CLINICAL REVIEW

Newer oral anticoagulants: A review of laboratory monitoring options and reversal agents in the hemorrhagic patient

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Purpose. Available evidence on laboratory monitoring of coagulation assays and reversal strategies for the management of hemorrhagic events associated with the newer anticoagulants is reviewed.

Summary. While there are no published studies with dabigatran and prothrombinase-induced clotting time (PiCT) and no chromogenic assays available to measure the anticoagulant effects, thrombin time and activated partial thromboplastin time (aPTT) may be used to detect the presence of dabigatran. Although ecarin clotting time is sensitive to elevated concentrations of dabigatran, only a small fraction of institutions have access to this assay. Rivaroxaban and apixaban prolong prothrombin time, dilute prothrombin time, aPTT, Heptest results, and PiCT to varying degrees, having the most-pronounced effects at higher concentrations. In contrast, the chromogenic anti-factor Xa assay proved to be sensitive to lower amounts of rivaroxaban and apixaban with less variability. Despite the expectations with these newer anticoagulants, the associated risk of bleeding is significant, and there are insufficient data depicting treatment options in emergency situations. Until four-factor prothrombin complex concentrates (PCCs) become available in the United States, the obtainable options are activated PCC, three-factor PCCs, and recombinant factor VIIa.

Conclusion. Although there is currently no gold standard of measurement for any of the newer anticoagulants, the published literature enables practitioners to evaluate the efficacy and sensitivity of a majority of these assays. Prohemostatic agents can be used in instances of severe, life-threatening hemorrhagic complications.

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The efficacy of anticoagulation therapy for the treatment of venous thromboembolism and the prevention of stroke and systemic embolism has been established over 50 years of clinical research.[1,2] Consequently, anticoagulants are used for a variety of conditions, including the avoidance of ischemic stroke in patients with atrial fibrillation, attenuation of thrombin generation in acute coronary syndromes, and prevention of early and late recurrences of thromboembolism and thrombus extension in patients with deep venous thrombosis (DVT) or pulmonary embolism (PE).[3-6] The long-established anticoagulant armamentarium consists of warfarin, unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs), and parenteral indirect activated factor Xa (FXa) inhibitors (e.g., fondaparinux). Despite the extensive efficacy demonstrated with these agents, their utility is limited by multiple factors. Warfarin interacts with a multitude of drugs and foods, has a delayed onset of action, has a narrow therapeutic range, requires routine therapeutic monitoring, and exhibits variability in patient response as influenced by genetic factors.[2] Limitations of UFH and LMWHs include the potential development of heparin-induced thrombocytopenia, with UFH requiring extensive monitoring and dosage adjustment. The only FXa inhibitor available for subcutaneous injection, fondaparinux, has a long half-life and lacks a specific reversal agent.[1] Aside from warfarin, these anticoagulants are avail-

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able only in injectable formulations. Furthermore, patients may experience significant hemorrhagic events secondary to these anticoagulants.[7]

Although there has been much support for the use of newer anticoagulants (e.g., dabigatran), bleeding complications still occur, and there is no specific antidote to reverse the effects of these agents. To date, numerous fatalities due to spontaneous gastrointestinal or intracranial hemorrhages associated with dabigatran have been reported.[8] While routine monitoring of laboratory test values is not required for patients receiving newer anticoagulants, there are certain circumstances, such as hemorrhagic events, for which measuring and quantifying these agents' degree of anticoagulation may be beneficial. However, there is a paucity of data regarding the monitoring considerations and management of bleeding for newer anticoagulants.

This article reviews the bleeding risks associated with newer anticoagulants, the potential laboratory measurements for monitoring their effects, and available evidence for management of bleeding associated with these agents.

Oral anticoagulants and bleeding frequency

Direct thrombin inhibitors. Direct thrombin inhibitors (DTIs) act directly with the thrombin moiety, as opposed to UFH, which initially interacts with antithrombin. DTIs bind to and inhibit the activity of soluble thrombin as well as thrombin bound to fibrin, thus limiting thrombus progression.[9-11] Similar to UFH, bivalirudin, argatroban, and lepirudin have short half-lives of less than 2 hours. If bleeding occurs, drug concentrations may quickly decrease once a dose is withheld. Just as UFH has the advantage of having a reversal agent available, ideally the same should exist for newer anticoagulants. There may be situations in which expedited reversal beyond just withholding a dose is necessary (e.g., when the effects of the anticoagulant may outlast the reversal agent used and even warrant a second dose of the reversal agent). Caution must be taken with these particular DTIs (bivalirudin, argatroban, and lepirudin), as their half-lives may be lengthened in patients with hepatic or renal impairment, depending on the drug's route of elimination. Dabigatran etexilate (dabigatran), a recently approved DTI for oral administration, has a half-life of 12–17 hours[12]; therefore, the bleeding risk associated with this particular agent may be more pronounced.

The Food and Drug Administration (FDA) approval of the labeling for dabigatran in atrial fibrillation stemmed from the Randomized Evaluation of Long-term Anticoagulant Therapy (RE-LY) trial, which compared the efficacy and safety of dabigatran versus warfarin in the prevention of stroke or systemic embolism in patients with nonvalvular atrial fibrillation.[13] The trial found that renal impairment and age of ≥80 years are associated with a higher bleeding risk.[14] Although the dabigatran etexilate 75-mg dosage has not been studied in the clinical setting, dabigatran etexilate 75 mg twice daily is recommended instead of 150 mg twice daily for patients with severe renal impairment (i.e., estimated creatinine clearance [CL_{cr}] of 15–30 mL/min).[15] In patients with a CL_{cr} of ≥50 mL/min, the yearly major bleeding rates for dabigatran were lower compared with warfarin. Because many of the risk factors that warrant treatment with dabigatran are likely to be found in the elderly, these patients should be assessed for hemorrhagic risks to help guide clinical decisions.[16] Factors that increase the risk of stroke, according to $CHADS_2$ (congestive heart failure, hypertension, age of ≥75 years, diabetes mellitus, prior stroke or transient ischemic attack) score, are also associated with an increased risk of bleeding in patients with atrial fibrillation receiving oral anticoagulants.[17]

Numerous serious adverse effects associated with dabigatran use have been reported.[8] The Australian regulatory authority issued a safety advisory regarding dabigatran due to increasing reports of hemorrhagic events. Of the total dabigatran adverse-event reports received by the Australian regulatory authority since 2009, a considerable amount involved patients older than 75 years.[18] The Australian regulatory authority recommended that kidney function be assessed before initiating therapy with dabigatran and that patients with a CL_{cr} of <30 mL/min not receive the medication. It also suggested that patients older than 75 years or patients with moderate renal impairment have their kidney function assessed at least once per year.[18] Soon after, the product labeling for dabigatran in the United States was revised to recommend that renal function be tested annually in patients with a CL_{cr} of <50 mL/min.[19] Further, the labeling urges physicians to use the dabigatran etexilate 75-mg twice daily dosage in patients with a CL_{cr} of 30–50 mL/min who are taking either dronedarone or systemic ketoconazole, as these two P-glycoprotein inhibitors potentiate dabigatran exposure similar to that observed in severe renal impairment.[19] Both Japan and New Zealand have also issued safety advisories for dabigatran attributable to numerous deaths related to hemorrhagic complications.[8,20]

FXa inhibitors. In addition to direct thrombin inhibition, FXa is a target for some newer anticoagulants. Fondaparinux is currently indicated for the treatment of DVT or PE and for DVT prophylaxis (including extended prophylaxis) in patients undergoing hip fracture surgery, hip replacement surgery, knee replacement surgery, or abdominal surgery.[21] In contrast to fondaparinux, the mechanism of action of rivaroxaban and apixaban is independent

of antithrombin, and both agents inhibit free and prothrombinase-bound FXa by binding directly to the catalytic site of this molecule.[22] This is especially noteworthy, as prothrombinase complex surpasses the activity of free FXa at activating prothrombin to thrombin by approximately 300,000-fold.[23] Rivaroxaban is indicated to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation and for the prophylaxis of DVT, which may lead to PE in patients undergoing knee or hip replacement surgery.[24] In the Rivaroxaban versus Warfarin in Nonvalvular Atrial Fibrillation (ROCKET AF) trial, which enrolled 14,264 patients who were followed for a median of 1.94 years, the rates of major bleeding were similar in both the rivaroxaban and warfarin groups (3.6% and 3.4%, respectively; $p = 0.58$).[25] Although the rate of intracranial bleeding was significantly lower in the rivaroxaban group (0.5% versus 0.7% per year; hazard ratio [HR], 0.67; 95% confidence interval [CI], 0.47–0.93; $p = 0.02$), gastrointestinal bleeding was more common with rivaroxaban than warfarin (3.2% versus 2.2%, $p < 0.001$). While rivaroxaban is currently approved for marketing in the United States, apixaban is expected to gain FDA approval for use in patients with atrial fibrillation in 2012, as clinical trial results have indicated its superiority over warfarin, based on lower rates of major bleeding and overall mortality compared with warfarin. The Apixaban versus Warfarin in Patients with Atrial Fibrillation (ARISTOTLE) trial followed 18,201 patients for a median of 1.8 years.[26] The trial revealed a greater rate of major bleeding in the warfarin group (2.13% per year with apixaban versus 3.09% per year with warfarin; HR, 0.69; 95% CI, 0.60–0.80; $p < 0.001$). The yearly rate of intracranial hemorrhage was also significantly reduced in the apixaban group compared with the warfarin group (0.33% and 0.80%, respectively; HR, 0.42; 95% CI, 0.30–0.58; $p < 0.01$).

Despite the expectations for the newer anticoagulants, their associated bleeding risk is significant, and data for treatment options in emergency situations such as hemorrhagic events are lacking. Complicating this dilemma, the mechanism of action of these novel agents differs from other currently available agents and may require different clotting assays to measure their individual levels of anticoagulation in plasma.

Coagulation assays

Based on the pharmacodynamic and pharmacokinetic predictability of dabigatran, rivaroxaban, and apixaban, routine monitoring has been considered unnecessary.[19,27-29] However, in the setting of hemorrhage or overdose, measuring the effect of these anticoagulants may be critical to guide reversal therapy.

Prothrombin time. Prothrombin time (PT), a commonly used assay in the clinical setting, is the time in seconds for plasma to coagulate after the addition of calcium and thromboplastin, an activator of the extrinsic coagulation pathway, to citrated plasma.[30] PT is most often used to monitor warfarin because of its inhibition of factors II, VII, and X.[2] Inhibitors or deficiencies of these coagulation factors prolong PT.[2] One of the disadvantages of the PT assay is the varying sensitivities of the available thromboplastin agents to the reduction in coagulation factors.[31] In order to correct for the variability of the thromboplastin reagent used, PT is converted to an International Normalized Ratio (INR) through a mathematical calculation that accounts for the manufacturer's International Sensitivity Index.[30] Once calculated, the INR can be evaluated without regard to the thromboplastin reagent used in the PT assay.

DTIs. In laboratory studies of healthy human volunteers, dabigatran caused a concentration-dependent increase in PT.[32] However, PT is relatively insensitive to the overall effects of dabigatran. In one study that used five different thromboplastin reagents to assess PT at trough and peak dabigatran concentrations, the INR never exceeded 1.2.[33] There was also a significant amount of variability in PT and the INR depending on the thromboplastin reagent used. Of note, supratherapeutic concentrations of dabigatran had more-pronounced effects on PT and the INR.[34]

Multiple case studies have shown that dabigatran can cause false elevations in point-of-care INR monitoring.[35] In one case, an elevation of 7.1 in the INR was reported, but the elevation was later confirmed to be 1.7.[36] The cause of the false elevation was not determined. Results from another study suggest that point-of-care measurement devices may report falsely elevated INRs versus traditional laboratory measurements.[37] Although PT is increased at supratherapeutic concentrations of dabigatran, the insensitivity and variability of the assay deem it undesirable to guide reversal therapy in the hemorrhaging patient.

FXa inhibitors. In contrast to fondaparinux, which has no effect on PT, rivaroxaban prolonged PT in a concentration-dependent, incremental manner through its inhibition of free and bound FXa in human studies.[21,38] At therapeutic concentrations, rivaroxaban has a relatively weak effect on PT[31]; however, the effect is more profound at higher concentrations.[39] Human studies have revealed a linear increase as well as a significant amount of variation in the PT depending on the thromboplastin reagent.[38,39] As with warfarin, converting PT to the INR may correct for this variation, but the variability between thromboplastin reagents cannot be diminished.[38,40] In fact, at higher concentrations of rivaroxaban, converting PT to the INR creates a wider range of variability compared

with PT.[39] The variation of PT and the INR seen with differing thromboplastin reagents may not allow for standardization, but these assays may be useful in certain clinical situations. The ability of rivaroxaban to prolong PT may prove beneficial in assessing patients with hemorrhagic complications. Similarly, apixaban prolonged PT in a concentration-dependent fashion.[39] In a study conducted by Barrett et al.[39] using human plasma samples, the effect of apixaban on PT varied significantly between the 12 thromboplastin reagents used, and the conversion of PT to the INR only increased this variability. Although not ideal, the availability of the PT assay in most hospitals throughout the United States gives this assay the potential to guide reversal therapy in the setting of hemorrhage.

Dilute PT. Although traditionally used for lupus anticoagulant screening, dilute PT (dPT) shows promise for the monitoring of the newer anticoagulants. In contrast to PT, the dPT assay utilizes a similar thromboplastin reagent that has been diluted. This dilution has been shown to increase the sensitivity of the assay and potentially create an environment that more closely resembles physiological conditions.[38,41]

DTIs. Wong et al.[41] tested the effect of dabigatran on multiple coagulation assays using an in vitro rabbit model and found that dPT correlated well with the antithrombotic effect of dabigatran and was significantly more sensitive to the effects of this anticoagulant compared with PT and activated partial thromboplastin time (aPTT). The use of dPT to measure the effect of this DTI shows much promise, yet more studies with this assay are warranted.

FXa inhibitors. When used to measure the in vitro effects of rivaroxaban in a set of human pooled plasma, dPT was prolonged in a concentration-dependent manner; yet, this prolongation varied depending on the thromboplastin reagent used.[38] Apixiban also causes a concentration-dependent prolongation of dPT. In fact, the results of one study involving rabbits showed that dPT was 10–20 times more sensitive to apixaban than PT.[42] Although the use of dPT to monitor rivaroxaban and apixaban shows potential, further testing is required to validate these results.

Thrombin time. The thrombin time assay, available in numerous hospitals, measures the activity of thrombin in plasma and can directly measure the activity of DTIs. A highly sensitive, linear, concentration-dependent response in thrombin time has been observed in patients taking single and multiple daily doses of dabigatran.[43] However, thrombin time may be too sensitive to the effects of dabigatran. In patients taking single doses of dabigatran etexilate 100 or 200 mg, the maximum mean plasma dabigatran concentrations observed were 82.2 and 161 ng/mL, respectively. At dabigatran concentrations exceeding 600 ng/mL, the maximum measurement of the coagulometer is exceeded.[32] Therefore, thrombin time is more appropriate for detecting the presence of dabigatran than quantifying the medication levels in the hemorrhagic patient. In contrast to dabigatran, the direct inhibition of rivaroxaban and apixaban on FXa makes thrombin time an undesirable assay to measure these anticoagulants. This hypothesis was proven in a rabbit model study in which these oral FXa inhibitors had no effect on thrombin time.[41]

Ecarin clotting time. The ecarin clotting time (ECT) assay directly measures thrombin generation. In this assay, coagulation is initiated with ecarin, a type of snake venom. Ecarin activates prothrombin, which in turn stimulates the thrombin precursor meizothrombin.[32]

DTIs. The ability of dabigatran to inhibit the activity of meizothrombin and subsequent clot formation results in the prolongation of ECT. A concentration-dependent linear response was observed in the ECT assay in patients treated with dabigatran etexilate 10–400 mg once daily or 50–400 mg three times daily. ECT was significantly more sensitive and precise than aPTT in these patients. Compared with thrombin time, ECT was more sensitive with higher concentrations of dabigatran (>600 ng/mL).[32,43]

FXa inhibitors. Unlike dabigatran, rivaroxaban does not prolong ECT, as it acts primarily on FXa.[44] Currently, there are no data on the effect of apixaban on ECT. However, due to the drug's activity on FXa and its similarity to rivaroxaban, apixaban is not expected to affect this coagulation assay.

aPTT. While aPTT is similar to PT, aPTT is reflective of the activity and presence of factors II, V, and VIII–XII and fibrinogen. Any anticoagulant that inhibits these factors will cause an increase in aPTT.[45] The aPTT assay is most commonly used to monitor heparin in the inpatient setting. Heparin affects aPTT in a linear fashion, allowing for dosage adjustments based on assay results.[46]

DTIs. aPTT is more sensitive to the effects of dabigatran, with minimal variability between reagents compared with PT.[33] Supratherapeutic doses of dabigatran in healthy men have been shown to curvilinearly prolong aPTT, though aPTT leveled out at dabigatran concentrations of >200 ng/mL.[32] This effect suggests that the assay may be insensitive to supratherapeutic concentrations of dabigatran. However, in a study conducted by Lindahl et al.[33] using healthy human plasma, a linear response in aPTT was observed at dabigatran concentrations exceeding 200 ng/mL. The greatest utility for the aPTT assay may be in the initial assessment of the hemorrhagic patient secondary to dabigatran use. Although aPTT has the potential to dictate trends in coagulation in patients taking dabigatran, the efficacy

of this assay to evaluate the effectiveness of reversal therapy requires further evaluation.

FXa inhibitors. One in vitro study of rivaroxaban showed a concentration-dependent prolongation of aPTT in pooled human plasma.[38] Comparable to PT, the effect of rivaroxaban on aPTT has been most prominently seen with supratherapeutic rivaroxaban concentrations, and the magnitude of this effect has varied with different reagents.[38] However, another study that utilized five different reagents to assess the effect of rivaroxaban on aPTT demonstrated a nonlinear dose response, with minimal variability among the reagents.[31] When comparing aPTT with PT, PT appears to be a more-sensitive method for assessing the effect of rivaroxaban.[38,47] Depending on the reagent, apixaban has been shown to prolong aPTT to varying degrees in a dose-dependent manner.[38,42] In a study by Wong et al.,[42] the apixaban concentration required to double aPTT was approximately two times that required to double PT, rendering PT the more-sensitive assay.

Heptest. A relatively new assay, the Heptest (American Diagnostica, Stamford, CT), is used to measure the inhibition of exogenous FXa. The Heptest assay is based on the ability of heparin to catalyze the inactivation of FXa.[48] However, the Heptest is not specific for FXa and can be influenced by agents that inhibit factor IIa.[49] The degree of FXa inhibition is directly proportional to the amount of heparin present. Although this test is indicated for the quantification of heparin, LMWHs, and heparinoids, studies have been performed to evaluate the effects of rivaroxaban and apixaban on this assay.[38,42]

DTIs. No studies have evaluated the effect of dabigatran on the Heptest. While argatroban prolongs the Heptest clotting time in a concentration-dependent manner, it did not correlate well after intrinsic and extrinsic activation in in vitro studies with normal human plasma, making it an undesirable assay to measure direct thrombin inhibition.[50]

FXa inhibitors. Rivaroxaban prolongs the Heptest clotting time in a dose-dependent, incremental manner.[38] In a study assessing the pharmacokinetics of rivaroxaban in the treatment of patients with DVT, rivaroxaban was found to have a direct response on the Heptest clotting time.[51] A decrease in the Heptest clotting time was observed with rivaroxaban concentrations of <0.2 μg/mL.[38] However, this paradoxical effect was not seen when the incubation time was shortened or when antithrombin-deficient plasma was used. Under these circumstances, the Heptest had a high sensitivity to rivaroxaban.[38] In one human model, apixaban also prolonged the Heptest coagulation time.[42] The amount of apixaban required to double the coagulation time is significantly less with the Heptest than with the PT and aPTT assays.[42] Therefore, the Heptest would be preferable to PT and aPTT assays for monitoring patients receiving apixaban.

Prothrombinase-induced clotting time. Although only approved for measuring the effects of UFH and LMWHs, prothrombinase-induced clotting time (PiCT) has been evaluated as a way to measure the anticoagulant effect of rivaroxaban. The PiCT assay is composed of a combination of FXa, phospholipids, and an enzyme that activates factor V. When patient plasma samples are added to these components, the result is activation of factor V. Subsequently, the coagulation time can be measured, and the effect of the anticoagulant can be assessed.[52]

DTIs. There are no published results concerning the effects of dabigatran on PiCT at this time. In a study by Fenyvesi et al., PiCT was found to be highly sensitive to the effects of argatroban and melagatran.[53]

FXa inhibitors. Rivaroxaban has a significant concentration-dependent relationship with PiCT.[52] Similar to the Heptest results, lower concentrations of rivaroxaban have been found to shorten coagulation time. However, this effect was not observed when the incubation period was eliminated, plasma was deficient of antithrombin, or human FXa was utilized.[38] With these adjustments, PiCT has been shown to be sensitive to low concentrations of rivaroxaban.[52] Although not available in all clinical settings, the high sensitivity of the PiCT test as well as the Heptest provides strong evidence for their use over aPTT and PT in measuring rivaroxaban activity. The PiCT test has not been studied with apixaban; however, based on their similar mechanisms of action, the effects of apixaban on PiCT are likely to be similar to those of rivaroxaban.

Chromogenic assays. The use of chromogenic anti-factor IIa assay for dabigatran and anti-FXa assay for rivaroxaban and apixaban may help measure the direct effects of these agents. While anti-FXa assays are widely available and commonly used in clinical practice, the use of anti-factor IIa chromogenic assays is limited. Chromogenic anti-FXa assays are currently used to monitor UFH and LMWHs in a variety of settings, including pediatrics, pregnancy, and renal dysfunction. In this assay, FXa is added to plasma containing an FXa substrate (e.g., heparin) that is tagged with a chromophore. The chromophore is cleaved by FXa, resulting in a color change.[54] The change in color is directly proportional to the concentration of FXa present in the assay. As these results can be compared with a standard assay with a known quantity of inhibitor, the amount of FXa inhibitor can be calculated.[54] Each of these standard assays is calibrated for a specific LMWH or UFH and cannot be used to assess the anticoagulation effect of other agents that have not been previously calibrated. For example, the effect of rivaroxaban

cannot be correctly measured if the assay is standardized for UFH.

DTIs. Although in development, chromogenic anti-factor IIa assays are not yet available to measure the effects of dabigatran. In a human study assessing the effects of lepirudin, the chromogenic assay displayed a highly sensitive linear correlation with lepirudin. Although not substantial, these data support the potential use of the chromogenic assay to measure the effects of this new anticoagulant.[55]

FXa inhibitors. In a study of 20 healthy volunteers who ingested 10 mg of rivaroxaban, the use of a chromogenic anti-FXa assay was accurate and precise. Unlike PT and aPTT, which vary depending on the thromboplastin reagent used, the results of the chromogenic assay were reproduced by nine laboratories.[56] Apixaban has also been shown to correlate well with the anti-FXa assay. The anti-FXa assay has been shown to be more accurate than the PT assay and INR values.[39] In a study by Becker et al.,[57] the chromogenic anti-FXa assay revealed a statistically significant linear correlation in patients with acute coronary syndrome taking apixaban ($r = 0.9669$, $p < 0.0001$). Whereas the PT and aPTT assays are less sensitive to apixaban, the anti-FXa apixaban assay was shown to be sensitive at much lower concentrations of apixaban.

Issues for consideration when selecting a coagulation assay

Overall, the new anticoagulants have varying effects on numerous coagulation assays (Table 1). While there are no published studies with dabigatran and PiCT and no chromogenic assays commercially available to measure the drug's anticoagulant effects, thrombin time and aPTT may be used to detect the presence of dabigatran in the hemorrhaging patient. Although ECT is sensitive at elevated concentrations of dabigatran, only a small fraction of institutions possess this assay. Until the ECT

Table 1.
Sensitivity and Utility of Current Coagulation Assays for Dabigatran, Rivaroxaban, and Apixaban[21,31-33,35-42,45,48-50,52,54,55,a]

Assay	Dabigatran		Rivaroxaban		Apixaban	
	Sensitivity	Utility	Sensitivity	Utility	Sensitivity	Utility
PT	Relatively insensitive	Not ideal; widely available	More sensitive at higher concentrations	Widespread availability makes PT useful	More sensitive at higher concentrations	Widespread availability makes PT useful
dPT	More sensitive than PT	Not widely available; lacks FDA approval	Variability between thromboplastin reagents	Not widely available; lacks FDA approval	More sensitive than PT	Not widely available; lacks FDA approval
Thrombin time	Too sensitive, inaccurate at high concentrations	Sensitivity limits utility in quantifying anticoagulation	No effect	Not useful	No effect	Not useful
Ecarin clotting time	Sensitive at all concentrations	Limited availability; lacks FDA approval	No effect	Not useful	Unlikely to have an effect	Not useful
aPTT	More sensitive than PT	Availability and sensitivity support use	Less sensitive than PT	Not ideal; widely available	Less sensitive than PT	Not ideal; widely available
Heptest	Does not correlate well	Not likely to be useful	Sensitive at low and high concentrations	Not widely available; lacks FDA approval	More sensitive than PT	Not widely available; lacks FDA approval
PiCT	Not studied	Not likely to be useful	Sensitive at a wide range of concentrations	Not widely available; lacks FDA approval	Not studied; likely to have an effect	Not widely available; lacks FDA approval
Chromogenic anti-factor IIa	Highly sensitive to lepirudin	Minimal data and not currently available	Accurate and precise	Most promising assay	More sensitive than PT and aPTT	Most promising assay

[a]PT = prothrombin time, dPT = dilute prothrombin time, FDA = Food and Drug Administration, aPTT = activated partial thromboplastin time, PiCT = prothrombinase-induced clotting time.

assay becomes widely available, aPTT is a practical selection to measure the anticoagulation effects of dabigatran, as this test is commonly available. Apixaban and rivaroxaban also have an effect on certain coagulation assays. These agents prolong PT, dPT, aPTT, and clotting time in the Heptest and PiCT to varying degrees, with the most pronounced effects occurring at higher concentrations. The chromogenic anti-FXa assay proved to be sensitive to lower amounts of rivaroxaban and apixaban and had less variable results, rendering it the preferred test to use when monitoring rivaroxaban and apixaban. However, most health care settings currently lack a standardized chromogenic assay calibrated for these agents. Although the utility of the dPT, Heptest, and PiCT assays has been established within the investigational setting, their limited availability and lack of widespread use in clinical practice present a challenge when trying to select the appropriate assay.

Management of bleeding complications

DTIs. The major complication associated with all anticoagulants, including the newer agents, is bleeding. Unlike warfarin, which is readily reversed with the use of several agents, there is not a specific antidote for dabigatran in the event of hemorrhaging or overdose. In vitro studies have evaluated the use of activated charcoal for cases of overdose, and the results are encouraging.[58] Activated charcoal should be administered within one to two hours of dabigatran ingestion to prevent absorption within the intestine. In the case of a major bleeding episode, health care practitioners should discontinue the anticoagulant, initiate supportive measures, and provide dialysis, as this method removes 60% of the drug in two to three hours.[19] Unfortunately, prompt dialysis may not be an option for unstable patients due to massive bleeding or those with large intracranial hemorrhages.[59] Nevertheless, hemodialysis has been successfully used to manage a patient experiencing massive dabigatran-associated bleeding after cardiac surgery.[60] Although the role of three-factor prothrombin complex concentrates (PCCs) in this arena has not been established, there is some experimental evidence to support the use of four-factor PCCs, activated PCCs (aPCCs) (i.e., antiinhibitor coagulant complex), and recombinant factor VIIa (rFVIIa). Four-factor PCC products contain factors II, VII, IX, and X, whereas three-factor products contain factors II, IX, and X, with lower concentrations of factor VII compared with the four-factor PCC products. aPCCs consist of a high quantity of clotting factors in their activated state. Feiba NF (Baxter Healthcare) is the only aPCC available for clinical use in the United States. It contains factors II, IX, and X, which are mainly nonactivated, and factor VII (mainly in the activated form).[61] The compositions of potential reversal agents (i.e., PCCs) are listed in Table 2.[61-68]

FXa inhibitors. In the setting of overdose or hemorrhage secondary to rivaroxaban or apixaban use, reversal of the anticoagulant effect of these agents is critical. As with dabigatran, there is currently no identifiable antidote for rivaroxaban or apixaban, and studies supporting a reversal strategy for these anticoagulants are limited. In the setting of overdose, activated charcoal may be administered to decrease the absorption of rivaroxaban or apixaban. Administration of activated charcoal 15 minutes after rivaroxaban ingestion resulted in a 65% decrease in the area under the concentration curve.[69] Per the manufacturers, activated charcoal should be given within eight hours of rivaroxaban ingestion and within three hours of apixaban ingestion.[69,70] Because PCCs, aPCC, and rFVIIa contain specific coagulation factors, they may be able to reverse the anticoagulant effects of rivaroxaban and apixaban. Though much of the data surrounding these newer anticoagulants may be inferred from data related to other indirect FXa inhibitors (i.e., fondaparinux, idraparinux), data are available depicting the possibility for bleeding reversal.[71-77] Although fondaparinux, rivaroxaban, and apixaban target FXa, indirect and direct FXa inhibitors have very different mechanisms of action, and the reversal of their anticoagulant effects may differ greatly based on the reversal agent used. Available data must be interpreted with caution, as the effect of each reversal agent on newer anticoagulants may prove to be more or less efficacious than with older anticoagulants. An antidote, PRT06445, is being developed in concert with the FXa inhibitor betrixaban.[78]

PCCs. *DTIs.* In an animal model study, the efficacy of PCCs was assessed in mice receiving dabigatran minutes after the induction of intracranial hemorrhage via surgical methods. The administration of a four-factor PCC (100 units/kg) 30 minutes after the initiation of intracranial hemorrhage reduced hematoma expansion and tail vein bleeding time.[79] A study conducted by Eerenberg et al.[62,66,71] revealed that a nonactivated four-factor PCC (50 units/kg) failed to decrease the anticoagulant effect of dabigatran, as it did not decrease the aPTT, thrombin time, or ECT in a group of healthy volunteers. The PCC used in this study, Cofact (Sanquin Blood Supply, Amsterdam), consists of factors II, VII, IX, and X and contains the natural anticoagulants protein C and S and antithrombin.[61,65,71] Depending on the amount or presence of these natural anticoagulants in the PCC formulation, there may be a variation in the reversal effect. It is unknown if other PCCs, including aPCC, would reveal a more or less favorable response than Cofact if used

to reverse the anticoagulant effects of dabigatran. Since this study was performed in healthy volunteers, a clinical trial involving patients with major bleeding events is needed to better extrapolate these findings to clinical practice.

FXa inhibitors. Although scarce, evidence supporting the efficacy of PCCs does exist in the reversal of certain FXa inhibitors.[72] Kaskadil (LFB Biomedicaments, Les Ulis, France; no longer available), a four-factor PCC containing factors II, VII, IX, and X, effectively reversed the anticoagulant effects of fondaparinux in rat models.[73] This PCC significantly decreased blood loss and bleeding time (defined as the time between the incision and complete arrest of bleeding) without a significant increase in arterial thrombosis. In another rat model study, a high-dose four-factor PCC (50 units/kg) (Beriplex, CSL Behring, Marburg, Germany) almost completely normalized bleeding time, while lower doses (25 units/kg) had no effect on bleeding time.[74] In this study, bleeding time was defined as the time taken for continuous blood flow to cease for more than 30 seconds (maximum observation time, 30 minutes). Thus, it can be theorized that the effect of this PCC is due to the repletion of these factors. Theoretically, these results could be applied to the newer FXa inhibitors because of their similar effects on FXa.

Eerenberg et al.[71] studied the effects of 50 units/kg of a four-factor PCC (Cofact) in 12 healthy male volunteers who received rivaroxaban 20 mg twice daily for five doses. PT values normalized to baseline immediately after PCC administration—an effect that was sustained for 24 hours. When evaluating the potential for rebound thrombosis, the mean ± S.D. endogenous thrombin potential (expressed as a percentage of the normal value) was increased above baseline in patients receiving the PCC versus placebo (114% ± 26% versus 41% ±

Table 2.
Composition of Prothrombin Complex Concentrates[61-68]

Prothrombin Complex Concentrate	Factor II	Factor VII	Factor IX	Factor X	Heparin	Human Antithrombin III	Protein C	Protein S	Protein Z
Feiba NF[a,b] (Baxter Healthcare)	1.3 IU/IU	0.9 IU/IU	1.4 IU/IU	1.1 IU/IU	…[c]	…	1.1 IU/IU	…	…
Profilnine SD[a] (Grifols Biologicals)	NMT 150 units/100 factor IX units	NMT 35 units/100 factor IX units	100 units	NMT 100 units/100 factor IX units	…	…	…	…	…
Bebulin[a] (Baxter Healthcare)	24–38 IU/mL	<5 IU/mL	24–38 IU/mL	24–38 IU/mL	<0.15 IU/IU factor IX	…	…	…	…
Beriplex P/N (CSL Behring UK Ltd)	19–40 IU/mL	10–25 IU/mL	20–31 IU/mL	25–51 IU/mL	0.5 IU/mL	0.6 IU/mL	21–41 IU/mL	12–34 IU/mL	…
Kanokad (Sanquin)	14–35 IU/mL	7–20 IU/mL	25 IU/mL	14–35 IU/mL	…	…	…	…	…
Octaplex (Octapharma AG)	14–38 IU/mL	9–24 IU/mL	25 IU/mL	18–30 IU/mL	5–12.5 IU/mL	…	13–31 IU/mL	12–32 IU/mL	…
Cofact (Sanquin)	14–35 IU/mL	7–20 IU/mL	25 IU/mL	14–35 IU/mL	…	<0.6 IU/mL	11–39 IU/mL	1–8 IU/mL	…

[a] Available in the United States. NMT = no more than.
[b] Activated product.
[c] Not in product.

6%). Thus, the benefit of bleeding reversal with a PCC must be weighed against the potential risk of rebound thrombosis. Results of this trial confirm those of previous animal model studies indicating that high-dose four-factor PCC (50 units/kg) is a potential reversal treatment for rivaroxaban in the setting of hemorrhage.[73,74] However, a four-factor PCC is not approved for marketing in the United States.

Currently, there are no published studies evaluating the use of PCCs for the reversal of apixaban overdose or bleeding events. Because of the similarities with the mechanism of action of rivaroxaban, these results could potentially be applied to apixaban; however, the exact response to a PCC in patients receiving apixaban is unknown.

Compared with PCCs, data regarding the use of aPCC for the reversal of FXa inhibitors are scarce. Blombäck et al.[75] assessed the effects of Feiba NF, an aPCC, on fondaparinux and apixaban in a fibrin network permeability model. Fibrin network permeability models assess the effects of anticoagulants on the fibrin structure. This in vitro study found that Feiba NF did not significantly reverse the permeability effects of this FXa inhibitor and that the agent was only effective with lower concentrations of fondaparinux and apixaban.

In another laboratory study, Feiba NF 20 or 40 units/kg significantly normalized thrombin generation in platelet-rich plasma samples from six healthy volunteers to whom fondaparinux had been administered.[76] The thrombin generation test measures the amount and speed of thrombin formation over time. The results of this study contradict the fibrin network permeability model while concurring that aPCC could be used in the reversal of apixaban and rivaroxaban. Lastly, a baboon study found that aPCC effectively reversed the effects of rivaroxaban; however, the reversal was transitory.[77] This evidence, coupled with the availability of Feiba NF in the United States, gives health care providers a potential agent with which to reverse the hemorrhagic complications of rivaroxaban and apixaban. On the other hand, as most of the human studies involved healthy volunteers, the ability to reverse the effects in a hemorrhagic scenario is unknown.

rFVIIa. *DTIs.* While small-scale animal studies and human case reports have evaluated the use of rFVIIa for the reversal of bleeding associated with newer anticoagulants, these reports have yielded conflicting results. Prolonged bleeding time and aPTT associated with dabigatran were significantly reduced when rFVIIa (0.1 or 0.5 mg/kg) was administered to anesthetized rats.[80] Another rodent study evaluating rFVIIa and aPCC revealed that rFVIIa produced less-pronounced effects on blood loss and bleeding time than did aPCC.[81] In that study, bleeding time was defined as the time from incision to first arrest of bleeding, assessed at intervals of 15 seconds. In a case report, Garber et al.[82] found that rFVIIa did not slow the progression of intracranial hemorrhage in a patient taking dabigatran etexilate 150 mg twice daily after a ground-level fall. In another case report, Warkentin et al.[60] concluded that the combination of high-dose rFVIIa and hemodialysis decreased dabigatran-associated bleeding after cardiac surgery. Further randomized controlled studies, preferably in patients taking dabigatran who are actively bleeding, are required to assess the effectiveness and optimal dose of rFVIIa. A Phase IV trial is ongoing to evaluate PCCs, aPCC, and rFVIIa for the reversal of anticoagulation with dabigatran in healthy volunteers.[83]

FXa inhibitors. Few studies have evaluated rFVIIa for the reversal of rivaroxaban or apixaban. Inferences regarding the use of this agent to reverse newer anticoagulants could be made from the limited studies with fondaparinux. In one randomized, placebo-controlled trial, 16 participants received 90 μg/kg of rFVIIa or placebo two hours after fondaparinux administration.[84] Thrombin generation time (i.e., the speed at which thrombin is generated in whole blood), aPTT, and PT were immediately normalized after rFVIIa administration. Further, rFVIIa reversed the decrease in thrombin generation caused by fondaparinux for up to six hours. The results of a small baboon study revealed that bolus injection and infusion of rFVIIa was effective in reversing the effects of rivaroxaban; however, the reversal effects were fleeting.[77] Although rFVIIa may be effective in reversing the effects of fondaparinux and rivaroxaban, more evidence of its utility in the reversal of newer anticoagulants is needed.

Plasmapheresis. Plasmapheresis, also referred to as plasma exchange, removes whole blood from the body and separates it into its components by centrifugation.[85] In this process, the plasma is exchanged with an alternative solution that is reinfused into the patient. To date, there are no high-quality clinical trials evaluating the use of plasmapheresis in patients who have experienced hemorrhagic events due to anticoagulant overdose.

Recombinant antidote. While aPCC, PCCs, or rFVIIa may aid in the reversal of rivaroxaban and apixaban, none are true antidotes. The ideal agent to reverse the effects of these new anticoagulants may be recombinant (r)-antidote, PRT064445, a recombinant catalytically inactive form of FXa, which is undergoing development in preclinical trials. In one rat model study with fondaparinux, r-antidote completely corrected blood loss and almost completely reversed the effects of rivaroxaban in animal models.[86] In rats treated with rivaroxaban, blood loss was decreased by almost 80% after administration of r-antidote.[78] The advent of r-antidote

may prove to be monumental for the reversal of FXa inhibitors.

Current status of reversal agents

While four-factor PCCs are not approved for use in the United States, there is evidence to support their use to reverse the effects of the newer anticoagulants. As a result, these agents may be considered viable reversal options in the future. Due to the lack of available evidence with three-factor PCCs, it is unknown whether these reversal agents will have the same encouraging results as four-factor PCCs. Until four-factor PCCs are available in the United States, the available options include aPCC, three-factor PCCs, and rFVIIa. In the setting of overdose, administration of activated charcoal may be used depending on the time of anticoagulant ingestion. Until a true antidote becomes available, such as r-antidote for the new oral FXa inhibitors, aPCC, three-factor PCCs, and rFVIIa will have to suffice to reverse the anticoagulant effects in the setting of hemorrhage and overdose.

Conclusion

Although there is currently no gold standard of measurement for any of the newer anticoagulants, the published literature enables practitioners to evaluate the efficacy and sensitivity of a majority of these assays. Prohemostatic agents can be used in instances of severe, life-threatening hemorrhagic complications.

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