

Coagulation for the clinician

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Coagulation physiology

The integrity of the circulation is maintained through the provision of a rapid, potent, but tightly localised coagulation response to vascular damage. There is, however, one extraordinary problem in the regulation of haemostasis – blood flows. Normal haemostasis is the ability of the haemostatic system to control activation of clot formation and clot lysis in order to prevent haemorrhage without causing thrombosis. It classically involves vasoconstriction, platelet adhesion and aggregation at the site of injury, leading to a plug formation. This is followed by fibrin formation consolidating the plug and rendering it stable. All of this occurs without clotting occurring elsewhere. Procoagulant products, whether clotting factors or platelets, must therefore be spatially localised to the site of damage, and/or kinetically controlled so that they are inactive when distant from the damaged vessel.

The blood coagulation cascade is usually initiated when sub-endothelial tissue factor is exposed/expressed to the blood flow following either damage or activation of the endothelium.¹⁻³ As a result, the haemostatic mechanism is invoked through a complex series of regulated events, involving interactions of blood components and tissue proteins, resulting in a spectrum from haemorrhage, through controlled haemostasis, to thrombosis (Fig. 1).

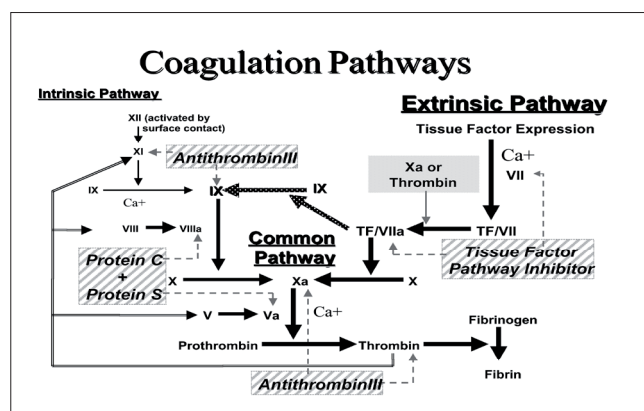


Fig. 1. The coagulation cascade.

A major insight into the functioning of the process of coagulation is that the tissue factor/factor VIIa complex of the classic ‘extrinsic pathway’ not only activates factor X of the ‘common pathway’ but also directly activates factor IX^{4,5} of the classic ‘intrinsic pathway’, making factor XII redundant. The entire response of clot formation has been characterised as a *cascade* or *waterfall* of enzymatic reactions, which result in α -thrombin converting soluble plasma fibrinogen to the

insoluble fibrin polymer,^{6,7} making the coagulation process a series of reactions in which the sequentially derived products have the capacity to amplify small stimuli to result in the rapid generation of large amounts of α -thrombin.⁸ Tissue factor, factor Xa and thrombin are pivotal, together with physiological controls (positive and negative feedback loops, and natural anticoagulants) that first enhance thrombin generation but then preserve vessel patency by limiting haemostatic plug formation to areas of injury. Abnormalities in these mechanisms can increase thrombosis risk.⁹

The classic pathways of blood coagulation

The ‘extrinsic pathway’ is the primary route of thrombin formation, through which the physiological haemostatic response occurs. It occurs through contact with a non-blood protein source. The physiological initiator of coagulation is *tissue factor*, which comes into contact with the blood at the site of vascular injury and combines with plasma serine protease, factor VII.^{10,11} Being a trans-membrane protein, it is *ipso facto* localised.¹² The tissue factor/factor VIIa (TF/VIIa) complex activates factor X to factor Xa. It can, however, also activate factor IX to IXa.^{4,5}

Tissue factor, the intrinsic membrane protein, is present on the surface of many cell types that are not normally in contact with the circulation (Fig. 2). However, it is exposed to blood when vascular damage occurs. Vascular damage will therefore lead to increasing levels of tissue factor at the site of injury. In association with the serine protease factor VIIa,¹³ it initiates the blood coagulation cascade, requiring no calcium.¹⁰⁻¹² The direct factor IX activation, while slower, forms exponentially increasing amounts of thrombin. Tissue factor is not released into the circulation in a soluble form during normal haemostasis. If there is a major injury there is a potential for it to be swept into the circulation, which may lead to disseminated intravascular coagulation (DIC).¹⁴ However, at the site of damage platelets interact with

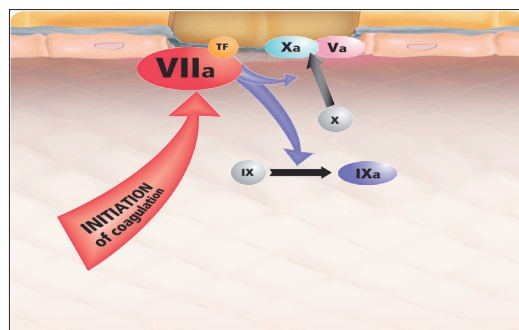


Fig. 2. The coagulation cascade – initiation phase.

components of the vessel wall at the same time that plasma factor VII comes into contact with tissue factor. The platelet plug thus prevents the unchecked release of the TF/VIIa complex.

The limited amounts of the serine protease factor Xa produced generate minute concentrations of thrombin, which partially activates platelets and cleaves the pro-cofactors factor V and factor VIII, generating the active cofactors factor Va and factor VIIIa, respectively.^{15,16} The extrinsic pathway catalyst is therefore responsible for factor X activation through both pathways.^{17,18} Furthermore, the efficiency of factor X activation by the factor VIIIa/IXa complex is significantly greater than the efficiency of the TF/VIIa complex. It is relevant that the factor IX-dependent route, in contrast to the direct activation of factor X by TF/VIIa, involves two enzyme-catalysed reactions in sequence and therefore provides extra amplification over the direct activation of factor X, but will take longer.¹⁹ The reactions occur on the platelet surface, but only on those platelets that are activated and involved at the site of vascular injury (see below); the response is therefore localised to the site of vascular damage. Multiple control mechanisms guard against procoagulant materials entering the general circulation and causing disseminated intravascular coagulation.²⁰ Obviously, since the latter condition exists, these safeguards can on occasion be overrun.

The pathway classically referred to as the 'intrinsic pathway' starts with the formation of factor XIIa.²¹ This can cleave prekallikrein to provide kallikrein,²² which in turn reciprocally activates factor XII.²³ Factor XIIa, in the presence of high-molecular-weight kininogen, converts factor XI to factor XIa, which in turn converts factor IX to factor IXa.²⁴ Factor IXa binds with its cofactor protein factor VIIIa, in the presence of calcium and the appropriate membrane surface, and activates factor X to factor Xa.²⁵

Factor VIIIa forms the *intrinsic factor Xase* complex with the serine protease, factor IXa, on a membrane surface provided by platelets, microparticles and endothelial and other cells²⁶ and activates factor X at a 50 - 100-fold higher rate than the factor VIIa-tissue factor complex,⁴ in turn creating a 'thrombin burst' that activates fibrin (Fig. 3).

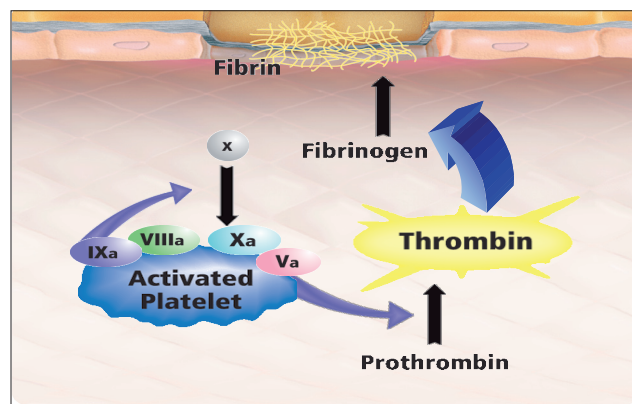


Fig. 3. The coagulation cascade – propagation phase.

Factor VIII circulates in association with von Willebrand factor in blood and is released from von Willebrand factor during activation of the factor VIII molecule by thrombin (Fig. 4). Factor Xa binds to its cofactor protein factor Va, in the presence of calcium and the appropriate membrane

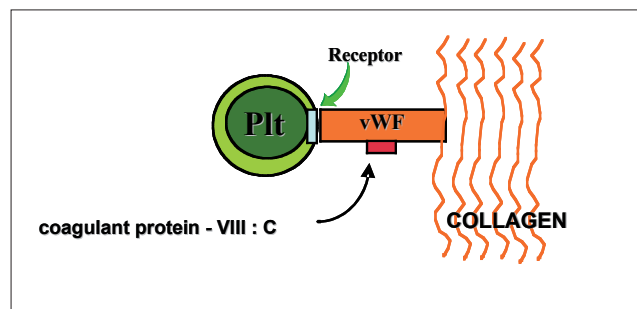


Fig. 4. Factor VIII: von Willebrand factor.

surface, and activates factor II (prothrombin) to IIa (α -thrombin).²⁷

The two soluble plasma proteins, factor V and factor VIII, participate in the formation of vitamin K-dependent protein complexes. Once formed they are unstable in solution, being inactivated by protein C.

The conversion of prothrombin to α -thrombin (or 'common pathway') is accomplished via a catalytic subunit, factor Xa in the presence of calcium ions, and a regulatory subunit, factor Va.^{28,29} Human factor X circulates in plasma and is converted to its active form, factor Xa, by both of the mechanisms above.

Factor V circulates in the blood, both in platelets (~20%) and as the soluble plasma protein (~80%). Since a deficiency of platelet factor V is associated with decreased factor Xa binding and a bleeding diathesis,³⁰ it is clear that the platelet pool of this protein plays a major physiological role. The factor Xa/Va complex activates prothrombin on an anionic phospholipid membrane, the platelet surface.

Thrombin generation is the central biochemical reaction in both normal haemostasis and thrombosis. The thrombin produced further amplifies its own generation through other important coagulation events such as activating factor XI³¹ and completing platelet aggregation,³² plus factor V and VIII activation.¹⁵ It also causes the transformation of soluble fibrinogen into insoluble fibrin,^{33,34} and the activation of factor XIII to factor XIIIa,³⁵ which stabilises the blood clot.^{36,37} Thrombin also participates in damping of its own production by complexing with thrombomodulin,³⁸ which is released by endothelial cells. These also participate in the activation of proteins C and S, which result in the inactivation of factors Va and VIIIa.³⁹ Residual serine proteases are inhibited by antithrombin III.⁴⁰⁻⁴⁵

At initiation of coagulation thrombin is generated at a relatively low rate, with partial activation of platelets and proteolysis of factor V, factor VIII, factor VIIa, factor IXa, factor Xa, and factor XIa are also generated.^{15,16,46}

Subsequently, in the presence of all procoagulant proteins at their mean plasma concentrations, the propagation phase of thrombin generation occurs, with the clot occurring at the inception of this phase. The characteristic features of the propagation phase are a prothrombin conversion at a high rate, completion of platelet activation, and solid clot formation^{15,16,37,46,47} (Fig. 5).

Platelets

Platelets form the platelet plug, playing a pivotal role in haemostasis with platelet membrane glycoproteins mediating binding to subendothelial tissue and aggregation into

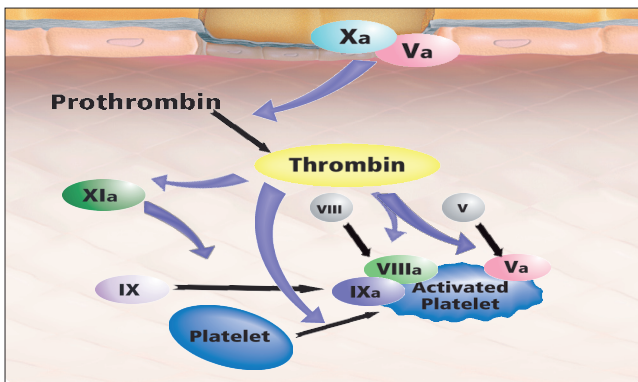


Fig. 5. The coagulation cascade – thrombin amplification.

haemostatic plugs.⁴⁸ Reduced platelet activity will result in a bleeding tendency, while stimulation of platelets can lead to thrombosis.⁴⁹ In addition, aggregated, activated platelets provide procoagulant phospholipid-equivalent surfaces upon which the complex-dependent reactions of the blood coagulation cascade are localised.^{50,51}

Platelets are small (2 μm diameter), non-nucleated blood cells, which are activated rapidly after blood vessel injury or blood exposure to artificial surfaces, providing haemostasis by four interconnected mechanisms: (i) adhering to sites of vascular injury or artificial surfaces; (ii) releasing compounds from their granules; (iii) aggregating together to form a haemostatic platelet plug; and (iv) providing a procoagulant surface for activated coagulation protein complexes on their phospholipid membranes.⁵²

The adhesion to the subendothelium is the initial step in platelet activation,⁵³ which is extremely rapid and is facilitated by von Willebrand factor binding to platelet surface glycoprotein Ib/IX/V.⁵⁴⁻⁵⁶ Platelets can also adhere to vascular wall associated fibrin or fibrinogen through interaction with platelet surface glycoprotein IIb/IIIa.^{57,58} After adhering to the subendothelium, platelets undergo activation leading to a shape change with development of pseudopods. Intracellular signalling processes lead to increased cytoplasmic calcium and initiation of a secretory release reaction, whereby products are released into the surrounding milieu.⁵⁹ Platelets also have numerous intrinsic glycoproteins embedded in the outer surface of their plasma membrane that are receptors for agonists, such as adrenaline, thrombin, collagen, and platelet-activating factor, which can initiate platelet aggregation⁶⁰ (Fig. 6).

As mentioned above, activated platelets also play a vital procoagulant role, linking platelet function and coagulation activation. Platelet membrane phospholipids undergo a

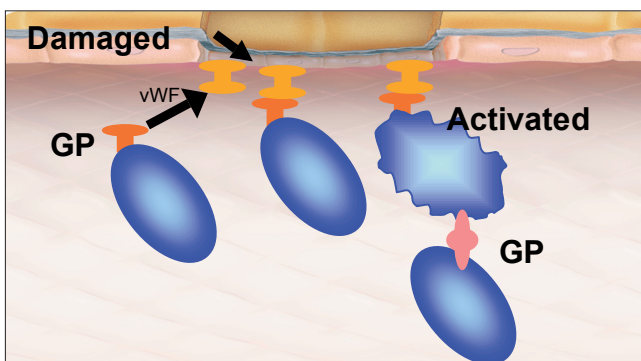


Fig. 6. The coagulation cascade – the platelet plug.

rearrangement during activation, providing a binding site for phospholipid-dependent coagulation complexes that activate both factor X and prothrombin,⁶¹ accelerating coagulation through the assembly of multi-enzyme complexes with great catalytic efficiency. The platelet surface membrane also interacts with factors Xa and Va to form the prothrombinase complex, as well as promoting the assembly of the factor IXa-VIIIa complex required for factor Xa activation.⁶² These interactions should not be overlooked, since they dramatically enhance enzymatic formation of both thrombin and factor Xa⁶³ and in the absence of membrane the formation of the complex is either abolished or impaired.^{64,65} Calcium is also an essential component for efficient complex formation.⁶⁶

Fibrin formation

Activated α -thrombin rapidly converts soluble plasma fibrinogen to an insoluble fibrin polymer by limited proteolysis, thus consolidating the haemostatic platelet plug. Fibrinogen is synthesised in the liver and is present in plasma and platelet α -granules. It is an acute-phase reactant: with maximal stimulation, fibrinogen synthesis can be increased 20-fold. Thrombin also activates the plasma transglutaminase, factor XIII,⁶⁷ which covalently cross-links the fibrin polymers both longitudinally and transversely.⁶⁸ Calcium enhances lateral fibre growth. This is then relatively resistant to proteolytic attack.

Alternative pathways

Factor XI activation

The alternative route of activation of factor XI via thrombin⁶⁹ is a positive feedback system, which explains both the requirement for factor XI and the lack of a requirement for factor XII. A reasonable scenario is therefore that tissue factor initiates coagulation, leading to fast thrombin production. Thrombin then feeds back, producing small amounts of factor XIa. The XIa in itself may then have a positive feedback on itself, thus leading to an increase in the rate of factor XI activation. When the initiation phase of thrombin generation is prolonged, either because of low tissue factor concentration or inhibitory effects of elevated concentrations of some proteins involved in blood coagulation, factor XI plays an important role.⁴⁷

Factor IX activation by TF/VIIa

Although factor XIa is an activator of factor IX in one of the simplest reactions of coagulation, requiring only calcium as a cofactor, as discussed above, the factor VIIa-tissue factor complex activates factor IX as well as activating factor X.^{4,5,70} This links the extrinsic pathway directly into the intrinsic one.

Factor VIII activation by thrombin and factor Xa

Factor VIII circulates as a complex with von Willebrand factor, and normally exists in an almost inactive state, requiring activation to factor VIIIa. Activation is due to the feedback action of either factor Xa or thrombin. Upon factor VIII activation by thrombin or factor Xa, it is released from von Willebrand factor, which enables binding of the activated cofactor.

Positive feedbacks

The positive feedbacks in coagulation play a major role in the architecture of the system: there are several feedback loops. In a positive feedback an enzyme formed later in the pathway acts to provide something that accelerates its own formation.

Thrombin/factor Xa

The known major feedback loops of clotting, all originating via either factor Xa or thrombin, result in the formation of both enzymes and activated cofactors. The production of thrombin is therefore rapid (≤ 1 minute) and self-reinforcing because of its feedback activation of numerous coagulation proteins (factor V, VIII, XI and VII)^{31,41,71,72} that contribute to thrombin production. This provides the threshold above which the 'thrombin burst' occurs.

Platelet activation

Platelets have numerous receptors for ligands ranging from fibrinogen, collagen, thrombin and thrombospondin to von Willebrand factor and fibronectin.⁶⁰ Of note is that most of the platelet activators exert their action by binding to specific receptors on the platelet surface membrane. A possible exception to this is activation by thrombin, one of the most potent platelet activators known.⁶³ Minor quantities of thrombin formed early in haemostasis, prior to coagulation,⁷³ bind to platelet receptors to elicit release and aggregation. Platelet aggregation itself also involves positive feedbacks through the release from platelets of platelet agonists such as adenosine diphosphate (ADP) and thromboxane A₂.

Factor VII activation

The major activator of factor VII is factor Xa, which generates factor VIIa. Although anionic phospholipid alone does support this reaction, it is much faster in the presence of tissue factor. Tissue factor therefore has two distinct cofactor roles in coagulation: enhancing the activity of factor VIIa on factors IX and X, and accelerating the feedback activation of factor VII by factor Xa. Factor VII can also be feedback-activated, but at lower rates, by the TF/VIIa complex and by thrombin and factor IXa.⁷⁴

Cofactor activation

Both major plasma cofactors, factors VIII and V, are activated in feedback loops. The principal activator of both is thrombin,⁷⁵ but both are also activated by factor Xa in the presence of anionic phospholipid.^{76,77} Factor VIII activation by factor Xa constitutes a feedback, but activation of factor V does not. It is possible that factor Xa action plays a significant role in the early phases of coagulation before significant thrombin generation.

Positive feedbacks in the presence of inhibitors

The enzymes catalysing positive feedbacks, thrombin and factor Xa, are also the major targets of plasma inhibitors, chiefly antithrombin III (ATIII). Inhibition may therefore be expected to play a role in the regulation of the feedbacks. Positive feedback loops, when subject to control by inhibitors such as ATIII, confer threshold properties on the system.⁷⁸

Below the threshold no response will occur, while above the threshold the response should be at or near maximum.

Negative feedbacks

Control of coagulation reactions

Several natural anticoagulant mechanisms are present, able to exert damping effects upon each step of the coagulation cascade.⁷⁹ Two types of inhibition exist in the regulation of thrombin production. The first depends on the plasma concentration of ample activated inhibitors, the major example being ATIII,⁸⁰ which is able to effectively neutralise all serine proteases produced during the blood coagulation process.⁴⁴ Possibly α_2 -macroglobulin is another minor player. In addition to this, protein C anticoagulant mechanisms will regulate activated cofactors.⁸¹ However, antithrombin III does appear to be the most quantitatively important.

The second type of inhibition, which is so far unique, is the tissue factor pathway inhibitor (TFPI), involving a negative feedback in which an inhibitory species is formed only when the system is activated. It can bind and inactivate both factor Xa and factor VIIa simultaneously.⁴⁵ TFPI is also an important regulator of the serine proteases, its principal targets being factor Xa and the factor VIIa-TF/factor Xa complex.⁴⁰

The dynamic inhibitory system is generated when thrombin binds to its cofactor thrombomodulin and activates protein C.³⁸ Activated protein C attenuates the coagulation reaction of factor V/Va and factor VIII/VIIIa.^{41,43}

The protein C system and antithrombin III have prominent effects on the propagation phase of thrombin generation.^{82,83} In the presence of both, TFPI and antithrombin III, the concentration of factor Xa (the limiting reactant of thrombin generation) is decreased. Additionally, antithrombin III also inhibits other serine proteases produced *in situ* during the reaction⁴⁴ (see Fig. 1). As a consequence of synergistic effects, thrombin generation becomes a threshold-limited process, influenced by relatively small changes in the concentrations of procoagulants and their inhibitors.⁷⁴

Therefore, a number of plasma proteins are able to inhibit serine proteases involved in coagulation, fibrinolysis, and kinin formation. These include ATIII, heparin cofactor II, α_2 -macroglobulin, α_1 -antitrypsin, tissue factor pathway inhibitor, protein C inhibitor, α_2 -antiplasmin, and plasminogen activator inhibitor-1 (PAI-1). The similarity in structure and mechanism of action of these proteins (with the exception of α_2 -macroglobulin) has led to the general term *serpins* for these serine protease inhibitors.⁸⁴

Antithrombin III, which is also known as antithrombin or heparin cofactor I, inhibits its target enzymes by forming 1:1 enzyme:inhibitor complexes in which the active site of the enzyme is blocked.⁸⁵ Factors IXa, Xa, XIa and XIIa are inactivated by ATIII in a manner similar to that outlined for thrombin.⁸⁶⁻⁸⁸ Although the interaction is reversible,⁸⁹ dissociation is probably too slow to be significant. Since the plasma ATIII concentration is in significant excess of the sum of the concentrations of all the coagulants, there is ample mass of ATIII available. However, despite the level being adequate in mass terms, the second-order kinetics of its action mean that the rates of inhibition of target enzymes are a function of its concentration, both in pure systems and plasma.⁹⁰⁻⁹²

Heparin cofactor II (HCII) is a serine protease inhibitor (serpin) that is synthesised by the liver and circulates in plasma. About 40% of the protein equilibrates with an

extravascular compartment and it has been detected in the intima of normal human arteries. It inhibits thrombin, but has no activity against other proteases involved in coagulation or fibrinolysis.⁹³

α_2 -**Macroglobulin** can inhibit numerous serine proteases, including thrombin, factor Xa, plasmin and kallikrein, albeit at a relatively modest rate compared with other inhibitors.⁸¹

Tissue factor pathway inhibitor is a serum component that has been shown to form a quaternary complex with factor Xa, factor VIIa and tissue factor in the presence of calcium and thereby suppresses the activity of the extrinsic cascade.^{94,95}

Protein C. A blood coagulation inhibitor, autoprothrombin IIa, is generated when thrombin is converted to prothrombin. The vitamin K-dependent protein, which was termed protein C, was subsequently found to be the same protein as autoprothrombin IIa.⁹⁶

Plasminogen activator inhibitor-1 plays the major physiological part in suppressing the function of tissue plasminogen activator as well as urokinase, thus preventing the activation of the fibrinolytic pathway. In contrast to most other serine protease inhibitors in plasma, the normal level of PAI-1 is quite low. PAI-1 can also inhibit other coagulation system proteases including plasmin, activated protein C and thrombin.^{97,98}

Monitoring of coagulation abnormalities

Normal haemostasis is the ability of the haemostatic system to control activation of clot formation and clot lysis in order to prevent haemorrhage without causing thrombosis. All this occurs with no clotting elsewhere. This basic definition of normal haemostasis, depending on several finely balanced mechanisms, shows that:

1. There are three systems involved simultaneously in haemostasis: the formation of clots, involving platelets and the coagulation pathways; this is opposed by the anticoagulant system, and thirdly, the fibrinolysis of clots once they have been formed.

2. The absence or presence of haemorrhage or thrombosis depends on a delicate balance between the activated procoagulants and anticoagulant systems, while the clot maturity, or early breakdown once formed, will depend on the fibrinolytic system. In other words, an excess of *activated* procoagulants will primarily result in thrombosis. On the other hand, an increased anticoagulant potential (antithrombin, protein C, etc.), or decrease in activation of procoagulant factors will result in haemorrhage. In addition, enhanced fibrinolysis will also lead to bleeding.

3. Given the intricate interactions between the three systems, any instrument *measuring coagulation endpoints may only be able to identify single points*, rather than the interaction between systems. This is because coagulation is a dynamic process dependent on the interaction of an array of factors rather than one determined by actual values of individual agents.

As the understanding of the normal mechanisms of coagulation grows, so does the ability to monitor haemostasis, becoming more specific and accurate as instrumentation techniques become simpler and more efficient, facilitating the monitoring of haemostasis in the clinical setting.⁹⁹ The haemostatic process of coagulation is extremely complex, with multiple interactions between factors, including the coagulation and fibrinolytic proteins, platelets, activators and

inhibitors. This is in order to avoid uncontrolled action in either direction, and is accomplished by numerous positive and negative inhibiting and activating feedback mechanisms. As a result, the actual *quantity* or *level* of an individual factor does not necessarily reflect its functional status. With this complicated and interactive dynamic system, the measurement of static endpoints will not reflect the dynamics of the system.

Any medical coagulopathy, as opposed to a surgical bleed, needs the actual underlying cause determined in order to direct the therapy appropriately. Traditionally, haematological tests like the prothrombin time (PT) and its derived value, the international normalised ratio (INR), activated partial thromboplastin time (aPTT), bleeding time and activated clotting time (ACT) (see below) have been done. However, while they are useful endpoint markers to identify the lack of a given factor, they do not measure the dynamic interaction between the different systems, as blood coagulation occurs efficiently on cell surfaces such as activated platelets, monocytes, and fibroblasts. Both PT and aPTT are highly artificial *in vitro* systems with major limitations.¹⁰⁰

Standard tests of coagulation

Most of the routine tests of coagulation are performed on blood that has had the coagulation cascade suspended through the addition of sodium citrate 3.8%, which removes the calcium from the blood. The sample is then spun down leaving only plasma, with almost no platelets. In order to measure the various tests of coagulation endpoints, calcium is added in abundance, as are the various factors used to initiate coagulation. As a result, these tests are generally useful for measuring absolute values, or endpoints. However, they will not measure the interaction between pro- and anticoagulant factors, because of the addition of excess initiating factors.

Prothrombin time (PT/INR)¹⁰¹

This test measures the overall efficiency of the extrinsic system. It was introduced as an assay of prothrombin in plasma before many of the factors participating in the coagulation reactions were identified. It is now used as a screening tool of the activities of fibrinogen, prothrombin and factors V, VII, and X. It is also used to monitor the oral anticoagulants, since these lower the levels of prothrombin, VII and X (Fig. 7).

The test is initiated by recalcifying citrated plasma in the presence of thromboplastin. Thromboplastin is added far in excess of normal levels and is a source of tissue factor in a

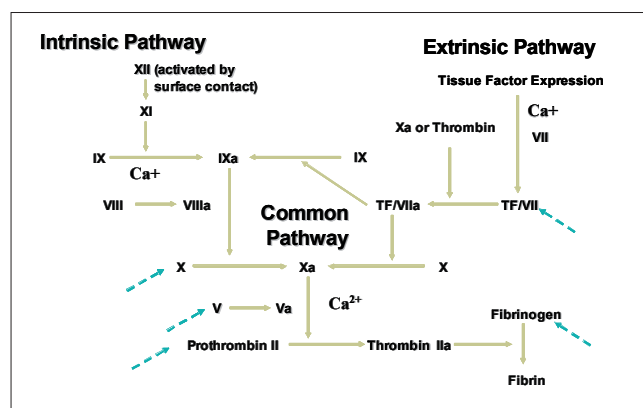


Fig. 7. The prothrombin time.

suspension of phospholipids. The time that elapses until the plasma clots is the PT and is predominantly determined by: (i) factor VIIa/tissue factor in the presence of phospholipids and calcium activating factor X; (ii) factor Xa/Va in the presence of phospholipids and calcium activating prothrombin; (iii) thrombin cleaving fibrinogen; and (iv) fibrin monomers polymerising.

The test has to be standardised and consists of taking blood in a ratio of 9 parts blood to 1 part 3.8% citrated blood. Inter-assay variability is increased by the variability of methods used to detect clot formation.

In order to interpret this, the prothrombin time ratio (PTR) needs to be calculated, i.e. the patient's result compared with the mean reference range. It then needs to be corrected for the slope of response of the assay to oral anticoagulants. This is the international sensitivity index (ISI). The INR is then calculated as $INR = PTR^{ISI}$. INRs shorter than the reference range are uncommon and have not been consistently correlated with any clinical situation. Calculation of the INR improves standardisation, but is still limited by variations in reagents and methods. As a measure of anticoagulation, the INR only has meaning for patients on a stable dose of anticoagulants; it therefore cannot be used in patients who have not been taking their anticoagulants for at least a week or in patients who have abnormal PTs for reasons such as liver disease. However, in practice the INR is used to test for intrinsic liver function in hepatitis, as factor VII is produced in the liver.

The ACL 200 analyser simultaneously determines the PT and fibrinogen using the PT-fibrinogen recombinant (PT-FIB) reagent. The normal INR range is 1.0 to 1.3.

Activated partial thromboplastin time^{102,103}

This is usually used as a screening test to identify acquired or inherited deficiencies or inhibitors of factors VIII, IX, and XI. Reduced activation of fibrinogen, prothrombin, or factors V and X can prolong the aPTT. It is also used to monitor heparin therapy (Fig. 8).

Again the test is initiated with citrated platelet-poor plasma being incubated and recalcified after the addition of a source of platelets or platelet substitute together with a contact activator and phospholipid to 'activate' coagulation. The time that elapses until the plasma clots is the aPTT. While the normal has been difficult to establish as there is great variability, hence the 'controlled activation' of citrated blood to give an endpoint, it is again important to note that the variability of coagulation results from subtle changes

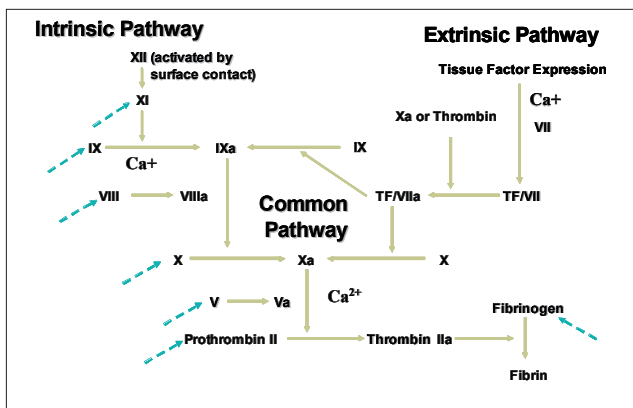


Fig. 8. The activated partial thromboplastin time (aPTT).

occurring in the dynamic coagulation system and that these are lost in the aPTT.

The aPTT is sensitive to heparin and is usually used to monitor anticoagulant therapy. It is noted that some patients with disseminated intravascular disease (DIC) or malignancies may have a shortened aPTT reflecting the *in vivo* state; however, a shortened aPTT or PT rarely point to significant findings of enhanced coagulation.

The ACL 200 analyser determines the activated partial thromboplastin time using the APTT reagent and calcium, giving the printout aPTT result in seconds, with the normal range being 26 to 40 seconds.

Red blood cell count (RBC)/platelet count¹⁰⁴

Using fresh EDTA anticoagulated blood, both red blood cell and platelet counts are analysed by a single optical cytometer after appropriate dilution of the blood sample with ADVIA 120 RBC/PLT reagent. The red blood cells are isovolumetrically sphered and lightly fixed with glutaraldehyde to preserve the spherical shape. Red cells and platelets are counted from the signals from a common detector with two different gain settings, with the platelet signals being amplified considerably more than the RBC signals. The haemoglobin concentration is calculated from the RBC count and the MCV.

The test is performed on the ADVIA[®] 120.

Bleeding time (BT)¹⁰⁵

This is a screening test for inherited or acquired disorders of platelet function and for von Willebrand's disease. It is measured as the time required for a standardised skin wound to stop bleeding. It is thought to be determined by the rate of platelet plug formation. It should only be performed by experienced operators and each centre should have its own reference range (~2.5 - 10 minutes). A lower platelet count can prolong the bleeding time and it may be affected by multiple other factors. It is not possible to reliably demonstrate abnormal bleeding in anyone with an acquired platelet dysfunction. The test has a poor sensitivity and specificity. The major conclusions of a position article on bleeding time are: (i) in the absence of a history of a bleeding disorder, the bleeding time is not a useful predictor of the risk of haemorrhage associated with surgical procedures; (ii) a normal bleeding time does not exclude the possibility of excessive haemorrhage associated with invasive procedures; and (iii) the bleeding time cannot be used to reliably identify patients who may have recently ingested aspirin or non-steroidal anti-inflammatory agents.¹⁰⁶

Activated clotting time¹⁰⁷

This is mostly used to monitor heparin anticoagulation. The result is available within minutes of starting the test, and it is therefore often used in a clinical situation. It is conceptually similar to the aPTT, except that fresh whole blood is tested rather than citrated plasma. Whole blood is added into a tube containing an activator, and the time that elapses until clot is formed is the ACT. This assay therefore depends on the same reactions as the aPTT plus the effects of platelets and any other cells that can influence coagulation. In this regard it reflects *in vivo* conditions much better. However, it only measures an endpoint and does not identify where the source of a coagulation abnormality rests.

Platelet aggregation¹⁰⁸

Platelet-rich plasma (PRP) is stirred and a suspension of collagen is added to it. As a result some of the platelets adhere to the collagen coating the fibres. After the adherence has occurred, the free platelets begin to swell and stick together, with the reaction accelerating until large platelet masses have formed. This is the process of *platelet aggregation*; it is distinct from the adherence to collagen although initiated by it. Platelet aggregation occurs when aggregation agents are added to PRP, which is continually being stirred. As aggregation proceeds, platelet clumping occurs. In the plasma, this reduces the optical density of the PRP allowing more light (infra red) to pass through. If all the platelets clumped, the resultant optical density would be equivalent to that of the platelet-poor plasma (PPP). The light transmittance through the PPP represents 100% and that of the PRP 0% aggregation. The aggregometer develops a voltage proportional to the transmittance of light through the plasma. This voltage is recorded on a strip chart recorder as a function of time. Since the optical channel is automated, a voltage representing the difference in transmittance between the PRP and PPP cuvettes is automatically developed and this voltage is registered on the strip chart recorder.

Tests on the whole blood aggregometer optical channel detect platelet aggregation in a 0.45 ml sample of PRP by measuring infrared light transmission through the sample.

Calculations. Provided that the recorder is run long enough to permit a plateau or a reversal of the responses to occur, a plot of the maximum aggregation achieved during the time verses the logarithm of the agonist concentration gives a characteristic sigmoidal curve.

Normal range. The platelets of normal subjects usually produce a single reversible primary wave with 1 $\mu\text{mol/l}$ ADP or less, a biphasic aggregation with 2.5 $\mu\text{mol/l}$, and a single irreversible wave at 5 - 10 $\mu\text{mol/l}$. A single-phase response is observed after a lag phase lasting not more than 1 minute with 1 and 4 $\mu\text{g/ml}$ of collagen. A single phase or biphasic response is seen with 1.2 mg/ml of ristocetin and biphasic aggregation is observed with 2 - 10 $\mu\text{mol/l}$ of adrenaline.

Interpretation of results. The length of time between the addition of the aggregating agent and the onset of the recorder's pen deflection provides a clue as to how the aggregation agent works: whether through directly aggregating platelets or stimulating the release of ADP. The slope of the curve allows estimation of the speed of aggregation while the course, maximum height and return to baseline provides information on the responsiveness of the platelets, from different sources, to the same stimulus.

ADP. Low concentrations of ADP (0.5 - 2.5 $\mu\text{mol/l}$) cause primary or reversible aggregation. At very low concentrations of ADP, platelets may disaggregate after the first phase. In the presence of higher concentrations of ADP an irreversible secondary wave of aggregation is associated with the release of dense and α -granules due to activation of the arachidonic acid pathway. Failure to aggregate with ADP is caused by aspirin consumption, storage pool disease (no α - or dense granules released), or thrombasthenia (due to a missing or defective platelet membrane glycoprotein).

Collagen. The aggregation response to collagen is preceded by a short 'lag' phase lasting 10 - 60 seconds. The duration of the lag phase is inversely proportional to the concentration

of the collagen used and to the responsiveness of the platelets tested. This phase is succeeded by a single wave of aggregation due to activation of the arachidonic pathway. Higher doses of collagen can cause sudden increase in intra-platelet calcium concentration, thus bypassing the prostaglandin pathway. The following causes defective or no response to collagen aggregating agents: aspirin consumption, thrombasthenia thromboxane synthetase deficiency or cyclooxygenase deficiency.

Ristocetin. Ristocetin reacts with von Willebrand factor and the membrane receptor to induce platelets to clump together (agglutination). It does not activate any of the three aggregation pathways or cause the initial granule release. Defective aggregation is caused by a glycoprotein deficiency or defect and is associated with disease.

Adrenaline. No shape change precedes aggregation, but the response thereafter resembles the ADP response. Such a response is usually obtained with concentrations of 2 - 10 $\mu\text{mol/ml}$. Some clinically normal people will fail to aggregate with adrenaline.

Antithrombin III (AT III)¹⁰⁹

AT III or heparin cofactor I is the major inhibitor of blood coagulation, and as such a deficiency is associated with a high risk of thromboembolic disorders. It is also essential for effective heparin therapy. By inhibiting the coagulation proteases, especially thrombin, factors Xa and IXa, AT III prevents uncontrolled coagulation and thrombosis. The assay is based on a chromogenic substrate and on factor Xa inactivation, with the method being consequence specific and not influenced by heparin cofactor II.

Antithrombin levels in patient plasma are measured automatically on the ACL Futura in two stages: (i) incubation of the plasma with factor Xa reagent in the presence of an excess of heparin; and (ii) quantification of the residual factor Xa activity with a synthetic chromogenic substrate.

The paranitroaniline released, monitored at 405 nm, is inversely proportional to the antithrombin III level in the test sample. The result is reported in percentage activity and is automatically calculated, with the method being linear from 10% to 150%. The normal range is 85 - 120%.

Thrombin-antithrombin complex (TAT)¹¹⁰

This is an enzyme immunoassay for the determination of human thrombin/antithrombin III complex. Enzygnost® TAT micro is an enzyme immunoassay for the quantitative determination of thrombin/antithrombin III complex in human plasma and is used for the diagnosis of disturbances in blood coagulation, which are associated with changes in the activity of the coagulation system. Persons predisposed to thrombosis and DIC are found to have elevated concentrations of TAT.

D-dimer (XDP)¹¹¹

D-dimer-containing moieties are formed by plasmin degradation of factor XIIIa cross-linked fibrin, with elevated levels being found in clinical conditions such as deep-vein thrombosis (DVT), pulmonary embolism (PE) and DIC.

For quantitative analysis using the ACL Futura the degree of agglutination is directly proportional to the concentration

of D-dimer in the sample and is determined by measuring the decrease of the transmitted light at 405 nm caused by the aggregates (turbidimetric immunoassay).

Quantitative test. The QXDP test is used and results are reported in ng/ml, with the interpretation of results being that agglutination will occur within 180 seconds for samples containing more than 250 ng/ml D-dimer. The mean level of D-dimer (XDP) in the healthy population is between 8 and 135 ng/ml and neat plasma from normal healthy individuals should not agglutinate. If no agglutination is observed a thrombotic condition is unlikely. The circulatory half-life of D-dimers is about 12 hours and therefore elevated levels can persist for some time after the active process has ceased.

Fibrinogen

This is measured using an IL Test™ PT-Fibrinogen Recombinant kit. The test is based on a combined assessment of the prothrombin time and fibrinogen level by the addition of recombinant rabbit thromboplastin to the plasma. The fibrinogen is quantified by relating the absorbance or light scatter during clotting to a calibrator

Catecholamines

Heparinised samples are immediately centrifuged at 4 000 rpm for 10 minutes, after which plasma is frozen at -70°C for batching. Plasma catecholamine concentrations are measured by electrochemical detection after separation with reverse-phase high-pressure liquid chromatography (using dihydrobenzylamine as internal standard). The coefficient of variation is 7.9% for noradrenaline and 8.7% for adrenaline; the limit of sensitivity is 20 pg/ml.

Whole-blood dynamic tests of coagulation

There are two tests using whole blood to measure clot formation rather than measuring end-points as is done in the standard tests.

Sonoclot

Sonoclot (Sonoclot R Coagulation Analyser, Sienco, Inc.) analysis provides a simple test of cellular and plasmatic coagulation properties.¹¹² It and the thrombelastograph (TEG) (see below) assess the coagulation process in whole blood and may therefore be physiologically more relevant than assays of isolated haemostatic components. Hypercoagulability is detected in a high proportion by both techniques, with the clotting rates of the Thrombelastograph and Sonoclot being significantly correlated.^{113,114} In addition, Sonoclot coagulation analyses correlate better with blood loss than aPTT and platelet count in the routine coagulation analyses.¹¹⁵

Thrombelastography (TEG)

The Thrombelastograph Haemostasis Analyser is a non-invasive diagnostic instrument designed to analyse the coagulation state of a blood sample in order to assess haemostasis conditions from haemorrhagic through thrombotic.

TEG design and operating principles

The TEG analyser's approach to the monitoring of patient haemostasis is based on two facts: (i) *the end result of the*

haemostasis process is a single product – the clot; and (ii) the clot's physical properties (rate, strength, and stability), which will determine whether the patient will have normal haemostasis, will haemorrhage or will develop thrombosis.

The TEG analyser involves two mechanical parts to measure the physical properties of the clot, a cup and a pin. The special stationary cylindrical cup holds the blood (0.36 ml) and is oscillated through an angle of $4^{\circ}45'$ at 37°C .

Each rotation cycle lasts 10 seconds. The pin is suspended in the blood sample by a torsion wire and is monitored for motion, which is either transduced mechanically to a heated stylus, moving across heat-sensitive graph paper on a chart recorder, or in the newer models converted by a mechanical-electrical transducer to an electrical signal, which can be monitored by a computer. When no clot exists, the motion of the cuvette does not affect the pin and a straight line is recorded. The torque of the rotating cup is only transmitted to the immersed pin after fibrin-platelet bonding has linked the cup and pin together. The strength of these fibrin-platelet bonds affects the magnitude of the pin motion, such that strong clots move the pin directly in phase with the cup motion. The magnitude of the output is therefore directly related to the strength of the formed clot. As the clot retracts or lyses, these bonds are broken and the transfer of cup motion is diminished.

The resulting haemostasis profile is a measure of the time it takes for the first fibrin strand to be formed, the kinetics of clot formation, the strength of the clot and dissolution of clot.

TEG assessment

The coagulation profile evaluated by the TEG is a graphic method of displaying a measure of the kinetics of clot formation and dissolution as well as of clot quality. It monitors shear elasticity, a physical property of a blood clot, and is therefore sensitive to all the interacting cellular and plasmatic components in the blood that affect the rate or structure of a clotting sample and its breakdown.

The clot's ability to perform useful mechanical work (the work of haemostasis) is a function of the net result of the interactive coagulation proteins and cellular elements involved in the process of haemostasis. In essence, the TEG analyser measures the ability of the clot to perform mechanical work throughout its structural development (Fig. 9).

A recent advance is computerisation of the TEG, which essentially gives greater accuracy and consistency than manual measurements by removing the human error from the measurements. The TEG pattern is divided into component variables. The various parameters of the TEG are explained below and reflected in Table I.

Reaction time. The time from the start of a sample run until the first significant levels of detectable clot formation (amplitude = 2 mm in the TEG tracing). This is the point at which most traditional coagulation assays reach their end-points. *r*-time is prolonged by anticoagulants and factor deficiencies and shortened by hypercoagulable conditions.

Achievement of a certain clot firmness. The time from the measurement of *r* (beginning of clot formation) until a fixed level of clot firmness is reached (amplitude = 20 mm). Therefore, *k*-time is a measure of the speed or clot kinetics to reach a certain level of clot strength. *k* is shortened by increased fibrinogen level and, to a lesser extent, by platelet

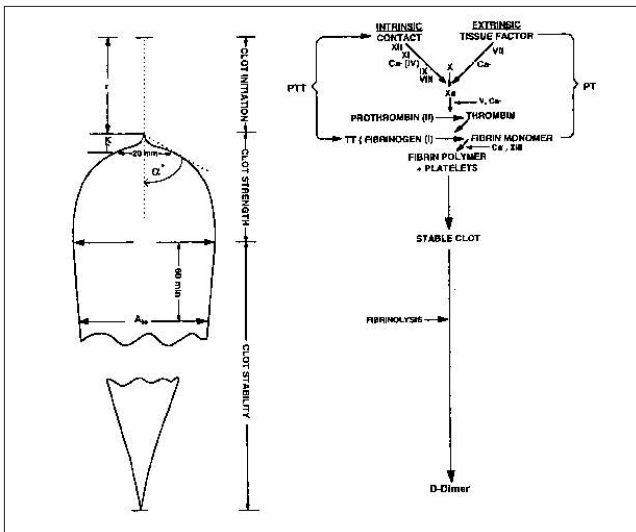


Fig. 9. TEG tracing parameters.

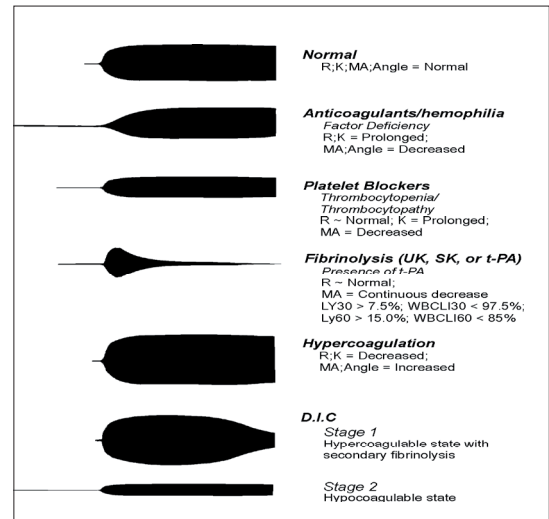


Fig. 10. Qualitative analysis.

TABLE I. TEG TRACING PARAMETERS

r	r-time is the period of time of latency from the time that the blood was placed in the TEG analyser until the initial fibrin formation
k	k-time is a measure of the speed to reach a certain level of clot strength
α	Alpha angle measures the rapidity of fibrin build-up and cross-linking (clot strengthening)
MA	Maximum amplitude is a direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot
LY30	LY30 measures the rate of amplitude reduction 30 minutes after MA and reflects clot stability.

TABLE II. STANDARD PARAMETER MEASUREMENT

	r (min)	k (min)	α-Angle (deg.)	MA (mm)
Native	15.5 - 23.0	5.5 - 10.5	22.0 - 38.0	47.0 - 58.0

function, and is prolonged by anticoagulants that affect both.

The kinetics of clot development. The α -angle is closely related to k -time, since they are both a function of the rate of polymerisation. The angle is more comprehensive than k -time, since there are hypocoagulable conditions in which the final level of clot firmness does not reach an amplitude of 20 mm (in which case k is undefined). Similar to k , α is enlarged by increased fibrinogen levels and, to a lesser extent, by platelet function, and is decreased by anticoagulants that affect both.

Maximum amplitude. This is the measurement of maximum strength of stiffness (maximum shear modulus) of the developed clot. Clot strength is the result of two components – the modest contribution of fibrin to clot strength and the much more significant contribution of the platelets.

The basic TEG measurements of kinetics, strength and stability of a clot can be determined by using a native whole blood sample. This has provided a sensitive method for monitoring conditions of hyper-, hypo- and normal coagulation, and fibrinolysis (Fig. 10). However, by using the measurements and established normal ranges and indices, the patterns can be quantified as to the degree of abnormality. Haemoscope provides reference normal ranges for the TEG (Table II).

The TEG therefore provides a quantitative and qualitative indication of the coagulation state of a blood sample by

measuring coagulation parameter and recording the kinetic changes in a sample of whole blood, plasma or platelet rich-plasma as it clots, retracts and breaks apart (lysis). As a dynamic measurement of coagulation, it reflects the interaction between cell surfaces and coagulation factors (pro- and anti-) involved in the localisation, amplification and modulation of coagulation functions, rather than endpoints.

It is a ‘near-patient’ coagulation test that poses many theoretical advantages over standard tests, not least of which is the yield of information relating to cumulative effects of several components of coagulation at a given instant.^{116,117} More recently, the contribution of platelets and soluble clotting components to clot strength has been the focus of clinical studies using TEG,¹¹⁸ for coagulation changes in both coagulation factors and platelets.¹¹⁹ It will also determine the degree of anticoagulation produced by low-molecular-weight heparin, with the r -time from the TEG correlating with serum anti-Xa concentration.¹²⁰ In summary, it can provide rapid information on coagulation status and specific treatment options within 30 minutes.¹¹⁶ It is the leading point-of-care monitor in many instances.¹²¹

Comparison of clinical tests

TEG has been widely used in a clinical setting,¹²² but there have been conflicting opinions on its validity as a stand-alone test. In an editorial in *Anesthesia and Analgesia*, Samama casts a critical light on TEG.¹²³ He observes that among other things, TEG is the leading point-of-care monitor for hypercoagulability^{113,121} and has become so popular that many groups consider it a self-sufficient coagulation test. However he goes on to state that ‘it is not a conventional hemostasis test, thus remaining a second-level test and therefore not reaching the standards acceptable of most academic hemostasis societies’. He agrees with Whitten¹²⁴ ‘that if thromboelastography is to be considered a promising hemostasis tool, its global management must be improved as it deserves a new evaluation leading to a final validation

or it will remain an expensive, non-validated point-of-care monitor'.¹²³

Conversely, TEG has been quoted as a proven useful research tool and compared with six common tests (the haematocrit, platelet count, fibrinogen, PT, aPTT and fibrin split products) by Zuckerman.¹²⁵ The results indicated that, although there is a strong relationship between the thromboelastographic variables and these common laboratory tests, thromboelastographic variables contain additional information on the haemostatic process. TEG has been used to confirm normal coagulation in the face of an abnormal prothrombin time.¹²⁶ Kang *et al.*¹²⁷ found the relationship between TEG and standard laboratory tests of coagulation to be similar to that of other researchers,^{125,128} with the greatest correlation occurring between *r*-time and aPTT, while McNicol *et al.*¹²⁹ found the relationship between standard tests of coagulation and TEG to be an inconsistent correlate. However, the authors comment that this is not surprising given that TEG variables are interdependent, measuring the interaction of the coagulation cascade and platelets in whole blood rather than specific endpoints in centrifuged plasma samples. The dynamic data provided by TEG allow far more appropriate replacement therapy.¹³⁰

TEG is a real-time monitor of global haemostasis, enabling readily available analysis of coagulation in a shorter period of time than laboratory coagulation tests would take under ideal circumstances, thus providing useful and prompt identification of coagulation disorders.¹³¹ It allows rapid intraoperative diagnosis and specific management,¹¹⁷ covering the entire field of haemostasis in the perioperative setting, from platelet function, to fibrinolysis. Davis and Chandler¹³² found in their study that of various assays tested, only abnormal TEG values, α -angle ($p < 0.01$) and *k*-time ($p < 0.04$), were associated with an increased risk of bleeding. Bleeding times were not predictive of an increased risk of post-biopsy bleeding. All prothrombin time, partial thromboplastin time and platelet count abnormalities were mild and none of these assays predicted bleeding. It was concluded that TEG was the best assay for detecting mild coagulation abnormalities associated with an increased risk of bleeding.

With the advent of computerisation in the past decade, TEG has evolved from a research laboratory tool into a compact processor, providing global information on the entire coagulation process. The battery of traditional coagulation tests is based on the isolated, static laboratory end-points and as a result they do not take into account the interactions within the clotting cascade and platelets in whole blood. Haemostasis is an integrated, interactive, dynamic, and extremely complex process involving coagulation proteins, activators, cellular elements and inhibitors, which is recorded by TEG,¹³³ while standard tests of coagulation stop measuring at the first onset of clot formation – in other words, at static endpoints of blood coagulation. This provides no information on the dynamics of clot formation, strength and stability. As a result, it is difficult to assess what the exact dynamic process for inhibition of clotting is.

In the clinical setting, TEG may be used to rapidly assess coagulation abnormalities in the initial assessment of coagulation in trauma patients.¹³⁴ When compared with platelet count and PT/aPTT only TEG was predictive of early transfusion ($p < 0.05$), leading to the conclusion that TEG is a rapid, simple test that can broadly determine coagulation abnormalities. TEG and Sonoclot analysis were 100% accurate in predicting bleeding after cardiopulmonary

bypass and significantly better predictors of postoperative haemorrhage than routine coagulation tests.¹³⁵ Essell *et al.*¹³⁶ tested the utility of TEG versus platelet studies (BT, platelet count, mean platelet volume) and standard coagulation tests (PT, aPTT, fibrinogen). The sensitivities of the BT (71.4%) and platelet count (100%) were similar to TEG (71.4%), but the specificity (89.3%) of TEG in predicting postoperative bleeding was greater than that of the bleeding time (78.5%) and platelet count (53.6%), suggesting that patients with a normal TEG who have a post-bypass bleed should not be given blood products empirically, but that a surgical cause should rather be excluded. Kang *et al.*¹²⁷ found TEG to be a reliable and rapid monitoring system during liver transplantation and Martin *et al.*¹³⁷ validate the role of TEG during paediatric liver surgery, finding a 100% sensitivity (8/8) and 73% specificity (8/11) in predicting increased postoperative bleeding. In a study comparing the sensitivity of the ACT, aPTT and TEG, the ACT was less sensitive to residual heparin anticoagulation than aPTT or TEG.¹³⁸ In addition TEG using heparinase cups to analyse anticoagulated blood during cardiac surgery is useful to guide treatment with haemostatic components. Twelve-hour chest tube losses were not different between groups despite the threefold reduction in the use of haemostatic products, showing that intra-operative monitoring of coagulation in the anticoagulated patient can be used to guide treatment.¹³⁹ TEG, by providing information on the interaction of all the coagulation precursors, gives more clinically useful information on coagulation than that available from the coagulation profile or the activated clotting time alone. TEG was a significantly better predictor (87% accuracy) of postoperative haemorrhage and need for re-operation than was the ACT (30%) or coagulation profile (51%),¹⁴⁰ with TEG providing diagnostic data within 30 minutes of blood sampling. TEG is also useful in assessing platelet function in the presence of thrombocytopenia¹⁴¹ and pre-eclampsia/eclampsia.¹⁴²

In screening for hypercoagulability, standard laboratory tests that may indicate hypercoagulability include a high platelet count, high levels of coagulation factors VII, VIII, IX, X and XI, or fibrinogen levels, low levels of protein S, protein C, antithrombin, and the presence of a lupus anticoagulant, while the TEG indicators of hypercoagulability are a short *r*-time and *k*-time, a large MA or an α -angle that is large and rapidly increasing. This has been noted with increased frequency in a variety of patient groups who are considered to be at increased risk of thrombosis, with the predictive value of the TEG being higher than other systems. Hypercoagulability may not be reflected by standard coagulation monitoring and may be predominantly influenced by increased platelet reactivity,¹⁴³ which needs to be measured as a dynamic test and not as an end-point.

Conclusion

Coagulation is a dynamic and intricate process that becomes more complicated to understand as more is discovered about the process. On the other hand, the clinical end-point is still 'Will the patient clot or not?' With the many interactions taking place concomitantly there is not one simple answer to establish the exact state of the coagulation system, but the dynamic coagulation tests like the TEG are definitely getting closer to providing an understanding. Certainly rapid bedside, or theatre, information on coagulation status with rapid analysis of appropriate therapy will go far in managing

the coagulation status, especially when taken in conjunction with the clinical findings.

Much of this work was based on my Ph.D. thesis and a chapter on 'Fluids and coagulation' currently in press. Some of the diagrams were courtesy of Mike James (based on slides from Novo Nordisc) and Eli Cohen (Haemoscope).

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