Potential usefulness of activated charcoal (DOAC remove®) for dRVVT testing in patients receiving Direct Oral AntiCoagulants

G. Jourdi, M. Delrue, A. Stepanian, J. Valaize, G. Foulon-Pinto, J. Demagny, J. Duchemin, F. Nedelec-Gac, L. Darnige, E. Curis, et al.

Potential usefulness ~~Utility~~ of activated charcoal (DOAC remove®) for dRVVT testing in patients receiving Direct Oral AntiCoagulants

Georges Jourdi[a*], Maxime Delrue[b], Alain Stepanian[b], Jessica Valaize[c], Geoffrey Foulon-Pinto[d], Julien Demagny[e], Jerome Duchemin[f], Fabienne Nedelec-Gac[c], Luc Darnige[g], Emmanuel Curis[h], Xavier Delavenne[i], Pascale Gaussem[g], Virginie Siguret[d+] & Isabelle Gouin-Thibault[j+]

[a] Université de Paris, U1140 Innovative Therapies in Haemostasis, INSERM, F-75006 Paris, France, Service d'Hématologie Biologique, AP-HP, Cochin Hospital, F-75014 Paris, France; [b] University Institute of Hematology, EA 3518, Saint-Louis Hospital, Paris, France, Service d'Hématologie Biologique, AP-HP, Lariboisière Hospital, F-75010 Paris, France; [c] Service d'Hématologie Biologique, CHU Pontchaillou, Rennes, France; [d] Université de Paris, U1140 Innovative Therapies in Haemostasis, INSERM, F-75006 Paris, France, Service d'Hématologie Biologique, AP-HP, Lariboisière Hospital, F-75010 Paris, France; [e] Service d'Hématologie Biologique, AP-HP, Lariboisière Hospital, F-75010 Paris, France; [f] Service d'Hématologie Biologique, AP-HP, Cochin Hospital, F-75014 Paris, France; [g] Université de Paris, U1140 Innovative Therapies in Haemostasis, INSERM, F-75006 Paris, France, Service d'Hématologie Biologique, AP-HP, Georges Pompidou European Hospital, F-75015 Paris, France; [h] Laboratoire de biomathématiques EA 7537, Faculté de Pharmacie, Université de Paris, France, Service de biostatistiques et informatique médicale SBIM, Saint Louis Hospital, AP-HP, Paris, France; [i] University of Lyon, Inserm U1059, Saint-Etienne, France, Laboratory of Pharmacology and Toxicology, CHU Saint-Étienne, Saint-Étienne, France; [j]University of Rennes 1, CIC-Inserm1414, Rennes, France, Service d'Hématologie Biologique, CHU Pontchaillou, Rennes, France.

[+] These authors share equal senior authorship.

*** Corresponding author:**

Dr Georges JOURDI

Service d'Hématologie Biologique, Hôpital Cochin & Inserm U1140 Innovative Therapies in Haemostasis

27 Rue du Faubourg Saint Jacques, F-75014 Paris, France.

Tel. +33 1 58 41 20 05

E-mail: georges.jourdi@aphp.fr

Fax number: +33 1 58 41 19 95

Abstract

Introduction: Lupus Anticoagulant (LA) testing using dilute Russell Viper Venom Time (dRVVT) is challenging in patients receiving Direct Oral AntiCoagulants (DOAC) due to potential false positive results. In a multicenter study, we evaluated the *in vitro* removal of DOAC by activated charcoal (DOAC remove®), allowing reliable dRVVT testing.

Materials and Methods: Patient samples were analyzed before and after treatment with DOAC remove®: 49 apixaban, 48 rivaroxaban, 24 dabigatran and 30 none. DOAC plasma concentrations were measured using anti-Xa or ~~anti-IIa~~ diluted thrombin time assays. In a subset of 28 samples, DOAC concentrations were also measured using HPLC-MS/MS following treatment with DOAC remove®. DRVVT was performed using STA-Staclot dRVVT Screen®/Confirm® (Stago) or LAC-Screening®/Confirm® (Siemens).

Results: Baseline median [min-max] concentrations were 94 [<20-479] for apixaban, 107 [<20-501] for rivaroxaban and 135 ng/mL [<20-792] for dabigatran; dRVVT screen ratio /confirm ~~normalized~~ ratio was positive in 47, 90 and 42 % of apixaban, rivaroxaban and dabigatran samples. Treatment with DOAC remove® did not affect dRVVT results in non-DOAC patients while it resulted in DOAC concentrations < 20 ng/mL in 82, 98 and 100 % of samples, respectively. Concentrations were < 5 ng/mL with HPLC-MS/MS in 5 out of 10, 8 out of 10 and 7 out of 8 samples, respectively. DOAC remove® corrected DOAC interference with dRVVT assays ~~allowed excluding LA~~ in 76, 85 and 95 % of the patients, respectively. ~~without affecting dRVVT results in non-DOAC patients.~~

Conclusion: For dRVVT testing in DOAC patients, we suggest the use of DOAC remove® for every rivaroxaban sample, whereas it might only be used in positive apixaban and dabigatran samples. A residual DOAC interference cannot be ruled out in case of persisting dRVVT positive results after treatment with DOAC remove®. ~~For those with persisting positive results, LA diagnosis using dRVVT remains questionable.~~

Key words

Antiphospholipid syndrome; apixaban; dabigatran; lupus anticoagulant; rivaroxaban

Abbreviations:

APS, antiphospholipid syndrome; DOAC, Direct Oral AntiCoagulants; dRVVT, dilute Russell viper venom time; dTT, diluted thrombin time; F, factor; HPLC-MS/MS, high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry; LA, lupus anticoagulant; LLOQ, lower limit of quantification; LMWH, low molecular weight heparin; PPP, platelet-poor plasma; TE, thromboembolic events; VKA, vitamin K antagonists;

1. Introduction

Direct oral anticoagulants (DOAC), including factor (F) Xa (xabans) and thrombin (dabigatran) inhibitors, are increasingly prescribed for treatment and prevention of arterial and venous thromboembolic events (TE). Although the majority of patients with TE should not be tested for thrombophilia during the anticoagulation period [1], screening assays including lupus anticoagulant (LA) testing may be useful in selected patients while they are still anticoagulated. Indeed, ~~The association of persistent (confirmed 12 weeks after an initial positive result) positive LA and/or anti-cardiolipin and/or anti-β_2 glycoprotein I antibodies (> 99th percentile of normal controls) [2,3] with a venous or arterial thrombosis, and/or obstetrical complications leads to the diagnosis of the antiphospholipid syndrome (APS) in accordance with Sydney criteria [4]. Triple positivity for these markers is associated with a significant higher risk of thrombosis than single or double positivity in APS or asymptomatic patients [5,6]. LA confers a high risk of recurrent TE after stopping anticoagulant therapy in patients with unprovoked TE [1,7]. In case of persistent LA, patients with TE should be on long-term anticoagulant treatment to avoid recurrence. Moreover, in case of triple positivity, they should be switched to vitamin K antagonist (VKA) if they were initially on DOAC.~~ LA results might ~~therefore~~ affect the choice of the anticoagulant drug and the treatment duration [1,2,3]. ~~Hence, LA testing is highly clinically relevant in selected patients [1] and will in all likelihood be requested in patients whilst they are on DOAC.~~ However, rivaroxaban, apixaban and dabigatran may interfere with LA testing ~~these assays~~ [4-6]. Their effect may vary according to the DOAC and the assays [7] making therefore the LA diagnosis challenging ~~the LA diagnosis~~.

~~Recommendation for LA detection is to perform two test systems with different analytical principles to maximize detection rates.~~ Dilute Russell viper venom time (dRVVT) is one of the recommended tests for LA detection [8-10]. ~~It consists on a direct activation of coagulation FX by a protease extracted from the venom of the Russell's viper in the presence~~

~~of calcium ions and phospholipids added at low (screen assay) or high (confirm assay) concentration. These assays are highly sensitive to LA but may be affected by DOAC treatment. Indeed,~~ Previous studies have shown false positive results in dRVVT assays performed in DOAC spiked plasma samples or in *ex vivo* samples drawn from DOAC patients [2,4,11-13] while Favaloro *et al.* have recently shown a potential for false negative dRVVT results in apixaban samples [6]. *In vitro* extraction of DOAC from *ex vivo* anticoagulated patient samples prior to plasma testing could be an option for a reliable LA diagnosis. Many options have already been described such as *in vitro* neutralization of DOAC with specific antidotes [6,14,15], ~~Idarucizumab neutralized dabigatran in *ex vivo* samples and allowed therefore the use of coagulation assays for the diagnosis of hemostasis disorders among which dRVVT [18,19]. However, it might lack availability and it would be a expensive solution. Andexanet-alpha would be likewise proposed for xabans samples, but in addition to the same drawbacks than idarucizumab use, its neutrality regarding coagulation in the absence of xabans is not yet well established [12]. Difficulties of LA diagnosis in DOAC patients could be circumvented by previous~~ *in vitro* drug adsorption using activated charcoal (DOAC remove®, DOAC stop™), ~~(which composition has not been disclosed by the manufacturer)~~ or sample filtration on DOAC filter (Stago) or Hemofilter® (Hemosafe) ~~as described in prior studies~~ [6,16-18]. ~~Usage~~ The potential usefulness of these devices for DOAC neutralization and subsequent LA diagnosis in treated patients is currently under investigation.

LA testing remains therefore ~~nowadays~~ problematic in DOAC patients with no clear guidance about how and when to conduct testing in such patients. ~~Moreover, the effect may vary according to the DOAC and the assays [23].~~ In a multicenter study, we hence sought 1/ to investigate the extent of interference of dabigatran, rivaroxaban, or apixaban with ~~LA testing using~~ dRVVT in plasmas from anticoagulated patients referred to us for LA detection and 2/ whether a neutralizing agent, DOAC remove®, could reduce the ~~number~~ proportion of false

positive results. Finally, based on our study results, we propose a diagnosis algorithm for dRVVT testing in DOAC patients ~~is proposed~~.

2. Materials and methods

2.1 Plasma samples

This non-interventional study was conducted in three university hospitals: Cochin (AP-HP, Paris, France), Lariboisière (AP-HP, Paris, France) and Pontchaillou (Rennes, France). In each center, blood samples ~~were sent to the routine hospital laboratories for LA testing following an episode of arterial or venous TE. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Blood samples were collected from 151 patients: 121 receiving DOAC and 30 patients not receiving DOAC. The latters, known as LA negative or positive, were used as controls. Blood was drawn by venipuncture in the antecubital vein and collected into 0.109 M buffered trisodium citrate (9:1 v/v) tubes (Greiner Bio One, Courtabœuf, France).~~ were collected into 0.109 M buffered trisodium citrate (9:1 v/v) tubes (Greiner Bio One, Courtabœuf, France) and referred to the hospital haematology laboratory for locally LA testing including dRVVT. A double centrifugation at 2500 *g* for 15 minutes at room temperature with plasma decantation in a second tube in between led to platelet-poor plasma (PPP) which was frozen at -80 °C until use. Just prior to experiments, PPP was thawed at 37 °C then processed within 2 hours. Overall, 154 samples were analyzed in the present study: 121 from patients receiving DOAC, 30 from patients not receiving DOAC known as dRVVT negative or positive, which were used as controls and 3 from patients with a known positive dRVVT for years receiving DOAC. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

2.2 DRVVT assays

Two different reagents were used: STA-Staclot dRVVT Screen® and Confirm® (Stago, Asnières-sur-Seine, France) in one center, and LAC Screening® and Confirmation® (Siemens Diagnostics, Saint-Denis, France) in two centers. They consist in a direct activation of coagulation FX by a protease extracted from the venom of the Russell's viper in the presence of calcium ions and phospholipids added at low (screen assay) or high (confirm assay) concentration. Pool Norm Plasma (Stago) or CryoCheck® (Cryopep, Montpellier, France) were used as reference plasma and run in each series~~, allowing the calculation of dRVVT screen, confirm and normalized ratios~~. Both are integrated tests with similar performances having screen/confirm results in the same order of magnitude in external quality control reports. Forty-eight samples were analyzed using LAC Screening® and Confirmation® and Pool Norm Plasma on STAR-Evolution analyzer (Stago), 44 using LAC Screening® and Confirmation® and CryoCheck® on CS-5100 (Siemens) and 29 using STA-Staclot dRVVT Screen® and Confirm® and Pool Norm Plasma on STAR-Evolution (Stago) . ~~Screen and confirm assays results were expressed as ratios of patient and reference plasma clotting times. Final results were expressed as a normalized ratio (*i.e.* screen ratio/confirm ratio).~~ Patient screen and confirm results were normalized *i.e* expressed as ratios against reference plasma results. Cut-off value was 1.20 for both screen ratio and screen ratio/confirm ~~normalized~~ ratio with both reagents as stated by the manufacturers and locally validated [8,9].

2.3 Direct oral anticoagulant measurement

Xabans and dabigatran concentrations were measured in plasma before and after treatment with DOAC remove® (see below) using specific anti-Xa (STA-Liquid anti-Xa) or ~~anti-IIa~~ diluted thrombin time (dTT) assays (Hemoclot Thrombin Inhibitor, Hyphen Biomed, Neuville-sur-Oise, France), respectively. The lower limit of quantification (LLOQ) was locally determined and was equal to 20 ng/mL for both assays in each center. In order to precisely measure the concentration below 20 ng/mL following treatment with activated charcoal, drug concentrations ~~of 28 arbitrarily chosen samples~~ were additionally measured in

a subset of samples (see *infra*) using a validated high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-MS/MS). The LLOQ of HPLC-MS/MS was 5 ng/mL [20].

2.4 Treatment with activated charcoal

Treatment with DOAC remove® (5-Diagnostics, Heuberg, Switzerland) was performed according to the manufacturer instructions. Briefly, one tablet of activated charcoal was added to one milliliter of plasma sample. Sample was gently mixed for 10 minutes at room temperature, then centrifuged for 2 minutes at 2500 *g*, 20 °C. Supernatant was thereafter spun for 1 minute at 2500 *g*, 20 °C to remove any residual activated charcoal particulate and supernatant plasma was tested again using dRVVT and specific anti-Xa or ~~anti-IIa~~ dTT ~~specific~~ assay.

2.5 Study design

dRVVT screen was performed in all patient samples. No additional assay was performed in samples tested negative while those tested positive (*i.e.* with LA screen ratio ≥ 1.20) were subsequently analyzed using confirm assay, and in parallel, were treated with DOAC remove® as previously described. Screen assays were repeated on charcoal treated samples along with the confirm assays if screen ratio remained above the cut-off value (≥ 1.20). The same samples were used for all steps of pre- and post-DOAC remove® treatment testing.

2.6 Statistical analysis

DOAC plasma concentrations ~~are~~ were expressed as median [min - max]. A paired t-test was used to compare anticoagulant concentrations, ~~as well as~~ screen and confirm ratios as well as ~~and~~ screen ratio/confirm ~~normalized~~ ratio before and after treatment with DOAC remove®. A p-value < 0.05 was considered statistically significant. All statistical analysis and graph representation were computed using the GraphPad Prizm 3.0 software.

3. Results

3.1 DOAC remove® does not interfere with dRVVT in the absence of DOAC

Thirty samples from non-DOAC treated patients were tested before and after treatment with activated charcoal. Before treatment with DOAC remove®, 10 were LA negative (*i.e.* screen ratio < 1.20), 10 were low LA positive with a screen ratio/confirm ratio between 1.20 and 1.50, and 10 were high LA positive with a screen ratio/confirm ratio above 1.50. DOAC remove® did not affect dRVVT results in either negative ($p = 0.8$), low ($p = 0.4$) or high ($p = 0.2$) positive LA samples (Figure 1) confirming therefore the neutrality of activated charcoal with regard to dRVVT screen and confirm assays in the absence of DOAC. Moreover, we also checked three additional rivaroxaban patients with a known positive LA for years before starting DOAC treatment. They still had LA positive results after charcoal treatment.

3.~~1~~2 Impact of DOAC on ~~LA testing using~~ dRVVT assays

dRVVT screen was performed in plasma samples from 121 DOAC patients referred for LA testing. Forty-nine patients received apixaban, 48 rivaroxaban and 24 dabigatran. DOAC plasma concentrations ranged from < 20 to 479, < 20 to 501 and < 20 to 792 ng/mL, respectively (Table ~~1~~). Screen ratio increased in a concentration-dependent manner in the presence of DOAC. Rivaroxaban and dabigatran appeared to have a more pronounced effect on the dRVVT screen than apixaban (Figure ~~1~~2). Screen ratio was positive (i.e. ≥ 1.20) in 80 % of apixaban, 98 % of rivaroxaban and 100 % of dabigatran samples (Figure 3~~Table 2~~). Samples with positive screen results were further tested using confirm assay: confirm ratio was increased to a less extent compared to screen ratio with all the three DOAC ($p \leq 0.005$; Figure ~~1~~2). Screen ratio /confirm ~~Normalized~~ ratio was positive in 47 % of apixaban, 90 % of rivaroxaban and 42 % of dabigatran samples (Figure 3).

3.~~2~~3 Effect of DOAC remove® on DOAC plasma concentrations

Activated charcoal significantly reduced the plasma concentration of apixaban ($p < 0.0001$), rivaroxaban ($p < 0.0001$) and dabigatran ($p = 0.0001$). Overall, 82 % of apixaban, 98 % of rivaroxaban and 100 % of dabigatran samples had DOAC plasma concentrations below the

LLOQ as assessed by specific anti-Xa or ~~anti-IIa~~ dTT assays (i.e. < 20 ng/mL; Table ~~1~~, Supplementary Figure) following treatment with DOAC remove®.

We wondered whether DOAC remove® completely adsorbed anticoagulant drugs from plasma samples or it only decreased their plasma concentrations below the LLOQ ~~of specific anti-Xa and anti-IIa assays (20 ng/mL)~~. Therefore, in a randomly selected subset of 28 samples (apixaban with median [min - max] initial concentrations of 144 [49 - 370] (n=10), rivaroxaban 140 [42 - 501] (n=10) and dabigatran 74 ng/mL [51-196]) (n=8), DOAC plasma concentrations were additionally measured with HPLC-MS/MS following treatment with DOAC remove®. Nine out of 10 rivaroxaban samples and all dabigatran samples had residual DOAC concentration < 20 ng/mL as assessed by specific anti-Xa or dTT ~~anti-IIa~~ assays, respectively; HPLC-MS/MS revealed residual DOAC concentration < LLOQ (*i.e.* 5 ng/mL) in 8 out of 10 rivaroxaban samples and 7 out of 8 dabigatran samples. Results were different for apixaban samples. While 9 out of 10 samples had residual concentration < 20 ng/mL (specific anti-Xa assay), only 5 out of 10 samples had a plasma concentration below 5 ng/mL with HPLC-MS/MS. Results are detailed in the supplementary Table. No association was observed between the original DOAC concentration and the adsorption efficacy of DOAC remove®.

3.~~3~~4 Effect of DOAC remove® on ~~LA testing using~~ dRVVT in DOAC samples

Following treatment with activated charcoal, drug interference with dRVVT screen was corrected ~~LA presence could be excluded~~ in 61, 69 and 67 % of apixaban, rivaroxaban and dabigatran samples ~~using dRVVT screen~~ (Figure ~~2~~3~~; Table 2~~). Following dRVVT confirm, ~~LA diagnosis was overall excluded~~ DOAC interference was corrected in 76, 85 and 95 % of patients, respectively (Figure ~~2~~3~~; Table 2~~). Consequently, ~~LA diagnosis~~ DOAC interference with dRVVT could not be ruled out ~~remained questionable~~ in 24, 15 and 5 % of apixaban (n = 49), rivaroxaban (n = 48) and dabigatran (n = 24) patients. Comparable results were obtained independently of the reagent/analyzer system used. ~~Importantly we also tested three additional~~

rivaroxaban patients with a known positive LA for years before starting DOAC treatment. They still had LA positive results after charcoal treatment.

3.4 Absence of DOAC remove® effect on dRVVT in the absence of DOAC

Thirty samples from non-DOAC treated patients were tested before and after treatment with activated charcoal. Before treatment with DOAC remove®, 10 had a screen ratio < 1.20 thus were LA negative, 10 were low LA positive with a normalized ratio between 1.20 and 1.50 and 10 were high LA positive with a normalized ratio above 1.50. DOAC remove® did not affect dRVVT results in either negative (p = 0.8), low (p = 0.4) or high (p = 0.2) positive LA samples (Figure 3) confirming therefore its neutrality with regard to dRVVT screen (n = 30) and confirm assays (n = 20) in the absence of DOAC.

4. Discussion

This real-life multicenter study provided evidence on the extent of interference of DOAC at different concentrations with LA testing using dRVVT in plasmas of anticoagulated patients and showed that DOAC remove® allowed reducing this interference from around 50 % for apixaban and dabigatran and 90% for rivaroxaban samples before DOAC remove® to 24, 5 and 15% after DOAC remove®, respectively. We confirmed that false positive results of dRVVT screen assays were obtained with the vast majority of samples drawn from rivaroxaban, apixaban or dabigatran patients. While normalized ratio following the confirm assay allowed LA exclusion in around 50 % of apixaban and dabigatran samples, 90 % of rivaroxaban samples remained positive in the absence of charcoal treatment. The highest DOAC concentrations tested in our study were 479, 792 ng/mL and 501 ng/mL, respectively. DOAC remove® allowed drug adsorption, resulting in plasma concentration below 20 ng/mL in almost 100 % of 51 rivaroxaban and 24 dabigatran samples. Concentrations were even below 5 ng/mL in at least 80 % of the 18 samples assessed with HPLC-MS/MS. It was less effective for the 49 apixaban samples, 82 % of which got below 20 ng/mL and only 50 % of

the 10 samples analyzed with HPLC-MS/MS were below 5 ng/mL. *In vitro* sample treatment with DOAC remove® allowed LA exclusion in 76, 85 and 95 % of apixaban, rivaroxaban and dabigatran samples with no effect on dRVVT results in the absence of DOAC.

While clear recommendations have been published for patients receiving heparin derivatives or VKA, there are still no guidelines regarding LA testing in patients taking DOAC. Indeed, sampling of patients just before low molecular weight heparin (LMWH) injection combined with the use of dRVVT reagents containing heparin neutralizer agent contribute to limit anticoagulant interference [3,10]. In patients receiving VKA, mixing patient plasma to a reference plasma in a 1:1 proportion is recommended when INR (international normalized ratio) is comprised between 1.5 and 3.0 to overcome the reduced functional factors effect even though the sensitivity to LA is reduced [3,10]. For samples from DOAC patients,

International experts suggest that dRVVT in samples from DOAC patients should be performed just before the next intake of the drug since it would less likely lead to false positive dRVVT results [21]. Nevertheless, DOAC interference cannot be entirely excluded, since it has been shown that false positive results may be observed in samples containing DOAC even at concentrations below the trough levels (i.e. DOAC plasma level just before the next intake of the drug) [22] or even at a level below the LLOQ of specific anti-Xa and dTT anti-IIa assays widely used in clinical laboratories may induce false positive results i.e around 20 ng/mL [7,23,24]. Owing to the high inter-individual variability of DOAC plasma level, a temporary interruption of DOAC treatment for 24 to 48 h or even longer could be required to ensure undetectable DOAC concentrations thus preventing false positive results [4,20,25-27]. However, this might be clinically unacceptable: patients with potential antiphospholipid syndrome are at high risk of thrombotic events on one hand, and in the day-to-day practice they may choose not to stop or forget to stop anticoagulant treatment on the other hand [28]. Our results from real-life DOAC patients confirmed that dabigatran as well as both xabans are likely to induce false positive dRVVT results even at low plasma concentrations. Remarkably,

~~rivaroxaban increased dRVVT ratios in a more potent manner compared to apixaban. Rates of FXa neutralization, 4-fold higher with rivaroxaban than apixaban, could explain the lower sensitivity of coagulation assays to the latter as it was previously proved with prothrombin time and thrombin generation assay [32,33].~~

A temporarily switch from DOAC to low molecular weight heparin (LMWH) might be suggested ~~for a reliable LA diagnosis using~~to avoid DOAC interference with dRVVT: nevertheless, it might be challenging to manage, all the more since a repeat of the assays is required in 12 weeks-time following a first positive dRVVT in order to establish the diagnosis of anti-phospholipid syndrome [29]. Some studies also recommend Taipan snake venom time, ecarin and textarin clotting times in DOAC patients, however these tests are not still widely available nor standardized in clinical practice [5,30,31].

Based on our results, we propose a diagnosis algorithm for LA testing using dRVVT in DOAC patients (Figure 4). Since at least 90 % of the screen ratio/confirm ~~normalized~~ ratio were positive before treatment with activated charcoal, rivaroxaban samples should be readily treated with DOAC remove® before assay performance. If dRVVT screen ratio is ≥ 1.20, dRVVT confirm should be performed and screen ratio/confirm ~~normalized~~ ratio calculated. In our study, this strategy resulted in negative dRVVT results in 85 % of rivaroxaban samples. As sample treatment with DOAC remove® requires manual input and is time consuming, dRVVT could be performed before treating apixaban and dabigatran samples with DOAC remove® as it resulted in negative dRVVT ~~allowed LA exclusion~~ in around 50 % of the cases, irrespectively of the initial DOAC plasma concentration. Remaining samples tested positive could afterwards be treated with DOAC remove® and dRVVT assays repeated. This algorithm ~~would~~ prevented the DOAC interference with dRVVT ~~allow LA exclusion using dRVVT~~ in 76 and 95 % of apixaban and dabigatran samples in our study. Of note, it remains at the discretion of the clinical ~~biologist~~ pathologist to readily treat apixaban and dabigatran samples as for rivaroxaban before any dRVVT testing. Whenever screen ratio/confirm ~~normalized~~

ratio is ≥ 1.20 after treatment with DOAC remove®, ~~LA diagnosis remains questionable~~ since a residual DOAC interference cannot be ruled out, and a switch from DOAC to LMWH would be mandatory for a reliable LA diagnosis using dRVVT.

Our study has some limitations. First, we solely evaluated the sensitivity of two commercially available dRVVT reagents ~~sensitivity~~ to DOAC before and after treatment with DOAC remove®. Therefore caution should be made on the generalization of our results to other dRVVT reagents without local validation of the process. It is to mention that the cut-off of 1.20 cannot be generalized and should be validated in each laboratory. Moreover, ~~Yet~~ LA diagnosis requires the association of a sensitive aPTT-based assay with dRVVT. DOAC interference with the former depends on the reagent used, therefore on phospholipid type and concentration. DOAC interference with dRVVT is less reagent-dependent than with aPTT: this is the reason why we focused on evaluating dRVVT sensitivity to DOAC molecules and DOAC remove® in this study [13,32]. ~~It is to mention that the cut-off of 1.20 cannot be generalized over the laboratories and should be likely validated in every laboratory.~~ Second, the potential interference of DOAC concentrations comprised between 5 and 20 ng/mL on dRVVT results after DOAC remove® ~~evaluation of DOAC concentration by HPLC-MS/MS in samples treated with DOAC remove®~~ treatment requires further confirmation ~~due~~ since only a limited number of samples had DOAC concentration measured using HPLC-MS/MS~~to the relative low number of tested samples~~: nevertheless, apixaban seems less susceptible to adsorption by activated charcoal compared to rivaroxaban and dabigatran. A potentially longer adsorption time might be required for a total neutralization of apixaban compared to other DOAC as recently suggested with DOAC-Stop™ [16]. Third, fewer data were obtained with dabigatran in comparison to xabans, therefore limiting conclusions regarding this DOAC, although the observed effects are consistent with previously reported trends [33]. Fourth, no mixing studies were performed with dRVVT screen and confirm since no

correction of dRVVT in the presence of active DOAC molecules in mixed samples is expected [13].

In conclusion, it~~It~~ is mandatory to interpret dRVVT results in DOAC patients with great caution in order to prevent false positive diagnosis and subsequent clinical consequences. DOAC remove® is a valuable tool that limits DOAC interference with this key assay as it was the case in ~~allowing LA exclusion in~~ 76, 85 and 95 % of apixaban, rivaroxaban and dabigatran samples included in our study ~~that presented 479, 501 and 792 ng/mL as the highest plasma concentrations, respectively~~. Nevertheless, complete adsorption of DOAC molecules did not occur in all samples, thus a residual DOAC interference cannot be ruled out ~~LA diagnosis using dRVVT remains questionable in DOAC samples with~~ in case of persisting dRVVT positive results after treatment with DOAC remove®.

Conflict of interest

I.G.-T. P.G. and V.S. received honoraria for participating in expert meetings on apixaban (Bristol-Myers Squibb/Pfizer), rivaroxaban (Bayer Healthcare AG) and dabigatran (Boehringer Ingelheim). The other authors declare no conflicts of interest.

Acknowledgements

We thank L. Mine, J. Demagny, A. Borgel and J. Gay for their excellent technical assistance.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] J.M.Connors, Thrombophilia testing and venous thrombosis, N. Engl. J. Med. 377 (2017) 1177-1787. DOI: 10.1056/NEJMra1700365

[2] G. Ruiz-Irastorza, M.J. Cuadrado, I. Ruiz-Arruza, R. Brey, M. Crowther, R. Derksen, D. Erkan, S. Krilis, S. Machin, V. Pengo, S. Pierangeli, M. Tektonidou, M. Khamashta, Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: report of a task force at the 13th International Congress on antiphospholipid antibodies, Lupus 20 (2011) 206-218. DOI: 10.1177/0961203310395803

[3] V. Pengo, G. Denas, G. Zoppellaro, S.P. Jose, A. Hoxha, A. Ruffatti, L. Andreoli, A. Tincani, C. Cenci, D. Prisco, T. Fierro, P. Gresele, A. Cafolla, V. De Micheli, A. Ghirarduzzi, A. Tosetto, A. Falanga, I. Martinelli, S. Testa, D. Barcellona, M. Gerosa, A. Banzato, Rivaroxaban vs warfarin in high-risk patients with antiphospholipid syndrome, Blood 132 (2018) 1365-1371. DOI: 10.1182/blood-2018-04-848333

[4] A. Hoxha, A. Banzato, A. Ruffatti, V. Pengo, Detection of lupus anticoagulant in the era of direct oral anticoagulants, Autoimmun. Rev. 16 (2017) 173-178. DOI: 10.1016/j.autrev.2016.12.010

[5] M.E. Martinuzzo, L.H. Barrera, M.A. D'Adamo, J.C. Otaso, M.I. Gimenez, J. Oyhamburu, Frequent False-positive results of lupus anticoagulant tests in plasmas of patients receiving the new oral anticoagulants and enoxaparin, Int. J. Lab. Hematol. 36 (2014) 144-150. DOI: 10.1111/ijlh.12138

[6] E. Favaloro, G. Gilmore, S. Arunachalam, S. Mohammed, R. Baker, Neutralising rivaroxaban induced interference in laboratory testing for lupus anticoagulant (LA): A comparative study using DOAC Stop and andexanet alfa, Thromb. Res. 180 (2019) 10-19. DOI: 10.1016/j.thromres.2019.05.013

[7] T. Flieder, M. Weiser, T. Eller, M. Dittrich, K. von Bargen, S. Alban, J. Kuhn, C. Knabbe, I. Birschmann, Interference of DOACs in different DRVVT assays for diagnosis of lupus anticoagulants, Thromb. Res. 165 (2018) 101-106. DOI: 10.1016/j.thromres.2018.03.009

[8] V. Pengo, A. Tripodi, G. Reber, J.H. Rand,T.L. Ortel, M. Galli, P.G. De Groot, Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. Update of the guidelines for lupus anticoagulant detection. Subcommittee on lupus anticoagulant/ antiphospholipid antibody of the scientific and standardisation committee of the international society on thrombosis and haemostasis, J. Thromb. Haemost. 7 (2009) 1737-1740. DOI: 10.1111/j.1538-7836.2009.03555.x

[9] D. Keeling, I. Mackie, G.W. Moore, I.A. Greer, M. Greaves, Guidelines on the investigation and management of antiphospholipid syndrome, Br. J. Haematol. 157 (2012) 47-58. DOI: 10.1111/j.1365-2141.2012.09037.x

[10] P.A. Wayne, Clinical & Laboratory Standards Institute, Laboratory Testing for the Lupus Anticoagulant, CLSI document H60-A (2014).

[11] J.R. Genzen, J.L. Miller, Presence of direct thrombin inhibitors can affect the results and interpretation of lupus anticoagulant testing, Am. J. Clin. Pathol. 124 (2005) 586-593. DOI: 10.1309/7RYUN5DRK4WCH3PM

[12] A. Hillarp, K.M. Gustafsson, L. Faxalv, K. Strandberg, F. Baghaei, I. Fagerberg Blixter, M. Berndtsson, T.L. Lindahl, Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays, J. Thromb. Haemost. 12 (2014)1545-1553. DOI: 10.1111/jth.12649

[13] E. Merriman, Z. Kaplan, J. Butler, E. Malan, E. Gan, H. Tran, Rivaroxaban and false positive lupus anticoagulant testing, Thromb. Haemost. 105 (2011) 385-386. DOI: 10.1160/TH10-08-0511

[14] M. Jacquemin, J. Toelen, J. Schoeters, I. van Horenbeeck, I. Vanlinthout, M. Debasse, M. Peetermans, T. Vanassche, K. Peerlinck, J. van Ryn, P. Verhamme, The addition of idarucizumab to plasma samples containing dabigatran allows the use of routine coagulation aassays for the diagnosis of hemostasis disorders, J. Thromb. Haemost. 13 (2015) 2087-2092. DOI: 10.1111/jth.13138

[15] M. Jacquemin, J. Toelen, L. Feyen, J. Schoeters, I. Van Horenbeeck, I. Vanlinthout, M. Debasse, T. Vanassche, K. Peerlinck, P. Verhamme, The adsorption of dabigatran is as efficient as addition of Idarucizumab to neutralize the drug in routine coagulation assays, Int. J. Lab. Hematol. 40 (2018) 442-447. DOI: 10.1111/ijlh.12807

[16] T. Exner, N. Michalopoulos, J. Pearce, R. Xavier, M. Ahuja, Simple method for removing DOACs from plasma samples, Thromb. Res. 163 (2018) 117-122. DOI: 10.1016/j.thromres.2018.01.047

[17] J. Favresse, B. Lardinois, L. Sabor, B. Devalet, J. Vandepapeliere, M. Braibant, S. Lessire, B. Chatelain, H. Jacqmin, J. Douxfils, F. Mullier, Evaluation of the DOAC Stop procedure to overcome the Effect of DOACs on Several thrombophilia screening tests, Thromb. Haemost. Open. 2 (2018) e202-e209. DOI: 10.1055/s-0038-1657785

[18] C. Bouvy, J. Evrard, R. Siriez, F. Mullier, J. Douxfils, D. Gheldof, Removal of DOACs from plasma: performance comparison and pre-analytical considerations of three different devices, 2018 ECTH meeting; P220.

[20] I. Gouin-Thibault, X. Delavenne, A. Blanchard, V. Siguret, J.E. Salem, C. Narjoz, P. Gaussem, P. Beaune, C. Funck-Brentano, M. Azizi, P. Mismetti, M.A. Loriot, Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin, J. Thromb. Haemost. 15 (2017) 273-283. DOI: 10.1111/jth.13577

[21] K.M.J. Devreese, T.L. Ortel, V. Pengo, B. De Laat, for the subcommittee on lupus anticoagulant/antiphospholipid antibodies, Laboratory criteria for antiphospholipid

syndrome: communication from the SSC of the ISTH, J. Thromb. Haemost. 16 (2018) 809-813. DOI: 10.1111/jth.13976

[22] R.C. Gosselin, D.M. Adcock, J. Douxfils, An update of laboratory assessment of direct oral anticoagulants (DOACs), Int. J. Lab. Hematol. 41 (2019) 33-39. DOI: 10.1111/ijlh.12992

[23] R. Bonar, E.J. Favaloro, S. Mohammed, M. Ahuja, L. Pasalic, J. Sioufi, K. Marsden, The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples, Pathology 48 (2016) 60-71. DOI: 10.1016/j.pathol.2015.11.025

[24] F. Ratzinger, M. Lang, S. Belik, P. Jilma-Stohlawetz, K.G. Schmetterer, H. Haslacher, T. Perkmann, P. Quehenberger, Lupus-anticoagulant testing at NOAC trough levels, Thromb. Haemost. 116 (2016) 116: 235-240. DOI: 10.1160/TH16-02-0081

[25] A. Godier, A.S. Dincq, A.C. Martin, A. Radu, I. Leblanc, M. Antona, M. Vasse, J.L. Golmard, F. Mullier, I. Gouin-Thibault, Predictors of pre-procedural concentrations of direct oral anticoagulants: a prospective multicentre study, Eur. Heart. J. 38 (2017) 2431-2439. DOI: 10.1093/eurheartj/ehx403

[26] S. Testa, A. Tripodi, C. Legnani, V. Pengo, R. Abbate, C. Dellanoce, P. Carraro, L. Salomone, R. Paniccia, O. Paoletti, D. Poli, G. Palareti, START-Laboratory Register, Plasma levels of direct oral anticoagulants in real life patients with atrial fibrillations: results observed in four anticoagulation clinics, Thromb. Res. 137 (2016) 178-183.

[27] A. Antovic, E.M. Norberg, M. Berndtsson, A. Rasmuson, R.E. Malmström, M. Skeppholm, J. Antovic, Effects of direct oral anticoagulants on lupus anticoagulant assays in a real-life setting, Thromb. Haemost. 117 (2017) 1700-1704. DOI: 10.1016/j.thromres.2015.12.001

[28] A.J. Goodwin, D.M. Adcock, Thrombophilia testing and venous thrombosis, N. Engl. J. Med. 377 (2017) 2297-2298. DOI: 10.1056/NEJMc1713797

[29] S. Miyakis, M.D. Lockshin, T. Atsumi, D.W. Branch, R.L. Brey, R. Cervera, R.H. Derksen, P.G. DE Groot, T. Koike, P.L. Meroni, G. Reber, Y. Shoenfeld, A. Tincani, P.G. Vlachoyiannopoulos, S.A. Krilis, International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. 4 (2006) 295-306. DOI: 10.1111/j.1538-7836.2006.01753.x

[30] G.M. Van Os, B. De Laat, P.W. Kamphuisen, J.C.M. Meijers, P.H.G. De Groot, Detection of lupus anticoagulant in the presence of rivaroxaban using Taipan snake venom time, J. Thromb. Haemost. 9 (2011) 1657-1659. DOI: 10.1111/j.1538-7836.2011.04395.x

[31] C. Pouplard, C. Vayne, C. Berthomet, E.A. Guery, B. Delahousse, Y. Gruel, The taipan snake venom time can be used to detect lupus anticoagulant in patients treated by rivaroxaban, Int. J. Lab. Hem. 39 (2016) e60-e63. DOI: 10.1111/ijlh.12611

[32] W.M. Halbmayer, G. Weigel, P. Quehenberger, J. Tomasits, A.C. Haushofer, G. Aspoeck, L. Loacker, M. Schnapka-Koepf, G. Goebel, A. Griesmacher, Interference of the new oral anticoagulant dabigatran with frequently used coagulation tests, Clin. Chem. Lab. Med. 50 (2012) 1601-1605. DOI: 10.1515/cclm-2011-0888

[33] R. Bonar, E.J. Favaloro, S. Mohammed, L. Pasalic, J. Sioufi, K. Marsden, The effect of dabigatran on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples, Pathology 47 (2015) 355-364. DOI: 10.1097/PAT.0000000000000252

Figure captions

Figure 1: Effect of DOAC remove® (activated charcoal) on dRVVT in the absence of DOAC. Non-DOAC patients' samples were tested using dRVVT screen and confirm before (open symbols) and after (closed symbols) treatment with DOAC remove®. Dashed line corresponds to the cut-off value of 1.20. LA positive + samples correspond to those having a screen ratio/confirm ratio ranged between 1.20 and 1.50 while LA positive ++ samples correspond to those with a screen ratio/confirm ratio > 1.50. No significant effect of DOAC remove® on either screen or confirm assays was observed.

Figure ~~1~~2: Impact of DOAC on dRVVT ratios. Samples from DOAC patients were tested using dRVVT screen and confirm. Screen and confirm ~~and normalized~~ ratios are plotted as a function of apixaban (A), rivaroxaban (B) or dabigatran (C) plasma concentration. Dashed line corresponds to the cut-off value of 1.20.

Figure ~~2~~3: Effect of DOAC remove® (activated charcoal) on LA testing using dRVVT in DOAC patients' samples. dRVVT screen and confirm ~~and normalized~~ ratios of plasma samples from DOAC patients were calculated before (open symbols) and after (closed symbols) treatment with DOAC remove®. ~~SR CR and NR correspond to screen confirm and normalized ratio, respectively.~~ Dashed line corresponds to the cut-off value of 1.20. * 3 dabigatran samples could not be tested with confirm assay following treatment with DOAC remove® due to insufficient sample volume.

Figure 4: Proposed algorithm for dRVVT testing in DOAC patients' samples. ~~SR and NR~~SR/CR corresponds to ~~screen and normalized~~ screen ratio/confirm ratio~~, respectively~~.

Tables

Table ~~1~~: DOAC plasma concentrations before and after treatment with DOAC remove®.

	before DOAC remove® Median concentration* [min-max] (ng/mL)	**after DOAC remove®** Median concentration* [min-max] (ng/mL)	**% of total neutralization (DOAC < LLOQ)**
apixaban (n = 49)	94 [<20-479]	< 20 [< 20-85]	82 %
rivaroxaban (n = 48)	107 [<20-501]	< 20 [< 20-45]	98 %
dabigatran (n = 24)	135 [<20-792]	< 20	100 %

*****DOAC concentrations measured using specific anti-Xa or dTT ~~anti-IIa~~ assays. LLOQ: lower limit of quantification (20 ng/mL).

Highlights

- DOAC induce false positive dRVVT results in a substantial proportion of patients.
- [DOAC] < 20 ng/mL after charcoal treatment may still interfere with dRVVT.
- After DOAC remove®, dRVVT was negative in 76 to 95% of samples upon DOAC drug.
- A diagnosis algorithm for dRVVT in DOAC samples including DOAC remove® is proposed.

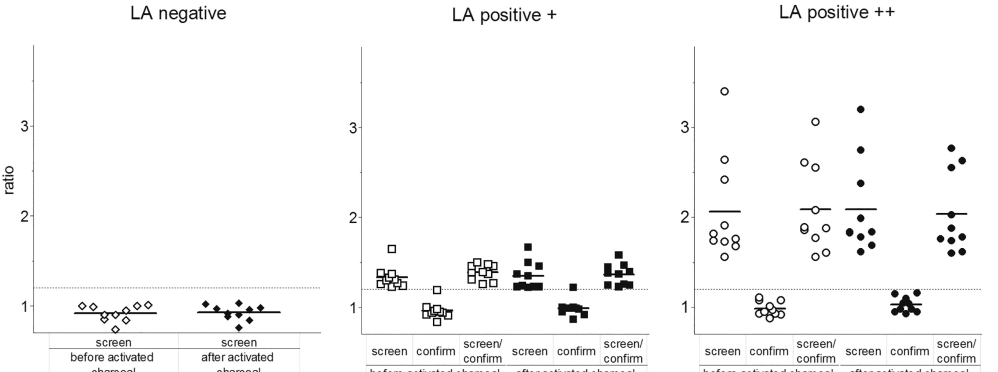

LA negative
LA positive +
LA positive ++
ratio
3
2
1
screen
before activated charcoal
screen
after activated charcoal
screen
confirm
screen/ confirm
screen
confirm
screen/ confirm
before activated charcoal
after activated charcoal

Figure 1

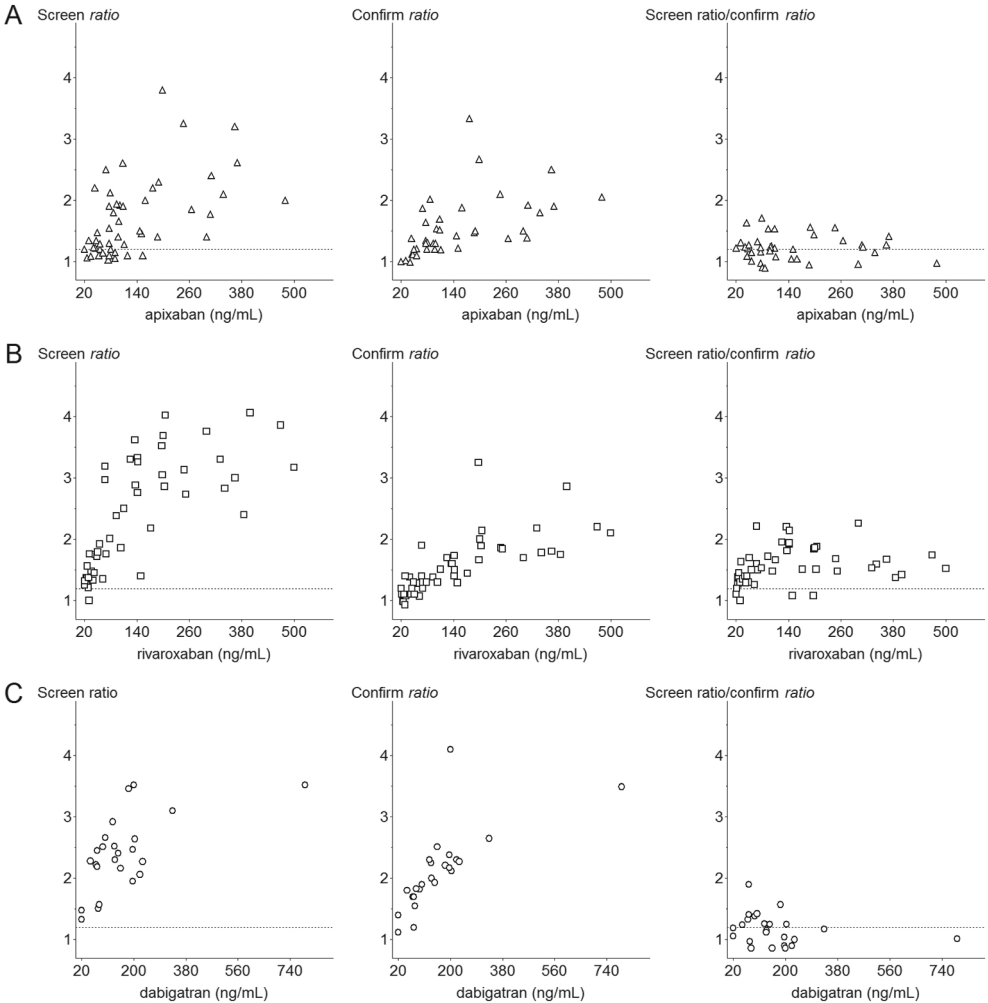
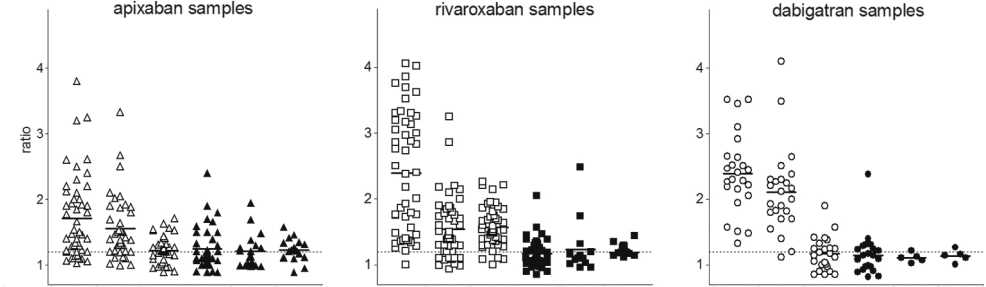

A
Screen ratio
Confirm ratio
Screen ratio/confirm ratio
apixaban (ng/mL)
B
rivaroxaban (ng/mL)
C
dabigatran (ng/mL)

Figure 2



ratio	screen	confirm	screen/ confirm	screen	confirm	screen/ confirm
> 1.20	39/49 (80%)		23/49 (47%)	19/49 (39%)		12/49 (24%)
	before activated charcoal			after activated charcoal		

screen	confirm	screen/ confirm	screen	confirm	screen/ confirm
47/48 (98%)		43/48 (90%)	15/48 (31%)		7/48 (15%)
before activated charcoal			after activated charcoal		

screen	confirm	screen/ confirm	screen	confirm	screen/ confirm
24/24 (100%)		10/24 (42%)	8/24 (33%)		1/21* (5%)
before activated charcoal			after activated charcoal		

Figure 3

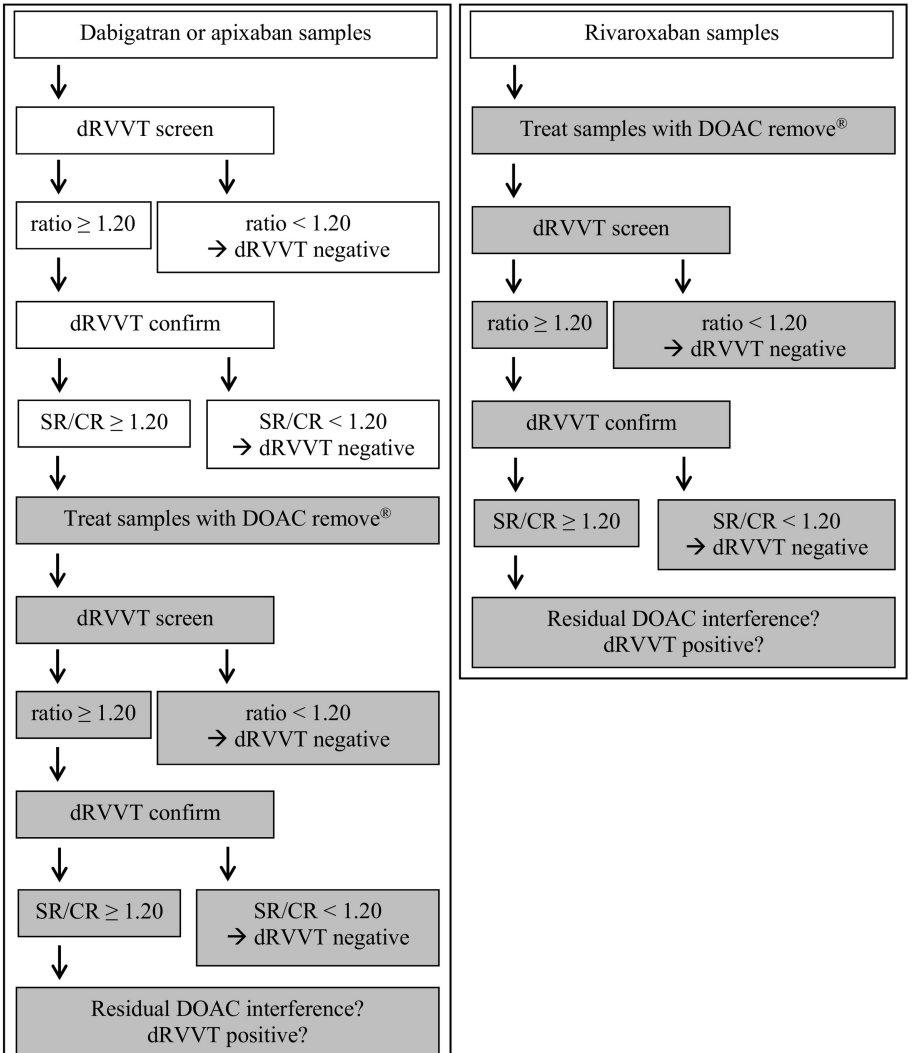

Dabigatran or apixaban samples
dRVVT screen
ratio ≥ 1.20
ratio < 1.20
→ dRVVT negative
dRVVT confirm
SR/CR ≥ 1.20
SR/CR < 1.20
→ dRVVT negative
Treat samples with DOAC remove®
dRVVT screen
ratio ≥ 1.20
ratio < 1.20
→ dRVVT negative
dRVVT confirm
SR/CR ≥ 1.20
SR/CR < 1.20
→ dRVVT negative
Residual DOAC interference?
dRVVT positive?
Rivaroxaban samples
Treat samples with DOAC remove®
dRVVT screen
ratio ≥ 1.20
ratio < 1.20
→ dRVVT negative
dRVVT confirm
SR/CR ≥ 1.20
SR/CR < 1.20
→ dRVVT negative
Residual DOAC interference?
dRVVT positive?

Figure 4

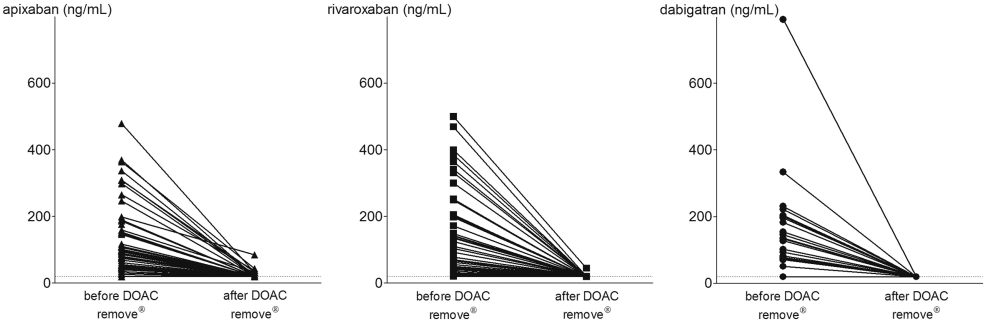

apixaban (ng/mL)
600
400
200
0
before DOAC remove®
after DOAC remove®
rivaroxaban (ng/mL)
600
400
200
0
before DOAC remove®
after DOAC remove®
dabigatran (ng/mL)
600
400
200
0
before DOAC remove®
after DOAC remove®

Figure 5