

General mechanisms of coagulation and targets of anticoagulants (Section I)

Position Paper of the ESC Working Group on Thrombosis – Task Force on Anticoagulants in Heart Disease

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Summary

Contrary to previous models based on plasma, coagulation processes are currently believed to be mostly cell surface-based, including three overlapping phases: initiation, when tissue factor-expressing cells and microparticles are exposed to plasma; amplification, whereby small amounts of thrombin induce platelet activation and aggregation, and promote activation of factors (F)V, FVIII and FXI on platelet surfaces; and propagation, in which the Xase (tenase) and prothrombinase complexes are formed, producing a burst of thrombin and the cleavage of fibrinogen to fibrin. Thrombin exerts a number of additional biological actions, including platelet activation, amplification and self-

inhibition of coagulation, clot stabilisation and anti-fibrinolysis, in processes occurring in the proximity of vessel injury, tightly regulated by a series of inhibitory mechanisms. "Classical" anticoagulants, including heparin and vitamin K antagonists, typically target multiple coagulation steps. A number of new anticoagulants, already developed or under development, target specific steps in the process, inhibiting a single coagulation factor or mimicking natural coagulation inhibitors.

Keywords

Anticoagulants, coagulation, tissue factor, heart disease, coronary heart disease, heart failure, atrial fibrillation

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Introduction

Drugs that interfere with blood coagulation (anticoagulants) are a mainstay of cardiovascular therapy. Despite their widespread use, there are still many unmet needs in this area, prompting the development of an unprecedented number of new agents. A Task Force of coagulation experts and clinical cardiologists appointed by the European Society of Cardiology (ESC) Working Group on Thrombosis will review the entire topic of anticoagulants in heart disease. The project is intended to follow and complement the recent Task Force document on the use of antiplatelet agents in cardiovascular disease (1), a previous comprehensive document on anticoagulants

in heart disease (2), and an updated summary on new anticoagulants (3).

Section I, presented here, provides

- (a) a general overview of coagulation in relation to the pathogenesis of thrombosis in heart disease;
- (b) an overview of current targets of anticoagulants;
- (c) epidemiological data on the use of anticoagulants in heart disease.

Future Sections will deal with parenteral anticoagulants (*Section II*), vitamin K antagonists (*Section III*), new anticoagulants in acute coronary syndromes (*Section IV*), and special situations (*Section V*).

Blood coagulation in relation to heart disease

Haemostasis

Under physiological conditions and with intact blood vessels, the haemostatic system maintains circulating blood in a fluid phase. Haemostasis, i.e. the arrest of haemorrhage preventing blood loss upon blood vessel damage – rapidly sealing the site of disruption in most cases – occurs through the concerted action of platelets, the coagulation system, and fibrinolysis, with the additional contribution of a vasomotor response. Haemostasis occurs through the rapid formation of an impermeable platelet and fibrin plug (haemostatic thrombus) at the site of injury. To prevent propagation of this platelet-fibrin thrombus into the vascular lumen, the activation of platelets and coagulation is localized to the site of injury. In addition, fibrin within the thrombus triggers its own dissolution by plasmin-mediated fibrinolysis, which further limits thrombus propagation. Maintenance of blood fluidity within the circulation and the ability to prevent blood loss after vessel injury reflects therefore a delicate balance among tightly regulated platelet function, coagulation and fibrinolysis (*haemostatic balance*) (4). Disturbances in the regulation of the balance may cause the formation and deposition of too little fibrin at the site of injury, resulting in impaired haemostasis – ultimately manifesting as bleeding – or enhanced fibrin formation and deposition – causing thrombosis (4, 5).

The initiation of coagulation: local exposure of tissue factor

Coagulation is initiated when tissue factor (TF), normally segregated from the flowing blood, is exposed to plasma, binding coagulation factor (F) VII/VIIa and forming a complex on cellular surfaces that triggers the coagulation cascade. TF (CD142), a transmembrane glycoprotein, is a member of the class II cytokine receptor superfamily, and functions both as receptor and essential cofactor for FVII and FVIIa. In the vessel wall, TF is constitutively expressed by vascular smooth muscle cells, adventitial fibroblasts and pericytes, the cells that surround blood vessels and large organs. This creates a haemostatic barrier that triggers coagulation when the integrity of the vessel wall is compromised. The expression of TF can also be induced in monocytes and, to some extent, in endothelial cells in response to various stimuli, including inflammatory cytokines, endotoxin, growth factors and oxidised/modified low-density lipoproteins (LDL). Such expression may lead or contribute to thrombosis under certain pathological conditions, such as sepsis and disseminated intravascular coagulation (6, 7). Total lethality in homozygous TF knock-out mice embryos provides convincing evidence that TF is indispensable for life. Different animal models have enabled the exploration of the role of TF in thrombosis. Mice expressing low TF have reduced thrombosis in a carotid artery injury model, where the vessel wall, mostly in the adventitial layer, provides the major source of TF (8). Mice lacking TF in smooth muscle cells also show reduced carotid arterial thrombosis (9) and inhibitors of the TF/FVIIa complex reduce

thrombosis in pigs (10), rabbits (11) and humans (12, 13). Altogether, these data suggest that inhibition of the initiation of coagulation at the level of TF/FVIIa may provide a novel approach for prevention of thrombotic events, although the bleeding risk connected with this approach is still largely unknown.

Beyond the role in haemostasis, the binary TF/FVIIa-complex and the ternary TF/FVIIa/FXa complex elicit intracellular signalling events that result in the induction of genes involved in diverse biological functions that include embryonic development, cell migration, inflammation, apoptosis and angiogenesis (14-17).

Circulating TF and tissue factor pathway inhibitor

In healthy individuals, TF is present in the bloodstream at very low concentrations, mainly localised to monocytes and to TF-bearing microparticles (MPs) derived from monocytes and platelets (18). MPs are cell membrane-derived fragments with a diameter of 0.1-1.0 µm that are released upon cell activation or during apoptosis (19). These MPs consist of proteins and lipids, and may contain DNA, mRNA and microRNA. Because they are cell membrane-derived, MPs express antigens on their surface similar to those of the parent cells from which they originate (20). By exposing phosphatidylserine and expressing TF on their surface, MPs can initiate and propagate coagulation (21). Increased numbers of TF-bearing MPs have been reported in patients with established cardiovascular disease and in those with cardiovascular risk factors, such as diabetes, dyslipidaemia, hypertension (20), as well as in patients with atrial fibrillation (22). Although it is unlikely that neutrophils are capable of *de novo* TF synthesis, TF-positive MPs may transfer TF to neutrophils (23).

Alternatively-spliced TF is another form of circulating TF. This TF derivative, which is formed upon splicing exon 4 directly to exon 6, lacks the transmembrane domain (24). Alternatively-spliced TF is produced by monocyte/macrophages, and has been postulated to play a role in atherothrombotic disease (18). However, without the membrane binding properties of TF, it has been shown to lack procoagulant activity (25), and is therefore unlikely to play a part in coagulation.

Tissue factor pathway inhibitor (TFPI), a Kunitz type inhibitor, is an important regulator of TF/FVIIa-induced coagulation. TFPI functions by neutralising the catalytic activity of FXa and, in the presence of FXa, by feedback inhibition of the TF/FVIIa complex (26). TFPI contains three Kunitz-type domains; the first binds to FVIIa and the second to FXa. The third C-terminal domain is involved in binding of TFPI to lipoproteins and to cell surfaces (27). Although the primary site of TFPI synthesis is the vascular endothelium (28), other cell types reported to synthesise TFPI include megakaryocytes/platelets and monocytes. *In vivo*, only 20% of TFPI is present in plasma, where it circulates in complex with low-density lipoproteins (LDL). A major pool of TFPI is associated with the endothelial surface and is rapidly released into the circulation after administration of heparin, or by thrombin or shear forces (29). Protein S serves as a cofactor for TFPI and enhances the rate of TFPI-mediated inhibition of FXa by 10-fold (30). Because of its high affinity for negatively-charged phospholipids,

protein S may increase the affinity of TFPI for the surface of activated platelets, thereby increasing the local concentration of TFPI (31). Because of its potential to downregulate coagulation, recombinant TFPI (tifacogin) was tested in patients with severe sepsis in the OPTIMIST trial. Unfortunately, treatment with tifacogin had no effect on all-cause mortality and was associated with an increased risk of bleeding (32). Nonetheless, tifacogin reduced mortality in patients with a normal international normalised ratio (INR) at baseline (32), raising the possibility that it may have potential in some patients.

A cell-based model of coagulation

Coagulation has been classically depicted in terms of an *extrinsic pathway* (initiated by TF/FVIIa), an *intrinsic pathway* (explaining coagulation occurring when plasma is in contact with negatively charged surfaces – contact phase activation), and a *common pathway*, proceeding after the activation of FX (33). In a more modern conception, however, the coagulation process in whole blood in contact with injured blood vessels consists of highly regulated reactions that take place on cell surfaces (34, 35). Coagulation thus occurs in three overlapping phases: initiation, amplification and propagation (36-38). The process starts on TF-exposing cells, and continues on the surfaces of activated platelets.

The initiation phase is localised to TF-bearing cells that are exposed after endothelial injury or are tethered to endothelial cells via adhesion molecules that are expressed when endothelial cells are activated. The proteolytic TF/FVIIa complex activates small amounts of FIX and FX. On TF-expressing cells, FXa then associ-

ates with FVa to form the prothrombinase complex (► Figure 1). FVa is derived from several sources: it is released from activated platelets adhering at injury sites, or it can come from plasma, where FV can be activated by thrombin or, less efficiently, by FXa. The prothrombinase complex cleaves prothrombin to generate small amounts of thrombin, the enzyme responsible for fibrin formation. The relative concentrations of TF/FVIIa complex and TFPI determine the duration of this initiation phase. When FXa is generated, it is bound by TFPI, and a quaternary complex with TF and FVIIa is then formed, which inhibits VIIa. In contrast to FXa, FIXa is not inhibited by TFPI, and is only slowly inhibited by anti-thrombin. FIXa moves from TF-bearing cells to the surface of activated platelets that localise at the injury site.

In the amplification phase, low concentrations of thrombin activate platelets adhering to the injury site, thereby inducing the release of FV and FVa from their α -granules. A positive feed-back loop is initiated, whereby thrombin activates circulating FV and releases FVIII from von Willebrand factor, and activates it. FVa and FVIIIa bind to platelet surfaces and serve as cofactors for the large-scale thrombin generation that occurs during the propagation phase. Thrombin also activates FXI bound to platelets (► Figure 1).

In the propagation phase, the FVIIIa/FIXa complex (termed “intrinsic tenase”) and the FVa/FXa complex (prothrombinase) assemble on the surface of activated platelets and accelerate the generation of FXa and thrombin, respectively. In addition, FXIa bound to the platelet surface activates FIX to form additional intrinsic tenase. FXa rapidly associates with FVa on the platelet surface, resulting in a burst of thrombin, which converts fibrinogen to

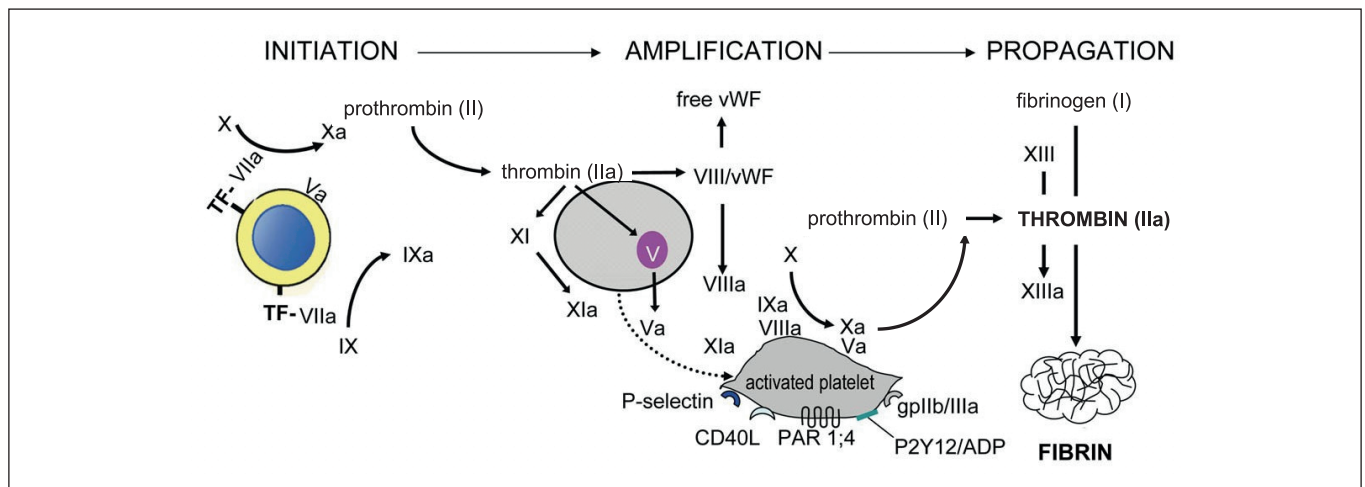


Figure 1: A scheme of current concepts on the coagulation process. The cell surface-based coagulation process includes three overlapping phases. In the initiation phase, upon vascular injury, tissue factor (TF)-expressing cells and microparticles are exposed to the coagulation factors in the lumen of the vessel, and thereby initiate thrombosis. Platelets, activated by vascular injury such as plaque rupture, are recruited and adhere to the site of injury. The TF/FVIIa complex activates coagulation factors IX to IXa and X to Xa, and trace amounts of thrombin are generated. In the amplification phase, this small amount of thrombin is a signal for further platelet acti-

vation and aggregation. On the surface of platelets, thrombin activates FV, FVIII and FXI. In the propagation phase, FVIIIa forms a complex with FIXa (Xase), and FVa forms a complex with FXa (prothrombinase) on the platelet surface, which accelerates the generation of FXa and thrombin, respectively. When FXa associates with FVa, it is protected from tissue factor pathway inhibitor (TFPI) and antithrombin (AT). In the propagation phase, a burst of thrombin is generated, which is sufficient for the clotting of soluble fibrinogen into a fibrin meshwork. A thrombus is thus formed.

fibrin. Soluble fibrin monomers polymerise to form fibrin protofibrils, which are stabilised by FXIIIa (which is also activated by thrombin), to form a solid fibrin network that in turn stabilises platelet aggregates to form a platelet/fibrin thrombus (► Figure 1). Because coagulation comprises a series of enzymatic processes, thrombin generation is the result of an amplifying cascade, with approximately one molecule of FXa generating approximately 1,000 molecules of thrombin (39), thus making upstream inhibition of coagulation, e.g. at the level of FXa, an attractive pharmacological target.

Thrombin serves a number of functions in addition to fibrin formation (► Figure 2), thus expanding the role of coagulation inhibitors, beyond such interference, to platelet activation and inflammation (see below).

Role of the contact phase

Hereditary deficiency of FXII (Hageman factor) or FXI, plasma proteases that initiate the intrinsic pathway of coagulation, has long been known to have a minimal impact on haemostasis. However it has been recently appreciated that such deficiency impairs thrombus formation and provides protection from vascular occlusive events (40). As the FXII-FXI pathway contributes to thrombus formation to a greater extent than to normal haemo-

stasis, pharmacological inhibition of these coagulation factors may offer the exciting possibility of anticoagulation therapies with minimal or no bleeding risk (40). Such concepts, however, have not yet been translated into human trials.

Natural anticoagulant mechanisms

Thrombin generation and fibrin formation occur rapidly at sites of vascular injury. To control and localise these processes, a number of inhibitory mechanisms are in place. Regulation of coagulation is exerted at multiple levels, either by enzyme inhibition or by modulation of the activity of the cofactors. Antithrombin, protein C and protein S are the most important regulators of coagulation. Together with TFPI and the fibrinolytic system, they constitute the main natural anticoagulant and antithrombotic mechanisms in the organism. Thus, patients with a familial deficiency in one or the other of these components tend to develop thromboembolic complications (thrombophilia). Knowledge of natural coagulation inhibitors is guiding the development of several new anticoagulants.

Most of the enzymes generated during activation of coagulation are inhibited by the serine-protease inhibitor antithrombin (AT), previously called AT III. AT preferentially inhibits free enzymes, whereas enzymes that are part of the intrinsic tenase or prothrombinase complexes are less accessible for inhibition. AT probably physiologically limits the coagulation process to sites of vascular injury and protects the circulation from liberated enzymes (33, 37). AT is, in itself, an inefficient inhibitor, but heparin and the heparin-like molecules that are present on the surface of endothelial cells stimulate its activity (see below).

Thrombomodulin (TM), a transmembrane molecule expressed on endothelial cells, binds thrombin, and the thrombin/TM complex activates protein C, a vitamin K-dependent proenzyme, to an active serine protease. The activated protein C (APC) anticoagulant system regulates coagulation by modulating the activity of the two cofactors, FVIIIa and FVa (33). The activation rate of thrombin-mediated protein C activation is slow, but is increased at least 100-fold when thrombin binds to TM. The rate increases another 20-fold when protein C binds to endothelial protein C receptor (EPCR), which presents protein C to the thrombin/TM complex for efficient activation, highlighting a mechanism for endothelial cell localisation of anticoagulation. Thus, thrombin (► Figure 2) has the capacity to express both procoagulant and anticoagulant functions depending on the context under which it is generated. At sites of vascular disruption, the procoagulant effects of thrombin are fully expressed. In contrast, with an intact vascular system, thrombin has an anticoagulant function since it binds to TM and activates protein C.

Another vitamin K-dependent cofactor protein, protein S, supports the anticoagulant activity of APC. In human plasma, about 30% of protein S is free, the remainder being bound to the complement regulatory protein C4b-binding protein. APC and free protein S form a membrane-bound complex, which can cleave FVIIIa and FVa, even when these are part of the fully assembled intrinsic tenase and prothrombinase complexes. *In vivo*, APC does not cleave intact FVIII because the binding of FVIII to von Willebrand

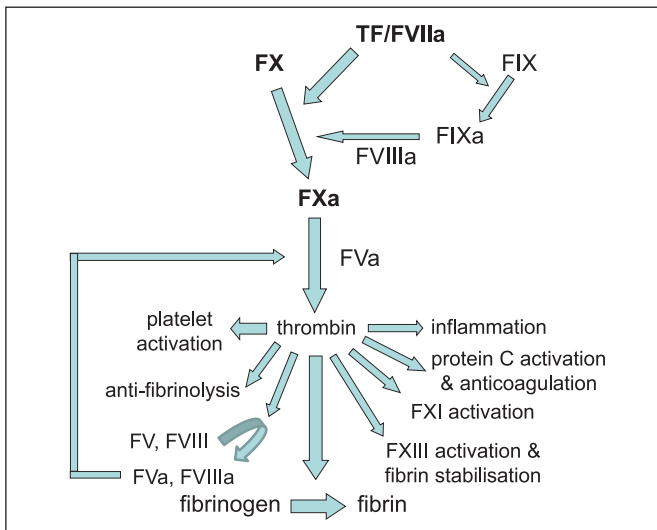


Figure 2: Multiple actions of thrombin. As the final coagulation enzyme, thrombin exerts multiple biological actions, only one of which, the best recognised over time, is the cleavage of fibrinogen to generate fibrin. In addition, by engaging protease-activated receptors (PARs)-1 and -4 present in platelets and multiple cell types, thrombin promotes platelet activation and aggregation; and exerts pro-inflammatory actions. Thrombin also amplifies clotting by activating coagulation FXI and the cofactors FV and FVIII into FVa and FVIIIa, respectively; and it stabilises clots by activating FXIII. Thrombin also exerts anti-fibrinolytic actions, through the activation of thrombin activatable fibrinolysis inhibitor (TAFI), providing a molecular link between coagulation and inhibition of fibrinolysis; thrombin promotes the activation of protein C and protein S, two natural vitamin K-dependent anticoagulant proteins that contain the coagulation process by inactivating FVa and FVIIIa.

factor prevents it from interacting with the phospholipid membranes. In contrast, APC is able to cleave FV, which binds phospholipids to a similar extent as FVa. The consequence of APC-mediated cleavage of FV is the generation of anticoagulant FV that functions in synergy with protein S as an APC cofactor in the degradation of FVIIIa. Thus, FV can function as a procoagulant and an anticoagulant cofactor. Procoagulant FVa is formed after limited proteolysis by thrombin or FXa, whereas anticoagulant FV activity is expressed by FV that has been cleaved by APC. The anticoagulant potential of FV may be particularly important in the regulation of the intrinsic tenase complex by APC and protein S. The physiological importance of the protein C system is shown by the severe thromboembolic disease that is associated with homozygous deficiency of protein C or S in both humans and mice (41). In both cases, the severe lethal thrombotic disease manifests shortly after birth. Mice lacking a functional TM or EPCR gene have even more severe disease and die during embryogenesis, even before development of a functional cardiovascular system (42). Recombinant forms of soluble TM (recomodulin and solulin) and of APC (drotrecogin) have been developed as anticoagulants (see below).

In addition to activation of protein C, the thrombin/TM-complex also activates the thrombin activatable fibrinolysis inhibitor (TAFI), a latent carboxypeptidase (43). Once activated, TAFI slows the rate of fibrin degradation by removing the C-terminal lysine residues from fibrin. Because these lysine residues serve as binding sites for tissue-plasminogen activator (t-PA) and plasminogen, their removal renders fibrin more resistant to lysis.

Decreased overall fibrinolytic potential, occurring in patients with congenital plasminogen, alpha2-antiplasmin or t-PA deficiency, or with high plasma levels of TAFI have been associated with the risk of venous thrombosis, whereas little evidence exists for their role in arterial thrombosis. Increased levels of plasminogen activator inhibitor (PAI)-1 have been conversely associated with arterial thrombosis (44).

Several fibrinolytic proteins have activities that extend beyond fibrinolysis, such as inflammation, vascular remodelling, and atherosclerosis (44).

Cross-talk between coagulation and inflammation

Coagulation and inflammation are integrated processes (45, 46). This cross-talk is highlighted by thrombus formation superimposed on ruptured atherosclerotic plaques, which contain an abundance of inflammatory cells, as well as by the increased prevalence of atherothrombosis (myocardial infarction) in inflammatory rheumatic diseases (47).

Coagulation proteases modulate inflammation by activating protease activated receptors (PARs), and by binding to other cell surface receptors, such as TM and EPCR (48, 49). PARs are a family of G protein-coupled receptors expressed on a variety of cells, including platelets, endothelial cells and leucocytes. Platelets express PAR-1 and -4, which serve as thrombin receptors. Thrombin binds to the extracellular domain of these receptors, where it cleaves a specific peptide bond, thereby generating a new N-termi-

nus that serves as a tethered ligand by folding back and interacting with the body of the receptor. In platelets, this induces platelet activation, the expression of P-selectin and CD40 ligand (CD40L), and the release of inflammatory cytokines and growth factors (49). Among its numerous biological functions, thrombin is chemotactic for leukocytes and promotes the expression of adhesion molecules on the surface of these cells (► Figure 2). Cross-talk between cells in platelet-leukocyte complexes occurs via P-selectin and CD40L, and leads to TF expression and further cytokine release. PAR-1 may also bind the ternary complex TF/FVIIa/FXa. In addition, APC bound to the endothelial protein C receptor (EPCR) on endothelial cells promotes anti-inflammatory and cytoprotective signalling through the activation of endothelial PAR-1 (50).

PAR-2 does not bind thrombin, but the TF/FVIIa complex and FXa can activate this receptor (51). Activation of PARs by the various coagulation proteases results in the upregulation of genes involved in inflammation, including interleukin (IL)-8 and tumour necrosis factor (TNF)- α . The TF/FVIIa complex also can initiate various intracellular signalling events, such as the activation of mitogen-activated protein kinase (MAPK) pathways and phosphatidylinositol-3 kinase (PI3K)/AKT. TF/FVIIa-induced signalling events can modulate cell fate and behaviour, rendering cells and tissues proliferative, pro-migratory, and resistant to apoptosis. Based on these findings, PAR inhibitors are under development and PAR-1-targeting drugs have undergone phase III clinical trial evaluation (52, 53).

In addition to the role of PARs in inflammation, additional cross-talk occurs at the level of FXa. This concept is highlighted by the recent demonstration that lufaxin, a FXa inhibitor from the salivary glands of blood-sucking arthropods, not only inhibits thrombosis in mice, but also attenuates oedema formation triggered by FXa injection into their paws (54).

Variable mechanisms of thrombosis in heart disease

Although thrombosis occurs because of excess activation of platelets and coagulation, distinct mechanisms underpin thrombosis in different heart diseases (55), offering opportunities for targeted antithrombotic strategies.

Arterial thrombosis, the leading cause of myocardial infarction, occurs in the vast majority of cases as a complication of atherosclerosis (atherothrombosis) through at least two different mechanisms: erosion of the endothelium or plaque rupture (56-58). Superficial erosion or desquamation of endothelial cells lining the plaque accounts for about 25% of all cases of fatal coronary thrombosis (59), while plaque rupture accounts for most of the remainder. When plaques rupture, there is an exposure of thrombogenic material from the core of the plaque to the flowing blood. Exposure of the lipid core, which is rich in TF, and of the underlying connective tissue matrix rich in collagen leads to activation of platelets and coagulation, and to the release of vasoactive substances, which induce thrombus formation and vasoconstriction.

Unless these processes are rapidly counteracted or an adequate collateral circulation is present in the heart, myocardial ischaemia and an acute coronary syndrome (ACS) may result. An occlusive thrombosis is most often found in ST-elevation myocardial infarction (STEMI), which is due to the complete interruption of coronary blood flow and the ensuing ischaemia in the dependant territory. In the case of mural thrombosis, there is more often a “waxing-and-waning” course of ischaemia, with the prevailing consequence of non-ST elevation ACS (NSTEMI-ACS) (56). While STEMI is characterised by propagation of thrombosis (red thrombus), making it susceptible – in most cases and if given early enough – to fibrinolytic treatments, in NSTEMI-ACS there is a minimal propagation component and thrombi are platelet-rich and largely resistant to fibrinolytic drugs (56). In the proximity of the ruptured plaque, thrombi possess both a platelet and a fibrin component, thus prompting the use of antiplatelet agents and anticoagulants as therapeutic strategies.

Contrary to atherothrombosis, where there is a prominent role of both platelets and coagulation, thrombosis in the left atrium/left atrial appendage in the setting of atrial fibrillation (AF) (60, 61) or in akinetic or dyskinetic areas of the left ventricle in the case of heart failure (62, 63) appears to be mostly caused by blood stasis and – to some extent – blood hypercoagulability (64). Consequently, these thrombi have a larger fibrin component than platelet component. Blood stasis is necessary but insufficient to increase the thromboembolic risk in AF, as demonstrated by the low risk in the absence of risk factors (65). The relative importance of coagulation over platelets in AF is highlighted by the fact that warfarin produces a greater reduction in stroke in such patients than either aspirin or aspirin plus clopidogrel (66). Likewise, apixaban also was superior to aspirin for stroke prevention (67).

Targets of anticoagulants

The targets of anticoagulants in current use or in development are depicted in ► Figure 3 and ► Figure 4, placing them in the context of the familiar traditional scheme of blood coagulation.

Heparins [unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH)] and vitamin K antagonists (VKA) are among the oldest anticoagulants in clinical use; their main sites of action are shown in ► Figure 3.

Heparin consists of a family of highly sulfated polysaccharide chains, ranging in molecular weight from 3,000 to 30,000 Dalton (Da) with a mean of 15,000 Da, which corresponds to about 45 saccharide units (68, 69). Only one third of the heparin chains have anticoagulant activity because they possess the unique pentasaccharide sequence that binds AT with high affinity (68, 69). With higher doses, however, heparin chains with or without a pentasaccharide sequence can activate heparin cofactor II, a second plasma cofactor (70). Heparin catalyses the inhibition of thrombin by AT by simultaneously binding to both AT (via its pentasaccharide sequence) and thrombin. Formation of this ternary heparin/AT/thrombin complex, which bridges the inhibitor and the enzyme together thereby accelerating their interaction (69), can only occur with heparin chains consisting of 18 or more saccharide units (about 5,400 Da). However, shorter pentasaccharide-containing heparin molecules can catalyse FXa inhibition by AT because this reaction does not require bridging (69). To some extent, heparin also catalyses the AT-mediated inhibition of other coagulation factors, including FVIIa, FIXa, FXIa, and FXIIa, and has other anticoagulant properties, the clinical relevance of which are uncertain (see Section II of this series for an extensive review).

Like UFH, LMWH exert their anticoagulant effects by activating AT and accelerating the rate at which it inhibits FXa and thrombin. Because only pentasaccharide-containing chains composed of at least 18 saccharide units are of sufficient length to bridge AT to thrombin, at least 50% to 75% of LMWH chains are too short to catalyse thrombin inhibition. However, these shorter chains retain the capacity to promote FXa inhibition, which does not require bridging. Consequently, LMWH preparations have greater capacity to promote FXa inhibition than thrombin in-

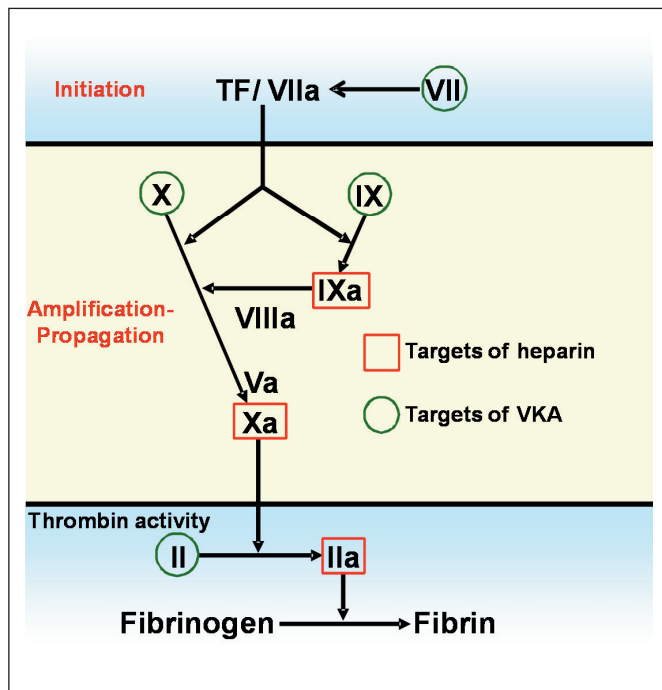


Figure 3: Targets of “classical” anticoagulants: heparin and vitamin K antagonists (VKA, e.g. warfarin). The sites of action of anticoagulants are depicted within the classical coagulation model, with the tissue factor (extrinsic) pathway, the contact phase (intrinsic) pathway (not shown), and the common pathway. Although probably not as close to reality as the cellular-based model depicted in Figure 2, this model allows an easier understanding of the sites of action of interfering drugs and the results of coagulation tests. Unfractionated heparin (UFH) inhibits both thrombin and FXa, as well as FIXa, FXIa and FXIIa, through a potentiation of the activity of the natural anticoagulant antithrombin (AT). Low-molecular-weight heparins (LMWH) inhibit FXa to a greater extent than thrombin. VKA exert their anticoagulant effect by interfering with the γ -carboxylation and thereby activation of the vitamin K-dependent coagulation factors II, VII, IX and X, but they also interfere with the activation of the natural anticoagulants protein C and protein S (here not shown, see text for details).

hibition, and have anti-FXa to anti-FIIa activity ratios that range from 2:1 to 4:1 depending on their molecular weight profiles (69). The mechanism of action and clinical applications of UFH and LMWH will be reviewed in detail in Section II of this series.

The coumarin derivatives VKA exert their anticoagulant effect by interfering with the γ -carboxylation and thereby activation of the vitamin K-dependent coagulation factors II, VII, IX and X, but they also interfere with the activation of the natural anticoagulants protein C and protein S (71). The mechanism of action and clinical applications of VKA will be reviewed in detail in Section III of this series.

Drugs that target the initiation phase of coagulation are under evaluation. These agents inhibit the activity of TF/FVIIa-complex and include tifacogin, recombinant nematode anticoagulant protein c2 (NAPc2) and recombinant active site-inhibited FVIIa (rFVIIai or ASIS) (► Figure 4). Despite promising results in *in vitro* or animal models, none of these TF/FVIIa inhibitors has reached clinical testing in heart disease.

Drugs that target the propagation phase of coagulation decrease thrombin generation. They include direct or indirect inhibitors of the proteases FIXa or FXa. Direct FIX inhibitors include monoclonal antibodies and aptamers, such as pegnivacogin. Recombinant human forms of activated protein C (drotrecogin) and soluble thrombomodulin (recomodulin and solulin) inactivate or promote the inactivation of FVa and FVIIIa. None of the above drugs has reached phase III clinical testing, and these agents will therefore not be reviewed in the following Sections.

Synthetic pentasaccharides mediate indirect, antithrombin-dependent, inhibition of FXa. Orally active direct FXa inhibitors have been licensed for short- and long-term indications. A potential advantage of direct FXa inhibitors over indirect inhibitors is their capacity to inhibit not only free FXa but also FXa within the prothrombinase complex.

Direct thrombin inhibitors (parenteral and oral) bind to thrombin and block its interaction with substrates, thereby preventing the formation of fibrin and activation of platelets, FV, FVIII, FXI, and FXIII. These drugs may also inhibit thrombin-induced intracellular signal transduction pathways, including thrombin-induced platelet activation. The direct thrombin inhibitors block thrombin bound to fibrin in addition to thrombin in plasma (72). The utility of oral direct FXa inhibitors and thrombin inhibitors in heart disease has been reviewed by this Task Force (3) and will also be the subject of Section IV of this series.

A rational classification of currently available anticoagulants, based on their route of administration and their mechanism of action is presented in ► Figure 5.

The use of anticoagulant therapy in heart disease – epidemiological data

Coronary heart disease

Anticoagulant therapies are an essential component of regimens used for ACS management. Population-based surveys conducted in Europe from 1999–2001 have shown that most (>90%) patients

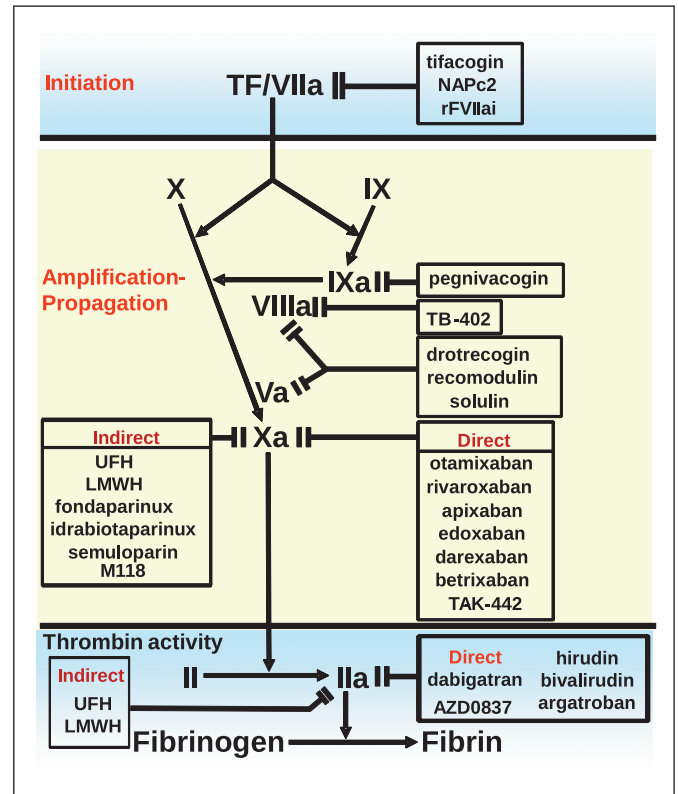


Figure 4: Targets of new anticoagulants. The sites of action of new anticoagulants are depicted within the classical coagulation model, with the tissue factor (extrinsic) pathway, the contact phase activation (intrinsic) pathway, and the common pathway. Although probably not as close to reality as the cellular-based model depicted in Figure 2, this model is easier to allow an understanding of the sites of action of interfering drugs and the results of coagulation tests. Direct thrombin inhibitors bind directly to thrombin and prevent fibrin formation as well as thrombin-mediated activation of FV, FVIII, FXI and FXIII. They also prevent thrombin-mediated activation of platelets, of inflammation, of anti-fibrinolysis, as well as of the anticoagulant protein C / protein S / thrombomodulin pathway. Parenteral direct thrombin inhibitors include hirudin, bivalirudin and argatroban. Oral direct thrombin inhibitors are pro-drugs that generate an active compound able to bind directly to the catalytic site of thrombin: examples include ximelagatran (withdrawn from development), as well as AZD0837, now under evaluation, and dabigatran etexilate. Drugs that target coagulation proteases involved in the amplification phase include agents that block FIXa (such as the DNA aptamer pegnivacogin), FVIIIa (TB-402) or jointly FVa/FVIIIa, cofactors that are critical for the generation of thrombin (drotrecogin, a recombinant form of human activated protein C; recomodulin and solulin, both recombinant soluble derivatives of human thrombomodulin). Blockers of the propagation phase include FXa inhibitors. At variance from the parenteral indirect FXa inhibitors, such as UFH, LMWH, and pentasaccharide derivatives (fondaparinux, idrabiotaparinux), which exert their effects equally on thrombin and on FXa (UFH), prevalently on FXa (LMWH) or exclusively on FXa (fondaparinux, idrabiotaparinux), all by potentiating the natural inhibitor antithrombin (AT), direct FXa inhibitors have direct, non-AT-mediated effects. A number of direct FXa inhibitors are in clinical trials. To target the initiation of coagulation, inhibitors towards the TF/FVIIa complex have been developed, such as recombinant TFPI (tifacogin), recombinant nematode anticoagulant protein c2 (NAPc2), active site-inhibited recombinant (r) FVIIa (rFVIIai) and monoclonal antibodies against TF. The arrows depict activation or generation. A line ending with two perpendicular short lines depicts inhibition.

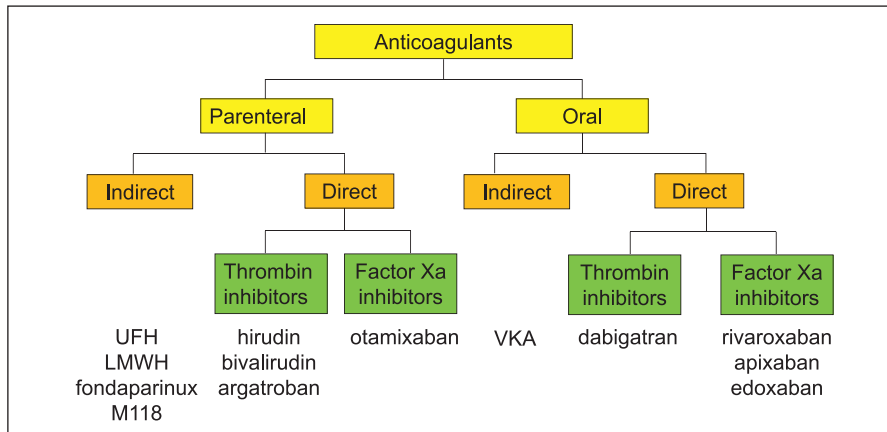


Figure 5: A rational classification of currently available anticoagulants, based on their route of administration (parenteral vs oral) and their mode of action.

presenting with ACS receive aspirin during hospital admission, and that most (~80%) also receive either UFH or a LMWH, with the proportions receiving these two forms of heparin approximately equal (73-75). The frequency of use of heparin varies little according to the presence/absence of ST-elevation on admission, or to the final diagnosis (Q-wave myocardial infarction [MI], non-Q-wave MI or unstable angina), but there is considerable variation in usage among European countries. At hospital discharge, most patients (>90%) receive an antithrombotic agent, generally the antiplatelet agent aspirin, but in one survey about 6% were prescribed warfarin and about 9% were given LMWH (75).

In a more recent European survey of prescribing habits in ACS, the multicentre, prospective, observational Acute Coronary Syndromes Registry analysed data of 11,823 consecutive hospital survivors of acute MI. Based on the initial analysis, guideline-adherent secondary prevention drug therapy was associated with an improved one-year survival. On multivariate analysis, chronic oral anticoagulation was the strongest predictor for not receiving aspirin (odds ratio [OR]: 19.6, 95% confidence interval [CI]: 15.9-24.0) at discharge (76). After adjustment for confounding factors, neither prior aspirin nor oral anticoagulant treatment were independent predictors for in-hospital mortality (77).

The EUROASPIRE-II study examined treatment of patients who had undergone a coronary procedure, such as coronary bypass surgery or percutaneous coronary intervention (PCI), or who had been hospitalised with an acute MI or myocardial ischaemia (78). On admission, about 50% of such patients were taking an antiplatelet agent, and 7% an anticoagulant (which was likely to be a VKA in most instances). At discharge, 90% were receiving an antiplatelet regimen, and 12% were on an anticoagulant regimen (which could have been a VKA or subcutaneous LMWH). There was evidence of variation in the frequency of use of anticoagulants among European countries.

Atrial fibrillation

Oral anticoagulant (OAC) therapy remains the best treatment strategy for the prevention of cardioembolism in AF. In a systematic review of “real-world” data, ischaemic stroke rates were higher

in AF patients receiving no therapy (median: 4.45/100 person-years; range: 0.25-5.90) or antiplatelet-therapy (median: 4.45/100 person-years; range: 2.0-10.0) compared with VKA-treated patients who were followed in an anticoagulation clinic (median: 1.72/100 person-years; range: 0.97-2.00), or were managed in the community setting (median 1.66/100 person-years; range: 0-4.90) (79). Interestingly, major bleeding rates in patients receiving antiplatelet or no therapy were similar to those in VKA-treated patients.

In a recent systematic review of 29 studies of patients with prior stroke/transient ischaemic attack (TIA) who should all have received OAC therapy according to published guidelines, under-treatment was reported in 25 studies, with 21 of 29 studies reporting OAC treatment levels below 60% (range 19%-81.3%) (80). In the same systematic review, high-risk subjects, essentially those with a CHADS₂ score ≥ 2 also were suboptimally treated, with seven of nine studies reporting treatment levels below 70% (range 39%-92.3%). Thus, there continues to be underuse of OAC therapy with VKA, such as warfarin, in AF patients at high risk for stroke.

Generally, the use of VKA therapy for stroke prevention in AF is increasing, whilst the proportion of untreated patients is decreasing and the proportion of antiplatelet therapy use remains static (81). Nonetheless, the percentage of patients receiving no therapy still ranges from 4% to 48% (median 18%), antiplatelet therapy from 10% to 56% (median 30%), and VKA therapy from 9% to 86% (median 52%), suggesting that many AF patients at moderate or high risk for stroke still receive suboptimal treatment based on contemporary guidelines. Also, the quality of anticoagulation control is highly variable across centers and countries (81), with likely important consequences, as poor time in therapeutic range can result in outcomes that are worse than if the patient is left untreated (82).

The Euro Heart Survey on Atrial Fibrillation examined prescribing patterns among European cardiology practices in 2003-2004 (83). Most patients surveyed had risk factors for stroke, and hence OAC treatment was indicated. About 80% of those with persistent or permanent AF, and 50% of those with paroxysmal AF received OAC treatment. Only 4% of patients with persistent or permanent AF did not receive any antithrombotic therapy. How-

ever, these rates are likely to overestimate the use of anticoagulants in general practice.

Epidemiological data on the risk of bleeding connected with the use of OAC (essentially VKA) have recently become available. Clearly, the risk of OAC-related bleeding in AF is multifactorial, and the highest risk period is when OAC treatment is initiated (84). The European Heart Rhythm Association recently published a position document, endorsed by the ESC Working Group on Thrombosis, which addresses the epidemiology and scope of the problem of bleeding in AF patients and provides an overview of established bleeding risk factors (85). Factors influencing bleeding are the modality of OAC therapy, i.e. usual care vs anticoagulation clinic vs self management; age; prior stroke; history of bleeding; anaemia; co-morbidities (hypertension, renal insufficiency, liver disease); the use of antiplatelet agents, non-steroidal anti-inflammatory drugs or drugs affecting the intensity of OAC; and alcohol abuse (85). Patient values and preferences in balancing the risk of bleeding against the risk of stroke, as well as awareness of the prognostic implications of bleeding, are important considerations in driving therapeutic choices (85).

Despite considerations about bleeding, the low rates of OAC prescription in patients with AF at increased risk of stroke are a major concern, as recently highlighted by the ESC AF Guidelines (86), and are an important driver for the use of novel oral anticoagulants in this condition (86).

Prosthetic heart valves

OAC (VKA) treatment is widely prescribed and used in patients with prosthetic heart valves, but irregularly recommended and used in patients with rheumatic mitral stenosis who are in sinus rhythm. There is a paucity of data on the consistency of OAC use and on how closely available recommendations are followed in different countries [see (87) and <http://americanheart.org/downloadable/heart/1150461625693ValvularHeartDisease2006.pdf>].

Only a limited number of case series have been published, and these provide inconclusive information on the pattern of use and optimal antithrombotic regimen for patients with prosthetic heart valves (88). European (89) and North American guidelines (90) differ on the recommendations for prescribing anticoagulants without (89) or with aspirin (90), respectively, likely reflecting different patterns of simultaneous use of VKA and aspirin in different parts of the world (see [91] for an in-depth discussion).

Heart failure

Heart failure is associated with an increased risk of venous thromboembolism, cardio-embolic stroke and sudden death, and indeed the latter has been associated with new coronary (thrombotic) occlusions in about 30% of patients (92). In a Cochrane systematic review exploring whether long-term oral anticoagulation reduced total deaths and/or major thromboembolic events in patients with heart failure, the evidence from randomised, controlled trials and observational studies found a reduction in mortality and cardiovascular events with anticoagulants compared with control or

placebo (93). Current evidence, however, does not support their routine use in heart failure patients who remain in sinus rhythm, as shown in a recently completed randomised controlled trial (94). One recent survey also did not find any significant – positive or negative – association of warfarin with mortality and hospitalisation (95).

Conclusions

Blood coagulation is an essential component of haemostasis and thrombosis. Coagulation is mostly a cell surface-based process offering multiple possibilities of interference. Besides classical anticoagulants – heparins and VKA – several new coagulation inhibitors, both parenteral and oral, are being developed and introduced in the market. At variance from classical anticoagulants, most of the new anticoagulants inhibit only a single step in the coagulation process. Multiple surveys confirm that parenteral anticoagulants are routinely used in ACS with or without PCI. The use of anticoagulants (essentially VKA) for long-term use is mostly reserved for the prophylaxis of cardioembolism in AF and with the use of prosthetic cardiac valves. Recent surveys show an increased use of such drugs in the setting of AF, but still far from the almost generalised use recommended by current treatment guidelines, a deficiency that may be addressed with the availability of the novel oral anticoagulants, which are more convenient to administer than VKA.

Conflicts of interest

Dr. De Caterina receives consultant and speaker fees from AstraZeneca, Bayer, Boehringer-Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, and Lilly; and research grants from AstraZeneca and Boehringer-Ingelheim. Dr. Husted receives advisory board or speaker fees from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, and Sanofi-Aventis; and research grants from AstraZeneca, Bayer, Pfizer, Boehringer Ingelheim, and Bristol-Myers Squibb. Dr. Wallentin receives consultant fees from Athera, Behring, Evolva, Portola, and Roche Diagnostics; and institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Merck, Pfizer, and Schering-Plough. Dr. Andreotti receives consultant or speaker fees from AstraZeneca, Bayer, Bristol-Myers Squibb, Pfizer, Daiichi-Sankyo, and Lilly. Dr. Huber receives speaker fees from AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi Sankyo, Eli Lilly, and The Medicines Company. Dr. Kristensen receives speaker fees from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck, Pfizer, and The Medicines Company. Dr. Lip receives speaker fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, and Sanofi-Aventis; and consultant fees from Astellas, AstraZeneca, Bayer, Biotronik, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Merck, Sanofi-Aventis, Portola, and Pfizer. Dr. Morais receives consultant fees from AstraZeneca, Bayer, Jaba Recordati, MSD, Lilly Portugal, and Merck. Dr. Siegbahn receives institutional grants from AstraZeneca and Boehringer Ingelheim. Dr. Verheugt receives consultant fees from Bayer,

Daiichi Sankyo, Pfizer, Eli Lilly, Merck, and The Medicines Company; and educational and research grants from Bayer, Boehringer Ingelheim, Eli Lilly, and Roche. Dr. Weitz receives consultant fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Johnson & Johnson, Janssen Pharmaceuticals, and Pfizer. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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