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Laboratory Measurement of the Anticoagulant Activity of the Target-specific Oral Anticoagulant Agents: A Systematic Review

Adam Cuker, MD[*], **Deborah M. Siegal, MD**[†], **Mark A. Crowther, MD, MSc**[†], and **David A. Garcia, MD**[‡]

[*]Department of Medicine and Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[†]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

[‡]Department of Medicine, University of Washington, Seattle, WA

Abstract

Background—The target-specific oral anticoagulant agents (TSOACs) do not require routine laboratory monitoring. However, laboratory measurement may be desirable in special situations and populations.

Objectives—This study's objective is to systematically review and summarize current evidence regarding laboratory measurement of the anticoagulant activity of dabigatran, rivaroxaban, and apixaban.

Methods—We searched PubMed and Web of Science for studies that reported a relationship between drug levels of dabigatran, rivaroxaban, and apixaban and coagulation assay results. Study quality was evaluated using Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2).

Results—We identified 17 eligible studies for dabigatran, 15 for rivaroxaban, and 4 for apixaban. For dabigatran, a normal thrombin time excludes clinically relevant drug concentrations. The activated partial thromboplastin time (APTT) and prothrombin time (PT) are less sensitive and may be normal at trough drug levels. The dilute thrombin time (R^2 0.92–0.99) and ecarin-based assays (R^2 0.92–1.00) show excellent linearity across on-therapy drug concentrations and may be used for drug quantification. In terms of rivaroxaban and apixaban, anti-Xa activity is

Corresponding author: Adam Cuker, MD, Hospital of the University of Pennsylvania, 3 Dulles, 3400 Spruce Street, Philadelphia, PA 19014, Telephone: (215) 615-6555, Fax: (215) 615-6599, adam.cuker@uphs.upenn.edu.

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linear (R^2 0.89–1.00) over a wide range of drug levels and may be used for drug quantification. Undetectable anti-Xa activity likely excludes clinically relevant drug concentrations. The PT is less sensitive (especially for apixaban); a normal PT may not exclude clinically relevant levels. The APTT demonstrates insufficient sensitivity and linearity for quantification.

Conclusions—Dabigatran, rivaroxaban, and apixaban exhibit variable effects on coagulation assays. Understanding these effects facilitates interpretation of test results in TSOAC-treated patients. More information on the relationship between drug levels and clinical outcomes is needed.

Keywords

apixaban; dabigatran; laboratory; monitoring; rivaroxaban

Dabigatran etexilate, an oral prodrug of the direct thrombin inhibitor dabigatran and the oral direct inhibitors of factor Xa, rivaroxaban and apixaban, are approved in the United States, Europe, and Canada to prevent stroke and systemic embolism in patients with nonvalvular atrial fibrillation (AF). They are also variably licensed for treatment of venous thromboembolism (VTE) and prevention of VTE after major orthopedic surgery (MOS) in certain jurisdictions. We refer to these agents collectively as target-specific oral anticoagulant agents (TSOACs) in this article. Synonymous terms preferred by other authors include direct-acting oral anticoagulant agents (DOACs) and new, novel, or nonvitamin K antagonist oral anticoagulant agents (NOACs) (1).

Unlike warfarin and other vitamin K antagonists (VKAs), the TSOACs are administered in fixed doses and do not require routine laboratory monitoring (2–4). However, measurement of their anticoagulant activity may be desirable in special clinical settings such as bleeding; the preoperative state; breakthrough thrombosis; suspected overdose, non-compliance, or drug interactions; and populations, including those with extremes in body weight and in the elderly and patients with renal insufficiency in whom there is a risk of drug accumulation. Assessment of anticoagulant effect may also be important in AF patients presenting with acute ischemic stroke prior to administration of thrombolytic therapy (5).

Numerous studies on use of coagulation assays for measurement of TSOAC activity have been published recently, though a systematic review has not been undertaken. The objective of our analysis was to summarize current evidence regarding laboratory measurement of the TSOAC anticoagulant activity and to provide evidence-based guidance to practicing cardiologists on the interpretation of coagulation tests in TSOAC-treated patients.

Methods

LITERATURE SEARCH

We performed a systematic review of the literature to examine current evidence for laboratory measurement of the TSOACs. A search of PubMed and Web of Science from inception through December 1, 2013, was undertaken separately for dabigatran, rivaroxaban, and apixaban using the following keywords: “Name of drug” AND ((laboratory measurement) OR laboratory monitoring)).

STUDY SELECTION

Articles were examined, first by title and abstract, then by review of the complete paper as indicated. Additional articles were sought by reviewing bibliographies. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the reference method for measurement of the plasma concentration of the TSOACs (6). Studies that reported the relationship between drug (or active metabolite) levels in human plasma, as measured directly using LC-MS/MS or indirectly using LC-MS/MS-validated calibration standards and one or more clinical coagulation assays were eligible for inclusion. We excluded animal studies, abstracts only, and non-English language publications.

DATA EXTRACTON

We extracted key characteristics from eligible studies and recorded them in an evidence table. These included: author, year of publication, setting, TSOAC (i.e., dabigatran, rivaroxaban, apixaban), reference method for measurement of drug levels, range of drug concentrations studied, test material (i.e., ex vivo patient plasma, ex vivo healthy control plasma, spiked normal plasma), dose (for studies using ex vivo plasma only), indication (for studies using ex vivo patient plasma only), number of samples (for studies using individual [i.e., unpooled] plasma only), coagulation assays and reagents, and descriptors of the relationship between drug level and coagulation assay (e.g., R^2 values, range of linearity, etc.).

QUALITY ASSESSMENT

Study quality was evaluated using Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2), a standardized tool for quality assessment of studies of diagnostic accuracy. The tool comprises 4 domains: patient selection, index test, reference standard, and flow and timing. Risk of bias is assessed across all domains; the first 3 domains are also assessed with respect to applicability to clinical practice (7).

Results

DABIGATRAN

Dabigatran etexilate, an oral non-peptide prodrug, is rapidly converted to the active drug dabigatran by ubiquitous esterases. Dabigtran directly inhibits both free and clot-bound thrombin. It has relatively poor bioavailability (~6.5%) and is eliminated predominantly by the kidneys (80%). In individuals with normal renal function, the half-life of dabigatran is 12 to14 hours. Prolonged clearance and bioaccumulation are observed in patients with renal insufficiency (8). In patients with nonvalvular AF and normal kidney function, the dose is 150 mg twice daily, which is reduced in patients with renal insufficiency.

Peak levels of dabigatran occur 2 to 3 hours after ingestion. Steady-state peak and trough concentrations in patients with AF and normal renal function taking dabigatran 150 mg twice daily are shown in Table 1 (8). Substantial interindividual variability in drug exposure is observed. In the PETRO (Prevention of Embolic and Thrombotic Events in Patients With Persistent Atrial Fibrillation) study, the range (5^{th} to 95^{th} percentile) in peak and trough

concentrations in patients taking 150 mg twice daily was 64 to 443 ng/ml and 31 to 225 ng/ml, respectively (9,10).

STUDY SELECTION—Our literature search yielded 160 articles. Nine additional references were identified from bibliographies. One hundred fifty-two articles were excluded: 135 did not report an original research study, 14 did not report drug levels measured by LC-MS/MS, and 3 were published as abstracts only. The remaining 17 articles (11–27) met eligibility criteria (Figure 1). Eligible studies were collectively conducted in 9 different countries across a range of dabigatran concentrations from 0 to 1886 ng/ml. Only 4 studies used ex vivo plasma from dabigatran-treated patients; the remainder involved ex vivo healthy volunteer plasma or normal plasma spiked in vitro with dabigatran as the test material (Table 2).

APTT—Twelve eligible studies reported a relationship between the activated partial thromboplastin time (APTT) and dabigatran levels. Three used ex vivo patient plasma, 2 ex vivo healthy volunteer plasma, and 7 normal plasma spiked with dabigatran in vitro (Table 2). Dabigatran prolonged the APTT in a concentration-dependent manner in both ex vivo and in vitro studies. The dose response was linear up to a concentration of 200 to 300 ng/ml, then flattened out at higher drug levels (17,28). This curvilinear relationship did not permit quantitative assessment of dabigatran levels, particularly at higher concentrations.

Commercial APTT reagents differ widely in their sensitivity to dabigatran. The APTT of plasma spiked with dabigatran 120 ng/ml ranged from 26.0 to 91.9 seconds in a cross-validation study of 9 different APTT methods (27). These findings suggest that coagulation laboratories should perform dose-response studies using calibration standards to determine the sensitivity of their particular APTT method to dabigatran and communicate the results to clinicians. The least sensitive reagents required a dabigatran concentration of ~400 ng/ml to produce a 2-fold prolongation in the APTT over control (19). The APTT may not be prolonged in the presence of typical on-therapy trough levels (Table 1), particularly if a relatively insensitive reagent is used. In a study of ex vivo plasma from patients taking dabigatran 150 mg twice daily, 18% of subjects had a normal APTT at trough (25). In another study of patients with AF, some samples had an APTT within the normal range despite dabigatran concentrations as high as 60 ng/ml (23).

PT/INR—Eleven studies reported a relationship between dabigatran levels and the prothrombin time/international normalized ratio (PT/INR). Eight used spiked plasma, 2 ex vivo patient plasma, and 1 ex vivo plasma from healthy volunteers (Table 2). Dabigatran prolonged the PT in a concentration-dependent manner, as defined by an exponential (i.e., nonlinear) relationship (17). The PT/INR was less sensitive to dabigatran than the APTT. In an ex vivo study of patients with AF, an INR of 1.2 or greater was only observed with dabigatran concentrations in excess of 400 ng/ml (23).

As with APTT, commercial PT reagents differ in their sensitivity to dabigatran. In a survey of 71 coagulation laboratories, the PT of plasma spiked to a concentration of 300 ng/ml ranged from 15.7 to 50.2 seconds, depending on the reagent (27). Prothrombin time may be measured using two assay types. The Quick method is influenced by the entire extrinsic and

common pathways of coagulation whereas the Owren method is affected only by factors II, VII, and X. In studies of spiked plasma, Quick PT reagents were more sensitive to dabigatran than Owren reagents (14,27).

Point-of-care devices are available for measuring the INR (POC-INR) in whole blood in VKA-treated patients. In one study assessing the relationship between dabigatran levels and a single POC-INR system, the POC system yielded INR values 2- to 4-fold higher than those obtained using a laboratory PT/INR method. Dabigatran concentrations greater than 500 ng/ml were beyond the POC system's limit of detection (22).

TT—Six studies reported a relationship between thrombin time (TT) and dabigatran levels (Table 2). The TT (in unmodified form) was inordinately sensitive in both ex vivo and in vitro studies. Depending on the reagent, dabigatran concentrations of as little as 25 and up to 150 ng/ml exceeded the limits of detection (15–17,28).

DILUTE TT—In the dilute TT assay, the excessive sensitivity of the TT is overcome by diluting the plasma sample (21). Seven studies reported a relationship between the dilute TT and dabigatran concentration (Table 2). Two studies used an in-house modification of the TT (15,20); 5 employed the HEMOCLOT Thrombin Inhibitor assay (HYPHEN-BioMed, Neuvillesur-Oise, France), a commercially available dilute TT test (17,21,23–25). Dabigatran prolonged the assay in a concentration-dependent manner. The relationship showed a high degree of linearity with R^2 values ranging between 0.92 and 0.99 in both in vitro and ex vivo studies. The lower limit of detection according to the manufacturer is 50 ng/ml (21). Two studies determined the assay to be less accurate and more variable at concentrations below 50 to 100 ng/ml (15,21). The dilute TT is currently not widely available; in a recent survey in Australia and New Zealand, only 9 of 592 laboratories reported using it (29).

ECARIN-BASED ASSAYS—Ecarin is a snake venom that cleaves prothrombin to form meizothrombin, an unstable intermediate of thrombin. Dabigatran inhibits the thrombin-like activity of meizothrombin (24). Two assays use ecarin as an activator: the ecarin clotting time (ECT) and ecarin chromogenic assay (ECA).

Six studies reported a relationship between ECT and dabigatran levels (Table 2). Both in vitro and ex vivo studies demonstrated a high degree of linearity with R^2 values ranging between 0.92 and 1.00. Loss of linearity was observed in 2 studies at dabigatran levels in excess of 470 to 500 ng/ml (17,19). A relationship between ECA and drug levels was reported in 4 studies (Table 2). The relationship was linear with R^2 values of 0.94 to 0.99 in in vitro and ex vivo samples. One study identified greater variability at dabigatran concentrations <50 ng/ml (23). The ECT and ECA are hampered by lack of standardization, variability in sensitivity to dabigatran among different lots of ecarin, and limited availability (17,20).

OTHER ASSAYS—Relationships between dabigatran level and the dilute PT, prothrombinase-induced clotting time (PiCT), and activated clotting time (ACT) were each reported in a single study. Both the dilute PT and PiCT evinced a complex nonlinear dose-

response curve (17). As with heparin, the ACT proved insensitive to lower concentrations of dabigatran. In an ex vivo study, the ACT was normal in 40% of trough samples despite on-therapy dabigatran levels (25).

RIVAROXABAN

Rivaroxaban is an oral inhibitor of free and clot-associated factor Xa through reversible, competitive interactions with its active site (30). Bioavailability following oral administration is dose dependent (80% to 100% following a 10 mg dose; 66% following a 20 mg dose). It is highly bound to plasma proteins (>90%) (31); plasma levels peak 2 to 4 hours following oral administration (32,33). Partially excreted by the kidneys (36%), rivaroxaban has a half-life of 6 to 13 hours depending on dose and age (31–35). Table 1 shows expected peak and trough plasma concentrations in patients with AF treated with 20 mg daily (36).

STUDY SELECTION—Our literature search yielded 134 unique rivaroxaban articles. Two additional references were identified from bibliographies. We excluded 121 articles: 108 did not report an original research study, 12 did not report a relationship between a coagulation assay and drug levels measured by LC-MS/MS, and 1 was published as an abstract only. The remaining 15 articles (27,37–50) met eligibility criteria (Figure 1). Rivaroxaban concentrations in eligible studies ranged from 0 to >1000 ng/ml. Four studies used ex vivo plasma from rivaroxaban-treated patients, one incorporated ex vivo plasma from healthy controls, and the remainder used normal plasma spiked in vitro with rivaroxaban (Table 3).

PT—We found 11 studies evaluating the effect of rivaroxaban on PT (Table 3). In general, rivaroxaban prolonged the PT in a concentration-dependent, linear fashion in plasma spiked with rivaroxaban and in plasma from patients receiving rivaroxaban for approved indications. On-therapy rivaroxaban concentrations showed a modest effect on the PT. Typical trough (41 to 60 ng/ml) and peak (219 to 305 ng/ml) concentrations increased PT by 6% to 19% and 50% to 135%, respectively (27,38,42,47). Assay sensitivity varied significantly among thromboplastin reagents. Inter-assay variability was reduced by use of an international sensitivity index (ISI) specific for rivaroxaban, but not by conversion to an INR used for monitoring VKA therapy (38,42). These observations suggest that coagulation laboratories should perform dose-response studies using calibration standards to determine the sensitivity of their particular PT method to rivaroxaban and communicate the results to clinicians.

APTT—Five studies evaluated the effect of rivaroxaban on APTT (Table 3). While rivaroxaban prolonged APTT in a dose-dependent manner, the overall relationship between rivaroxaban concentration and APTT prolongation was nonlinear with studies reporting conflicting data regarding the concentration ranges over which nonlinearity was most pronounced (27,41,45). Similar to PT results, there was significant variability among reagents and among individual laboratories in a multicenter study (27,45). Hillarp et al. reported that the APTT assay was insensitive at the lowest drug level studied (25 ng/ml) (41).

ANTI-Xa ACTIVITY—Ten studies assessed the effect of rivaroxaban on anti-Xa activity (Table 3). In general, the studies showed a linear, concentration-dependent relationship between rivaroxaban concentration and anti-Xa activity over a wide range of concentrations (e.g.. 20–660 ng/ml) when measured using a standard curve generated with rivaroxaban calibrators and controls with R^2 values ranging from 0.95 to 1.00 (39,43,44,49,50). The correlation was less robust at concentrations <100 ng/mL (49). However, Samama and colleagues demonstrated that low rivaroxaban concentrations could be measured with a modified anti-Xa test using less diluted samples (46). Investigators found a greater degree of assay imprecision at higher rivaroxaban concentrations (800 ng/ml) in one study (45). In a multicenter study, both intra- and interlaboratory precision were satisfactory except at the lower limit of detection (20 ng/ml); use of a centrally distributed reagent reduced interlaboratory variability (46). Mathematical modeling also decreased interassay variability resulting from different sensitivities of individual reagents to rivaroxaban (40). When commercial anti-Xa assays were used with unfractionated or low-molecular-weight heparin (LMWH) calibrators (rather than rivaroxaban calibrators), the relationship remained linear up to a rivaroxaban concentration of 500 ng/ml (38,41).

OTHER ASSAYS—The relationship between rivaroxaban concentration and the dilute PT, dilute Russell viper venom time (dRVVT), and PiCT was evaluated in a single study (38). Researchers uncovered a linear, dose-dependent relationship between rivaroxaban and the dilute PT. Rivaroxaban increased the dRVVT ratio (expressed as ratio vs. baseline) in a concentration-dependent, but nonlinear manner. At low concentrations of rivaroxaban (<200 ng/ml), there was a paradoxical shortening of PiCT, whereas PiCT was prolonged in a concentration-dependent fashion at higher concentrations.

APIXABAN

Like rivaroxaban, apixaban is a small, orally available, direct inhibitor of coagulation factor Xa (51). It has 50% bioavailability and, in healthy volunteers, reaches its maximum plasma concentration approximately 3 hours after ingestion. Apixaban is highly protein-bound in plasma and concomitant food intake has little impact on its pharmacokinetics (52). Metabolized through multiple routes, apixaban is less dependent on renal clearance than dabigatran and rivaroxaban. In persons with normal renal function, apixaban has a half-life of approximately 12 hours (53). As measured by LC-MS/MS, the expected steady-state concentrations of apixaban have been published by Frost et al. (52) and are shown in Table 1.

STUDY SELECTION—Our literature search for apixaban yielded 70 articles; 3 additional references were identified from bibliographies. Sixty-nine articles were excluded: 68 did not report an original research study and 1 was published as an abstract only. The remaining 4 articles (37,54–56) met eligibility criteria (Figure 1). Eligible studies collectively evaluated apixaban across a range of concentrations from 0 to 2500 ng/ml (Table 4).

PT/INR—We found 3 studies that reported the relationship between the PT and apixaban levels. One study used both spiked normal plasma and ex vivo plasma from apixaban-treated patients (37), another used spiked plasma as well as ex vivo plasma from healthy volunteers

taking apixaban (55), and the third study included only spiked normal plasma samples (56) (Table 4). For the 2 studies that used ex vivo plasma, the relationship was linear in one (37) and curvilinear in the other (55). Correlation was modest with R^2 values of 0.36 and 0.41, respectively. Across in vitro and ex vivo samples and for a variety of reagents, the PT was inadequately sensitive to apixaban, not only below, but also above the expected trough concentration of 50 ng/ml.

APTT—Only one study compared apixaban concentrations to APTT (Table 3), which was measured using 10 different APTT reagents in normal plasma samples spiked with 10 different concentrations of apixaban. The sensitivity of the APTT was unacceptably low; all assays yielded a ratio of 1.1 times control when the spiked apixaban concentration was 100 ng/ml (i.e., twice the expected trough concentration).

ANTI-Xa ACTIVITY—Three studies compared anti-Xa activity measurements to the plasma concentration of apixaban (Table 4). In 2 of the studies (37,54), ex vivo patient samples were used while the third study (56) included only spiked samples of normal plasma. In general, the relationship was linear at all apixaban concentrations, with R^2 values ranging from 0.89 to 0.97. Available evidence suggests that an anti-Xa assay calibrated with LMWH standards will also correlate linearly with apixaban concentrations (37,54).

STUDY QUALITY

Assessment of study quality using QUADAS-2 criteria (7) highlighted several recurrent methodologic concerns among eligible studies (Table 5). Many studies used in vitro samples or ex vivo samples from healthy controls rather than ex vivo patient samples. Concern about the applicability of these studies to clinical practice within the patient selection domain was judged to be high. Some studies examined assays not widely available to clinicians (e.g., ECA, ECT, dilute TT). Concern regarding applicability of these studies to clinical practice across the index test domain was judged to be high. Because data correlating plasma TSOAC levels and clinical outcomes are scarce, concern about the applicability of the reference standard (plasma drug concentration) to clinical practice was judged to be high for all eligible studies.

Discussion

This systematic review sought to examine evidence for laboratory measurement of the anticoagulant activity of dabigatran, rivaroxaban, and apixaban. Although data on the relationship between plasma TSOAC levels and clinical outcomes are beginning to emerge (57), there is, as yet, no evidence that routine monitoring or dose titration will improve outcomes. Nevertheless, measurement may be useful in three circumstances: 1) to determine if very high levels are present (in the case of suspected excess effect; e.g., due to overdose or bioaccumulation); 2) to determine if drug is present in typical on-therapy ranges (e.g., in the case of suspected therapeutic failure); and 3) to determine if any clinically relevant drug effect is present (e.g., in the case of bleeding or planned invasive procedures). An ideal assay would thus show adequate linearity, sensitivity, and reproducibility to enable quantification across a broad range of drug levels. Apart from LC-MS/MS, a test generally

restricted to select reference laboratories, no single coagulation assay meets these idealized standards. Therefore, it is important for clinicians to be aware of how coagulation tests perform at TSOAC concentrations below, within, and above typical on-therapy ranges (Central Illustration).

DABIGATRAN

The effect of dabigatran on various coagulation assays is summarized in the Central Illustration. The TT is exquisitely sensitive to dabigatran. A normal TT excludes the presence of clinically relevant drug levels; however, the assay is too sensitive for quantification within and above the on-therapy range. The dilute TT, ECT, and ECA show a high degree of linearity at drug levels >50 ng/ml and are thus useful for quantification across the entire on-therapy range. They may be unreliable at concentrations below this threshold. The APTT is relatively insensitive to dabigatran; a normal APTT may not exclude clinically relevant below or on-therapy drug levels. The curvilinear response of APTT at higher drug levels does not permit accurate quantification. The PT has even poorer sensitivity and may be normal within much of the on-therapy range.

RIVAROXABAN

Anti-Xa activity measured using chromogenic substrates and rivaroxaban or heparin/LMWH calibrators correlates linearly with rivaroxaban over a wide range of concentrations (20 to 660 ng/ml) (Central Illustration). When rivaroxaban calibrators are used, anti-Xa assays can provide a quantitative measure of rivaroxaban concentration. A negative anti-Xa assay likely excludes clinically relevant rivaroxaban levels. Although rivaroxaban prolongs the PT, assay results vary markedly with different thromboplastin reagents. A normal PT does not rule out the presence of clinically significant below or within on-therapy rivaroxaban concentrations; however, a prolonged PT qualitatively indicates the drug's presence. The APTT is not suitable for measuring rivaroxaban due to the nonlinear relationship with rivaroxaban concentration, poor sensitivity, and significant variability between reagents.

APIXABAN

Although both the PT and APTT may be prolonged in the presence of apixaban, neither is sufficiently sensitive to exclude the presence of clinically relevant on-therapy drug concentrations (Central Illustration). Anti-Xa activity measurements demonstrate a strong linear correlation with apixaban concentration; the absence of detectable anti-Xa activity (whether the standard curve is established with apixaban or LMWH) likely excludes the presence of physiologically important apixaban activity.

SUGGESTIONS AND COMPARISON WITH GUIDANCE DOCUMENTS

Recommendations for laboratory measurement of the TSOACs differ by drug and clinical objective. The findings of our systematic review support the suggestions summarized in Table 6. These suggestions align with recommendations provided in drug labels and published guidance documents, with two notable exceptions. First, we found strong evidence from studies of ex vivo patient samples that a normal APTT does not definitively exclude on-therapy dabigatran concentrations (23,25). This observation is at variance with

guidelines from the American Society of Hematology (ASH) and the British Committee for Standards in Haematology (BCSH), which state that a normal APTT is likely to exclude therapeutic intensity dabigatran (58) or contribution of dabigatran to bleeding (59). Second, we found that a normal PT does not exclude clinically relevant rivaroxaban levels. The BCSH statement, in contrast, comments that a normal PT ratio with most reagents excludes therapeutic intensity rivaroxaban (58). The ASH and BCSH statements were published in 2011 and 2012, respectively. Discrepancies between our findings and these statements may reflect availability of new information since their publication regarding a wider variety of test reagents and their sensitivity to the TSOACs.

LIMITATIONS

Quality assessment highlighted several key limitations of eligible studies (Table 5). First, on-therapy ranges (Table 1) were derived from pharmacokinetic analyses. We resisted the term “therapeutic range” because data on how these ranges correlate with clinical outcomes are sparse, though they are beginning to emerge (57). Second, many eligible studies used either ex vivo or spiked plasma samples from healthy controls rather than ex vivo samples from TSOAC-treated patients. Reassuringly, results obtained with patient samples generally aligned with those from healthy controls. Third, we identified only 4 eligible apixaban studies, just 2 of which used ex vivo patient samples. Because apixaban was the most recent TSOAC to receive regulatory approval, we expect additional studies of its laboratory measurement will be forthcoming. Such studies are also needed for TSOACs not yet approved in North America and Europe (e.g., edoxaban).

CONCLUSIONS

A relatively large number of published studies have assessed the relationship between coagulation tests and levels of dabigatran, rivaroxaban, and apixaban. Each drug produces unique effects on coagulation assays. Our systematic review provides guidance to the clinician on how to use and interpret coagulation test results in TSOAC-treated patients. Further studies are needed to define the relationship between drug levels, coagulation test results, and clinical outcomes.

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ABBREVIATIONS AND ACRONYMS

ACT	activated clotting time
AF	atrial fibrillation
APTT	activated partial thromboplastin time
LC-MS/MS	liquid chromatography/tandem mass spectrometry
PT/INR	prothrombin time/international normalized ratio

TSOAC	target-specific oral anticoagulant agent
TT	thrombin time
VKA	vitamin K antagonist

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PERSPECTIVES

Competency In Medical Knowledge 1

The prothrombin time (PT) and activated partial thromboplastin time (APTT) do not show sufficient sensitivity or linearity for quantification of dabigatran, rivaroxaban, or apixaban. A normal PT and/or APTT may not exclude clinically relevant anticoagulant effects of these drugs.

Competency in Medical Knowledge 2

A normal thrombin time likely excludes clinically relevant plasma levels of the direct thrombin inhibitor, dabigatran. The dilute thrombin time and ecarin-based assays may be used to measure dabigatran activity.

Competency In Medical Knowledge 3

An anti-Xa assay using drug-specific calibrators may be used to measure the activity of the factor Xa inhibitors rivaroxaban and apixaban.

Competency In Patient Care

The target-specific oral anticoagulants dabigatran, rivaroxaban and apixaban do not require laboratory monitoring of coagulation during routine clinical use, but measurement of their anticoagulant effect may be desirable in certain circumstances.

Translational Outlook

Development of laboratory assays for measurement of the anticoagulant activity of these target-specific oral anticoagulants is a high priority. Ideally, these assays should be sufficiently sensitive to detect all clinically relevant drug concentrations, show linearity across a wide range of concentrations to permit quantification, and be reproducible and simple to perform at the point of care.

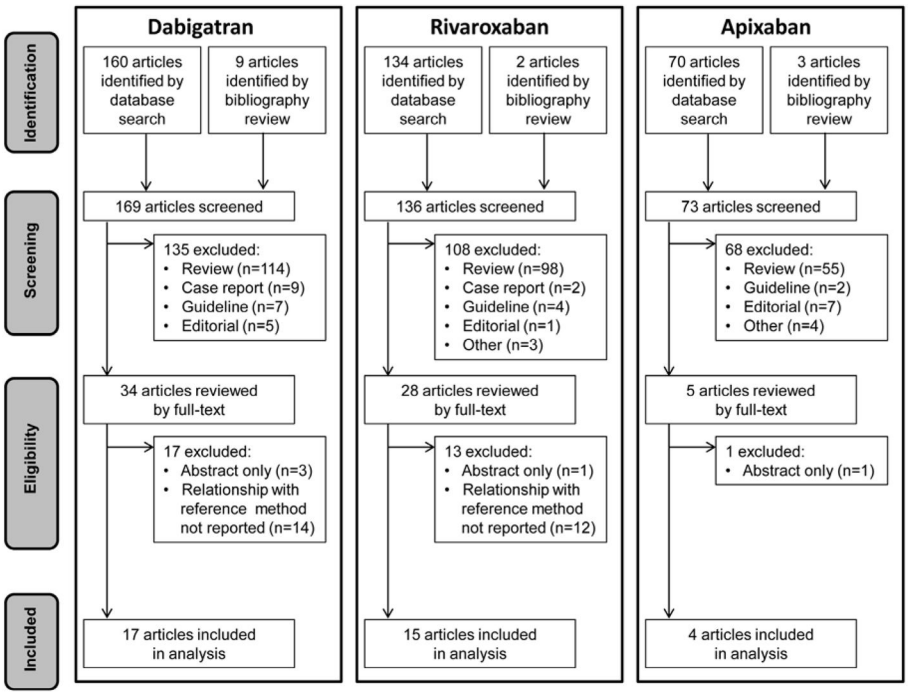
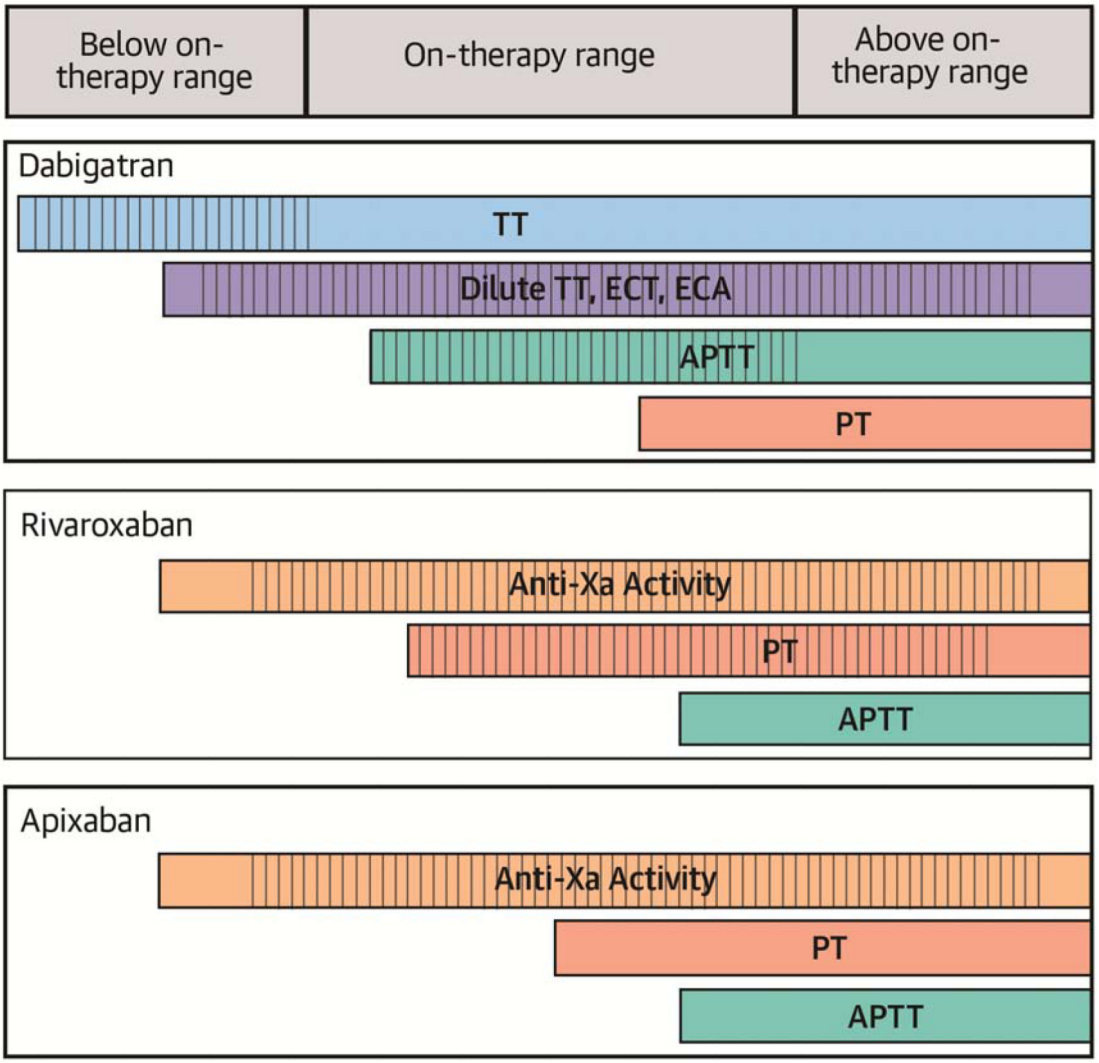


FIGURE 1. PRISMA Diagram

This PRISMA flow diagram illustrates dabigatran, rivaroxaban, and apixaban literature searches



CENTRAL ILLUSTRATION. Sensitivity and Linearity of Coagulation Assays to Below, Within, and Above Typical On-therapy Concentrations of Dabigatran, Rivaroxaban, and Apixaban

Red bars and vertical hatching correspond to the approximate range of detectability (i.e., sensitivity) and linearity, respectively, of each assay to below, within, and above typical ontherapy concentrations of dabigatran, rivaroxaban, and apixaban. Ranges are approximations and may vary based on choice of reagent. Typical on-therapy drug levels are shown in Table 1.

APTT = activated partial thromboplastin time; ECA = ecarin chromogenic assay; ECT = ecarin clotting time; PT = prothrombin time; TT = thrombin time.

TABLE 1

Expected Steady-state Peak and Trough Concentrations of Dabigatran, Rivaroxaban, and Apixaban Derived from Pharmacokinetic Studies.

Drug	Dose	Study Population	Peak (ng/ml)	Trough (ng/ml)	References
Dabigatran	150 mg BID	Patients with AF	184*	90*	9,10
Rivaroxaba	20 mg QD	Patients with AF	274†	30†	33
Apixaban	5 mg BID	Healthy male volunteers	129‡	50‡	52

Only standard doses approved for atrial fibrillation are shown.

*median;

†median estimated by mathematical modeling;

‡mean

AF = atrial fibrillation; BID = twice daily; QD = once daily.

TABLE 2

Characteristics of Eligible Dabigatran Studies

Reference	Setting	Range of Drug Concentration (ng/ml)	Test Material	Indication (n)	Dose (mg)	Coagulation Test	R^2
10	Germany	NR	Ex vivo patient plasma	MOS (287)	12.5–300 BID; 150–300 QD	APTT ECT	NR NR
11	Germany	NR	Ex vivo healthy volunteer plasma	NA (80)	10–400 SD; 50–400 TID	APTT ECT PT/INR TT	0.85 0.92–0.96 0.83–0.85 0.60–0.86
12	Germany	NR	Ex vivo healthy volunteer plasma	NA (35)	150 BID	APTT ECT	NR NR
13	Sweden	0–1000	Spiked normal pooled plasma	NA (NA)	NA	APTT PT/INR	NR NR
14	USA	0–500	Spiked normal pooled plasma	NA (NA)	NA	APTT Dilute TT TT	0.95* 0.98 NR
15	USA, UK, Canada	25–500	Spiked normal pooled plasma	NA (NA)	NA	APTT PT/INR TT	0.96 0.99 NR
16	Belgium	4.7–943	Spiked normal pooled plasma	NA (NA)	NA	APTT Dilute PT Dilute TT ECA ECT PiCT PT/INR TT	0.96–0.99 NR 0.99 0.99 1.00 NR NR NR
17	Austria	0–480	Spiked normal pooled plasma	NA (NA)	NA	APTT PT/INR	NR NR
18	Germany	0–1000	Spiked normal pooled plasma	NA (NA)	NA	APTT ECT PT/INR	NR NR NR
19	New Zealand	30–960	Spiked normal pooled plasma	NA (NA)	NA	Dilute TT	NR
20	Germany	0–1886	Spiked normal pooled plasma	NA (NA)	NA	Dilute TT	0.99
21	Germany	50–1400	Spiked healthy volunteer plasma	NA (4)	NA	POC-INR PT/INR	NR NR
22	Sweden	<0.5–586	Ex vivo patient plasma	AF (70)	NR	APTT Dilute TT ECA PT/INR	0.58 0.97 0.96 0.48

Reference	Setting	Range of Drug Concentration (ng/ml)	Test Material	Indication (n)	Dose (mg)	Coagulation Test	R^2
23	USA	0–500	Ex vivo patient plasma Spiked normal pooled plasma	AF (NR) VTE (NR) NA (NA)	150 BID NA	Dilute TT ECA	0.96–0.97 0.99
24	USA	18–487	Ex vivo patient plasma	AF (29) VTE (6)	NR	ACT APTT Dilute TT ECA ECT PT/INR TT	0.71 0.74–0.82 0.92 0.94–0.99 0.98 0.60–0.86 0.75–0.97
25	Sweden	0–985.5	Spiked normal pooled plasma	NA (NA)	NA	PT/INR	NR
26	Finland	120–300	Spiked normal pooled plasma	NA (NA)	NA	APTT PT/INR TT	NR NR NR

* Relationship described by second order polynomial regression.

ACT = activated clotting time; APTT = activated partial thromboplastin time; ECA = ecarin chromogenic assay; ECT = ecarin clotting time; INR = international normalized ratio; MOS = major orthopedic surgery; NA = not applicable; NR = not reported; PiCT = prothrombinase-induced clotting time; POC = point of care; PT = prothrombin time; SD = single dose; TT = thrombin time; VTE - venous thromboembolism; other abbreviations as in Table 1.

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TABLE 3

Characteristics of Eligible Rivaroxaban Studies

Reference	Setting	Range of Drug Concentration (ng/ml)	Test material	Indication (n)	Dose (mg)	Coagulation Test	R^2
36	USA, Canada	<10 – >1000	Spiked normal pooled plasma	NA (NA)	NA	PT/INR	NR
37	France	0–1000	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa APTT Dilute PT DiluteRVVT PiCT POC-INR PT/INR	NR 0.90–0.98 0.97–0.99 0.99 NR 1.00 0.99–1.00
38	France	NR	Ex vivo patient plasma	MOS (166)	10 QD	Anti-Xa APTT PT/INR	0.97–1.00 0.75 0.47
39	Germany	25–900	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa	NR
40	Sweden	0–1000	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa APTT PT/INR	NR NR NR
41	Italy	100–700	Spiked normal pooled plasma	NA (NA)	NA	PT/INR	NR
42	Switzerland	40–150	Ex vivo healthy volunteer plasma Spiked normal pooled plasma	NA (20) NA (NA)	10 QD NA	Anti-Xa	0.98–0.99
43	Germany	1.9–283.0	Ex vivo patient plasma	MOS (80)	10 QD	Anti-Xa	1.00
44	Netherlands	0–1000	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa APTT PT/INR	NR 1.00 1.00*
45	Europe, North America	20–662	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa	NR
46	Europe, North America	19–643	Spiked normal pooled plasma	NA (NA)	NA	PT/INR	NR
47	Netherlands	0–800	Spiked normal pooled plasma	NA (NA)	NA	PT/INR	1.00
48	Belgium	NR	Ex vivo patient plasma	AF, VTE (52)	NR	Anti-Xa PT/INR	0.95 0.58–0.66
26	Europe	60–305	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa APTT PT/INR	NR NR NR
49	France	NR	Ex vivo patient plasma	MOS (41)	10 QD	Anti-Xa PT/INR	0.99 NR

*Relationship described by second order polynomial regression.

RVVT = Russell viper venom time; other abbreviations as in Tables 1 and 2.

TABLE 4

Characteristics of Eligible Apixaban Studies

Reference	Setting	Range of Drug Concentration (ng/ml)	Test Material	Indication (n)	Dose (mg)	Coagulation test	R^2
36	USA, Canada	<10 – >1000	Ex vivo patient plasma Spiked normal pooled plasma	VTE (348) NA (NA)	5–10 BID, 20 QD NA	Anti-Xa PT/INR	0.88–0.89 0.36
53	Global	1–933	Ex vivo patient plasma	ACS (1691)	5–20 QD	Anti-Xa	0.97
54	USA	0–2500	*Ex vivo* healthy volunteer plasma Spiked healthy volunteer plasma	NA (39) NA (21)	20 SD NA	PT/INR	0.41
55	France	0–1000	Spiked normal pooled plasma	NA (NA)	NA	Anti-Xa APTT PT/INR	NR NR NR

ACS = acute coronary syndrome; other abbreviations as in Table 2.

TABLE 5

Study Quality Assessment Using QUADAS-2 Criteria

Reference	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Dabigatran Studies							
10	L	U	U	L	L	H	H
11	NA	U	U	L	H	H	H
12	NA	U	U	L	H	H	H
13	NA	U	L	L	H	L	H
14	NA	U	L	L	H	H	H
15	NA	L	L	L	H	L	H
16	NA	U	L	L	H	H	H
17	NA	U	L	L	H	L	H
18	NA	U	L	L	H	H	H
19	NA	U	L	L	H	H	H
20	NA	U	L	L	H	H	H
21	NA	U	L	L	H	L	H
22	U	U	U	L	L	H	H
23	U	U	U	L	L	H	H
24	U	U	U	L	L	H	H
25	NA	U	L	L	H	L	H
26	NA	U	L	L	H	L	H
Rivaroxaban Studies							
26	NA	U	U	L	H	L	H
36	NA	U	U	L	L	L	H
37	NA	U	L	L	H	H	H
38	U	U	U	L	L	L	H
39	NA	U	L	L	H	L	H
40	NA	U	L	L	H	L	H
41	NA	U	L	L	H	L	H

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Reference	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
42	NA	L	L	L	H	L	H
43	U	U	U	L	L	L	H
44	NA	U	L	L	H	L	H
45	NA	L	L	L	H	L	H
46	NA	L	L	L	H	L	H
47	NA	U	L	L	H	L	H
48	U	U	U	L	L	L	H
49	U	U	U	L	L	L	H
Apixaban Studies							
36	L	U	U	L	L	L	H
53	L	U	U	L	H	L	H
54	NA	U	U	L	H	L	H
55	NA	L	L	L	H	L	H

H = high; L = low; U = unclear; other abbreviations as in Table 2.

TABLE 6

Suggestions for Laboratory Measurement of Target-specific Oral Anticoagulant Agents

	Clinical Objective					
	Determine Whether Clinically Relevant Below On-therapy Drug Levels are Present		Estimate Drug Levels Within On-therapy Range		Determine Whether Above On-therapy Drug Levels are Present	
Drug	Suggested Test	Interpretation	Suggested Test	Interpretation	Suggested Test	Interpretation
Dabigatran	TT	A normal TT likely excludes clinically relevant drug levels	Dilute TT, ECA, ECT	-	APTT, Dilute TT, ECA, ECT	A normal APTT likely excludes excess drug levels. Only dilute TT, ECA, and ECT are suitable for quantitation.
Rivaroxaban	Anti-Xa	Normal anti-Xa activity likely excludes clinically relevant drug levels	Anti-Xa	-	Anti-Xa, PT	A normal PT likely excludes excess drug levels. Only anti-Xa is suitable for quantitation.
Apixaban	Anti-Xa	Normal anti-Xa activity likely excludes clinically relevant drug levels	Anti-Xa	-	Anti-Xa	-

Suggestions for laboratory measurement of the anticoagulant activity of dabigatran, rivaroxaban, and apixaban based on clinical objective. Typical on-therapy drug levels are shown in Table 1. Abbreviations as in Table 2.