Fibrin clot properties and their modulation in thrombotic disorders

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Summary
Accumulating evidence indicates that accelerated formation of fibrin clots composed of compact, highly-branched networks with thin fibres which are relatively resistant to plasmin-mediated lysis can be commonly observed in patients with venous or arterial thrombosis. This review discusses characteristics of fibrin clot structure and function in patients with various thromboembolic manifestations, in particular myocardial infarction, ischaemic stroke and venous thromboembolism, based on the publications till December 2013. Moreover, factors will be presented that in vivo unfavourably determine altered fibrin clot properties in thrombotic disorders and modalities that can improve clot phenotype.

Keywords
Fibrin clot, fibrinolysis, thrombosis

Introduction
Fibrin constitutes the primary structural protein of blood clots and intravascular thrombi in all locations. Fibrin formation and its functions are essential for both haemostasis and the pathologic thrombosis (1). Fibrinogen, the soluble fibrin precursor, is a complex multifunctional 340-kDa glycoprotein of 45 nm in length that is composed of two copies each of three polypeptide chains (AaBβγ), linked together by 29 disulfide bonds. The central E region contains the N termini of all six chains. The chains in the form of two coiled-coils composed of three chains represent two symmetrical globular D domains containing the β- and γ-nodules formed by the carboxy termini of the Bβγ- and γ-chains, respectively. The C-terminal segment of the Aa chain goes through the D region, and folds back to join the coiled-coil for several residues (Aa residues 213 to 610). About 10% of fibrinogen molecules contain γ-chains, in which the four C-terminal residues are replaced with a 20-residue sequence with a large negative charge and they occur predominantly as γA/γ' heterodimers (1–3).

Fibrin formation is initiated by limited proteolysis of fibrinogen by thrombin. Thrombin splits off two pairs of fibrinopeptides A and B from the N termini of the Aa and Bγ chains, respectively, in the central nodule, leading to the exposure of binding sites ‘A’ and ‘B’. There are two stages of polymerisation of fibrin monomers, namely the formation of half-staggered and double-stranded protofibrils through binding of knobs ‘A’ and ‘B’ in the central nodule of fibrin monomer to complementary holes ‘a’ and ‘b’ in the γ- and β-nodules, respectively, of another monomer and the assembly of protofibrils into fibres through lateral aggregation promoted mainly by intermolecular αC: αC interactions between protofibrils and probably also by interactions between both α- and γ-chains (3, 4). Fibrin fibres are on average composed of thousands of protofibrils arranged side-by-side; e.g. a fibre with a thickness of 400 nm consists of about 5,000 fibrin molecules in diameter (5). However, the fibre growth within a clot is highly heterogeneous and consequently some fibres reach an early stationary state at much smaller diameters (5). Fibrin fibres are characterised by the largest extensibility observed for protein fibres; they can be strained 180% (2.8-fold extension) without sustaining permanent lengthening, and they can be strained up to 525% (average 330%) before rupturing (6).

The stability of blood clots in response to mechanical forces imposed in vivo mainly by the blood flow is regulated by the kinetics of dissociation of the knob-hole bonds until the crosslinking activity of activated factor (F) XIIIa takes place. When thrombin and a protransglutaminase, FXIII are bound to fibrin, thrombin efficiently activates FXIII which catalyses the formation of ε-(γ-glutamyl)lysyl covalent bonds between γ-γ, γ-α, and α-α chains of adjacent fibrin molecules, together with the incorporation of several proteins, including α2-plasmin inhibitor and plasminogen activator inhibitors into a fibrin clot, substantially increasing fibrin clot resistance to enzymatic degradation and mechanical deformation (7). Fibrinogen also specifically binds a variety of other proteins, including fibronectin, albumin, thrombospondin, von Willebrand factor, fibulins, fibroblast growth factor-2, vascular endothelial growth factor, and interleukin-1.

The mechanical properties of any branched network depend on the network architecture and the mechanical properties of the individual fibres (2). The mechanical properties of the individual
fibrin monomers such as the coiled-coil connectors, the folded globular nodules, and the relatively unstructured αC regions largely contribute to the fibrin clot mechanics (8). Since fibrin clot networks have extensibilities of 100 to 200%, lower than individual fibres (6), which involve two mechanisms to extend, first aligning then stretching fibres, it is likely that clot rupture does not arise from the rupture of individual fibres, but rather the branch points.

Architecture of a fibrin clot can be characterised by 1) the fibre diameter largely determined by lateral aggregation, and typically estimated using clot permeability under different hydrostatic pressures and 2) the size of the pores in the fibre network, which is most commonly assessed from the density of fibres in dehydrated clots on scanning electron microscopy (SEM) images. In purified systems, there is a positive correlation between fibre diameter and the pore size in a fibrin clot.

Fibrinolysis represents a major mechanism of clot removal that aims at maintaining blood flow. The fibrinolytic system, with the zymogen plasminogen binding to fibrin together with tissue-type plasminogen activator (tPA) to promote plasmin generation, results in digestion of fibrin at specific lysine residues. Effective fibrinolysis is crucial in maintaining the vessel patency. There is compelling evidence that the fibrin clot architecture is a potent regulator of the rate of clot lysis since it determines the distribution of lytic enzymes within a fibre network leading to much slower lysis of denser clots (9). Paradoxically, in vitro a modulatory effect of fibrin structure on fibrinolysis results from slower tPA-induced plasminogen activation on looser fibrin meshes (10). The rate of activation of plasminogen is faster in the presence of fibrin.

Importantly, in vivo fibrin clots are subjected to modulatory effects of flow, cellular blood components, endothelial cells, and a number of circulating proteins and other molecules that can bind to fibrin and possibly alter clot characteristics. Moreover, in vivo fibrinogen and fibrin are subjected several posttranslational modifications including oxidation, phosphorylation etc. known to alter clot structure as shown at least in vitro (11). Consequently, structural and functional differences between plasma fibrin clots and those made from purified fibrinogen have been well documented. For example, fibrin network formed from citrated plasma is composed of thicker fibres making looser meshes compared with that formed from purified fibrinogen (12). This results in higher permeability and accelerated fibrinolysis at least in part due to more efficient transport of fibrinolytic agents through a fibrin clot. In plasma like in purified systems fibre diameter determines fibrinolytic rate induced by either urokinase or t-PA, and fibrinolytic rate is increased with increasing diameter (13).

Several lines of evidence have shown that the structure and function of fibrin clots formed from the patient’s plasma differ from those of clots formed from plasma obtained from healthy individuals (▶Figure 1). Rapidly accumulating evidence indicates that most cases of arterial and venous thrombotic events share a common prothrombotic fibrin clot phenotype, which is typically characterised with the formation of more compact fibrin meshworks of impaired permeability and lysisability (14–16). Logically, many well-established and emerging prothrombotic risk factors, both genetic and environmental, should negatively affect the fibrin component of thrombi and/or thromboemboli, largely by increasing dense fibrin content and rendering it more resistant to removal or, on the contrary, susceptible to fragmentation (▶Figure 1). Unfortunately, despite much experimental and clinical effort in recent years, the rules that govern changes in fibrin clot properties in a variety of human clinical entities associated with thrombotic risk remain elusive in many aspects. This review summarises the current knowledge (up to December 2013) on the links of fibrin clot formation, structure and degradation with thrombosis at various locations with emphasis on clinical implications of fibrin clot characteristics in humans.

Flow and fibrin clot structure

Not surprisingly, the velocity of blood flow and wall shear rates displaying huge differences among blood vessels of various calibre and type (10–100+ in the veins and 500–1500 in the arteries, with up to 10,000 at the site of critical stenosis) have been found to significantly influence fibrin clot structure. Given evidence that fibrin monomers and larger structures dissociate from fibrin fibres, the flow of blood may remove those fragments that dissociate, increasing the rate of turnover, which is of importance in particular at higher shear rates typical for the arteries during an early phase of clot formation (17). Fibrin fibres are aligned in the direction of flow, which impacts the elastic properties of the clot (18–20). Fibrin fibres are more resistant to stretch than to flexion, and hence alignment of the fibres in the direction of flow will increase stiffness of the clot in that direction. In vitro studies on the association of flow with fibre diameter yielded inconsistent results from a neutral effect (18) to a major impact indicating that faster flow enhances the fibre thickness and formation of bundles in the direction of flow, with thinner fibres interconnecting the thicker ones perpendicularly and such clots display a marked tensile stiffness making them probably more resistant to deformation (19). Flow modulates the kinetics of both fibrin monomer formation and polymerisation through depletion of fibrin monomers leading to impaired fibre formation at higher shear rates (20). This effect of flow is, however, almost abolished by enhanced thrombin production which increases local fibrin monomer concentrations thus supporting fibre formation (20). Recently, it has been shown that shear strengthens fibrin clots through a “catch-slip” mechanism of the knob-hole interactions (21).

Changes in fibre diameter likely affect plasmin generation and clot susceptibility to lysis. Thick fibres support faster plasmin formation, although thin individual fibres are dissolved at a higher rate than thick ones, like coarse networks of thick fibres (22). Flow studies performed on tissue factor (TF)-bearing human dermal fibroblasts suggested that thick fibres under flow lyse more slowly, while thin fibres running perpendicular to flow vectors lyse more rapidly, leading to heterogeneous pattern of lysis within a clot (19).

It is uncertain to what extent in vitro observations reflect the in vivo situation in the arteries and veins. Analysis of thrombotic material removed from the right atrium and pulmonary (lobar
and segmental) arteries of a patient with high-risk pulmonary embolism showed that distally located thrombi are richer in densely-packed fibrin fibres aligned along the flow vector compared with the proximal ones and the atrial thrombus (23). Thrombi obtained during thrombectomy of markedly stenosed epicardial arteries of patients in the acute phase of myocardial infarction (MI) have commonly thick fibrin bundles up to a thickness of 1 μm, which, however, in contrast to in vitro experiments on the cells with high expression of TF (19), are aligned frequently at angles of 20–30 degrees to the direction of blood flow; this likely mirrors highly turbulent blood flow at sites of stenosis (24). This supports the view that in vivo, local disturbances in blood flow, predominantly at sites of eccentric large stenosis, have a marked effect on fibrin clot structure, which could be different from that visualised in vitro.

**Cells and fibrin clot structure**

In vivo, activation of blood coagulation and fibrin formation occurs on a surface predominantly activated platelets or injured vascular wall, which might suggest an impact of “surface-related” factors on fibrin structure. In the presence of human fibroblasts incubated with FXa, FVa, prothrombin and fibrinogen, or plasma, in the absence of platelets, it has been demonstrated that the fibrin network became looser when the distance from the cell surface increases, although surprisingly, fibre thickness was similar and consequently, the most compact networks with a relative resistance to lysis can be observed close to the cell surface (25). In contrast to platelet studies, in the fibroblast model neither αvβ3, αIIbβ3, nor other RGD-binding integrins caused the spatially-dependent morphology seen in clots formed during in situ thrombin generation.

Figure 1: Factors that change normal plasma fibrin clot phenotype to prothrombotic phenotype and diseases or abnormalities in which prothrombotic fibrin clot characteristics have been reported (based on [14–16]).
This suggests that a major mechanism causing the spatially heterogeneous fibrin clot morphology is likely attributable to differential rates of thrombin generation at and above the cell surface (25).

Incubation of fibroblast, smooth muscle cells (SMCs), and unstimulated and tumour necrosis factor (TNF) α-stimulated human umbilical vein endothelial cell (HUVECs) monolayers with recalcified, normal plasma demonstrated that loose fibrin networks are observed on unstimulated endothelial cells in contrast to other cell types (26). Moreover, αIIbβ3 determines fibrin structure in platelet-rich clots, whereas integrin interactions play a minor role in clots formed by fibroblasts, SMCs, and HUVECs (25). In this model, extravascular cells and TNFα-stimulated HUVECs produce stable fibrin networks resistant to lysis (26). Additional implications of these findings are that the differences mediated by various prothrombotic cells in fibrin structure may be responsible partly for vascular bed-specific fibrin deposition.

Permeability and degradation of clots formed from the whole blood were 6.7– and 38-fold lower, respectively, compared with plasma clots, which could result from occlusion of the network pores by red blood cells and a consistent increase in thrombin generation due to platelets and red blood cells inducing formation of a tighter clot (27). Matt dense fibrin networks around erythrocytes have been visualised and enhanced oxidative stress as a major contributor to this effect has been suggested (28).

Analysis of fibrin clots formed at increasing red blood cell counts shows a decrease in fibre diameter, with its increase following inhibition of platelet glycoprotein (GP) IIA/IIb, while red blood cells impair clot lysis, with no effect of aggregation inhibition (29).

Other cell-derived modifiers of fibrin clot structure are histones and DNA. It has been demonstrated that the addition of histone-DNA complexes to fibrin results in thicker fibres, increased clot stability and rigidity, along with significant prolongation of clot lysis related to binding of large fibrin degradation products (30). The effects of DNA and histones alone are subtle and suggest that histones affect clot structure whereas DNA changes the way clots are lysed (30).

Increasing evidence indicates that microparticles (MPs) exert multiple prothrombotic actions (31). Compared with controls, monocyte-derived MPs supported faster fibrin formation, 38% higher fibrin network density and higher clot stability (3.8-fold higher turbidity in the presence of tPA) while platelet-derived MPs have no effect on fibrin clot structure (32).

The strongest evidence links the occurrence of DVT with prolonged clot lysis time (CLT) determined in an assay introduced by Lisman et al. (34) in 2001, in which blood clotting is triggered by TF in the presence of phospholipid vesicles and fibrinolysis is activated by addition of recombinant tPA to citrated plasma. In the Leiden Thrombophilia Study (LETS), a population-based case-control study on risk factors for DVT, hypofibrinolysis determined using this assay has been shown in subjects following the first episode, resulting in a two-fold increased risk for values above the 90th percentile in well-matched healthy controls (35). In patients following a first VTE episode, the main (77%) contribution to such hypofibrinolysis provided elevated plasminogen activator inhibitor 1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), α1-antiplasmin as well as lower prothrombin and plasminogen, with a negligible, or none, effect of tPA, fibrinogen, factor VII, factor X, and factor XI (36). A potential contributor to hypofibrinolysis in VTE might be impaired profibrinolytic effect of activated protein C possibly mediated in part by weaker inactivation of PAI-1 activity and enhancement of prothrombin activation, combined with altered clot structure by increasing the relative mass of fibrin within thrombi (37–40).

Recently, Traby et al. (41) have reported that there is a weak association between CLT and risk of VTE recurrence in women only, who experienced a first unprovoked VTE without cancer or thrombophilia and were followed for an average of 46 months after anticoagulation withdrawal. In a a population-based case-control Multiple Environmental and Genetic Assessment (MEGA) study, hypofibrinolysis alone (the fourth quartile of the CLT values) increased thrombosis risk about two-fold relative to individuals with the shortest CLT and this risk rose 20 times in oral contraceptive users, 10 times in immobilised subjects and eight times in Factor V Leiden carriers, with no effect of the combination of hypofibrinolysis and the prothrombin 20210A mutation (42). Using a global assay of fibrinolysis with the use of the eguobulin fraction added to a clot made from plasminogen-rich bovine fibrin, it has been demonstrated that fibrinolytic capacity is impaired in VTE patients below 50 years even when compared with young stroke survivors after adjustment for multiple confounders including PAI-1 (43). Impaired fibrinolysis has also been identified as a risk factor of a rare thrombosis, Budd-Chiari syndrome, with a 3.4 higher risk in subjects with the least efficient fibrinolysis largely associated with elevated PAI-1 activity, but not with TAFI (44). Together, slower fibrin lysis represents a novel risk factor for VTE, particularly in younger age categories.

In 2009 it was demonstrated that abnormalities in plasma fibrin clot features other than hypofibrinolysis can be detected in VTE patients (45). In patients following a first unprovoked VTE episode, without acquired or genetically determined thrombotic risk factors, including thrombophilia, it has been shown that plasma fibrin clots contain smaller pores compared to those made from plasma obtained from healthy individuals and patients’ relatives (> Figure 2a); however, fibrils are formed after similar lag phases (45). Interestingly, less compact fibrin structure associated with faster clot lysis seems to characterise subjects who have experienced PE, regardless of the presence of concomitant DVT or not.

**Clinical implications**

**Fibrin clots and venous thrombosis**

The pathogenesis of venous thromboembolism (VTE) encompassing deep-vein thrombosis (DVT) and pulmonary embolism (PE) is complex and multifactorial. Thromboemboli removed from the pulmonary arteries in acute PE consistently show the dominance of fibrin (up to 80%) over blood cells and platelets, with very compact structures in distal arteries and looser networks at the periphery of thrombi (23, 33).
It might be speculated that a constellation of higher clot permeability, and shorter lysis time, found in PE patients compared with DVT, contributes to clot fragmentation and the subsequent embolisation (45). Genetic background of abnormal fibrin clot properties in VTE has been supported by the observation that the clot phenotype is intermediate in relatives of VTE patients, between those seen in controls and VTE patients (45). Patients with DVT after 5–12 months of anticoagulant therapy displayed not only 20–30% lower clot permeability, but also 25% longer lysis time and 8% lower rates of the D-dimer release from fibrin clots compared with controls (46). The presence of non-severe thrombophilia, the localisation of DVT (proximal/distal), or time since VTE were not associated with fibrin clot characteristics (46). Importantly, the presence of residual vein thrombosis (RVT) was associated with shorter lag phase, higher maximum absorbancy, lower clot permeability and prolonged lysis time (46). Faster formation of denser fibrin clot displaying impaired lysability in DVT complicated by RVT has been found to be largely driven by small apo(a) isoforms and higher lipoprotein(a) levels (47). Unfavourable fibrin clot phenotype appears to be also involved in a higher risk of postthrombotic syndrome regardless of RVT (Undas A, unpublished data).

Until recently, it has been unclear whether prothrombotic clot phenotype is associated with the risk of recurrent VTE. Our preliminary study addressing this issue has shown that in patients aged 18 to 65 years with a history of first-ever VTE, during a median of 30 months of follow-up recurrent VTE was associated with not only older age, overweight and idiopathic thrombosis, but also with lower clot permeability, prolonged lysis time, faster fibrin formation, and greater maximum absorbance, indicating thicker fibrin fibres, all determined following anticoagulation cessation (48). Importantly, this study suggests that unfavourably altered fibrin clot characteristics measured following discontinuation of anticoagulant therapy could help identify patients at risk for recurrent VTE.

One might suspect that this phenotype represents a common denominator of acquired, inherited and biochemical prothrombotic risk factors for VTE. Importantly, heterozygous form of FV Leiden, the most prevalent genetic risk factor for VTE, is associated with impaired efficiency of lysis in apparently healthy women below 50 years, which is independently predicted by the TAFI activity, and it has no significant effect on the fibrin network structure, reflected by clot permeability (49).

Deficiencies of natural anticoagulants including antithrombin, protein C and protein S, might have an effect on clot structure. Indeed, reduced clot permeability and lysability has been reported in a patient with antithrombin deficiency with normalisation of permeability upon addition of antithrombin to plasma (50). While testing only CLT in Kindred Vermont I, a pedigree at a high thrombosis risk, partially attributable to a type I protein C deficiency, it has been observed that contra-intuitively, protein C-deficiency is linked to shorter CLT; however, prolonged CLT increased the risk of VTE in non-deficient family members (51). Interestingly, this study showed that the heritability of CLT is 42–52% and is in part explained by the prothrombin 20210A mutation (51).

Antiphospholipid syndrome (APS), a systemic autoimmune disease associated with thrombotic complications, most frequently with VTE, has been shown to display lower clot permeability, shorter lag phase, prolonged clot lysis time with lower maximum rate of the increase in D-dimer levels released from plasma clots (52). Patients with “double”- or “triple” antibody positivity had less permeable plasma clots compared with those with one positive antibody, with no difference in clot lysis or fibrin variables between primary and secondary APS (52). Of note, patients with this thrombophilia who experienced PE formed plasma fibrin clots of higher permeability and lysability than those with DVT alone (52).

In patients with previous PE and no pulmonary hypertension, mild hyperhomocysteinaemia has been reported to determine prolonged TAFI-mediated clot lysis and hyperhomocysteinemic patients had a three-fold higher risk to have an impaired fibrinolysis (53). Growing evidence links abnormal fibrin clot properties to the pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH), a severe complication of acute VTE. In 2006 it was
reported that fibrin clots made from fibrinogen purified from patients with CTEPH are partially resistant to plasmin-mediated lysis as compared to healthy individuals (54). This observation suggests that structural or functional abnormalities in fibrinogen molecules and the subsequent fibrin properties may contribute to the development of CTEPH by prolonged presence within the pulmonary arteries and stimulation of remodelling of the thrombi into fibrotic intravascular material. Interestingly, analysis of purified fibrinogen and gene sequencing of patients with CTEPH disclosed a relatively high incidence of inherited dysfibrinogenaemias (5 of the 33 patients) characterised by abnormal fibrin clot structure and lysis (55). Moreover, Marsh et al. (56) have reported that in patients with these CTEPH-associated dysfibrinogenaemias, there are low clot turbidity, decreased porosity, and fibrinolytic resistance, combined with disorganised fibrin network composed of thinner fibres and more extensive fibre branching. They concluded that abnormal clot architecture and fibrinolytic resistance may contribute to incomplete clot resolution in this form of CTEPH.

Fibrin clots and myocardial infarction (MI)

Acute intraluminal coronary artery thrombus formation resulting mostly from atherosclerotic plaque rupture results in a blood flow cessation in an infarct-related artery area leading to ST-segment elevation MI (STEMI). Scanning electron microscopy of intracoronary thrombi from STEMI patients (Figure 3) showed that thrombus composition evolves over time during the acute phase of MI and fibrin content increased from 48.4% in thrombi collected <3 hours from symptom onset to 66.9% in those collected after 6 hours (h), whereas platelet content decreased from 24.9% to 9.1% (57). Ischaemic time, but not fibrinogen and C-reactive protein (CRP), was the only predictor of thrombus composition with a two-fold increase in fibrin content per each ischaemic hour (57).

Compared to stable coronary artery disease (CAD), acute coronary events (ACS) have been associated with the formation of less permeable and lysable clots in plasma drawn within the first 12 h from the onset of chest pain (58). Moreover, plasma clots in patients with acute MI contained thicker fibres and began polymerisation faster than those of stable angina patients matched for potential confounders (Figure 2B). In contrast to stable angina, clot permeability and fibrinolysis in acute MI patients were determined by the degree of oxidative stress and stimulation of inflammation (58). A potent modulator of fibrin clot properties in acute MI is acute hyperglycaemia that has been reported to impair efficiency of fibrinolysis, with no effect on clot permeability (59).

A history of MI has also been shown to be linked with prothrombotic fibrin clot characteristics, including increased stiffness and shorter fibres of impaired lysability in young patients as compared to healthy controls (60). Patients aged below 50 years after a first MI had longer CLT, which was associated with body mass index, blood pressure and CRP (61), with a two-fold increase after adjustments, while older patients displayed no such association (61). A role of genetics in the clot structure and function has been supported by the observation that first-degree relatives of patients with MI have similar, but milder alterations in fibrin structure (62). A global test performed in 800 MI cases versus controls showed that abnormal haemostasis in platelet-poor plasma, reflected either as an attenuated fibrinolytic capacity or the resulting increase of fibrin formation, was associated with increased MI risk (63). Altered plasma fibrin clot structure/function, including reduced clot permeability and susceptibility to lysis, characterise also patients with a specific form of MI induced by stent thrombosis (64).

Gender-associated differences in the association between fibrin clot phenotype and MI have been demonstrated. For example, the RATIO case-control study performed in young women showed that impaired fibrinolysis is associated with an increase in risk of MI, especially when combined with oral contraceptive use and cigarette smoking (65).

Among comorbidities associated with both unfavourable plasma fibrin clot properties and elevated risk of MI, of importance are end-stage renal failure and diabetes. Impaired endogenous thrombolysis reflected by prolonged clot lysis was associated with 4-fold higher risk of the composite endpoint of cardiovascular death, non-fatal MI, or stroke (66). A small prospective study demonstrated an increased risk of arterial thrombotic events associated with reduced clot permeability and lysability in subjects with end-stage renal disease during a three-year follow-up (67). Type 2 dia-

![Figure 3: Scanning electron microscopic (SEM) images showing fibrin-rich thrombi obtained from coronary arteries of patients with STEMI within 6 h since chest pain onset. a) Relatively thin fibrin fibres aligned in the direction of arterial blood flow. b) Dense fibrin network close to the surface of a damaged atherosclerotic plaque. Magnification 3500x.](image-url)
betes markedly impairs fibrin clot phenotype in association with the level of glycaemia control and disease duration (68, 69).

It has been postulated that abnormalities in fibrin clot properties observed in MI are largely driven by the effects of increased oxidative stress and thrombin generation, supported by prothrombotic actions of various molecules released following platelet activation like some proteins, e.g. platelet factor 4, or beta-thromboglobulin, and possibly polyphosphates (11, 14, 58, 70). Different levels of oxidation of fibrinogen result in various changes in clot characteristics ranging from impaired fibrin formation to the formation of a dense network of thin fibres (11, 71). Recently, enhanced oxidation has been shown to lead to prothrombotic alterations in fibrin clots after activation by thrombin and delayed fibrin lysis induced by plasmin and t-PA, with abnormal linear and non-linear mechanical properties of oxidised fibrin gels (72). It has been postulated that methionine oxidation of specific residues may be related to hindered lateral aggregation of protofibrils in fibrin gels (67). Serum F2-isoprostanes, produced upon non-enzymatic arachidonic acid peroxidation, as well as a specific platelet activation marker, beta-thromboglobulin, have been shown to correlate with clot permeability and fibrinolysis in patients with acute MI (58). Given a substantial increase in thrombin formation in MI and consistent data showing that clots formed in the presence of high thrombin concentrations are composed of thin fibres and are relatively resistant to fibrinolysis (73), a role of this factor in promoting prothrombotic fibrin clot phenotype appears potent. This is supported by significant associations between thrombin generation and clot characteristics measured in plasma-based assays (68).

**Fibrin clots and ischaemic stroke**

Precerebral or cerebral artery occlusions account for three fourths of acute strokes and thrombotic material may originate from diverse sources, including venous "paradoxical" sites, intracardial left-sided cavities, or superimposed thrombi on atherosclerotic lesions in the aorta and carotid arteries. The histological analysis of thromboemboli retrieved by endovascular mechanical extraction from the middle cerebral artery and intracranial carotid artery of patients with acute ischaemic stroke showed random fibrin fibres mixed with platelet deposits with a marked heterogeneity (74). It is estimated that fibrin constitutes about 60% of the retrieved thrombi obtained from stroke patients and the most common type of the thrombi (44%) is fibrin-dominant (75). There was no correlation between thrombus histopathology with stroke severity, functional outcomes, stroke aetiology, and unlike MI, with the timing of clot extraction (75).

Acute ischaemic stroke within the first 72 h of symptom onset, like acute MI, is associated with reduced plasma clot permeability by 30% and prolonged lysis time by 11%, coupled with faster fibrin formation by 8% and larger plasma fibrin clot mass by 17%, as compared to healthy controls (76). Patients with acute stroke and concomitant CAD had significantly prolonged clot lysis when compared to those without history of CAD. Fibrin clot compaction correlated with neurological deficit both on admission and at discharge of patients admitted for acute ischaemic stroke (76).

Patients who survived ischaemic stroke have been reported to display abnormal plasma fibrin clot properties (Figure 2C). The first report on such association was published in a study on patients with cryptogenic stroke (77). More compact plasma clots resistant to lysis were shown when clots were generated ex vivo from plasma samples obtained 3-19 months after the event (77). Scanning electron microscopy of fibrin clots showed increased fibre diameter and density in stroke patients (77). Interestingly, lower clot permeability and reduced fibrinolysis observed within the first 24 h since the onset of stroke symptoms remained unaltered after 60 days from the event suggesting that hypofibrinolysis is a persistent feature of ischaemic stroke (78).

Since 15% of ischaemic strokes occur in patients with atrial fibrillation (AF) and they are mostly caused by thrombotic material formed within the left atrium, in 90% in its appendage, unfavourably clot properties might also increase the risk of cerebral infarction. It has been shown that patients with chronic AF and previous stroke have prolonged CLT combined with higher TAFI antigen than the subjects without history of this complication (79). Of note, CLT, PAI-1, TAFI activity, and soluble thrombomodulin were positively correlated with CHA2DS2-VASc scores, but not with time from thrombotic event to blood collection (79). Similarly, lower clot permeability in association with CHA2DS2-VASc scores has been observed in AF patients without cerebrovascular events (A. Undas, unpublished data).

Plasma fibrin clots from APS patients who experienced stroke and/or MI were less permeable and were lysed faster compared with those with VTE alone (52). This study is the first to show that APS is associated with prothrombotic plasma fibrin clot phenotype, with worse characteristics in patients following arterial thrombosis.

Analysis of CLT in the presence of TF and phospholipid vesicles demonstrated that prolonged fibrinolysis was not associated with an increase in risk of ischaemic stroke, whereas hyperfibrinolysis, in particular oral contraceptive user and current smokers four-fold increased this risk in young women (65). Some investigators have suggested that, since in terms of aetiology MI is largely thrombotic by nature, while ischaemic stroke is not, association between clot phenotype measured ex vivo and stroke will be more complex and heterogeneous.

Fibrin content within thromboemboli in acute ischaemic stroke could affect the outcomes of the current therapy. Experimental studies in a porcine model have suggested that cerebral arteries occluded by fibrin-rich clot demonstrated a significantly lower recanalisation rate and a longer mean recanalisation time than did arteries occluded by erythrocyte-rich clots (80). The angiographic outcome of mechanical thrombectomy can be influenced by the histology of the occluding thromboembolus (80).

Overall, atherothrombotic and AF-associated ischaemic stroke as well as that observed in some thrombophilic patients are linked with prothrombotic alterations in fibrin structure and function, indicating common mechanisms leading to cerebrovascular and coronary thromboembolic episodes. Most likely, all types of ischaemic strokes cannot share similar fibrin characteristics.
Fibrin clots and other thrombotic disorders

Patients following retinal vein occlusion have been reported to display abnormal clot characteristics including faster formation of significantly less permeable and poorly lysable dense plasma fibrin clots (81). Prolonged CLT in association with endogenous thrombotic potential in such patients has been confirmed recently (82). Another pathology associated with the risk of blindness is diabetic retinopathy, in which unfavorable fibrin clot phenotype has also been observed suggesting its pathophysiologic role in this disorder (83).

Thrombosis at unusual location might also develop in subjects that form denser and poorly lysable fibrin clots. For example, a particularly reduced plasma clot permeability and susceptibility to tPA-mediated lysis has been reported in a patient with thrombus in the aorta complicated by distal embolisation (84).

Fibrin clots and inflammatory diseases

The molecular processes of inflammation and thrombosis are closely intertwined. Prothrombotic fibrin clot phenotype characterised by low permeability and susceptibility to lysis has been observed in systemic inflammatory disorders including rheumatoid arthritis (85), chronic obstructive pulmonary disease (86) and inflammatory bowel disease (87). A major mechanism underlying associations between inflammation and abnormal fibrin clot properties is likely to result from binding of CRP with fibrinogen and fibrin (88). This is supported by a significant correlation between serum CRP and plasma clot permeability as well as clot lysability not only in inflammatory diseases (85–87), but also in atherosclerotic vascular disease (89, 90). CLT also correlates with CRP as shown by De Lange et al. (91). Interaction between fibrin clot function and inflammatory disorders is an additional aspect given evidence that fibrin and also fibrin degradation products modulate the inflammatory response by affecting leukocyte migration and cytokine production, with proinflammatory effects for most products and a potent anti-inflammatory effect observed for the Bβ15–42 fragment (92). It is worth noting that low-grade inflammation affects fibrin clot properties in a different way compared with endotoxaemia, which induced enhanced fibrinogen consumption followed by downregulation of fibrinolysis (93).

Fibrin clots and non-thrombotic pathologies

Patients with abdominal aortic aneurysm (AAA) typically associated with thrombi within the widened aorta have shown to display smaller plasma clot pore size with its stepwise reduction from controls via small to large AAA (94). Moreover, the lag phase for plasma clot formation and lysis time were prolonged in a stepwise manner, with no impact of thrombin generation (94). Interestingly, structure of clots made from purified fibrinogen was similar in AAA patients and controls, which provided additional evidence for a potent modulatory effect of other plasma factors or circulating molecules (94). It remains to be established whether the fibrin alterations in patients with AAA contribute to clinical outcomes, including thromboembolic complications.

Formation of denser and poorly lysable plasma clots have been shown to increase fibrin accumulation on the surface of human retinal vessel wall, which might indicate that prothrombotic fibrin clot phenotype predisposes to faster progression of aortic stenosis, the most common valvular defect in the elderly (95).

Another common disease associated with increased risk of thromboembolic events is hyperthyroidism, which has also been demonstrated to be linked to 25% higher clot maximum absorbance and 12% longer clot lysis time compared with controls, and these unfavourable alterations correlated with free thyroxine (96).

During a long-term follow-up, the progression in both patients with peripheral arterial disease and Buerger's disease, or thromboangiitis obliterans, has been observed to be associated with about 15% lower clot permeability and 11% prolonged clot lysis (97).

Growing evidence suggests that in a majority of the diseases associated with the risk of thromboembolism, prothrombotic clot

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Table 1: Interventions that could improve fibrin clot structure and stability (13, 92–100).

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<tr>
<td>Smoking cessation</td>
<td>↓ oxidative stress</td>
</tr>
<tr>
<td>Treatment with antidiabetic agents (insulin and metformin)</td>
<td>↓ glycation of fibrinogen and plasminogen ↓ inflammatory state</td>
</tr>
<tr>
<td>Statin use (simvastatin and atorvastatin)</td>
<td>↓ thrombin generation ↓ inflammatory state</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>↓ acetylation of fibrinogen</td>
</tr>
<tr>
<td>Blood pressure lowering therapy</td>
<td>↓ inflammatory state</td>
</tr>
<tr>
<td>Administration of folic acid</td>
<td>↓ homocysteinylation of fibrinogen</td>
</tr>
<tr>
<td>Administration of polyunsaturated omega-3 fatty acids</td>
<td>↓ thrombin generation ↓ oxidative stress</td>
</tr>
<tr>
<td>Anticoagulant therapy (vitamin K antagonists, heparins, fondaparinux, dabigatran, rivaroxaban)</td>
<td>↓ thrombin generation</td>
</tr>
</tbody>
</table>

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phenotype can be found, although indirect effects of acquired factors of varying intensity, in particular enhanced inflammatory state, account usually for this phenotype.

**Beneficial changes in fibrin clot phenotype**

As shown in Table 1, a number of lifestyle modifications and therapeutic interventions have been reported to favourably modify fibrin clot properties (14). The magnitude of the post-intervention changes in most fibrin clot variables has been estimated at 10–20% of the baseline values.

Among medications (apart from anticoagulants), the strongest evidence from several groups shows favourable alterations in clot structure following administration of low-dose aspirin largely through acetylation of fibrinogen (98, 99). There are also data suggesting improved clot permeability and lysability in response to cholesterol-lowering statins (100) and anti-hypertensive agents, in particular angiotensin-converting enzyme inhibitors (101). Lowering homocysteine during administration of folic acid can also improve plasma fibrin clot properties (102). In diabetic patients better glycaemia control also improves fibrin clot properties through several mechanisms, including reduced glycation of fibrinogen and other proteins like plasminogen (99, 103). Limited data suggest that smoking cessation (104) and administration of polyunsaturated omega-3 fatty acids (105) might also increase clot permeability and susceptibility to lysis.

**Conclusions**

An increasing number of epidemiological and case-control studies indicate that various clinical entities manifest as venous or arterial thromboembolic events are associated with certain so-called prothrombotic structural characteristics of a fibrin clot, reflected by denser networks that are formed quickly and are relatively resistant to lysis, thus proving additional argument for the concept of clinically relevant substantial similarities in the pathophysiology of thrombosis occurring in the venous and arterial vascular beds (106, 107). There are, however, some differences between fibrin characteristics observed in various thrombotic diseases, in particular larger clot porosity and facilitated lysis in PE compared with DVT. Clot properties in thrombotic disease are predominantly modulated by multiple environmental factors that undergo dynamic changes over time largely in response to vascular injury and its systemic consequences. Of note, in vivo important modulators of fibrin characteristics and efficiency of lysis represent blood flow conditions and cells that are in contact to blood. Clot structure and its key function, susceptibility to enzymatic lysis and mechanical deformation, influence the persistence of thrombi and their removal from the vessels, and might contribute to clinical outcomes, for instance recurrent VTE. Further experimental and clinical studies on modulation of clot stability are needed to allow novel treatment options to alter fibrin clot properties in vivo and thereby potentially prevent or limit thrombotic disorders that are associated with substantial morbidity and mortality.

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**Conflicts of interest**

None declared.

**References**

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