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Direct Oral Anticoagulants (DOACs) in the Laboratory: 2015 Review

D.M. Adcock [a,*], R. Gosselin [b]

[a] Colorado Coagulation, Laboratory Corporation of America® Holdings, Englewood, CO, United States
[b] University of California, Davis Health System, Sacramento, United States

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ABSTRACT

Direct oral anticoagulant therapies, including direct anti-Xa and thrombin inhibitors have recently been introduced and may have advantages over vitamin K antagonists such as warfarin. This review describes briefly the clinical utility and mechanism of action of these agents. Detailed information is provided on effect of these agents on routine assays including the APTT and PT as well as their impact on specialty laboratory assays. Also included are the use of drug specific assays and a discussion of alternative methods to determine relative drug concentration, such as evaluating drug calibrators in APTT and PT assays and using heparin calibrated anti-Xa assays to measure direct Xa inhibitors.

Contents

Introduction . . . 7
Laboratory Assays and DOACS - An Overview . . . 8
Dabigatran – Routine Screening Assays . . . 8
Dabigatran – Drug Specific Assays . . . 9
Dabigatran - Interference in Specialty Coagulation Assays . . . 9
Anti-Xa DOACs- Routine Screening Assays . . . 10
Direct Anti-Xa DOAC – Drug Specific Assays . . . 10
Anti-Xa DOAC - Interference in Special Coagulation Assays . . . 10
Conclusion . . . 11
Conflict of Interest Statement . . . 11
References . . . 11

Introduction

Oral anticoagulant agents recently approved in both the United States (US) and Europe include dabigatran etexilate (Pradaxa®, Boehringer-IngelheimPharma GmbH & Co., Ingelheim, Germany), rivaroxaban (Xarelto® - Janssen and Bayer HealthCare), apixaban (Eliquis, Bristol-Myers Squibb/Pfizer), and edoxaban (Savaysa, Daiichi Sankyo, Inc). Dabigatran is a direct inhibitor of thrombin while rivaroxaban, apixaban, and edoxaban are direct inhibitors of activated factor X (FXa). This new class of anticoagulants has been referred to as non-vitamin K or novel oral anticoagulants (NOACs), target-specific oral anticoagulant agents (TSOACs), or direct oral anticoagulant agents (DOACs). The International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) for the control of anticoagulation recommends the term DOACs [1].

The most common clinical indications for these rapid-acting anticoagulant drugs includes stroke prevention in non-valvular atrial fibrillation, thromboprophylaxis in hip or knee replacement surgery, and for the treatment as well as secondary prevention of venous thromboembolic disease (VTE), including deep venous thrombosis and pulmonary embolus. It is estimated that 3 million individuals in the US suffer atrial fibrillation, as many as 900,000 could be affected with

* Corresponding author at: Colorado Coagulation, 8490 Upland Dr., Englewood CO 80112, United States. Tel.: +1 720 568 4328; fax: +1 720 568 4314.
E-mail address: adcockd@labcorp.com (D.M. Adcock).

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VTE annually, and approximately 300,000 undergo hip and 700,000 knee replacement yearly [2–4]. In clinical trials, DOACs been shown to be at least as effective as warfarin, but with a reduced incidence of intracranial hemorrhage [5–10]. As each of these agents has predictable pharmacodynamics, pharmacokinetics and wide therapeutic windows, routine therapeutic monitoring is not required [9,11]. Although therapeutic ranges have not been validated by the pharmaceutical companies that manufacturer DOACs, information about drug concentration is available in select FDA summary reports and some published studies [12–16].

DOACs are rapidly-acting, target-specific anticoagulants that inhibit both free and bound activated serine proteases, unlike heparin that can inhibit only free proteases [17,18]. This is of clinical importance because bound thrombin and FXa retain activity. For example, activated factor X (FXa) within the prothrombinase complex (bound FXa) is 300,000 fold more efficient in converting prothrombin to thrombin, than is circulating (free) FXa. The ability to inactivate bound serine proteases makes the anticoagulant action of DOACs more robust than warfarin or heparin. DOACs have relatively short half- lives and multiple clearance mechanisms including both hepatic and renal clearance, although dabigatran is cleared exclusively through the kidneys. Given the many advantages of DOACS, their use may be favored over both warfarin and heparin and it is likely that over time DOACs will be prescribed to millions of patients annually [10].

Laboratory Assays and DOACS - An Overview

DOACs present unique challenges with both routine screening as well as specialty assays of coagulation, and, although routine monitoring is not required, drug specific assays to measure plasma concentration are available. Effect on routine and specialty assays as well as assays to monitor DOACs will be discussed in subsequent sections.

Routine coagulation screening assays, including the prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin clotting time (TCT) are widely available on a routine and emergent basis in most clinical laboratories. These assays are not a reliable measure of DOAC anticoagulant effect. This is because the sensitivity of the PT and APTT varies considerably based on reagents used, as well as the specific DOAC being measured [19–24]. [Fig. 1] Given this, PT or APTT results (in seconds), in the presence of a given concentration of DOAC, cannot be standardized across laboratories [21–23]. Furthermore, because DOACs inhibit both free and bound serine proteases, a given prolongation of the clotting time, such as PT in seconds, when a patient is on warfarin, does not equate to the same level of anticoagulation when a patient has the same prolongation of PT but is on a direct Xa DOAC [25]. Patients can be fully anticoagulated on apixaban for example, with only a slight elevation of the PT [26]. The traditional TCT is exquisitely sensitive to the presence of dabigatran, with even trough levels resulting in "no clot detected" with some reagent systems [27]. Direct Xa DOACs will not prolong the TCT.

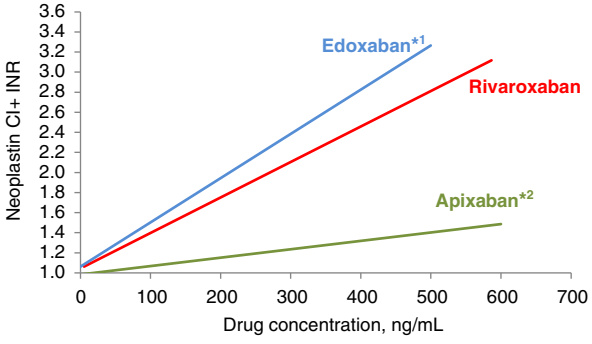

Fig. 1. Difference sensitivity of a single PT reagent (Neoplastin CI +, Diagnotisca Stago, Parisppany, NJ) to anti-Xa DOAC enriched pooled normal plasma. (Edoxaban data extrapolated from reference [15]; apixaban data unpublished observation DMA and RG, January 2015).

Although laboratory monitoring is not required when patients are administered DOACs, there are several clinical situations where determination of the level of anticoagulation may be of value, such as a patient experiencing hemorrhage or thrombosis, or requiring an emergent surgical procedure while on therapy [19,20]. DOAC concentrations can be accurately measured using a variety of laboratory methods [19, 20,23,28,29]. Mass spectrometry, when calibrated with each drug to be measured, is considered the gold-standard method and demonstrates good accuracy and precision over a broad concentration range although this test is not widely available [28]. More rapid methods including the dilute thrombin time, ecarin methods and chromogenic anti-Xa assays are potentially suitable means to measure DOACs, but must employ calibrators and controls specific for (or referenced against) the DOAC being measured [19,20,22,28,30]. Despite their availability, problems associated with existing assays used to quantitate DOACs include lack of: 1) FDA- approved DOAC calibrators or kits, 2) validated expected therapeutic plasma concentrations, and 3) knowledge of plasma concentrations associated with increased thrombotic or hemorrhagic risk. Furthermore, clot-based and chromogenic assays demonstrate variation between instrument/reagent systems, and also lack specificity [30,31].

If drug-specific assays are not available, it has been recommended that the relative sensitivity of a laboratory's PT and APTT to various types and concentrations of DOACs be determined locally. To accomplish this, commercially available calibrator material specific for the drug to be measured is assayed in the local laboratory against routine APTT and PT assays. This practice has been proposed by both the British Committee for Standards in Haematology (BCSH) as well as the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, although published studies verifying this practice were not available when these recommendations were made [32,33]. We used drug specific calibrators to assess PT and APTT DOAC sensitivity and compared this to sensitivity determined using well-characterized patients samples with drug concentrations measured by LC/MS-MS. With the exception of manufactured dabigatran calibrator evaluated using 2 APTT reagents (SynthASil and PTT-A), the use of drug-specific calibrators over-estimated reagent sensitivity compared to sensitivity determined using patients samples [34]. A patient's level of anticoagulation may be greatly underestimated when response to APTT and/or PT is based on a manufacturer's calibrator rather than samples from patients actually on drug. [Fig. 2A-C] This discrepancy likely reflects variation in the citrate concentration of the manufactured calibrators compared to that used for APTT and PT assays or may be due to the lyophilization process applied to the calibrator material.

Other laboratory methods, such as thromboelastographic measurements or endogenous thrombin potential assays have also been explored in patients taking DOAC, but their clinical use is not widely appreciated [20,35].

Dabigatran – Routine Screening Assays

The APTT is more responsive to dabigatran than is the PT while the TCT is exquisitely responsive. The APTT, however, cannot reliably distinguish therapeutic from subtherapeutic levels of dabigatran. In a study evaluating 7 APTT reagents (including 2 of the most common reagents used in the US, Actin FS and SynthasIL), we demonstrated that 18% of patients had a normal APTT despite measureable on-therapy dabigatran levels using LC-MS/MS [27]. This finding does not support the recommendation in the "Practice Guide" from the American Society of Hematology adapted in part from the American College of Chest Physicians Evidence-Based Clinical Practice Guideline on Antithrombotic and Thrombolytic Therapy (9th Edition), which states that in a patient on dabigatran who is bleeding, "a normal APTT is an indicator that