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ORIGINAL ARTICLE

Direct oral anticoagulants-Remove versus Taipan snake venom time for detection of a lupus anticoagulant in patients taking oral direct factor Xa inhibitors

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Abstract

Background: The optimal method of detecting a lupus anticoagulant (LA) for patients taking direct factor Xa inhibitor (DFXaI) direct oral anticoagulants (DOACs) remains controversial. Methods include charcoal adsorption of the DOACs to allow testing with the activated partial thromboplastin time (APTT) and dilute Russell viper venom time (dRVVT), or use of the DFXaI-insensitive Taipan snake venom time (TSVT) and Ecarin time (ET) assays on neat plasma.

Objectives: The objective was to compare the utility of APTT and dRVVT analysis following DOAC Remove against TSVT/ET on untreated plasma for LA detection in spiked plasmas and routine clinical samples for patients on DFXaIs.

Patients/methods: Various LA-negative and LA-positive samples were assayed by APTT, dRVVT, and TSVT/ET, and then separately spiked with rivaroxaban, apixaban, and edoxaban calibrators to a concentration of ~190 ng/ml and the assays repeated on spiked plasma before and after DOAC Remove treatment. Testing of 284 consecutive samples from DFXaI-anticoagulated patients by APTT/dRVVT and TSVT/ET before and after DOAC Remove treatment was undertaken.

Results: In the spiking model, we found that both TSVT/ET and DOAC Remove strategies generally distinguished LA-negative and LA-positive samples, but some false-positive LA results occurred. In the investigation of 284 consecutive patient samples on DFXaIs, the percentage agreement for LA detection in neat samples tested by TSVT/ET versus APTT and dRVVT after DOAC Remove treatment was 90% (Cohen kappa 0.12).

Conclusion: Our data highlight uncertainty and disagreement for testing LA in patients on DFXaI. Further studies are required.

KEYWORDS
antiphospholipid syndrome, charcoal adsorption, DOAC Remove™, factor Xa inhibitor, lupus anticoagulant, Taipan snake venom time

Essentials

- Testing for a clotting tendency (lupus anticoagulant) whilst on anticoagulants is challenging.
- Patient samples were tested for lupus anticoagulant whilst on anticoagulants using two methods.
- These methods (charcoal adsorbtion v the Taipan Snake Venom time) showed complex results.
- Agreement between the methods was poor and further studies are needed within this area.

1 | INTRODUCTION

The use of direct oral anticoagulants (DOACs) has proliferated because of the convenience of fixed dosing and safety profile.[1] There is a need to detect a subset of high-risk patients with thrombosis and antiphospholipid syndrome (APS) who may benefit from a vitamin K antagonist rather than DOACs.[2,3] Because of interference on haemostasis assays, testing for lupus anticoagulant (LA) for patients taking DOACs is not generally advised; however, to do so would aid diagnostic accuracy for APS while maintaining the convenience of uninterrupted anticoagulation.[4-6] Two assays of differing analytical principles are required to maximize LA detection rates, which are commonly an LA-sensitive activated partial thromboplastin time (APTT) and the dilute Russell viper venom time (dRVVT).[7] Several strategies are available to mitigate the effects of DOAC on hemostasis tests and thus potentially detect an LA; temporarily withhold the DOAC to allow "off-therapy testing," plasma DOAC adsorption methods using activated charcoal (e.g., DOAC Remove, 5-diagnostics, Quadratech, Switzerland, and DOAC Stop, Haematex Research), plasma filter technology, or use of the direct factor Xa inhibitor (DFXaI)-insensitive assays Taipan Snake Venom Time (TSVT) screening test and ecarin time (ET) confirmatory test.[8-10] In 2020, the British Society for Haematology published an addendum to its APS guidance that recommended APTT- or dRVVT-based tests "should not be used to detect LA on samples from patients taking DOAC when there is a detectable drug level."[11] In view of the controversy regarding the optimal laboratory methodology of investigating patients on DOACs, we investigated a strategy of DOAC-Remove treatment before APTT and dRVVT (our first-line standard LA tests[12]) compared with using TSVT/ET on plasma samples from patients taking DFXaI DOACs, where LA investigation had been requested by the clinical team.

2 | METHODS

Sample preparation and storage was conducted in accordance with International Society on Thrombosis and Haemostasis (ISTH) guidance.[6] All LA testing was performed on an ACL TOP 750 (Werfen, Bedford, MA, USA). In this study, only DFXaI DOACs were investigated because the direct prothrombin activation of Taipan and ecarin venoms bypasses FXa inhibition but not thrombin inhibition, rendering dabigatran an interferent in TSVT and ET.[13]

2.1 | Spiking experiments

Ten separate pools of confirmed LA-negative patients (each with 10 unique patients, with equal amounts of plasma) from patients with normal clotting times and not known to be anticoagulated were produced using convenience samples of previously tested patients. Ten separate pools of LA-positive patients (each with 10 unique patients, with equal amounts of plasma), confirmed using the established dRVVT and APTT test ratio procedures in the laboratory as well as displaying TSVT/ET test ratio positivity, were created for spiking experiments (i.e., pools were positive by all three assays). These pools are referred to as samples in the rest of the manuscript. Coagulation factor levels were not measured in any samples; however, in the LA-negative patients, normal clotting tests exclude a significant deficiency, and LA-positive samples came from previously investigated patients known to the laboratory. The LA-negative and two LA-positive plasmas of the First World Health Organization (WHO) International Reference Panel for LA (13/172) (National Institute for Biological Standards and Control), and CRYOcheck LA-positive control and weak LA-positive control were also assessed (Precision Biologic). The WHO LA-positive reference plasmas and Precision Biologic LA-positive control plasmas displayed positivity for LA using all three LA testing systems (APTT, dRVVT, and TSVT/ET assays). Data were aggregated for analysis for the LA-positive and LA-negative samples. Samples were spiked to a concentration of approximately 190 ng/ml of DFXaI by combining with calibrators for rivaroxaban (Werfen), apixaban (Werfen), and edoxaban (Hyphen BioMed). Baseline levels for each sample were conducted on samples that had been combined with an equal volume of 0 ng/ml calibrator to negate the issue of dilutional effect on spiked sample results. Calibrators were shown to be negative for LA on all testing methods. Samples were assessed with the LA assays before spiking (using the combination of 0 ng/ml calibrator and sample), once spiked, and after anticoagulant removal using DOAC Remove. In total there were 11 negative LA samples (10 patient pools and WHO LA-negative references plasma) and 14 positive LA samples (10 patient pools [all triple positive for APTT test ratio, dRVVT test ratio, and TSVT/ET test ratio], 2 WHO LA-positive reference plasmas, and 2 CRYOcheck LA-positive plasmas).

2.2 | Testing on patient samples

Testing was performed on consecutive residual plasma samples where the DFXaI anticoagulant was listed on the test request form

from 15 August 2019 until 1 September 2020. All LAs had been requested as part of routine care.

2.3 | Laboratory assays

Dilute Russell viper venom time was assessed using HemosIL dRVVT Screen and Confirm (Werfen) reagents, reflex testing for confirmation with dRVVT confirm reagent was performed when the dRVVT screen ratio exceeded 1.2. APTT test ratio was performed using a combination of APTT reagents: APTT-SP (LA sensitive) (Werfen) and Dade Actin FS (LA-insensitive) (Siemens Healthineers). TSVT/ET (Diagnostic Reagents Ltd) was performed in accordance with the manufacturer's instructions as previously described.[13] TSVT/ET test ratio and APTT test ratio were tested as integrated testing systems. Ratios for all assays were calculated using the reference range mean clotting times.[13,14] Test ratios were calculated as follows: [Patient screen clotting time/Mean reference range clotting time]/[Patient confirm clotting time/Mean reference range confirm clotting time]. Cutoffs are given in Table 1.

2.4 | DFXaI removal from plasma

Samples were treated with DOAC Remove as previously described.[12]

2.5 | Statistical analysis

2.5.1 | Spiking experiments

Ratios of LA assay results for neat vs spiked/anticoagulant-removed were produced, with 95% confidence intervals (CI); data were normally distributed. Using a null hypothesis of a ratio of 1, where there is no difference between the control groups, in this case, the neat sample and the spiked/anticoagulant removed sample. If the CI includes or crosses 1, there is insufficient evidence to conclude that the groups are statistically significantly different.

2.5.2 | Patient sample evaluation

Cohen Kappa was used to assess agreement between samples where TSVT/ET test ratio was performed on neat samples or where the APTT/dRVVT test ratios had been performed after DOAC Remove. For Cohen kappa, <0.20 is poor agreement, 0.21–0.40 is fair, 0.41–0.60 is moderate, 0.61–0.80 is good, and 0.81–1.00 is very good.

Descriptive statistics have been used. Analysis was performed with Microsoft Excel 2010 (Microsoft).

3 | RESULTS

3.1 | Spiking experiments

The results are presented in Table 1. Where LA-negative samples became LA positive after either spiking or DOAC Remove, details on individual samples are described. No LA-positive samples became LA negative after spiking or DOAC Remove; therefore, individual data on samples are not described but rather the mean effect of spiking/DOAC Remove on the LA assays.

In LA-negative samples, rivaroxaban significantly elevated dRVVT screen (11/11 samples), dRVVT test ratios (11/11 samples), and APTT test ratios (11/11 samples). All samples (11/11) returned to within the reference range for dRVVT screen ratio after DOAC Remove treatment; however, 7/11 did not return to reference range for APTT test ratio. For the TSVT/ET assays, 1/11 and 0/11 samples were falsely positive by the TSVT ratio and test ratio before and 4/11 and 2/11 after DOAC Remove, respectively.

In LA-positive samples, rivaroxaban increased the mean dRVVT screen ratio, which after DOAC Remove was not fully returned to baseline. Mean APTT test ratio was numerically higher after spiking, although this was not statistically significant. Rivaroxaban elevated the mean TSVT ratio (including after DOAC Remove); however, there was no significant change in the mean TSVT/ET test ratio.

In LA-negative samples, apixaban factitiously elevated the dRVVT screen (9/11 samples) but not test ratio (1/11 samples) and after DOAC Remove; all dRVVT screen ratios returned to the reference range. The mean APTT test ratio was numerically lower after apixaban (and DOAC Remove) but this was not statistically significant. For the TSVT/ET assays, 1/11 and 1/11 samples were falsely positive by the TSVT ratio and test ratio before and 3/11 and 2/11 after DOAC Remove, respectively.

In LA-positive samples, apixaban increased the mean dRVVT screen ratio, which was not fully returned to baseline after DOAC Remove. The mean APTT test ratio was not affected by apixaban. The mean TSVT screen was elevated with apixaban and DOAC Remove further elevated this; mean TSVT/ET test ratios were increased in spiked samples including after DOAC Remove.

In LA-negative samples, edoxaban factitiously elevated the dRVVT screen ratio (10/11 samples) and 9/11 returned to normal after DOAC Remove; all of these, however, were negative on dRVVT test ratio. The APTT test ratio was numerically lower after edoxaban (and DOAC Remove) but this was not statistically significant. For the TSVT/ET assays, 1/11 and 1/11 samples were falsely positive by the TSVT ratio and test ratio before and 3/11 and 2/11 after DOAC Remove, respectively.

In LA-positive samples, edoxaban numerically but not statistically significantly increased the mean dRVVT screen ratio (because of the wide 95% CI of the neat/spiked ratio). The mean dRVVT screen ratio after DOAC Remove was similar (1.81 in spiked plasma vs 1.80 after DOAC Remove). Mean TSVT screen ratios were similar for neat versus spiked plasma; however, DOAC Remove significantly increased the mean TSVT screen ratio. Mean TSVT/ET test ratios appeared unaffected.

TABLE 1 Spiking experiments for LA-negative and LA-positive plasma samples, WHO reference plasmas, and CRYOcheck control plasmas, subjected to anticoagulant removal

Drug	Sample type	Test	Neat (mean value)	Spiked (mean value)	Post spiking and drug removal[b] (mean value)	Neat vs spiked ratio (mean value)	Neat vs spiked ratio 95% CI	Neat vs anticoagulant removed ratio[b] (mean value)	Neat vs anticoagulant removed ratio 95% CI
Rivaroxaban	LA negative	dRVVT screen ratio	0.98	2.38	0.99	0.44	0.30–0.58	1.00	0.95–1.05
		dRVVT test ratio[a]	-	1.90	-	-	-	-	-
		APTT test ratio	1.09	1.50	1.24	0.73	0.78–0.98	0.87	0.77–0.97
		TSVT ratio	1.00	1.04	1.09	0.97	0.84–1.10	0.92	0.79–1.05
		TSVT/ET ratio	1.05	0.99	1.06	1.07	0.92–1.22	1.00	0.87–1.13
	LA positive	dRVVT screen ratio	1.57	4.72	1.80	0.33	0.29–0.37	0.87	0.84–0.90
		dRVVT test ratio[a]	1.69	2.60	1.77	0.62	0.53–0.71	0.95	0.90–1.00
		APTT test ratio	1.32	1.61	1.42	0.85	0.69–1.01	0.94	0.88–1.00
		TSVT ratio	1.48	1.58	1.71	0.94	0.90–0.98	0.87	0.82–0.92
		TSVT/ET ratio	1.64	1.72	1.73	0.96	0.92–1.00	0.96	0.89–1.03
Apixaban	LA negative	dRVVT screen ratio	0.98	1.38	1.05	0.73	0.60–0.86	0.97	0.92–1.02
		dRVVT test ratio[a]	-	0.90	-	-	-	-	-
		APTT test ratio	1.09	1.04	1.02	1.04	0.95–1.13	1.07	0.95–1.19
		TSVT ratio	1.00	1.03	1.08	0.97	0.86–1.08	0.93	0.81–1.05
		TSVT/ET ratio	1.05	0.99	1.02	1.07	0.95–1.19	1.04	0.90–1.18
	LA positive	dRVVT screen ratio	1.57	2.64	1.79	0.60	0.57–0.98	0.88	0.85–0.91
		dRVVT test ratio[a]	1.69	1.36	1.74	1.19	1.11–1.27	0.97	0.93–1.01
		APTT test ratio	1.32	1.36	1.32	0.98	0.92–1.04	1.00	0.96–1.04
		TSVT ratio	1.48	1.61	1.77	0.92	0.86–0.98	0.83	0.79–0.87
		TSVT/ET ratio	1.64	1.82	1.81	0.91	0.84–0.98	0.91	0.85–0.97
Edoxaban	LA negative	dRVVT screen ratio	0.98	1.78	1.12	0.58	0.41–0.75	0.89	0.74–1.04
		dRVVT test ratio[a]	-	1.05	1.05	-	-	-	-
		APTT test ratio	1.09	0.95	1.01	1.14	1.02–1.26	1.08	0.99–1.17
		TSVT ratio	1.00	1.04	1.08	0.96	0.85–1.07	0.93	0.81–1.05
		TSVT/ET ratio	1.05	0.99	1.02	1.06	0.96–1.16	1.04	0.90–1.18
	LA positive	dRVVT screen ratio	1.57	1.81	1.80	0.88	0.56–1.20	0.87	0.83–0.91
		dRVVT test ratio[a]	1.69	1.70	1.77	1.00	0.95–1.05	0.96	0.91–1.01
		APTT test ratio	1.32	1.33	1.43	0.99	0.97–1.01	0.93	0.87–0.99
		TSVT ratio	1.48	1.49	1.69	0.99	0.98–1.00	0.88	0.84–0.92
		TSVT/ET ratio	1.64	1.64	1.71	1.00	0.99–1.01	0.96	0.90–1.02

Note: Mean drug concentrations for spiked samples were 191 ng/ml for rivaroxaban, 188 ng/ml for apixaban, and 186 ng/ml for edoxaban. All samples showed a result of 0 ng/ml on baseline samples and 0.03 ng/ml on post-DOAC Remove drug concentrations. Test cutoffs for positives: dRVVT screen ratio >1.19, dRVVT test ratio >1.23, APTT test ratio 1.19, TSVT ratio >1.11, TSVT/ET ratio >1.12. LA-negative samples, $n = 11$; LA-positive samples, $n = 14$.

Abbreviations: APTT, activated partial thromboplastin time; CI, confidence interval; dRVVT, dilute Russell viper venom time; ET, ecarin time; F, factor; LA, lupus anticoagulant; TSVT, Taipan snake venom time; WHO, World Health Organization.

[a]Anticoagulant removal for direct FXa inhibitor samples was with DOAC Remove.

[b]dRVVT test ratio only required if dRVVT screen ratio was elevated.

3.2 | Testing on patient samples in routine clinical care

A summary of the DFXaI concentrations in patient samples is shown in Table 2. A total of 284 samples were available for analysis, the majority of which (182) were rivaroxaban-treated patients. Several samples that were LA negative by TSVT ratio (mean 1.06) appeared to become LA positive (with a prolonged TSVT ratio; mean 1.15) confirmed by TSVT/ET test ratio (mean 1.16) following DOAC Remove treatment (28/182 samples in rivaroxaban patients, 10/88 in apixaban patients, and 1/14 in edoxaban patients became positive after DOAC Remove). We did not test coagulation factors or anti-Xa levels to further investigate this. For samples that were LA positive both before and after DOAC Remove, the mean TSVT ratios were 1.13 and 1.21,

TABLE 2 Plasma anticoagulant levels in patient samples and comparison of LA-positivity before and after anticoagulant removal in TSVT/ET and APTT test ratio/dRVVT

			TSVT/ET testing[b]		APTT test ratio/dRVVT[a]	
Anticoagulant	Number of samples	DFXaI level (ng/ml) Mean (median, range)	Before anticoagulant removal Number LA +ve (%)	After anticoagulant removal Number LA +ve (%)	Before anticoagulant removal Number LA +ve (%)	After anticoagulant removal Number LA +ve (%)
Rivaroxaban	182	275 (315, 75–474)	10 (5)	38 (21)	Total: 126 (69) dRVVT positive: 120 APTT test ratio: 3 dRVVT and APTT test ratio: 3	Total: 10 (5) dRVVT positive: 4 APTT test ratio: 6 dRVVT and APTT test ratio: 0
Apixaban	88	220 (222, 21–427)	7 (8)	17 (19)	Total: 8 (9) dRVVT positive: 4 APTT test ratio: 3 dRVVT and APTT test ratio: 1	Total: 5 (7) dRVVT positive: 2 APTT test ratio: 2 dRVVT and APTT test ratio: 1
Edoxaban	14	179 (164, 11–345)	2 (14)	3 (21)	1 (7) dRVVT positive: 1 APTT test ratio: 0 dRVVT and APTT test ratio: 0	0 (0)

Note: DFXaI samples were treated with DOAC Remove.

Abbreviations: +ve, positive; APTT, activated partial thromboplastin time; DFXaI, direct factor Xa inhibitor; dRVVT, dilute Russell viper venom time; ET, ecarin time; LA, lupus anticoagulant; TSVT, Taipan snake venom time.

[a]Positive TSVT/ET tests had a prolonged TSVT and confirmation with the TSVT/ET ratio.

[b]Recorded as LA positive if either or both of APTT test ratio or dRVVT test ratio were consistent with the presence of a LA.

respectively, which was further reinforced by the TSVT/ET test ratio means, which were 1.15 and 1.20 before and after DOAC Remove. All ETs were within reference range and not affected by DOAC Remove. Samples tended to become LA negative by APTT/dRVVT test ratio after treatment with DOAC Remove: a reduction by 116/182 in rivaroxaban samples, 3/88 in apixaban samples, and 1/14 in edoxaban samples. For the APTT and dRVVT assays, 135/284 (49%) of patients were positive before anticoagulant removal and 15 (5%) positive for LA after anticoagulant removal. Analysis of the performance of the TSVT/ET test ratio on neat plasma versus the APTT /dRVVT test ratios on DOAC Remove- treated plasma is shown in Table 3. This was performed because in a diagnostic service, this is the evaluation that is important to inform concordance between testing strategies. The percentage agreement of the tests for all DFXaI was 90%, with a Cohen kappa of 0.12, which is poor agreement. For all the DFXaIs, there was agreement for LA negativity in 253 samples, positivity by TSVT/ET test ratio in 16 samples, positivity by APTT/dRVVT test ratio in 12 samples, and positivity for both methods (i.e., TSVT/ET test ratio or APTT/dRVVT test ratios) in three samples. For LA-positive samples (a total of 31), Table 4 demonstrates the combinations of positivity seen between the TSVT/ET, dRVVT, and APTT assays.

4 | DISCUSSION

The present study sought to compare two analytical strategies for circumventing DFXaI interference in LA assays and has two important findings. The first is that DOAC Remove has complex effects on plasma in an *in vitro* plasma spiking model using DFXaIs; the second finding is that there is poor agreement between the TSVT/ET and DOAC Remove strategies for detecting an LA in patient samples. There is significant potential for false-positive or false-negative results in clotting assays used to detect a LA in patients on DFXaIs.[6,7]

Spiking LA-negative plasma with DFXaI revealed complex and differential effects for the dRVVT, APTT, and TSVT/ET assays for rivaroxaban, apixaban, and edoxaban (Table 1). Although use of DOAC Remove was generally able to distinguish true- versus false-positive LA, not all assays always returned to the reference range in the LA-negative spiked samples treated with DOAC Remove (e.g., dRVVT screen ratio with edoxaban; TSVT ratio with rivaroxaban, apixaban and edoxaban; TSVT/ET test ratio with rivaroxaban; APTT test ratio for rivaroxaban; described in Results). Incomplete DOAC removal by charcoal adsorbents has been previously described, mainly for high DFXaI levels, and is a limitation of this strategy unless DFXaI assays can be performed to evidence complete removal.[15-17] LA-positive samples remained positive after spiking with all DFXaI, and dRVVT screen ratios were further prolonged, which would be expected. The most striking results were seen with TSVT/ET testing, in which TSVT ratios became more elevated on spiking with rivaroxaban and apixaban, which has not been reported in other studies, and were further prolonged after DOAC Remove treatment with all three DFXaIs. Despite this, spiked and postdrug removal TSVT/ET test ratios were unaffected compared with neat results with rivaroxaban and edoxaban, although they were significantly different with apixaban.

TABLE 3 LA status agreement between TSVT/ET on neat plasma vs APTT/dRVVT test ratio after anticoagulant removal

		APTT and dRVVT test ratio[b] (DOAC Remove)		Percentage agreement (%)	Cohen kappa
		Negative	Positive		
TSVT/ET testing[a] (neat plasma)					
Rivaroxaban	Negative	163	9	90	0.05
	Positive	9	1		
Apixaban	Negative	78	3	91	0.29
	Positive	5	2		
Edoxaban	Negative	12	0	-	-
	Positive	2	0		
All DFXaI	Negative	253	12	90	0.12
	Positive	16	3		

Note: DFXaI samples were treated with DOAC Remove.

Abbreviations: APTT, activated partial thromboplastin time; DFXaI, direct factor Xa inhibitor; dRVVT, dilute Russell viper venom time; ET, ecarin time; LA, lupus anticoagulant; TSVT, Taipan snake venom time.

[a]Recorded as LA positive if either or both of APTT test ratio or dRVVT testing were consistent with the presence of a LA.

[b]Positive TSVT/ET tests had a prolonged TSVT and confirmation with the TSVT/ET ratio.

TABLE 4 LA status agreement between TSVT/ET on neat plasma, dRVVT test ratio, and APTT test ratio after anticoagulant removal (with DOAC Remove) on patient samples found to be LA positive from Table 3

LA assay	Number of positive samples
APTT	8
TSVT/ET	16
dRVVT	3
APTT + TSVT	0
dRVVT + APTT	1
dRVVT + TSVT/ET	3
TSVT/ET + APTT + dRVVT	0

Abbreviations: APTT, activated partial thromboplastin time; DFXaI, direct factor Xa inhibitor; dRVVT, dilute Russell viper venom time; ET, ecarin time; LA, lupus anticoagulant; TSVT, Taipan snake venom time.

There is no immediately obvious explanation for this phenomenon, and further studies would be needed to ascertain any direct interactions between Taipan venom, DFXaIs, and DOAC Remove in the presence of LA. Overall, our data highlight differences in effects (and confounding) of DOAC Remove with different LA assays and DFXaI highlighting the need for further research in this area.

A total of 284 residual samples from consecutive DFXaI anticoagulated patients in routine clinical care were retested by dRVVT and APTT test ratios after anticoagulant removal, and by TSVT/ET test ratio, to assess for effects on routine diagnostic practice where many patients are being tested for LA for the first time. The lower percentages of LA-positive results before DOAC Remove treatment in patients on apixaban and edoxaban compared with rivaroxaban reflect the spiking experiments on LA-negative plasmas. As anticipated from previous reports and mirrored in the spiking experiments, the DOAC Remove procedure markedly reduced the numbers of LA-positive results with dRVVT and APTT, particularly for rivaroxaban. Crucially, frequency of positivity was similar between dRVVT and APTT after DOAC Remove treatment and TSVT/ET test ratio before DOAC Remove treatment, suggesting a degree of diagnostic concordance and accuracy, although the accuracy could only be confirmed with subsequent testing at least 12 weeks later, ideally off anticoagulation. The rise in TSVT/ET test ratio positivity after DOAC Remove treatment reflects the spiking experiments and emphasizes that samples treated for anticoagulant removal with an adsorbent before dRVVT and APTT analysis should not be used for TSVT/ET testing, which should be performed on untreated plasma. Overall, the percentage agreement for TSVT/ET test ratio (on neat plasma) versus LA after DOAC Remove-treated plasma was 90% (Cohen kappa 0.12; poor agreement) in this study of 284 DFXaI samples of which the majority were rivaroxaban.

The TSVT has been previously investigated as a method to detect an LA in patients on DFXaIs.[10,13,18,19,20,21] The advantage of a TSVT/ET assay is that no preanalytical inactivation step is necessary for LA detection on DFXaI because prothrombin activation bypasses the effects of factor Xa inhibitors.[13] The ISTH has previously issued guidance within this area and concluded that there was "no conclusive independent evidence reported on their diagnostic efficacy" and that studies had small numbers of patients mostly on rivaroxaban with challenges in kit standardization and availability likely to limit widespread utility and generalizability.[6] This has recently been challenged in a multicenter, multiplatform publication by the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies, which validated the TSVT/ET for patients taking

DFXaI.[13] What remains unclear is whether the discrepancy in LA detection between the techniques we have investigated are because of (i) complex effects of plasma manipulation by DOAC Remove; (ii) differential sensitivity of the TSVT/ET, APTT, and dRVVT for detecting LA (because of the heterogenous nature of LA antibodies, the sensitivity of the TSVT/ET for all LA was 72% and 78% in confirmed APS cases and 87% in triple-positive [i.e., LA, cardiolipin, and beta-2-glycoprotein I positive] APS cases in the recent ISTH SSC publication regarding this[13]); and (iii) confounding of the anticoagulant effects on assays or combinations thereof. In theory, the dRVVT/APTT could be more sensitive to detecting an LA compared with TSVT/ET because there are two assays; however, this was not borne out in the comparison of the two techniques (Table 3). In an ideal validation scenario, a large number of LA-negative samples off anticoagulation would be collected and then subsequent further sample collection would be undertaken after starting DOACs. This would be compared with a large number of people with a positive LA off anticoagulation who had a further sample collected after initiating DOAC therapy. These samples could then be subject to investigation using different LA detection methods. The prospect of such a validation seems remote because of the inherent difficulties in performing such a study. The Clinical and Laboratory Standards Institute advises comparison of methods that this is performed in at least 50 negative and 50 positive samples.[22]

This study has limitations. It is a single-center study reflecting our local assays and cannot be generalized beyond our center, and dabigatran was not studied. We have not performed mixing studies as suggested in ISTH guidance because of a lack of available plasma; likewise, factor assays have not been assessed because the Dade Actin FS APTT has been assessed for each patient to ensure no prolongation has been noted.[7,23,24] We have not correlated the assay results with clinical details of the patient samples (e.g., presence of cardiolipin/beta-2-glycoprotein I antibodies and history of obstetric morbidity/thrombosis) and we have no prior knowledge of their LA status off anticoagulation previously. This study did not aim to correlate the assays with patient phenotype but investigated the assay performance for DOAC Remove plus dRVVT/APTT versus TSVT/ET. We also performed spiking experiments with pooled samples (which may introduce confounding) and *in vitro* results may not relate to *in vivo* effects. A strength of the work is that we knew the exact DFXaI patients were taking, with the mean DFXaI concentration being 275, 220, and 179 ng/ml for rivaroxaban, apixaban, and edoxaban, respectively, for patient samples. These are within the expected "peak concentrations" on treatment and therefore our results represent testing where there is maximal DFXaI interference with assays.[25] Our study did not seek to address how DOAC Remove dealt with different DFXaI concentrations but rather with the evaluation of dRVVT/APTT after DOAC Remove versus the TSVT/ET on neat plasma. Additionally, we did not investigate other charcoal adsorbents (e.g., DOAC Stop), which may not be equivalent to DOAC Remove; a comparative study is required as different adsorbents may have adsorbents have variable capacity, specificity, and kinetics and should not be generalized. Nevertheless, we provide novel data on the comparability and performance of TSVT/ET versus DOAC Remove plus dRVVT/APTT in routine patient care albeit in a specialized hemostasis laboratory.

5 | CONCLUSION

In conclusion, we found, first, that in a spiking experiment, DOAC Remove generally distinguished LA-negative versus LA-positive samples though there were some false-positive LA results, and second, LA detection by APTT/dRVVT after DOAC Remove versus testing neat plasma samples for TSVT/ET in patients on DFXaI showed inconsistent results between the two methods. A proposed algorithm could be the testing for an LA on neat plasma by the TSVT/ET (to avoid the confounding effects of charcoal inactivation) and then proceed to DOAC Remove only if the TSVT/ET is negative. This would avoid the initial plasma manipulation step; however, we should recognize that proceeding straight to charcoal absorption is a more straightforward approach.[13] The controversial issue surrounding the optimal detection of an LA for patients taking DOACs remains and our data highlight discrepancies between methodologies; further studies are required in this area.

RELATIONSHIP DISCLOSURE

Danielle White has nothing to declare. Gary W. Moore reports consultancy fees from Technoclone. Martin Besser reports speakers fee from STAGO and advisory boards for Novartis, Cosmopharma, and Werfen. Stephen MacDonald reports support to attend educational meetings from Sysmex, Werfen, and Stago. Will Thomas reports speakers fees from Bayer, Pfizer, Alexion, and Portola and advisory board for Daiichi Sankyo.

AUTHOR CONTRIBUTIONS

D.W. and W.T. designed the study, wrote the manuscript, and performed data analysis. D.W. performed the laboratory work. M.B., G.W.M., and S.M. had intellectual input and approved the final draft of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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