Neutralisation of Rivaroxaban Induced Interference by DOAC Stop and Andexanet Alfa

Elysse Dean[1], Roslyn Bonar[1] and Emmanuel J Favaloro[2]

[1] RCPAQAP, St Leonards, Sydney, NSW, Australia;
[2] Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Sydney Centres for Thrombosis and Haemostasis, NSW Health Pathology, Westmead Hospital, Westmead, NSW, Australia.

Introduction

Rivaroxaban is a widely utilised direct oral anticoagulant (DOAC), indicated for thrombosis treatment and prevention. Rivaroxaban binds to Factor Xa, affecting many haemostasis assays, including activated protein C resistance (APCR) and activated partial thromboplastin time (APTT) based factor assays, including factor VIII (FVIII) and IX (FIX). Andexanet alfa is a clinical 'antidote' for anti-Xa agents[1], including rivaroxaban, neutralising activity *in vivo* and *in vitro*. DOAC Stop is a commercial product that neutralises *in vitro* activity of DOACs[2]. We investigated whether rivaroxaban would induce false positive APCR and/or falsely reduced FVIII & FIX activity, and whether this could be neutralised by andexanet alfa or DOAC Stop.

Method

Four lyophilised plasma samples were prepared as indicated in Table 1. The samples were distributed to all laboratories performing APCR and/or FVIII & FIX testing in RCPAQAP surveys, labelled with the sample IDs below to create a blind survey. Participants were asked to perform their normal assays, and interpret results as usual.

Table 1. Contents of samples.

Sample ID	Referred to as	Contents
APC-19C/FAC-19C	Normal sample	Pool of normal plasma
APC-19D/FAC-19D	Rivaroxaban sample	Sample C spiked with rivaroxaban
APC-19A/FAC-19A	DOAC Stop sample	Sample D treated with DOAC Stop
APC-19B/FAC-19B	Andexanet alfa sample	Sample D treated with andexanet alfa

Results

The Pefakit and Coatest reagents are unaffected by the presence of rivaroxaban. The sample with DOAC-Stop appears to have neutralised the effect of rivaroxaban in the majority of kits that were affected by rivaroxaban. The ProC ACR users reported a reduced ratio for both the andexanet alfa and rivaroxaban samples. ProC Global users reported unexpected results, where even the control (normal) sample had a reduced ratio (Figure 1).

Figure 1. APCR interpretations by reagent kit.

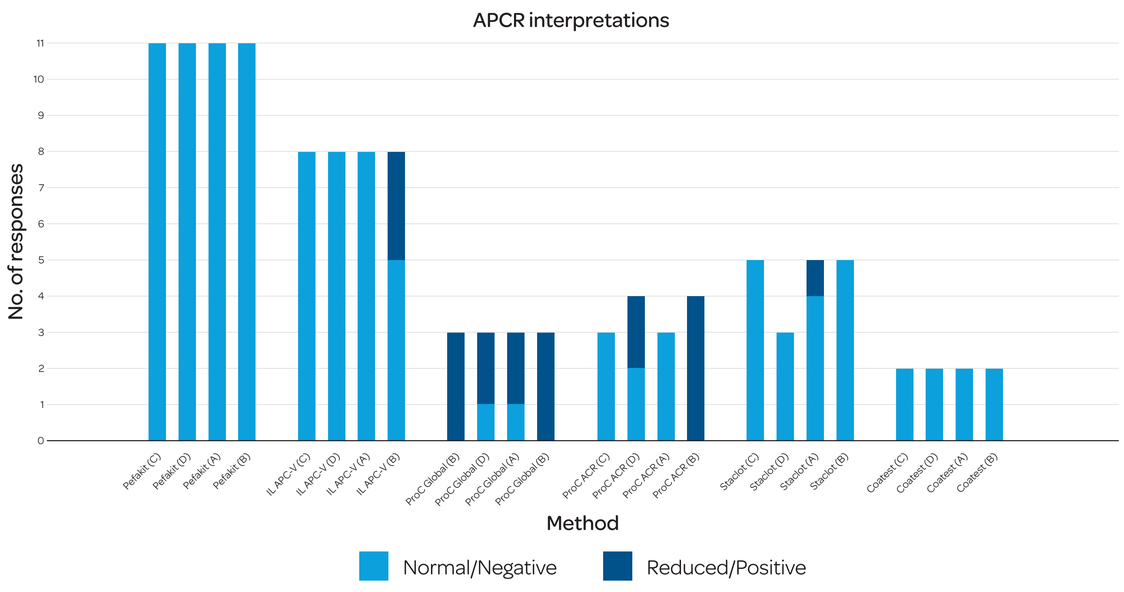


Figure 2 illustrates the effect of rivaroxaban on the measured level of FVIII and FIX in the conventional factor assay procedure. A false reduction in the level of both FVIII and FIX is observed due to the influence of rivaroxaban. Similar results for each assay were produced, with a 43% reduction in FVIII (from the normal sample to the rivaroxaban sample), and a 44% reduction for FIX. Excluding a few outlier results, the sample containing rivaroxaban produced FVIII results below the normal cut off of 50%, giving rise to 'false' abnormal results for 95% of participants. For the FIX assay, 56% of participants recorded this 'false' abnormal interpretation for the rivaroxaban sample (Figure 3). This effect of rivaroxaban has been neutralised by the addition of DOAC Stop, with samples producing borderline/normal assay levels for both FVIII and FIX respectively, similar to the normal sample. The samples spiked with andexanet alfa display a two-tiered response in Figure 2 and Figure 3, with one group indicating neutralisation of the rivaroxaban, and another group recording minimal change.

Figure 2. FVIII and FIX results by sample.

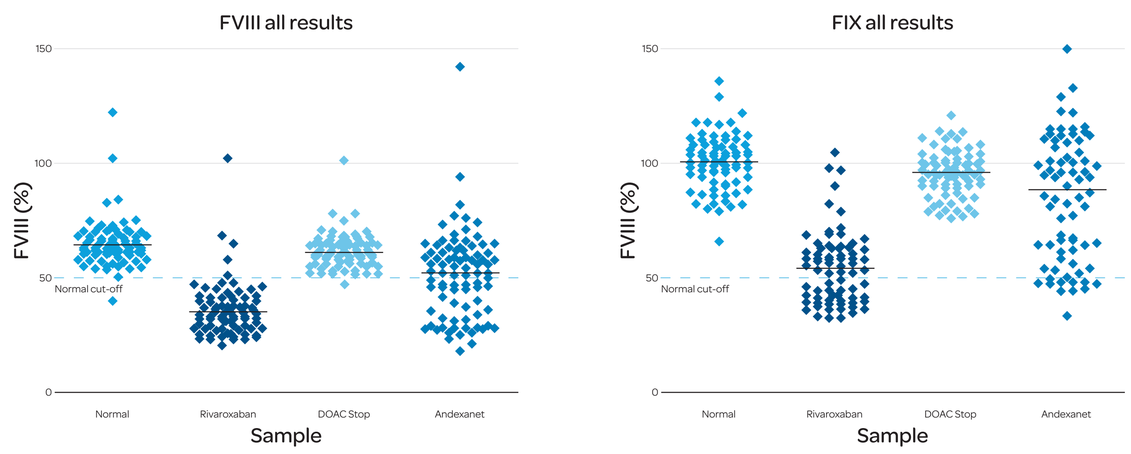
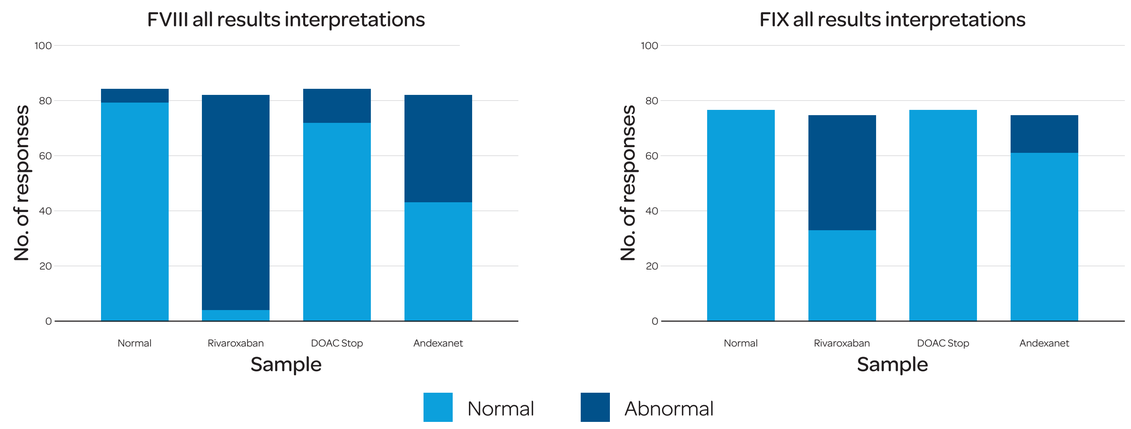


Figure 3. FVIII and FIX interpretations by sample.



Discussion

For most reagents, the APCR results were not affected by the addition of rivaroxaban. We cannot explain the anomalous results shown by the ProC Global users, where the normal sample indicated false positive APCR interpretations for all 3 users. Thus we did not consider the other ProC Global results. The assay most sensitive to rivaroxaban was the ProC ACR assay, which is based on a RVVT procedure. RVVT methods are generally very sensitive to interference by rivaroxaban[3]. The IL APC-V and ProC ACR reagents unexpectedly produced more false positives with the andexanet alfa sample than rivaroxaban alone.

Rivaroxaban greatly affected the levels of FVIII and FIX, lowering the factor level by almost half, which lead to a false diagnoses of low FVIII and/or FIX. This 'false positive' occurred for 95% and 56% of the participants assessing FVIII and FIX respectively. Although both factors were similarly reduced by rivaroxaban (43% and 44%), the median of the FIX normal sample was higher at 100.8%, hence many results were still above the normal cut off of 50%.

The DOAC Stop sample was able to successfully neutralise the effects of rivaroxaban, with assay results and interpretations similar to those of the normal sample for both FVIII and FIX. On the other hand, the addition of andexanet alfa did not completely neutralise the rivaroxaban, with a decrease in the level of FVIII and FIX still observed. The two tiered pattern in the andexanet samples is due to a reagent sensitivity with the Siemens Actin FS APTT reagent giving lower results for the majority of these users. Further, the majority of Siemens calibrator users reported a decreased factor level compared to the rest of the group.

Conclusion

In the rivaroxaban samples, most APCR assays appeared largely unaffected, while FVIII & FIX assays showed a substantive reduction. Where assays were affected, DOAC Stop neutralised rivaroxaban effects. Andexanet alfa did not completely neutralise the rivaroxaban effect, for APCR or FVIII & FIX, and unexpectedly lead to higher rates of false APCR than rivaroxaban alone.

References

1. Siegal DM, Curnutte JT, Connolly SJ, Lu G, Conley PB, Wiens BL, Mathur VS, Castillo J, Bronson MD, Leeds JM, Mar FA, Gold A, Crowther MA. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. N Engl J Med. 2015 Dec 17;373(25):2413-24.
2. Exner T, Michalopoulos N, Pearce J, Xavier R, Ahuja M. Simple method for removing DOACs from plasma samples. Thromb Res. 2018 Mar;163:117-122.
3. Favaloro EJ, Gilmore G, Arunachalam S, Mohammed S, Baker R. Neutralising rivaroxaban induced interference in laboratory testing for lupus anticoagulant (LA): A comparative study using DOAC Stop and andexanet alfa. Thromb Res. 2019 Aug;180:10-19.

RCPAQAP
The Royal College of Pathologists of Australasia
Quality Assurance Programs

FIND OUT MORE **RCPAQAP.COM.AU**
1300 78 29 20 | haematology@rcpaqap.com.au
NATA Accredited Proficiency Testing Scheme Provider. Number 14863 Accredited to ISO/IEC 17043:2010