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UDC:

DOI: https://doi.org/10.2298/VSP210217101K

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
INFLUENCE OF DOACS AND DOAC REMOVE® ON COAGULATION ASSAYS DURING THROMBOPHILIA TESTING IN DOAC TREATED PATIENTS

UTICAJ DOAK I DOAC REMOVE® NA KOAGULACIONE TESTOVE U TOKU TESTIRANJA TROMBOFILIJA KOD PACIJENATA LEČENIH PRIMENOM DOAK

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Abstract

Background/Aim. Direct oral anticoagulants (DOACs) administration has a significant interference on coagulation assays. Our study was conducted in order to evaluate the effect of DOACs and DOAC Remove® on coagulation assays during thrombophilia testing.

Methods. In the period of January 2019 to the end of June 2020 30 DOAC treated patients
tested for thrombophilia, due to venous thromboembolism (VTE), 14 females and 16 males aged 23 to 63 years (median age 47.6 years), were included in the study. Thrombophilia testing was performed using DOAC Remove® tablets (activated charcoal). The results before and after DOAC Remove® were compared. **Results.** Positive LA results were observed in 20% apixaban, 100% dabigatran, and 70% rivaroxaban treated patient, while in samples after DOAC Remove® the LA positivity was observed only in one from the apixaban group. Before DOAC Remove®, the APC-R ratio was measurable in 40% dabigatran, and 80% rivaroxaban treated patients, while, after using DOAC Remove® the APC-R was measurable in all cases. Comparing the results obtained from the samples before and after DOAC Remove®, a difference was noted in relation to all dRVVT tests, except for the dRVVT ratio in the apixaban group. Clot-based methods for detection of the APC resistance are significantly affected by dabigatran, and less by rivaroxaban. **Conclusion.** DOAC was practically inactivated after the addition of the DOAC Remove®, which made it possible to perform analyses for the LA and APC-R testing freely and obtain relevant results. **Key words: DOAC, interference on coagulation assays, thrombophilia testing, DOAC Remove®**

**Apstrakt**

**Uvod/Cilj.** Primena direktnih oralnih antikoagulansa (DOAK) uzrokuje ometanje u izvođenju testova koagulacije. Naše istraživanje je sprovedeno kako bi se procenio efekat DOAK i DOAC Remove® tableta (aktivni ugalj) na testove koagulacije tokom ispitivanja trombofilije. **Metode.** U periodu januar 2019. - jun 2020. godine, uključeno je 30 pacijenata lečenih DOAK-om, testiranih na trombofiliju zbog venskog tromboembolzma (VTE), 14 žena i 16 muškaraca, starosti od 23 do 63 godine (medijana 47,6 godina). Ispitivanje trombofilije izvršeno je upotrebom DOAC Remove® tableta (aktivni ugalj). Upoređeni su rezultati pre i posle DOAC Remov®. **Rezultati.** Pozitivni rezultati za ispitivani LA dobijeni su kod 20% pacijenata lečenih apiksabanom, 100% dabigatranom i 70% rivaroksebanom, a u uzorcima nakon DOAC Remov® pozitivnost na LA dobijena je samo kod jednog iz apixaban grupe. Pre DOAC Remov®, odnos APC-R bio je merljiv kod 40% pacijenata koji su lečeni dabigatanom i 80% rivaroksabanom, dok je nakon upotrebe DOAC Remov® APC-R bio merljiv u svim slučajevima. Upoređujući rezultate dobijene iz uzoraka pre i posle DOAC Remov®, primećena je razlika u odnosu na sve dRVVT
testove, osim dRVVT u grupi lečenih apixabanom. Na koagulacionu metodu za otkrivanje APC rezistencije značajno utiče dabigatran, a manje rivaroxaban. **Zaključak.** DOAK je praktično inaktivisan nakon dodavanja DOAC Remove® tableta, što je omogućilo dobijanje relevantnih rezultata za ispitivan LA i test rezistencije na APC.

**Ključne reči:** DOAK, ometanje u izvođenju koagulacionih testova, ispitivanje trombofilije, DOAC Remove®

**Introduction**

The new class of anticoagulants has been referred as novel oral anticoagulants (NOACs). With regard to the mechanism of action they are target-specific oral anticoagulant agents and such as aimed at inhibiting a specific coagulation factor, so dabigatran is a direct inhibitor of thrombin, while rivaroxaban, apixaban, and edoxaban are direct inhibitors of activated factor X (FXa). The International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) for the control of anticoagulation recommends the term Direct oral anticoagulants (DOACs) (1). DOACs are increasingly being used as an alternative to warfarin in the treatment of venous thromboembolism. However, the introduction of DOACs brought new challenges for all those involved in laboratory testing and assessing of thrombophilia presence. These challenges include DOACs interference on coagulation (clot-based) assays, that can lead to false-positive or -negative results (depending on drug concentration) (2–5). Particularly problematic thrombophilia tests include clotting assays for proteins C and S, lupus anticoagulant (LA) and activated protein C resistance (APC-R) (6-9). The most recent guidance from the Clinical and Laboratory Standards Institute (CLSI), published in 2014, gave a firm conclusion that LA testing was not recommended in DOACs treated patients (10). With regard to the results of the recently published study all DOACs led to a prolongation in both dilute Russel’s Viper Venom Time (dRVVT) based screen and confirm assays used for LA detection (11). As a result of the extent prolongation of the dRVVT screen influenced by DOAC, mixing studies (ratio 1:1) recommended by the guidelines, could not remove this interference, as it can do with Vitamin K antagonist (VKA). Therefore, inhibition from the DOAC would still be present (12).

Despite this interference on coagulation assays, clinicians still continue to request thrombophilia testing for DOAC treated patients. The correct interpretation of
thrombophilia testing results obtained in DOAC treated patients is mandatory to prevent misclassification and subsequent clinical consequences (6).

This study was conducted in order to evaluate the effect of DOACs and DOAC Remove® on coagulation assays during thrombophilia testing among our DOAC treated patients.

**Methods**

*Patients*

In the period of January 2019 to the end of June 2020, 30 DOAC treated patients tested for thrombophilia, due to venous thromboembolism (VTE), 14 females and 16 males aged 23 to 63 years (median age 47.6 years), were included in the study. In all of them thrombophilia testing was performed from a sample before and after the addition of DOAC Remove® tablets (activated charcoal). The results obtained before and after DOAC remove were compared. One of DOACs treated patients from the apixaban group was known to be LA positive. LA positive test was diagnosed in this case upon low molecular weight heparin in the period of the first VTE event.

*Laboratory analysis*

*Patient samples treated with DOAC Remove®*

In all subjects, whole blood was collected 2-12 hours after DOACs intake into buffered sodium citrate tubes (containing 1/10 volume sodium citrate stock solution at 0.129 mmol/L; Vacutest, Kima) by sterile, atraumatic venipuncture. Two samples were taken for each patient. One was prepared by standard method to obtain platelet poor plasma for coagulation assays used in the thrombophilia testing and denoted as sample before DOAC Remove®. The second sample was prepared using DOAC Remove® tablets. According to the instruction, 1 mL of previously prepared plasma sample was mixed gently for 5 minutes with DOAC Remove® tablet and then centrifuged two minutes at 2000g. The supernatant obtained after centrifugation, denoted as sample after DOAC Remove®, was used for coagulation assays.

Thrombophilia testing included: screening tests, Activated partial thromboplastin time (APTT) and Prothrombin time (PT); determination of Antithrombin (AT), Protein C (PC) and Protein S (PS) activity, presence of Lupus anticoagulant (LA) and Activated protein C resistance (APC-R). All assays were performed using Siemens Healthcare Diagnostics reagents on a BCS XP system (Siemens, Marburg, Germany) according to the
manufacturer’s instructions. Pathromptic SL and Thromborel S were used for APTT and PT testing, respectively. Activity of natural inhibitors was measured using Berichrom ATIII (anti-IIa based) and Innovance Antithrombin (anti-Xa based) for AT, Berichrom Protein C for PC and Innovance Free Protein S Ag for PS. Presence of LA was evaluated using an integrated assay based on dilute Russel’s viper venom time (dRVVT) tests that utilize dRVVT LA screen reagent (LA1) and dRVVT LA confirm reagent (LA2). Results for LA1, LA2 and LA Ratio were expressed in accordance with CLSI recommendations published in 2014 (10) as normalized ratios and a value 1.2 was used as cut-off value. APC-R was determined using the Russell Viper Venom Time (RVVT)-based functional clotting test (ProC Ac R assay). APC-R lower than 1.8 was considered pathological. Additionally, genotyping of FV Leiden and FII G20210A mutations was carried out in all study participants. The mutations were detected by polymerase chain reaction (PCR) followed by digestion with specific restriction enzymes (MnlI for FV Leiden and HindIII for FII G20210A, NEBiolabs). Normal and mutated alleles were distinguished by the size of the restriction fragments, using electrophoresis on polyacrylamide gels. For one AT deficient patient revealed in routine thrombophilia testing the PCR analysis for SERPINC 1 gene was performed afterwards. In order to minimize the effect of acute thrombosis on thrombophilia testing, the blood samples were collected after 6-8 weeks after the acute thrombosis. With regard to the defined indication for the thrombophilia testing, patients who developed their first thrombosis before age of 50 were included in the study.

Institutional approval for the study was granted by the Local Research Ethics Committee in accordance with the internationally accepted ethical standards and each patient signed the informed consent form.

Statistical analysis

Distribution of analyzed data was tested. Description of data was done using median and interquartile range (IQR). Differences between groups’ data were evaluated using Mann-Whitney U test and Fisher’s exact test. P value less than 0.05 was considered as statistically significant.

The Statistical Package for Social Sciences 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

Results
A clinical data of the study participants (age, sex, thrombotic manifestation, comorbid condition and DOAC doses) are presented in a Table 1.

Analysis of the results obtained among 30 DOAC treated patients, denoted as samples before DOAC Remove®, and after DOAC Remove®, are shown in Figures 1, 2 and 3. Positive LA results were observed in 20% apixaban, 100% dabigatran, and 70% rivaroxaban treated patients. In samples after DOAC Remove® the LA positivity was observed only in one from the apixaban group. Before DOAC Remove®, the APC-R ratio was measurable in 40% dabigatran, and 80% rivaroxaban treated patients, while, after using DOAC Remove® the APC-R was measurable in all cases. With regard to the APC-R test results, PCR tests confirmed the obtained APC-R findings, the patients tested did not have the F V Leiden mutation.

Comparison of the coagulation assays results obtained before and after applying DOAC Remove® showed significant difference with regard to the APTT only in the dabigatran group. The significant difference was observed in all DOAC treated groups in relation to dRVVT screen (p = 0.012, p < 0.001, p < 0.001), and dRVVT confirm (p = 0.016, p = 0.005, p < 0.001), whereas the difference in dRVVT ratio was observed among rivaroxaban and dabigatran treated patients, p < 0.001. In relation to the evaluation of biological activity of natural inhibitors there were no differences between samples before and after DOAC Remove® (Table 2).

One patient from the apixaban group after treatment with DOAC Remove® was confirmed as AT deficient patient. Using anti-IIa assay before applying DOAC Remove®, AT activity of 73% was obtained, while after applying DOAC Remove® it was 61%. Using anti-Xa assay before applying DOAC Remove®, AT activity of 106% was obtained, while after applying DOAC Remove® it was 54%. Repeated analyses of a new sample taken after one month, showed similar results. Genetic analyses have confirmed the presence of AT deficiency type I.

**Discussion**

The results obtained from the samples denoted before DOAC Remove® showed that the reliability of the tests used in the detection of LA and APC-R was uncertain. Most patients on dabigatran (100%) or rivaroxaban (70%) and 20% on apixaban appeared to be LA positive, while in half of those on dabigatran and up to 20% on rivaroxaban, the APC-R
test was not measurable. The results obtained from the samples before DOAC Remove® confirmed that DOACs administration has a significant interference on coagulation assays, mostly to those that are based on the APTT principle. In relation to the standard coagulation tests, the analysis showed that APTT is most responsive to dabigatran. With regard to the specific assays, all DOACs interfere with most APTT-based assays that were used in the detection of LA, while clot-based methods for detection of the APC resistance are significantly affected by dabigatran, and less by rivaroxaban.

Comparing the results obtained from the samples before and after DOAC Remove®, a difference was noted in relation to all dRVVT tests, except for the dRVVT ratio in the apixaban group. Favarolo et al explained the difference between DOACs in LA testing that was confirmed in our study too. They showed that rivaroxaban affected the screen more than the confirm, leading to higher RVVT ratios, while apixaban affected the confirm more than the screen, leading to lower RVVT ratios (11).

We have to note that in the apixaban group one patient was diagnosed as AT deficient patient. In this particular case, in the sample before DOAC Remove® we found a discrepancy in the level of measured AT activity since that anti-Xa assay provided a normal AT level, while after DOAC Remove® both assays showed reduced AT activity. Ząbczyk et al. showed that treatment with rivaroxaban and apixaban overestimates AT activity measured by FXa- but not FIIa-based assay in AT-deficient individuals (13). Due to the mechanism of inhibition (via FIIa or FXa) DOACs may interfere with AT activity tests that are based on the same principles. Therefore, the FIIa-based assay is preferred in those patients who are treated with rivaroxaban or apixaban (14).

On the other hand, the analysis of PCR results confirmed the obtained findings of APC-R test, obtained from samples denoted as after DOAC Remove®. We have to emphasize that in relation to the uncertain findings of APC-R test, we can always instruct the patient to perform PCR in order to confirm or exclude the FV Leiden mutation, while in the detection of LA we do not have such a possibility. It should be noted that the findings of the tests used in the evaluation of the LA presence are very significant, given its impact on the recurrent thrombosis risk, which is important for clinicians in assessing of the anticoagulant therapy duration. Therefore the use of in vitro drug adsorption with activated charcoal (DOAC Remove® or DOAC Stop™) is useful tool for DOACs neutralization and subsequent LA diagnosis in DOACs treated patients. This is supported by recently
published studies showing that activated charcoal effectively reduced plasma DOAC concentrations leading to appropriate dRVVT results in up to 97% of VTE patients (15, 16). The authors of the second mentioned study suggest the use of DOAC Remove® for every rivaroxaban sample and in positive apixaban and dabigatran samples (16). Likewise, Favresse et al. suggested the use of the DOAC-Stop® treatment in clinical practice to avoid potential misclassifications and clinical consequences (17).

Among the limitation of our study that should be discussed is missing data of DOAC concentration in plasma before and after DOAC removal. However, less than 1% of laboratories that perform routine coagulation screening tests, PT or APTT, could carry out the measurement of DOACs (5). It is known that DOACs have predictable pharmacokinetic and stable pharmacodynamics profile, so their administration does not require coagulation monitoring or measurement of concentration and dose adjustment. Peak plasma levels for DOACs are reached after 2–5 hours (h) after administration and their half-life is between 7–14 h, while the minimum effect is observed directly before the next drug administration at least 18–24 h after the last dose (4,6,15). Recent studies reported that addition of activated charcoal tablet has been able to neutralize the highest likely clinical concentrations of apixaban, dabigatran, rivaroxaban and edoxaban, and to provide measurable and appropriate results for routine screening and specialty coagulation tests in patients taking DOACs. At the same time, these products showed minimal influence to non-DOAC plasmas (16, 18). Kopytek et al. demonstrated that DOAC-Remove reduced DOAC concentrations of apixaban (<3–7 ng/mL, P < 0.0001) and dabigatran (all <5 ng/mL, P < 0.0001), while rivaroxaban concentrations were abolished at almost 100% (all <3 ng/mL, P < 0.0001) (9). Moreover, Slavik et al. using reference HPLC-MS/MS method reported that half-tablet of DOAC-Stop® added to each 0.5mL of plasma removed apixaban from 97.1%, dabigatran from 99.5% and rivaroxaban from 97.9% of participants’ plasmas, leaving in some samples residual concentrations of DOACs that do not affect coagulation (19). In another study by Favarese et al. DOAC-Stop® treatment has been shown to be efficacious in neutralizing DOACs in patient samples removing DOACs to residual levels lower than the limit of quantification of the corresponding DOAC assays (17).

Conclusion
DOACs was practically inactivated after the addition of the DOAC Remove® tablets in the prepared plasma samples, which made it possible to perform analyses for the LA and APC-R testing freely and obtain relevant results. Since DOACs laboratory monitoring is not routinely performed in most clinical laboratories the use of activated charcoal tablets to neutralize DOACs in samples collected immediately prior to re-dosing may be an optimal approach to enable the coagulation testing and the correct interpretation of results in patient taking DOACs.

Acknowledgements: This study was supported by grant 173008 from the Ministry of Education, Science and Technological Development, Serbia.

Conflict of interest statement: None declared

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Table 1. Patient characteristics

| Apixaban | Rivaroxaban | Dabigatran |
|----------|-------------|------------|
|          |             |            |
|          |             |            |
| Current age, Range (median) | 43-63 (48.3) | 23-63 (43.4) | 40-61 (51.2) |
|---------------------------|--------------|--------------|--------------|
| Sex M/F                   | 6/4          | 7/3          | 3/7          |
| Thrombosis localization   |              |              |              |
| DVT/PE                    | 4**          | 7***         | 4*           |
| PE                        | 3*           | 2            | 4*           |
| DVT                       | 3*           | 1*           | 2*           |
| Age of the first VTE      | 41-49 (45.6) | 19-50 (37.8) | 40-49 (45.8) |
| Risk factors for the first VTE |         |              |              |
| Surgery                   | 2            | 2            | -            |
| Hormonal therapy          | 2            | 1            | 3            |
| CA                        |              |              | 1            |
| Concomitant diseases      |              |              |              |
| CA                        | 2            | 1            | 1            |
| Hypertension              | 1            |              |              |
| Arrhythmia                | 1            |              |              |
| DOAC doses                |              |              |              |
| 2 Pts 2x 5 mg             | 20 mg        | 2x150 mg     |
| 8 Pts 2x 2.5 mg           |              |              |

M - male, F - female, DVT - Deep Venous Thrombosis, PE - Pulmonary Embolism, VTE - Venous Thromboembolism, * patient with recurrent thrombosis, CA - breast cancer, DOAC - Direct Oral Anticoagulants, Pts - Patients

Figure 1. Results for dRVVT ratio of tested patients.
Data are shown as individual dot plots with median and interquartile range (IQR) ratios for dRVVT ratio (y-axis). The samples are denoted as before and after DOAC Remove® (x-axis).

Figure 2. Results for APC-R of tested patients.

Data are shown as individual dot plots with median and interquartile range (IQR) ratios for APC-R (y-axis). The samples are denoted as before and after DOAC Remove® (x-axis).
The dashed horizontal line presents cut-off for APC-R (1.8) to define negative vs positive test results. ★ - unmeasurable data
Figure 3. Results for dRVVT screen, dRVVT confirm and dRVVT ratio of tested patients. Data are shown as individual dot plots with median and interquartile range (IQR) ratios for dRVVT screen (A), dRVVT confirm (B) and dRVVT ratio (C) (y-axis). The patient groups are presented in relation to DOACs drugs (apixaban, rivaroxaban, dabigatran), before and after DOAC Remove® (x-axis). The dashed horizontal line presents cut-off for dRVVT ratio (1.2) to define negative vs positive test results.
Table 2. Coagulation assays obtained from plasma samples before and after the addition of DOAC Remove® tablets

| Coagulation assay | Apixaban | Rivaroxaban | Dabigatran |
|-------------------|----------|-------------|------------|
|                   | (median) | IQR         | (median)   | IQR         | (median) | IQR         |
| APTT (s)          | B D R    | 31.2        | 5.15       | 31.85       | 8.875    | 41.15       | 5.5        |
|                   | A D R    | 28.5        | 2.6        | 29.7        | 3.5      | 30.05       | 3.5        |
|                   | p        | 0.101       | 0.054      | <0.001      |          |             |            |
| PT (%)            | B D R    | 108         | 13         | 104.5       | 18.25    | 95.5        | 19.25      |
|                   | A D R    | 120         | 14         | 110.5       | 12.25    | 105         | 20.5       |
|                   | p        | 0.477       | 0.173      | 0.054       |          |             |            |
| Fibrinogen        | B D R    | 3.7         | 1.55       | 3.6         | 0.95     | 3.55        | 0.725      |
|                   | A D R    | 3.35        | 1.275      | 3.05        | 1.1      | 3.4         | 0.7        |
|                   | p        | 0.343       | 0.172      | 0.544       |          |             |            |
| dRVVT screen (s)  | B D R    | 45.8        | 9.125      | 51.75       | 26.57    | 80.4        | 27.7       |
|                   | A D R    | 38.2        | 4.75       | 36.95       | 4.833    | 37.5        | 3.275      |
|                   | p        | 0.012       | <0.001     | <0.001      |          |             |            |
| dRVVT confirm (s) | B D R    | 39.55       | 5          | 39.05       | 6.85     | 54.95       | 25.62      |
|                   | A D R    | 35          | 2.65       | 34.55       | 6.15     | 35.6        | 3.025      |
|                   | p        | 0.016       | 0.005      | <0.001      |          |             |            |
| dRVVT ratio       | B D R    | 1.17        | 0.108      | 1.28        | 0.478    | 1.41        | 0.255      |
|                   | A D R    | **1.04      | 0.135      | 1.025       | 0.057    | 1.05        | 0.043      |
|                   | p        | 0.112       | <0.001     | <0.001      |          |             |            |
| AT (%)            | B D R    | 99          | 19         | 99.5        | 19.75    | 100         | 17.25      |
|                   | A D R    | #93         | 30.5       | 93          | 11.75    | 99.5        | 19.75      |
|                   | p        | 0.132       | 0.161      | 0.762       |          |             |            |
|        | PC (%) |         |         |         |         |         |
|--------|--------|---------|---------|---------|---------|---------|
|        | B D R  | B D R   | A D R   | A D R   | p       |         |
|        | 124    | 120     | 47      | 44.5    | 0.536   |         |
|        | 91     | 92.5    | 29.5    | 31.25   | 0.97    | 0.97    |
|        | 29.5   | 118.5   | 33.75   | 32      |         |         |
| PS (%) |         |         |         |         |         |         |
|        | B D R  | B D R   | A D R   | A D R   | p       |         |
|        | 94     | 93      | 16.5    | 13.5    | 0.965   |         |
|        | 84.5   | 82.5    | 36.75   | 37.75   | 0.91    | 0.97    |
|        | 109    | 108     | 35      | 33.5    |         |         |
| APC-R  |         |         |         |         |         |         |
|        | B D R  | B D R   | A D R   | A D R   | p       |         |
|        | 5.300  | 5.600   | 1.3     | 0.9     | 0.873   |         |
|        | 4.1    | 4.2     | 1.3     | 1.175   | 0.657   |         |
|        | 4      | 4.7     | 4       | 3       | 0.62    |         |

APC-R (Activated protein C resistance), AT (Antithrombin), APTT (Activated partial thromboplastin time), dRVVT (dilute Russel’s viper venom time), PC (Protein C), PS (Protein S), PT (Prothrombin time), IQR (Interquartile range), p (Mann-Whitney U test), B D R (before DOAC® remove), A D R (after DOAC® remove).

* PCR tests confirmed that one patient treated with rivaroxaban was carrier of FII G20210A mutation.

** One patient with positive LA and #one with AT deficiency were diagnosed in the apixaban group.