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STATE OF THE ART REVIEW
Thrombosis and antithrombotic treatment

Platelet biology and function: plaque erosion vs. rupture

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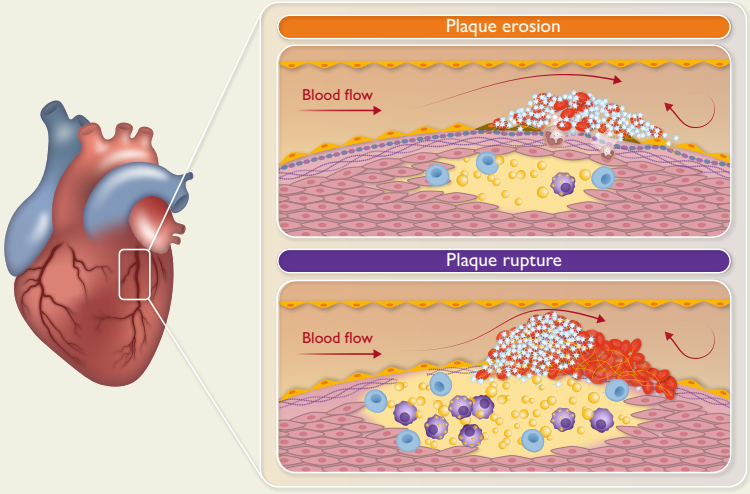
Abstract

The leading cause of heart disease in developed countries is coronary atherosclerosis, which is not simply a result of ageing but a chronic inflammatory process that can lead to acute clinical events upon atherosclerotic plaque rupture or erosion and arterial thrombus formation. The composition and location of atherosclerotic plaques determine the phenotype of the lesion and whether it is more likely to rupture or to erode. Although plaque rupture and erosion both initiate platelet activation on the exposed vascular surface, the contribution of platelets to thrombus formation differs between the two phenotypes. In this review, plaque phenotype is discussed in relation to thrombus composition, and an overview of important mediators (haemodynamics, matrix components, and soluble factors) in plaque-induced platelet activation is given. As thrombus formation on disrupted plaques does not necessarily result in complete vessel occlusion, plaque healing can occur. Therefore, the latest findings on plaque healing and the potential role of platelets in this process are summarized. Finally, the clinical need for more effective antithrombotic agents is highlighted.

Graphical Abstract



Schematic representation of the differences in thrombus formation induced by either erosion or rupture of coronary atherosclerotic plaques.

Keywords Plaque rupture • Plaque erosion • Thrombus formation • Platelet activation

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Introduction

Following vascular injury, matrix components and vascular smooth muscle cells (VSMCs) that are typically inaccessible to blood are exposed to the circulation, leading to the activation of the haemostatic system to prevent further blood loss. Within this process, platelets are the first to respond by recognizing and binding to von Willebrand factor (vWF), exposed collagen, and other matrix components. Upon binding, platelets become activated and release autocrine and paracrine mediators from their granules, which will further enhance platelet incorporation into the growing blood clot or thrombus.[1] In addition, platelet activation is augmented by exposure to the endocrine mediators norepinephrine and epinephrine, which are highly increased during myocardial infarction (MI).[2] Different platelet populations with specialized functions can be recognized within a thrombus as a consequence of environmental factors as well as intrinsic platelet factors (inherited from the megakaryocyte).[1] While megakaryocytes residing in the lungs display a more inflammatory phenotype compared to bone marrow megakaryocytes,[3,4] it is unknown whether platelets derived from these megakaryocytes also differ in their contribution to haemostatic and atherothrombotic processes.

Also, thrombin generation takes place as a consequence of the activation of the extrinsic and intrinsic coagulation pathways resulting in fibrin formation and further platelet activation. These two processes, platelet activation and coagulation, are required for the formation of a stable haemostatic plug.[1,5] In atherothrombotic pathologies, the haemostatic system is triggered by the atherosclerotic lesion, i.e. in a situation of endothelial injury but not blood loss. The result is the formation of a thrombus within the vessel, culminating in the development of acute coronary artery disease (CAD).

In this review, we discuss plaque phenotypes and its implications for thrombus composition. Further, we provide an overview of important mediators in plaque-induced platelet activation and subsequent thrombus formation. Lastly, we consider plaque healing and the potential role of platelets herein.

Plaque composition and phenotype

Atherosclerotic lesions develop over time starting with adaptive thickening of the arterial vessel wall that gradually advances into atheromatous, fibrous, and fibroatheromatous plaques.[6] For a detailed overview on plaque development and the role of platelets therein, we refer to detailed reviews.[7–10]

Advanced plaques can erode or rupture, leading to arterial thrombosis and tissue ischaemia[10–12] (*Graphical Abstract*). The composition of an atherosclerotic plaque will determine whether it is prone to rupture or to erode. Ruptured plaques are typically thin-cap fibroatheroma with a large lipid core that can show signs of recent intra-plaque haemorrhage.[13,14] Upon rupture, the fissure extends into the plaque exposing the highly thrombogenic lipid rich, necrotic core to the circulation. In ruptured plaques, macrophages and foam cells are abundantly present and are typically present at the margins of the rupture.[13–15] Also, T cells are commonly detected at the luminal surface near the fission, although in smaller numbers than macrophages.[13,15] Interestingly, at the site of macrophage and T-cell accumulation, generally no VSMCs are detected, and rupture usually does not occur in VSMC-rich areas.[13] Furthermore, extensive inflammatory reactions contribute to the degradation of the extracellular matrix.[16] In contrast, eroded plaques consist for a major part of VSMCs and are rich in proteoglycans (e.g. versican) and hyaluronan at the site of erosion.[14,17] Erosion of the endothelial layer will leave the underlying VSMCs and extracellular matrix (e.g. proteoglycans, collagen) exposed to the circulation. In eroded plaques, inflammation is limited with a sparse distribution of macrophages and T cells,[15,18] while accumulation of neutrophil extracellular traps (NETs) has been reported. Neutrophils and NETs locate in plaques with superficial erosion within the atheroma and at the luminal surface at the site of endothelial denudation or the thrombus.[19,20] However, recent work has shown that they can also be found in ruptured plaques.[21] Peptidyl arginine deiminase 4 (PAD4)-mediated NET formation is reported to activate endothelial cells (ECs) and induce tissue factor (TF) production,[19,22] via interleukin (IL)-1α and cathepsin G. Further, neutrophils and NETs colocalize with Toll-like receptor 2 (TLR2) expression in plaques rich in VSMCs. In combination with *in vitro* findings showing potentiation of TLR2-induced endothelial stress by neutrophils, these findings suggest an important role for TLR2 and neutrophils in superficial plaque erosion.[20] Further, calcification of eroded plaques tends to be less than for ruptured plaques[14] and is restricted to areas with speckled calcifications in contrast to ruptured plaques that have areas with diffuse or fragmented calcifications.[18] In general, the atherosclerotic burden is smaller for eroded plaques compared to ruptured plaques.[14,18]

It is estimated that in ~40% of patients, the culprit lesion responsible for acute coronary syndrome (ACS) is eroded rather than ruptured.[23] Advances in intravascular imaging techniques have improved plaque phenotyping, resulting in a higher prevalence of eroded plaques than reported in autopsy studies. Nonetheless, the prevalence of plaque erosion has increased independent of the improvements made in diagnostic modalities. Better and earlier primary prevention of traditional risk factors (e.g. LDL, smoking, hypertension) has contributed to a gradual shift in plaque phenotype towards superficial erosion.[23] Plaque erosion is typically associated with a non-ST-elevation MI (NSTEMI) presentation of ACS, while plaque rupture is more common in ST-elevation MI (STEMI) patients.[23] Additionally, patients with erosion of the culprit plaque are more likely to have a favourable prognosis than those with plaque rupture.[24] In a small cohort of ACS patients, patients with plaques with an intact fibrous cap had a lower overall occurrence of major adverse cardiovascular events (MACEs) during follow-up and a better MACE-free survival than patients with ruptured plaques.[25] It is suggested that the more favourable prognosis of patients with eroded plaques is in part due to a lower complexity and smaller overall atherosclerotic burden of the eroded lesions; lower plasma levels of LDL cholesterol, triglyceride, and C-reactive protein; and a lower prevalence of traditional risk factors.[23,24] Although most studies only categorize coronary plaques as either eroded or ruptured, a recent transcriptomics study demonstrated five distinct plaque subsets associated with clinical outcomes. The expression levels of genes associated with extracellular matrix organization, glycolysis, and transforming growth factor (TGF)-β signalling were higher in the cluster containing the highest rate of ischaemic samples.[26] Interestingly, this cluster showed high similarity to pathways found in carotid plaques associated with clinical symptoms.[26] The same subset exhibited a higher prevalence of pathways and cell types traditionally associated with two distinct plaque phenotypes, the 'stabilizing' phenotype characterized by the abundance of smooth muscle cell-specific genes and the 'destabilizing' phenotype characterized by the abundance of macrophage specific genes.[26] Novel studies using single-cell mass spectrometry and RNA sequencing provided further insight into the immune cell content of the plaque microenvironments and their relation to plaque stability.[27–30] Depuydt *et al.*[29] showed that plaques contain various T-cell populations, pro-inflammatory macrophages, and foam cell-like macrophages. Plaque stability was mainly affected by pro-inflammatory

Table 1 General overview of the differences in plaque, patients, and thrombus characteristics between eroded and ruptured plaques

	Plaque erosion	Plaque rupture	References
Plaque composition	Little or no lipid core	Large lipid pool	13–17
	Endothelial denudation	Greater necrotic core	
	Smooth muscle cells rich	Thin fibrous cap	
	Matrix rich (proteoglycan, glycosaminoglycan, hyaluronic acid)	Abundant monocytes, macrophages, and foam cells	
	Neutrophil predominance	Greater plaque burden	
Plaque location			
LAD	40%–66%	40%–46%	32–36
RCA	22%–30.6%	35%–59%	32–36
LCX	8.1%–14.9%	9.2%–15.6%	32–36
Sex and age differences[a]			
Female	<50 years:	<50 years:	37–39
	74.0%–77.1%	22.9%–26.0%	
	>50 years:	>50 years:	
	30.3%–44.1%	55.9%–69.7%	
Male	<50 years:	<50 years:	37–39
	45.6%–65.2%	34.8%–53.4%	
	>50 years:	>50 years:	
	18.1%–43.4%	56.5%–81.9%	
Clinical presentation	NSTEMI	STEMI	23
Thrombus composition	Non-occlusive	Occlusive	40–43
	Platelet rich	Platelet and fibrin rich	
	RBCs poor	Abundant RBCs	
	NETs present		

LAD, left anterior descending artery; LCX, left circumflex artery; NETs, neutrophil extracellular traps; NSTEMI, non-ST elevation myocardial infarction; RBC, red blood cells; RCA, right coronary artery; STEMI, ST elevation myocardial infarction.
[a]Only post-mortem studies.

macrophages, although functional B cells contributed as well.[28] In plaques from symptomatic patients, abundant CD4+ T cells, activated and differentiated T cells, and exhausted T cells were observed,[27] although activated T cells and IL-1β-positive macrophages were present in plaques from asymptomatic patients too.[27] These results indicate the presence of multiple, partially overlapping plaque phenotypes with distinct cellular signatures; however, novel studies are warranted for further characterization.

While atherosclerotic plaque formation can take place throughout the vessels, branching points of the vascular tree are the most susceptible locations. These regional differences can be explained by the differences in rheological conditions as atherosclerosis tends to develop in regions with flow disturbances, lower shear, and oscillatory flow.[31] Studies showed different prevalence of plaques and their subsequent phenotypes within the coronary circuit (*Table 1*).[32–36]

Whether sex influences plaque phenotype is not straightforward. Post-mortem studies reported a prevalence of around 75% for plaque erosion in women younger than 50 years who suffered sudden coronary death, while for men in the same age category numbers range from 35% to 65%,[37–39] demonstrating an increased prevalence of plaque erosion in women compared with men (*Table 1*). This was further confirmed using optical coherence tomography (OCT) imaging in patients with stable CAD or ACS.[44] In contrast, the study by Kim *et al.*[45] showed no significant sex difference in plaque phenotype analysed by OCT in ACS patients, although their study demonstrated higher frequency of plaque erosion in younger (<50 years) vs. older (>70 years) women. This relative higher frequency of plaque rupture in older female patients may be partially explained by the loss of anti-atherosclerotic effects of oestrogen during and post-menopause. Interestingly, oestrogen does not appear to affect plaque erosion.[46]Therefore, insight into oestrogen levels as well as potential hormone replacement therapy can be informative when assessing the relation among age, sex, and plaque phenotype.

Platelet response to plaque phenotype and consequences for clot composition

The different phenotypes of eroded and ruptured plaques also affect the structure and composition of the thrombus formed at the culprit lesion. Thrombi associated with ruptured plaques tend to be rich in fibrin and erythrocytes and to a lesser extent in platelets, giving them a typical 'red' appearance.[40] On the other hand, eroded plaques provoke the formation of white thrombi, predominantly rich in platelets, but poor in erythrocytes.[40] Insights into the composition of thrombi are crucial, as this will determine the mechanical properties of the clot in terms of stability and embolization, as well as resistance to lysis.[47] Here, an increased platelet and erythrocyte content and denser fibrin network are related to a higher resistance to lysis.[47] The heterogeneous composition of plaque-associated thrombi is well illustrated by thrombi obtained by aspiration thrombectomy from STEMI patients, where relatively more fibrin and platelets are present in the outer layer, while erythrocytes and polyhedrocytes are relatively more abundant in the inner core of the thrombus.[41] Moreover, thrombus composition changes over time. Studies on (aspirated) thrombi from culprit arteries from patients suffering from STEMI or sudden cardiac death showed that a longer ischaemic period correlates with increased fibrin and decreased platelet content.[42,43] Erythrocyte and leukocyte contents were unaffected by ischaemic time.[43]

Differences in thrombus composition in response to plaque rupture or erosion occur at multiple levels. In the following paragraphs, the influence of local haemodynamics, matrix composition, soluble factors, and platelet interactions with other cell types on thrombus composition is discussed, as illustrated in *Figures 1* and *2*.

Haemodynamic alterations

By causing endothelial dysfunction, haemodynamic disturbances drive atherosclerotic plaque development, but also in later stages, haemodynamic alterations contribute to plaque erosion[49] and rupture.[50] The rate of these flow disturbances depends on the asymmetry of the plaque (i.e. concentric vs. eccentric). Concentric plaques exhibit minimal alterations during the cardiac cycle, while eccentric plaques can undergo significant changes as their shoulder region (i.e. the junction between the plaque and the adjacent plaque-free vessel wall)

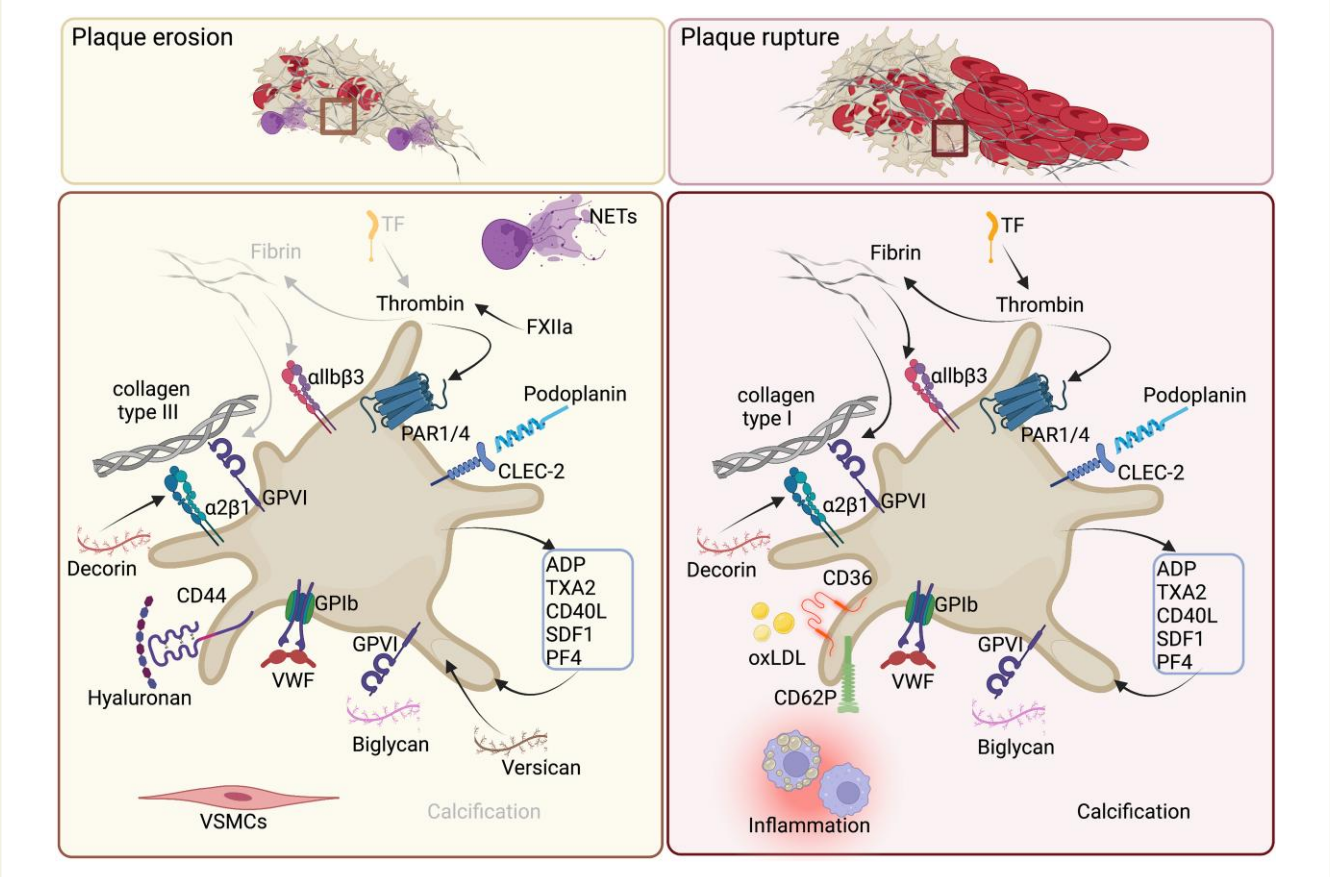

Figure 1 Platelet response towards prominent matrix components and soluble factors in either plaque erosion or rupture. Overview of mechanisms responsible for platelet activation and consequent thrombus formation. For more information, see *Matrix components* and *Soluble factors*. ADP, adenosine diphosphate; CD36, cluster of differentiation 36; CD40L, cluster of differentiation 40 ligand; CD44, cluster of differentiation 44; CD62P, P-selectin; CLEC-2, C-type lectin-like type II; FXIIa, activated coagulation factor XII; GPIb, glycoprotein Ib; GPVI, glycoprotein VI; integrin α2β1; integrin αIIbβ3; NETs, neutrophils extracellular traps; oxLDL, oxidized low density lipoprotein; PAR1/4, protease activated receptor 1/4; PF4, platelet factor 4; SDF-1, stromal cell derived factor 1; TF, tissue factor; TXA2, thromboxane A2; VSMCs, vascular smooth muscle cells; VWF, von Willebrand factor. Figure was created with Biorender.com

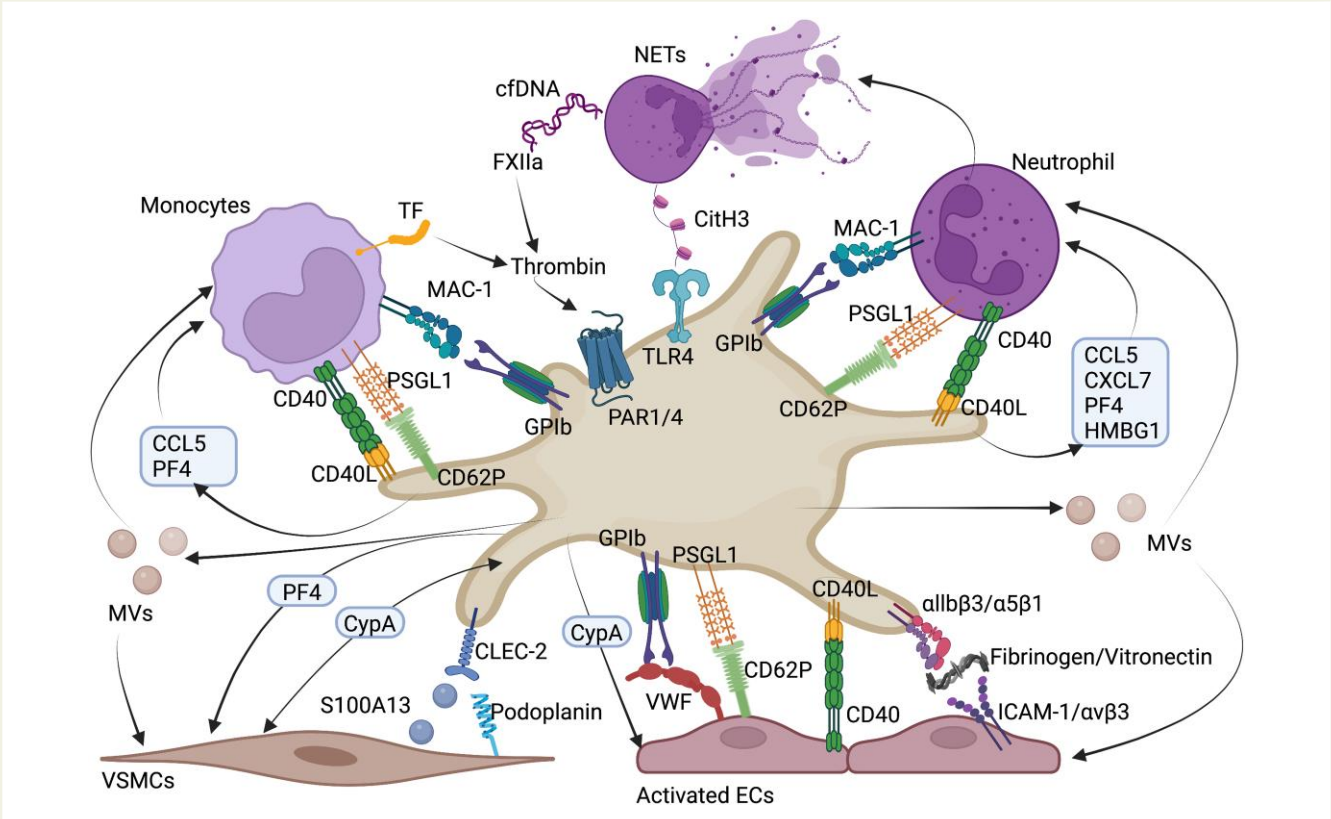

Figure 2 Interactions of platelets with leukocytes, endothelial cells, and vascular smooth muscle cells in atherosclerosis. Overview of ligand–receptor interactions and soluble important factors in the crosstalk between platelets and leukocytes (neutrophils, monocytes, and T cells), endothelial cells, and vascular smooth muscle cells.[48] CCL5, chemokine (C-C motif) ligand 5, CD40, cluster of differentiation 40, CD40L, cluster of differentiation 40 ligand, CD62P, P-selectin, CitH3, citrullinated histone H3, cfDNA, cell free DNA, CLEC-2, C-type lectin-like type II, CypA, cyclophilin A, CXCL7, chemokine (C-X-C motif) ligand 7, ECs, endothelial cells, FXIIa, activated coagulation factor XII, GPIb, glycoprotein Ib, HMGB1, high mobility group box 1 protein, ICAM-1, intracellular adhesion molecule 1, integrin αIIbβ3, integrin α5β1, integrin αvβ3, MAC-1, macrophage-1 antigen, MVs, microvesicles, NETs, neutrophil extracellular traps, PAR1/4, protease activated receptor 1/4, PF4, platelet factor 4, S100A13, S100 calcium binding protein 13, TLR4, toll-like receptor 4, VSMCs, vascular smooth muscle cells, VWF, von Willebrand factor. Figure was created with Biorender.com

bends, increasing the risk for plaque disruption/rupture.[51] The majority (80%) of plaques observed in patients with coronary atherosclerosis exhibited eccentricity.[52,53] Previous studies demonstrated that during ACS, regions of plaque rupture often coincide with elevated levels of endothelial shear stress and that the circumferential stress is centred at the shoulder region in coronary atherosclerosis.[54–56]

Also, platelet adhesion and aggregation are affected by local shear microgradients.[57,58] Thereby, local disturbances in haemodynamics shape plaque-induced thrombus formation (*Figure 3*). *In silico* modelling has shown that—using a straight vessel model—platelets adhered and aggregated to the injured site in a homogeneous fashion, leading to an even coverage.[59] With increasing stenosis, thrombus formation gradually became more skewed towards the distal part of the injured stenotic site.[59] At 70% stenosis, platelet aggregation was predominantly initiated at the apex of the stenosis, and the thrombus was located at the distal and downstream parts of the injured site, leaving most of the upstream injured site uncovered.[59] In both *in vitro* and *in silico* models of vessel stenosis, the highest wall shear stress can be found at the apex of the stenosis, which is also the initiation and anchor point of platelet aggregation.[58,60] Depending on the level of turbulence, the thrombus either grows along the post-stenosis channel wall or lines up along the post-stenotic recirculation zone.[58,60] The rate at which thrombi grow at these pathological shear rates is strongly accelerated compared to physiological arterial shear rates,[60,61] with post-stenotic deceleration amplifying the rate of platelet aggregation.[58] Also stenosis length and surface roughness affect platelet adhesion and activation.[62] An increasing stenosis length results in enhanced priming of platelets for activation *in vitro*.[63]

Matrix components

As demonstrated by *in vitro* experiments, the thrombogenic surface strongly determines the type of thrombus formed.[64] Whereas the basement membrane of healthy vessels consists for a major part of collagen Type IV,[65] atherosclerotic lesions consist predominantly of collagen Types I and III.[17] Importantly, significant heterogeneity in collagen composition exists between the different plaque phenotypes. Stable lesions contain a mixture of collagen Types I and III with an accumulation of Type I in the fibrous cap.[17] In contrast, in regions of plaque erosion Type III predominates, while in ruptured plaques at the site of cap thinning, attenuated strands of Type I can be found.[17] In terms of thrombogenic properties, the different types of collagen support platelet adhesion and activation to a different extent depending on the differential contribution of the platelet collagen receptors glycoprotein VI (GPVI) and α2β1.[66] However, *in vitro* whole blood perfusion

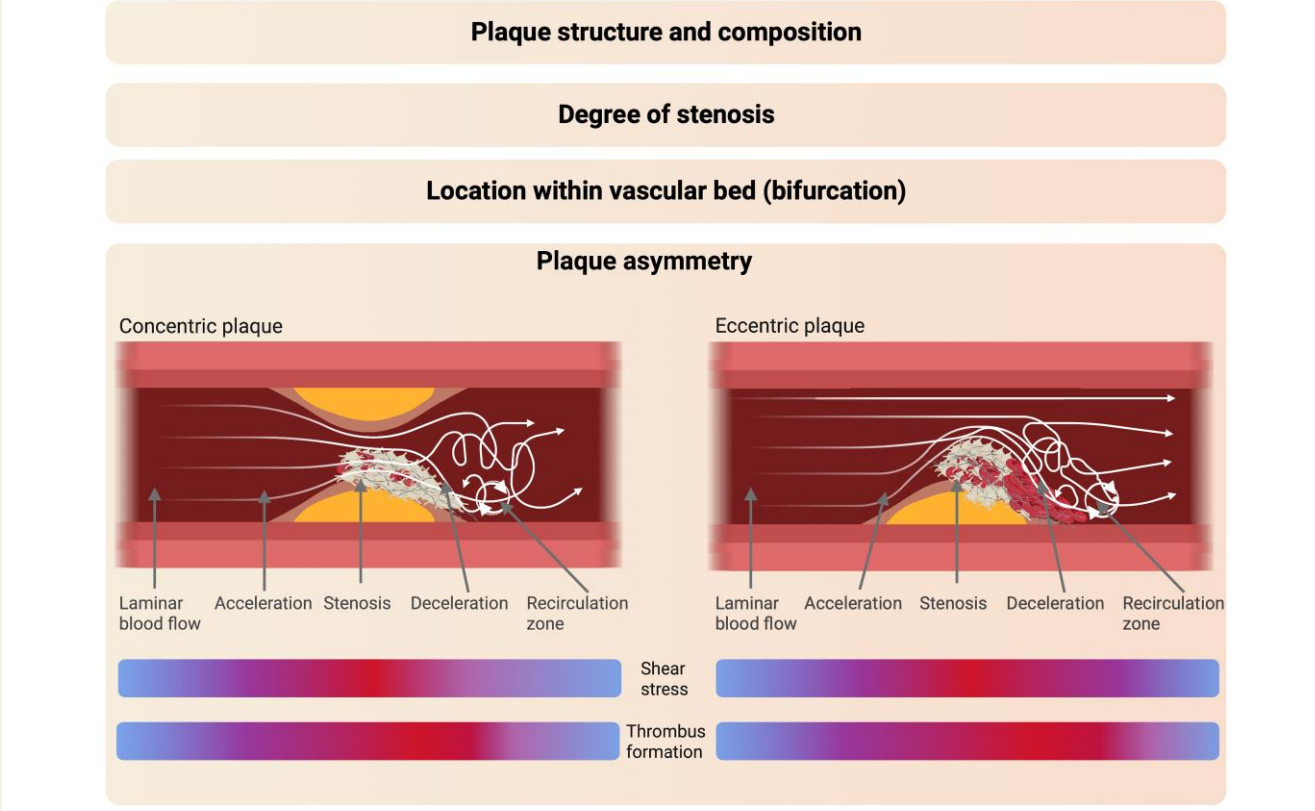

Figure 3 Factors influencing local haemodynamics and contributing to plaque-induced thrombus formation. Schematic representation of factors that have a local effect on haemodynamics and shape thrombus formation. For more information, see *Haemodynamic alterations*. Figure was created with Biorender.com

experiments over human atherosclerotic plaque homogenate showed a great dependency on GPVI as blockage of GPVI effectively inhibited thrombus formation. Inhibition of α2β1, however, was without effects.[67–70] Notably, plaque composition and structure are not taken into account in these experimental set-ups.

In contrast to stable or ruptured plaques, eroded plaques are typically rich in hyaluronan and proteoglycans like versican.[17] Interestingly, in eroded plaques, the receptor for hyaluronan cluster of differentiation 44 (CD44) is highly expressed by VSMCs at the plaque/thrombus interface, whereas weakly expressed in platelets.[17] Although hyaluronan has anti-inflammatory and antithrombotic properties when incorporated into the glycocalyx, the opposite is true for hyaluronan within atherosclerotic plaques.[71] Platelet binding to hyaluronan has been reported;[72] however, to which extent hyaluronan actively supports platelet adhesion and activation is unknown. Alternatively, platelets express hyaluronidase 2 and thereby contribute to degradation of hyaluronan into fragments which are pro-inflammatory and angiogenic.[73] A higher (platelet) hyaluronidase 2 expression has also been observed in patients with ACS compared to patients with stable angina, with a significantly higher expression in patients with ruptured vs. intact plaques.[74,75] In case of versican, it has been shown that versican isolated from human aortic tissue can support platelet adhesion, especially at low shear, in a GPIbα- and integrin αIIbβ3-independent manner.[76] Moreover, co-coating of versican with collagen Types I and III significantly enhanced thrombus formation.[76] The proteoglycans, decorin and biglycan, can also support platelet adhesion and activation,[77,78] although their non-differential expression[17] does not argue for a relatively larger contribution to thrombus formation on eroded vs. ruptured plaques.

Within advanced atherosclerotic lesions, an up-regulated expression of C-type lectin-like type II (CLEC-2) ligands has been detected,[79,80] with podoplanin expression in human atherosclerotic lesions[79] and S100A13 in murine plaques.[80] Whether expression of these ligands is differentially regulated in eroded vs. ruptured plaques is unknown. In a rat model of vascular occlusion, gene transfer of human podoplanin resulted in the formation of occlusive thrombi at the site of endothelial overexpression.[81] However, thrombus formation on coated human plaque material was not affected by blockage of CLEC-2, suggesting that other matrix interactions are more important in humans.[82]

In healthy arteries, TF expression is predominantly restricted to the adventitial fibroblasts.[83] However, atherosclerotic lesions exhibit a significant up-regulation of procoagulant TF, expressed and released by activated macrophages and VSMCs.[83,84] This up-regulation can be triggered by exposure to modified LDLs or pro-inflammatory cytokines, such as IL-1, interferon (IFN)-γ, and tumour necrosis factor (TNF)-α.[85] This strong up-regulation in activity of the extrinsic coagulation pathway results in thrombin-mediated platelet activation and fibrin formation, where the expression level of TF is positively associated with thrombus size.[86] Although TF has a dominant role in plaque-induced coagulation, factor XIIa ensures thrombus stability as shown by an enhanced embolization rate upon plaque rupture in mice injected with a Factor XIIa inhibitor as well as an impaired plaque-induced thrombus formation *in vitro* using blood from Factor XII deficient mice and patients.[87]

That TF contributes more to thrombus formation in plaque rupture than in plaque erosion is reflected by the relatively higher fibrin content of thrombi formed on ruptured plaques than on eroded plaques.[40] Moreover, in ruptured plaques, fibrin deposition as a result of intraplaque haemorrhage has been observed in regions rich in macrophages and cholesterol clefts as well as in the necrotic core.[88] With fibrin supporting platelet adhesion and aggregation under flow,[89] fibrin deposits within plaques could thereby serve as scaffold for thrombus formation.[88] Furthermore, GPVI–fibrin interactions contribute to platelet procoagulant activity and GPVI modulates clot structure, resulting in the formation of a denser and less permeable fibrin network.[90]

Soluble factors

Plaque-induced thrombus formation is highly dependent on platelet autocrine and paracrine feed-forward signalling. This is for example well-illustrated in an *in vitro* stenosis model where inhibition of adenosine diphosphate (ADP) and thromboxane A2 (TXA2) signalling significantly reduced platelet aggregation post-stenosis.[91] Also, the stabilizing role of platelet $P2Y_{12}$ receptors in thrombus formation was shown in an *in vivo* mouse model of plaque rupture.[92] $P2Y_{12}$ inhibition or deficiency resulted in the formation of smaller thrombi that more easily embolized.[92] In healthy blood vessels, the endothelium contributes to the continuous conversion of adenosine triphosphate and ADP into adenosine by adenosine diphosphatase (ADPase). Typically, ADPase activity reduces with increasing severity of atherosclerosis.[93] The importance of vascular ADPase activity in limiting thrombus formation has been shown in a rat model of thrombosis, where over-expression of human E-NTPDase in VSMCs significantly inhibited photochemically induced thrombus formation.[94]

Circulating vWF, supporting GPIbα-mediated platelet rolling, is also a key in plaque-induced thrombus formation. von Willebrand factor is synthesized and released by ECs as ultralarge multimers (ultra-large von Willebrand factor [ULVWF]), but upon inflammatory triggers its expression is significantly up-regulated.[95] Also in stenotic regions, vWF expression is increased, and thrombus formation post-stenosis is highly vWF dependent.[91] In physiological conditions, the protease ADAMTS13 cleaves ULVWF into smaller, less thrombogenic fragments.[96] Pro-inflammatory cytokines like TNFα, IFNγ, and cluster of differentiation 40 ligand (CD40L), important in atherosclerosis, have been shown to reduce ADAMTS13 expression in ECs *in vitro*.[97] Moreover, it has been reported that CAD patients had significantly lower plasma ADAMTS13 levels and activity.[96] Consequently, cultured ECs exposed to plasma obtained from CAD patients showed significantly increased platelet tethering to ULVWF as well as an up-regulation of ULVWF, demonstrating the functional impact of the reduction in plasma ADAMTS13 in CAD patients.[96] Along the same line, it has been shown that MMP13, a matrix metalloproteinase significantly up-regulated in atherosclerotic plaques,[98] can cleave vWF resulting in the generation of fragments that potently support platelet adhesion.[99] Furthermore, these vWF fragments induced the formation of larger thrombi on a collagen surface, suggesting that by cleaving vWF, MMP13 can augment the thrombotic response of the atherosclerotic plaque.[99]

Upon activation, platelets are a major source of plasma CD40L.[100] *In vivo* mouse models of atherosclerosis have shown that CD40L plays an instrumental role in the development of atherosclerotic plaques as it accelerates plaque development and progression.[101] Interestingly, platelet CD40L does not appear to be involved in atherogenesis but rather contributes to atherothrombosis.[102] Here, CD40L has a stimulatory effect on collagen and plaque-induced thrombus formation, illustrated by the reduction in thrombus size in $CD40L^{-/-}$ mice[103] as well as the increased aggregate size upon the supplementation of CD40L.[104] Another chemokine highly up-regulated in smooth muscle cells, ECs, and macrophages within atherosclerotic plaques is stromal cell-derived factor-1 (SDF-1).[105] Also platelet expression levels of SDF-1 are up-regulated in ACS patients, although no difference was observed between NSTEMI and STEMI patients.[106] SDF-1 can activate platelets as well as enhance collagen- and plaque-induced platelet activation and thrombus formation in a CXCR4-dependent manner, resulting in increased TXA2 generation and granule release.[105,107,108] In line with *in vitro* findings, $SDF\text{-}1^{-/-}$ mice exhibited a prolonged time to vessel occlusion in an arterial thrombosis model as thrombus growth and stability were reduced without impacting haemostasis.[108] Interestingly, systemic treatment of mice with SDF-1 resulted in the stabilization of atherosclerotic plaques as the accumulation of smooth muscle progenitor cells was enhanced.[109] However, in a cohort of patients with stable CAD or ACS, a high platelet surface expression of SDF-1 was significantly associated with the composite of all-cause death, MI, and/or stroke,[110] suggesting that the negative effects outrule the plaque stabilizing effects of SDF-1.

Oxidized LDL (oxLDL) is a key in the development of atherosclerotic lesions, but besides its activating effects on the endothelium and immune system, oxLDL affects platelet reactivity too.[111] Platelet activation upon oxLDL exposure is triggered via cluster of differentiation 36 (CD36) signalling, illustrated by a reduction in platelet aggregation and granule secretion in hyperlipidaemic $CD36^{-/-}$ mice.[112,113] Furthermore, genetic knockout of CD36 corrected the prothrombotic phenotype of hyperlipidaemic $ApoE^{-/-}$ mice as time to vessel occlusion and tail bleeding times were comparable with those of wild-type mice.[112] Given the high degree of oxLDL accumulation within atherosclerotic plaques, especially upon plaque rupture where the circulation is in direct contact with the lipid core, platelets can be exposed locally to high concentrations of oxLDL. Moreover, platelets can contribute to oxidation of LDL, thereby fuelling oxLDL-induced activation.[114] Further, cholesterol deposited within the atherosclerotic plaque can crystallize, leading to the expansion of the plaque and potential disruption of the cap by sharp-edged crystals.[115,116] Upon contact with the circulation, these cholesterol crystals can further amplify the platelet response[117,118] and support coagulation in a complement-dependent manner.[119] In human carotid and coronary plaques, a significant association between thrombus formation and crystal content was observed, with a higher crystal content resulting in an increased risk of atherothrombosis.[116]

Platelet interactions with other cell types

In arterial thrombi of patients with MI, neutrophils are the most abundant leukocyte subset. Through direct interactions, platelets and neutrophils interact and activate each other (*Figure 2*). Cytokines and chemokines held within platelet granules can recruit and activate neutrophils, but also neutrophils can lead to platelet activation through, e.g., NET formation.[120] Studies have found the resulting platelet-neutrophil aggregates to independently predict atherothrombotic events.[121]

Within both eroded and ruptured plaques, neutrophils and NETs were mostly observed within the thrombus and at the thrombus–plaque interface. Moreover, neutrophils and NETs were scarce in intact lesions, suggesting that, in humans, their role is limited in stages of atherosclerosis in which thrombus formation has not yet occurred.[21] Given their direct and indirect prothrombotic effects, NETs can augment the thrombotic response once incorporated into the plaque and growing thrombus. Vice versa, activated platelets can also elicit NET formation (*Figure 2*).[122] The central role of NETs and its mediator PAD4 in atherothrombosis was shown in a mouse model that mimics aspects of plaque erosion, where bone marrow PAD4 deficiency was associated with a decrease in endothelial injury, smaller thrombi, and lower luminal TF levels.[19] Loss of

(endothelial) nuclease activity could potentially also contribute to neutrophil activation and subsequent NET formation, in addition to platelet activation.[123] Whether neutrophils and NETs have a differential contribution to thrombus formation driven by plaque rupture or erosion in humans requires more investigation.

Although not as abundant as neutrophils, other leukocyte subtypes in plaques can interact with platelets and affect platelet activation. Monocytes and T cells bind to platelets, mostly through similar mechanisms as for platelet–neutrophil interactions (*Figure 2*).[124,125] Given the subtle differences in abundance of the various leukocyte subtypes associated with plaque vulnerability,[28,29] platelet activation and subsequent thrombus formation may be differentially influenced.

Whereas the interaction between healthy ECs and platelets is actively prevented, activated ECs in atherosclerotic plaques support platelet adhesion and activation through a plethora of mechanisms. Also here, activated platelets can enhance endothelial dysfunction by secretion of granular cargo (*Figure 2*). In addition to direct cell–cell contact or soluble mediators, extracellular vesicles derived from platelets effectuate another way of intercellular communication. Extracellular vesicle generated from platelets with specific phenotypes may have either protective or detrimental effects on endothelial integrity.[126,127] A recent study showed that platelets trigger endothelial activation through platelet matrix metalloproteinase 2 (MMP-2)-mediated activation of endothelial PAR1, indicating that platelet MMP-2 is involved in the early stages of atherogenesis. Moreover, in a cohort of CAD patients, it was observed a positive correlation between platelet MMP-2 expression and the degree of carotid artery stenosis at ultrasound imaging.[128] As MMP-2 activity has been linked to plaque instability and rupture,[16,28] platelet MMP-2 activity may contribute to the progression of stable atherosclerotic plaques towards ruptured plaques.

VSMCs within atherosclerotic lesions switch phenotype and thereby alter plaque characteristics.[129] As their phenotype changes, the thrombogenic potential of the VSMC may also change as recently shown for calcified VSMCs.[130] Which mediators are involved are largely unknown, although a role for CLEC-2–S100A13 interactions has been shown in mice (*Figure 2*).[80]

Plaque and thrombus healing

Thrombus formation on disrupted plaques is a critical process in the onset of acute cardiovascular events, but it does not always lead to complete vessel occlusion with subsequent acute symptomatic events. In ACS, a non-occlusive or transiently occlusive thrombus most often results in NSTEMI, while STEMI patients more commonly present with a more stable and occlusive thrombus.[131] This can be seen in the different prevalence of healed plaques in ACS patients which is indicative for previous non-occlusive thrombotic events.

Healed plaques are identified by multi-layers of different optical characteristics observed at sites previously associated with thrombi formed through an acute event and which have undergone a so-called healing process. The first matrix layer consists of a platelet- and fibrin-rich thrombus, infiltrating VSMCs, and macrophages, and eventually the plaque becomes fully re-endothelialized upon complete healing.[132,133] The healed plaque may undergo further destabilization and eventual healing without manifesting in clinical symptoms as shown by autopsy studies.[134,135] Clinical manifestation of this process (i.e. ACS) is believed to be the result of disruption of the delicate balance between atherosclerotic plaque activation and passivation.[133]

Autopsy studies revealed that 32%–61% of sudden coronary death was associated with a healed plaque phenotype contributing to narrowing of the lumen.[134,135] In patients with coronary syndrome, the rate of healed plaques varies between 3% and 90% depending mainly on the detection method and clinical presentation.[136–141] In the last decades, healed plaques have been associated with higher luminal stenosis,[138,139,142] higher vulnerability,[142,143] but not with thrombus burden.[142] Interestingly, newly formed healed plaques have been shown to have a larger thrombus burden and lower luminal stenosis which gradually increases during the healing process.[136]

The concept of the dynamic nature of the process behind plaque rupture and healing occurring in ACS has been supported by the presence of differently matured thrombi in STEMI patients. This thrombus maturation process allows the thrombus to evolve gradually over a period of days or weeks before fully obstructing the lumen.[134,144] During this maturation/healing process, clot composition changes from a platelet and fibrin rich fresh thrombus into a lytic and eventually organized thrombus with infiltrated VSMCs and/or ECs. The occurrence of these aged thrombi was first recognized in an autopsy study of young adults who died from sudden coronary occlusion.[144] Histopathological analyses on aspirated thrombi from STEMI patients showed a prevalence of aged (i.e. >1 day old) thrombi of 7%–63%, whereas fresh thrombi (i.e. <1 day old) had a prevalence of 34%–76%.[36,134,145–151] In a more in-depth analysis, it was shown that lytic thrombi (thrombi aged 1–5 days) and organized thrombi (thrombi aged >5 days) displayed comparable occurrence rates (17%–35% vs. 7%–36%, respectively), indicating that acute coronary occlusion frequently marks the final stage of thrombotic events that took place in the preceding days or weeks.[36,145–147,150] To reinforce the notion of an active process, thrombi consisting of both freshly formed and lytic or organized components were observed in 7% of STEMI patients.[146,151]

Platelets play a significant role in both the development of arterial plaques and the process of wound healing, suggesting their potential involvement in plaque healing. However, the mechanism behind this process is not yet well understood. Studies have demonstrated that plaque healing occurs as a result of VSMC proliferation, triggered by growth factors such as platelet-derived growth factor BB and platelet TGF-β, as well as mitogens (e.g. thrombin) that are released during acute thrombosis.[12,152] *In vivo* studies in mice have shown that activated platelets adhering to an injured arterial wall can facilitate the recruitment of bone marrow-derived VSMC progenitor cells from circulating blood through the expression of P-selectin and SDF-1.[153–155]

Therapeutic implications and novel antiplatelet targets

Dual antiplatelet therapy consisting of aspirin with a $P2Y_{12}$ inhibitor is the cornerstone of initial management of ACS, whether or not combined with anticoagulant therapy and followed by an invasive approach.[156] Especially in the first month after an ACS event, the benefit of antithrombotic therapy outweighs the risk of bleeding. However, over time the bleeding risk can increase relative to the thrombotic risk.[156] In addition, antithrombotic treatment, in particular anticoagulant treatment, has been shown in a meta-analysis to be associated with a higher risk of intraplaque haemorrhage in carotid atherosclerotic plaques,[157] a characteristic of unstable plaques.[158] For patients with chronic coronary syndromes (CCS), including stable CAD or an ACS event >1 year ago, current guidelines for those in sinus rhythm recommend single antiplatelet therapy, while dual antiplatelet therapy (DAPT) or low-dose dual antithrombotic therapy (aspirin and rivaroxaban) should be considered in patients with a high ischaemic risk but low bleeding risk.[159] Active and forthcoming clinical trials evaluate more potent $P2Y_{12}$ inhibitors, combinations of antiplatelet agents and direct oral anticoagulants in addition to antiplatelet therapy in ACS and CCS patients (*Table 2*).

Table 2 Ongoing and forthcoming clinical trials in acute coronary syndrome and chronic coronary syndrome patients

Trial number	Trial name	Study population	Interventions	Primary outcome measures	Phase
NCT03331484	CAPITAL PCI AF	ACS patients with AF ($n = 40$)	Ticagrelor plus rivaroxaban	Composite of TIMI bleeds	3
NCT03357874	TROUPER	ACS patients with CKD ($n = 514$)	Clopidogrel vs. ticagrelor	Rate of MACE	3
NCT04718025	ELECTRA-SIRIO	ACS patients ($n = 4500$)	Low-dose ticagrelor plus aspirin vs. low-dose ticagrelor plus placebo vs. standard dose ticagrelor plus aspirin	Bleeding, death from any cause, non-fatal MI, or non-fatal stroke	3
NCT05162053	PK/PD Study of Vicagrel and Clopidogrel in healthy subjects with different CYP2C19 metabolizers	Healthy subjects with different CYP2C19 metabolizers ($n = 128$)	Cross-dosing of vicagrel and clopidogrel in different metabolizer groups	Inhibition of platelet aggregation, platelet reactivity, maximum plasma concentration of vicagrel and clopidogrel, AUC over a dosing interval	1
NCT05233124	OVER-TIME	ACS patients with coronary artery ectasia ($n = 60$)	DAPT of aspirin plus clopidogrel vs. clopidogrel monotherapy plus low-dose rivaroxaban	Composite of cardiovascular death, recurrent MI, and repeated vascularization, composite of minor and major bleeding events	2
NCT05577988	ADEN	ACS patients ($n = 2468$)	Stop aspirin for ticagrelor or prasugrel vs. aspirin or clopidogrel guided by genetic testing	Rate of combined major and minor bleeding events	3
NCT05638867	NOAC therapy guided by PARIS Risk Score and D-dimer in patients with ACS after PCI	ACS patients with high ischaemic risk ($n = 3944$)	Aspirin plus clopidogrel plus rivaroxaban for 3 months followed by DAPT vs. aspirin plus clopidogrel after PCI	MACCE	3
NCT05779059	PROTEUS	ACS patients (NSTEMI, unstable angina) ($n = 50$)	Initial ticagrelor and switch to prasugrel at Day 45 or initial prasugrel and switch to ticagrelor at Day 45	Platelet reactivity	3
NCT05825573	ARGONAUT	Patients with intra-cardiac thrombus ($n = 340$)	VKA vs. DOAC	Net clinical benefit	3
NCT05093790	A study to evaluate BMS-986141 added on to aspirin or ticagrelor or the combination, on thrombus formation in a thrombosis chamber model in participants with stable coronary artery disease and healthy participants	Stable CAD ($n = 55$)	Ticagrelor plus BMS-986141 vs. aspirin plus BMS-986141 vs. ticagrelor plus aspirin plus BMS-986141 vs. BMS-986141	Change from baseline in thrombus area post-treatment BMS-986141	2
NCT05122455	Effects of edoxaban on platelet aggregation in patients with stable CAD	Stable CAD ($n = 70$)	Aspirin vs. aspirin plus edoxaban, followed by clopidogrel monotherapy vs. clopidogrel plus edoxaban, followed by edoxaban monotherapy	Platelet aggregability	2/3

Includes clinical trials (Phase 1–3) based on ClinicalTrials.gov for platelet aggregation inhibitors and anticoagulants. Selection criteria disease: acute coronary syndrome ACS, (stable) coronary artery disease (CAD), chronic coronary syndrome (CCS).

AF, atrial fibrillation; CKD, chronic kidney disease; CYP2C19, cytochrome P450 2C19; DAPT, dual antiplatelet therapy; DOAC, direct oral anticoagulant; MACE, major adverse cardiovascular event; MACCE, major adverse cardiac and cerebrovascular event; MI, myocardial infarction; NOAC, novel oral anticoagulants; NSTEM, non-ST-elevation myocardial infarction; PCI, percutaneous coronary intervention; PK/PD, pharmacokinetic/pharmacodynamic; STEMI, ST-elevation myocardial infarction; TIMI, thrombolysis in myocardial infarction; VKA, vitamin-K antagonist.

Despite antithrombotic therapy, recurrent thrombotic events are reported in patients with CAD underscoring the clinical need for more effective drugs that prevent thrombosis without compromising haemostasis. Especially upon plaque rupture, TF/FXIIa-induced thrombin generation will contribute to platelet activation. The findings that thrombin generation remains high after ACS[160] and thrombin signalling via platelet PAR1/4 is preserved in patients on DAPT[161] indicate that antagonism of these receptors is of interest. Since PAR1 inhibition is associated with significant bleeding, PAR4 is seen as a promising new therapeutic target.[162] The small molecule BMS-986141 blocks PAR4 and is currently assessed in a Phase 2 clinical trial (NCT05093790) in CCS patients (*Table 2*).

GPVI plays an important role in platelet activation via the interaction with collagen, present in both eroded and ruptured plaques, as well as in enhancing thrombus growth via binding to fibrin. The recombinant GPVI-Fc complex revacept and the humanized Fab fragment ACT017 have been designed to block GPVI. In a Phase 2 trial (ISAR-PLASTER), revacept did not reduce the incidence of MI in patients with stable ischaemic heart disease undergoing PCI.[163] ACT017 is now assessed in a Phase 2/3 trial (NCT05070260) on top of standard therapy in patients with acute ischaemic stroke, awaiting further investigation in patients with CAD. In ACS patients, a higher fraction of procoagulant platelets (exposing phosphatidylserine) was reported.[164] This procoagulant response is typically induced by combined stimulation of GPVI and thrombin receptors, meaning that inhibition of GPVI signalling might be beneficial in lowering the procoagulant activity of platelets and subsequent thrombin generation. Furthermore, ACS patients in the acute phase present with a higher fraction of highly reactive young platelets,[165] and GPVI expression is increased in young platelets.[166]

Other promising antiplatelet targets include vWF, GPIbα, 5-hydroxytryptamine receptor subtype 2A, protein disulfide isomerase, P-selectin, phosphoinositide 3-kinase β, and phosphodiesterase-3, as reviewed elsewhere.[162]

Future perspectives

In patients with ACS, the phenotype and location of the plaque in the coronary circuit determine the extent of platelet activation and structural composition of the thrombus. Advancements in technology gradually enable characterization of multiple plaque phenotypes beyond eroded and ruptured plaques, and these phenotypes can be mechanistically linked to the process of thrombus formation and the specific platelet populations involved. Future research focused on in-depth characterization of thrombus architectures is essential for targeting the main pathophysiological factors and thereby optimizing the efficacy of antithrombotic therapy in ACS patients. In our opinion, the development and application of atherothrombosis models (*in vivo, ex vivo, in silico*) based on different plaque phenotypes is crucial. These models allow the testing of novel platelet inhibitors (e.g. GPVI inhibitors) and/or anticoagulants either alone or on top of standard therapy.

Since patients with ACS are often of advanced age and present with comorbidities, the complexity of antithrombotic management has increased and should be more tailored to individual characteristics of patients. Considering the importance of inflammatory processes in the pathophysiology of the main processes of atherothrombosis (plaque formation, progression, and rupture), it has also been suggested to include anti-inflammatory drugs in the prevention of cardiovascular atherothrombotic events. This combination of antithrombotic and anti-inflammatory approaches might be a promising treatment strategy for patients with ACS.

Supplementary data

Supplementary data are not available at *European Heart Journal* online.

Declarations

Disclosure of Interest

H.S. is a shareholder of Coagulation Profile.

Data Availability

No data were generated or analysed for or in support of this paper.

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