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Background and aim

There is a laboratory and clinical need to know the impact of anticoagulants on diagnostic tests to avoid misinterpretation of results. Although the labeling documents of these medicines (i.e. EMA summary of products characteristics, FDA prescribing information) provide some information about the influences of each DOAC (factor Xa inhibitors including apixaban, edoxaban, rivaroxaban and betrixaban and factor IIa inhibitor dabigatran) on diagnostic tests, these are usually limited to some of the most common tests, with few information about the reagents used and not head to head comparison. This study aims to compare the impact of DOACs on a large panel of hemostasis diagnostic tests and provide practical recommendations for clinicians.

Methods

The impact of increasing concentrations of DOACs on a normal pooled plasma has been assessed on several thrombophilia testing, including assays for antiphospholipid syndrome, protein C, protein S and antithrombin activity, measurements of clotting factors levels and the detection of activated protein-C resistance with a large panel of reagents. The results are compared and discussed with data obtained from the literature.

Significant impacts of DOACs on hemostasis diagnostic tests

Overall, factor Xa and factor IIa inhibitors significantly affect clot-based hemostasis diagnostic tests resulting in false-positive or false-negative results. Impacts on thrombin-based hemostasis diagnostic tests are observed with dabigatran but not with anti-Xa and conversely for FXa-based hemostasis diagnostic tests (Figure 1-2). No impact was observed with antigenic or chromogenic methods for the assessment of protein S and C activity (Data not showed).

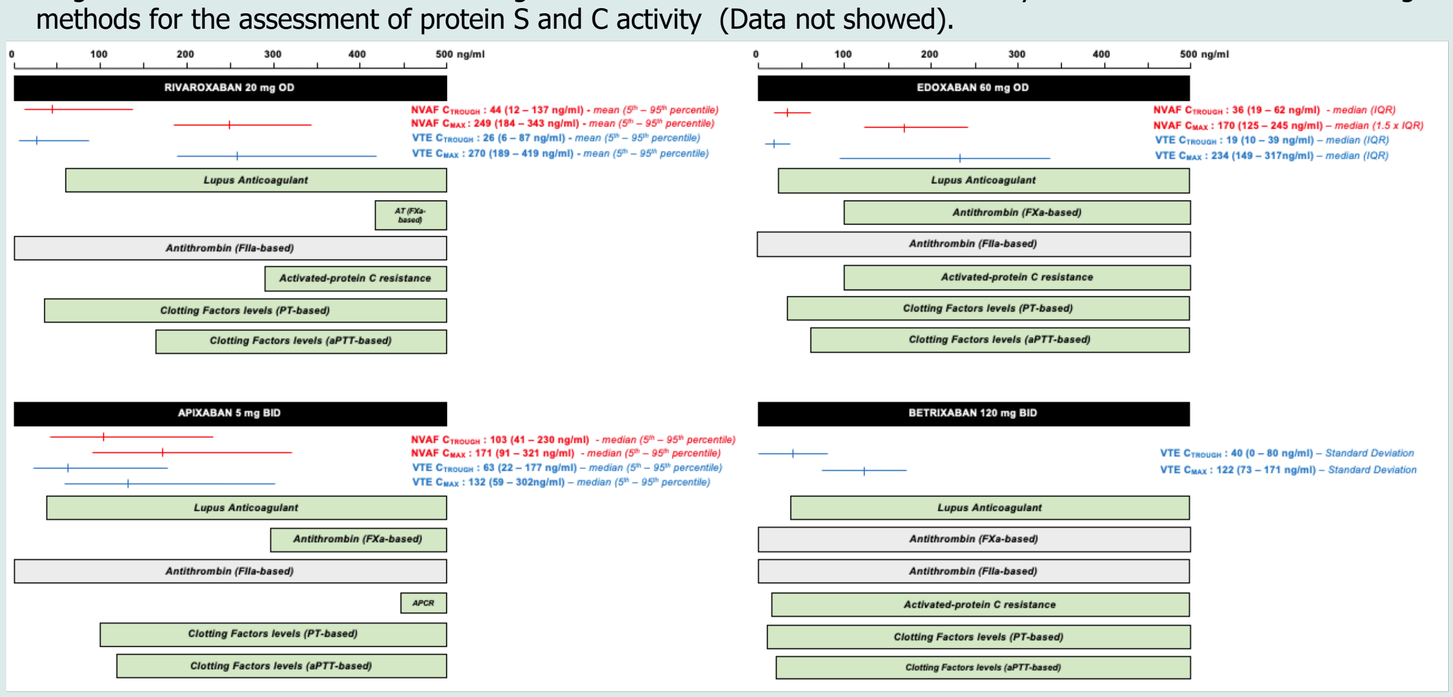

Figure 1: Impact of factor Xa inhibitors related to indications on diagnostic assays. Laboratory testing of factor Xa inhibitors and their impacts on diagnostic assays. Red and blue lines represent plasma concentrations at peak and trough in NVAF and VTE, respectively. Green boxes represent ranges of concentrations that can impact diagnostic assays and lead to misdiagnosis while grey boxes represent unaffected assays. For clotting factors measurement, boxes represent the dynamic range of quantitation (the dynamic range of quantitation is defined as range covering from the lowest observed limit of quantification to the maximal concentration tested or supposed) of PT and aPTT for sensitive reagents. Ctrough: Minimum plasma concentration during the dosing interval; Cmax: maximum plasma concentration during the dosing interval; VTE: Venous thromboembolism; NVAF: Non-valvular atrial fibrillation; OD: once daily; bid: twice daily; APCR: Activated Protein C Resistance. Notes: Data on plasma concentrations were extracted from J. Douxfils et al. for apixaban, edoxaban, rivaroxaban and from R. Siriez et al. and SmPC for betrixaban.

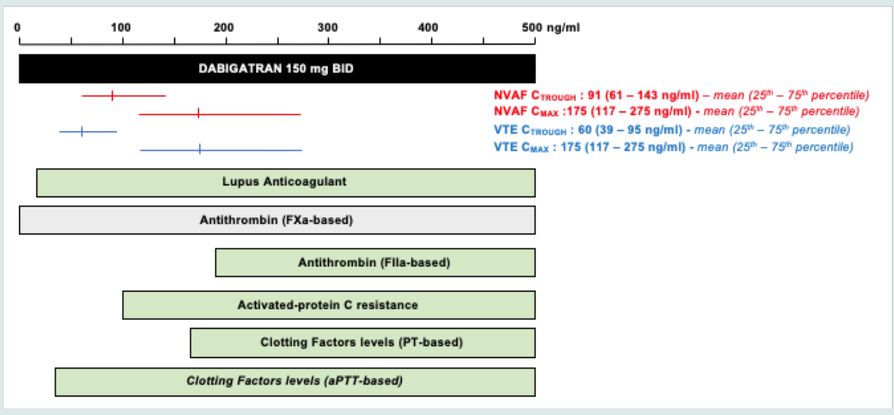

Figure 2: Impact of factor IIa inhibitors related to indications on diagnostic assays. Laboratory testing of factor IIa inhibitors and its impacts on diagnostic assays. Red and blue lines represent plasma concentrations at peak and trough in NVAF and VTE, respectively. Green boxes represent ranges of concentrations that can impact diagnostic assays and lead to misdiagnosis while grey boxes represent unaffected assays. For clotting factors measurement, boxes represent the dynamic range of quantitation (the dynamic range of quantitation is defined as range covering from the lowest observed limit of quantification to the maximal concentration tested or supposed) of PT and aPTT for sensitive reagents. Ctrough: Minimum plasma concentration during the dosing interval; Cmax: maximum plasma concentration during the dosing interval; VTE: Venous thromboembolism; NVAF: Non-valvular atrial fibrillation; OD: once daily; bid: twice daily. Notes: Data on plasma concentrations were extracted from Douxfils et al., Favaloro et al., Gessoni et al. and Bonar et al.

Providing recommendations to deal with impacts of DOACs on hemostasis diagnostic tests is a clinical challenge since these impacts depend on i.) the anticoagulant agent; ii.) the plasma level of anticoagulant and iii.) the sensitivity of the tests and the reagents/methodologies used.

- Lupus Anticoagulant: LA are immunoglobulins binding phospholipids and protein providing a disruption of coagulation process prolonging phospholipid-dependent coagulation tests, as APTT. Recommendation is to use two different tests for the detection of LA such as a sensitive APTT assays or a dilute Russell viper venom tests (dRVVT) :
  - aPTT : aPTT with low phospholipids (PL) concentrations making them very sensitive to LA is performed with a classical aPTT-based test with higher amount of PL to correct the clotting time by saturating the antibodies.
  - DRVVT : The venom from Daboia russelii is used as an activator of the endogenous factor X presents in the sample. The test is performed with two different concentrations of added phospholipids. A screen test is first performed with a low concentration of phospholipids to suspect the presence of LA antibodies. If the screen ratio between the patient's clotting time and a reference pool plasma is at least equal to 1.2 then a confirmatory test, with high concentration of phospholipids, is performed against versus the reference pool plasma.

  An impact on both tests could be observed since these tests required FXa and FIIa. Furthermore, an impact on diagnostic is explained by an increased sensitivity of the screen reagent due to the low concentration of PL. The presence of DOAC will impact results of screen and confirm assays in different ways that will provide a ratio screen:confirm higher than 1.2 inducing unnecessary investigations.

- Activated Protein C Resistance: Several assays exist to assess the APC-R:
  - The first assay used the ratio between baseline aPTT and aPTT in presence of purified exogenous APC to inactivates factor Va and factor VIIIa. The presence of APC results in an increased clotting time of the APTT in samples without FV Leiden mutation. By contrast, in a sample with mutation the prolongation in the clotting time is less marked. Nevertheless, any factors that could impact aPTT assays (LA, anticoagulants treatments, …) could false this interpretation.
  - Second assays improved sensitivity and specificity by diluting plasma sample with FV-deficient human plasma (1:4) but was still influence by LA and oral anticoagulants.
  - Third generation assay used Russell's viper venom from Daboia Russelli to overcome the influence of contact pathway but without dilution in FV-deficient human plasma, low FII, FV as well as fibrinogen concentrations, anticoagulants may interfere.
  - Finally, researchers developed plasma based functional clotting assay with the replacement of FXa by noscarin, a FVa-dependent and phospholipid-independent prothrombin activator isolated by Notechis scutatus scutatus. A dilution with FV-deficient plasma (1:4) is performed to overcome to factor deficiencies/elevations and the absence of phospholipids in the reagent avoid impacts of lupus anticoagulants. On one hand, the assessment of APC-R based on aPTT and using Factor V-deficient plasma seem to be impacted by DOACs contrary to prothrombinase-based assay as the Pefakit APC-R factor V Leiden which is only impacted by edoxaban and dabigatran, due to their impact on thrombin, according Douxfils et al. and Favresse et al. On the other hand, Hillarp et al. showed that edoxaban do not impact Pefakit ACP-R factor V Leiden. Theses contrary results could be explained by the high lot-to-lot variability of phospholipids concentrations or by the slight anti-thrombin activity of edoxaban.

- Antithrombin level measurement: An assessment of antithrombin activity can be performed by immunological or functional assays. Functional AT assays are chromogenic assays based on the principle of FIIa (human or bovine) or FXa (human) inhibition. The antithrombin measurements using FIIa-based chromogenic assays (e.g. Berichrom ATIII® or Stachrom ATIII®) are not influenced by direct Xa inhibitors (apixaban, edoxaban, rivaroxaban and betrixaban) and the antithrombin measurements using FXa-based chromogenic assays (e.g. Coamatic LR® or HemosIL® Liquid Antithrombin) are not influenced by dabigatran. Immunological assays are not impacted by the presence of DOACs.

- Clotting factors levels: The evaluation of factors from extrinsic pathway (FVII, FX, FII, FV) use a PT-based clotting method unlike the evaluation of intrinsic pathway factors (FVIII, FIX, FXI and FXII) which required an aPTT-based clotting method. Given the fact that chronometric tests are severely impacted by DOACs, the presence of one of these drugs impact the measurement of clotting factors leading to an underestimation of the levels of clotting factors. Although the impact is less pronounced when the dilution is high, a significant decrease in the accuracy of the test is noticed. Moreover, the measurement of clotting factor should be performed with insensitive PT- or aPTT-reagents , or known to be less sensitive (*e.g.* Dade®Innovin® for PT and C.K-Prest® for aPTT are less sensitive to the presence of DOAC). The ionic force, the pH of the buffer solution, the source of activator or even the phospholipids composition could be parameters that impact on the sensitivity of a particular assay.

Conclusion

Interpretation of hemostasis diagnostic tests results should be done with caution in patients on DOACs since many of them may be impacted. The rational use of appropriate reagents for diagnostic tests must be performed according to the present DOAC for antithrombin level measurement or activated protein C resistance. For clotting factors levels measurements, the use of DOAC-insensitive PT or aPTT reagents with adapted dilution of samples should be considered. Finally, the use of a device/chemical compound able to remove or antagonize the effect of DOACs or the development of new diagnostic tests insensitive to DOACs should be considered to further minimize the risk of false results for lupus anticoagulant.

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