Mechanical clot detection	Stago STA-R Max	Stago Staclot APC-R	No
15	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Sysmex CS 5100	Pefakit APC-R Factor V Leiden	No
16	Not Reported	Not Reported	RVVT + FV deficient	Optical clot detection	Sysmex CN 6000	Pefakit APC-R Factor V Leiden	No
17	>300	Negative	Staclot	Mechanical clot detection	Stago STA-R Max	Stago Staclot APC-R	No

Conclusion

All the Pefakit users obtained a normal ratio in 2016, which suggests that there may have been a formulation change that has enhanced the assay's sensitivity to DOAC. The elevated APTT observed with Dabigatran underscores the need for greater awareness that DOACs can artificially raise APCR ratios. Utilising a DOAC neutraliser can remove this interference, leading to reliable results and interpretations. This in vitro study using EQA samples, although limited, offers some evidence that haemostasis reagents are now more sensitive to DOAC interference than they were seven years ago.

References:

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