STATE OF THE ART ISTH 2017

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The coagulation system in atherothrombosis: Implications for new therapeutic strategies

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Abstract

Clinical manifestations of atherosclerotic disease include coronary artery disease (CAD), peripheral artery disease (PAD), and stroke. Although the role of platelets is well established, evidence is now accumulating on the contribution of coagulation proteins to the processes of atherosclerosis and atherothrombosis. Coagulation proteins not only play a role in fibrin formation and platelet activation, but also mediate various biological and pathophysiologic processes through activation of protease-activated-receptors (PARs). Thus far, secondary prevention in patients with CAD/PAD has been the domain of antiplatelet therapy, however, residual atherothrombotic risks remain substantial. Therefore, combining antiplatelet and anticoagulant therapy has gained more attention. Recently, net clinical benefit of combining aspirin with low-dose rivaroxaban in patients with stable atherosclerotic disease has been demonstrated. In this review, based on the State of the Art lecture "Clotting factors and atherothrombosis" presented at the ISTH Congress 2017, we highlight the role of coagulation proteins in the pathophysiology of atherothrombosis, and specifically focus on therapeutic strategies to decrease atherothrombotic events by optimization of vascular protection.

KEYWORDS
anticoagulation, antiplatelet therapy, atherosclerosis, atherothrombosis, coagulation, inflammation

Essentials
- A State of the Art lecture "Clotting factors and atherothrombosis" was presented at the ISTH congress 2017.
- Coagulation proteins are not only involved in hemostasis, they also play a role in atherogenesis.
- Inhibition of coagulation proteins could potentially protect the vessel wall against progression of atherosclerosis.
- Combining antiplatelet and anticoagulant therapy has been demonstrated to improve cardiovascular outcomes in patients with stable atherosclerotic disease.

1 | INTRODUCTION

In the broad category of arterial thrombosis, one can distinguish thrombosis related to atherosclerosis, called atherothrombosis, and arterial thromboembolism, eg, as a consequence of atrial fibrillation. Clinical manifestations of atherothrombosis occur in the heart (coronary artery disease [CAD], myocardial infarction), the peripheral arterial vasculature (peripheral artery disease [PAD]) and in the brain (ischemic stroke). Traditionally,

the treatment of atherothrombotic disease consists of antiplatelet therapy, whereas treatment of arterial (and venous) *thromboembolism* is based on oral anticoagulants (either vitamin K antagonists [VKA] or direct oral anticoagulants [DOACs], also known as non–vitamin-K oral anticoagulants [NOACs]). This differentiation is based on the important role of platelets in the etiology of the progression from atherosclerotic plaques to atherothrombotic occlusion.[1] Atherosclerosis is characterized by endothelial dysfunction and chronic inflammation.[2,3] Binding of monocytes to the activated endothelial cells of the arterial vessel wall and their subsequent translocation and differentiation into macrophages that internalize oxidized lipoproteins results in the formation of macrophage foam cells. These foam cells induce several processes, such as vascular smooth muscle cell (VSMC) proliferation, migration, and fibrous cap formation, eventually leading to the formation of a fatty streak.[4] Atheroma, composed of a mixture of lipid in foam cells, smooth muscle cells, and a fibrin cap, forms slowly growing atherosclerotic plaques in the lumen of the artery. Once this atherosclerotic plaque ruptures, collagen and tissue factor (TF) are exposed and trigger the activation of platelets and the coagulation cascade, respectively, thus leading to atherothrombotic occlusion of the artery. Although the role of platelets in this whole process is well established,[1] evidence is now accumulating on the contribution of coagulation proteins to the processes of atherosclerosis and atherothrombosis. In this review, which is based on the State of the Art lecture "Clotting factors and atherothrombosis" at the ISTH Congress 2017 in Berlin, we focus on the contribution of the coagulation system on atherogenesis and consecutive atherothrombotic events, explore the implications and potentials for treatment, and discuss recent and future developments in treatment of different clinical manifestations of atherothrombotic disease.

2 | PATHOPHYSIOLOGY

2.1 | Mechanisms of thrombosis in atherosclerosis

The pathogenesis of atherothrombotic events starts with disruption of an atherosclerotic plaque, thereby exposing thrombogenic material to the blood.[5] Thrombus formation is driven by coordinated platelet and thrombin generation pathways. Platelets adhere to exposed subendothelial collagen and von Willebrand factor (vWF), become activated, and then release ADP and thromboxane A2 (TXA2), which activate other platelets. Platelet activation induces conformational changes in glycoprotein (GP) IIb/IIIa, which increases the affinity for its ligands fibrinogen and vWF, and thereby mediates platelet aggregation. Simultaneously with platelet activation and aggregation, the transmembrane receptor TF in the plaque binds to factor VII(a) in blood and coagulation is triggered, resulting in thrombin generation. Thrombin is a key enzyme in that it not only converts fibrinogen into fibrin, but also is a potent platelet agonist via activation of protease-activated receptor (PARs)-1 and 4. Furthermore, thrombin also drives several feedback loops that include activation of factors V, VIII, and XI to further amplify thrombin generation; eventually, factor XIII is activated to cross-link fibrin to stabilize the clot. The amplification phase of coagulation requires a phospholipid surface providing phosphatidylserine (PS) on which the intrinsic tenase (a complex of factor IXa and factor VIIIa) and prothrombinase (factor Xa and factor Va) complexes assemble.[6] This phospholipid surface is primarily provided by activated platelets at the site of plaque rupture, and thereby, the platelet pathway and thrombin pathway are connected.

Pathological examination of coronary artery thrombi removed by thrombectomy or collected after autopsies of fatal myocardial infarction, has shown that thrombi in coronary arteries often have a white, platelet-rich head, which forms at the site of plaque rupture, while its distal extension is red in color, reflecting its fibrin- and erythrocyte-rich tail, which forms when blood flow is reduced.[5,9,10] Platelets dominate this process in the first 3-4 hours, whereas fibrin becomes the dominant component at a later time point. These studies confirm that both platelet and thrombin pathways are involved in thrombus formation on an atherosclerotic background.

2.2 | Associations of coagulation factors with atherosclerotic plaque progression

Numerous coagulation proteins have been implicated in proinflammatory conditions, such as atherosclerosis. The presence of TF, considered the primary physiologic trigger of the coagulation cascade, in atherosclerotic lesions was shown by studies of Wilcox et al.[11,12] TF was found on the membrane of macrophages and vascular smooth muscle cells, where it co-localizes with factor VII. Several clinical studies[13–15] indicate that levels of TF are significantly higher in lesions obtained from patients with unstable angina or myocardial infarction than in those from patients with stable coronary artery disease. The role of these coagulation proteins in atherosclerotic lesions was more extensively studied by Borissoff et al., who examined the presence and activity of relevant coagulation proteins in early and stable advanced atherosclerotic lesions.[16] TF, thrombin, factor X, and FXII activities were significantly higher in early atherosclerotic lesions than in stable advanced atherosclerotic lesions. Furthermore, endogenous thrombin potential and thrombin-antithrombin (TAT) complex values indicated a procoagulant profile of early atherosclerotic lesions as compared to stable advanced atherosclerotic lesions. Even in subclinical atherosclerotic disease, such as individuals with increased carotid intima media thickness (IMT), a relation between TF and IMT as marker of early atherosclerosis has been documented.[17]

The presence of coagulation components in atherosclerotic lesions suggests at least a role of these coagulation proteins in plaque thrombogenicity. Taking it one step further, the question rises whether some of these coagulation proteases like thrombin and factor Xa really drive atherogenesis up to the point of atherothrombosis. The fact that coagulation proteins are more present in early atherosclerotic lesions compared to advanced atherosclerotic lesions,[16] supports an important role for these coagulation proteins in the initial development of atherosclerosis, rather than involvement limited to thrombus formation in unstable plaques solely. If so, one would expect some sort of protective effect of factor deficiencies like hemophilia against atherosclerotic disease. Although mouse models suggest that hypocoagulability in hemophilia protects against atherosclerosis, studies in patients with a factor VIII or IX deficiency clearly show that these patients have the same degree

of atherosclerosis burden as the general population.[18,19] When explaining these apparently conflicting findings between mice and hemophilia patients, the use of factor concentrates in humans should be taken into account, although supplementation will never be complete. The signal of lower cardiovascular mortality in patients with hemophilia,[20] albeit not significant (standardized mortality ratio 0.51, 95% CI 0.24-1.09), might not be explained by a reduction in atherosclerotic burden, but by reduced thrombus formation due to hypocoagulability, possibly in combination with increased plaque stability due to reduced thrombin generation.[19] More recently, factor XI deficiency was shown to slow down atherogenesis in mice, with a prominent reduction of macrophage infiltration in the atherosclerotic lesions, as marker of reduction of inflammatory activity in the vessel wall of these mice.[21] Moreover, another coagulation protein of the contact activation system, factor XII, has recently been shown to play an important role in atherosclerotic lesion formation by functioning as a strong inducer of pro-inflammatory cytokine responses in macrophages.[22] Factor XIIa may under certain conditions not only contribute to arterial thrombus formation, but also contribute to inflammation through the activation of the inflammatory kallikrein-kinin system, as reviewed by Nickel et al.[23]

2.3 | PAR activation

Besides their activity in coagulation, the TF-VIIa complex, factor Xa and thrombin each can signal through activation of PARs, and thereby, coagulation and inflammatory pathways are connected, as illustrated in Figure 1. Via PAR-activation, the serine proteases from the clotting system are engaged in several processes, including leucocyte transmigration, vascular remodeling, angiogenesis, and inflammation, which
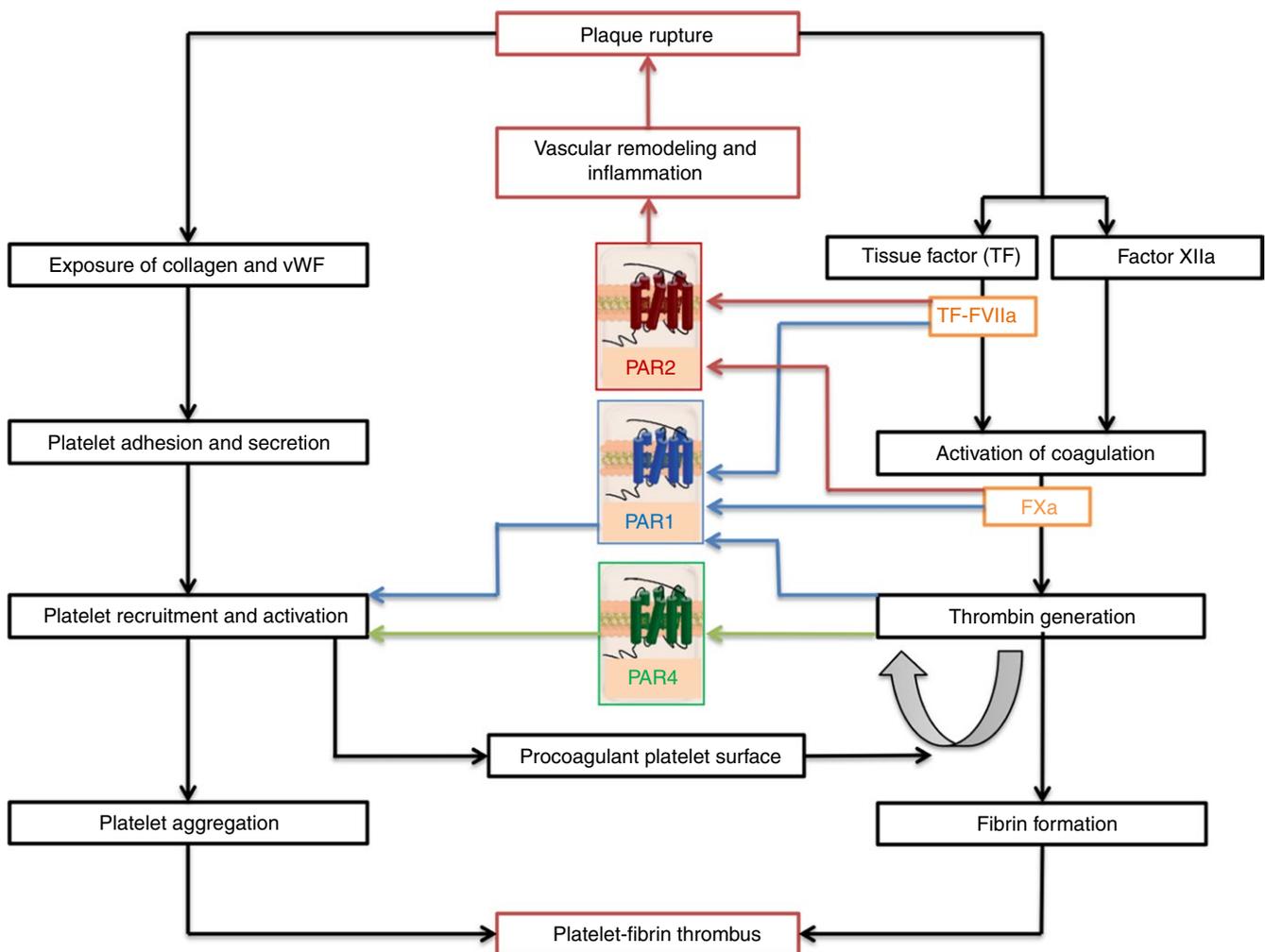

FIGURE 1 Pathways involved in arterial thrombus formation. An atherothrombotic event starts with disruption of an atherosclerotic plaque, thereby exposing collagen, vWF, TF, and factor XII to the blood. A dual pathway of platelet activation/aggregation and coagulation activation ultimately leads to formation of an arterial thrombus, composed of platelets and fibrin. The interplay between thrombin and platelets is illustrated via the activation of PARs, and via the procoagulant platelet surface as a site for assembly of the tenase and prothrombinase complexes, required for the propagation phase of coagulation. The activation of distinct PARs by plasma proteases initiates a series of cellular responses, including platelet activation and several proatherogenic processes such as vascular remodeling and inflammation. Red arrows represent PAR2-mediated processes, blue arrows represent PAR1-mediated processes, green arrows represent PAR4-mediated processes. Adapted from Weitz, JI. *Thromb Haemost* 2014;112:924–31[7] and Ten Cate, H. *Thromb Haemost* 2017;117:1265–71.[8] PAR, protease-activated receptor; TF, tissue factor; vWF, von Willebrand factor

all contribute to the initial development of atherosclerosis.[24] With the exception of PAR2, all PARs are cleaved by thrombin, whereas factor Xa initiates cellular responses through PAR2 and, to a lesser extent, PAR1 activation.[25,26] PAR1 is not only a potent platelet agonist, it also induces several proatherogenic cellular responses. Depending on the conditions of PAR activation, such as thrombin concentration, duration of activation, and the location and conformation of the PAR receptor, thrombin can have opposing effects on a cell, as was reviewed in greater detail by Posma et al.[27] PAR2, a receptor for factor Xa, but not for thrombin, seems to be a key player in vascular remodeling and inflammation.[25] Thus, the direct cellular effects of thrombin and factor Xa are responsible for several proatherogenic processes and these additional actions of thrombin and the other coagulation proteins open opportunities for therapeutic modulation. In particular, the potential to attenuate thrombo-inflammation by direct anticoagulants in the setting of secondary prevention of atherothrombotic events (see below), seems of interest.[28]

3 | BIOMARKERS IN CARDIOVASCULAR DISEASE

Both isolated coagulation proteins and coagulation activation markers like D-dimer, TAT, and prothrombin fragment 1.2 have been associated with arterial cardiovascular disease in numerous prospective studies and meta-analyses, and this has been extensively reviewed by Lowe et al.[29] For example, plasma fibrinogen shows a strong and consistent association (OR 1.78, 95% CI 1.69-1.86) with coronary heart disease, although this may partially reflect its inflammatory marker status, as its concentration is increased under the influence of pro-inflammatory mediators such as interleukin-6.[30] Also more integral tests, like in vitro thrombin generation tests, have been studied in relation to cardiovascular disease.[31] Risks for (recurrent) cardiovascular events are highly variable among individual patients, and from that perspective, the use of biomarkers could potentially help to identify patients at greatest risk to stratify therapy accordingly. Although there is no evidence linking thrombin production to cardiovascular mortality, the downstream fibrin split product D-dimer is associated with cardiovascular events, including mortality in patients with PAD.[32] Contrary to venous thromboembolism (VTE), where the association of D-dimer levels with VTE-risk is strong enough to use it in clinical risk prediction, thus far, the associations of coagulation biomarkers with arterial thrombosis have not added significantly to risk prediction of cardiovascular events. Recently, a biomarker-based prediction model in patients with coronary heart disease (CHD) was published; the "ABC-CHD" model (Age, Biomarkers, Clinical variables).[33] However, only cardiac biomarkers, like N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity troponin T (hs-TnT) and LDL-cholesterol have been incorporated in this prediction model. Besides cardiac biomarkers, markers of inflammatory activity, such as high-sensitivity C-reactive protein (hsCRP) or interleukin (IL)-6, or markers of hypercoagulability, like D-dimer levels, could be prognostic in the setting of patients with established arterial cardiovascular disease.

Future studies using a combination of these different groups of biomarkers may increase the accuracy of risk prediction, and should explore their role in personalizing antithrombotic strategies in high-risk cardiovascular disease patients.

4 | THERAPEUTIC STRATEGIES IN ATHEROTHROMBOTIC DISEASE

Based on the associations between the coagulation system and atherothrombosis, and above mentioned experimental studies, evidence is accumulating that the coagulation system plays an important role in atherogenesis, plaque (in)stability and consecutive atherothrombotic complications. Hence, if thrombin generation and other coagulation proteins like factor Xa play such an important role, does intervention in thrombin generation then modify atherosclerosis and thrombotic events? What clinical evidence do we have to support the idea that we should not only focus on platelet inhibition to prevent atherothrombotic disease, but also add some form of anticoagulant therapy?

Classically, secondary prevention of coronary events is the domain of antiplatelet therapy, either monotherapy with aspirin or dual antiplatelet therapy (DAPT) with ADP receptor blockers. Several alternative options for the "standard antiplatelet regimen" with aspirin plus clopidogrel have emerged in recent years. More potent platelet P2Y12 ADP receptor blockers, prasugrel and ticagrelor, have been introduced in patients with coronary artery disease (CAD).[34,35] Both were associated with significantly reduced rates of ischemic events, but with an approximately 30% increase in the risk of major bleeding. These newer antiplatelet agents have been implicated as standard of care in international guidelines, especially for acute coronary syndrome (ACS) patients with low estimated bleeding risks.[36–39] Extended duration of dual therapy with ticagrelor and aspirin beyond the first year in patients with prior myocardial infarction reduced the composite endpoint of cardiovascular death, stroke, and myocardial infarction, however, this benefit comes with a significant increase in major bleeding and no overall mortality benefit.[40] Despite intensification of antiplatelet therapy in recent years, there remains an approximately 10% risk for recurrent ischemic events at 1 year after coronary events.[34,35] Even higher seems the risk for cardiovascular events in patients with symptomatic peripheral artery disease (PAD)[41,42]; current guidelines[43,44] recommend monotherapy with aspirin or clopidogrel to reduce the risk of myocardial infarction (MI), stroke, or vascular death, but despite the use of antiplatelet therapy an approximately 20% risk for recurrent ischemic events, rehospitalization or death at 1 year remains.[41] In PAD patients, ticagrelor was not superior to clopidogrel in reducing major adverse cardiovascular events (MACE) and acute limb ischemia.[45] According to analysis of PAD patients in the CHARISMA trial, DAPT vs aspirin alone was not able to reduce this high risk for recurrent events in PAD patients, whereas the risk of minor bleeding was increased.[46] In a subgroup analysis of patients with a history of myocardial infarction and concomitant PAD in the PEGASUS trial, the combination of ticagrelor and aspirin reduced both MACE and major

adverse limb events (MALE) compared to aspirin alone.[47] However, in this subgroup of patients with concomitant PAD and CAD treated with ticagrelor and aspirin, the incidence of MACE during 3 years follow-up was still as high as 15.2%. Thus, despite antiplatelet therapy, high morbidity and mortality remains in all patients with atherosclerosis, with the highest rates in those with PAD. Moreover, patients with PAD or CAD often have polyvascular disease, and those patients have an even higher risk of morbidity and mortality than patients with only one affected vascular bed.[41,42]

With blockade of the TXA2 pathway and the P2Y12 receptor, platelets can still be activated by thrombin, via the PAR-1 receptor. In an attempt to further optimize antiplatelet therapy, the selective PAR-1 antagonist vorapaxar was developed. Vorapaxar blocks thrombin-mediated platelet activation, but does not inhibit other modes of thrombin activity, such as fibrin formation, protein C activation, and PAR-4 activation.[48] The combination of three antiplatelet agents in stable ACS patients in the TRA-2°P-TIMI 50 study, involving aspirin, clopidogrel, and the PAR-1 inhibitor vorapaxar, led to significant reduction in rates of ischemic cardiovascular events, but at the price of increased major bleeding.[49] In a subgroup analysis of PAD patients, the addition of vorapaxar to standard therapy with aspirin and/or clopidogrel did not reduce the risk of CV death, myocardial infarction, or stroke when compared with placebo.[49,50] However, it did result in significant risk reduction in limb outcomes, such as acute limb ischemia, (non-) urgent peripheral revascularization and hospitalization. However, again, intensification of antiplatelet therapy was accompanied by an increased risk of bleeding, especially intracranial bleeding.[50] Hence, according to current guidelines, the overall clinical benefit of vorapaxar added to standard antiplatelet therapy in PAD patients is uncertain.[43] Nevertheless, the beneficial effect of vorapaxar on prevention of both urgent revascularizations (ie, acute limb ischemia due to atherothrombosis) and non-urgent revascularizations (ie, progression of atherosclerotic disease) raises the question whether there could be an additional non-platelet-mediated effect of vorapaxar on the vascular endothelium. Since PAR-1 on endothelial cells and VSMCs mediates mitogenic effects,[27,51] vorapaxar might be effective in reducing vascular remodeling and consecutive progression of atherosclerosis.[50]

Standard treatment of patients at risk of (recurrent) atherothrombotic disease with antiplatelet agents exclusively, allows the thrombin pathway to have a persistent proatherogenic and prothrombotic effect. While platelet activation can persist despite antiplatelet treatment via thrombin-induced activation of PAR1 and PAR4, activation of PAR2 by factor Xa and the TF-VIIa complex may promote a proinflammatory condition in the vessel wall. Therefore, ongoing research focuses on identifying the optimal "dual-pathway" approach; a combination of antiplatelet and anticoagulant agents, maximizing the efficacy in reduction of thrombotic events with smallest risk of bleeding.

Until recently, vitamin K antagonists were the only available oral anticoagulants evaluated for long-term treatment of patients with coronary artery disease. Their efficacy in reducing myocardial infarction and stroke was quite convincingly demonstrated in the Warfarin and Aspirin ReInfarction Study (WARIS)[52] and the Anticoagulants in the Secondary Prevention of Events in Coronary Thrombosis (ASPECT) study.[53] However, despite efficacy in the setting of secondary prevention of cardiovascular disease, the combination of aspirin and warfarin increased bleeding and did not reduce mortality.[54-56] Moreover, after introduction of DAPT, therapy with VKA in the setting of ACS, or percutaneous coronary intervention (PCI) was practically abandoned due to more effective protection of DAPT, in particular against instent thrombosis.[57,58] In patients with PAD, the combination of aspirin and warfarin did not reduce major cardiovascular complications and markedly increased bleeding.[59] However, a post hoc analysis that excluded patients with fatal bleeding and hemorrhagic stroke from the co-primary outcome demonstrated risk reductions that were more favorable with combination therapy than with antiplatelet therapy alone, suggesting that the excess bleeding neutralized the potential benefit of combination therapy in PAD patients.[59]

Nonetheless, the results with warfarin had provided proof-of-principle that inhibition of coagulation may be of additional benefit in atherothrombotic disease, a process previously thought to be predominantly platelet-driven. Therefore, after the introduction of direct oral anticoagulants (DOACs), several phase II and later two phase III trials evaluated their role in prevention of recurrent ischemia in stabilized ACS patients. The APPRAISE-2 trial (APixaban for Prevention of Acute Ischaemic Events) was terminated early after recruitment of almost 7400 patients, because of an increase in major bleeding with apixaban at a dose of 5 mg twice daily, without a reduction in recurrent ischemic events.[60] However, the ATLAS-2 trial (Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome) was carried to completion and met its primary objective.[61] In this study, 15,526 patients with a recent acute coronary syndrome were randomized to receive twice-daily doses of either 2.5 mg or 5 mg of rivaroxaban or placebo, for the majority of patients (93%) on top of DAPT with aspirin and a thienopyridine. The two doses of rivaroxaban significantly reduced the primary efficacy endpoint, a composite of MI, death from cardiovascular causes or stroke, with the 2.5 mg B.I.D. dose also showing a survival benefit. Nevertheless, both doses of rivaroxaban increased the rates of major bleeding and intracranial hemorrhage. Even the lower dose of rivaroxaban (2.5 mg B.I.D.) tested in ATLAS-2 was still associated with a three-fold increase in major bleeding and a doubling of the rate of intracranial hemorrhage. However, there was no significant increase in fatal bleeding, and the lower dose of rivaroxaban reduced overall and cardiovascular mortality, thereby resulting in survival benefit. Because of these findings, rivaroxaban 2.5 mg B.I.D. is licensed for this indication in Europe. Although promising, the exact role for DOACs in ACS management seems to be complicated, due the increased bleeding risk, uncertainty about the competing antithrombotic options in post-ACS patients and uncertainty about the combination with other platelet inhibitors than clopidogrel, such as prasugrel, ticagrelor, and vorapaxar.[58]

Nevertheless, the findings with rivaroxaban in ATLAS-2 have opened new avenues for research, because they provide a proof of concept that anticoagulants may complement current antiplatelet drugs to reduce ischemic events in patients with atherosclerotic disease. Thus, several phase II and phase III studies are underway,

studying the role of DOACs in patients with atherothrombotic disease. To overcome the increased bleeding risk of triple therapy in ATLAS-2, the GEMINI-ACS-1 trial was set up to explore the safety of a dual pathway antithrombotic therapy approach with low-dose rivaroxaban (2.5 mg B.I.D), in place of aspirin, together with a P2Y12 inhibitor (clopidogrel or ticagrelor) in 3037 post-acute ACS patients.[62] Compared to patients in the standard treatment arm with aspirin and a P2Y12 inhibitor, patients in the rivaroxaban plus P2Y12 inhibitor arm had similar rates of clinically significant bleeding. Although clearly undersized for ischemic events, the composite ischemic endpoint of cardiovascular death, myocardial infarction, stroke or stent thrombosis was similar in both groups.

Very recently, results of the COMPASS-trial (Cardiovascular OutcoMes for People using Anticoagulation StrategieS) were published.[63] This study tested the hypothesis of anticoagulation as a superior strategy as compared to antiplatelet therapy alone in secondary cardiovascular prevention.[64] Following a planned interim analysis the Data Monitoring Committee recommended to stop the trial early as the primary endpoint, a composite of cardiovascular death, stroke or MI, had reached its prespecified criteria for superiority. Among patients with stable cardiovascular disease, either CAD or PAD, those assigned to rivaroxaban 2.5 mg B.I.D. plus aspirin had a 24% risk reduction for the composite of cardiovascular death, stroke or myocardial infarction compared to those assigned to aspirin alone (HR 0.76,

TABLE 1 Schematic presentation of different therapeutic strategies in patients with stable coronary artery disease (CAD) (unless indicated as "post-ACS") and stable peripheral artery disease (PAD), with their outcomes on major adverse cardiovascular and limb events (MACE, MALE) and bleeding

Therapeutic strategy	Stable coronary artery disease (CAD); outcome		Stable peripheral artery disease (PAD); outcome				Bleeding complications in PAD and CAD patients	
	MACE	ref.	MACE	ref.	MALE	ref.	Bleeding	ref.
Aspirin	↓	[96]	↓/–	[96,97]	↓	[98]	↑	[96–98]
Monotherapy compared to aspirin								
Clopidogrel	↓	CAPRIE [99]	↓	CAPRIE [99]	n/a	n/a	↑	CAPRIE [99]
Ticagrelor	n/a	n/a	↓[b]	EUCLID [45]	–[b]	EUCLID [45]	↑	EUCLID [45]
Rivaroxaban 5 mg B.I.D.	–	COMPASS [63,66]	–	COMPASS [63,65]	↓	COMPASS [63,65]	↑↑	COMPASS [63,65,66]
Combination therapy compared to aspirin								
Aspirin + clopidogrel	↓	CHARISMA [100]	–	CHARISMA [46]	–	CHARISMA [46]	↑↑	CHARISMA [46,100]
Aspirin + ticagrelor	↓↓	PEGASUS-TIMI 54 [40]	↓↓[a]	PEGASUS-TIMI 54 [47]	↓↓[a]	PEGASUS-TIMI 54 [47]	↑↑	PEGASUS-TIMI 54 [40,47]
Aspirin/DAPT + vorapaxar	↓↓↓	TRA2°P-TIMI 50 [49]	–	TRA2°P-TIMI 50 [49,50]	↓↓	TRA2°P-TIMI 50 [49,50]	↑↑↑	TRA2°P-TIMI 50 [49,50]
Aspirin + VKA	↓↓↓	WARIS, ASPECT [52,53]	–	WAVE [59]	↓	WAVE [59]	↑↑↑	WARIS, ASPECT [52,53] WAVE [59]
DAPT + rivaroxaban 2.5 mg B.I.D. (post-ACS)	↓↓↓↓	ATLAS ACS 2-TIMI 51 [61]	n/a	n/a	n/a	n/a	↑↑↑	ATLAS ACS 2-TIMI 51 [61]
Aspirin + rivaroxaban 2.5 mg B.I.D.	↓↓↓↓	COMPASS [63,66]	↓↓↓↓	COMPASS [63,65]	↓↓↓↓	COMPASS [63,65]	↑↑	COMPASS [63,65,66]

Study population, control groups, and definition of primary efficacy and safety outcomes are highly variable between different studies. This table is based on the authors' interpretation of clinical trials and meta-analyses, and is clearly not based on head-to-head comparisons of the different therapeutic strategies.

(↓) to (↓↓↓↓) indicates modest to strong decrease in MACE/MALE.

(↑) to (↑↑↑↑) indicates modest to strong increase in bleeding complications.

(–) indicates no beneficial effect compared to aspirin monotherapy.

(↓/–) indicates contradictory results.

[a]In patients with concomitant PAD and CAD.

[b]No direct comparison with aspirin monotherapy, but compared to clopidogrel monotherapy.

ACS, acute coronary syndrome; B.I.D., bis in die, twice a day; DAPT, dual antiplatelet therapy; MACE, major adverse cardiovascular events; MALE, major adverse limb events; n/a., not available and/or not applicable; ref., reference; VKA, vitamin K antagonist.

95% CI 0.66-0.86). Although there was an increase in major bleeding (HR 1.70, 95% CI 1.40-2.05), there was no significant difference in intracranial or fatal bleeding between these two groups. Treatment with a combination of very-low dose rivaroxaban and aspirin provided a clear net clinical benefit, both in patients with CAD and PAD. For the latter, a group of patients with either peripheral artery disease or carotid artery disease, not only major adverse cardiovascular events (MACE) were significantly reduced, but there was also a significant reduction in major adverse limb events (MALE) and amputations for patients treated with the combination of rivaroxaban and aspirin.[65] As described above, no other pharmaceutical therapy in PAD patients had shown such a clear reduction in both cardiovascular and limb events thus far (Table 1). The relative risk reduction with combination therapy compared to aspirin alone was demonstrated in all patient groups, including patients with carotid artery disease, PAD, or CAD.[65,66]

5 | PLEIOTROPIC EFFECTS OF ANTICOAGULANTS

What is at the basis of this beneficial effect of adding low-dose rivaroxaban to aspirin? It is likely that the benefit of factor Xa and thrombin inhibition in this setting reflects not only attenuation of coagulation, but also inhibition of thrombin-mediated platelet activation, and suppression of several processes leading to atherogenesis, vascular inflammation, and plaque instability. Thus, DOACs might offer "vascular protection" that goes beyond thrombosis prevention. The term vascular protection refers to the spectrum of effects that are potentially mitigated by inhibiting the sequelae of inflammation, cell proliferation, tissue remodeling, destabilization of atherosclerotic plaques, ultimately leading to plaque rupture and atherothrombosis. The observation in COMPASS that the benefit of the combination was evident right from the start might reflect atherosclerotic plaque stabilization. However, the curves continue to divert throughout the whole study, suggesting that not only existing atherosclerotic lesions are stabilized, but also pro-inflammatory activities in the development of atherosclerosis are inhibited. Such additional effects of DOACs have been strongly suggested by several preclinical models. In animal studies, dabigatran reduced the size of atherosclerotic lesions and enhanced plaque stability.[67,68] Comparable animal studies with direct Xa inhibitors showed similar beneficial effects on atherosclerosis.[69,70] These beneficial effects of DOACs on atherosclerosis and atherothrombosis are thought to be due to inhibition of thrombin's PAR-mediated signaling.[24,27] These pleiotropic effects have also been suggested in other models, such as in myocardial ischemia-reperfusion models, where rivaroxaban improved cardiac function through the reduction of inflammation in the left ventricle of mice.[71] Factor Xa is thought to elicit a pro-inflammatory response by directly activating PAR-2 receptors, whereas inhibition of factor Xa, and thereby attenuating thrombo-inflammation, might be part of the beneficial effect of rivaroxaban in this setting. Whether such pleiotropic effects would also appear in the human vasculature is not yet elucidated. Nevertheless, the marked reduction in major adverse limb events and amputations in

the PAD subset from the COMPASS trial may hint towards an effect on atherosclerosis rather than "simply" on thrombosis.[65] However, this hypothesis of vascular protection does not explain why it was just the combination arm in COMPASS that was superior to aspirin, and the rivaroxaban only arm was not. Two possible explanations come to mind when trying to explain these findings. COMPASS-PAD showed a significant reduction in MALE in the rivaroxaban monotherapy arm compared to the aspirin monotherapy arm, however, for MACE only a non-significant trend (HR 0.86, 95% CI 0.69-1.08) was reported, which might have been a result of premature termination of the COMPASS trial. The second explanation may be potential synergism of the combination of two drugs that act on different pathways; anti-thrombotic (aspirin), anticoagulant (rivaroxaban), and anti-inflammatory (both). Future studies should further explore the opportunities and challenges of combined anticoagulant and antiplatelet agents in prevention and treatment of patients with atherosclerotic disease. These studies should focus on the identification of highest risk patients, for example with polyvascular bed involvement, who are likely to benefit the most of optimization of antithrombotic strategies.

6 | PRECAUTIONS

Anticoagulation, and thus inhibition of coagulation proteases, not only attenuates fibrin formation, but may also influence other biological and pathophysiologic processes. For example, besides impairing the function of several coagulation factors, the VKAs inhibit vascular vitamin K-dependent proteins, such as Matrix Gla Protein and osteocalcin, resulting in calcification of arteries.[72] However, the clinical importance of VKA-induced vascular calcifications remains unclear, because the evident beneficial effect, due to the high efficacy of VKA in stroke prevention, may outweigh the potential harmful effects of VKA-associated vascular calcification. Another point of concern, is that pathology studies have suggested that the use of VKAs is an independent risk factor for plaque instability in patients with coronary artery disease[73] and stroke[74] due to intraplaque hemorrhage. Whether DOACs have the same negative effect on plaque stability is currently unknown. Conflicting results have been published on the risk of acute myocardial infarction (MI) with the direct thrombin inhibitor dabigatran. While some meta-analyses of randomized trials[75,76] and a recently published large retrospective cohort study[77] concluded that the use of dabigatran was associated with an increased risk of MI, a post-hoc analysis of revised data from the RELY trial[78] and other meta-analyses did not confirm this finding.[79] The potential underlying mechanisms of the suggested increase in MI associated with dabigatran is not clear. Some speculated that the combination of TF- and contact-activation-generated thrombin at the site of coronary plaque rupture might overwhelm the local concentrations of dabigatran.[80] Ex vivo, plasma samples from warfarin-administered patients generated lower peak thrombin levels than those from dabigatran-administered patients, and the authors speculate that the reduced ability of dabigatran to counter the high concentrations of thrombin that are generated by TF after a ruptured atherosclerotic plaque might explain the difference in MI in patients on dabigatran compared to warfarin.[81]

7 | FUTURE PERSPECTIVES

The results of the COMPASS trial might change the field of antithrombotic treatment in patients with atherosclerotic disease. Future studies will have to focus on further implementation and challenges of combined anticoagulant and antiplatelet agents, for example a head-to-head comparison between the addition of a second antiplatelet drug vs a very low dose of a factor-Xa inhibitor to aspirin. Moreover, these future studies should focus on the identification of patient groups that are likely to benefit the most of combining anticoagulant and antiplatelet treatment.

Besides, the search for potential new anticoagulants will continue. The contribution of the proteins of the contact system (factors VIII, IX, XI, XII, prekallikrein, and high-molecular-weight kininogen) to the process of atherothrombosis have recently gained more attention.[82] Not only fibrin formation and the strength of the fibrin clot is stimulated by several steps of the contact system, it also attenuates the fibrinolytic pathway.[83] Currently, specific inhibitors against factors XIIa and XIa are being studied as potential therapeutic targets for prevention of thrombosis.[23,84,85] Factor XII deficient humans have a normal hemostatic capacity, while patients lacking factor XI only have a mild trauma-induced bleeding disorder. Contrary to their role in normal hemostasis, animal models revealed a more important role of FXIIa-driven coagulation in arterial thrombosis,[86] and furthermore, factor XIIa contributes to inflammation through the activation of the inflammatory bradykinin-producing kallikrein-kinin system.[22,23] Besides attenuation of coagulation, factor XI deprivation has also been shown to slow down atherogenesis in apoE/factor XI double knockout mice.[21] Thus, pharmacological inhibition of factors XI(a) and XII(a) interferes with both thrombosis and inflammation, and targeting the FXIIa-driven contact system may eventually be a safe therapeutic strategy, potentially with additional beneficial anti-inflammatory and anti-atherogenic effects.

Although the link between inflammation and atherosclerosis is known for several decades,[2,3] only very recently clinical data demonstrating a direct benefit of targeting inflammation in patients with atherosclerotic disease have become available. Results of the CANTOS trial showed that in patients with prior myocardial infarction with elevated biomarkers of inflammation (hsCRP), anti-inflammatory therapy with canacinumab (an IL-1β blocker) reduced the incidence of recurrent cardiovascular events.[87] However, due to safety concerns (more fatal infections) and costs, it is currently unclear whether canakinumab or other anti-inflammatory therapies will ultimately be used in high-risk patients with atherosclerotic disease.

8 | ISTH BERLIN REPORT

Lind and colleagues report that incident VTE was associated with progression of existing carotid atherosclerotic lesions, but not with new plaques in an analysis from the Tromsø study.[88] Importantly, this effect was not mediated by hs-CRP, suggesting that it is probably the hypercoagulability associated with VTE that drives atherosclerosis, which could also explain the increased risk of arterial thrombotic events following incident VTE.[89]

Novel preclinical data were reported by Owens and colleagues showing that PAR-2 is a relevant mediator of proatherogenic effects; PAR-2 deficient mice had smaller aortic plaques, possibly due to combined effects on cholesterol efflux from macrophages and attenuation of VSMC activity.[90] While coagulation proteases like factors VIIa and Xa may be important ligands in PAR-2 mediated effects, in the context of atherosclerosis this link needs further exploration. Finally, van Gorp et al. investigated warfarin as compared to dabigatran in effects on atherogenesis, showing that only dabigatran attenuated atherogenesis.[91] Posthuma and colleagues showed that rivaroxaban not only inhibited atherogenesis but even diminished existing lesions, the mechanisms of which need further study.[92]

Data on hemostasis biomarkers of cardiovascular disease were reported by Komarov et al., showing that addition of D-dimer to well-known scoring systems in patients after elective PCI could improve the diagnostic capacity to predict MACE in these patients.[93] Soluble thrombomodulin (sTM) was studied as a biomarker of endothelial injury in CAD patients by Komanasin and colleagues, reporting that raised plasma sTM was associated with increasing coronary artery stenosis.[94] Finally, Eggebrecht and colleagues studied the effects of DOACs compared to VKAs on several biomarkers of cardiovascular structure and function in individuals with documented cardiac dysfunction. Although this analysis demonstrated differential relations between these biomarkers in individuals with DOACs vs VKAs, fibrinogen, the only hemostasis biomarker included, did not appear to be different between treatment groups.[95]

9 | CONCLUSION

Contrary to what was previously thought, the coagulation system plays an important role in the development of thrombotic complications in patients with established atherosclerotic disease. Coagulation activation and generation of thrombin not only promotes fibrin production and platelet activation, but seems to be involved in atherogenesis, mainly through PAR-activation. This has important implications for treatment of patients with atherosclerotic disease, like CAD and PAD, who have high residual risk of atherothrombotic events, despite classical management with antiplatelet therapy. The results of the COMPASS trial provide clinical evidence of the concept that combined antiplatelet and anticoagulant treatment provides net benefit in secondary prevention of cardiovascular complications. Future studies will have to explore further opportunities of attenuating thrombo-inflammation and optimizing vascular protection in atherosclerotic disease.

RELATIONSHIP DISCLOSURES

rpth research & practice in thrombosis & haemostasis

AUTHOR CONTRIBUTIONS

R.H. Olie reviewed the literature and drafted the manuscript. P.E.J. van der Meijden and H. ten Cate critically revised the manuscript.

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