Hemostasis testing: What is the impact of direct oral anticoagulants?

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July 2019—Prevention and treatment of venous thromboembolic disease is accomplished through the use of anticoagulant agents, which are prescribed for millions of Americans annually. A revolution in anticoagulant use has occurred over the last decade, as direct oral anticoagulants (DOACs) were introduced to the market. The new agents have a number of advantages over warfarin, the traditionally administered oral agent, which is a vitamin K antagonist (VKA). DOAC advantages include a lower incidence of major bleeding, predictable pharmacokinetic profiles, and no need for routine monitoring in the average patient. While in the United States VKAs remain the most popular anticoagulant prescribed, use of DOACs, specifically rivaroxaban and apixaban, is rapidly approaching 50 percent. Dabigatran, a direct thrombin inhibitor, has modest use despite being the first DOAC to receive Food and Drug Administration approval. The remaining DOACs, specifically the more recently approved edoxaban and betrixaban along with rivaroxaban and apixaban, are inhibitors of activated factor X (FXa), known as direct FXa inhibitors.

As DOACs do not require laboratory monitoring and because they may not affect the activated partial thromboplastin time (aPTT) and prothrombin time (PT) ("Direct oral anticoagulants and APTT, PT results: the risk of normal results in patients on therapy," CAP TODAY, July 2018), there may be a lack of appreciation that these agents can have significant impact on hemostasis testing. Even though these drugs can interfere with testing, laboratory evaluation for bleeding and thrombotic disorders is often performed while patients are on anticoagulant therapy (see "Effect of various anticoagulants on commonly used coagulation assays," page 26).

DOACs function as anticoagulants by inhibiting serine proteases, specifically thrombin and FXa, both of which are vital components of the coagulation cascade. These drugs therefore may affect commonly used global coagulation assays. DOACs can also interfere in a number of coagulation assay methodologies, causing assays to be spuriously elevated, spuriously decreased, as well as falsely positive (Gosselin RC, Adcock DM. *J Thromb Haemost.* 2016;14[5]:886–893). It is important to understand when the DOAC effect is causing a result to be truly abnormal or the DOAC impact causes a factitious laboratory result.

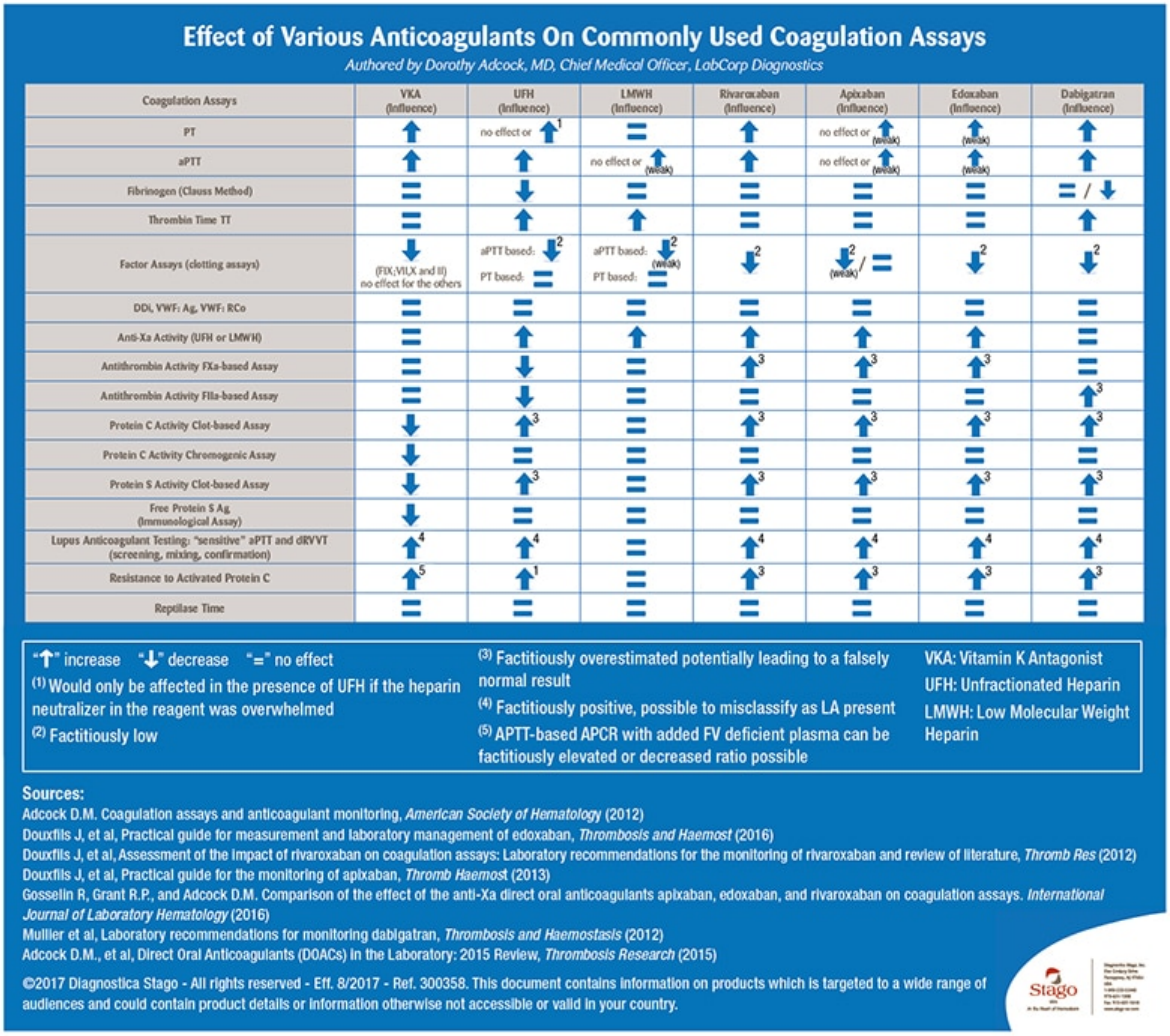
Different aPTT and PT reagents show varying responsiveness to each of these agents depending on the specific DOAC as well as the various components of the reagents (for example, activator, buffer, phospholipid) (Samuelson BT, et al. *Chest.* 2017;151[1]:127–138). In general, direct thrombin inhibitor drugs tend to prolong the aPTT more than the PT while direct FXa inhibitor drugs prolong the PT to a greater extent than the aPTT. Likewise, one-stage aPTT or PT dependent factor activity and inhibitor (for example, Bethesda) assays may be affected by DOAC presence, and the degree of interference depends on reagent responsiveness, the specific DOAC present, and DOAC concentration (Adcock DM, et al. *Am J Clin Pathol.* 2013;139[1]:102–109; Mani H. *Int J Lab Hematol.* 2014;36[3]:261–268). DOAC-affected one-stage factor activity results are spuriously low and may or may not demonstrate non-parallelism while false-positive inhibitor titers may be reported. If an anticoagulated patient presents with acute bleeding and a hemostasis workup is performed, not knowing that a DOAC is present or the impact that the DOAC can have on assays may result in the patient being falsely labeled as factor deficient with the presence of a factor inhibitor, such as a factor VIII inhibitor. To lend credence to this concept, a report on a series of cases of factor VIII inhibitors that developed in patients on DOAC therapy was submitted for publication in an international laboratory hematology journal but rejected due to the fact that the low factor activity and positive inhibitor results were factitious. The approach to a patient bleeding related to DOAC therapy versus a factor VIII inhibitor would be significantly different.

Chromogenic factor activity assays based on FXa generation (i.e. factor VIII, IX, and X assays) may be falsely underestimated by direct FXa inhibitors but not by direct thrombin inhibitor agents. Chromogenic FXIII activity will be spuriously underestimated in the presence of dabigatran but not direct FXa inhibitors.

Thrombin time assays are exquisitely sensitive to the presence of dabigatran such that prolongation may be evident even at trough levels while direct FXa inhibitor agents have no effect on the thrombin time (Adcock DM, et al. *Am J Clin Pathol.* 2013;139[1]:102–109). The effect of dabigatran on measuring functional fibrinogen is method-dependent, but most fibrinogen assays are not affected by any of the DOACs (Favaloro EJ, et al. *Blood Transfus.* 2017;15[6]:491–494).

Tests used to screen and confirm the presence of a lupus anticoagulant that are based on the aPTT or Russell's viper venom may be variably affected by the presence of any DOAC, leading to falsely elevated or false-positive lupus anticoagulant results. aPTT-based screening and phospholipid neutralization methods are generally affected more commonly by dabigatran than by direct FXa inhibitors, while both classes of DOACs impact RVV-based phospholipid neutralization and screening assays. DOAC therapy therefore can cause false-positive lupus anticoagulant results, and this can greatly impact therapy as lupus anticoagulants are generally treated using long-term anticoagulation with VKA and not DOACs.

Thrombophilia testing may be affected by the presence of DOACs depending on the underlying test methodology. Chromogenic antithrombin activity assays that are based on FXa generation are overestimated in the presence of direct FXa inhibitor drugs, and assays based on FIIa generation are overestimated by dabigatran. Clot-based protein C and S assays are falsely overestimated by all DOACs. A deficiency of antithrombin or protein C or S could potentially be missed in DOAC-treated patients. In this population, chromogenic protein C and free protein S antigen assays are recommended due to their lack of DOAC interference. Assays for activated protein C resistance (APCr) are typically overestimated in the presence of any DOAC masking protein C resistance. However, with certain methodologies, such as a prothrombinase-based APCr assay, the levels of dabigatran typically evident when treated with a standard dose can elevate results to the extent that an abnormal APCr result is falsely extended into the normal range (Gessoni G, et al. *Blood Transfus.* 2017;15[6]:562–567).



All DOAC-affected thrombophilia and lupus anticoagulant assays may be impacted by even trough levels of DOACs, depending on the specific assay and the specific DOAC present (Gosselin RC, et al. *Int J Lab Hematol.* 2019;41[suppl 1]:33–39). To obtain accurate results, hemostasis testing should be performed four to five days after DOAC therapy is discontinued. When anticoagulant therapy cannot or should not be discontinued and testing must be completed, other options must be entertained. Testing may be sent to a laboratory that offers methods not interfered with by DOACs (for example, a chromogenic antithrombin based on FXa rather than FIIa inactivation for a patient on dabigatran or a free protein S antigen rather than a clot-based protein S activity). Another consideration is to switch anticoagulant therapy from DOAC to low-molecular-weight heparin for a transient period. Although not FDA approved, adsorbent agents (primarily using activated charcoal) have been developed, often in the form of tablets, that can be added to plasma and following centrifugation remove DOACs from the plasma sample. These tablets tend to remove even high concentrations of DOACs, but there appears to be a modest increase in thrombin generation noted in post-treatment of normal plasma (DOAC naive) samples (Kopatz WF, et al. *Thromb Res.* 2018;170:97–101).

Anticoagulation using DOACs in preference to warfarin is increasing in popularity. These anticoagulants can significantly affect many hemostasis assays, often causing results to be spurious. In general, DOACs may cause the results of factor activity assays to be spuriously reduced, factor inhibitor assays to be spuriously positive, lupus anticoagulant assays to be spuriously positive, and thrombophilia testing to be spuriously normal. Such factitious results may have a negative impact on patient care. Laboratorians can play an important role in helping clinicians order appropriate testing in patients on DOACs as well as in assisting in the proper interpretation of results.

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