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Title **International multi-centre, multi-platform study to validate Taipan snake venom time as a lupus anticoagulant screening test with ecarin time as the confirmatory test: Communication from the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies**

Running head Validation of Taipan/ecarin lupus anticoagulant tests

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Essentials

- Therapeutic anticoagulation interferes with assays for lupus anticoagulant (LA) detection

- Taipan and ecarin LA assays are insensitive to warfarin and direct factor Xa inhibitors
- Taipan screen/ecarin confirm showed 78.2% sensitivity 95.0% specificity for LA in known APS
- The Taipan and ecarin assay pairing was validated for LA testing

Abstract

Background: Lupus anticoagulant (LA) assays are compromised in anticoagulated patients, and existing strategies to overcome the interferences have limitations. The prothrombin-activating Taipan snake venom time (TSVT) screening test and ecarin time (ET) confirmatory test are innately insensitive to vitamin K antagonists (VKA) and direct factor Xa inhibitors (DFXaI).

Objectives: Validate standardised TSVT/ET reagents for LA detection, in a multi-centre, multi-platform study.

Patients/Methods: Six centres from four countries analysed samples with TSVT/ET from 81 non-anticoagulated patients with LA, patients with established antiphospholipid syndrome (APS) and proven persistent LA who were either not anticoagulated (n=120) or were anticoagulated with VKAs (n=180) or DFXaIs (n=71). Additionally, 339 non-anticoagulated LA-negative patients, and 575 anticoagulated non-APS patients (172 VKA, 403 DFXaI) were tested. Anticoagulant spiking experiments were performed and 112 samples containing potential interferences (i.e. direct thrombin inhibitors) were tested. Results were evaluated against locally derived cut-offs. Imprecision was evaluated.

Results: Cut-offs were remarkably similar despite use of different analysers and donor populations. Cut-offs for TSVT ratio, ET ratio, percent correction and normalised TSVT ratio/ET ratio ranged between 1.08-1.10, 1.09-1.12, 9.3%-14.8% and 1.10-1.15 respectively. Coefficients of variation for TSVT and ET ratios were ≤5.0%. TSVT/ET exhibited sensitivity, specificity, negative and positive predictive values of 78.2%/95.0%/86.3%/91.5% respectively with established APS as the LA-positive population, and 86.9%/95.0%/76.8%/97.4% respectively with triple-positive APS. Interference was seen with direct thrombin inhibitors, unfractionated heparin and low molecular weight heparins, but not VKAs or DFXaIs.

Conclusions: TSVT/ET are validated for LA detection in non-anticoagulated patients and those on VKAs or DFXaIs.

Keywords: antiphospholipid syndrome

dilute Russell's viper venom time
ecarin time,
lupus anticoagulant
Taipan snake venom time
validation

1. INTRODUCTION

Antiphospholipid syndrome (APS) is defined by the presence of persistent antiphospholipid antibodies (aPL) in patients with vascular thrombosis or pregnancy morbidity.[1] These clinical manifestations are non-specific for APS so diagnosis is reliant on accurate laboratory detection of three criteria aPL, anticardiolipin antibodies (aCL), antiβ2glycoprotein I antibodies (aβ2GPI), and lupus anticoagulants (LA).[2] Solid-phase assays are used to detect aCL and aβ2GPI whilst no assay specific for LA exists, their detection relying on differences in antibody behaviour in a medley of phospholipid-dependent coagulation assays.[3] Simultaneous presence of all three criteria antibodies, so-called triple-positivity, puts patients at high risk of thrombosis and recurrence.[2]

Antibody heterogeneity means that no single coagulation assay type is sensitive for all LA and two test systems of differing analytical principles are required to maximise detection rates, usually dilute Russell's viper venom time (dRVVT), and an LA-responsive activated partial thromboplastin time (APTT).[2-6] As with many coagulation-based assays seeking to isolate specific abnormalities, standard use and interpretation of LA assays assumes no other causes of elevated clotting times are present. This is, of course, not always the case and the thrombotic nature of APS prompts many requests for LA detection after initiation of anticoagulant treatment.[3,7] Therapeutic targets of different anticoagulants and varying assay principles dictate whether, and to what extent, a given anticoagulant and dose will interfere with a given assay type. This is further complicated by variable responses to anticoagulants between same-principle reagents from different manufacturers, arising predominantly from compositional variation.[9-11]

Those issues significantly complicate LA detection such that testing anticoagulated patients is discouraged because the possibility of false-positive and -negative results is high.[7,11-14] There are, however, clinical and research settings where attempting to detect LA during anticoagulation is warranted or desirable and strategies exist to minimise or eliminate assay interference.[7,8] The main approaches are mixing tests to correct the effects of vitamin K antagonists (VKA),[5-7,15] reagent-integral heparin neutralisers,[14] and pre-analytical removal of direct oral anticoagulants (DOAC) from plasma using adsorbent material.[11] However, mixing tests reduce sensitivity to LA,[7,16] heparin neutralizers are effective only up to a certain level of

heparin,[14] and incomplete removal, and assay interferences in samples from non-DOAC treated patients, have been reported with DOAC adsorbents.[17]

Assays employing snake venom prothrombin activators that are insensitive to the effects of VKAs, and by design will bypass direct factor Xa inhibitors (DFXaI), have been described but are not widely used, partly due to limited availability of standardised reagents.[18-22] Although a modest record of single-centre evaluations exists, predominantly focussing on reagent performance in VKA-anticoagulated patients,[15,18-20,23-27] and recent studies on rivaroxaban-anticoagulated patients,[21,22,28] there are no large collaborative studies that could promote recommendations to employ these assays in routine diagnostic repertoires. The present ISTH SSC-endorsed multi-centre study seeks to validate the Taipan snake venom time (TSVT)/ecarin time (ET) pairing with the only currently available standardised versions of the reagents for LA testing, in non-anticoagulated patients, and patients anticoagulated with VKAs or DFXaIs.

2. MATERIALS AND METHODS

The participating laboratories were: Haemostasis Laboratory, The Royal London Hospital, London, UK (Laboratory 1), Coagulation Laboratory, Ghent University Hospital, Ghent, Belgium (Laboratory 2), Department of Hemostasis, University Hospital of Tours, Tours, France (Laboratory 3), Diagnostic Haemostasis and Thrombosis Laboratories, Viapath Analytics, Guy's & St. Thomas' Hospitals, London, UK (Laboratory 4), Haematology Department, Alfred Health, Melbourne, Australia (Laboratory 5), Department of Haematology, Specialist Haemostasis Unit, Cambridge University Hospitals NHS Trust, United Kingdom (Laboratory 6).

2.1 Patient samples

Each laboratory identified plasma samples from storage repositories and/or ongoing testing that fulfilled criteria for cohorts of LA-positive and LA-negative patients, for subsequent testing with TSVT/ET. There were three categories for LA-positive patients; (i) non-anticoagulated patients with LA, and ideally, patients with established APS and persistent LA whose current testing confirmed the LA (ii) patients anticoagulated with a VKA for established APS and persistent LA (iii) patients anticoagulated with a DFXaI for established APS and persistent LA. Routine LA assay results were available for the anticoagulated patients since they can evidence presence of LAs in situations where interferences are overcome. Four categories encompassed LA-negative patients; (iv) patients with thrombosis, pregnancy morbidity, or APS/aPL-associated conditions, who had tested negative for LA (v) patients on VKA anticoagulation for

reasons other than APS (vi) patients on DFXaI anticoagulation for reasons other than APS (vii) non-APS patients whose samples contained potential interferents of TSVT and/or ET. Results for concurrent routine coagulation screening and aCL and aβ2GPI assays were provided where available. It was accepted that there may not be historical LA testing for samples in categories (v), (vi) and (vii). Participant laboratories were asked, where possible, to aim for a minimum of 20 samples in each category except for category (iv), where numbers closer to 50 was the aspiration.

2.2 Lupus anticoagulant assays

Each laboratory employed ISTH SSC guideline-compliant sample preparation and storage procedures and dRVVT and APTT assay performance and diagnostic interpretation, and for aCL and aβ2GPI assays.[4,29] For LA assays, the dRVVT reagents used by four laboratories were STA®-Staclot® dRVV Screen and Confirm (Diagnostica Stago, Asnières-sur-Seine, France), one used Siemens LA1 and LA2 (Siemens Healthineers, Marburg, Germany), and one used HemosIL® dRVVT Screen and Confirm (Instrumentation Laboratory, Bedford, USA). Four laboratories used PTT-LA® (Diagnostica Stago) as the APTT screening test, two of which used Staclot-LA® (Diagnostica Stago) as the confirmatory test and two used addition of Bio/Data™ Lupus Anticoagulant Confirmation Reagent (Bio/Data Corporation, Horsham, USA). One laboratory used Cephen LS and LR (HYPHEN BioMed, Neuville-sur-Oise, France) as APTT screen and confirm respectively, and one used HemosIL® APTT-SP (Instrumentation Laboratory) and Dade Actin FS (Siemens Healthineers) as APTT screen and confirm respectively.

2.2.1 Taipan snake venom time and ecarin time assays

Taipan snake venom time was performed with Diagen Taipan Venom (Diagnostic Reagents Ltd, Thame, UK), and Diagen Bell and Alton Platelet Substitute (Diagnostic Reagents Ltd) diluted 1:6 in imidazole buffer as the phospholipid component. The Taipan venom reagent contains calcium chloride and the platelet substitute reagent has been previously recommended and employed as LA-sensitive in dilute form.[30] Ecarin time was performed with Diagen *Echis* Venom (Diagnostic Reagents Ltd). Each laboratory adapted the manufacturer's instructions for relative amounts of reagent and plasma volumes and incubation times for LA testing on their routine coagulation analysers. The Diagen reagents were kindly supplied by the manufacturer. Each laboratory employed their routine LA-negative and LA-positive control samples when testing with TSVT/ET.

Briefly, TSVT was performed with one volume each of plasma and dilute phospholipid incubated at 37°C for 60s, and then two volumes of Taipan venom added and timed to clot. Ecarin time was performed by

adding two volumes of *Echis v*enom to one volume of plasma pre-incubated at 37°C for 60s. Clotting times for TSVT and ET were converted to normalized ratios via locally derived reference range (RR) mean clotting times as denominators.[6,8,25,31-33] Phospholipid dependence was determined using the percent correction formula, calculated as [(TSVT ratio - ET ratio)/TSVT ratio] x 100, and the normalised screen/confirm ratio (NSCR), calculated as TSVT ratio/ET ratio.

2.2.2 Analytical platforms

Laboratories 1 and 4 used Sysmex CS2100i and CS2000i (Sysmex UK, Milton Keynes, UK) automated coagulation analysers respectively. Laboratories 2 and 5 used Stago STA-R Evolution® and Stago STA-R Max2® (Diagnostica Stago) automated coagulation analysers respectively, and Laboratory 3 used a semi-automated Stago STart®4 coagulometer (Diagnostica Stago). Laboratory 6 used an Instrumentation Laboratory ACL TOP 750 (Instrumentation Laboratory) automated coagulation analyser.

2.3 Reference ranges

Laboratories were asked to identify a minimum of 40 normal plasmas from healthy, non-anticoagulated, adult donors to generate local TSVT and ET RR mean clotting times, and RRs for TSVT ratio, ET ratio, percent correction, and NSCR. In view of difficulties in accessing sufficient donors for accurate 99th percentile cut-offs, data were first assessed for Gaussian distribution to permit parametric evaluation, particularly where donor numbers were <100.[34] The Kolomogorov-Smirnov normality test was used, with p=<0.05 being taken to show a significant departure from normality. Transformations were attempted where p=<0.05 or to improve values. Outlier assessment employed the Tukey method. Analyse-it® software (Analyse-it Software Ltd, Leeds, UK) was used for statistical analyses.

2.4 Imprecision

Intra-assay precision for TSVT and ET ratios was assessed from assaying locally employed normal and LA-positive control plasmas a minimum of 8 times each in the same run. Inter-assay precision was assessed with data from the same plasmas obtained from the different runs performed during the study, numbers of which varied between each centre.

2.5 Lupus anticoagulant reference plasmas

Participant laboratories were supplied with three sets of the 1st WHO International Reference Panel for Lupus Anticoagulant (13/172), kindly supplied by NIBSC (Potters Bar, UK). Each panel consists of three freeze-dried human plasmas comprising an LA-negative plasma (12/148), a moderate LA-positive plasma

(12/150), and a strong LA-positive plasma (12/152). Each laboratory analysed their plasma sets with TSVT and ET on three separate days, one set per day.

2.6 Anticoagulant-spiked normal plasmas

Interference in TSVT and ET by DOACs was assessed by assaying commercial DOAC assay calibration plasma sets separately containing rivaroxaban, apixaban, edoxaban and dabigatran in a range of batch-specific concentrations. Calibrator sets were STA®-Rivaroxaban Calibrator (Diagnostica Stago), STA®-Apixaban Calibrator (Diagnostica Stago), BIOPHEN™ Edoxaban Calibrator (HYPHEN BioMed, Neuville-sur-Oise, France), and BIOPHEN™ Dabigatran Calibrator (HYPHEN BioMed).

Interference in TSVT and ET by heparins and heparinoids was assessed by spiking separate aliquots of a normal control plasma, Coagulation Control N (Technoclone, Vienna, Austria), with either unfractionated heparin (UFH), enoxaparin, dalteparin, tinzaparin, or danaparoid at final anti-Xa levels of 0.25, 0.50 and 1.00 IU/mL, and fondaparinux at 0.25, 0.50 and 1.00 mg/L. The spiking experiments were performed only at Laboratory 4.

3. RESULTS

Each laboratory was supplied with spreadsheets to record their historical results and study results, which were returned to the initiating centre (laboratory 4) for centralised analysis of TSVT and ET testing.

3.1 Reference ranges

Table 1 shows RR data. Reference range mean clotting times for TSVT ranged between 30.4–36.8s in Laboratories 1-5, with Laboratory 6 an outlier at 22.8s. The range for ET was 14.6–21.2s. Despite differences in RR mean clotting times, conversion to normalized ratios generated similar RRs between each centre. Other than ET ratio from Laboratory 5, all other RRs did not deviate significantly from normality, some requiring transformations (Table 1.) Consequently, and because not all centres could procure >100 normal donors, RRs were derived parametrically as ±2 standard deviations of the mean,[5,6,8,33,34] upper limits being used as cut-offs. A right-skewed distribution prevented successful transforms for ET ratio from Laboratory 5 and the cut-off was taken as 99th percentile. The manufacturer's RRs for TSVT and ET ratios are 0.93–1.10 and 0.90–1.11 respectively, LA-positivity being defined as TSVT ratio >1.10 which is corrected by ≥10% by the ET ratio.

Lupus anticoagulant detection is predicated on normal samples and those with non-LA abnormalities not exhibiting a significant difference between screen and confirm results. A two-tailed paired t-test (p <0.05) was performed on each TSVT ratio and ET ratio normal donor population pair to evidence whether their TSVT and ET ratios were significantly different. The p-values are given in Table 1.

3.2 Imprecision

Intra-assay and inter-assay coefficients of variation (%) for TSVT and ET ratios with locally employed normal and LA-positive controls are in Table 2.

3.3 Anticoagulant-spiked normal plasmas

The TSVT and ET results for anticoagulant-spiked normal plasmas are shown in Table 3. Percent correction and NSCR are given where TSVT ratio was elevated. Other than a slightly elevated ET ratio with 496 ng/mL edoxaban, all TSVT and ET ratios for rivaroxaban, apixaban, edoxaban and fondaparinux, the anticoagulants specifically targeting FXa, were normal. Dabigatran elevated TSVT and ET ratios at all concentrations.

All TSVT ratios were increased by UFH whilst ET ratios were unaffected, leading to false-positive interpretations at every concentration. The low molecular weight heparins (LMWH) elevated TSVT ratio at most concentrations, albeit to a lesser degree than UFH, whilst all ET ratios were normal, giving false-positive interpretations whenever TSVT ratio was elevated. Danaparoid elevated TSVT and ET ratios similarly at 1.00 IU/mL, so no false-positive interpretation ensued.

The TSVT and ET ratios for the normal plasma used for heparin and heparinoid spiking were 0.98 and 0.94 respectively.

3.4 Patient samples

Data on known LA-positive patients are given in Table 4. In view of difficulties in obtaining samples from non-anticoagulated patients with established APS, results for non-anticoagulated patients are sub-divided into those with established APS and persistent LA, or patients not diagnosed as APS with either a persistent LA or the sample being tested was an isolated finding and/or first test for LA. Known triple-positive patients from each cohort are shown, which are not additional to the totals given in the preceding column but a sub-population of them. Based on an elevated TSVT screening test ratio accompanied by elevated NSCR, TSVT/ET analysis detected 326/452 (72.1%) of all LA, 290/371 (78.2%) of

known persistent LA in patients with established APS, and 152/175 (86.9%) of LA in triple-positive patients. Percent correction achieved positive interpretations in all these samples except for three non-anticoagulated patients who each had NSCR of 1.16 (cut-off 1.15) but 14.0% correction (cut-off 14.4%), and two DFXaI anticoagulated patients with NSCRs of 1.17/1.16 (cut-off 1.14) and 14.5%/13.9% corrections respectively (cut-off 14.8%). There was no correlation between INR and TSVT ratio in VKA-anticoagulated patients with established APS (Spearman r = 0.097). All ET ratios were normal as they were unaffected by the LA that were elevating the TSVT ratios.

Table 5 shows data from LA-negative patients. Just 6/339 (1.8%) of non-anticoagulated LA-negative patients were LA-positive via elevated TSVT ratio and NSCR. One of these was subsequently LA-positive by dRVVT at a later date and another had elevated IgG aCL in the same sample. Of note is a pre-liver transplant patient with TSVT ratio/ET ratio/NSCR of 1.80/1.69/1.07 respectively and 6.1% correction, thus not giving a false LA-positive interpretation. Forty of 575 (7.0%) non-APS patients anticoagulated with VKAs or DFXaIs were LA-positive by TSVT/ET.

Based on these results, sensitivity, specificity, positive predictive value, negative predictive value and accuracy for LA testing by TSVT/ET were 72.1%/95.0%/87.6%/87.3/87.4% respectively. Excluding LA-positive patients where APS was not yet established, values were 78.2%/95.0%/86.3%/91.5%/90.1% respectively. Assessing only triple-positive patients as the LA-positive population, values were 86.9%/95.0%/76.8%/97.4%/93.4% respectively.

Laboratories 1, 2, 4 and 5 submitted 55/25/2/30 samples respectively containing potential interferences with TSVT and/or ET assays. Thirty five of 37 dabigatran samples had elevated TSVT ratios, the two with normal results had undetectable dabigatran and were likely trough samples.[35] Seven with elevated TSVT ratio did not generate elevated NSCR or percent correction despite a cross-over in dabigatran levels with samples that did generate false-positive interpretations. Four of those without a positive interpretation had dabigatran levels >400 ng/mL (410/697/700/1050 ng/mL), mirroring the spiking experiments with the higher dabigatran concentration. All patients on argatroban had elevated TSVT ratios but only two generated positive interpretations. The argatroban levels were 179/368 ng/mL with respective NSCRs of 1.26/1.41 (cut-off 1.15). For the other argatroban samples, TSVT ratio tended to be lower than ET ratio; TSVT range 2.62-3.49 (Mean/Median 2.92/2.95), ET range 2.54-7.32 (Mean/Median 4.14/3.96). Figure 1 plots argatroban concentration vs TVST/ET NSCR indicating a greater increase in ET clotting times compared to TSVT clotting times as argatroban concentration increases. All patients with UFH anti-Xa

levels >0.33 IU/mL generated false-positive TSVT/ET, as per the spiking experiments. The other two UFH samples were from the same patient with liver disease and INR 2.0, one with normal TSVT ratio/anti-Xa 0.20 IU/mL, the other with elevated TSVT but normal NSCR and anti-Xa 0.27 IU/mL.

In contrast to the spiking experiments, nine patients with enoxaparin ≥0.50 IU/mL (0.69-0.93 IU/mL) had normal TSVT ratios. One other, with anti-Xa 0.62 IU/mL, had a slightly elevated TSVT ratio of 1.11 (cut-off 1.08) but concordant ET ratio, of 1.13. Five other patients on unspecified LMWHs were LA-positive by TSVT/ET, one of which had severe liver disease and deranged coagulation screen. Most other samples either generated normal TSVT ratios or concordantly elevated TSVT and ET ratios. The patient with FII 13.7IU/dL had minimally elevated INR and APTTr, 1.3/1.4 respectively, yet TSVT ratio/ET ratio/NSCR were 1.56/1.29/1.21 respectively, with 17.0% correction.

3.5 Lupus anticoagulant reference plasmas

The TSVT/ET results for the WHO reference plasmas are given in Table 7. The plasmas were consistently correctly classified as LA-negative or -positive.

Notably, all laboratories encountered little or modest difference in apparent potency of the moderate and strong positive reference plasmas. To further investigate this phenomenon, TSVT screening test ratios were plotted against dRVVT and APTT screening test ratios for samples from non-anticoagulated, LA-positive patients from all laboratories where TSVT/ET testing was also LA-positive (Figure 2). Whilst there was a trend for TSVT ratio to increase with increasing dRVVT and APTT ratios, TSVT ratios were generally lower. TSVT ratio ranged from 1.09–1.90 (Mean 1.29, Median 1.25), dRVVT from 1.14–6.34 (Mean 2.18, Median 1.89), and APTT from 0.89–5.06 (Mean 2.27, Median 2.02). Of the TSVT/ET-positive samples, 4/117 (3.4%) were positive by dRVVT alone in routine testing, one of which was triple positive, 4/117 (3.4%) were positive by APTT alone, three of which were triple positive, and 109/117 (93.2%) were positive by both dRVVT and APTT.

4. DISCUSSION

Although testing for LA in anticoagulated patients is broadly discouraged, it is necessary in certain clinical situations and for full antibody profile characterisation in research studies and registries.[7] The main analytical strategies to reduce or eliminate anticoagulant interference in LA assays do permit accurate LA detection within their own limitations, yet those limitations mean that enhancing current practice can improve our ability to detect LA in this difficult clinical scenario.

The oscutarin C fraction of Coastal Taipan (*Oxyuranus scutellatus*) venom is a phospholipid- and calcium-dependent, FV-independent, serine protease prothrombin activator, and the ecarin fraction of Indian Saw-Scaled viper (*Echis carinatus carinatus*) venom is a co-factor independent metalloproteinase prothrombin activator.[36] Diluting the phospholipid in TSVT renders the assay LA-sensitive, facilitating operation as a screening test,[9,36] whilst absence of phospholipid in ET makes it impossible for LA to affect clotting times, permitting operation as a prothrombin-activated confirmatory test.[18,20,36] Some LA achieve a degree of resistance to the swamping effect of concentrated phospholipid confirmatory reagents,[4] the most extreme examples generating false-negative interpretations,[37] but this cannot occur with ET, which has improved performance over high-phospholipid confirmatory reagents.[20] Both venoms can activate the undercarboxylated prothrombin produced on VKA anticoagulation, to thrombin or meizothrombin, so normal results are obtained in VKA-anticoagulated patients without LA.[15,19,20,27,36] Recent studies confirmed that direct prothrombin activation makes both assays rivaroxaban-insensitive.[21,22] The present study aimed to validate TSVT/ET for LA detection in non-anticoagulated patients, and those anticoagulated with VKAs or DFXaIs, in a multi-centre setting, to widen diagnostic possibilities, particularly in anticoagulated patients.

The study employed the only commercially available TSVT and ET reagents specifically formulated by the manufacturer to be used together for LA detection. Each laboratory adapted the manufacturer's assay performance instructions to their routine coagulation analysers to maintain analytical parity. Reference ranges and cut-offs for LA assays should be locally derived and were generated at each laboratory to account for differences in analysers, operators and local patient populations.[4-6,25,38,39] Although ISTH LA detection guidelines recommend 99th percentile cut-offs it is problematic for many laboratories to source sufficient normal donors for an accurate 99th percentile estimation (>100). Furthermore, other guidelines and studies indicate that, in common with routine coagulation screening assays, LA assay population distributions are commonly Gaussian, or can be made so by data transformation, and that parametric evaluation can be applied on lower donor numbers.[5,6,8,33,34,40,41] All but one of the RRs were Gaussian or successfully transformed, so cut-offs were taken as mean +2SD, equating to 97.5th percentile for normally distributed data.[5,6,34] The marked right skew for ET ratio at Laboratory 5 prevented successful transformation, and since there were >100 donors, a 99th percentile cut-off was used and was similar to cut-offs in the other centres. Reference range mean clotting times for TSVT in laboratories 1–5 varied by 6.4s, and for ET in all laboratories, by 6.2s. Given that three laboratories employed automated photo-optical clot detection, two employed automated mechanical clot detection and one employed semi-

automated mechanical clot detection, and that different normal donor plasmas were used, the modest differences in clotting times are unremarkable other than for TSVT in Laboratory 6.[25,39,41] The difference was most likely because Laboratory 6 was the only one using an Instrumentation Laboratory analyser and that clotting times were obtained from the second derivative of the clot waveform. As previously described,[25,39,40] converting TSVT and ET clotting times to ratios reduced between-laboratory differences. Consequently, RRs and cut-offs were remarkably similar to each other and those of the manufacturer. Cut-offs for TSVT ratio/ET ratio/NSCR differed by up to 0.03/0.04/0.05 respectively. The Gaussian distributions were reflected in close mapping of NSCR cut-offs to percent correction cut-offs i.e. NSCR cut-off of 1.12 for Laboratory 6 equates to the percent correction cut-off of 12.1%. Consequently, there were few interpretive discrepancies between NSCR and percent correction, which occurred in 5/325 (1.5%) samples with LA that were also TSVT/ET positive, where percent corrections were just below cut-offs and corresponding NSCRs just above.

Within-assay and between-assay imprecision values were all ≤5.0% for TSVT ratio and ET ratio, which are within expected limits for clotting assays on automated and semi-automated analytical platforms.[42-45]

Although anticoagulant-spiked plasmas do not always directly reflect results of *ex vivo* samples, particularly for UFH,[46,47] they are nonetheless useful in assessing effects on a given assay.[11,46] As previously reported for rivaroxaban,[21,22] and theoretically anticipated for apixaban and edoxaban, all TSVT and ET ratios were normal at all DFXaI concentrations in spiked plasmas, except a slightly elevated ET ratio at the highest edoxaban level. An apparent albeit slight dose response was noted for TSVT and ET with increasing edoxaban concentration. No elevated TSVT ratios occurred and the coefficients of variation for TSVT and ET ratios within the concentration range were 2.8% and 3.2% respectively, and thus within the ranges of assay imprecision. The other DFXaI, fondaparinux, had no effect on TSVT or ET. Being a direct inhibitor of thrombin and meizothrombin, dabigatran elevated TSVT and ET at every concentration, and ET ratios were sufficiently lower than TSVT ratios to generate false-positive interpretations at 30ng/mL and 255ng/mL but not 468 ng/mL where ET ratio was higher than TSVT ratio. Ecarin has a linear response to dabigatran over a broad range,[48,49] including supratherapeutic levels, these results suggesting the relationship is less linear or has a different slope with TSVT. Elevated TSVT ratios at all UFH concentrations reflects the predominantly anti-IIa activity of UFH. The ET ratios were normal because steric hindrance prevents the heparin-antithrombin complex from inhibiting the meizothrombin generated by ecarin.[49,50] Consequently, false-positive interpretations occurred at all UFH levels. The predominantly anti-Xa activity of LMWHs manifested as lower TSVT ratios than with UFH, which were normal at 0.25IU/mL enoxaparin

and dalteparin. Normal ET ratios persisted with LMWHs so all elevated TSVTs were accompanied by false-positive interpretations. The higher TSVT ratios with tinzaparin, and TSVT elevation at 0.25IU/mL, most likely reflect it's lower anti-Xa/anti-IIa ratio than the other LMWHs.[51] Although danaparoid is a glycosaminoglycan mixture it has a high anti-Xa/anti-IIa ratio,[52] so the dose response with both TSVT and ET, sufficient to elevate both at 1.00IU/mL, was surprising, although percent correction and NSCR were not positive. The most likely cause is inhibition via the dermatan sulfate component of danaparoid, which promotes thrombin and meizothrombin inhibition when in complex with heparin cofactor II.[53] It is a minor component of danaparoid, which likely explains why TSVT and ET ratios were only elevated at the highest concentration.

There is no gold standard against which LA results can be verified as no single assay can detect every LA, so performance of TSVT/ET in detecting known LA necessarily employed the dRVVT and APTT pairing as a pseudo-gold standard comparator. A large body of evidence attests to the diagnostic efficacy of the dRVVT and APTT pairing, yet it is not infallible due to between-manufacturer variability of same-type reagents,[6,25,38,41,54,55] and that some LA preferentially manifest in other assays.[6,18,19,27,36,56-59] This introduces a selection bias potentially disadvantaging assays evaluated against dRVVT and APTT as they may, in part, be sensitive to different antibody sub-populations. Taking that into account, the 72.1% sensitivity of TSVT/ET to all the LA was good and comparable to, or better, than that reported for some dRVVT reagents in comparison studies.[25,55,60,61] Sensitivity rose to 78.2% when LA-positive samples from patients not yet proven to have APS were excluded, and was higher again at 86.9% for LA only from triple positive patients. Only 13/175 (7.4%) of triple-positive patients were LA-negative by TSVT/ET compared to 61/371 (16.4%) non-triple positive APS patients. A possible explanation comes from a recent report indicating that aβ2GPI exert LA activity through interference with FV activation, to which oscutarin C and ecarin would be insensitive, whilst prothrombin-directed antibodies compete for phospholipid binding sites,[62] and other recent studies report that LA activity in high-risk patients is largely attributable to prothrombin-directed antibodies.[63,64] This high sensitivity of TSVT/ET for the clinically significant LA in triple positive patients provides a vehicle for LA detection in patients already anticoagulated with VKAs or DFXaIs at a diagnostic juncture where antibody profiling is clinically and prognostically valuable.[2,65] Whilst TSVT/ET did not detect all LA from triple positive patients, not all were positive in both dRVVT and APTT, which we have reported previously.[41] Although single positivity is associated with fewer clinical events and less recurrence, single positivity via LA can be clinically significant.[2,66] Particularly for patients anticoagulated with VKAs where mixing tests with dRVVT and APTT can generate false-negatives,[4-7,15,16] TSVT/ET provides a viable route to detection of LA in single positive patients when anticoagulated with VKAs, or DFXaIs. The

lack of correlation between INR and TSVT ratio was unsurprising given the ability of oscutarin C to activate descarboxyprothrombin. Since FII is less depleted than FX during VKA therapy, it seems that the relatively low FII level is sufficient for coagulation to proceed.[67]

Previous studies on TSVT/ET testing report high specificity, which in large part is due to direct FII activation bypassing most of the coagulation reactions, and minimal or absent cofactor requirements for oscutarin C and ecarin. Consequently, mixing tests are rarely performed with TSVT/ET as the only factor deficiencies requiring correction are FII and fibrinogen.[19-22,26-28] Both deficiencies are rare in the population requiring LA detection except the acquired FII deficiency of VKA therapy, to which both venoms are insensitive anyway, whilst the LA-hypoprothrombinemia syndrome is occasionally encountered.[68] The advent of integrated testing with dRVVT has led to recognition that an elevated screening test accompanied by a normal confirmatory test reduces the need for mixing tests,[5,6,8,69-71] and that assays more LA-specific require mixing tests less often.[72] The innate specificity of the cofactor-independent ET, where even potent LA cannot interfere, makes it an ideal confirmatory test.[36] The results from patients with reduced FII or fibrinogen, or deranged coagulation due to liver disease, further emphasise specificity of TSVT/ET as all but two (discussed below) did not generate false-positive results and demonstration of the presence of an inhibitor was unnecessary. As would be anticipated, the main cause of elevated ET ratios was direct thrombin inhibitors, where TSVT/ET testing would anyway be postponed, so most other samples generated normal ET ratios. The current ISTH SSC guideline acknowledges that the CLSI guideline prioritises confirmatory tests over mixing tests, omitting the latter when there is no evidence of other causes of elevated clotting times or phospholipid dependence is already demonstrated by screen and confirm discordance,[4,6] an approach also recommended in the BSH guidelines.[5] The ISTH SSC guideline recommends simultaneous mix and confirm performance in response to an elevated screen to permit interpretation even when mixing tests are negative, which has been shown to improve detection rates in a separate study.[71] For these reasons, plus limited volumes of stored plasmas, mixing tests were not performed. The testing of samples previously negative for LA, and from non-APS VKA- or DFXaI-anticoagulated patients, corroborated previous reports by generating specificity of 95.0%. Only 1.8% of LA-negative non-anticoagulated patients were TSVT/ET positive, whilst 7.0% of non-APS anticoagulated patients were TSVT/ET positive. Given that some of the anticoagulated patients were being treated for thromboses without previous testing for aPL, and that TSVT/ET has been shown to detect LA unreactive in dRVVT and APTT, at least some of the apparent false-positives may have be genuine LA.[19,27,56] Nonetheless, specificity was calculated by treating them as false-positives when they were, strictly speaking, merely dRVVT and APTT negative.

Argatroban was not included in spiking experiments but the patient data indicate potential for false-negatives as argatroban concentration increases. The two samples with positive NSCRs went against this trend and may have been genuine positives, but that could not be confirmed by taking them off argatroban to re-test as historical samples were used. A striking finding was that, in contrast to false-positives in spiking experiments, nine patients with enoxaparin >0.5IU/mL returned normal TSVT ratios, and another with elevated TSVT ratio had normal NSCR. This suggests that TSVT/ET can at least be employed as a negative predictor of LA in enoxaparin anticoagulation. Other, unspecified LMWHs did generate false-positives so TSVT/ET testing must be approached with caution in such patients. In concordance with spiking experiments, UFH samples gave predominantly false-positive TSVT/ET results. TSVT reagents containing heparin neutralisers would alleviate this problem. Although *ex vivo* dabigatran samples did not entirely mirror spiking experiments, in that there was cross-over between levels generating concordant or discordant TSVT and ET ratios, direct thrombin inhibition precludes use of these assays in the presence of dabigatran. Most non-anticoagulant potential confounders either had normal TSVT ratios or concordant ET ratios where TSVT was elevated, including four samples with fibrinogen <1.0 g/L, two immunodepleted FII deficient plasmas and a FII deficiency of 19.6IU/dL. Five patients with elevated INRs due to liver disease had normal TSVT, reflecting that some forms of liver disease result in synthesis of undercarboxylated FII.[73] Those with concordantly elevated TSVT and ET reflect a similar response of both assays to reduced synthesis of normal FII. Given that most samples with reduced FII had concordantly elevated TSVT and ET, the patient with FII 13.7IU/dL and TSVT higher than ET was likely a genuine LA where the ET reflected the reduced FII alone, and TSVT resulted from a combination of LA and FII deficiency. This an example of one of the rare occasions where a TSVT mixing test could be useful by confirming the presence of an inhibitor. The patient on an unspecified and unquantified LMWH with liver disease and deranged coagulation screen, including markedly elevated thrombin time, had TSVT ratio 1.32, ET ratio 1.16, 12.1% correction and NSCR 1.14. Whilst these results could represent an LA they could also be due to multiple abnormalities compromising LA detection.

The LA reference panel plasmas were correctly classified by every lab on every occasion of testing yet the minimal differences between TSVT elevations for moderate and strong LA plasmas were unexpected. Despite broadly correlating, patient data revealed that TSVT ratios tend to be lower than dRVVT or APTT in a given sample. The assay has good sensitivity to LA but elevations manifest within a narrower range than dRVVT or APTT. It is well known that some LA can appear more potent in one of dRVVT or APTT than the other, or may be positive in only one assay, so it is presence not potency that secures diagnosis.

Additionally, this may reflect differences in antibody populations given that TSVT and ET may be more sensitive to prothrombin-directed antibodies.[62]

In summary, TSVT/ET showed good sensitivity to LA in non-anticoagulated patients and those anticoagulated with VKAs and DFXaIs, but testing is compromised in the presence of heparins and direct thrombin inhibitors. Previous reports of high specificity for TSVT were corroborated with larger sample numbers, and some apparent false-positives could equally have been genuine LA that were unreactive with dRVVT or APTT, suggesting a potential role as second line testing in non-anticoagulated patients.[27,56,74] Anticoagulation with DOACs has become the standard of care for patients with a first unprovoked thrombosis yet results from clinical trials, however limited they may be, have led the European Medicines Agency to recommend avoiding DOAC anticoagulation in APS, particularly in triple positive patients.[7,75-79] Not only does this make accurate LA detection in anticoagulated patients more important,[7] but the resultant emphasis on a return to VKA anticoagulation for APS creates an analytical niche which TSVT/ET testing is best placed to fill since adsorbents do not mitigate for the effects of VKAs and mixing tests with dRVVT or APTT are prone to false-negatives. In common with all LA screening tests, TSVT in isolation cannot detect every LA, so a negative result does not exclude LA but a positive result is diagnostic and the search for LA, with less reliable assays, need go no further.

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CONFLICTS OF INTEREST

G.W. Moore reports consultancy fees from Technoclone. All other authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

G.W. Moore initiated and designed the study, identified samples, interpreted data, performed statistical analyses, and wrote the manuscript, and was employed at St. Thomas' Hospital when the laboratory work for that site was performed. P.O. Jones identified samples, performed testing, interpreted data, and undertook the spiking experiments. S. Platton and N. Hussain performed testing and interpreted data, and S. Platton identified samples and collated data. D. Davies and W. Thomas identified samples and

interpreted data, and D. Davies performed testing. J. Rigano identified samples, performed testing and interpreted and collated data. C. Pouplard identified samples, performed testing and interpreted and collated data. E. Gray prepared and characterised the WHO LA reference panel plasmas. KMJ Devreese identified samples, performed testing, and interpreted and collated data. All authors reviewed, critically revised and accepted the manuscript.

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TABLE 1. Reference interval statistics for TSVT and ET parameters for each centre

	Lab 1	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**
N	120	41	51	70	102	48
Analyser	Sysmex CS2100i	Stago STA-R Evolution®	Stago STart®4	Sysmex CS2000i	Stago STA-R Max2®	IL ACL TOP 750
TSVT						
RR mean TSVT (s)	36.8	35.1	30.4	35.1	33.5	22.8
Mean TSVT ratio	1.00	1.00	1.00	1.00	1.00	1.00
Median TSVT ratio	0.99	0.99	1.00	1.00	1.00	1.01
Transform	Box-Cox	None	None	None	None	None
KS *p*-value	0.374	0.538	0.754	0.720	0.211	0.340
Outliers	0	1	1	0	0	1
±2SD of mean	**0.89 – 1.10**	**0.92 – 1.08**	**0.91 – 1.09**	**0.91 – 1.09**	**0.92 – 1.08**	**0.90 – 1.10**
ET						
RR mean ET (s)	19.0	16.5	21.2	20.3	18.4	14.6
Mean ET ratio	1.00	1.00	1.01	1.00	1.00	1.00
Median ET ratio	1.00	1.00	1.01	0.99	0.99	0.99
Transform	Box-Cox	None	Reciprocal	None	-	None
KS *p*-value	0.501	0.050	0.335	0.462	<0.05	0.062
Outliers	3	1	1	1	0	2
±2SD of mean	**0.90 – 1.09**	**0.86 – 1.12**	**0.88 – 1.12**	**0.91 – 1.09**	**>1.10***	**0.92 – 1.08**
% correction						
Mean % correction	0.13	0.45	1.18	0.17	-0.03	0.05
Median % correction	-0.12	1.08	1.71	0	0.5	0.50
Transform	Exponential function	None	Exponential function	None	None	None
KS *p*-value	0.895	0.858	0.174	0.926	0.288	0.906
Outliers	2	1	0	2	1	0
±2SD of mean	**-13.2 – 14.4**	**-10.6 – 11.5**	**-7.6 – 10.1**	**-8.9 – 9.3**	**-14.8 – 14.8**	**-12.0 – 12.1**
Normalised TSVT/ET ratio						
Mean TSVT/ET ratio	1.00	1.00	1.00	1.00	0.99	1.00
Median TSVT/ET ratio	1.00	1.01	1.01	1.00	1.00	1.01
Transform	Box-Cox	None	Exponential function	None	None	None
KS *p*-value	0.416	0.642	0.284	0.949	0.955	0.883
Outliers	1	1	1	2	1	0

2-tail paired t *p*-value	0.990	0.955	0.886	0.953	0.973	0.820
±2SD of mean	**0.87 – 1.15**	**0.89 – 1.12**	**0.90 – 1.11**	**0.91 – 1.10**	**0.86 – 1.14**	**0.88 – 1.12**

* Data could not be transformed to achieve Gaussian distribution, the value given is the 99th percentile cut-off.

Abbreviations: ET, ecarin time; KS, Kolmogorov-Smirnoff; Lab, Laboratory; RR, reference range; SD, standard deviation; TSVT, Taipan snake venom time

TABLE 2. Imprecision data for TSVT ratio and ET ratio

TSVT ratio	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**
Intra-assay CV (%) NPP	2.4	0.8	3.2	0.6	1.4	1.3
Intra-assay CV (%) LA-positive control	2.3	4.8	ND	1.5	2.1	1.5
Inter-assay CV (%) NPP	4.0	4.6	ND	2.8	1.9	3.8
Inter-assay CV (%) LA-positive control	2.5	5.0	1.9	4.0	1.3	3.6
ET ratio						
Intra-assay CV (%) NPP	0.5	1.0	4.2	3.5	1.2	0.1
Intra-assay CV (%) LA-positive control	2.5	1.4	2.7	3.5	0.9	0.1
Inter-assay CV (%) NPP	1.5	3.9	ND	3.6	1.1	1.7

Inter-assay CV (%)	2.3	3.6	3.4	3.9	0.9	3.7
LA-positive control						

Abbreviations: CV, coefficient of variation; ET, ecarin time; LA, lupus anticoagulant; ND, not done; NPP, normal pooled plasma; TSVT, Taipan snake venom time

TABLE 3. TSVT/ET results on anticoagulant-spiked normal plasmas

Anticoagulant	TSVT ratio (Cut-off >1.09)	ET ratio (Cut-off >1.09)	% correction (Cut-off >9.3)	NSCR (Cut-off >1.11)

Rivaroxaban	48 ng/mL	0.94	1.02	-	-
	247 ng/mL	0.94	1.04	-	-
	472 ng/mL	0.90	0.83	-	-
Apixaban	90 ng/mL	0.91	0.86	-	-
	227 ng/mL	0.89	0.83	-	-
	452 ng/mL	0.94	0.95	-	-
Edoxaban	26 ng/mL	0.95	1.03	-	-
	51 ng/mL	0.99	1.05	-	-
	107 ng/mL	0.98	1.06	-	-
	255 ng/mL	1.01	1.08	-	-
	496 ng/mL	1.02	**1.12**	-	-
Dabigatran	30 ng/mL	**1.38**	**1.23**	**10.9**	**1.12**
	255 ng/mL	**3.47**	**2.49**	**28.2**	**1.39**
	468 ng/mL	**4.31**	**4.65**	-7.9	0.93
UFH	0.25 IU/mL	**1.15**	0.89	**22.6**	**1.29**
	0.50 IU/mL	**1.56**	0.89	**42.9**	**1.75**
	1.00 IU/mL	**2.00**	0.92	**54.0**	**2.17**
LMWH	0.25 IU/mL	1.05	0.89	-	-
(enoxaparin)	0.50 IU/mL	**1.19**	0.89	**25.2**	**1.34**
	1.00 IU/mL	**1.42**	0.93	**34.5**	**1.53**
LMWH	0.25 IU/mL	1.04	0.95	-	-
(dalteparin)	0.50 IU/mL	**1.22**	0.94	**23.0**	**1.30**
	1.00 IU/mL	**1.48**	0.99	**33.1**	**1.49**
LMWH	0.25 IU/mL	**1.13**	0.96	**15.0**	**1.18**
(tinzaparin)	0.50 IU/mL	**1.36**	0.97	**28.7**	**1.40**
	1.00 IU/mL	**1.63**	1.00	**38.7**	**1.63**
Danaparoid	0.25 IU/mL	1.02	0.97	-	-
	0.50 IU/mL	1.07	1.04	-	-
	1.00 IU/mL	**1.23**	**1.17**	4.9	1.05
Fondaparinux	0.25 mg/L	0.92	0.93	-	-
	0.50 mg/L	0.99	0.94	-	-
	1.00 mg/L	0.97	0.94	-	-

Values above cut-offs are in bold. Abbreviations: ET, ecarin time; LMWH, low molecular weight heparin; NSCR, normalized screen/confirm ratio; TSVT, Taipan snake venom time; UFH, unfractionated heparin

TABLE 4. TSVT/ET results on LA-positive plasmas

Lab	Patient category		LA positive by TSVT/ET		Triple positives		Anticoagulant information	Patients LA-negative by TSVT/ET
1	LA-positive:	not anticoagulated	8/18	(44%)	None	-	-	5 x SLE with persistent LA, 4 x persistent LA, 1 x first test for LA
	APS with persistent LA:	not anticoagulated	36/52	(67%)	33/42	(79%)	-	9 x triple positive APS, 7 x APS with persistent LA
		VKA anticoagulated	20/29	(69%)	12/14	(86%)	INR range 1.4 - 6.3	2 x triple positive APS, 7 x APS with persistent LA
		DFXaI anticoagulated	13/17	(76%)	10/11	(91%)	7 x Riv. (22-531ng/mL) 7 x Apix. (23-555ng/mL) 3 x Edox. (40-45ng/mL)	1 x triple positive APS (Riv.), 3 x APS with persistent LA (1 each of Riv., Apix., Edox.)
2	LA-positive	not anticoagulated	6/11	(55%)	2/3	(67%)	-	1 x ALL, 1 x SLE/triple positive, 1 x previous DVT/PE, 1 x TIAs, 1 x CVA (follow-up sample was LA-negative – transient)
	APS with persistent LA:	not anticoagulated	1/1	(100%)	1/1	(100%)	-	-
		VKA anticoagulated	6/7	(86%)	3/3	(100%)	INR range 2.01 - 3.94	2 x APS with persistent LA
3	LA-positive:	not anticoagulated	5/18	(28%)	1/1	(100%)	-	2 x persistent LA, 11 x isolated LA
	APS with persistent LA:	not anticoagulated	4/7	(57%)	1/3	(33%)	-	2 x triple positive APS, 1 x APS with persistent LA
		VKA anticoagulated	6/7	(86%)	1/1	(100%)	INR range 1.6 – 2.4	1 x APS with persistent LA
		DFXaI anticoagulated	0/1				1 x Riv. (<30ng/mL)	1 x APS with persistent LA (Riv.)
4	LA-positive:	not anticoagulated	16/21	(76%)	13/16	(81%)	-	1 x SLE/triple positive, 1 x SLE, triple positive/dRVVT -ve, 1 x SLE with persistent LA, 1 x triple positive, 1 x first test for LA
	APS with persistent LA:	not anticoagulated	11/16	(69%)	7/8	(88%)	-	5 x APS with persistent LA, 1 x triple positive APS
		VKA anticoagulated	101/111	(91%)	42/43	(98%)	INR range 1.4 – 6.1	1 x triple positive APS, 10 x APS with persistent LA
		DFXaI anticoagulated	27/34	(79%)	11/11	(100%)	26 x Riv. (levels not done) 7 x Apix. (levels not done)	7 x APS with persistent LA (Riv.)

							1 x Edox. (level not done)	
5	LA-positive:	not anticoagulated	1/8	(13%)	None	-	-	2 x DVT, 2 x stroke, 1 x PE, 1 x SLE, 1 x recurrent miscarriage
	APS with persistent LA:	not anticoagulated	23/32	(72%)	1/2	(50%)	-	1 x triple positive APS, 9 x APS with persistent LA
		VKA anticoagulated	9/10	(90%)	2/2	(100%)	INR range 2.0 - 6.2	1 x APS with persistent LA
		DFXaI anticoagulated	11/12	(92%)	None	-	8 x Riv. (79-273ng/mL) 4 x Apix. (90-194ng/mL)	1 x APS with persistent LA (Apix.)
6	LA-positive:	not anticoagulated	0/5	(0%)	None	-	-	5 x persistent LA but asymptomatic
	APS with persistent LA;	not anticoagulated	6/12	(50%)	3/5	(60%)	-	2 x triple positive APS, 2 x APS with persistent LA, 2 x SLE + APS with persistent LA
		VKA anticoagulated	14/16	(88%)	8/8	(100%)	INR range 2.3 – 4.1	2 x APS with persistent LA
		DFXaI anticoagulated	2/7	(29%)	1/1	(100%)	5 x Riv. (224-360ng/mL) 2 x Apix. (131 & 270ng/mL)	4 x APS with persistent LA (Riv.), 1 x APS with persistent LA (Apix.)

Abbreviations: ALL, acute lymphoblastic leukaemia; Apix, apixaban; APS, antiphospholipid syndrome; CVA, cerebrovascular accident; DFXaI, direct factor Xa inhibitor; dRVVT, dilute Russell's viper venom time; DVT, deep vein thrombosis; Edox, edoxaban; ET, ecarin time; FVL, factor V Leiden; INR, international normalised ratio; LA, lupus anticoagulant; Lab, Laboratory; PE, pulmonary embolism; Riv, rivaroxaban; SLE, systemic lupus erythematosus; TSVT, Taipan snake venom time; VKA, vitamin K antagonist

TABLE 5. TSVT/ET results on LA-negative plasmas

Lab	Patient category	LA+ve by TSVT/ET		Anticoagulant information	Patients LA-positive by TSVT/ET
1	LA-negative not anticoagulated	0/149		-	None
	Non-APS, on VKA	15/100	(15%)	INR range 1.5 – 5.0	3 x VTE, 2 x CVST, 2 x DVT, 2 x PE, 2 x recurrent DVTs, 1 x recurrent strokes, 2 x Behçets Disease, 1 x CLL/DVTs
	Non-APS, on DFXaI	5/150	(3%)	50 x Riv. (17 - 519ng/mL) 50 x Apix. (6 - 396ng/mL) 50 x Edox. (2 - 508ng/mL)	1 x PE (Apix. 132 ng/mL), 3 x AF (Apix. 66, 233 & 265 ng/mL), 1 x DVT (Edox. 14ng/mL)
2	LA-negative not anticoagulated	0/22		-	None
	Non-APS, on VKA	1/20	(5%)	INR range 2.0 - 3.9	1 x PE
	Non-APS, on DFXaI	5/20	(25%)	12 x Riv. (129 - 604ng/mL)* 3 x Apix. (124 - 688ng/mL)* 5 x Edox. (61-236ng/mL)*	1 x AF/scleroderma (Riv. level ND), 1 x liver cirrhosis (Riv. level ND), 1 x massive PE (Apix. 119ng/mL), 1 x AF/heterozygous FVL/recurrent thrombosis (Edox. 235ng/mL), 1 x AF/TIAs (Edox. 236ng/mL)
3	LA-negative not anticoagulated	0/29		-	None
	Non-APS, on VKA	0/12		INR range 1.5 – 2.8	None
	Non-APS, on DFXaI	0/2		2 x Riv. (<30ng/mL)	None
4	LA-negative not anticoagulated	2/40	(5%)	-	1 x arterial thrombosis (subsequently LA-positive by dRVVT), 1 x myelitis/raised IgG aCL
	Non-APS, on VKA	2/10	(20%)	INR range 1.8 – 4.0	1 x AF/lymphoma, 1 x recurrent PE
	Non-APS, on DFXaI	5/23	(36%)	19 x Riv. (levels ND) 4 x Apix. (levels ND)	5 x AF (Riv.)
5	LA-negative not anticoagulated	4/48	(8%)	-	1 x DVT, 1 x scleroderma , 1 x PE, 1 x stroke
	Non-APS, on VKA	0/18		INR range 2.0 - 6.2	None
	Non-APS, on DFXaI	0/24		13 x Riv. (108 - 592ng/mL) 11 x Apix. (97 - 526ng/mL)	None
6	LA-negative not anticoagulated	0/51		-	None

Non-APS, on VKA	0/12		INR range 1.5 – 3.8	None
Non-APS, on DFXaI	7/184	(4%)	123 x Riv. (41 - 609ng/mL) 53 x Apix. (21 - 427ng/mL) 8 x Edox. (3 - 310ng/mL)	5 x PE (Riv. 41, 152, 322, 372,ng/mL, Edox. <50ng/mL), 1 x DVT (Riv. 331ng/mL), 1 x CTEPH (Riv. 192ng/mL)

Abbreviations: aCL, anticardiolipin antibodies; AF, atrial fibrillation; Apix, apixaban; APS, antiphospholipid syndrome; CLL, chronic lymphocytic leukaemia; CTEPH, chronic thromboembolic pulmonary hypertension; CVST, cerebral sinovenous thrombosis; DFXaI, direct factor Xa inhibitor; dRVVT, dilute Russell's viper venom time; DVT, deep vein thrombosis; Edox, edoxaban; ET, ecarin time; FVL, factor V Leiden; INR, international normalised ratio; LA, lupus anticoagulant; Lab, Laboratory; PE, pulmonary embolism; ND, not done; Riv, rivaroxaban; TIA, transient ischemic attack; TSVT, Taipan snake venom time; VKA, vitamin K antagonist. *DFXaI levels were not available for all samples, ranges are given for those that were available.

TABLE 6. TSVT/ET results in plasmas containing potential interferences

TSVT/ET results	**Clinical data**	**Laboratory data**
Normal TSVT ratio	9 x on enoxaparin	anti-Xa 0.69 - 0.93 IU/mL
	5 x liver disease	INRs 1.3, 1.5, 1.6, 2.1, 2.3
	2 x DIC	INR 1.7 APTTr 1.8 Fib. 3.4g/L; INR 2.4 APTTr 3.0 Fib. 0.6g/L
	2 x false LA-positive by APTT due to elevated CRP	-
	2 x thrombolysis	Normal INR/APTTr, Fib. 0.8g/L; INR 1.9 APTT 54.2s TT 17.9s
	2 x FX deficient plasma (Stago & Helena)	-
	2 x on dabigatran	1 x Dabigatran <20 ng/mL; 1 x level not done but TT 19.0s
	1 x liver disease/UFH	anti-Xa 0.20 IU/mL, INR 2.0
	1 x atypical HUS (not anticoagulated)	INR 1.8 APTT 54.5s TT 19s
	1 x ischemic gut	INR 5.4 APTT 106.9s TT 19.8s
Elevated TSVT ratio Normal NSCR	19 x on argatroban	Argatroban levels: 339 – 2296 ng/mL (from 14 samples)
	7 x on dabigatran	Dabigatran levels: <20 - 1050 ng/mL (from 6 samples)
	5 x liver disease	INRs 1.3, 1.6, 2.3, 4.4, 5.0
	2 x FII deficient plasma (Stago & Helena)	-
	1 x on enoxaparin	anti-Xa 0.62 IU/mL
	1 x dysfibrinogenemia	Clauss fib. 0.36 g/L
	1 x FII deficiency	FII 19.6 IU/dL

	1 x myeloma	INR 2.5 APTTr 1.8 Fib. 0.34
	1 x liver disease/UFH	anti-Xa 0.27 IU/mL, INR 2.0
Elevated TSVT ratio	28 x on dabigatran	Dabigatran levels: <20 – 343 ng/mL (from 19 samples)
Elevated NSCR	11 x on UFH	anti-Xa 0.33 – 1.40 IU/mL
	4 x on unspecified LMWH	anti-Xa levels: 0.22, 0.53, 0.82, 0.94, IU/mL
	2 x on argatroban	Argatroban levels: 179, 368 ng/mL
	1 x FII deficiency	FII 13.7 IU/dL
	1 x liver failure/unspecified LMWH	INR 2.0 APTT 54.5s TT 70.5s, anti-Xa not done

Abbreviations: APTT, activated partial thromboplastin time; APTTr, activated partial thromboplastin time ratio; CO, cut-off; DIC, disseminated intravascular coagulation; ET, ecarin time; Fib., fibrinogen; FII, factor II; FX, factor X; HUS, haemolytic uraemic syndrome; INR, international normalized ratio; ; ET, ecarin time; FVL, factor V Leiden; INR, international normalised ratio; LA, lupus anticoagulant; LMWH, low molecular weight heparin; TSVT, Taipan snake venom time; TT, thrombin time; UFH, unfractionated heparin. Patients with liver disease were not anticoagulated unless stated. Where APTT and TT are given in seconds, the cut-offs are 38s and 19s respectively.

TABLE 7. TSVT/ET results on LA reference panel plasmas

Laboratory	Reference plasma	Mean TSVT ratio	Mean ET ratio	Mean % correction	Mean NSCR
1	LA negative	1.00	1.00	-0.3	1.00

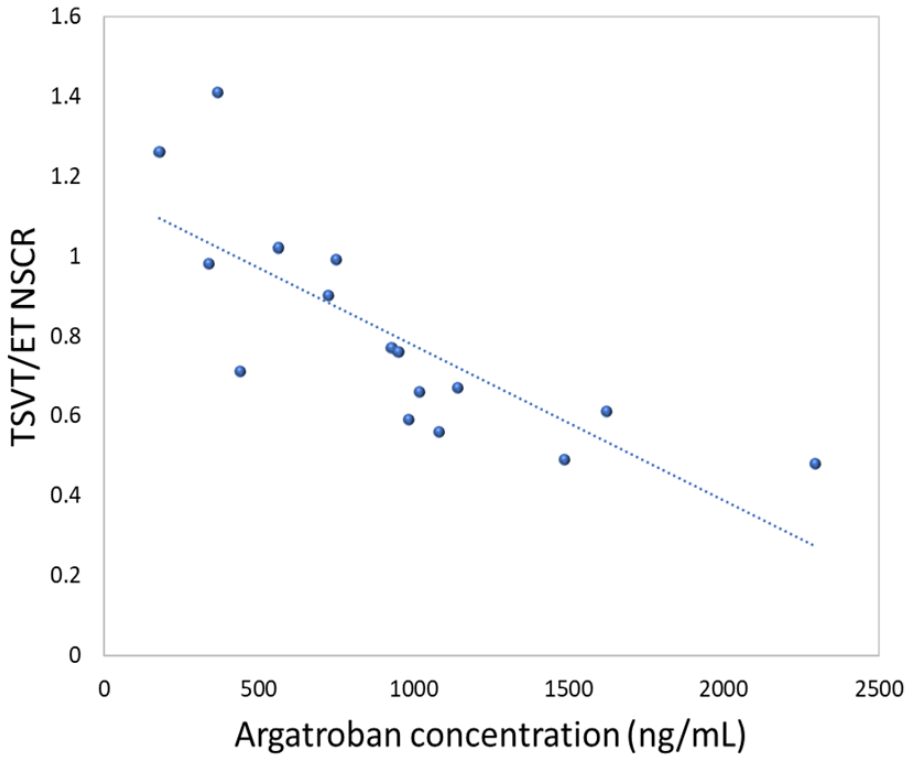
	Moderate LA positive	**1.23**	1.04	**16.0**	**1.19**
	Strong LA positive	**1.31**	1.05	**19.6**	**1.25**
2	LA negative	1.01	1.00	0.65	1.01
	Moderate LA positive	**1.23**	1.05	**14.4**	**1.17**
	Strong LA positive	**1.30**	1.06	**18.4**	**1.23**
4	LA negative	0.94	0.96	-2.1	0.98
	Moderate LA positive	**1.28**	1.00	**21.8**	**1.28**
	Strong LA positive	**1.37**	1.02	**25.3**	**1.34**
5	LA negative	1.03	0.99	3.7	1.04
	Moderate LA positive	**1.20**	1.01	**17.9**	**1.19**
	Strong LA positive	**1.24**	1.00	**19.4**	**1.24**
6	LA negative	1.03	1.02	1.03	1.01
	Moderate LA positive	**1.27**	1.08	**15.1**	**1.18**
	Strong LA positive	**1.44**	1.01	**29.7**	**1.42**

All results given are mean values derived from three results, each from a separate run, except for Laboratory 2, which are derived from two results from separate runs. The plasmas were not analysed at Laboratory 3. Values above cut-offs are in bold. Abbreviations: ET, ecarin time; LA, lupus anticoagulant; NSCR, normalized screen/confirm ratio; TSVT, Taipan snake venom time

Figure legends

FIGURE 1. Argatroban concentration vs TSVT/ET normalized screen/confirm ratio.

FIGURE 2. Comparison of TSVT screen ratio with dRVVT screen ratio and APTT screen ratio in non-anticoagulated LA-positive patients for samples LA-positive by TSVT/ET. (A) TSVT vs dRVVT (B) TSVT vs APTT



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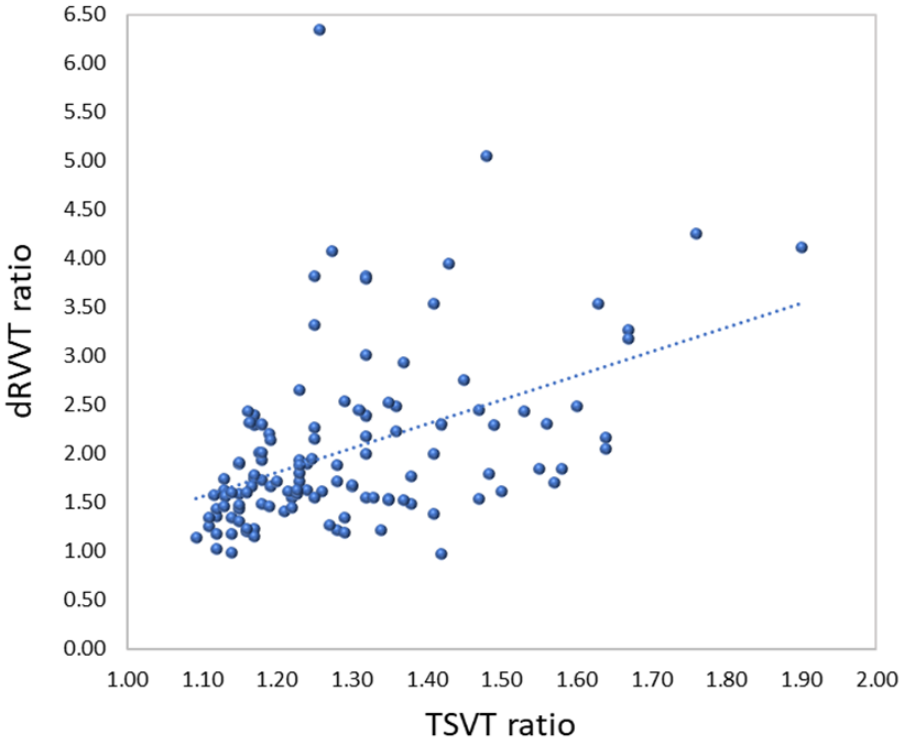

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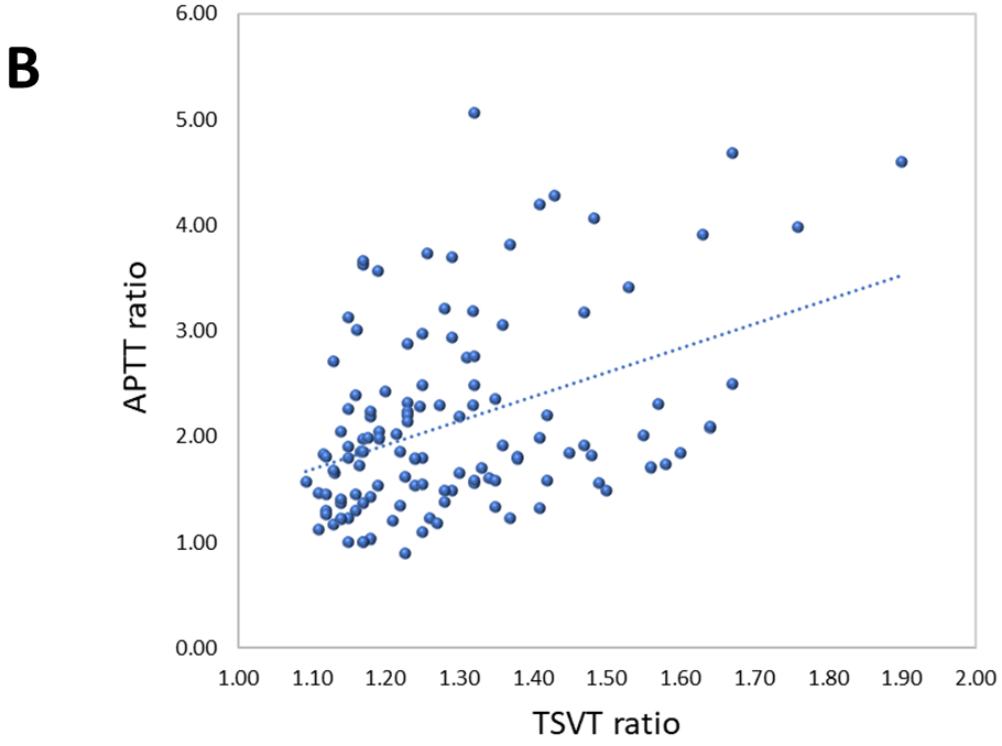

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