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Fibrinolysis in acute and chronic cardiovascular disease

Noppadol Kietsiriroje MD[1,2], Robert A.S. Ariëns PhD[1], Ramzi A. Ajjan MD, PhD[1*]

[1]*Leeds Institute of Cardiovascular and Metabolic Medicine, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom*

[2]*Endocrinology and Metabolism Unit, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla, Thailand*

[*]**Corresponding author**

Ramzi A. Ajjan, MD, FRCP, MMed.Sci, PhD

Professor in Metabolic Medicine

The LIGHT Laboratories

Leeds Institute of Cardiovascular and Metabolic Medicine

Faculty of Medicine and Health, University of Leeds.

Clarendon Way, Leeds LS2 9DA, United Kingdom

e-mail: r.ajjan@leeds.ac.uk

Phone number: 0113-343 7475

ORCID iD: 0000-0002-1636-3725

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Abstract

The formation of an obstructive thrombus within an artery remains a major cause of mortality and morbidity worldwide. Despite effective inhibition of platelet function by modern antiplatelet therapies, these agents fail to fully eliminate atherothrombotic risk. This may well be related to extensive vascular disease, beyond the protective abilities of the treatment agents used. However, recent evidence suggests that residual vascular risk in those treated with modern antiplatelet therapies is related, at least in part, to impaired fibrin clot lysis. In this review, we attempt to shed more light on the role of hypofibrinolysis in predisposition to arterial vascular events. We provide a brief overview of the coagulation system followed by addressing the role of impaired fibrin clot lysis in acute and chronic vascular conditions, including coronary artery, cerebrovascular and peripheral vascular disease. We also discuss the role of combined anticoagulant and antiplatelet therapies to reduce the risk of arterial thrombotic events, addressing both efficacy and safety of such an approach. We conclude that impaired fibrin clot lysis appears to contribute to residual thrombosis risk in individuals with arterial disease on antiplatelet therapy, and targeting proteins in the fibrinolytic system represents a viable strategy to improve outcome in this population. Future work is required to refine the antithrombotic approach by modulating pathological abnormalities in the fibrinolytic system and tailoring therapy according to the need of each individual.

List of abbreviations

A2AP	alpha-2 antiplasmin
ACS	acute coronary syndrome
C	complement
CVD	cardiovascular disease
DAPT	dual-antiplatelet therapy
GTT	global thrombosis test
Lp(a)	lipoprotein (a)
MACE	major adverse cardiovascular events
MI	myocardial infarction
PAD	peripheral artery disease
PAI-1	plasmin-activator inhibitor 1
PAP	plasmin-antiplasmin complex
PAR	protease-activated receptor
STEMI	ST-elevated myocardial infarction
TAFI	thrombin activatable fibrinolysis inhibitor
TEG	thromboelastography
TF	tissue factor
t-PA	tissue-type plasminogen activator

1 Introduction

Despite significant advances in management, cardiovascular disease (CVD) remains a major cause of mortality worldwide.[1] Myocardial infarction (MI) and cerebrovascular thrombotic events usually follow atherosclerotic plaque rupture, or erosion, which activates the cellular and protein arms of coagulation culminating in thrombus formation. Initially, exposure of subendothelial collagen rapidly mediates platelet adhesion and aggregation through platelet alpha2beta1 and glycoprotein VI interactions with collagen, and glycoprotein Ib interactions with von Willebrand factor bound to collagen. Subsequently, activation of coagulation driven by tissue factor (TF) expressed in the exposed core of the plaque generates thrombin for the formation of the fibrin network, that is necessary to provide a scaffold for the blood clot.[2]

The molecular processes involved in coagulation activation and fibrinolysis have been reviewed in detail previously[3-5] and only a brief description is provided here (**Error! Reference source not found.**). Following plaque rupture, TF is exposed, binds factor (F) VII, promoting proteolysis and activation to FVIIa (TF or extrinsic pathway). TF/FVIIa complex subsequently cleaves traces of FIX (contact or intrinsic pathway) and FX (common pathway) into FIXa and FXa. The latter is activated by both TF/VIIa and FIXa, and (later together with FVa) converts prothrombin (FII) into thrombin. The slowly accumulated thrombin during the initiation phase further converts several factors including FXI, FVIII and FV, amplifying the activation of coagulation cascade, resulting in increased thrombin generation (positive feedback). Sufficient amounts of thrombin are then generated, which is able to convert fibrinogen to form fibrin fibres.[3] Finally, thrombin-activated plasma transglutaminase FXIIIa catalyses the formation of covalent crosslinks between adjacent D-regions of fibrin monomers, increasing the stability of fibrin clot by tightening its structure and increasing resistance to fibrinolysis.[6] Additionally, thrombin activates platelets through its protease-activated receptor (PAR) 1 and 4, contributing the formation of the platelet plug.[7]

The formation of fibrin also directly triggers the activation of fibrinolysis by which insoluble fibrin is degraded into small fragments (fibrin degradation products). This interaction is pivotal to ensuring that clotting does not extend within the vessel thus maintaining patency. Upon conversion of fibrinogen to fibrin, conformational changes take place exposing binding sites of plasminogen and tissue-type plasminogen activator (t-PA), while these sites are normally cryptic on fibrinogen.[8] Plasmin, that is generated through activation of fibrinolytic pathway, degrades fibrin by cleaving the αC-regions first, followed by the coiled-coil region that connects the E- and D-regions in fibrin (**Error! Reference source not found.**).[5]

A balance between clot formation and endogenous fibrinolysis, therefore, is mandatory to restore vascular patency and prevent serious vascular occlusion. Fibrinolysis also plays a key role in wound healing by degrading the blood clot when it is no longer needed, and by promoting angiogenesis as appropriate. The impairment of endogenous fibrinolysis, on the other hand, increases the risk of pathological vascular occlusion leading to cardiovascular events. In this review, we delineate the pathological alterations in the fibrinolytic system in CVD and discuss possible therapeutic targets that can be developed to reduce the risk of atherothrombotic events.

2 Regulation and alteration of fibrinolysis

The initiation of fibrinolysis commences when fibrinogen is cleaved to form fibrin fibres. The conformational changes that ensue after fibrin formation initially expose t-PA and plasminogen binding sites in the αC-region near the D-region (**Error! Reference source not found.**),[9] consequently leading to the formation of ternary complex between plasminogen, t-PA and fibrin, culminating in plasmin activation. When fibrin is degraded, additional binding sites (such as C-terminal lysine residues on αC-region) for plasminogen and t-PA become

available, thus accelerating the fibrinolytic process (Figure 2).[8] Endogenous regulators, therefore, are necessary to limit this seemingly ceaseless process. However, pathological alteration of these regulators might lead to impaired fibrinolysis, which increases the risk for CVD.

Several case-control studies have shown an association between impaired fibrin clot lysis and vascular disease, but it was not until recently that longitudinal studies have been conducted, which demonstrated that reduced fibrin clot lysis is an independent predictor of adverse vascular outcome. Sumaya et al have shown, in a large acute coronary syndrome cohort of 4354 individuals, that fibrin clot lysis predicts cardiovascular mortality within the first year of the event.[10] This was quickly followed by another publication showing that fibrinolysis can determine major adverse cardiac outcome, 496 patients with ST-elevated myocardial infarction (STEMI).[11] Others have shown that fibrin clot lysis does not only predict outcome following arterial occlusion but can also be used to predict outcome following venous occlusive disease.[12] Therefore, fibrin clot lysis appears to be an important modulator of clinical outcome and represents a credible therapeutic target to reduce the risk of vascular occlusion and improve clinical outcome.

In this section, we will review the regulation of fibrinolysis and the impact of each parameter discussed on fibrin degradation.

2.1 Clot structure

The thickness of fibrin fibres determines density and pore size of fibrin networks. Thinner fibres are usually associated with denser fibrin clots having smaller pores and this kind of fibrin structure impedes the diffusion of plasminogen and t-PA, thus resulting in delayed fibrinolysis.[13-15] Two studies exploring clot architecture in coronary artery disease patients

demonstrated that *ex vivo* clots from these individuals are less permeable with slower lysis time than those from controls.[16,17]

Thrombin generation influences the architecture of clots. Thrombin cleaves short peptides from the N-termini of the Aα- and Bβ-chains, termed FpA and FpB respectively (**Error! Reference source not found.**), allowing assembly of fibrin through interactions of newly exposed binding sites ("knobs") in the E-region with constitutively expressed binding pockets in the D-region ("holes"). This interaction first generates double stranded protofibrils, which subsequently aggregate laterally to form fibrin fibres that branch into a three-dimensional elastic fibrin network (**Error! Reference source not found.**). An increase or a decrease in thrombin concentration lead to smaller or larger pore sizes, facilitating lower or higher rate of fibrinolysis, respectively.[18,19]

Another determinant of fibrin clot structure is plasma levels of fibrinogen with higher levels resulting in more compact clots.[20] In addition to quantitative changes in fibrinogen, qualitative alterations in this molecule, such as glycation, phosphorylation, oxidation, and other post-translational modifications lead to changes in clot structure,[21] which in turn affect resistance to fibrinolysis.

2.2 Transglutaminase factor XIII (FXIII) crosslinking and alpha-2 antiplasmin

FXIII is a transglutaminase enzyme that forms a covalent bond between γ-γ, γ-α, and α-α of adjacent fibrin molecules,[22] thus stabilizing the fibrin network. Alpha-2 antiplasmin (A2AP), largely produced by the liver, is a strong plasmin inhibitor and is crosslinked by FXIII into the fibrin network, which increases resistance to fibrinolysis by inhibiting plasmin and forming plasmin-antiplasmin complex (PAP) (**Error! Reference source not found.**).[23]

Yet, the association between A2AP level and CVD is still controversial. One study measured plasma levels of fibrinolytic proteins in 555 male survivors of first MI and 635 controls, and found that levels of A2AP were independently associated with MI risk.[24] Two large cohort studies enrolled 5201 and 6391 patients without baseline CVD also showed that elevated PAP levels were associated with increased risk of MI or death,[25,26] whereas the study in patients with coronary disease failed to demonstrate an association between PAP levels and cardiovascular death.[27] To further add to the confusion, in patients surviving MI, PAP levels were inversely correlated with repeat coronary events.[28] However, we need to be cautious in our interpretations as plasma levels of PAP may not reflect vascular risk which may instead be related to the amount of A2AP incorporated into fibrin networks. Indeed, an earlier study in type 2 diabetes patients, a high vascular risk group, has shown increased incorporation of A2AP into diabetic clots,[29] which correlated with diabetes control measured as HbA1c levels. A subsequent study in type 1 diabetes individuals has again shown increased incorporation of A2AP into diabetes clots,[30] and therefore future studies are required to understand the relationship between the amount of plasmin inhibitor present in clots and predisposition to vascular events.

2.3 Thrombin activatable fibrinolysis inhibitor

Thrombin activatable fibrinolysis inhibitor (TAFI), a zymogen in plasma produced by the liver, can be activated by interaction between thrombin and thrombomodulin complex, located on the endothelial cell surface. Activated TAFI (TAFIa) exerts its fibrinolytic inhibitory effect by cleaving off C-terminal lysine residues from the fibrin surface, thereby limiting the rate of t-PA mediated plasminogen activation (**Error! Reference source not found.**).[31]

The association between TAFI levels and increased risk of CVD is still inconclusive. Case-control studies suggested that high TAFI antigen levels are associated with coronary or

178 peripheral artery atherosclerosis,[32,33] whereas other studies proposed that lower levels of TAFI,
179 due to genetic polymorphisms, are associated with increased risk of MI.[34-37] In addition, the
180 AtheroGene study, enrolling patients with coronary disease, demonstrated that a ratio between
181 activated/inactive TAFI, but not its total level, is associated with cardiovascular death.[38] Taken
182 together, the activation of TAFI rather than its total level may contribute to increased risk of
183 CVD, but further studies are required to confirm this.

184 **2.4 Plasminogen-activator inhibitor 1**

185 Plasminogen activator inhibitor-1 (PAI-1) mitigates fibrinolysis activity, under normal
186 physiological conditions, by inhibiting t-PA in a 1:1 stoichiometric ratio, preventing plasmin
187 formation on the fibrin surface (**Error! Reference source not found.**).

188 Largely produced and secreted by activated platelets, levels of PAI-1 in thrombi are up
189 to 30 times higher than that in plasma,[39] suggesting a key role for PAI-1 in stabilising the clot
190 and making it resistant to lysis. PAI-1 is also synthesized by various cells (including endothelial
191 cells, smooth muscle cells, and adipocytes) and upregulated in different conditions deemed
192 high-risk for CVD such as insulin resistance, obesity and diabetes.[40,41]

193 Plasma levels of PAI-1 correlate with clot lysis time in healthy volunteers as well as high
194 vascular risk patients.[42,43] The role of PAI-1 in the thrombotic process is further highlighted in
195 animal studies showing that PAI-1 inhibition increases recombinant t-PA mediated
196 thrombolysis response and, thus, decreases thrombus extension in experimental thrombosis
197 models.[44]

198 The prognostic value of PAI-1 as a biomarker for cardiovascular outcomes has been
199 inconsistent, [45] related to a number of possible factors including: i) PAI-1 levels are confounded
200 by multiple risk factors (e.g. insulin resistance, obesity, exercise, diet and smoking); ii)

circadian variation of PAI-1 levels that is not always taken into account, iii) different PAI-1 assays and sample handling, and iv) plasma levels of PAI-1 may not necessarily represent protein levels near the obstructive thrombus.[41]

Recently, Song and colleagues performed a well-conducted systematic meta-analysis of 14 studies and observed an association between PAI-1 and coronary heart disease. From the Mendelian randomization analyses, the authors concluded that plasma PAI-1 levels predict coronary heart disease risk (OR 1.22 per unit increase of log-transformed PAI-1; 95%CI: 1.01, 1.47).[46]

2.5 Complement 3 and 5 as a substrate for plasmin

The complement (C) pathway is a complex innate immune system activated in a cascade-like fashion, in which C3 is a central protein for this activation. Plasminogen can directly bind to C3 and C5, thus inhibiting complement activation.[47] Likewise, C3 and C5 can be a substrate for plasmin therefore, competitively prevent plasmin from cleaving fibrin (**Error! Reference source not found.**).

Our previous study has demonstrated the impact of C3 on fibrinolysis in patients with type 1 diabetes and matched healthy controls.[48] We found that plasma levels of C3 correlated with clot lysis time, and C3 was directly responsible for prolonging lysis time with an effect that seemed to be enhanced in diabetes. Moreover, C3 plasma levels have shown a correlation with fibrin clot lysis in a large cohort of type 2 diabetes patients (n=837), with an effect similar to the classical antifibrinolytic factor PAI-1.[43] Interestingly, an *in vitro* study has recently indicated that the activation of C3 and C5 alters clot structure with thinner fibres and prolonged lysis time, and inhibition of C3 and C5 activation can restore both clot density and prolongation of lysis time.[49]

The prognostic value of C3 for prediction of cardiovascular event, however, has not been widely investigated. In one study in patients with stable coronary artery disease, C3 levels were significantly higher than controls, and shown to be an independent predictor of coronary artery disease.[50]

2.6 Lipoprotein (a)

It has been widely accepted that Lipoprotein (a) [Lp(a)] is an independent risk factors for CVD. Abundant evidence derived from meta-analyses, large observational studies, Mendelian randomization studies, and genome-wide association studies supports a relationship between increased circulating Lp(a) concentrations and atherosclerotic CVD.[51]

Although the precise pathological mechanisms for this link are not fully understood, one suggested mechanism is related to the sequence homology between Lp(a) and plasminogen, possibly resulting in competitive inhibition of fibrin(ogen) binding thus inhibiting plasmin activation.[52] Elevation of Lp(a) alters clot structure thus reducing fibrin clot permeability, which in turn affects fibrinolysis.[16,53] Additionally, Lp(a) stimulates PAI-1 expression and production by endothelial cells, further compromising the fibrinolytic process.[54,55]

However, this concept has been recently challenged by demonstrating that clot lysis time was not affected by Lp(a) levels using in vitro studies.[56] Therefore, the precise mechanisms for the association between Lp(a) and fibrinolysis are yet to be determined.

3 Clinical importance of impaired fibrinolysis in patients with CVD

In the second part of this review, we address the clinical relevance of altered fibrin clot lysis in individuals with acute or chronic vascular disease. To ensure adequate coverage of this area, we searched the literature from PubMed®, MEDLINE®, and EMBASE® databases using search terms as follow: "fibrin clot, lysis time or fibrinolysis" and "acute coronary syndrome/myocardial infarction/coronary disease, or stroke/cerebrovascular disorder, or peripheral artery disease". Titles and abstracts were screened to select relevant literature. Only those written in English and full text provided were selected. Only case-control or prospective studies investigating a link between altered clot structure or lysis time and CVD or outcomes were reviewed. All studies included in this review are summarised in Table 1 and Table 2.

It is worth noting that the heterogeneity of patients in various studies and the different assays carried out make it difficult to compare results across study populations. The measurements of fibrinolytic activity mentioned in this section can be divided into investigating two main blood components; i) platelet rich whole blood clots using thromboelastography (TEG) (low shearing force condition) and global thrombosis test (GTT) (high-shearing force condition); and ii) platelet poor plasma clots employing turbidimetric analysis and euglobulin clot lysis time. Additionally, it should be reminded that TEG is a technique measuring mainly clot strength and is not sensitive to assess clot lysis. The details of each test have been concisely reviewed elsewhere.[57]

3.1 Acute CVD

This can be divided into conditions affecting the coronary, cerebrovascular or peripheral arteries, each discussed separately below.

3.1.1 Acute coronary syndrome

Initial studies investigating fibrinolysis in individuals with coronary artery disease were observational and a study with a small number of acute coronary syndrome (ACS) patients

(n=40) showed denser clot structure, lower clot permeability, faster clot polymerization and prolonged lysis time, compared with 40 healthy controls.[58] Prospective studies followed that have shown that impaired fibrinolysis is associated with adverse vascular outcome in individuals with acute ACS. (Table1).

A study involving 270 patients has shown that increased clot strength measured by TEG can predict recurrent ischaemic events in ACS patients who underwent primary percutaneous coronary intervention.[59]

Three other prospective studies have investigated the effect of prolonged clot lysis time observed by the automated point-of-care GTT in 300, 496 and 82 patients, (Table 1) presenting with ACS or STEMI. These studies clearly demonstrated that prolonged lysis time strongly predicts major adverse cardiovascular events (MACE) or cardiovascular death.[11,60,61] Besides, STEMI patients with lysis time <1000s had a better chance for spontaneous reperfusion (Table 1).[11,60]

Analyses from the PLATO substudy which enrolled over 4354 ACS patients also concluded that each 50% increment in plasma clot lysis time, measured by turbidimetric analysis, is associated with 36% increased risk of 12-month cardiovascular death and this association remained significant after adjustment for inflammatory and prognostic biomarkers. Likewise, each 50% increase in maximum turbidity was associated with cardiovascular death but this association lost significance after adjustment for clinical and biochemical vascular markers (Table 1).[10]

Hence, results from these studies have delivered an essential message that impaired fibrinolysis represents a residual risk factor for cardiovascular events in patients with ACS, despite modern dual antiplatelet therapy.

3.1.2 Acute ischaemic stroke

A case-control study that enrolled 45 patients admitted for acute ischaemic stroke within 72 hours of onset, showed that plasma clots from these patients were less porous and more compact with longer lysis time than those from healthy controls.[62] A prospective study of 74 patients with acute ischaemic stroke receiving thrombolytic therapy showed that less porous clots with longer lysis time predicted adverse neurological response at 3-month (Table 1).[63] Generally, acute ischaemic stroke studies have been scarce with limited number of individuals and therefore robust conclusions cannot be made.

3.1.3 Acute limb ischaemia or critical limb ischaemia

A small case-control study included 43 patients with history of acute limb ischaemia, referred for further invasive treatments, demonstrated that plasma clots from the patients had lower clot permeability and higher thrombin generation whereas there were no differences in maximum clot formation or clot lysis time, compared to 43 healthy controls.[64] Another case-control study enrolled 85 critical limb ischaemia patients who underwent endovascular therapy and had symptomatic restenosis in treated segment over 12-month follow-up. The control group in this particular study consisted of 47 age-, sex- and cardiovascular risk-matched patients with peripheral artery disease (PAD). This work demonstrated that plasma clots from critical limb ischaemia patients had lower clot permeability and prolonged clot lysis time (Table 1).[65] In addition, the authors concluded that critical limb ischaemia patients with restenosis had a 3.3-fold higher rate of the adverse composite events (re-intervention, major amputation and cardiovascular death) compared to controls.

3.2 Chronic stable CVD

In chronic stable CVD, an increase in clot strength and prolonged clot lysis time are associated with increased risk of vascular events (summarized in Table 2). However, the lack

of large prospective studies makes it difficult to decipher whether altered clot structure contributes to further events or if it is simply associated with other factors responsible for the enhance risk.

3.2.1 Stable coronary artery disease (CAD)

Patients with stable CAD usually have *ex vivo* plasma clots that are more compact and display increased resistance to lysis compared with healthy controls.[66,67] More interestingly, among those with stable CAD, a history of previous MI can be associated with increased clot density and prolonged lysis time (Table 2).[68] The TRIP study also displayed an association between platelet-fibrin clot strength, measured by TEG, and symptoms of patients with CAD. In this study, 171 patients with CAD including 67 with asymptomatic stable CAD, 71 with stable angina and 33 with unstable disease, showed that patients with unstable angina exhibited the strongest clots followed by those with stable angina while individuals with asymptomatic disease had the weakest clots.[69]

Results from two prospective studies have affirmed that increased platelet-rich fibrin clot strength measured by TEG predicts recurrent ischaemic events within 2 years in patient with stable CAD (Table 2).[70,71] However, the effect of prolonged clot lysis time on the increased risk of cardiovascular events seems to be less profound than in ACS. The study by Neergaard-Petersen and colleagues followed 786 patients with stable CAD (90% had previous MI) over 3.1 years, and found that only area under the curve (AUC) of clot formation and lysis predicted composite cardiovascular outcomes, but not maximum turbidity or lysis time alone (Table 2).[72] However, the study had relatively small sample size with only 70 (9%) events occurring during the 3.1 years follow-up period. Thus, it was likely that the maximum turbidity or lysis time was underpowered to predict outcomes when analysed separately but the

combined analysis (that essentially includes these two measures) had enough power to show an association.

It is important to stress that TEG studies that measure clot strength do not necessarily reflect fibrinolysis potential, which can be influenced by additional factors.

3.2.2 Previous history of ischaemic stroke

A number of case-control studies have indicated that patients with previous ischaemic stroke produce more compact clots that are resistant to lysis, when compared to healthy controls (Table 2).[67,73-75] There is only one prospective study observing recurrent ischaemic stroke or transient ischaemic attack events in 218 patients with extra- or intracranial artery stenosis who underwent stenting. Using TEG, 18 patients who developed events showed stronger platelet-fibrin clots compared with those who remained free of an event (Table 2).[76] However, 9 out of 18 events occurred within 7 days of stenting, the result therefore should be regarded as a short-term effect of impaired fibrinolysis on stent re-thrombosis rather than a long-term effect.

3.2.3 Peripheral artery disease (PAD)

Two small case-control studies have shown that clots produced from plasma of patients with peripheral artery disease were more compact and resistant to lysis than matched controls, corresponding to other chronic CVD (Table 2).[77,78] Undas and colleagues investigated *ex vivo* clots from 106 patients with PAD, aged $\leq$70 years and found that clots from patients had lower permeability, higher density and prolonged lysis time, compared to matched controls.[77] Similarly in another study by Okraska-Bylica and colleagues, clots from 31 younger patients with premature CAD, aged $\leq$55 years, also showed reduced clot permeability and prolonged lysis time compared with matched controls.[78] Additionally, our works also demonstrated that

ex vivo clots derived from 106 male first-degree relatives and 34 male patients with intermittent claudication had higher density and prolonged lysis time, compared to healthy age-matched controls (Table 2).[79,80]

4 Potential adjunct therapies to target hypofibrinolysis in patients with CVD

From the evidence presented above, it appears that residual thrombosis risk in individuals with vascular disease may be ameliorated by addressing the hypofibrinolytic environment, in addition to the use of antiplatelet therapies. In this section, we explore the latest in adjunctive antithrombotic therapies, added to existing antiplatelets, to further reduce residual vascular risk and improve outcome in individuals with CVD. It should be noted that current chronic therapies for hypofibrinolysis target clot lysis indirectly by making the fibrin network more susceptible to lysis and agents that directly affect one or multiple proteins in the fibrinolytic system are not yet available for clinical use.

4.1 Factor Xa inhibitors

Inhibiting FXa in the coagulation cascade leads to decreased thrombin generation,[81] consequently resulting in reduced clot formation, together with the generation of fibrin networks that are less compact with increased susceptibility to lysis. Moreover, factor Xa inhibitors possibly enhance fibrinolysis by interfering with TAFI activation.[82] Therefore, the use of a FXa inhibitor in addition to standard antiplatelet therapy may offer additional benefits in patients with CVD. However, it remains unclear whether the additional benefits of combined antiplatelet/anticoagulant are related to reduction in fibrin clot formation, enhanced fibrinolysis, or a combination of the two.

386 The EDOC-APT study tested the effects of 30 and 60 mg of edoxaban on clot kinetics in
387 75 CAD patients taking aspirin and clopidogrel.[83] The authors concluded that edoxaban
388 delayed thrombin generation in a dose-dependent manner but did not affect maximum clot
389 firmness measured by TEG. Another ongoing similar study (NCT03775746) on 150 patients
390 with ACS is testing the effects of low-dose rivaroxaban and clopidogrel, in addition to aspirin,
391 on fibrinolytic status measured by GTT.[84]

392 In the ATLAS ACS 2-TIMI 51 study, a total of 15,526 high-risk ACS patients were
393 randomly assigned to receive low-dose rivaroxaban 2.5 mg twice daily, rivaroxaban 5 mg twice
394 daily, or placebo, on top of standard antiplatelet therapy, and were then followed for a median
395 of 13.1 months.[85] Compared with placebo, both 2.5 and 5 mg twice daily of rivaroxaban
396 significantly reduced MACE [HR 0.84 (95% CI: 0.72–0.97) and HR 0.85 (95% CI: 0.73–0.98),
397 respectively] at the cost of increased major TIMI bleeding [HR 3.46 (95% CI: 2.08–5.77) and
398 HR 4.47 (95% CI: 2.71–7.36), respectively]. Low-dose rivaroxaban also reduced
399 cardiovascular death whereas rivaroxaban 5 mg twice failed to show an effect. Based on these
400 data, low-dose rivaroxaban in high-risk patients on top of DAPT (aspirin and clopidogrel) or
401 aspirin alone has been approved by the European Medicines Agency. Nevertheless, these
402 results were not reproduced in another similar study using Apixaban 5 mg daily in addition to
403 DAPT or aspirin alone, in patients with high-risk ACS (the APPRAISE-2 study). This trial was
404 prematurely terminated because of an increase in major bleeding events with apixaban in the
405 absence of a counterbalancing reduction in recurrent ischemic events, after a median follow-
406 up of 241 days.[86]

407 In chronic stable CVD, the recently published COMPASS study has opened a new
408 opportunity for the use of low-dose rivaroxaban added to aspirin in high-risk patients with
409 stable CVD (older than 65 years, or atherosclerosis involving $\geq$2 vascular beds, or $\geq$2

additional risk factors).[87] In brief, 27,395 patients with high-risk stable coronary artery or peripheral artery diseases were randomly assigned to low-dose rivaroxaban (2.5g mg twice daily) plus aspirin, rivaroxaban (5 mg twice daily), or aspirin (100 mg daily). During a mean follow-up of 23 months, the composite cardiovascular death, stroke, or MI was significantly lower in low-dose rivaroxaban plus aspirin, compared to aspirin alone [HR 0.76 (95%CI:0.66-0.86), p<0.001], with a trade-off from higher major bleeding risk [HR 1.7 (95%CI: 1.4-2.05), p<0.001]. The combination of rivaroxaban and aspirin also offered all-cause mortality reduction by approximately 18% and net clinical benefits by 20%.[87] Interestingly, in a subgroup analysis of PAD patients, combination therapy showed reduction in amputations, demonstrating that the benefits are not only related to reduction of coronary events but also CLI.[88]

Based on the ATLAS ACS 2-TIMI 51 and COMPASS studies, dual pathway inhibition by low-dose rivaroxaban (inhibiting thrombin generation) and antiplatelet therapy synergistically reduces residual cardiovascular risk in patients with high-risk ACS or high-risk stable coronary artery or peripheral artery diseases,[89] however, the role of this dual combination is not applicable for ischaemic stroke patients, which was one of the exclusion criteria in both studies.

4.2 Direct thrombin inhibitor (dabigatran)

Dabigatran directly binds to active sites of thrombin, inhibiting its action and also promotes fibrinolysis through reduction in TAFI activation.[90] In one study measuring *ex vivo* plasma clots from patients with atrial fibrillation by turbidimetric analysis, dabigatran and factor Xa inhibitors delayed clot formation and modestly decreased clot firmness.[91] In contrast, another study scrutinising thrombin generation and clot firmness in whole-blood and plasma by using TEG from 8 heathy volunteers, demonstrated that while both FXa inhibitors and

dabigatran effectively prolonged thrombin generation, neither altered clot firmness.[92] These findings resembled the results from another a double-blinded, placebo, randomized controlled trial (RCT) that included 35 CAD patients on DAPT with aspirin and clopidogrel. Again, dabigatran significantly decreased thrombin activity and delayed fibrin clot formation, without affecting clot structure or fibrinolysis.[93] Importantly, the inconsistency of the dabigatran effect in studies is perhaps related to variation in the methodologies used, particularly when different triggers are used to stimulate clot formation (i.e tissue factor vs thrombin) which, in turn, invalidates attempts at comparing results from different studies.

Unfortunately, the use of dabigatran with antiplatelet therapy in patients with stable CAD or ACS has been a disappointment. The RE-LY study investigated the use of dabigatran to prevent ischaemic events in patients with atrial fibrillation and unexpectedly showed a possible increased risk of MI, despite the reduction in composite stroke and embolic events.[94] Results from the phase II RE-DEEM study, dabigatran was associated with a dose-dependent increase in bleeding events without reduction in MACE.[95] Finally, a meta-analysis included over 30,000 patients from 7 trials demonstrated that dabigatran was associated with an increased risk of MI and ACS indicating that this drug is not a viable option for those with coronary artery disease.[96]

4.3 Potential future therapies directly targeting clot lysis

As mentioned above, endogenous antifibrinolytic proteins, in pathological alterations, are associated with increased risk of CVD. Modulating their functional activity may, therefore, enable novel therapeutic options to alleviate residual thrombosis risk in patients with CVD.

Inhibition of TAFI was considered as a putative target to enhance fibrinolysis. The discovery of thrombin-thrombomodulin interaction activating TAFIa had drawn attention from pharmaceutical companies to develop compounds inhibiting TAFIa;[97] however, a limited number of drug candidates (AZD9684 from AstraZeneca and UK-396082 from Pfizer)

458 eventually entered clinical studies which were later discontinued for unknown reasons.[98]
459 Another potential approach of TAFIa inhibitors relied on antibodies or nanobodies, yet *in vivo*
460 data are still lacking.[99]

461 For decades, researchers have been attempting to develop small molecule inhibitors or
462 antibodies against PAI-1. Some of antibodies have previously shown the inhibitory effect *in*
463 *vivo* in animal models (TM5275, MA-33H1F7 and MA-MP2D2),[100,101] whereas most recent
464 nanobodies have been initially tested in an *in vitro* clot lysis assay.[102] Tiplaxtinin (or PAI 039)
465 is one of the most studied small molecule inhibitors, proven the PAI-1 inhibiting efficacy in
466 several animal models.[103] Nevertheless, none of these has successfully advanced further into
467 clinical studies.[104,105] Recently, an innovative strategy using heterodimer bi-specifically against
468 TAFI and PAI-1 has been tested in a thrombosis animal model emerging the concept of multi-
469 target inhibitors.[106]

470 A2AP is another possible drug target, with a number of different approaches investigated
471 to inhibit protein function including antibodies, plasmin inhibitor mutants, use of synthetic N
472 and C terminal peptides and inhibition of plasmin inhibitor cleavage. However, none of these
473 approaches so far progressed further to clinical trials.[99] After 20 years of relative inactivity,
474 treatment targeting A2AP has been reactivated via a recent study by Singh et al, showing that
475 the injection of A2AP inactivating antibody in adjunct to recombinant t-PA successfully
476 potentiated clot dissolution without increased bleeding in an *in vivo* model of acute pulmonary
477 emboli.[107] Human studies using this approach have been recently undertaken but the work was
478 stopped prematurely and a full report is awaited with interest (NCT03001544).

479 Even though Eculizumab (Soliris®, Alexion Pharma GmbH, Switzerland), a drug
480 inhibiting the terminal (C3 and C5) complement system, has been approved for the treatment
481 of series of inflammatory and autoimmune diseases,[108] the role of C3 or C5 inhibitor in

fibrinolysis has not yet been widely tested. The early work from our institute has shown that high affinity fibrinogen-binding and C3-specific conformational proteins (Affimers®, Avacta, Cambridge, UK) are able to abolish C3-induced prolongation of clot lysis *ex vivo* by interfering C3-fibrinogen interaction.[109] Yet it remains to be seen whether this approach can be advanced further for clinical trials in the future.

5 Conclusion and future directions

It is now without doubt that prolonged fibrinolysis represents a risk factor for adverse vascular outcome, even when aggressive antiplatelet therapies are in place. A number of factors are responsible for altered fibrin clot lysis, including quantitative and qualitative changes in different coagulation proteins. These can affect fibrinolysis indirectly by altering structure of the clot, secondary to raised fibrinogen levels or presence of post-translational modification of the protein.[20,21] Fibrinolysis can also be directly affected due to raised levels of antifibrinolytic proteins such as PAI-1,[42,43] increased incorporation of A2AP into the clot,[29] or increased glycation of plasminogen.[110]

Importantly, recent evidence indicates that impaired fibrinolysis is a key factor responsible for atherothrombotic events in high risk patients, even when adequately covered with modern antiplatelet therapies. Therefore, the hotly-debated area of "residual thrombosis risk" appears to be related, in large part, to impaired fibrin clot lysis. Consequently, targeting compromised fibrin clot lysis in these individuals represents a legitimate therapeutic approach. Indeed, studies have generally shown that combining antiplatelet with anticoagulant therapies reduces the risk of arterial vascular occlusion but usually at the expense of increased bleeding events, making widespread use of this strategy problematic. However, studies have used combination

505 treatment without an attempt to “individualise” such dual therapy and limit to those at the
506 highest risk. This is due to the fact that tangible strategies to stratify patients at particular risk
507 of impaired fibrinolysis have not made it into clinical practice. Therefore, developing and
508 validating an assay that can be routinely used to determine fibrinolytic efficiency will help to
509 facilitate patient stratification and tailor anti-thrombotic therapy accordingly. It must be
510 acknowledged that current chronic antithrombotic therapies that target the protein phase of
511 coagulation do not directly affect the fibrinolytic system but exert their effects indirectly, which
512 may explain the limited clinical benefit, which is largely related to increased bleeding risk.
513 Therefore, more work is needed to develop therapies that target the fibrinolytic system with a
514 particular focus on modulating the pathological abnormalities thus maximising anti-thrombotic
515 benefits while minimising bleeding risks. For example, inhibiting A2AP and/or PAI-1 in
516 individuals with diabetes and vascular disease is likely to be more effective that targeting FX
517 activity by offering a more favourable benefit:risk ratio, given the focus on known pathological
518 abnormalities in this metabolic condition. Finally, we will also need a reliable test that can
519 monitor response to a particular antithrombotic agent, akin to anti-hypertensive and anti-
520 hyperlipidaemic therapies, which will further refine our approach and allow a more structured
521 strategy to the management of thrombosis risk.

522 Anti-thrombotic therapies have made great progress over the past few decades and this
523 trend is likely to continue. The next steps will require the development of more targeted
524 therapies and expansion of individualised strategies of patients care, in order to ensure the best
525 clinical outcome in population with high vascular risk.

526 **6 Conflicts of interest**

527 All authors have no conflict of interest to be declared.

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8 Author contributions

NK was responsible for drafting and writing of the manuscript, searching of literature and interpreting of data. RASA and RAA was responsible for the drafting and writing the manuscript and critical revision of important intellectual content. All authors approved the version to be published.

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10 Figure legends

Figure 1 Overview of coagulation and fibrinolysis activation. Tissue injury exposes collagen enabling platelet adhesion and activation through platelet alpha2beta1 (GPIa/IIa), glycoprotein VI (GPVI) and glycoprotein Ib (GPIb) interacting with von Willebrand factor (vWF). Simultaneously, tissue factor (TF) is exposed activating factor (F) VII to form VIIa/TF complex (extrinsic pathway of coagulation). The VIIa/TF complex subsequently activates a number of proteins in the contact ('intrinsic') and common pathways of coagulation. The activation of these pathways ultimately leads to the accumulation of thrombin which, in turn, stimulates platelet activation via protease-activated receptor 1 or 4 (PAR1/4) and further amplifies activation of coagulation factors in the intrinsic and common pathways. An adequate amount of thrombin finally converts fibrinogen into fibrin and forms double stranded protofibrils. Thrombin also activates FXIII to stabilise the fibrin by crosslinking. The protofibrils then aggregate further into fibrin fibres that generate a network, trapping blood components, before forming a mature thrombus. The fibrinolysis starts after, and even during, fibrin network formation which uncovers binding sites for plasminogen and tissue-plasminogen activator (t-PA), enabling plasmin activation and start of the fibrinolytic process.

Figure 2 Regulation and alteration of fibrinolysis. Plasmin degradation of fibrin exposes C-terminal lysine (lys) residue – the docking site for plasminogen (Plg) and tissue plasminogen activator (t-PA) on α chain of fibrinogen, commencing fibrinolysis. This plasmin-induced proteolysis is seemingly endless and requires other endogenous regulators to limit this process. The interaction between thrombin and thrombomodulin (TM), located on endothelial cells, activates thrombin activatable fibrinolysis inhibitor (TAFI) which cleaves lys residue off the fibrin surface, compromising plasminogen binding and fibrinolysis. Activated platelets release plasmin-activator inhibitor 1 (PAI-1) to inhibit t-PA. Moreover, alpha 2 antiplasmin (α2AP) is crosslinked into fibrin and inhibits Plg. Complement (C) 3 is a substrate for plasmin which competitively prevent plasmin from cleaving fibrin. Lipoprotein (a) [Lp(a)] has similar homology to plasminogen, competitively binding to fibrinogen, thus, preventing plasmin activation. Lp(a) also stimulates PAI-1 secretion by endothelial cells.

11 Tables

Table 1 Summary of studies investigated the impacts of altered fibrinolysis in patients with acute cardiovascular disease.

Study	Population	Patients	Controls	Lab technique	Outcome	Main findings		Other comments
						Clot strength	Lysis time	
ACS: Case-control studies								
Undas A. (2008)[58]	40 patients with ACS admitted to the coronary care unit within the first 12 h after the onset	40 ACS patients	40 stable angina patients	1.Clot permeability 2.Turbidimetric analysis 3.Plasma clot lysis assay 4.SEM	Clot properties	Patients with ACS had denser clot structure observed by SEM, lower clot permeability ($p = 0.001$), faster fibrin	Patients with ACS had prolonged fibrinolysis time ($p < 0.0001$)	

						polymerization (p = 0.008),		
Mirjam E. (2008)[111]	426 men surviving the first MI, aged below 70 years old, excluded the use of anticoagulants	426 men surviving the first MI	646 men without a history of MI	Clot LT measured by turbidimetric analysis	Risk of MI of the 4th quartile of clot LT, compared to the 1st quartile	*NA*	overall OR 1.0 (95%CI: 0.6-1.5) Age <50 years OR 1.8 (95%CI: 0.7-4.8) Age ≥50 years	OR adjusted for age,BMI, smoking status, presence of diabetes, blood pressures, lipid parameters, and C-

							OR 0.7 (95%CI: 0.4-1.1)	reactive protein.
ACS: Prospective studies								
Kreutz RP. (2017)[59]	270 Patients underwent PCI (15.6% STEMI, 22.2% NSTEMI, 24.8% unstable angina, 35.9% stable angina)	142 patients with TEG-MA≥35.35 mm	128 patients with TEG-MA <35.35 mm	Whole blood TEG	First recurrent MI or CVD death, mean follow-up 2.9 years	HR 3.8 (95%CI: 1.7-8.3, p=0.001)	*NA*	

Saraf S. (2010)[61]	300 patients with hospitalized ACS	69 baseline LT≥3000s	231 baseline LT<3000s	GTT	12-month MACE	*NA*	HR 2.52 (95%CI: 1.34-4.71, p=0.004)	No association between OT and MACE
					12-month CV death	*NA*	HR 4.2 (95%CI: 1.13-15.62, p=0.033)	
Farag M. (2019)[11]	496 patients presenting with STEMI for primary PCI	70 baseline LT≥2500s	426 baseline LT<2500s	GTT	12-month MACE	*NA*	HR 9.1 (95%CI: 1.34-4.71, p<0.001)	LT was not altered by standard of care treatment including DAPT and was
					12-month CV death	*NA*	HR 18.5 (95%CI: 7.69-44.31, p<0.001)	

								unchanged at 30 days
Christopoulos C. (2017)[60]	82 patients presenting with STEMI for primary PCI	11 baseline LT≥3000s	71 baseline LT<3000s	GTT	12-month MACE	*NA*	HR 3.31 (95%CI: 1.02-10.78, p=0.045)	LT<1000 s was associated with spontaneous reperfusion
					12-month CV death	*NA*	HR 4.17 (95%CI: 0.99-17.51, p=0.05)	
Sumaya S. (2018)[10]	4354 moderate- to high-risk ACS patients	1082 in Quartile 4 (LT >888s)	1098 in Quartile 1 (LT<564s)	Turbidimetric analysis	12-month CV death or MI	*NS*	HR 1.48 (95%CI: 1.06-2.06, p=0.027)	50% increase in LT was associated with 17% increased
					12-month CV death	*NS*	HR 1.92 (95%CI: 1.19-3.1, p<0.001)	

								risk of CV death/MI and 36% CV death alone
AIS: Case-control studies								
Undas A. (2010)[62]	45 patients with AIS admitted within the first 72 h after the onset	45 patients	45 age- and sex-matched healthy control	1.Clot permeability 2.Turbidimetric analysis	Clot properties	AIS patients had clots with 30.5% less porous ($p<0.0001$), 20.5% more compact ($p<0.0001$),	AIS patients had clots with 10.8% longer lysis time ($p=0.001$)	

						17.1% higher clot mass ($p<0.0001$), 10.2% increased overall fiber thickness ($p<0.0001$)		
AIS: Prospective studies								
Bembenek JP. (2017)[63]	74 Patients admitted up to 4.5 h since AIS onset, eligible	44 patients with good outcome at 3-month (mRS 0-2)	30 patients with poor outcome at 3-month	1.Clot permeability 2.Turbidimetric analysis	Baseline clot properties	Patients with poor outcome had clots with lower baseline permeability ($p<0.01$)	Patients with poor outcome had clots with Prolonged baseline LT ($p=0.01$)	

	for thrombolysis							
ALI/CLI: case-control studies								
Karpińska IA.[64]	43 patients referred for ALI treatment, off anticoagulant at least 3months since the onset	43 patients	43 controls without history of ALI or vascular event	1.Clot permeability 2.Turbidimetric analysis	Clots properties	Patients with ALI exhibited 13.4% lower permeability, p=.001)	*NS*	
Nowakowski T.[65]	85 patients with CLI and symptomatic	85 patients with CLI and	47 PAD patients, age-, sex-	1.Clot permeability	Clots properties	CLI patients with restenosis had clots with	CLI patients with restenosis had clots with	During a 24 months follow-up

	restenosis after receiving percutaneous angioplasty within 12 months	symptomatic restenosis	and CV risk matched controls (with CLI =32, without CLI =15)	2.Turbidimetric analysis		9.5% lower permeability ($p<0.001$)	12.4% prolonged LT	the composite re-intervention, amputation or CV death occurred in 63.5% of CLI patients with restenosis and 19.1% of controls ($p<0.001$)

ACS = acute coronary syndrome; AIS = acute ischaemic stroke; ALI = acute limb ischaemia; CLI = critical limb ischaemia; mRS = modified Rankin Scale; GTT = global thrombosis test; TEG = thromboelastography; TEG-MA = TEG maximum amplitude; LT = lysis time; OT =

occlusion time; MACE = major adverse cardiovascular event; CV= cardiovascular; *NA* = not applicable; *NS* = not significant; MI = myocardial infarction; STEMI= ST-elevated myocardial infarction; NSTEMI = ; non ST-elevated myocardial infarction PCI=percutaneous coronary intervention; SEM = scanning electron microscopy;

Table 2 Summary of studies investigated the impacts of altered fibrinolysis in patients with chronic stable cardiovascular disease.

Study	Population	Patients	Controls	Lab technique	Outcome	Main findings		Other comments
						Clot strength	lysis time	
Stable CAD: Case-control studies								
Reddel CJ. (2013)[66]	56 patients with stable angina	56 patients	73 heathy volunteers	Turbidimetric analysis	Overall coagulation property (OCP) Overall fibrinolysis property (OFP)	CAD patients had higher OCP ($p<0.001$)	CAD patients had lower OFP and LT ($p<0.001$)	

Siegerink B. (2012) [67]	380 young women age 18-50 years, diagnosed with arterial thrombosis (205 with MI and 175 with ischaemic stroke)	205 MI patients	638 age-matched women	Turbidimetric analysis	LT	*NA*	75.2±25 min vs 64.4±14 min (no p-value provided)	The study contained several biases from confounders, drop-out rate and blood sample collection

Tantry US. (2010)[69]	171 patients with asymptomatic stable CAD, stable angina, and unstable angina	33 unstable angina patients	71 stable angina patients and 67 asymptomatic CAD patients	TEG	TEG-MA_{ADP}	Unstable angina patients produced the strongest clots compare to asymptomatic and stable angina groups ($p<0.001$ and 0.02, respectively) Stable angina patients had stronger clots	*NA*	Data presented in figures

						than asymptomatic one (p=0.02)		
Neergaard-Petersen S. (2013)[68]	177 patients with stable CAD with at least 50% stenosis	116 CAD with MI	61 CAD without MI	Turbidimetric analysis	Clot properties	MA [median (IQR)] 0.48 (0.41; 0.52) vs. 0.42 (0.38; 0.50) p = 0.02	LT [median (IQR)] 552 (498; 756) vs. 519 (468; 633)	CAD with MI patients were older and had lower LDL cholesterol

							seconds p = 0.02	
				SEM	clot structure	Thinner fibres (mean±SD) 119.7±27.5 vs. 127.8±31.1 nm p = 0.003	*NA*	
Stable CAD*: *Prospective studies								
Neergaard-Petersen S. (2020)[72]	786 patients with stable CAD with at	197 in quartile 1 (Clot AUC =121-195)	196 in quartile 4 (Clot AUC= 585-2005)	Turbidimetric analysis	MACE	Clot AUC Adjusted HR: 2.4 (1.2–4.8), p=0.01		Clot AUC represents the net effect

	least 50% stenosis, 90% had prior MI					MA Adjusted HR: 1.8 (0.9-3.7), p=*NS*	LT Adjusted HR: 1.6 (0.8-3.0), p=*NS*	between OCP and OFP
Hou X. (2017)[71]	759 patients underwent elective PCI, excluded Acute MI within 48h	58 patients with an ischaemic event in 2 years	701 patients without ischaemic event	TEG	Whole blood clot strength	TEG-MA_{ADP} 40.8 ± 10.1 mm vs 26.7 ± 13.7 mm, p<0.001	*NA*	TEG-MA >34 mm predicted ischaemic events after PCI
Gurbel PA. (2009)[70]	84 patients underwent elective PCI,	21 patients in quartile 4	63 patients in quartile 1-3	TEG	Ischaemic events	Ischaemic events Q4 = 48%	*NA*	TEG-$MA_{ADP}\geq$68mm (quartile3-

	excluded Acute MI within 48h	(TEG-$MA_{ADP}\geq$71mm)	(TEG-MA_{ADP}<71mm)		within 2 years (MACE and hospitalised angina)	vs Q1 = 13%, p=0.02 Q2 = 15%, p=0.03 Q3 = 30%, p=0.24		4) probably predicted ischaemic events
Previous ischaemic stroke: Case-control studies								
Anžej S. (2007)[73]	Patients with previous AIS, age <45 years old	44 patients	46 Healthy control	Turbidimetric analysis	OCP and OFP	*NS*	Patients had lower OFP (p<0.001)	
Undas A. (2009)[74]	147 patients with or	147 patients	120 healthy controls	1.Clot permeability	Clot properties	Patients had clots with	Patients had clots	

	without patent foramen ovale and a history of first-ever ischemic stroke			2.Turbidimetric analysis 3.SEM		lower permeability (p<0.0001), faster fibrin polymerization (p<0.0001), and increased fibre diameter and density	with prolonged clot LT (p<0.0001)	
Vučković BA. (2010)[75]	60 ischaemic stroke patients	60 patients	30 age- and sex-matched health controls	Euglobulin clot lysis time	LT	*NA*	219.7±78.8 min vs 183.5±58.2 min (p=0.005)	

	(90% had stroke within 1year)							
Siegerink B. (2012) [67]	380 young women age 18-50 years, diagnosed with arterial thrombosis (205 with MI and 175 with ischaemic stroke)	175 ischaemic stroke patients	638 age-matched women	Turbidimetric analysis	LT	*NA*	68.1.2±36.3 min vs 64.4±14 min (no p-value provided)	The study contained several biases from confounders, drop-out rate and blood sample collection

Previous ischaemic stroke: prospective studies								
Wang B. (2017)[76]	218 patients who underwent stenting for extracranial or intracranial artery (70-99%) stenosis	18 patients with recurrent ischaemic stroke or TIA events (9 events occurred within 7 days)	200 patients without ischaemic event	TEG	Baseline TEG-MA_{ADP}	41.57±15.10 vs 33.50±13.86 mm (p=0.020)	*NA*	The incidence of ischemic events in patients with MA_{ADP} >49.95 mm was markedly higher than in patients with MAADP ≤49.95 mm (20.8% vs

								6.7%, p=0.018).
PAD: Case-control studies								
Undas A. (2011)[77]	106 patients with PAD (ABI≤0.9) aged ≤70 years	106 patients	106 age-, sex- and CV risk matched controls	1.turbidimetric analysis 2. clot permeability	Clot properties	Patients had clots with 18.8% lower clot permeability (p = 0.005), 35.3% faster fibrin polymerizatio n (p<0.001),	Patients had clots with 30.6% prolonged clot lysis time (p = 0.003)	

						22.4% higher MA ($p<0.001$)		
Okraska-Bylica A. (2012)[78]	31 patients with symptomatic premature PAD (ABI$\leq$0.9) patients aged $\leq$55 years old,	31 patients	40 age- and sex-matched controls	1.turbidimetric analysis 2. clot permeability	Clot properties	Patients had clots with 32% lower clot permeability ($p<0.001$)	Patients had clots with 7% longer clot lysis time ($p = 0.004$)	
Bhasin N. (2008)[79]	106 male first-degree relatives of	106 male first-degree relatives	107 healthy male, age-	1. turbidimetric analysis	Clot properties	Male first-degree relatives had	Male first-degree relatives	

	male patients with intermittent claudication		matched controls	2. clot permeability 3. LSCM		clots with higher MA and fibre thickness on LSCM (p<0.001)_	had slower clot lysis velocity on LSCM (p=0.018)	

CAD = coronary artery disease; PAD = peripheral artery disease; TIA = transient ischaemic attack; ABI = ankle-brachial index; OCP = overall coagulation property; OFP = overall fibrinolysis property; MA = maximum absorbance; AUC = area under the curve; LT = lysis time; TEG = thromboelastography; TEG-MA_{ADP} = TEG-maximum amplitude of ADP-induced platelet-fibrin clot strength; LSCM, laser scanning confocal microscopy; MACE = major adverse cardiovascular event; CV= cardiovascular; *NA* = not applicable; *NS* = not significant; MI = myocardial infarction; SEM = scanning electron microscopy.