


Noncanonical Effects of Oral Thrombin and Factor Xa Inhibitors in Platelet Activation and Arterial Thrombosis

Amin Polzin^{1,*} Lisa Dannenberg^{1,*} Manuela Thienen^{2,3} Martin Orban^{2,3} Georg Wolff¹
Thomas Hohlfeld⁴ Tobias Zeus¹ Malte Kelm¹ Tobias Petzold^{2,3} 

¹ Department of Cardiology, Pulmonology, and Vascular Medicine, Cardiovascular Research Institute Düsseldorf, Medical Faculty of the Heinrich Heine University Düsseldorf, Düsseldorf, Germany

² Department of Cardiology, LMU München, Munich, Germany

³ DZHK (German Centre for Cardiovascular Research), Munich Heart Alliance, Munich, Germany

⁴ Institut of Pharmacology and Clinical Pharmacology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Address for correspondence Tobias Petzold, MD, Medizinische Klinik und Poliklinik I, Klinikum der Universität München, Marchioninstraße 15, D-81377 Munich, Germany
(e-mail: tobias.petzold@med.uni-muenchen.de).

Thromb Haemost 2021;121:122–130.

Abstract

Nonvitamin K oral anticoagulants (NOACs) or direct oral anticoagulants comprise inhibitors of factor Xa (rivaroxaban, apixaban, edoxaban) or factor IIa (dabigatran). Both classes efficiently interfere with the final or penultimate step of the coagulation cascade and showed superior net clinical benefit compared with vitamin K antagonists for prevention of thromboembolic events in patients with AF and for prevention and therapy of deep vein thrombosis and pulmonary embolism. None the less, accumulating data suggested, that there may be differences regarding the frequency of atherothrombotic cardiovascular events between NOACs. Thus, the optimal individualized NOAC for each patient remains a matter of debate. Against this background, some basic and translational analyses emphasized NOAC effects that impact on platelet activity and arterial thrombus formation beyond inhibition of plasmatic coagulation. In this review, we will provide an overview of the available clinical and translational evidence for so-called noncanonical NOAC effects on platelet activation and arterial thrombosis.

Keywords

- ▶ anticoagulation
- ▶ anticoagulants
- ▶ myocardial infarction
- ▶ platelet aggregation

Introduction

In patients with atrial fibrillation (AF), guidelines recommend nonvitamin K oral anticoagulants (NOACs) over vitamin K antagonists (VKAs) for prevention of thromboembolic complications.¹ Furthermore, NOAC has become the anticoagulant of choice for the treatment of venous thromboembolic events.²

In particular, patients with AF are on high risk for atherothrombotic events due to the presence of coronary artery

diseases (CADs) and the optimal NOAC for each patient remains a matter of debate. To date, inhibitors of factor Xa (FXa) (rivaroxaban, apixaban, edoxaban) or FIIa (dabigatran) are available. All of them were shown to have a superior net clinical benefit as compared with VKA.^{3–8} However, landmark trials showed differences regarding the frequency of atherothrombotic, ischemic events between NOACs. For the oral thrombin inhibitor dabigatran, but also for ximelagatran (active metabolite: melagatran), whose approval was withdrawn due to hepatotoxicity, clinical randomized studies revealed an increased risk of myocardial infarction (MI)

* Both authors contributed equally.

received

March 31, 2020

accepted after revision

August 1, 2020

published online

September 17, 2020

© 2020, Thieme. All rights reserved.

Georg Thieme Verlag KG,

Rüdigerstraße 14,

70469 Stuttgart, Germany

DOI <https://doi.org/>

10.1055/s-0040-1716750.

ISSN 0340-6245.

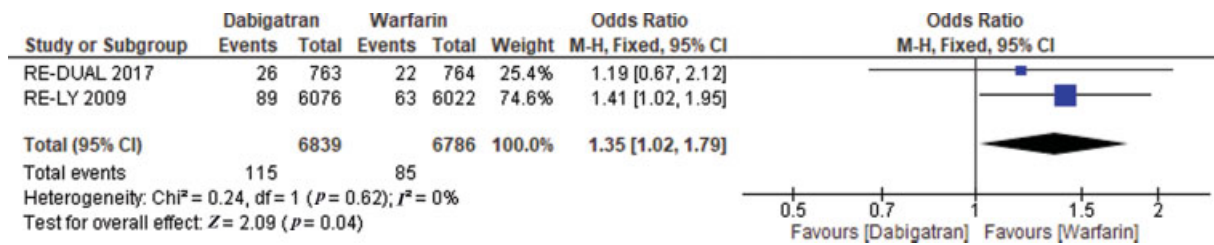


Fig. 1 Meta-analysis of risk of myocardial infarction under oral factor IIa (FIIa) inhibition. Individual and summary odds ratios with 95% confidence intervals for myocardial infarction in studies comparing FIIa inhibition to vitamin K antagonist (VKA) treatment in patients with atrial fibrillation. M-H, Mantel–Haenszel estimate; I² describes heterogeneity among studies; fixed, fixed-effects model.

(►Fig. 1) in patients.^{5,9,10} In contrast, FXa inhibition by rivaroxaban and apixaban showed a numerical decrease in MI rates (►Fig. 2) compared with VKA.^{3,4,6,11,12}

This was surprising and unexpected. Additionally, it seems counterintuitive given that all NOACs target either the penultimate or final step within the coagulation cascade. In contrast to such canonical effects that ultimately interfere with thrombin-induced platelet activation and coagulation-related events, this review focuses on “noncanonical” NOAC effects occurring during primary and secondary hemostasis, but independently of catalytic active thrombin.

Thrombin formation results from the sequential activation of an ensemble of serine proteases. Depending on the nature of coagulation trigger, the extrinsic or intrinsic coagulation pathway is initiated. Both are finally capable of activating FX to FXa. Catalytic active FXa cleaves FII (prothrombin) to FIIa (thrombin) which in turn processes FI cleavage (fibrinogen) and fibrin formation. In the context of atherothrombosis, thrombin takes a central role in mediating various effects which were recently reviewed.¹³ Being formed within seconds at sites of atherosclerotic plaque rupture, thrombin is the key multiplier of a locally forming prothrombotic milieu. Its antithrombotic effects, for example, by activation of protein C, are negligible in the acute setting. Given the plethora of parallel occurring interwoven thrombin-mediated effects at site of injury, two key functions that mediate thrombin’s prothrombotic activity have to be highlighted. First, as mentioned before, thrombin cleaves fibrinogen into fibrin, thereby contributing to the stabilization of the forming clot. Second, thrombin acts as a potent inducer of platelet activation, aggregation, and reactivity,^{14,15} which is associated with an increased risk of MI.^{16,17} Mechanistically,

thrombin triggers platelet activation either through proteolytic activation of protease-activated receptor (i.e., PAR-1, PAR-4) or through binding to glycoprotein (GP) Iba the most abundantly expressed platelet thrombin receptor.¹⁸ Of note, the latter mechanism is less potent compared with PAR-induced signaling.

In addition to their serial occurring actions within the coagulation cascade, thrombin and FXa exhibits various pleiotropic effects^{19,20} which are reviewed elsewhere and are beyond the scope of this review.

Given the high substrate specificity and binding affinity of NOACs, it is tempting to speculate that the clinically observed differences of ischemic events were due to so far unknown, coagulation independent and thus noncanonical NOAC effects on platelet function and platelet-driven arterial thrombosis. At sights of atherosclerotic plaque rupture, platelets become immediately recruited as first responders and contribute by different mechanism to deleterious thrombus formation.²¹ Against this background and the insufficiently understood role of NOACs in the context of platelet activation and arterial thrombosis, several in vitro and in vivo animal studies were initiated.

Oral Thrombin Inhibitors

Since RE-LY, the oral thrombin inhibitor dabigatran (dabigatran etexilate) is known to sufficiently prevent stroke in patients with AF. However, a debate if it enhances risk of MI emerged as the MI rate was higher in dabigatran-treated patients as compared with VKA in RE-LY for both dose regimens (110 and 150 mg twice daily).⁵ A repeated analysis

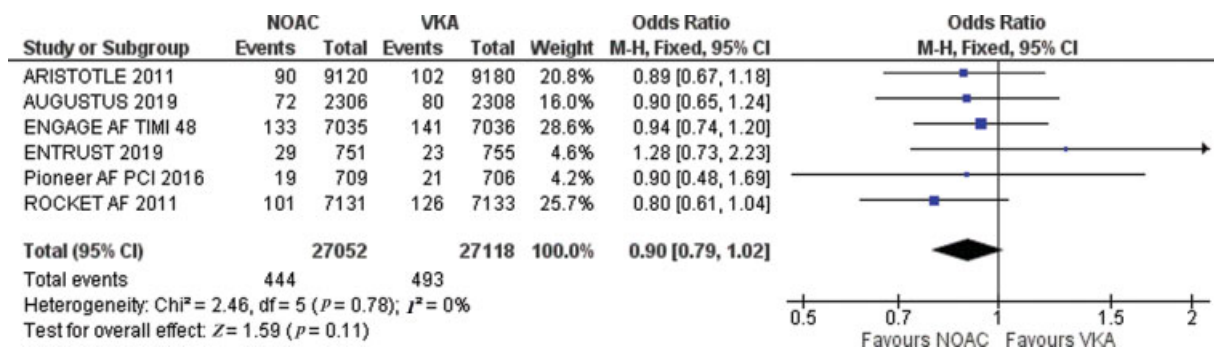


Fig. 2 Meta-analysis of risk of myocardial infarction under oral factor Xa (FXa) inhibition. Individual and summary odds ratios with 95% confidence intervals for myocardial infarction in studies comparing FXa inhibition to vitamin K antagonist (VKA) treatment in patients with atrial fibrillation. M-H, Mantel–Haenszel estimate; I² describes heterogeneity among studies; fixed, fixed-effects model.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

of RE-LY requested by the Food and Drug Administration revealed four hitherto unreported MIs and added 28 silent MI events.^{22,23} Adding these data, the difference in MI rate was not statistically significant anymore. Moreover, a meta-analysis of 11 randomized controlled trials (RCTs) comparing dabigatran to VKA recently revealed a 41% higher risk of MI in dabigatran-treated patients as compared with VKA.¹⁰ Another landmark study investigated dabigatran in a population with pre-existing CAD and thus an enhanced MI risk. RE-DUAL investigated dabigatran (110 or 150 mg) and P2Y12-inhibition in comparison to triple therapy with VKA, aspirin, and P2Y12-inhibition in AF patients who undergo percutaneous coronary interventions (PCIs).⁹ As expected, patients receiving dual therapy with dabigatran (110 and 150 mg) had fewer bleeding events. However, again a numerical increase toward higher MI rate was observed. Especially in patients treated with 110 mg twice daily. This was interesting, as sample size was substantially smaller (RE-DUAL PCI: 2,725 patients, RE-LY: 18,113 patients). Additionally, Gaubert et al recently investigated the occurrence of ischemic endpoints in acute coronary syndrome (ACS) patients with dabigatran versus VKA medication. In this real-world data as well, it could be shown that dabigatran was associated with an increased thrombotic risk shown by a higher incidence of major adverse cardiovascular events.²⁴

However, a recent network analysis did not reveal differences in ischemic outcome of major adverse cardiovascular events comparing different regimen with oral anticoagulants and antiplatelet therapy.²⁵ Furthermore, a recent observational study²⁶ and a meta-analysis merging observational studies and RCTs did not find a difference in risk of MI.²⁷ However, limitations of observational studies such as patient selection, confounders, unknown internationalized normalized ratios in VKA patients, socioeconomic and health care access differences, etc. may have biased the results. Moreover, in the study that merged observational studies and RCTs,²⁷ differences between observational studies and RCTs were striking.

A second line of evidence comes from translational and experimental studies (► **Table 1**) that aimed to dissect the underlying mechanism of an increased rate of ischemic events under dabigatran. Olivier et al investigated platelet reactivity in patients on chronic dabigatran treatment. The authors revealed that dabigatran therapy was associated with an increased thrombin receptor-activating peptides (TRAPs)-induced platelet aggregation in a dose-dependent manner.²⁸ Interestingly, this effect was not present in patients with additional antiplatelet therapy as shown in a follow-up study of the same group.²⁹ Moreover, we recently conducted flow chamber experiments and revealed enhanced platelet adhesion and thrombus formation on human atherosclerotic plaque material in dabigatran-treated patients and could confirm an increased reactivity.³⁰ This effect depended on augmented GPIIb α signaling, downstream of von Willebrand factor (vWF) binding. Using site-specific blocking antibody or mice deficient in the extracellular domain of GPIIb α , we could show that an altered thrombin GPIIb α interaction is responsible for the prothrombotic ef-

fect.³⁰ In contrast, a recent analysis conducted by Trabold et al did not observe increased vWF/ristocetin-induced platelet aggregation.³¹ To discuss potential reasons of the discrepant results, it is crucial to appreciate the different experimental setups used in either study. Trabold et al investigated platelets and platelet-rich plasma from healthy volunteers spiked with dabigatran and other divalent thrombin inhibitors, while our group used whole blood from patients under chronic therapy. In the chronic setting, we observed increased level of catalytic inactive thrombin that may favor the formation of thrombin dabigatran complexes. Beyond this noncanonical chronic effect, we and others also observed alteration of platelet function after single-dose treatment. By conducting time series analysis in AF patients with new-onset dabigatran medication, we found an increase in platelet reactivity upon TRAP treatment following a single-dose treatment. There was no influence on aggregation induced by other agonists like adenosine diphosphate (ADP), collagen, or arachidonic acid, respectively.³²

Thrombin mediates cleavage of its receptor, platelet PAR, from the platelet surface.³³ Chen et al revealed in first in vitro analyses that dabigatran attenuates thrombin-induced PAR-1 activation, internalization, and cleavage dose-dependently.³⁴ In accordance, PAR-1 surface expression increased again by prolonged incubation with inactivated thrombin.³⁴ In addition, we could show that in vivo inhibition of thrombin by dabigatran led to increased surface expression of PAR-1 and PAR-4 on platelets measured by flow cytometry.³² In an animal model of diabetes, it could furthermore be shown that long-term dabigatran treatment enhances PAR-4 expression.³⁵ Moreover, atherosclerosis-related mechanisms and occurrence of coronary lipid deposits could be revealed as further nondirect effects of dabigatran that might explain the enhanced risk for MI as well.³⁵

However, other studies did not find increased platelet reactivity by dabigatran. Zemer-Wassercug et al measured platelet aggregation by multiple electrode aggregometry (MEA) in 17 patients and did not show a significant increase of platelet reactivity by dabigatran.³⁶ However, most patients were on additional antiplatelet medication potentially repealing prothrombotic effects.³⁰ This goes in line with results of Franchi et al and data from our group. Franchi et al investigated platelet reactivity in presence of dabigatran and dual antiplatelet medication. No alterations of platelet reactivity measured by light transmission aggregometry (LTA) and MEA were detected either.³⁷ In vitro data from our studies also found, that addition of aspirin abrogated augmented platelet adhesion and thrombus formation under flow conditions in blood from dabigatran-treated patients.³⁰

Taken together, dabigatran was shown to increase the rate of MI in RCTs as well as in real-world data. Mechanistically, dabigatran shows some prothrombotic effect by enhancing PAR expression and augmenting GPIIb α signaling predominantly under flow conditions. These translational findings might contribute to the increased rate of MI in dabigatran-treated patients. In this context, the influence of antiplatelet medication remains to be insufficiently understood and requires further investigation.

Factor Xa Inhibitors

In contrast to FIIa inhibition, no increased MI rates were seen in clinical studies investigating FXa inhibitors. Rivaroxaban and apixaban showed a trend toward a reduced incidence of MI in their first landmark trials.^{3,4} In AF patients post-PCI (PIONEER/AUGUSTUS), again a numerical reduction of MI was found^{11,12} compared with triple therapy with VKA. However, so far it remains unclear whether this trend holds true for all FXa inhibitors in the presence or absence of antiplatelet therapy, or whether substance-specific effects exist. In edoxaban-treated patients, full dose edoxaban medication in ENAGE-AF was associated with a small reduction of MI.⁶ In contrast, ENTRUST enrolled patients undergoing PCI with concomitant antiplatelet therapy; however, edoxaban-treated patients did not show a reduced rate of MI as compared with VKA therapy.³⁸ Of note, while discussing these nonsignificant numerical differences of MI frequencies, it has to be considered that none of the NOAC-PCI trials was sufficiently powered to detect differences in ischemic events. Hence, large-scale clinical trials are urgently needed, given the increasing portion of patients treated with NOAC-based dual antithrombotic regimes.

Beside stroke prevention in AF patients and treatment of deep vein thrombosis/pulmonary embolism, a novel concept of low-dose anticoagulation on top of antiplatelet therapy entered the field of antithrombotic regimes. Vascular dose FXa inhibition with rivaroxaban (2.5 mg twice daily) was initially investigated in a high-risk cohort of patients presenting with ACS without AF. ATLAS ACS-2-TIMI 51 revealed a reduction of MI and stroke by vascular dose rivaroxaban on top of dual antiplatelet therapy with aspirin and clopidogrel.³⁹ Moreover, the COMPASS trial showed that vascular dose rivaroxaban on top to aspirin improved cardiovascular outcome in patients with stable CAD and peripheral artery disease patients.^{40,41}

The use of vascular dose rivaroxaban might also be an alternative to established antiplatelet regimes given the varying pharmacodynamic responses toward antiplatelet medication between individuals.^{42–45} However, a balanced clinical decision making is required before initiation of such antithrombotic regime, considering the increased risk for major bleedings.

Although beyond the focus of this review, some rather unexpected results came from a clinical study investigating the optimal antithrombotic regimen in patients with transcatheter aortic valve implantation (TAVI). The Galileo trial recently investigated FXa inhibition by rivaroxaban, in a reduced dose of 10 mg once daily, on top of aspirin in TAVI patients without AF.⁴⁶ Surprisingly the risk of death or thromboembolic complications was higher compared with the standard antiplatelet-based therapy with clopidogrel, leading to a premature termination of this trial.⁴⁶ Interestingly, subclinical leaflet-motion, as potential surrogate marker for thrombotic valve alteration, was reduced under rivaroxaban as shown in an accompanying imaging study.⁴⁷ Similar findings were made in an earlier animal study that suggested that rivaroxaban might be superior to standard

therapy after mechanical valve surgery in terms of valve thrombus and platelet deposition.⁴⁸ Unexpected results came also from the Re-Align study, that aimed to investigate dabigatran in patients after mechanical heart valve surgery. Increased rates of thromboembolic and bleeding complications compared with VKA regimen were observed.⁴⁹ Further studies like ATLANTIS and ENVISAGE-AF trials are ongoing, investigating apixaban and edoxaban in patients with AF and TAVI.⁵⁰ Thus, the type of NOAC and dose regimen seems to be crucial.⁵¹ None the less in patients with bioprosthetic valves and AF, a full-dose NOAC treatment seems to be as efficient and safe as VKA.⁵²

Summarizing the clinical available data, it is suggested that FXa inhibition reduces ischemic events in the different scenarios of atherothrombosis. Triggered by these clinical observations, several *in vitro* studies investigated the effects of FXa inhibition on platelet activation and coagulation. Perzborn et al were able to show that rivaroxaban reduced tissue factor-induced thrombin formation and platelet aggregation.⁵³ Another group observed the same inhibitory effect on thrombin generation and subsequent platelet aggregation for dabigatran and rivaroxaban underscoring the conventional—canonical—efficiency of NOACs to inhibit coagulation and thrombin-driven platelet activation.⁵⁴

As shown before for dabigatran, some experimental and translational studies revealed noncanonical effects on platelet function by FXa inhibitors (► **Table 1**). Nehaj et al demonstrated that PAR-1-mediated platelet aggregation was reduced in AF patients 2 hours after FXa intake.⁵⁵ Given that TRAP-6 activates platelets though PAR-1 independently of thrombin generation, this data suggested a noncanonical effect. Along this line, we recently described a novel antiplatelet effect of rivaroxaban⁵⁶ by performing time series as well as a cross-sectional analysis in patients treated with FXa inhibitors for stroke prevention under AF.⁵⁶ Rivaroxaban reduced ADP, collagen, TRAP, and plaque material-induced platelet aggregation in whole blood and platelet-rich plasma independently from thrombin. However, these antiplatelet effects required the presence of plasma components⁵⁶ to enable FXa *de novo* formation and subsequently FXa inhibition by rivaroxaban. Furthermore, rivaroxaban treatment attenuated thrombus formation under arterial flow conditions and altered thrombus composition with reduced fibrinogen content on human atherosclerotic plaque material. As underlying mechanism, we could reveal that FXa acts as direct platelet agonist by activating PAR-1 signaling, triggering phospholipase C and PI3K activation as key signaling events during platelet activation. As suggested by other studies before, we found a similar effect for apixaban suggesting a FXa group antiplatelet effect.⁵⁶

Additional evidence for noncanonical FXa effects comes from an earlier study by Al-Tamimi et al. They showed that FXa inhibitors attenuate platelet collagen receptor GPVI shedding, thereby reducing soluble GPVI concentrations.⁵⁷ GPVI known as platelet main collagen receptor,^{58,59} mediates platelet activation at the site of atherosclerotic plaque rupture that come along with the exposure of highly thrombogenic subendothelial matrix proteins. At sites of thrombus

formation, GPVI signaling capacity is regulated by receptor shedding to prevent excessive platelet activation and thrombus formation.⁶⁰ While several metalloproteinase were identified to cleave GPVI and generate a soluble ectodomain, a coagulation-induced FXa-driven, thrombin-independent shedding mechanism was described.⁵⁷ Against this background, Pignatelli et al⁶¹ found reduced, soluble GPVI levels, as marker for reduced platelet activation in patients under FXa inhibition. Whether this mechanism will indeed reduce platelet activation *in vivo* needs to be proven. Further evidence for noncanonical FXa effects, comes from a recent study using *in silico* docking modeling. This study proposed a direct interaction of rivaroxaban with GPVI. Thereby rivaroxaban inhibits thromboxane biosynthesis and oxidative stress generation by Nox-2 following GPVI activation by convulxin.⁶³ Importantly, in all three scenarios FXa-mediated antiplatelet effects were independent from catalytic active thrombin.

None the less, some other studies did not find any effect of FXa treatment on platelet aggregation in either whole blood or platelet-rich plasma. Zemer-Wassercug et al investigated rivaroxaban-antiplatelet effects but could not reveal any antiplatelet effects as measured by MEA, P-selectin expression, platelet deposition, and RANTES levels.³⁶ However, the interpretation of this study is limited as a significant percentage of patients was on concomitant aspirin therapy. Furthermore, Steppich et al conducted a time series analysis in 38 apixaban- or rivaroxaban-treated patients. Thrombin generation, aggregation measured by MEA, and expression of thrombospondin, β -thromboglobulin, and soluble P-selectin were investigated. Whole blood aggregation did not change during the time course. However, thrombin generation as well as expression of thrombospondin were reduced.⁶² Bánovčín et al evaluated platelet aggregation measured by LTA in 9 rivaroxaban- and 12 apixaban-treated AF patients in a time series analysis to compare responses at baseline peak

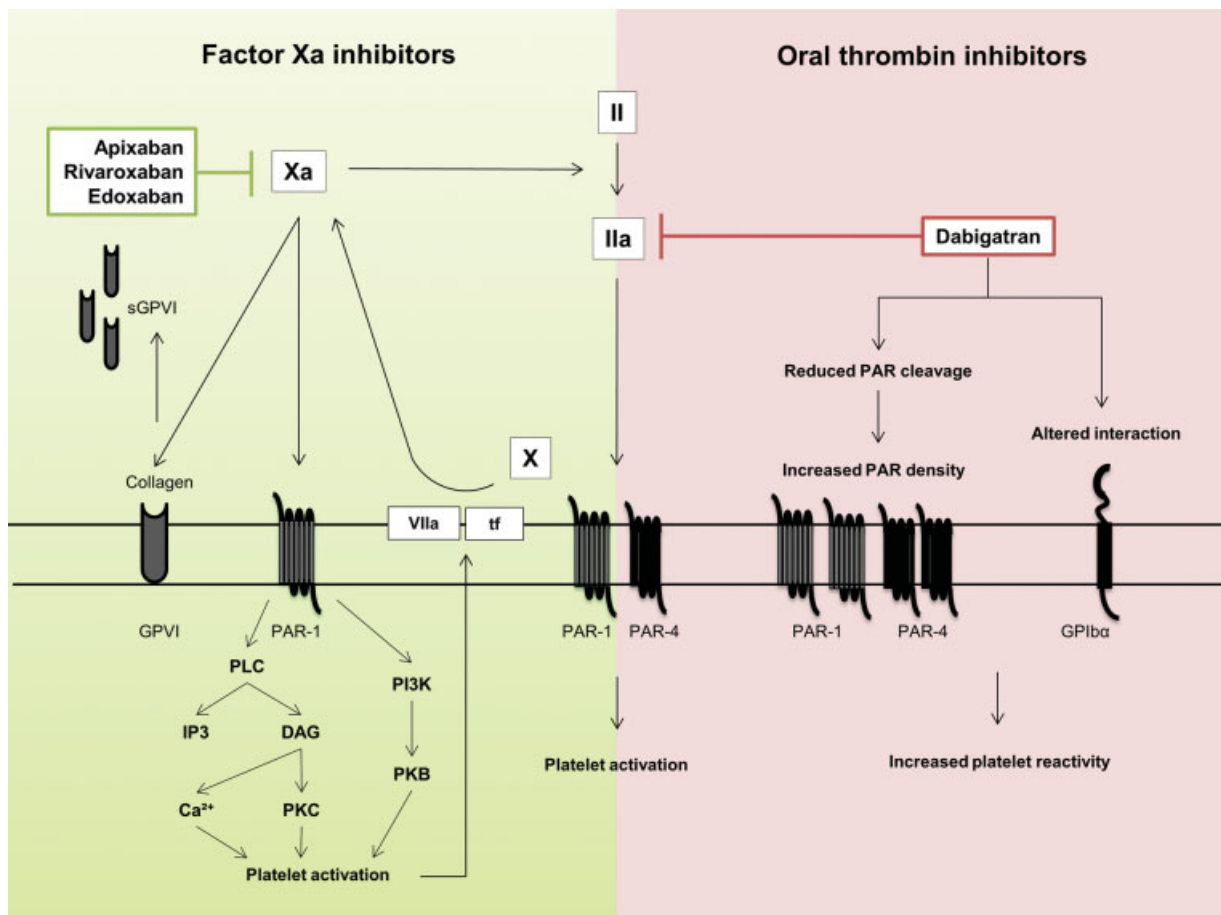


Fig. 3 Noncanonical mechanisms of nonvitamin K oral anticoagulants (NOACs) on platelet function. Factor Xa (FXa) inhibitors trigger antiplatelet effects by reducing FXa-mediated platelet activation of protease-activated receptor-1 (PAR-1). PAR-1 cleavage by FXa activates platelets via the phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K) pathways. In the presence of plasma, platelet activation (e.g., during aggregation) by various agonists induces FXa *de novo* formation by augmenting tissue factor (tf) exposure, finally triggering cleavage of FX. Another mechanism suggests that FXa inhibitors can directly interact with platelet glycoprotein (GP) VI thereby reducing NOX-2-mediated reactive oxygen species (ROS) production and platelet activation. Furthermore, FXa was shown to proteolytically cleave GPVI, yielding a soluble GPVI ectodomain (sGPVI). Reduced plasma levels of sGPVI in the presence of FXa inhibitors reflect reduced systemic platelet activation. Oral thrombin inhibitor treatment induces prothrombotic effects on platelet function by reducing cleavage of surface expressed PAR receptors, yielding augmented PAR receptors surface expression level. Moreover, dabigatran leads to an altered thrombin-GPIIb interaction, triggering increased platelet reactivity and thrombus formation downstream of von Willebrand factor (vWF) binding.

Table 1 Summary of experimental study results and main outcome variables

	Ref.	Mode	Dose	Study object	Duration	Assay	Outcome
Human	28	ts + cs	DABI 75, 110 or 150 mg 2 ×/d; RIVA 10, 15 or 20 mg 1 ×/d	Patients (AF)	Chronic	MEA (TRAP)	Increased aggregation under DABI (dose-dependent)
	32	ts	DABI 110 or 150 mg	Patients (AF)	Single dose	LTA (TRAP, ADP, collagen, AA)	Increase in platelet reactivity only upon TRAP
	36	ts	DABI 110 or 150 mg 2 ×/d or RIVA 15 or 20 mg 1 ×/d	Patients (AF)	Chronic	MEA (+Impact-R shear-induced platelet deposition, P-selectin, plasma RANTES levels)	No increase in platelet reactivity under DABI
	37	ts	DABI 150 mg 2 ×/d	Patients (CAD)	Chronic	LTA + MEA	No change in platelet reactivity under DABI + DAPT
	30	cs	DABI 150 mg 2 ×/d or blood from DABI (150 mg 2 ×/d) patients spiked with 1 mM ASA	Patients (AF) + healthy	Chronic	Flow chamber + MEA	Enhanced platelet adhesion under DABI, which can be abrogated by ASA
	55	ts	RIVA 15 mg 1 ×/d or APIXA 2, 5, or 5 mg 2 ×/d	Patients (AF)	Chronic	LTA (TRAP)	Reduced aggregation 2 h after RIVA or APIXA intake
	61	cs	APIXA 10 mg/d or RIVA 20 mg/d	Patients (AF)	Chronic	Enzyme immunoassay	Reduced soluble GPVI levels under FXa inhibition
	62	ts	APIXA (5 mg 2 ×/d) or RIVA (20 mg 1 ×/d)	Patients (AF)	Chronic	Thrombin generation, MEA, expression of thrombospondin, P-selectin	No effect on platelet reactivity under RIVA or APIXA
	63	ts	APIXA or RIVA	Patients (AF)	Chronic	LTA (ADP, epinephrine, collagen)	No change in aggregation independent of plasma levels
	56	ts + cs	RIVA 20 mg	Patients (AF) + healthy	Chronic + single dose	LTA, MEA, flow chamber on human atherosclerotic plaque	RIVA reduces arterial thrombosis by inhibition of FXa-driven platelet activation
	29	ts	DABI 75, 110, or 150 mg 2 ×/d, RIVA 10, 15, or 20 mg 1 ×/d; +/- ASA or clopidogrel	Patients (AF + VTE)	Chronic	MEA (ADP and AA)	No impact of NOACs on anti-platelet effects of ASA + clopidogrel
	64	cs	RIVA (dosing unknown)	Patients (VTE + AF)	Chronic	sCD40L, PFA4, TXA2	Similar levels of sCD40L + PFA4, but increased peak TXA2 levels in RIVA patients
	65	ts	RIVA 20 mg 1 ×/d	Patients (VTE)	Chronic	LTA + P-selectin	No intraindividual effects on platelet reactivity of RIVA at peak and trough plasma level
	31	Ex vivo	Platelets + PRP spiked with DABI (500 nM) or lepirudin (200 µg/mL)	Healthy	Single dose	LTA (ristocetin)	No increase in aggregation under DABI or lepirudin
	53	Ex vivo	PRP spiked with RIVA (15 or 30 ng/mL), ticagrelor (1 or 3 µg/mL), or both	Healthy	Single dose	LTA (TF)	RIVA reduced platelet aggregation
	54	Ex vivo	Citrated blood spiked with DABI (25–800 nM/L) or RIVA (25–800 nM/L)	Healthy	Single dose	SINNOWA, endogenous thrombin potential	RIVA + DABI reduce thrombin generation + platelet aggregation
	57	Ex vivo	PRP spiked with RIVA 1 µg/mL	Healthy	Single dose	Coagulation-induced sGPVI generation measured by ELISA	FXa inhibitors attenuate GPVI shedding and reduce soluble GPVI concentrations
	68	Ex vivo	Platelets spiked with RIVA 15–60 ng/mL	Healthy	Single dose	Docking simulation analysis	Direct interaction of RIVA with GPVI
	66	ts	RIVA 10 mg	Healthy	Single dose	MEA (AA, TRAP, ristocetin, ADP)	No influence of Xa inhibition on platelet aggregation
		34	Ex vivo	Thrombin preincubated with DABI (0–30 nM)	Human thrombin	Single dose	PAR-1 cleavage analysis; FACS
Animal	30	cs	DABI 37.5 mg/kg bw	Mouse	Single dose	Wire injury model	Prothromogenic, increased thrombus stability under DABI
	35	cs	DABI 1 or 0.3 mg/g bw	Rat	Chronic	Immunohistochemistry	Enhanced PAR-4 expression under DABI
	48	cs	RIVA 2 mg/kg 2 ×/d	Pig	Chronic	Model of mechanical aortic valve prosthesis	RIVA was more effective than enoxaparin for short-term thromboprophylaxis

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; AF, atrial fibrillation; APIXA, apixaban; ASA, acetylsalicylic acid; bw, body weight; CAD, coronary artery disease; cs, cross-sectional; DABI, dabigatran; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; PAR-1, protease-activated receptor-1; PFA4, platelet factor 4; PRP, platelet-rich plasma; RIVA, rivaroxaban; sCD40L, soluble CD40 ligand; sGPVI, soluble glycoprotein VI; TF, tissue factor; TRAP, thrombin receptor activating protein; ts, time series; TXA2, thromboxane A2; VTE, venous thromboembolism.
 Note: Chronic > 7 days and single dose < 7 days.

and trough plasma level but did not show a significant alteration of platelet responses.⁶³

In the context of recurrent venous thromboembolism (VTE), a different disease entity requiring long-term anticoagulation, a small study (total 8 patients: 7 recurrent VTE, 1 AF) analyzed the impact of rivaroxaban on platelet activation markers. While soluble CD40 ligand and platelet factor 4 did not differ compared with healthy controls, peak thromboxane A₂ levels in patients with rivaroxaban medication⁶⁴ were increased. Though the underlying molecular mechanism remains elusive, the authors postulate a prothrombotic effect of rivaroxaban. Another recent study performed time series analysis of platelet function in VTE patients on rivaroxaban and after cessation of anticoagulation. Schultz et al did not find any significant intraindividual effects on platelet reactivity (i.e., LTA aggregation and P-selectin) of rivaroxaban peak and trough plasma level. However, the authors revealed a trend toward a reduced P-selectin expression under rivaroxaban treatment.⁶⁵ A study by Schoerengerhofer et al analyzed diurnal variation in platelet reactivity by MEA following 3 days of intermediate dose (10 mg) rivaroxaban treatment, but did not find any impact on platelet reactivity.⁶⁶

Limitations

While reviewing the current knowledge we found partially contradicting reports regarding the effects of FIIa and FXa inhibitors on platelet function in the context of atherothrombosis. Although the origin of these discrepancies remained in parts elusive, some aspects have to be considered while interpreting the data. An important potential confounder is given by comparing data generated from patients' samples with samples isolated from healthy controls that were spiked with active NOAC in vitro. In particular, as NOAC effects might be more prominent in patient samples what might exhibit altered platelet baseline activation due to disease-specific effects. Patient samples isolated from anticoagulated AF patients show alterations of the coagulation system and platelet parameters.⁶⁷ On the other hand, patient samples often show a broad variability, due to various known and unknown interindividual confounders, that are difficult to compensate for in small-scale translational studies. Thus, time series analysis should be preferred over cross-sectional analysis. Next, considering the (expected) rather mild effects of NOAC on platelet function it seems crucial to use the same experimental setup and conditions (i.e., anticoagulants used, sample transport time, centrifugation steps, platelet concentration, etc.). Hence, standardized, multicenter translational studies may help to improve the reproducibility and generalizability of data.

Conclusion

In this review, we focused on noncanonical effects of oral thrombin and FXa inhibitors in platelet activation and arterial thrombosis (–Fig. 3). Translational studies identified different prothrombotic noncanonical effects under FIIa

inhibitor treatment, yielding increased platelet reactivity. In contrast, different noncanonical mechanisms resulting in reduced platelet reactivity and thrombus formation in patients treated with FXa inhibitors were described. Still, these noncanonical effects are complex and not completely understood and it seems likely that additional so far unknown mechanisms exist. Hence, an optimal individualized NOAC therapy requires further standardized translational analysis and a careful definition of secondary and safety endpoints in large-scale clinical trials.

Authors' Contributions

A.P., L.D., and T.P. wrote the initial draft of the manuscript and later finalized the manuscript. M.K. gave valuable input to the manuscript. G.W., M.T., M.O. T.Z., and T.H. revised the manuscript.

Funding

This work was supported by the Forschungskommission of the Medical Faculty of the Heinrich Heine University [No. 18–2019 to A.P.; No. 2019–29 to L.D., No. 2018–32 to G.W.] and by the German Research Foundation (PO 2247/2–1 and SFB1116 to A.P and PE2704/2–1 to T.P.), by Deutsche Herzstiftung (F/04/19 shared with Dr. Lüsebrink).

Conflict of Interest

None declared.

References

- 1 Kirchhof P, Benussi S, Kotecha D, et al; ESC Scientific Document Group. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J* 2016;37(38):2893–2962
- 2 Konstantinides SV, Meyer G, Becattini C, et al; The Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): the Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). *Eur Respir J* 2019;54(03):54
- 3 Patel MR, Mahaffey KW, Garg J, et al; ROCKET AF Investigators. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011;365(10):883–891
- 4 Granger CB, Alexander JH, McMurray JJ, et al; ARISTOTLE Committees and Investigators. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011;365(11):981–992
- 5 Connolly SJ, Ezekowitz MD, Yusuf S, et al; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009;361(12):1139–1151
- 6 Giugliano RP, Ruff CT, Braunwald E, et al; ENGAGE AF-TIMI 48 Investigators. Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2013;369(22):2093–2104
- 7 Olimpieri PP, Di Lenarda A, Mammarella F, et al. Non-vitamin K antagonist oral anticoagulation agents in patients with atrial fibrillation: insights from Italian monitoring registries. *Int J Cardiol Heart Vasc* 2020;26:100465
- 8 Coleman CI, Peacock WF, Bunz TJ, Alberts MJ. Effectiveness and safety of apixaban, dabigatran, and rivaroxaban versus warfarin in patients with nonvalvular atrial fibrillation and previous stroke or transient ischemic attack. *Stroke* 2017;48(08):2142–2149

- 9 Cannon CP, Bhatt DL, Oldgren J, et al; RE-DUAL PCI Steering Committee and Investigators. Dual antithrombotic therapy with dabigatran after PCI in atrial fibrillation. *N Engl J Med* 2017;377(16):1513–1524
- 10 Artang R, Rome E, Nielsen JD, Vidaillet HJ. Meta-analysis of randomized controlled trials on risk of myocardial infarction from the use of oral direct thrombin inhibitors. *Am J Cardiol* 2013;112(12):1973–1979
- 11 Gibson CM, Mehran R, Bode C, et al. Prevention of bleeding in patients with atrial fibrillation undergoing PCI. *N Engl J Med* 2016;375(25):2423–2434
- 12 Lopes RD, Heizer G, Aronson R, et al; AUGUSTUS Investigators. Antithrombotic therapy after acute coronary syndrome or PCI in atrial fibrillation. *N Engl J Med* 2019;380(16):1509–1524
- 13 Ten Cate H, Hemker HC. Thrombin generation and atherothrombosis: what does the evidence indicate? *J Am Heart Assoc* 2016;5(08):5
- 14 Chan NC, Weitz JI. Antithrombotic agents. *Circ Res* 2019;124(03):426–436
- 15 Niewiarowski S, Thomas DP. Platelet aggregation by ADP and thrombin. *Nature* 1966;212(5070):1544–1547
- 16 Mangiacapra F, Colaiori I, Ricottini E, et al. Impact of platelet reactivity on 5-year clinical outcomes following percutaneous coronary intervention: a landmark analysis. *J Thromb Thrombolysis* 2018;45(04):496–503
- 17 Dannenberg L, Metzen D, Zako S, et al. Enhanced platelet reactivity under aspirin medication and major adverse cardiac and cerebrovascular events in patients with coronary artery disease. *Pharmacology* 2020;105(1-2):118–122
- 18 Dubois C, Steiner B, Kieffer N, Reigner SC. Thrombin binding to GPIIb/IIIa induces platelet aggregation and fibrin clot retraction supported by resting alphaIIb beta3 interaction with polymerized fibrin. *Thromb Haemost* 2003;89(05):853–865
- 19 Esmon CT. Targeting factor Xa and thrombin: impact on coagulation and beyond. *Thromb Haemost* 2014;111(04):625–633
- 20 Spronk HM, de Jong AM, Crijns HJ, Schotten U, Van Gelder IC, Ten Cate H. Pleiotropic effects of factor Xa and thrombin: what to expect from novel anticoagulants. *Cardiovasc Res* 2014;101(03):344–351
- 21 Jackson SP. Arterial thrombosis—insidious, unpredictable and deadly. *Nat Med* 2011;17(11):1423–1436
- 22 Connolly SJ, Ezekowitz MD, Yusuf S, Reilly PA, Wallentin L; Randomized Evaluation of Long-Term Anticoagulation Therapy Investigators. Newly identified events in the RE-LY trial. *N Engl J Med* 2010;363(19):1875–1876
- 23 Hohnloser SH, Oldgren J, Yang S, et al. Myocardial ischemic events in patients with atrial fibrillation treated with dabigatran or warfarin in the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial. *Circulation* 2012;125(05):669–676
- 24 Gaubert M, Resseguier N, Laine M, Bonello L, Camoin-Jau L, Paganelli F. Dabigatran versus vitamin K antagonist: an observational across-cohort comparison in acute coronary syndrome patients with atrial fibrillation. *J Thromb Haemost* 2018;16(03):465–473
- 25 Kuno T, Ueyama H, Takagi H, et al. Meta-analysis of antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention. *Am J Cardiol* 2020;125(04):521–527
- 26 Lee CJ, Gerds TA, Carlson N, et al. Risk of myocardial infarction in anticoagulated patients with atrial fibrillation. *J Am Coll Cardiol* 2018;72(01):17–26
- 27 Wei AH, Gu ZC, Zhang C, et al. Increased risk of myocardial infarction with dabigatran etexilate: fact or fiction? A critical meta-analysis of over 580,000 patients from integrating randomized controlled trials and real-world studies. *Int J Cardiol* 2018;267:1–7
- 28 Olivier CB, Weik P, Meyer M, et al. TRAP-induced platelet aggregation is enhanced in cardiovascular patients receiving dabigatran. *Thromb Res* 2016;138:63–68
- 29 Olivier CB, Weik P, Meyer M, et al. Dabigatran and rivaroxaban do not affect AA- and ADP-induced platelet aggregation in patients receiving concomitant platelet inhibitors. *J Thromb Thrombolysis* 2016;42(02):161–166
- 30 Petzold T, Thienel M, Konrad I, et al. Oral thrombin inhibitor aggravates platelet adhesion and aggregation during arterial thrombosis. *Sci Transl Med* 2016;8(367):367ra168
- 31 Trabold K, Makhoul S, Gambaryan S, van Ryn J, Walter U, Jurk K. The direct thrombin inhibitors dabigatran and lepirudin inhibit GPIIb/IIIa-mediated platelet aggregation. *Thromb Haemost* 2019;119(06):916–929
- 32 Achilles A, Mohring A, Dannenberg L, et al. Dabigatran enhances platelet reactivity and platelet thrombin receptor expression in patients with atrial fibrillation. *J Thromb Haemost* 2017;15(03):473–476
- 33 Nieman MT, Schmaier AH. Interaction of thrombin with PAR1 and PAR4 at the thrombin cleavage site. *Biochemistry* 2007;46(29):8603–8610
- 34 Chen B, Soto AG, Coronel LJ, Goss A, van Ryn J, Trejo J. Characterization of thrombin-bound dabigatran effects on protease-activated receptor-1 expression and signaling in vitro. *Mol Pharmacol* 2015;88(01):95–105
- 35 Scridon A, Mărginean A, Huțanu A, et al. Vascular protease-activated receptor 4 upregulation, increased platelet aggregation, and coronary lipid deposits induced by long-term dabigatran administration - results from a diabetes animal model. *J Thromb Haemost* 2019;17(03):538–550
- 36 Zemer-Wassercug N, Haim M, Leshem-Lev D, et al. The effect of dabigatran and rivaroxaban on platelet reactivity and inflammatory markers. *J Thromb Thrombolysis* 2015;40(03):340–346
- 37 Franchi F, Rollini F, Cho JR, et al. Effects of dabigatran on the cellular and protein phase of coagulation in patients with coronary artery disease on dual antiplatelet therapy with aspirin and clopidogrel. Results from a prospective, randomised, double-blind, placebo-controlled study. *Thromb Haemost* 2016;115(03):622–631
- 38 Vranckx P, Valgimigli M, Eckardt L, et al. Edoxaban-based versus vitamin K antagonist-based antithrombotic regimen after successful coronary stenting in patients with atrial fibrillation (EN-TRUST-AF PCI): a randomised, open-label, phase 3b trial. *Lancet* 2019;394(10206):1335–1343
- 39 Mega JL, Braunwald E, Wiviott SD, et al; ATLAS ACS 2–TIMI 51 Investigators. Rivaroxaban in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;366(01):9–19
- 40 Eikelboom JW, Connolly SJ, Bosch J, et al; COMPASS Investigators. Rivaroxaban with or without aspirin in stable cardiovascular disease. *N Engl J Med* 2017;377(14):1319–1330
- 41 Anand SS, Caron F, Eikelboom JW, et al. Major adverse limb events and mortality in patients with peripheral artery disease: the COMPASS trial. *J Am Coll Cardiol* 2018;71(20):2306–2315
- 42 Polzin A, Dannenberg L, Sansone R, et al. Antiplatelet effects of aspirin in chronic kidney disease patients. *J Thromb Haemost* 2016;14(02):375–380
- 43 Polzin A, Kelm M, Zeus T. Impaired clopidogrel antiplatelet effects and age: young patients at risk. *Int J Cardiol* 2015;187:216–218
- 44 Polzin A, Afzal S, Balzer J, Rassaf T, Kelm M, Zeus T. Platelet reactivity in MitraClip patients. *Vascul Pharmacol* 2016;77:54–59
- 45 Polzin A, Dannenberg L, Sophia Popp V, Kelm M, Zeus T. Antiplatelet effects of clopidogrel and aspirin after interventional patent foramen ovale/atrium septum defect closure. *Platelets* 2016;27(04):317–321
- 46 Dangas GD, Tijssen JGP, Wöhrle J, et al; GALILEO Investigators. A controlled trial of rivaroxaban after transcatheter aortic-valve replacement. *N Engl J Med* 2020;382(02):120–129

- 47 De Backer O, Dangas GD, Jilaihawi H, et al; GALILEO-4D Investigators. Reduced leaflet motion after transcatheter aortic-valve replacement. *N Engl J Med* 2020;382(02):130–139
- 48 Greiten LE, McKellar SH, Rysavy J, Schaff HV. Effectiveness of rivaroxaban for thromboprophylaxis of prosthetic heart valves in a porcine heterotopic valve model. *Eur J Cardiothorac Surg* 2014;45(05):914–919
- 49 Eikelboom JW, Connolly SJ, Brueckmann M, et al; RE-ALIGN Investigators. Dabigatran versus warfarin in patients with mechanical heart valves. *N Engl J Med* 2013;369(13):1206–1214
- 50 Van Mieghem NM, Unverdorben M, Valgimigli M, et al. Edoxaban versus standard of care and their effects on clinical outcomes in patients having undergone transcatheter aortic valve implantation in atrial fibrillation—rationale and design of the ENVISAGE-TAVI AF trial. *Am Heart J* 2018;205:63–69
- 51 Aimo A, Giugliano RP, De Caterina R, Non-Vitamin K. Non-vitamin K antagonist oral anticoagulants for mechanical heart valves: is the door still open? *Circulation* 2018;138(13):1356–1365
- 52 Andrade JG, Meseguer E, Didier R, Dussault C, Weitz JI. Non-vitamin K antagonist oral anticoagulants in atrial fibrillation patients with bioprosthetic valves. *Expert Rev Cardiovasc Ther* 2018. Doi: 10.1080/14779072.2018.1475229
- 53 Perzborn E, Heitmeier S, Laux V. Effects of rivaroxaban on platelet activation and platelet-coagulation pathway interaction: in vitro and in vivo studies. *J Cardiovasc Pharmacol Ther* 2015;20(06):554–562
- 54 Wan H, Yang Y, Zhu J, et al. An in-vitro evaluation of direct thrombin inhibitor and factor Xa inhibitor on tissue factor-induced thrombin generation and platelet aggregation: a comparison of dabigatran and rivaroxaban. *Blood Coagul Fibrinolysis* 2016;27(08):882–885
- 55 Nehaj F, Sokol J, Ivankova J, et al. First evidence: TRAP-induced platelet aggregation is reduced in patients receiving xabans. *Clin Appl Thromb Hemost* 2018;24(06):914–919
- 56 Petzold T, Thienel M, Dannenberg LK, et al. Rivaroxaban reduces arterial thrombosis by inhibition of FXa driven platelet activation via protease activated receptor-1. *Circ Res* 2020;126(04):486–500
- 57 Al-Tamimi M, Grigoriadis G, Tran H, et al. Coagulation-induced shedding of platelet glycoprotein VI mediated by factor Xa. *Blood* 2011;117(14):3912–3920
- 58 Massberg S, Gawaz M, Grüner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med* 2003;197(01):41–49
- 59 Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* 2008;28(03):403–412
- 60 Bender M, Stegner D, Nieswandt B. Model systems for platelet receptor shedding. *Platelets* 2017;28(04):325–332
- 61 Pignatelli P, Pastori D, Bartimoccia S, et al. Anti Xa oral anticoagulants inhibit in vivo platelet activation by modulating glycoprotein VI shedding. *Pharmacol Res* 2016;113(Pt A):484–489
- 62 Steppich B, Dobler F, Brendel LC, et al. Effect of the FXa inhibitors Rivaroxaban and Apixaban on platelet activation in patients with atrial fibrillation. *J Thromb Thrombolysis* 2017;43(04):490–497
- 63 Bánovčín P Jr, Škorňová I, Samoš M, et al. Platelet aggregation in direct oral factor Xa inhibitors-treated patients with atrial fibrillation: a pilot study. *J Cardiovasc Pharmacol* 2017;70(04):263–266
- 64 Murphy PT, Grace S, Glavey S, Quinn J. Rivaroxaban may increase platelet activation in vivo via thromboxane A2. *Circ Res* 2019;125(02):e9
- 65 Schultz NH, Holme PA, Bjørnsen S, Henriksson CE, Sandset PM, Jacobsen EM. The impact of rivaroxaban on primary hemostasis in patients with venous thrombosis. *Platelets* 2020;31(01):43–47
- 66 Schoergenhofer C, Schwameis M, Brunner M, et al. Assessing the influence of diurnal variations and selective Xa inhibition on whole blood aggregometry. *Scand J Clin Lab Invest* 2015;75(06):531–536
- 67 Makowski M, Smorag I, Makowska J, et al. Platelet reactivity and mean platelet volume as risk markers of thrombogenesis in atrial fibrillation. *Int J Cardiol* 2017;235:1–5
- 68 Cammisotto V, Carnevale R, Nocella C, et al. Nox2-mediated platelet activation by glycoprotein (GP) VI: Effect of rivaroxaban alone and in combination with aspirin. *Biochem Pharmacol* 2019;163:111–118