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Full Length Article

Interference of DOACs in different DRVVT assays for diagnosis of lupus anticoagulants

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ABSTRACT

Objective: Determination of lupus anticoagulants (LA) is an important, but still challenging test in the diagnosis of antiphospholipid syndrome (APS). This is especially the case in patients using one of the direct oral anticoagulants (DOACs).

The aim of our study was to examine the influence of these drugs on DRVVT assays from two companies (in each case: screening test, confirming test and calculated ratio) and on aPTT and lupus-sensitive aPTT.

Methods: We used plasma samples from healthy volunteers spiked with the DOACs dabigatran, rivaroxaban and apixaban (0, 10, 30, 50, 100 ng/mL) for testing. Furthermore, samples from patients receiving a DOAC were investigated. The plasma concentrations of the DOACs were determined using ultra-performance liquid chromatography/electrospray ionization-tandem mass spectrometry (UPLC-MS/MS).

Results: Depending on type and concentration, all the DOACs resulted in pathological values in the DRVVT screening assays. In samples spiked with apixaban, no influence on the DRVVT normalized ratio of the two assays was observed, but 7 to 15% of samples from patients receiving apixaban displayed pathological values. In contrast, up to 71% of dabigatran-spiked samples showed normalized ratio values above the cut-off, whereas there was no influence in the patients' samples. In both spiked and patient samples containing rivaroxaban, the DRVVT assays were influenced.

Conclusion: LA diagnostics should, under DOAC therapy, be limited to situations in which time-critical evaluation is warranted. It is crucial to take into account the finding that even samples containing DOAC concentrations below the limit of detection of the drugs may lead to false-positive DRVVT measurements.

1. Introduction

Antiphospholipid syndrome (APS) is characterized by several clinical criteria, such as venous and/or arterial thrombosis and/or abortion. In addition, the lupus anticoagulants (LA) as laboratory criteria for determining antiphospholipid antibodies (APA) are necessary for classification of APS in accordance with the Sydney criteria [1,2].

APA are a heterogeneous group of antibodies which bind to negatively-charged phospholipids (PL) and therefore affect phospholipid-dependent coagulation tests. The critical, currently known corresponding antibodies anti-cardiolipin and anti-beta2-glycoprotein-1 could be detected in a solid phase assay. Furthermore, their presence could be determined in coagulation tests like aPTT (with low concentration of PL, aPTTL) and DRVVT (dilute Russell's viper venom time). No single test is sensitive to all LA, and two test systems with differing analytical principles should be employed to maximize detection rates (SSC guidelines) [1,3].

The DRVVT test is commercially available and widely used in clinical laboratories. It is believed to be specific for detecting LA in patients at high risk of thrombosis [4,5]. Therefore, DRVVT is an important tool in laboratory diagnosis of LA. The activator in the DRVVT test is extracted from the venom of the Russell's viper. The coagulant in the venom directly activates factor X, which turns prothrombin into thrombin in the presence of factor V and phospholipids. Normally, this

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procedure is divided into two steps: a screen test with low PL concentration and a confirm test with high PL concentration. A meaningful value to detect the existence of an LA is the ratio of both tests.

The second widely used test for LA is the aPTTL with low PL concentration of a paired aPTT regent with relatively high PL concentration or otherwise known to be relatively insensitive to LA.

Both analyses (DRVVT and aPTTL) need FXa and FIIa for clotting. Accordingly, the tests may be influenced by all FXa (e.g. apixaban and rivaroxaban) and FIIa inhibitors (e.g. dabigatran). It is well known that direct oral anticoagulants (DOACs) at peak concentrations produce false positive results in DRVVT assays, as well as in aPTT/aPTTL assays. Thus, common recommendations suggest that these laboratory investigations should be performed at trough level before the next intake of DOACs [6].

The aim of our study was to evaluate these recommendations by examining the influence of DOACs on DRVTT screen, DRVTT confirm and DRVVT normalized ratio, as well as on aPTTL and aPTT. For this, plasma samples were spiked with low concentrations of DOACs according to their trough levels. In a second stage, we took a sample of patients undergoing treatment with DOACs to confirm the results of the first stage.

2. Material and methods

2.1. Study design

Institutional Review Board approval was obtained from the ethics committee of Bad Oeynhausen (Reg.-No. 13/2013). All the procedures complied with the guidelines of the Institutional Review Board for the blood transfusion service.

Blood was collected using an 18-gauge butterfly with tubing and the corresponding tubes from Kabe Labortechik GmbH (Primavette S®) (Nümbrecht-Elsenroth, Germany). Citrated blood was collected in 2.9 mL tubes containing 290 μL sodium citrate (100 mmol/L). Blood was centrifuged for 10 min at 2500*g*. Plasma was transferred to a new tube and centrifuged for a second time under the same conditions. Coagulation analyses were performed either with fresh plasma or after storage at −80 °C.

For spiking experiments, plasma from 30 healthy volunteers showing negative results for APA and in the DRVVT assays was used. For each drug, plasma from 10 volunteers was spiked with 5 different concentrations of each DOAC (0 ng/mL, 10 ng/mL, 30 ng/mL, 50 ng/mL, 100 ng/mL).

In this study, 136 patients undergoing DOAC therapy were investigated (Fig. 1). For some patients, samples with different concentrations of the drug were included. This resulted in a total number of 229 plasma samples. Five patients were excluded because of positive antibodies in anti-cardiolipin or anti-beta2-glycoprotein-1 assays, eight patients were excluded due to insufficient plasma volume to perform all assays and 39 patients (55 samples) were excluded because of concomittant medication of low molecular weight heparin or unfractionated heparin. In all remaining patients (n = 84, 154 samples), no antibodies against cardiolipin (EUROIMMUN AG, Germany) or beta2-glycoprotein-1 (EUROIMMUN AG, Germany) were detected. It could be supposed that at the time of blood sampling no patients were suffering from APS.

2.2. Anticoagulants

The DOAC solutions were prepared as previously described [7]. In brief, stock solutions of dabigatran, rivaroxaban and apixaban (all: ALSACHIM (Illkrich, France)) were prepared using 50% methanol for dabigatran and 10% DMSO for rivaroxaban and apixaban. Further dilutions were performed using distilled water.

2.3. Assays

The measurements were performed using an ACL TOP 700 analyzer (Instrumentation Laboratory, Germany). All laboratory tests were performed according to the usual procedures and standards.

For the coagulation assays, aPTT SynthASil reagent and aPTTL APTT-SP reagent were used (Instrumentation Laboratory, Germany). DRVVT tests were performed with reagent from IL (DRVVT (IL)) (Instrumentation Laboratory, Germany) and STAGO (DRVVT (STA)), (Stago Deutschland GmbH).

2.4. DRVVT assays

A DRVVT assay is divided into two tests - a screen and a confirm test - which results in a normalized ratio (screen/confirm = ratio). In both assays coagulation is activated with Russell's viper venom. The toxin activates the factors V and X directly. The difference between the screen and the confirm test is to be found in the concentration of phospholipids. The screen test contains a low, and the confirm test a high amount of phospholipids. In case of an LA, the APA binds to the phospholipids, which results in a prolonged clotting time. The corresponding confirm test has a shorter clotting time because of the higher concentrations of phospholipids. The measured time of a patient sample for the screen test is divided by a measured time of reference plasma for the screen test. If the ratio of this test is 1.2, the confirm test should be performed. The procedure for the confirm test is equal to the screen test. Finally, the screen ratio is divided by the confirm ratio, which results in a normalized ratio. The cut-off for the normalized ratio is 1.2 for both assays (IL & STA), as stated by the companies and validated in our laboratory after measurement of 20 normal donors.

2.5. Mass spectrometry

Quantitative measurements of apixaban, dabigatran and rivaroxaban in plasma were performed according to the previously described method using ultra-performance liquid chromatography coupled with electrospray ionization-tandem mass spectrometry [8].

3. Results

3.1. Spiking experiments

In the first part of the study, we investigated specimens from ten healthy volunteers for each drug (Tables 1A, 2A). With the spiked plasma we evaluated the influence of the DOACs on the DRVVT and aPTTL/aPTT assays. All DOAC-free samples showed DRVVT normalized ratio values within the reference range, as well as normal aPTT. Furthermore, no antibodies against cardiolipin or beta2-glycoprotein-1 were detected. Therefore, it could be assumed that at the time of blood sampling none of the volunteers showed a laboratory pathological marker for the diagnosis of LA.

All DOACs induced a prolongation in both DRVVT screen tests in a dose-dependent way, compared to the corresponding samples without DOAC.

Apixaban induced a moderate prolongation of the DRVVT screen and DRVVT confirm for both assays in the same way. Therefore, no influence could be seen in total in the DRVVT normalized ratio (Table 1A). Only a slight influence of apixaban could be observed using aPTTL. For aPTTL, only 10% of samples spiked with 100 ng/mL of the drug showed pathological values (norm: 25–37 s).

Using dabigatran, a strong prolongation of both the DRVVT screen and DRVVT confirm could be observed. In the DRVVT (IL) assay, the DRVVT normalized ratio was only above the cut-off in 12% of cases. Using the DRVVT (STA) assay, an increased (28%) number of pathological samples in the DRVVT normalized ratio was seen (Table 1A). APTT and aPTTL increased with higher dabigatran concentrations in a