

Influence of New Anticoagulants on Coagulation Tests

White Paper

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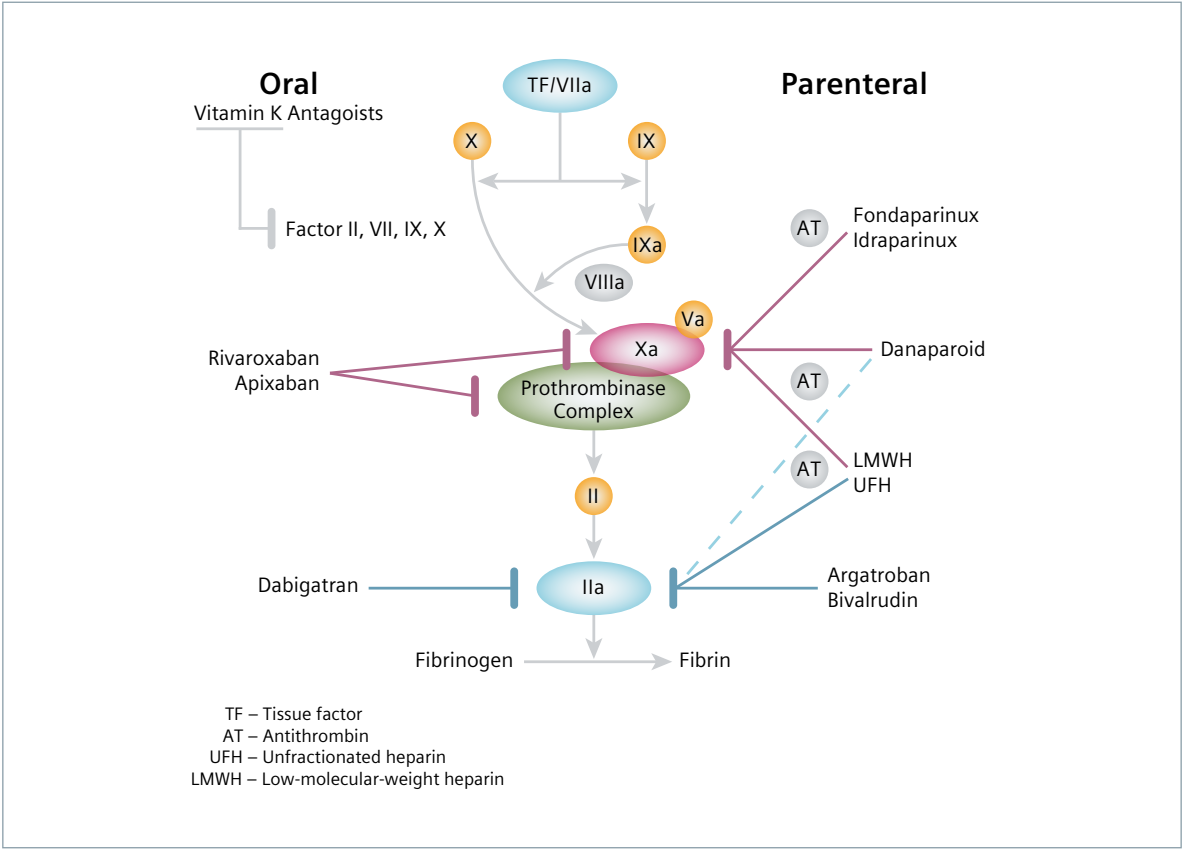
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Anticoagulants are used for prevention and treatment of thromboembolic disorders. Patients undergoing procedures such as total hip replacement are placed on thromboprophylactic agents, usually vitamin K antagonists such as warfarin or unfractionated or low-molecular-weight heparins. The disadvantages of these agents are well documented. Warfarin has numerous interactions with diet and other medications, requires complex individualized dosing, and has a delayed onset of action. Unfractionated heparins and low-molecular-weight heparins (LMWH), which require parenteral administration, pose a risk of heparin-induced thrombocytopenia, osteoporosis, and

hemorrhage. Such significant drawbacks make these agents problematic and inconvenient for clinicians and patients alike.

The side effects of conventional anticoagulants have prompted research into novel drugs that offer such advantages as oral mode of administration, more predictable anticoagulant response, greater specificity with no requirement for antithrombin action, and no need for routine patient monitoring. As shown in Figure 1, which depicts the various phases of hemostasis, the search for new anticoagulants has focused largely on using specific molecules to directly inhibit clotting factors within the clotting pathway, such as thrombin or activated factor X (FXa).

Figure 1. Targets of anticoagulants



Rivaroxaban and dabigatran belong to the new generation of oral anticoagulants, which have been in clinical use for more than two years. Their primary indications are for prevention of thromboembolic events after elective hip- or knee-replacement surgery and for prevention of stroke and systemic embolism in patients with atrial fibrillation.

Coagulation assays are commonly performed after anticoagulant administration in the clinic, typically as routine postoperative laboratory controls. Several studies have assessed the *ex vivo* and *in vitro* dose-dependent effect of the new anticoagulants in healthy individuals and patients.

This paper summarizes the pharmacology of the new anticoagulants and describes their influence on coagulation tests.

New Anticoagulants

Direct Thrombin Inhibitors

Because of its key role in the coagulation cascade, thrombin is one of the main targets in the development of direct-acting anticoagulants. Unlike heparins, direct thrombin inhibitors (DTIs) are small molecules that inhibit thrombin directly, with no need for a cofactor. DTI action results in specific binding of free and fibrin-bound thrombin, thereby preventing fibrin formation, thrombin-mediated activation of FV, FVIII, FXI, and FXIII, and thrombin-induced platelet aggregation. Since their chemical structure differs completely from that of heparin, DTIs do not interfere with heparin-induced thrombocytopenia type II (HIT-II) antibodies.

For years, three parenteral DTIs were available: lepirudin (Refludan®), bivalirudin (Angiox®), and argatroban (Novastan®).

The first oral DTI—ximelagatran (Exanta®)—was taken off the market in February 2006, 1.5 years after approval, because of side effects.

Dabigatran (Pradaxa®)

In March 2008, dabigatran was approved by the EMEA as an oral alternative to heparins after hip- and knee-replacement surgery. In August 2011, dabigatran was also licensed for prevention of stroke and systemic embolism in patients with atrial

fibrillation by the European authorities, after approval for this indication was received in October 2010 in the U.S.

Dabigatran etexilate is the prodrug of dabigatran that selectively and reversibly inhibits both free and clot-bound thrombin by binding to the active site of the thrombin molecule. The recommended dose for dabigatran etexilate is 220 mg once daily except in the elderly (75 or older) or patients with moderate renal impairment, in which case a dose of 150 mg q.d. is recommended. Its stable and reproducible pharmacokinetics is an advantage over conventional oral anticoagulants; it can be administered with fixed dosing and has no significant food or drug interactions. The time to maximum plasma concentration is 1.25–2.5 hours, and its half-life is about 12–14 hours. The drug is neither metabolized nor induced or inhibited by the cytochrome P450 drug-metabolizing enzymes. There are no specific reversing agents for dabigatran.

Indirect and Direct Factor Xa Inhibitors

Factor Xa is the other main target in the development of direct-acting anticoagulants. As with thrombin, factor Xa is a coagulation factor that acts at the convergence point of the intrinsic and extrinsic pathways in the blood coagulation system.

Indirect factor Xa inhibitors, such as fondaparinux, exert their anti-thrombotic effect by binding to antithrombin; therefore, their efficacy depends on the circulating level of antithrombin. They are parenteral agents and cannot be administered orally.

Rivaroxaban is the first orally administered direct factor Xa inhibitor. It selectively inhibits free and clot-associated factor Xa activity and has been licensed as an oral alternative to heparins after hip- and knee-replacement surgery since October 2008. Rivaroxaban was first approved in the U.S. in November 2011 for stroke prophylaxis in patients with atrial fibrillation. Apixaban, a newer oral factor Xa inhibitor, was approved for use after hip- and knee-replacement surgery in May 2011 in Europe. Other oral direct factor Xa inhibitors in advanced development include edoxaban or darexaban, undergoing phase III study, and betrixaban, which has passed phase II clinical testing.

Danaparoid (Orgaran®)

The indirect factor Xa inhibitor danaparoid sodium is a mixture of partially depolymerized glucosaminoglycans. Antithrombin-mediated danaparoid catalyzes the inactivation of factor Xa. There is also an antithrombin and heparin cofactor II-mediated inhibition of thrombin. However, the ratio of anti-Xa to anti-IIa activity is more than 22:1. The substance is in clinical use for thrombosis prophylaxis, and, because of its low cross-reactivity with heparin-platelet factor 4, it is also suitable for treatment of immune HIT-II.

Fondaparinux (Arixtra®)

This synthetic pentasaccharide, an indirect factor Xa inhibitor, acts by specific binding to antithrombin; it has no effect on thrombin or non-specific plasma protein binding. It exhibits almost 100% bioavailability after subcutaneous application. The drug is eliminated via renal filtration, and its half-life is about 17 hours. In vitro studies show no cross-reactivity with HIT antibodies.

Idraparinux

Due to its irreversible binding to antithrombin, this highly sulfated pentasaccharide is characterized by a long half-life of approximately 80 hours. This is very convenient for patients, because a once-weekly subcutaneous administration is sufficient. However, no antidote is available for idraparinux. A biotinylated form of the drug, currently in clinical trials, allows inactivation by avidin (a tetrameric biotin-binding protein).

Rivaroxaban (Xarelto®)

Rivaroxaban is both selective and reversible. Its time to maximum plasma concentration is 30 minutes to 3 hours, and its half-life has been reported to be 3–9 hours. Rivaroxaban provides concentration-dependent inhibition of factor Xa with high potency and selectivity and dose-dependent inhibition of tissue factor Xa. It is metabolized by CYP3A4 in the CYP450 system. However, 33% of the active substance is eliminated unchanged to 70% renal and 30% fecal. Rivaroxaban does therefore interact with the CYP450 system, specifically with CYP3A4. No relevant effects of extreme body weight, age, or gender on the pharmacological profile of this drug have been observed.

Rivaroxaban binds not only to free factor Xa but also to factor Xa bound in the prothrombinase complex.

Apixaban (Eliquis®)

Apixaban is a selective, reversible, direct, orally administered inhibitor of factor Xa. Its time to maximum plasma concentration is 30 minutes to 2 hours, and its half-life is 8–15 hours. Apixaban shows moderate selectivity for clot-bound factor Xa versus free factor Xa and also inhibits thrombin generation. Apixaban is absorbed in the gastrointestinal tract. Bioavailability is 50%. In the liver, the substance is oxidized to a phenol derivative, in which metabolism via cytochrome P450 plays a minor role. Elimination is 25% renal and, primarily, biliary excretion. The recommended dose is 2.5 mg orally twice daily.

Pharmacological Profiles

Table 1 lists brief pharmacological profiles of these new substances, which differ mainly in their bioavailability and metabolism.

Table 1. Overview of approved new anticoagulants (EU; Oct. 2011)

Anticoagulation	Substance (drug)	Application	Half-life Time (Time to Peak Concentration)	Elimination	Approved Indications	Monitoring
Direct thrombin inhibitors	Dabigatran (Pradaxa®)	Oral, twice daily	14–17 h (2–4 h)	Renal	VTE prophylaxis in elective hip- or knee-replacement surgery Stroke prevention in atrial fibrillation	Not required Options: Diluted thrombin time, ecarin time, anti-FIIa assay (requires calibration with dabigatran)
	Argatroban (Argatra®, Novastan®)	Intravenous	25 min	Hepatic	Prophylaxis Therapy of HIT-II	Mandatory APTT target: 1.5–3.0 fold Chromogenic ECT anti-FIIa assay (requires calibration with argatroban)
	Bivalirudin (Angiox®)		25 min	Renal	Acute coronary syndrome (ACS) Planned for early intervention Percutaneous coronary intervention (PCI)	ACT recommended Target: ACT >115 sec
Direct FXa inhibitors	Rivaroxaban (Xarelto®)	Oral, once daily	7–11 h, (2–4 h)	Renal and hepatic	VTE prophylaxis in elective hip- or knee-replacement surgery U.S.: Stroke prevention in atrial fibrillation	Not required Option: Anti-FXa assay (requires calibration with rivaroxaban)
	Apixaban (Eliquis)	Oral, twice daily	8–15 h, (0.5–2 h)	Renal and biliary	VTE prophylaxis in elective hip- or knee-replacement surgery	Not required Option: Anti-FXa assay (requires calibration with apixaban)
Indirect FXa inhibitors (require antithrombin)	Fondaparinux (Arixtra®)	Intravenous; subcutaneous, once daily	17 h, (25 min)	Renal	VTE therapy VTE prophylaxis ACS (without intervention)	Not required Option: Anti-FXa assay (requires calibration with fondaparinux)
	Danaparoid (Orgaran®)	Intravenous; subcutaneous, twice daily	7–8 h (4–5 h)	Renal	VTE therapy and prophylaxis VTE therapy and prophylaxis in HIT-II	Recommended for patients with renal impairment who weigh >90 kg Anti-FXa assay (requires calibration with danaparoid)

Advantages and Challenges

The new anticoagulants are considered to be more convenient and simpler to use than conventional agents because monitoring and dose adjustment are not required.

A more clinically relevant advantage of these new substances is that their binding to the coagulation factor is reversible. Therefore, they provide better controllability and significantly lower risk of bleeding compared to irreversible inhibitors such as hirudin.

A significant disadvantage is that there are no antidotes with which to rapidly reverse the new drugs' effects if required. Another challenge is that the mode of action of these new anticoagulants can alter or skew the results of coagulation tests. Clinicians must be aware of the impact particular anticoagulants have on the different types of coagulation assays to avoid misinterpreting test results.

Coagulation Assays

Clotting Assays

Clotting tests are still the most often performed assays in the hemostasis laboratory. In clotting tests, the time between the addition of a thromboplastin reagent and the formation of the fibrin clot is measured. The increasing viscosity and turbidity of the sample allow the detection of clot formation using mechanical or optical end-point detection.

Chromogenic Methods

The introduction of synthetic chromogenic substrates was a milestone for the investigation of individual coagulation inhibitors. The chromogenic assay allows a direct determination of the activity of the substrate.

The determination of inhibiting activity of anticoagulants on factor Xa is one of the most traditional chromogenic substrate methods. In this assay, activation of factor X in the sample is induced with one factor X-activating enzyme. The activated factor is directly detected with a chromogenic substrate.

Preliminary studies suggest that the anti-factor Xa chromogenic assays are potential candidate assays for measuring rivaroxaban blood concentrations, if required.

Prothrombin Time

The prothrombin time (PT) clotting assay is a screening assay for the function of the extrinsic pathway. It is used to obtain an overview of factors VII, X, V, thrombin, and fibrinogen.

Activated Partial Thromboplastin Time

In contrast to the PT assay, the activated partial thromboplastin time (APTT) is a screening test for the intrinsic system and its factors: kininogen, prekallikrein, XII, XI, IX, VIII, X, V, and thrombin. In the preincubation/preactivation phase of this test, antithrombin/heparin complexes can inactivate thrombin and its positive feedback reactions.

Thrombin Time

Thrombin time (TT) is a clotting screening test for fibrinogen polymerization and is performed by adding a low concentration of thrombin to plasma. This leads to formation of fibrin. The TT assay is a functional test of fibrinogen concentration and fibrin formation.

Fibrinogen (Clauss method)

The determination of fibrinogen is also one of the routine parameters in coagulation testing. A frequently used method is the method according to Clauss, based on the addition of an excess of thrombin to plasma.

Derived Fibrinogen

The total increase of turbidity during the prothrombin time (PT) is directly proportional to the concentration of fibrinogen. Therefore, the PT-derived fibrinogen assay is widely used as an alternate method for measurement of fibrinogen.

Antithrombin

Antithrombin can be determined by measuring the inhibitory effect on either thrombin or FXa as target enzyme.

Protein C

The coagulometric protein C assay is based on an APTT reaction in which the activated protein C inactivates the accelerators FVa and FVIIIa, prolonging the clotting time with increasing protein C levels. The chromogenic protein C assay allows a direct determination of the enzymatic activity.

Protein S

In the dRVVT-based coagulometric protein S assay, the clotting time recorded is proportional to the protein S level.

Lupus Anticoagulants (LA)

For determination of LA, the ratio between a dRVVT (dilute Russell viper venom test) low in phospholipids (LA screen) and a dRVVT rich in phospholipids (LA confirm) is measured.

ProC Global Assay and APC Resistance (APCR)

ProC® Global is an APTT-based screening assay for the protein C system that determines the ratio of a dilute APTT in the presence of protein C activation versus no protein C activation.

ProC AcR is a similar, more specific assay for determination of APCR/FV Leiden that uses a dRVVT in the presence and absence of a protein C activator.

Influence of New Anticoagulants on Coagulation Assays

The stable and reproducible pharmacokinetics of the new oral anticoagulants is an advantage over conventional agents, allowing them to be administered with fixed dosing and requiring no laboratory monitoring of coagulation parameters. Determination of concentrations of oral anticoagulants such as rivaroxaban or dabigatran may only be helpful in circumstances such as overdose, in patients with a hemorrhagic or thromboembolic event during treatment, in those with deteriorating renal function, or in patients who require urgent surgery.

However, the presence of new anticoagulants such as direct thrombin and FXa inhibitors causes a significant prolongation of the clotting reaction, which in turn can produce altered and potentially misleading results in routine coagulation tests.

For example, there have been several *in vitro* reports indicating that dabigatran considerably alters the results of routine coagulation assays. *Ex vivo* interference between rivaroxaban and global coagulation assays at different time points after drug administration in patients has also been investigated. In hospitals, these alterations are observed in the results of routine postoperative laboratory coagulation assays, raising concerns about potential bleeding risk in patients.

Because of the possibility of altered, misleading results, clinicians must be able to accurately interpret coagulation parameters in patients taking, for example, rivaroxaban or dabigatran. Detailed knowledge about these drugs' effects on routine coagulation assays is critical and essential.

Assays for measurement of PT are influenced with an increase in INR or seconds by direct thrombin and factor Xa inhibitors in a concentration-dependent manner. Conversely, indirect factor Xa inhibitors such as LMWHs, which react via antithrombin/heparin complexes, do not influence the PT. Rivaroxaban and dabigatran prolong the prothrombin time, with the sensitivity dependent on the reagent being used. Nevertheless, PT is not sensitive enough to detect clinically relevant changes in drug concentration.

The APTT is more sensitive for heparin or hirudin, and prolongation of APTT occurs with increasing direct factor Xa or thrombin inhibitors. In healthy volunteers, APTT and modified PT were dose-dependently prolonged and correlated with the determined plasma concentrations of apixaban.

Factor Xa inhibitors have no impact on the TT, but thrombin inhibitors, both direct and indirect, lead to a prolongation of TT. Thus, a modified diluted thrombin time and the ecarin clotting time (ECT) are highly sensitive tests for dabigatran.

Factor Xa inhibitors do not influence the determination of fibrinogen using the Clauss method, which is based on the addition of an excess of thrombin to plasma. However, direct thrombin inhibitors may lead to a falsely reduced fibrinogen concentration on some test assays. The influence of anticoagulants in the PT-derived fibrinogen assay as an alternative method for fibrinogen measurement is similar to the influence on assays for measurement of PT.

Table 2 gives an overview of influences of different classes of anticoagulants on coagulation parameters, based on *in vitro* studies. In general, all affected assays respond in a dose-dependent manner, with therapeutic doses having a stronger influence than prophylactic doses.

Table 2. Influence of anticoagulants on routine coagulation assays

	Direct Thrombin Inhibitors	Direct FXa Inhibitors	Indirect FXa Inhibitors
PT in sec and INR ^{1,2}	↑	↑	No
APTT ²	↑	↑	No
Thrombin time	↑ ↑	No	No
Fibrinogen (Clauss)	No / ↓	No	No
Multifibren U	↓ ↓	No	No
Derived fibrinogen	No / ↓	No / ↓	No
D-dimers	No	No	No

Thrombophilia screening is indicated in certain patients presenting with DVT or PE. However, in many cases, patients are already on anticoagulation treatment before the sample for the thrombophilia panel is drawn. Because both traditional and new anticoagulants target the key enzymes of the coagulation cascade—thrombin or FXa—any anticoagulant therapy interferes with certain thrombophilia tests.

Antithrombin can be determined by measuring the inhibitory effect on either thrombin or FXa for the target enzyme. Depending on the target enzyme used, the presence of thrombin inhibitors or FXa inhibitors will result in false-high antithrombin levels.

Presence of direct thrombin and FXa inhibitors cause an overestimation of protein C or S levels in clotting assays. Alternatively, the enzyme protein C can be measured directly after activation in a chromogenic assay, which is not influenced by the presence of any anticoagulant. LA screen and confirm are prolonged in the presence of direct thrombin or FXa inhibitors; the effect on the LA screen/confirm ratio cannot be predicted. In ProC Global and APC Resistance (APCR) assays, the clotting times are generally prolonged by the presence of direct thrombin and FXa inhibitors, with an unpredictable effect on the ratio.

1. If PT is used for monitoring, results must be reported in seconds, because the INR system is not valid for any of the new anticoagulants.
2. If PT or APTT are used for monitoring, the differing responsiveness of reagents to the various drugs must be considered.

Table 3 gives an overview of influences of different classes of anticoagulants on thrombophilia parameters, based either on in vitro studies or on what would be expected on the basis of the assay principle. In general, all affected assays respond in a dose-dependent manner, with therapeutic doses having a stronger influence than prophylactic doses.

With the introduction of these new anticoagulants, it is now even more important that samples for thrombophilia testing be obtained before introducing anticoagulant therapy.

Table 3. Influence of anticoagulants on thrombophilia assays

		Direct Thrombin Inhibitors	Direct FXa Inhibitors	Indirect FXa Inhibitors
Antithrombin	FIIa-based assay	↑	No	No
	FXa-based assay	No	↑	No
Protein C ¹	Coagulometric	↑	↑	No
	Chromogenic	No	No	No
Protein S activity,	Coagulometric ¹	↑	↑	No
DRVVT assay for lupus anticoagulant diagnostics, LA screen and confirm	Clotting Times	↑	↑	No
ProC Global, APCR ¹	Clotting Times	↑	↑	No
FXIII	Chromogenic determination, FXIII activation via thrombin ¹	↓	No	No

1. Expected trend based on assay principle.

Selected Literature

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