Europace (2016) **18**, 955–964
doi:10.1093/europace/euv030

REVIEW

Anticoagulation reversal in the era of the non-vitamin K oral anticoagulants

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Received 14 October 2014; accepted after revision 29 January 2015; online publish-ahead-of-print 26 March 2015

In recent years, non-vitamin K oral anticoagulants (NOACs) have emerged as an alternative to warfarin for the prevention and treatment of thrombo-embolic disease. Large randomized trials have demonstrated that these agents, which act by directly targeting thrombin (dabigatran) and factor Xa (rivaroxaban, apixaban, and edoxaban), are at least as effective as warfarin, with lower rates of bleeding and fewer interactions with food and drugs. In addition, NOACs have a more predictable anticoagulant effect, allowing a fixed dose regimen and obviating the need for routine anticoagulation monitoring. Since the introduction of NOACs, one of the major concerns for clinicians has been the lack of specific agents to reverse their anticoagulant effect in case of life-threatening haemorrhagic complications or emergency surgery, which have limited their use in patients deemed at a higher risk of bleeding. New specific antidotes (e.g. idarucizumab, andexanet alfa, and ciraparantag) show promising data, and may soon become available for clinical use. In this article, we review the pharmacology of these agents, the incidence and outcomes of haemorrhagic complications, the available strategies for anticoagulation reversal, and the more recent advances for the development of specific antidotes.

Keywords Anticoagulation • Bleeding • Reversal • Antidote • Non-vitamin K oral anticoagulants • Warfarin • Dabigatran • Rivaroxaban • Apixaban • Edoxaban • Andexanet alfa • Idarucizumab • Ciraparantag

Introduction

For more than five decades, warfarin, a vitamin K antagonist, was the only oral anticoagulant available for prevention and treatment of thrombo-embolic disease. However, despite its efficacy, warfarin has several limitations, including a narrow therapeutic window, slow onset and offset of action, need for strict anticoagulation monitoring, and numerous food and drug interactions. A major advance in the prevention of stroke has been the introduction in recent years of non-vitamin K oral anticoagulants (previously referred to as new or novel oral anticoagulants or NOACs[1]), which act by targeting specific components of the coagulation cascade such as thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, and edoxaban).[2] The first agent receiving approval by the Food and Drug Administration (FDA) was dabigatran (Pradaxa®, Boehringer Ingelheim) in 2010, followed by rivaroxaban (Xarelto®, Bayer) in 2011, and apixaban (Eliquis®, Pfizer and Bristol-Myers Squibb) in 2012. Edoxaban (Lixiana®, Daiichi Sankyo) is approved in Japan since April 2011 and is currently under review by the US FDA.

Non-vitamin K oral anticoagulants are at least as effective as warfarin with similar or lower rates of bleeding and fewer interactions with food and drugs. In addition, they have a more predictable anticoagulant effect, allowing a fixed dose regimen and obviating the need for routine anticoagulation monitoring. One of the potential drawbacks of NOACs since their introduction has been the absence of an antidote to reverse anticoagulation in case of life-threatening bleeding or emergency surgery. However, this will soon change as specific agents that counteract the effects of NOACs are under development and promising results of Phase 2 trials have been recently announced (*Figures 1* and *2*).

In this review article, we provide an overview the pharmacology of these agents, the incidence and outcomes of haemorrhagic complications, the available strategies for anticoagulation reversal in case of bleed, and the more recent advances for the development of specific antidotes.

Overview of warfarin-related bleeding

Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors II, VII, IX, and X. These factors are synthesized in the liver as precursor forms and are activated by carboxylation of specific glutamic acid residues which require vitamin K as a cofactor. Since factor

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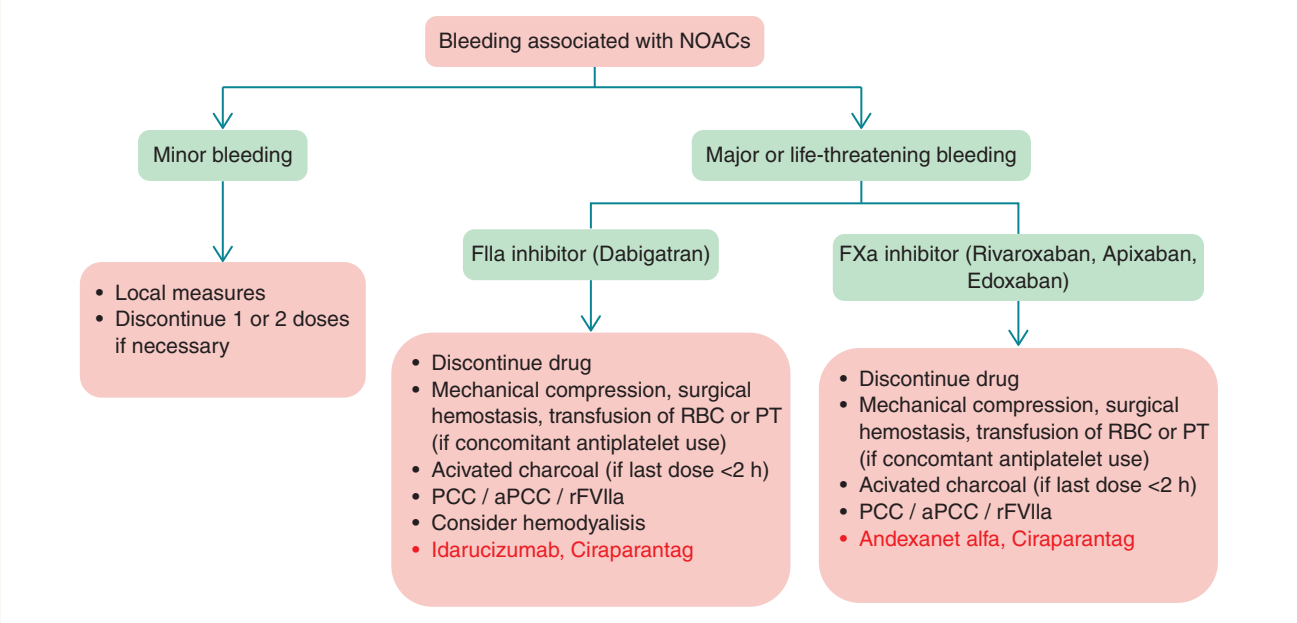

Figure 1 Management of bleeding associated with NOACs.

II (prothrombin) has a half-life of 60–72 h, the onset of action of warfarin is slow and the maximum anticoagulant effect may not be achieved before 4–5 days.[3] It has high bioavailability, is rapidly absorbed from the gastrointestinal (GI) tract, and reaches maximal blood concentrations about 90 min after oral administration.[3] Warfarin circulates bound to plasma proteins and is mainly metabolized by cytochrome P450 2C9, which explains part of its multiple drug-to-drug interactions.[4]

Pooled data from randomized trials estimate the annual risk of major bleeding associated with warfarin at around 1–2%,[5] but the rates may be higher (~3–4%) in community-based studies, which typically include older patients with more comorbidities and often poor dose management.[6,7] The most devastating complication of warfarin is intracranial haemorrhage (ICH), which presents with larger haematoma volumes and worse clinical outcomes than spontaneous ICH.[8,9] It has been estimated that and up to 10% of patients with warfarin-related major bleeding will die within 30 days and fatality associated with ICH approaches 50%.[10] The main determinant of bleeding is the intensity of anticoagulant effect and the risk of ICH doubles for every increase of 1 point in the INR.[11]

In case of life-threatening bleeding, efforts should be directed towards the rapid reversal of anticoagulation. Vitamin K is necessary to permit the *de novo* synthesis of coagulation factors; and a dose of 5–10 mg IV is able to normalize the INR in most patients; however, the effect is slow (~24 h).[12] In consequence, vitamin K is not sufficient as a sole measure and should be associated with aggressive replacement of coagulation factors. Available options include: fresh frozen plasma (FFP), prothrombin complex concentrates (PCCs), and recombinant-activated factor VII (rFVIIa; Novoseven®). Fresh frozen plasma has been the most widely used product for coagulation factor replacement, but large volumes are required to achieve normalization of the INR added to the risk of allergic reactions and transmission of infections.[13] In contrast, PCC contains much higher amounts of coagulation factors and three different products are commercially available: 3-factor PCC (contains factors II, IX, and X; Profilnine SD® and Bebulin VH®), 4-factor PCC (factors II, VII, IX, and X; Octaplex®, Beriplex®, and Cofact®), and activated PCC (APCC, coagulation factors in activated form; FEIBA®). Prothrombin complex concentrates achieve a more rapid reversal of the INR than FFP[14–16] and one prospective observational study also found improved outcomes, given by less major haemorrhage, haematoma expansion, and better 3-month functional outcomes.[17] A risk of thrombosis of 1.8% for 4-factor PCC and 0.7% for 3-factor PCC has been reported.[18] The evidence for rFVIIa is less clear. Data from small case series show reductions in the INR to variable degrees[19–21] but the use of rFVIIa failed to reduce blood loss in a human experimental model of punch biopsy-induced bleeding.

Based on the available evidence, current guidelines recommend 4-factor PCC associated with vitamin K 5–10 mg IV for patients with warfarin-associated major bleeding.[13] It is of note, however, that despite a rapid INR correction the prognosis of warfarin-associated ICH remains poor, with a high risk of haematoma expansion and in-hospital mortality.[22,23]

Dabigatran

Pharmacology

Dabigatran etexilate is a potent, direct, competitive inhibitor of thrombin, both free and bound to fibrin. It is a pro-drug with a bioavailability of 6% that after oral administration is rapidly converted to dabigatran by serum esterases. Dabigatran has a serum half-life of 12–17 h, with plasma levels peak around 2 h after ingestion, and 80% of the drug is excreted by the kidneys[24] (*Table 1*).

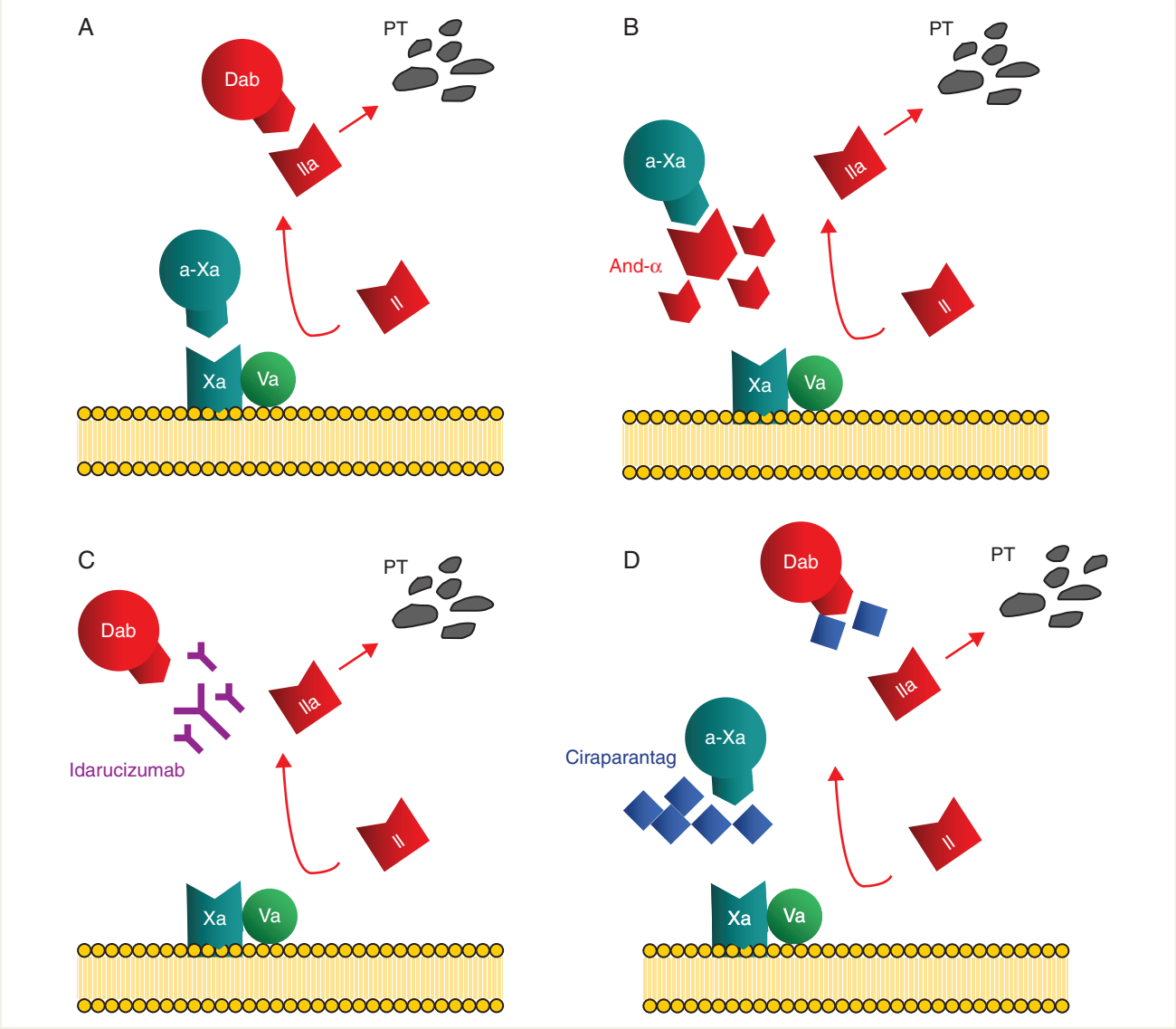

Figure 2 Mechanism of NOACs and their antidotes. (*A*) The prothrombinase complex, consisting of FXa and FIIa, catalyses the conversion of prothrombin (II) to thrombin (IIa), leading to fibrin generation and platelet aggregation. Dabigatran and FXa inhibitors (a-Xa) directly inhibit thrombin and FXa, respectively. (*B*) Andexanet alfa (And-α) is a modified inactive recombinant FXa that binds circulating FXa inhibitors, allowing native FXa to convert prothrombin to thrombin and restaure the coagulation cascade. (*C*) Idarucizumab (aDabi-Fab) is a humanized antibody fragment that binds to dabigatran, preventing it from binding to thrombin and neutralizing its anticoagulant effect. (*D*) Small synthetic molecule ciraparantag competitively binds the NOACs, restoring activity of blocked coagulation factors.

The RE-LY (Randomized Evaluation of Long-Term Anticoagulant Therapy) trial showed that dabigatran at a dose of 150 mg BID was superior to warfarin in preventing stroke and systemic embolism with a similar risk of major bleedings.[25] The benefit of dabigatran has also been demonstrated for prevention of thromboembolism after knee or hip arthroplasty[26,27] and for treatment of acute venous thrombo-embolic events.[28] Its use is not recommended in patients with mechanic heart valves based on the RE-ALIGN (Randomized, Phase II Study to Evaluate the Safety and Pharmacokinetics of Oral Dabigatran Etexilate in Patients after Heart Valve Replacement) trial, which was terminated prematurely because of an excess of thrombo-embolic and bleeding events among patients in the dabigatran group.[29]

Risk of bleeding

In the RE-LY study, the rate of major bleeding was 3.36% in the warfarin group, when compared with 2.71% per year in the group that received 110 mg of dabigatran ($P = 0.003$) and 3.11% per year in the group that received 150 mg of dabigatran ($P = 0.31$).[25] There was a significantly higher rate of major GI bleeding with dabigatran at the 150 mg dose, but life-threatening and intracranial bleeding were more frequent with warfarin than with either dose of dabigatran. The outcome of ICH was also better in the dabigatran group, with lower rates of fatal bleeds,[30] and among patients requiring urgent surgery, dabigatran was associated with similar rates of perioperative bleeding compared with warfarin.[31] Data

Table 1 Pharmacokinetic characteristics of new oral anticoagulants

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Dosing				
Non-valvular AF	150 mg BID	20 mg QD	5 mg BID	60 mg QD
DVT prophylaxis	220 mg QD	10 mg QD	2.5 mg BID	30 mg QD
DVT/PE treatment	150 mg BID	15 mg BID for 21 days, then 20 mg QD	10 mg BID for 7 days, then 5 mg BID	60 mg QD after initial therapy with heparin
Molecular weight (Da)	628	436	460	548
Target	II	Xa	Xa	Xa
Bioavailability (%)	6	63–79	66	50
T_{max} (h)	2–3	2–4	1–3	1–3
$T_{1/2}$ (h)	12–17	7–13	8–15	9–11
Protein binding (%)	35	95	87	54
Metabolism	80% renal 20% liver	1/3 renal 2/3 liver	25% renal 75% faecal	35% renal 63% liver
Interactions	P-gp inhibitors	CYP3A4 inhibitors P-gp inhibitors	CYP3A4 inhibitors P-gp inhibitors	CYP3A4 inhibitors P-gp inhibitors
Approved indications	Prevention of stroke and systemic embolism in non-valvular AF	Prevention of stroke and systemic embolism in non-valvular AF	Prevention of stroke and systemic embolism in non-valvular AF	In Japan for VTE prophylaxis after hip and knee replacement
	VTE prophylaxis after hip and knee replacement	VTE prophylaxis after hip and knee replacement	VTE prophylaxis after hip and knee replacement	

AF, atrial fibrillation; DVT, deep venous thrombosis; PE, pulmonary embolism; T_{max}, time to maximal concentration; $T_{1/2}$, half-life; VTE, venous thromboembolism.

from meta-analysis confirm a reduction of major bleeding [relative risk (RR) 0.88, 95% confidence interval (CI) 0.78–0.98][32] and ICH with dabigatran compared with vitamin K inhibitors.[32,33]

After dabigatran was first approved in the US in 2010, the FDA received an unusually high number of reports of bleeds associated with the use of the drug. The Mini-Sentinel database was examined to compare intracranial and GI haemorrhage in new users of dabigatran and warfarin from October 2010 to December 2011. Results showed that bleeding rates associated with dabigatran use during that period did not appear to be higher than those associated with warfarin and concluded that the large number of reported cases of bleeding associated with dabigatran probably was explained by the biased tendency to report adverse effects with newly released drugs, the so-called Weber effect.[34]

Other post-market studies have also addressed the safety of dabigatran in the real-world. A large Danish cohort including 4.978 patients on dabigatran found no evidence of excessive bleeding compared with warfarin.[35] Gastrointestinal bleeding was lower with dabigatran 110 mg BID and similar with dabigatran 150 mg BID, whereas ICH was lower with both dabigatran doses. Similarly, an FDA communication revealed the results of an observational cohort study of Medicare beneficiaries that compared dabigatran and warfarin over a 27-month period (October 2010–December 2012).[36] This study included more than 134 000 elderly patients (18 205 patient-years of dabigatran data), whereby dabigatran was associated with a lower risk of ischaemic stroke, ICH, and death and an increased risk of major GI bleeding compared with warfarin, in a propensity score matched analysis.

Another study by Hernandez *et al.*[37] using the same dataset came to a different conclusion. This was based on a much smaller cohort (631 patient-years of dabigatran data) with a selected sample of 5% random Medicare beneficiaries with newly diagnosed AF initiating dabigatran or warfarin, and had a shorter study period (11 months, between October 2010 and October 2011). Dabigatran was associated with a higher incidence of major bleeding [Hazard ratio (HR) 1.30; 95% CI 1.20–1.41], a higher risk of GI bleeding (HR 1.58; 95% CI 1.36–1.83), but a lower risk of ICH (HR 1.85; 95% CI 1.64–2.07).

More post-marketing data may be needed to better understand the risks associated with real-world use, especially among high-risk patients.

Measurement of anticoagulation effect

Although routine monitoring is not required for dose adjustment of NOACs, in specific situations some laboratory tests may help to assess for the presence of anticoagulation effect (overdose, acute bleeding, or in the event of an urgent surgery). For dabigatran, the most sensitive test is thrombin time (TT) that measures the direct activity of thrombin.[38] The ecarin clotting time (ECT) and TT determined by Hemoclot® test also directly measures thrombin inhibition, but are less sensitive. Among routine coagulation tests, the activated partial thromboplastin time (aPTT) can provide a qualitative assessment of dabigatran anticoagulant activity, but the correlation is not linear, especially at supratherapeutic levels.[38,39] None of the previous tests can accurately assess the intensity of anticoagulation *per se*.

Management

General measures

Until an antidote becomes available for clinical use, supportive care remains the mainstay of treatment in the event of haemorrhagic complications. The drug must be temporarily discontinued, the source of

bleeding investigated and general supportive measures should be adopted, including mechanical compression, surgical or endoscopic haemostasis, and replacement of fluids and blood products.[40–43] Activated charcoal can be used if recent overdose of the medication is suspected (<2 h).[44] Haemodialysis can also be considered for removal of the drug, especially, in patients with renal impairment. In a study of six patients with end-stage renal disease on haemodialysis that received a 50 mg dose of dabigatran, 62% of circulating drug was removed after 2 h and 68% after 4 h.[45]

Haemostatic agents

In a rat-tail model of template bleeding, APCC at a dose of 50 or 100 U/kg significantly reduced prolongation of bleeding time (BT) associated with high-dose dabigatran (1 μmol/kg bolus + 0.5 μmol/kg/h infusion for 25 min).[46] Similarly, in a murine ICH model 4-factor PCC 100 U/kg prevented haematoma expansion associated with dabigatran.[47] Studies in humans have reported dissimilar results. Using thrombin generation (TG) tests, Marlu *et al.*[48] showed *in vitro* reversal of the anticoagulant effect of dabigatran with 4-factor PCC and especially FEIBA when added to blood samples of healthy volunteers. Prothrombin complex concentrate corrected the endogenous thrombin potential, whereas FEIBA also corrected the thrombin peak, lag-time and time to peak. The first *in vivo* study in humans was conducted by Eerenberg *et al.*,[49] who investigated the effect of 4-factor PCC to revert the anticoagulant effect of dabigatran and rivaroxaban. In this randomized, placebo-controlled study, 12 healthy male volunteers received dabigatran 150 mg BID or rivaroxaban 20 mg daily for 2.5 days, followed by either a bolus of 50 IU/kg PCC or a similar volume of saline. After a washout period, the groups were crossed over and received the other anticoagulant following the same protocol. Dabigatran increased the aPTT, ECT, and TT, but PCCs failed to reverse these anticoagulant effects.

Recombinant FVIIa (0.1 or 0.5 mg/kg) has shown to reduce BT and prolongation of aPTT associated with dabigatran (1 μmol/kg bolus + 0.5 μmol/kg infusion for 25 min) in a rate-tail model of bleeding.[46] In contrast with PCC, however, rFVIIa failed to reduce haematoma expansion or mortality in a murine ICH model.[47] Its use has been reported to manage dabigatran-associated post-cardiac surgery bleeding,[50] but the evidence for this recommendation is weak.

Antifibrinolytic medications such as tranexamic acid or aprotinin have proved ineffective in reducing BTs with other direct thrombin inhibitors and are not recommended in patients taking dabigatran.[51]

Antidotes

Although supportive measures and replacement of coagulation factors with PCC or rFVIIa may be life-saving and remain today the keystone of management, the optimal strategy in case of anticoagulant-related bleeding is the use of antidotes to specifically target and inactivate the antithrombotic agent.

A specific antidote for dabigatran, idarucizumab, has been developed by Boehringer Ingelheim. The molecule is a humanized antibody fragment that binds to dabigatran with an affinity ~350 times greater than thrombin, preventing it from binding to thrombin and neutralizing its anticoagulant effect.[52,53] *In vivo* studies in rats have shown that dabigatran levels of ~200 ng/mL are neutralized within 1 min of the injection of an intravenous bolus of idarucizumab.[52] In humans, the results from the first Phase 1 study were presented at the 2013 American Heart Association Scientific Sessions.[53] In this randomized, double-blind, placebo-controlled study of 145 healthy male volunteers, investigators evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of the antibody fragment. In a first step, the tolerability of the antibody fragment was tested as an intravenous infusion of rising doses of up to 8 g. In a second step, the potential for reversal of dabigatran-induced anticoagulation was evaluated, with 5-min infusions using three different doses (1, 2, and 4 g) following pre-treatment with dabigatran (220 mg BID for 3 days). The anticoagulant effect of dabigatran and its reversal were assessed using diluted TT (Hemoclot® DTI assay), TT, aPTT, ECT, and activated clotting time (ACT). Dabigatran prolonged clotting times of all coagulation markers and there was a dose-dependent reversal with increasing doses of the antidote. Thrombin time was reversed from a ratio of up to 14-fold over baseline to <2-fold. Reversal was complete and sustained in seven of nine subjects administered 2 g and in all subjects administered 4 g. A Phase 3 study, the RE-VERSE AD (A Study of the Reversal Effects of Idarucizumab on Active Dabigatran) is already under way in patients with dabigatran-induced bleeding or requiring emergency surgery.

Another reversal agent is ciraparantag (PER977), a small, synthetic, water-soluble, cationic molecule developed by Perosphere Inc. that binds to unfractionated heparin, low-molecular-weight heparin, fondaparinux, dabigatran and to the new factor Xa inhibitors through hydrogen bonding and charge–charge interactions.[54] In tromboelastographic assays (*ex vivo*) and in a rat-tail-transection bleeding model, ciraparantag has demonstrated to completely reverse the effect of dabigatran, rivaroxaban, apixaban, edoxaban, fondaparinux, and heparin.[54] Studies in humans treated with dabigatran are not yet available.

Direct factor Xa inhibitors: rivaroxaban, apixaban, and edoxaban

Pharmacology

Rivaroxaban and apixaban are the two direct inhibitors of factor Xa currently approved in North America and Europe, whereas Edoxaban is approved in Japan since April 2011. These agents reversibly inhibit free and clot-bound factor Xa, thus preventing the conversion of prothrombin to thrombin and subsequent fibrin clot formation. They have a high bioavailability, ranging from around 50% in apixaban, 62% in edoxaban, to 100% in rivaroxaban when taken with food.[55] The main difference is the once-daily dosing for rivaroxaban and edoxaban, compared with the twice-daily dosing for apixaban (*Table 1*).

Rivaroxaban, apixaban, and edoxaban are equally or more effective than enoxaparin for the prevention of thrombo-embolic events after orthopaedic surgery.[56–62] Rivaroxaban is non-inferior to enoxaparin followed by warfarin in patients with established venous thrombosis, with similar occurrence of major or relevant bleedings.[63] Apixaban and edoxaban were also non-inferior to enoxaparin with significantly less bleeding.[64,65]

In non-valvular AF, both rivaroxaban in the ROCKET AF (Rivaroxaban Once Daily Oral Direct factor Xa Inhibitor Compared With Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation) and edoxaban in the ENGAGE AF-TIMI 48 (Effective Anticoagulation with Factor Xa Next Generation in Atrial Fibrillation—Thrombolysis in Myocardial Infarction 48) showed non-inferiority compared with warfarin in the prevention of stroke or systemic embolism.[66,67] On the other hand, using the same primary endpoint, the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial showed that apixaban was superior to warfarin.[68] All three factor Xa inhibitors exhibited a similar or better safety profile compared with warfarin, with equivalent or lower rates of major and intracranial bleeding. Apixaban and low-dose edoxaban (30 mg) were also associated with reduced all-cause mortality.[67,68]

Risk of bleeding

When used for thromboprohylaxis after hip or knee replacement, the risk of major bleeding associated with rivaroxaban fluctuates between 0.3 and 0.6% if the drug is administered at a dose of 10 mg daily for a period of 2–6 weeks.[56,57] In the ROCKET AF study, the annual rates of major bleeding were similar in the rivaroxaban and warfarin groups (3.6 and 3.4%, respectively; $P = 0.58$).[66] Interestingly, the rates of ICH were significantly lower in the rivaroxaban vs. the warfarin group (0.5 vs. 0.7% per year; $P = 0.02$), whereas GI bleeding was more common in the rivaroxaban group (3.2 vs. 2.2, $P < 0.001$), a fact attributed to the presence of active drug in the GI lumen, which may induce or exacerbate bleeding from local lesions. Meta-analysis of major clinical trials comparing rivaroxaban with warfarin have confirmed a similar rate of major bleeding with both drugs (RR 1.01, 95% CI 0.89–1.16)[32] and reduced risk of ICH with rivaroxaban.[32,33]

With apixaban, the annual rate of major bleeding was lower than with warfarin in the ARISTOTLE trial (2.13 vs. 3.09% per year; HR 0.69; 95% CI 0.60–0.80; $P < 0.001$), with less ICH (0.33 vs. 0.80% per year; HR 0.42, 95% CI 0.30–0.58), but similar rate of GI bleeds (0.76 vs. 0.85% per year, respectively; HR 0.86, 95% CI 0.70–1.15).[69] In addition, the outcomes of ICH were better with apixaban, leading less often to hospitalization, medical or surgical intervention, transfusion, or change in antithrombotic therapy.

In the ENGAGE AF-TIMI 48, the high and low doses of edoxaban were associated with lower rates of major bleeding compared with warfarin (2.75 and 1.61% per year vs. 3.73% per year).[67]

Measurement of anticoagulation effect

All factor Xa inhibitors prolong the PT and usually a normal value excludes a residual anticoagulation effect due to these drugs, but the sensitivity depends on the reagents used.[70] A more specific indicator of the anticoagulant effect is the measurement of anti-factor Xa activity by chromogenic assays, but these are not available in most care facilities and there are no data on a cut-off to clearly define a safety margin.[41,71]

Management

General measures

Same as with dabigatran, discontinuation of the drug and general support measures are the most important steps in case of major or life-threatening bleeding associated with direct factor Xa inhibitors.[40–42,72] Activated charcoal may reduce apixaban exposure by 50 and 28%, respectively, when administered at 2 and 6 h post dose.[73] Because of high plasma protein binding, rivaroxaban and apixaban are not dialyzable (*Tables 2* and *3*).

Haemostatic agents

In vitro, PCC, APCC, and rFVIIa resulted partially effective in reversing rivaroxaban-induced anticoagulation. Perzborn *et al.*[74] showed that APCC and rFVIIa were more effective than PCC in reversing prolongation of PT, CT, and TG lag time, but reversal was not complete, reaching a plateau with maximal effect of ~50%.

In animal models, PCC, APCC, and rFVIIa have also shown potential to improve laboratory parameters of coagulation, but the effects on BT are variable.[75,76] In a murine model of ICH associated with rivaroxaban FFP, PCC, and rFVIIa prevented excess haematoma expansion.[77]

In the previously cited study by Eerenberg *et al.*,[49] rivaroxaban induced a prolongation of the activated PT (15.8 ± 1.3 vs. 12.3 ± 0.7 s at baseline; $P < 0.001$) that was immediately and completely

Table 2 Strategies for anticoagulation reversal in bleeding associated with warfarin and new oral anticoagulants

	Warfarin	Dabigatran	Rivaroxaban, apixaban, and edoxaban
General measures	Drug discontinuation, mechanical compression, surgical haemostasis, transfusional support	Drug discontinuation, mechanical compression, surgical haemostasis, transfusional support	Drug discontinuation, mechanical compression, surgical haemostasis, transfusional support
Activated charcoal	Consider if last dose <2 h	Consider if last dose <2 h	Consider if last dose <2 h
Haemodialysis	No benefits (highly protein bound)	Removes 62–68% of circulating drug	No benefits (highly protein bound)
Coagulation factors	PCC (25 U/kg, repeat if necessary) FFP (10–15 ml/kg) rFVIIa (90 ug/kg)	PCC (25 U/kg, repeat if necessary) rFVIIa (90 ug/kg)	PCC (25 U/kg, repeat if necessary) or FEIBA (50 IE/kg, max 200 IE/day) rFVIIa (90 ug/kg)
Specific inhibitors	Vitamin K (5–10 mg IV)	Idarucizumab (Phase 1) Ciraparantag (preclinical)	Andexanet alfa (Phases 1–3) Ciraparantag (Phase 1)

PCC, prothrombin complex concentrates; rFVIIa, recombinant-activated factor VII.

Table 3 Comparison of specific antidotes for NOACs

Agent	Idarucizumab (Boehringer Ingelheim)	Andexanet alfa (Portola Pharmaceuticals)	Ciraparantag (Perosphere)
Target	Dabigatran	FXa inhibitors (Rivaroxaban, Apixaban, Edoxaban, Betrixaban)	Dabigatran, FXa inhibitors (Rivaroxaban, Apixaban, Edoxaban, Betrixaban), Fondaparinux, heparin
Structure	Humanized antibody fragment	Recombinant human FXa, catalytically inactive	Synthetic small molecule (512 Da)
Mechanism	Non-competitive binding to Dabigatran with 350 times greater affinity than thrombin	Binds competitively to direct FXa inhibitors	Binds to heparins and oral FXa and IIa inhibitors through hydrogen bonding
In vitro studies	Reversal of prolonged clotting time induced by Dabigatran	Complete and dose-dependent reversal of Rivaroxaban, Apixaban and Betrixaban in human plasma	Complete reversal of anti-Xa activity of Rivaroxaban, Apixaban and Edoxaban
Animal models	Reduction in blood loss and mortality in a porcine liver trauma model	Reduced blood loss induced by Rivaroxaban in mouse (tail transection) and rabbit (liver laceration) models	Decreased bleeding in a rat-tail transaction model
Clinical trials	Phase 1: Immediate, complete and sustained reversal of Dabigatran-induced anticoagulation in healthy humans Phase 3: Ongoing (RE-VERSE AD)	Phase 1: Dose-dependent reversal of Rivaroxaban in healthy volunteers Phase 2: Rapid reversal of Rivaroxaban and Apixaban. Ongoing trial with Edoxaban Phase 3: Rapid reversal of Apixaban (ANNEXA-A). Ongoing trial with Rivaroxaban (ANNEXA-R) and planned trial with Edoxaban (ANNEXA-E)	Phase 1: Rapid and sustained reversal of edoxaban

reversed by 4-factor PCC (12.8 $\pm$ 1.0 s; $P < 0.001$). A similar effect was observed on endogenous thrombin potential, which was inhibited by rivaroxaban (51 $\pm$ 22 vs. 92 $\pm$ 22% at baseline; $P = 0.002$) and normalized with 4-factor PCC (114 $\pm$ 26%; $P < 0.001$). Although these results are promising, it has to be considered that they correspond to improvement in laboratory parameters and the effects of PCC or rFVII have not been yet investigated in patients with bleeding events induced by rivaroxaban.

There is limited information regarding the effect of coagulation factor concentrates on apixaban-induced anticoagulation. Available data have shown that PCC, rFVIIa, and fibrinogen concentrates improve several laboratory parameters, but they failed to reverse bleeding in a rabbit model.[78,79]

Antidotes

Andexanet alfa, an injectable antidote for factor Xa inhibitors, has been recently developed by Portola Pharmaceuticals. This recombinant protein is a modified form of factor Xa that is catalytically inactive but retains high-affinity binding to factor Xa inhibitors.[80] Andexanet alfa reverses immediately and in a dose-dependent manner, the anticoagulant effect of rivaroxaban, apixaban, and edoxaban, and in two animal models the antidote reduced bleeding by >85%.[80,81]

In a Phase 1 study, 32 healthy volunteers were randomized to 4 intravenous doses of andexanet alfa or placebo, and rivaroxaban was added to plasma samples *ex vivo*.[82] The antidote reversed anti-factor Xa in a dose-dependent manner and no thrombotic events were reported. In December of 2013, the results of a Phase 2, double-blind, placebo-controlled trial were presented at the American Society of Hematology meeting.[83] In this study, 18 healthy volunteers were treated with rivaroxaban at an oral dose of 20 mg daily for 6 days and then given either an intravenous bolus of andexanet alfa or placebo on day 6. The doses of 210 and 420 mg reversed the anti-factor Xa activity by 32 and 51%, respectively. In addition, rivaroxaban-induced inhibition of thrombin generation and prolongation of both PT and ACT were also partially reversed by andexanet alfa in a dose-dependent manner. The drug was well tolerated and there were no thrombotic events or severe adverse effects. The results of andexanet alfa for apixaban were announced at the 2013 International Society on Thrombosis and Hemostasis congress.[84] In this randomized, placebo-controlled, double-blind, Phase 2 study, 27 healthy volunteers were treated on days 1–6 with *apixaban* 5 mg BID and then randomized in a 6:3 ratio to intravenous andexanet alfa (in three different dose: 90, 210, or 420 mg) or saline on day 6, 3 h after receiving the last *apixaban* dose. Results demonstrated a dose-dependent reversal of the anticoagulant activity of *apixaban*. Two minutes after administration of 420 mg of andexanet alfa ($n = 6$), the anticoagulant activity of *apixaban* decreased by >95% as measured by anti-factor Xa activity. Similarly, the 210 mg dose reduced anti-factor Xa activity by 80% compared with saline ($n = 9$). An observation from the aforementioned studies was the fact that anticoagulation returns to pre-treatment levels within few hours after a bolus infusion of the antidote and therefore a constant infusion of the drug may be needed to reverse anticoagulation for longer periods.

More recently, the results of a Phase 3 study, the ANNEXA-A (Andexanet Alfa a Novel Antidote to the Anticoagulant Effects of FXA Inhibitors-Apixaban) trial was presented at the American Heart Association 2014 Scientific Sessions.[85] In the first part, 33 patients on apixaban were randomized to andexanet alfa bolus

400 mg IV vs. placebo. The antidote administration resulted in rapid normalization of anti-factor Xa and thrombin levels, with peak effect observed a couple of minutes after completion of the bolus. In the absence of a maintenance infusion, anti-factor Xa levels gradually increased by 25% by 1 h and were similar to placebo by 2 h. There were no serious adverse events reported, including thrombotic events. The second part of the study, in which the intravenous bolus will be followed by a 2-h infusion or placebo, is under way and results are soon expected. Another Phase 3 study, known as ANNEXA-R (Andexanet Alfa a Novel Antidote to the Anticoagulant Effects of FXA Inhibitors-Rivaroxaban), is also ongoing and a third one, the ANNEXA-E (Andexanet Alfa a Novel Antidote to the Anticoagulant Effects of FXA Inhibitors-Edoxaban), is planned.

Finally, the preliminary data obtained with the universal antidote ciraparantag are promising. The molecule effectively reverses bleeding associated with factor Xa inhibitors in animal models of external and internal bleeding.[54,86,87] In addition, the results of the first human, Phase I study has been recently published. In this study, a single intravenous dose of ciraparantag (100 and 300 mg) 3 h after the administration of edoxaban restored the whole-blood clotting time to baseline levels in 10 min or less and the effect was sustained for 24 h.[88] Unlike andexanet alfa, ciraparantag showed no evidence of procoagulant activity, as assessed by measurement of d-dimer, prothrombin fragment 1.2, and tissue factor pathway inhibitor and by whole-blood clotting time. This, in addition to its broad spectrum, may represent a potential advantage over andexanet alfa. Possible related adverse effects were dysgeusia and transient perioral and facial flushing.

Conclusion

Non-vitamin K oral anticoagulants represent the new drug generation for prevention and treatment of thrombo-embolic disease. Owing to their proven efficacy and predictable anticoagulant effect, without need for routine monitoring, these agents have gradually started to replace warfarin for several indications. One of the major concerns for clinicians has been the lack of specific reversal agents for the management of severe haemorrhagic complications. However, a new line of antidotes specific for these drugs is at different stages of development and their release in the near future seems to be imminent. It is expectable that the availability of such antidotes will dissipate the still existing concerns about NOACs in the medical community and lead to their full incorporation and into clinical practice.

Conflict of interest: G.Y.H.L. is consultant for Bayer, Astellas, Merck, Astra-Zeneca, Sanofi Aventis, Biotronik, BMS/Pfizer, Portola, and Boehringher Ingelheim; and has been on the speakers bureau for Bayer, BMS/Pfizer, Boehringher Ingelheim, and Sanofi Aventis.

References

1. Husted S, de Caterina R, Andreotti F, Arnesen H, Bachmann F, Huber K *et al.* Non-vitamin K antagonist oral anticoagulants (NOACs): no longer new or novel. *Thromb Haemost* 2014;**111**:781–2.
2. De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F *et al.* Position paper of the ESC Working Group on thrombosis-task force on anticoagulants in heart disease. *Thromb Haemost* 2013;**109**:569–79.
3. Hirsh J, Dalen J, Anderson DR, Poller L, Bussey H, Ansell J *et al.* Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest* 2001;**119**:8S–21S.
4. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G *et al.* Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). *Chest* 2008;**133**:160S–98S.
5. Atrial Fibrillation Investigators. Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation: analysis of pooled data from five randomized controlled trials. *Arch Intern Med* 1994;**154**:1449–57.
6. Van der Meer FJM, Rosendaal FR, Van der Broucke JP, Briet E. Bleeding complications in oral anticoagulation therapy. *Arch Intern Med* 1993;**153**:1557–62.
7. Jackson SL, Peterson GM, Vial JH, Daud R, Ang SY. Outcomes in the management of atrial fibrillation: clinical trial results can apply in practice. *Intern Med J* 2001;**31**:329–36.
8. Flaherty ML, Tao H, Haverbusch M, Sekar P, Kleindorfer D, Kissela B *et al.* Warfarin use leads to larger intracerebral hematomas. *Neurology* 2008;**71**:1084–9.
9. Cucchiara B, Messe S, Sansing L, Kasner S, Lyden P. Hematoma growth in oral anticoagulant related intracerebral hemorrhage. *Stroke* 2008;**39**:2993–6.
10. Guerrouij M, Uppal CS, Alklabi A, Douketis JD. The clinical impact of bleeding during oral anticoagulant therapy: assessment of morbidity, mortality and post-bleed anticoagulant management. *J Thromb Thrombolysis* 2011;**31**:419–23.
11. Singer DE. Risk factors for intracranial hemorrhage in outpatients taking warfarin. *Ann Intern Med* 1994;**120**:897–902.
12. Garcia DA, Crowther MA. Reversal of warfarin: case-based practice recommendations. *Circulation* 2012;**125**:2944–7.
13. Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ *et al.* Evidence-based management of anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012;**141**:e152S–84S.
14. Fredriksson K, Norrving B, Strömblad LG. Emergency reversal of anticoagulation after intracerebral hemorrhage. *Stroke* 1992;**23**:972–7.
15. Demeyere R, Gillardin S, Arnout J, Strengers PFW. Comparison of fresh frozen plasma and prothrombin complex concentrate for the reversal of oral anticoagulants in patients undergoing cardiopulmonary bypass surgery: a randomized study. *Vox Sang* 2010;**99**:251–60.
16. Boulis NM, Bobek MP, Schmaier A, Hoff JT. Use of factor IX complex in warfarin-related intracranial hemorrhage. *Neurosurgery* 1999;**45**:1113–9.
17. Frontera JA, Gordon E, Zach V, Jovine M, Uchino K, Hussain MS *et al.* Reversal of coagulopathy using prothrombin complex concentrates is associated with improved outcome compared to fresh frozen plasma in warfarin-associated intracranial hemorrhage. *Neurocrit Care* 2014;**21**:397–406.
18. Dentali F, Marchesi C, Pierfranceschi MG, Crowther M, Garcia D, Hylek E *et al.* Safety of prothrombin complex concentrates for rapid anticoagulation reversal of vitamin K antagonists: a meta-analysis. *Thromb Haemost* 2011;**106**:429–38.
19. Skolnick BE, Mathews DR, Khutoryansky NM, Pusateri AE, Carr ME. Exploratory study on the reversal of warfarin with rFVIIa in healthy subjects. *Blood* 2010;**116**:693–701.
20. Deveras RAE, Kessler CM. Reversal of warfarin-induced excessive anticoagulation with recombinant human factor VIIa concentrate. *Ann Intern Med* 2002;**137**:884–8.
21. Lin J, Hanigan WC, Tarantino M, Wang J. The use of recombinant activated factor VII to reverse warfarin-induced anticoagulation in patients with hemorrhages in the central nervous system: preliminary findings. *J Neurosurg* 2003;**98**:737–40.
22. Dowlatshahi D, Butcher KS, Asdaghi N, Nahirniak S, Bernbaum ML, Giulivi A *et al.* Poor prognosis in warfarin-associated intracranial hemorrhage despite anticoagulation reversal. *Stroke* 2012;**43**:1812–7.
23. Zubkov AY, Mandrekar JN, Claassen DO, Manno EM, Wijdicks EF, Rabinstein AA. Predictors of outcome in warfarin-related intracerebral hemorrhage. *Arch Neurol* 2008;**65**:1320–5.
24. Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor xa inhibitors in development. *Clin Pharmacokinet* 2009;**48**:1–22.
25. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A *et al.* Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009;**361**:1139–51.
26. Eriksson BI, Dahl OE, Büller HR, Hettiarachchi R, Rosencher N, Bravo ML *et al.* A new oral direct thrombin inhibitor, dabigatran etexilate, compared with enoxaparin for prevention of thromboembolic events following total hip or knee replacement: the BISTRO II randomized trial. *J Thromb Haemost* 2005;**3**:103–11.
27. Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP *et al.* Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. *Lancet* 2007;**370**:949–56.
28. Ginsberg JS, Davidson BL, Comp PC, Francis CW, Friedman RJ, Huo MH *et al.* Oral thrombin inhibitor dabigatran etexilate vs North American enoxaparin regimen for

prevention of venous thromboembolism after knee arthroplasty surgery. *J Arthroplasty* 2009;**24**:1–9.
29. Eikelboom JW, Connolly SJ, Brueckmann M, Granger CB, Kappetein AP, Mack MJ *et al.* Dabigatran versus warfarin in patients with mechanical heart valves. *N Engl J Med* 2013;**369**:1206–14.
30. Hart RG, Diener HC, Yang S, Connolly SJ, Wallentin L, Reilly PA *et al.* Intracranial hemorrhage in atrial fibrillation patients during anticoagulation with warfarin or dabigatran: the RE-LY trial. *Stroke* 2012;**43**:1511–7.
31. Healey JS, Eikelboom J, Douketis J, Wallentin L, Oldgren J, Yang S *et al.* Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) randomized trial. *Circulation* 2012;**126**:343–8.
32. Dentali F, Riva N, Crowther M, Turpie AG, Lip GY, Ageno W. Efficacy and safety of the novel oral anticoagulants in atrial fibrillation: a systematic review and meta-analysis of the literature. *Circulation* 2012;**126**:2381–91.
33. Chatterjee S, Sardar P, Biondi-Zoccai G, Kumbhani DJ. New oral anticoagulants and the risk of intracranial hemorrhage: traditional and Bayesian meta-analysis and mixed treatment comparison of randomized trials of new oral anticoagulants in atrial fibrillation. *JAMA Neurol* 2013;**70**:1486–90.
34. Southworth MR, Reichman ME, Unger EF. Dabigatran and postmarketing reports of bleeding. *N Engl J Med* 2013;**368**:1272–4.
35. Larsen TB, Rasmussen LH, Skjøth F, Due KM, Callréus T, Rosenzweig M *et al.* Efficacy and safety of dabigatran etexilate and warfarin in "real-world" patients with atrial fibrillation: a prospective nationwide cohort study. *J Am Coll Cardiol* 2013;**61**:2264–73.
36. Graham DJ, Reichman ME, Wernecke M, Zhang R, Southworth MR, Levenson M *et al.* Cardiovascular, bleeding, and mortality risks in elderly medicare patients treated with dabigatran or warfarin for non-valvular atrial fibrillation. *Circulation* 2015;**131**:157–64.
37. Hernandez I, Baik SH, Piñera A, Zhang Y. Risk of bleeding with dabigatran in atrial fibrillation. *JAMA Intern Med* 2015;**175**:18–24.
38. van Ryn J, Stangier J, Haertter S, Liesenfeld KH, Wienen W, Feuring M *et al.* Dabigatran etexilate—a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost* 2010;**103**:1116–27.
39. Stangier J, Rathgen K, Stähle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *Br J Clin Pharmacol* 2007;**64**:292–303.
40. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH *et al.*; ESC Committee for Practice Guidelines-CPG; Document Reviewers. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation--developed with the special contribution of the European Heart Rhythm Association. *Europace* 2012;**14**:1385–413.
41. Heidbuchel H, Verhamme P, Alings M, Antz M, Hacke W, Oldgren J *et al.*; European Heart Rhythm Association. European Heart Rhythm Association Practical Guide on the use of new oral anticoagulants in patients with non-valvular atrial fibrillation. *Europace* 2013;**15**:625–51.
42. Vílchez JA, Gallego P, Lip GY. Safety of new oral anticoagulant drugs: a perspective. *Ther Adv Drug Saf* 2014;**5**:8–20.
43. Huisman MV, Lip GY, Diener HC, Brueckmann M, van Ryn J, Clemens A. Dabigatran etexilate for stroke prevention in patients with atrial fibrillation: resolving uncertainties in routine practice. *Thromb Haemost* 2012;**107**:838–47.
44. Suryanarayan D, Schulman S. Potential antidotes for reversal of old and new oral anticoagulants. *Thromb Res* 2014;**133**:S158–66.
45. Stangier J, Rathgen K, Stähle H, Mazur D. Influence of renal impairment on the pharmacokinetics and pharmacodynamics of oral dabigatran etexilate: an open-label, parallel-group, single-centre study. *Clin Pharmacokinet* 2010;**49**:259–68.
46. van Ryn J, Ruehl D, Priepke H, Hauel N, Wienen W. Reversibility of the anticoagulant effect of high doses of the direct thrombin inhibitor dabigatran, by recombinant Factor VIIa or activated prothrombin complex concentrate (abstract 0370). *Haematologica* 2008;**93**:148.
47. Zhou W, Schwarting S, Illanes S, Liesz A, Middelhoff M, Zorn M *et al.* Hemostatic therapy in experimental intracerebral hemorrhage associated with the direct thrombin inhibitor dabigatran. *Stroke* 2011;**42**:3594–9.
48. Marlu R, Hodaj E, Paris A, Albaladejo P, Cracowski JL, Pernod G. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban: a randomised crossover ex vivo study in healthy volunteers. *Thromb Haemost* 2012;**108**:217–24.
49. Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 2011;**124**:1573–9.
50. Warkentin TE, Margetts P, Connolly SJ, Lamy A, Ricci C, Eikelboom JW. Recombinant factor VIIa (rFVIIa) and hemodialysis to manage massive dabigatran-associated postcardiac surgery bleeding. *Blood* 2012;**119**:2172–4.
51. Elg M, Carlsson S, Gustafsson D. Effects of agents, used to treat bleeding disorders, on bleeding time prolonged by a very high dose of a direct thrombin inhibitor in anesthesized rats and rabbits. *Thromb Res* 2001;**101**:159–70.
52. Schiele F, van Ryn J, Canada K, Newsome C, Sepulveda E, Park J *et al.* A specific antidote for dabigatran: functional and structural characterization. *Blood* 2013;**121**:3554–62.
53. Glund S, Stangier J, Schmohl M, De Smet M, Gansser D, Lang B *et al.* A specific antidote for dabigatran: immediate, complete and sustained reversal of dabigatran induced anticoagulation in healthy male volunteers (abstract). *Circulation* 2013;**128**:A17765.
54. Laulicht B, Bakhru S, Lee C, Baker C, Jiang X, Mathiowitz E *et al.* Small molecule antidote for anticoagulants (abstract). *Circulation* 2012;**126**:10021.
55. Lau YC, Lip GY. Which drug should we use for stroke prevention in atrial fibrillation? *Curr Opin Cardiol* 2014;**29**:293–300.
56. Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK *et al.* Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med* 2008;**358**:2765–75.
57. Lassen MR, Ageno W, Borris LC, Lieberman JR, Rosencher N, Bandel TJ *et al.* Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. *N Engl J Med* 2008;**358**:2776–86.
58. Lassen MR, Raskob GE, Gallus A, Pineo G, Chen D, Portman RJ. Apixaban or enoxaparin for thromboprophylaxis after knee replacement. *N Engl J Med* 2009;**361**:594–604.
59. Lassen MR, Raskob GE, Gallus A, Pineo G, Chen D, Hornick P. Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. *Lancet* 2010;**375**:807–15.
60. Lassen MR, Gallus A, Raskob GE, Pineo G, Chen D, Ramirez LM. Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. *N Engl J Med* 2010;**363**:2487–98.
61. Fuji T, Wang CJ, Fujita S, Kawai Y, Nakamura M, Kimura T *et al.* Safety and efficacy of edoxaban, an oral factor Xa inhibitor, versus enoxaparin for thromboprophylaxis after total knee arthroplasty: the STARS E-3 trial. *Thromb Res* 2014;**134**:1198–204.
62. Fuji T, Fujita S, Kawai Y, Nakamura M, Kimura T, Kiuchi Y *et al.* Safety and efficacy of edoxaban in patients undergoing hip fracture surgery. *Thromb Res* 2014;**133**:1016–22.
63. Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS *et al.* Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med* 2010;**363**:2499–510.
64. Agnelli G, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M *et al.*; AMPLIFY Investigators. Oral apixaban for the treatment of acute venous thromboembolism. *N Engl J Med* 2013;**369**:799–808.
65. Büller HR, Décousus H, Grosso MA, Mercuri M, Middeldorp S, Prins MH *et al.* Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med* 2013;**369**:1406–15.
66. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W *et al.* Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011;**365**:883–91.
67. Giugliano RP, Ruff CT, Braunwald E, Murphy SA, Wiviott SD, Halperin JL *et al.* Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2013;**369**:2093–104.
68. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M *et al.* Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011;**365**:981–92.
69. Hylek EM, Held C, Alexander JH, Lopes RD, De Caterina R, Wojdyla DM *et al.* Major bleeding in patients with atrial fibrillation receiving apixaban or warfarin: The ARISTOTLE Trial (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation): Predictors, Characteristics, and Clinical Outcomes. *J Am Coll Cardiol* 2014;**63**:2141–7.
70. Tripodi A. Measuring the anticoagulant effect of direct factor Xa inhibitors. Is the anti-Xa assay preferable to the prothrombin time test? *Thromb Haemost* 2011;**105**:735–6.
71. Barrett YC, Wang Z, Frost C, Shenker A. Clinical laboratory measurement of direct factor Xa inhibitors: anti-Xa assay is preferable to prothrombin time assay. *Thromb Haemost* 2010;**104**:1263–71.
72. Turpie AG, Kreutz R, Llau J, Norrving B, Haas S. Management consensus guidance for the use of rivaroxaban--an oral, direct factor Xa inhibitor. *Thromb Haemost* 2012;**108**:876–86.
73. Wang X, Mondal S, Wang J, Tirucherai G, Zhang D, Boyd RA *et al.* Effect of activated charcoal on apixaban pharmacokinetics in healthy subjects. *Am J Cardiovasc Drugs* 2014;**14**:147–54.
74. Perzborn E, Heitmeier S, Laux V, Buchmüller A. Reversal of rivaroxaban-induced anticoagulation with prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated factor VII *in vitro*. *Thromb Res* 2014;**133**:671–81.

75. Godier A, Miclot A, Le Bonniec B, Durand M, Fischer AM, Emmerich J *et al.* Evaluation of prothrombin complex concentrate and recombinant activated factor VII to reverse rivaroxaban in a rabbit model. *Anesthesiology* 2012;**116**:94–102.
76. Perzborn E, Gruber A, Tinel H, Marzec UM, Buetehorn U, Buchmueller A *et al.* Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. *Thromb Haemost* 2013;**110**:162–72.
77. Zhou W, Zorn M, Nawroth P, Bütehorn U, Perzborn E, Heitmeier S *et al.* Hemostatic therapy in experimental intracerebral hemorrhage associated with rivaroxaban. *Stroke* 2013;**44**:771–8.
78. Martin AC, Le Bonniec B, Fischer AM, Marchand-Leroux C, Gaussem P, Samama CM *et al.* Evaluation of recombinant activated factor VII, prothrombin complex concentrate, and fibrinogen concentrate to reverse apixaban in a rabbit model of bleeding and thrombosis. *Int J Cardiol* 2013;**168**:4228–33.
79. Escolar G, Fernandez-Gallego V, Arellano-Rodrigo E, Roquer J, Reverter JC, Sanz VV *et al.* Reversal of apixaban induced alterations in hemostasis by different coagulation factor concentrates: significance of studies in vitro with circulating human blood. *PLoS One* 2013;**8**:e78696.
80. Lu G, DeGuzman FR, Hollenbach SJ, Karbarz MJ, Abe K, Lee G *et al.* A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa. *Nat Med* 2013;**19**:446–51.
81. Hollenbach S, Tan S, DeGuzman F, Malinowski J, Hutchaleelaha A, Inagaki M *et al.* PRT064445 reverses rivaroxaban induced antiacogulation in a rabbit liver laceration treatment model (abstract). *Eur Heart J* 2013;**34**:24.
82. Crowther MA, Kitt M, McClure M, Sinha U, Lu G, Karbarz M *et al.* Randomized, double-blind, placebo-controlled single ascending dose pharmacokinetic and pharmacodynamic study of PRT064445, a universal antidote for factor Xa inhibitors (abstract). *Arterioscler Thromb Vasc Biol* 2013;**33**:A10.
83. Crowther MA, Mathur V, Kitt M, Lu G, Conley P, Hollenbach S *et al.* A phase 2 randomized, double-blind, placebo-controlled trial demonstrating reversal of rivaroxaban-induced anticoagulation in healthy subjects by andexanet alfa (PRT064445), an antidote for FXa inhibitors (abstract). *Blood* 2013;**122**:A3636.
84. Crowther MA, Kitt M, Lorenz T, Mathur V, Lu G, Hutchaleelaha A *et al.* A phase 2 randomized, double-blind, placebo-controlled trial of PRT4445, a novel, universal antidote for direct and indirect Factor Xa inhibitors (abstract). *J Thromb Haemost* 2013;**11**:AS20.1.
85. Crowther M, Levy G, Lu G, Leeds J, Barron L, Conley P *et al.* ANNEXA^{TM} A: a Phase 3 randomized, double-blind, placebo-controlled trial, demonstrating reversal of apixaban-induced anticoagulation in older subjects by Andexanet alfa (PRT064445), a universal antidote for factor Xa (fXa) inhibitors (abstract). *Circulation* 2014;**130**:2105–26.
86. Bakhru S, Laulicht B, Jiang X, Chen L, Grosso M, Morishima Y *et al.* Reversal of anticoagulant-induced bleeding in external and internal bleeding models by PER977, a small molecule anticoagulant antidote (abstract). *Circulation* 2014;**130**:A19361.
87. Hollenbach S, Lu G, DeGuzman F, Tan S, Pratikhya P, Pandey A *et al.* Andexanet-alfa and PER977 (Arapazine) correct blood loss in a Rabbit Liver Laceration Model – only andexanet reverses markers of fXa-mediated anticoagulation (abstract). *Circulation* 2014;**130**:A14657.
88. Ansell JE, Bakhru SH, Laulicht BE, Steiner SS, Grosso M, Brown K *et al.* Use of PER977 to reverse the anticoagulant effect of edoxaban. *N Engl J Med* 2014;**371**:2141–2.