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The effect of DOAC-Stop® on several oral and parenteral anticoagulants

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Dear Editors,

DOAC-Stop® is a hydrophobic binding agent, containing activated charcoal, which can absorb the direct oral anticoagulants (DOACs) from plasma samples *in vitro* (1). It neutralised the effect of apixaban, edoxaban, rivaroxaban and dabigatran on routine coagulation assays, such as activated partial thromboplastin time (aPTT) and prothrombin time (PT)/international normalised ratio (INR) (1-3). It has been proposed that DOAC-Stop® not only can identify which samples with prolonged coagulation results actually contain the DOACs (1), but might also aid in estimating the DOAC concentration by calculating a "correction ratio" of aPTT before/after DOAC-Stop® treatment, if the reagents are adequately sensitive to the DOACs (4). DOAC-Stop® reduced the rate of false positive lupus anticoagulant assays (5, 6), thus can allow to perform thrombophilia testing without stopping DOAC treatment. A study performed on the thrombin generation assay showed that DOAC-Stop® eliminated the interference of dabigatran with the calibrators (7) and there are some reports that other low molecular weight anticoagulants (e.g. argatroban and lepirudin) were extracted by DOAC-Stop® (2).

However, it has been suggested that DOAC-Stop® might prolong the aPTT results (8), while in another study the normal plasma treated with DOAC-Stop® showed hypercoagulability on the thrombin generation assay, compared to the same untreated plasma (7). The aim of this study was to explore the effect of DOAC-Stop® on several oral and parenteral anticoagulants using standard and research-based coagulation assays.

A pool of normal platelet poor plasma (NPP) was obtained from anonymised citrated samples with normal PT and aPTT, after separation and double centrifugation (***Table S1***). There were two batches of NPP: the first was used to spike with apixaban (182 and 265 ng/ml), edoxaban (151 and 220 ng/ml), rivaroxaban (241 and 339 ng/ml), enoxaparin (0.93 and 1.68 IU/ml), and bivalirudin (13.3 and 26.4 μg/ml); the second with argatroban (3.06 and 6.16 μg/ml), dabigatran

(203 and 318 ng/ml), and fondaparinux (1.62 and 2.16 µg/ml). A pool of warfarinised platelet poor plasma (WPP) was obtained from anonymised citrated samples of outpatients receiving warfarin. Three pools of WPP were collected from samples with INR values in different ranges and final plasma pool INR values were 2.1, 3.2 and 4.2. Aliquots were stored at -80°C until analysis.

DOAC-Stop® (Haematex Research, Australia) was added to the thawed aliquots, according to the protocol detailed in ***Table S1***. All the results after DOAC-Stop® treatment were compared to the same untreated plasma, processed according to the same protocol (except for the addition of DOAC-Stop®). The following assays were performed (***Table S2***): anti-Xa, dilute thrombin time, PT/INR, aPTT, dRVVT for lupus anticoagulant, thrombin generation (measured using the calibrated automated thrombography [CAT] with 5pM tissue factor [TF]), native thromboelastography (TEG), fibrinogen (using the Clauss method), and one-stage factor assays (II, VII, VIII, IX, X, XI, XII). The CAT and the TEG were performed in duplicate, while the other assays were performed once. All samples were processed and analysed in March-June 2019.

The normal ranges of our NPP on the CAT and the TEG were obtained from multiple runs of the two batches of NPP (8 times each in different plates for the CAT, 10 times each in different channels for the TEG), before spiking with anticoagulants or processing with DOAC-Stop®, and calculated as mean ±1.96SD. Normalisation of CAT or TEG results after DOAC-Stop® treatment was defined as values within the normal ranges of the unspiked and unprocessed NPP.

The Wilcoxon matched-pairs signed-ranks test was used to compare treated vs untreated samples in the DOAC category (including apixaban, edoxaban, rivaroxaban, dabigatran) and in the NPP/WPP category. The statistical program STATA/SE v.12 (StataCorp LP, College

Station, Texas, USA) was used for analysis, considering statistically significant two-tailed p values <0.05.

After DOAC-Stop® treatment, the direct factor Xa inhibitors and the direct thrombin inhibitors were undetectable. No changes were observed in the concentrations of the indirect factor Xa inhibitors (***Table S3***). Normalisation of PT and aPTT was observed for the direct factor Xa inhibitors and for dabigatran. Bivalirudin and argatroban did not reach a complete normalisation of aPTT, which can be explained by their high concentrations resulting in very prolonged aPTT in the untreated samples. DOAC-Stop® did not produce any effect on the indirect factor Xa inhibitors, the NPP or the WPP (***Table S3***). The false positive lupus anticoagulant results obtained with rivaroxaban were normalised with DOAC-Stop® (***Table S4***).

On the CAT, DOAC-Stop® completely normalised the direct factor Xa inhibitors, while a partial normalisation was seen for the direct thrombin inhibitors (***Table S5***). After DOAC-Stop® treatment, both the DOAC-spiked plasma and the NPP/WPP showed a decrease of lag time and time-to-peak, and an increase of endogenous thrombin potential (ETP) and thrombin peak (***Figure 1***).

On the TEG, after DOAC-Stop® treatment a variable degree of normalisation was observed for the direct factor Xa inhibitors and the direct thrombin inhibitors (***Table S6***). The DOAC-spiked plasma showed a significant decrease of R time and K time, and a significant increase of angle and maximum amplitude (MA). NPP/WPP showed, instead, an increase of R time and non-significant changes of K time, angle and MA (***Figure 2***).

Decreased concentrations of coagulation factors levels were observed in the DOAC-spiked plasma (***Table S7***), because these assays are PT-based (II, VII, X) or aPTT-based (VIII, IX, XI, XII); thus, the effect of DOAC-Stop® was seen as a normalisation of these results. Low

factors II, VII, IX, X levels were observed in the WPP, because the synthesis of these factors is affected by warfarin. However, NPP/WPP showed a significant reduction of factors VIII, IX, X, XI, XII levels with DOAC-Stop® (***Figure 3***).

This study evaluated the effect of DOAC-Stop® treatment on a wide spectrum of oral and parenteral anticoagulants, using several coagulation assays and a rigorous protocol for sample processing. We confirmed the potential of DOAC-Stop® to absorb the DOACs, as shown by plasma concentrations below the lower limit of detection of the corresponding assays, normalisation of PT/INR, aPTT and lupus anticoagulant. DOAC-Stop® absorbed also the parenteral direct thrombin inhibitors argatroban and bivalirudin, while no effect was observed on the indirect factor Xa inhibitors.

DOAC-Stop® obtained more variable results on the CAT and the TEG, without complete normalisation of the DOAC-spiked plasma. Previously, Kopatz et al showed a complete normalisation of the CAT traces up to DOACs concentration 80 ng/ml (7); however, DOAC-Stop® has never been assessed on the TEG before. CAT results from NPP/WPP suggested hypercoagulability, as previously reported (7, 9), whereas TEG results suggested hypocoagulability. Factor assays results showed a reduction of factors involved in the intrinsic coagulation pathway, suggesting that DOAC-Stop® might be activating coagulation through the contact activation pathway, with consumption of these factors. Therefore, the different results of CAT (hypercoagulability) and TEG (hypocoagulability) could be explained by the different sensitivity of these two assays. The native TEG (performed without kaolin or TF) is more sensitive to the contact activation pathway (10), whereas the CAT (performed with TF) is more sensitive to the extrinsic pathway (11). However, two previous studies did not find any significant change in factors VIII (3, 12), VII and X (12) in non-anticoagulated plasma after DOAC-Stop® treatment, while others reported significant reduction of fibrinogen, protein S (9)

and tissue factor pathway inhibitor (7, 9). Nonetheless, our findings suggest a certain level of DOAC-Stop® binding/activation and raised concerns on its use in routine clinical practice, due to possible effects on patient plasma with more pronounced coagulation factors alterations. Furthermore, De Kesel and Devreese recently reported that treatment of plasma not containing DOACs with DOAC-Stop® may prolong the aPTT and the dRVVT assays (8). In our study, despite the reduction of certain factors levels, we did not see any relevant increase in the aPTT, a finding which can be explained by the different sensitivity of our aPTT reagent.

The main limitations of this study include the low number of samples tested, the fact that most of the assays were not performed in duplicate and the fact that only therapeutic concentrations of the DOACs were tested. In addition, our findings cannot be generalised to other assays or reagents, thus being hypothesis generating only. While the use of a pooled plasma does not allow to observe interindividual differences in the response to DOAC absorbing agents (9), it is an ideal substrate to evaluate and compare the profile of the different anticoagulant classes.

In conclusion, these results can contribute to the knowledge of the effects of DOAC-Stop® and provide the background for further research studies, in order to better assess the safety of this binding agent.

Competing interests: The authors have no relevant conflicts of interest to declare in relation to this study.

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Author contributions: NR: Conceptualization, Methodology, Investigation, Formal Analysis, Data curation, Writing – Original Draft. KV, KH, PG, CG, DZ: Investigation, Writing – Review & Editing. SK, MM, WA: Supervision, Writing – Review & Editing. AG: Conceptualisation, Methodology, Supervision, Writing – Review & Editing.

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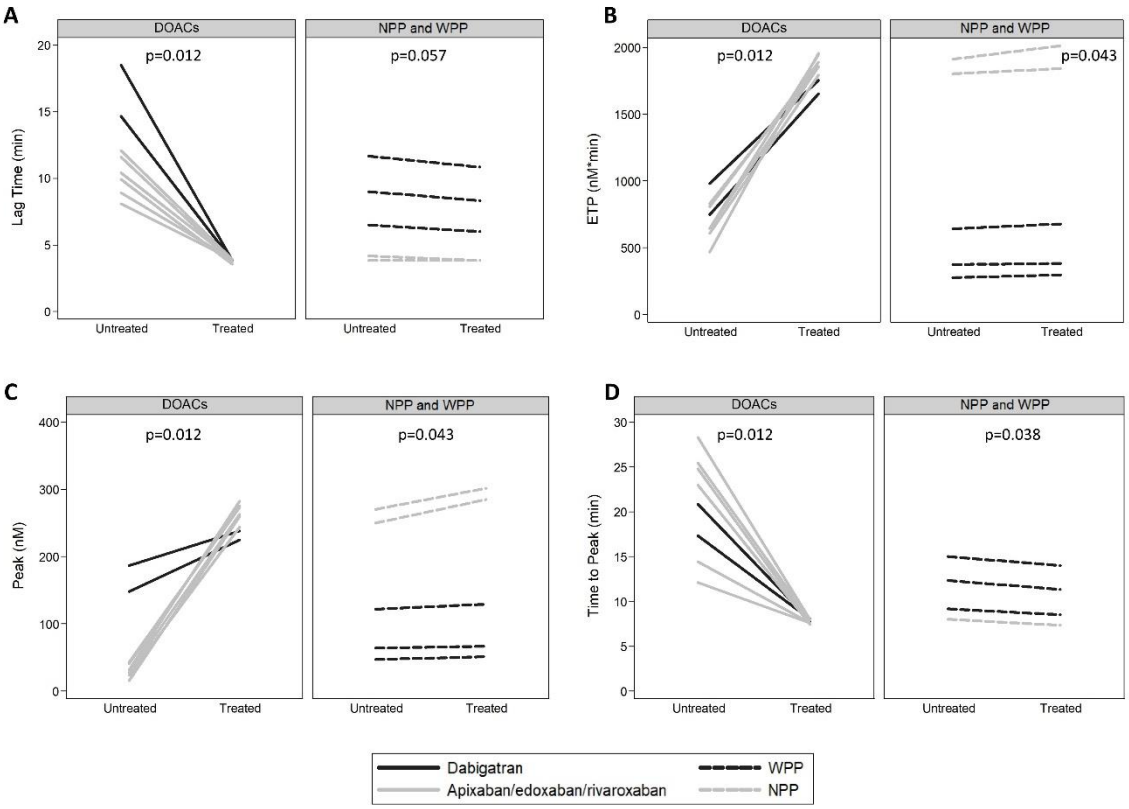
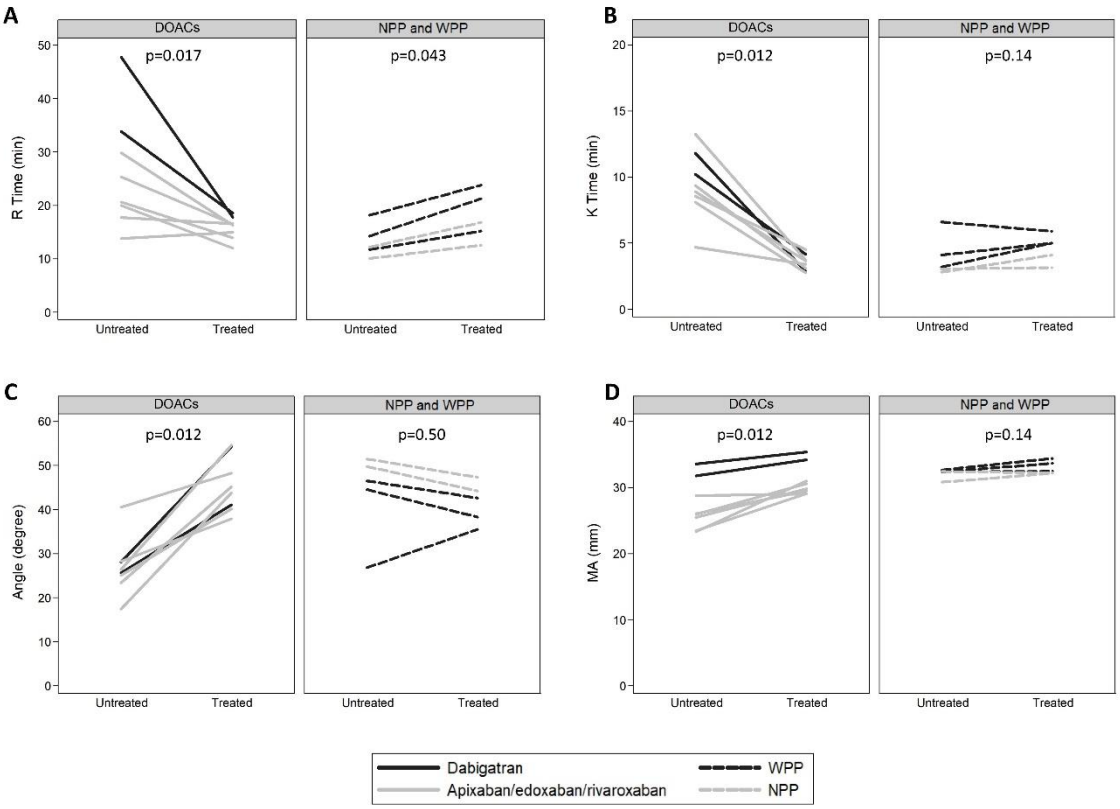
Figure 1. The effect of DOAC-Stop® treatment on calibrated automated thrombography



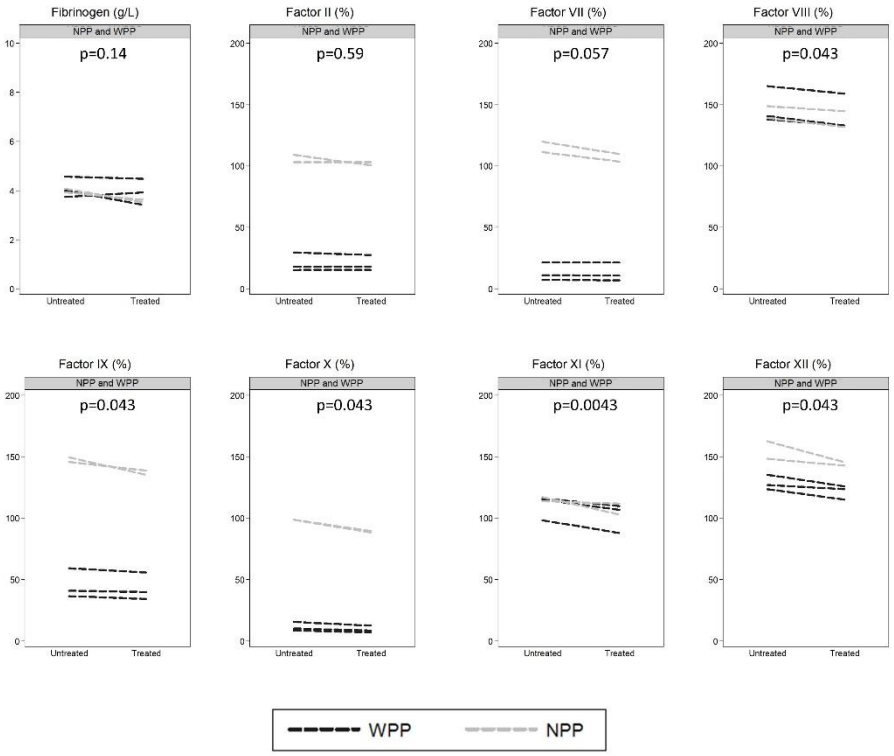
Legend: DOACs = direct oral anticoagulants, NPP = normal platelet poor plasma, WPP = warfarinised platelet poor plasma

Figure 2. The effect of DOAC-Stop® treatment on thromboelastography



Legend: DOACs = direct oral anticoagulants, NPP = normal platelet poor plasma, WPP = warfarinised platelet poor plasma

Figure 3. The effect of DOAC-Stop® treatment on factor assays



Legend: NPP = normal platelet poor plasma, WPP = warfarinised platelet poor plasma