

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Clinical Application of Fibrinolytic Assays

---

Dominic Pepperell, Marie-Christine Morel-Kopp and  
Chris Ward

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/57316>

---

## 1. Introduction

Haemostasis is a complex balance between thrombus formation and fibrinolysis. Research into bleeding and thrombotic conditions has led over many years to a detailed knowledge of the role of the components of coagulation, and subsequently many clinical applications have been developed for the testing of platelets, clotting factors and coagulation inhibitors. However, the same cannot be said for the components of fibrinolysis. Fibrinolytic research over the last 30 years has not resulted in the translation of basic science into routine clinical tests of fibrinolytic factors [1, 2], except for D-dimer assays, which are an indirect marker of both thrombosis and fibrinolysis. This has occurred partly due to the difficulties with individual fibrinolytic factor assays, but also due to the inherent limitation of using a single factor assessment to quantify the complex and dynamic process of fibrinolysis.

An ideal assay that could provide an overall or 'global' assessment of haemostasis would take into account the interactions between the proteins of the coagulation and fibrinolytic pathways, blood cellular components and the vessel wall. Such an assay does not currently exist, but refinements of old techniques with updated technology and the development of new global assays have brought improvements in this regard. The ability to assess fibrinolysis is a major benefit of some of these global assays, and has led renewed interest in this field.

### *Potential clinical applications*

There are multiple potential clinical situations in which the ability to detect hypo- or hyperfibrinolysis could in theory be useful. Hypofibrinolysis is a risk factor for thrombosis. Venous thromboembolism (VTE) is a common condition, with an incidence of approximately 1 in 1000 adults [3]. A potential benefit of detecting a fibrinolytic defect would be to identify individuals at higher risk of a first event, which could lead to different management strategies particularly in clinical scenarios such as pregnancy and the peri-operative period where the risk is already

known to be higher. This is relevant because currently only approximately one quarter of patients have a detectable inherited or acquired thrombophilia on testing, and 20% of patients have no cause at all found for their first VTE [3-5]. In addition, recurrent VTE events occur in approximately 20-30% of patients within 5 years [4, 6], and identification of hypofibrinolysis as a risk factor might be useful in informing the duration of anticoagulation. Hypofibrinolysis may also be important in arterial thrombosis such as myocardial infarction, and the ability to detect patients who are at increased risk of infarction or stent occlusion due impaired clot lysis would be an important clinical application [7].

Hyperfibrinolysis can result in an increased bleeding risk during surgery and invasive procedures. As it is associated with increased morbidity and mortality, identifying hyperfibrinolysis is a key aim for fibrinolytic assays [8]. Particularly in major procedures such as liver transplant, cardiac valve repair or revascularisation surgery, challenges to the haemostatic system are complex and continuously evolving, and the ability to rapidly detect hyperfibrinolysis in these patients has led to an improvement in their care [8-10]. Recently, attention has turned to the need for measuring fibrinolysis in trauma patients since the publication of a large multinational study revealed improvements in survival with the early administration of an anti-fibrinolytic drug [11]. Increased fibrinolysis can also be detected in some patients with inherited bleeding conditions such as haemophilia A, indicating that anti-fibrinolytics may also benefit these patients. Finally, disseminated intravascular coagulation (DIC) is a syndrome with abnormal activation of the both coagulation and fibrinolytic systems. Identifying hyperfibrinolysis in suspected cases could assist in the diagnosis of DIC, which otherwise requires complex scoring systems [12].

In this chapter we review assays of the individual factors of the fibrinolytic system and global tests which provide an overall assessment of the fibrinolysis. In each section we will outline the assay itself and its strengths and weakness, before reviewing the literature regarding its use and current or future clinical applications.

## 2. Fibrinolytic factors

### 2.1. Plasmin and $\alpha$ 2-plasmin inhibitor

The proteolytic enzyme plasmin is the main mechanism through which intravascular fibrin thrombi are degraded. It circulates as its inactive form, plasminogen, at a plasma concentration of approximately 2  $\mu$ mol/L and is activated intravascularly by tissue plasminogen activator (tPA) [13, 14]. The localization to intravascular thrombi and formation of the active protease occurs more readily after both plasminogen and tPA are bound to fibrin. Any free plasmin is bound by its primary physiological inhibitor,  $\alpha$ 2-plasmin inhibitor. This circulates at levels of approximately 1  $\mu$ mol/L in normal plasma (table 1), and neutralizes plasmin so rapidly that the active enzyme is undetectable in plasma. Assays have therefore been aimed at measuring plasminogen,  $\alpha$ 2-plasmin inhibitor or the plasmin- $\alpha$ 2-plasmin inhibitor (PAP) complex.

Plasminogen activity has been most frequently measured using a functional chromogenic assay, but antigenic assays are also available. In the functional assay, an extrinsic activator,

streptokinase, which acts on free as well as bound plasminogen, is added to a patient's plasma to convert all of the plasminogen to plasmin, which then acts on a chromogenic substrate [14, 15]. Several commercial kits are available. Where low activity levels are found, plasminogen antigen concentrations can be measured by enzyme-linked immunosorbent assays (ELISA) or nephelometry to distinguish between lack of plasminogen (type 1 deficiency) and dysplasminogenemia (type 2 defects) [15, 16]. PAP complexes have been assayed by ELISA kits [17, 18], and chromogenic assays have also been used to detect unbound  $\alpha$ 2-plasmin inhibitor activity [19].

|   | Plasma concentration |               | Site of Synthesis                       | Plasma half life (approximate) |
|---|----------------------|---------------|---|--------------------------------|
|   | ( $\mu$ g/ml)        | (mol)         |   |                                |
| <b>Plasminogen</b>                            | 200                  | 2 $\mu$ mol/L | Liver                                   | 1.8 – 2.7 days                 |
| <b>Plasmin</b>                                | Undetectable         | -             | -                                       | Very Brief                     |
| <b><math>\alpha</math>2-plasmin inhibitor</b> | 70                   | 1 $\mu$ mol/L | Liver                                   | 12 hours                       |
| <b>tPA</b>                                    | 0.005                | 70 pmol/L     | Vascular endothelium                    | 5 minutes                      |
| <b>PAI-1</b>                                  | 0.02                 | 400 pmol/L    | Vascular endothelium, Liver, Adipocytes | 2 hours                        |
| <b>TAFI</b>                                   | 5                    | 75 nmol/L     | Liver, Megakaryocytes                   | 10 minutes                     |

**Table 1.** Synthesis, plasma concentration and half life of fibrinolytic factors [13, 20, 21]

### *Congenital deficiency and venous thrombosis*

The clinical consequences of low plasminogen levels have been studied in patients with congenital deficiency. The prevalence of plasminogen deficiency in the general population has been estimated at 0.3% in a large Scottish cohort of blood donors [22], and 4.3% in a Japanese study [23]. The higher prevalence in the latter population is due to the high frequency of a specific plasminogen gene mutation (Ala6012Thr) in East Asian populations causing type 2 deficiency, with similar rates of type 1 disease in each study. Surprisingly, no definite relationship between either type of deficiency and arterial or venous thrombosis has been found [15, 16, 22, 23]. A modestly increased risk suggested by the combined findings of family studies was not statistically significant [15], and has not been confirmed in larger population-based research [22, 23]. Even in severe homozygous plasminogen deficiency there is no excess of thrombotic events; instead the main clinical phenotype is an accumulation of 'ligneous' fibrin-rich, pseudomembranous lesions in the conjunctiva and less commonly in other mucosal membranes [16]. The reason for a lack of thrombotic phenotype is unclear. It may be that residual plasminogen activity in these homozygous patients (the majority display between 4% and 51% activity) is enough to prevent the small vessel thrombosis seen in mouse gene knock-out models where there is no detectable plasminogen [24, 25], or that alternative fibrinolytic proteases released from neutrophils degrade fibrin [26].

Despite the evidence from congenital deficiency states, a small number of studies have investigated plasminogen or  $\alpha$ 2-plasmin inhibitor levels as a risk factor for venous thrombosis in non-deficient patients. A large case-control study of 770 patients and 743 controls (the MEGA study) found that modest correlations between thrombosis and plasminogen or  $\alpha$ 2-plasmin inhibitor were lost after adjustment for markers of inflammation and Body Mass Index [27], and the prospective cohort LITE study found no relationship with PAP complexes [18]. A small series of patients with Budd-Chiari syndrome were found to have a slight but statistically significant decrease in plasmin inhibitor levels [28].

#### *Arterial thrombosis*

Some large cohort and case-control studies have investigated an association between fibrinolytic proteins and myocardial infarction (MI). High plasminogen levels have surprisingly been consistently associated with a modest elevated risk of myocardial infarction [29-31]. However only one of these studies adjusted for inflammation or smoking [31], which along with elevated cholesterol are potential confounding factors, and the association with MI was lost. An alternative explanation may be that plasmin is known to contribute to the instability of atherosclerotic plaques by activating matrix metalloproteinases [32]. Alpha-2-plasmin inhibitor was also investigated, no association with MI being found in the prospective cohort ECAT study [29], and a slight positive correlation in the retrospective case-control SMILE study [31].

#### *Hyperfibrinolytic states*

Raised levels of PAP complexes and decreased levels of  $\alpha$ 2-plasmin inhibitor have been described in hyperfibrinolytic states, such as acute coagulopathy of trauma [reviewed in 17, 33], disseminated intravascular coagulation (DIC) [34] and the coagulopathy of acute promyelocytic leukaemia [reviewed in 35]. However, no clinical applications have been established, for example as a risk factor of severity.

Rare congenital  $\alpha$ 2-plasmin inhibitor deficiency has been described [reviewed in 36]. Homozygous deficiency results in a severe bleeding disorder due to increased fibrinolysis with a similar phenotype to congenital haemophilia or Factor XIII deficiency. Conventional haemostasis screening tests are normal, and a functional  $\alpha$ 2-plasmin inhibitor assay is required to make the diagnosis.

#### *Summary*

In summary, research has not resulted in any clinical applications of assays of plasminogen,  $\alpha$ 2-plasmin inhibitor or PAP complexes in the investigation of fibrinolytic activity despite their central role, perhaps because they circulate at relatively high concentrations and are generally not rate-limiting factors [13]. In rare cases of congenital deficiency of these proteins, functional assays can be used to make the diagnosis.

## **2.2. Tissue Plasminogen Activator (tPA) and Plasminogen Activator Inhibitor-1 (PAI-1)**

Both tPA and PAI-1 have been investigated as markers of fibrinolytic activity. After stimulation, tPA is released locally from endothelial cells and activated platelets, and binds to the

fibrin surface of the developing thrombus where it catalyses the formation of plasmin. Once bound, it is protected from its principle inhibitor PAI-1, which circulates in plasma and is also secreted by platelets [13, 37]. Free tPA has a plasma half life of approximately 5 minutes due to the action of PAI-1 and simultaneous clearance by the liver [13]. Therefore resting levels of unbound active tPA in plasma are very low and may be measured by ELISAs that detect both the active form and the tPA – PAI-1 complex. PAI-1 levels have been investigated using functional assays and various ELISAs, which utilise a range of monoclonal antibodies with varying sensitivities for unbound PAI-1 and the tPA-PAI-1 complex [38].

There are several issues that complicate the results of these assays. The first is that measuring resting plasma levels of tPA detects mainly inactive complexes with PAI-1, and so raised levels of tPA antigen may indicate inhibited fibrinolysis rather than increased fibrinolytic potential [2]. An inverse relationship between antigen and fibrinolytic activity has even been found [39, 40]. To overcome this, some investigators have measured acute tPA release following stimulation such as prolonged venous occlusion by a tourniquet. However, it is not clear whether this is a better reflection of the physiological situation [7]. In addition, plasma levels of PAI-1 do not reflect its true contribution in inhibiting fibrinolysis as the majority of PAI-1 is released at the site of thrombus by activated platelets [37, 41] and plasma and platelet pools of PAI-1 vary independently of each other [42].

Levels of PAI-1 and tPA are affected by other factors. PAI-1 in particular has many non-fibrinolytic functions, and may be raised in diabetes mellitus and insulin resistance [reviewed in 2]. Both tPA and PAI-1 are acute phase reactants and elevated with raised lipids and pregnancy [29, 31]. Furthermore, there is marked diurnal variation in their levels, being higher in the morning [43].

#### *Arterial and venous thrombosis*

The association between tPA and PAI-1 antigen levels with arterial disease and thrombosis has been investigated in multiple large studies with conflicting results, described in two recent reviews [2, 7]. Although some studies have found increased tPA and PAI-1 levels to be associated with an increased risk of arterial disease or recurrent events [reviewed in 44, 45], some major publications including the Framingham study have reported no association [30, 46], and in two studies even a trend to decreased risk was found in subjects with elevated PAI-1 [47, 48]. The use of assays measuring tPA release has not resulted in any clarity, increased levels being associated with major atherothrombotic events in one study [49] and the inverse in another [29]. These confusing results may be partly accounted for by confounding factors demonstrated by studies that have adjusted for a range other risk factors such as diabetes, cholesterol, obesity, and inflammation. In the ECAT, SMILE and Young Finn studies, apparent associations between PAI-1 or tPA and arterial risk were lost when other these other factors were included in the analyses [29, 31, 50].

The data on tPA and PAI-1 as risk factors for venous thrombosis has similarly failed to find any clinically useful associations [reviewed in 1, 2]. Some prospective studies have found no differences in antigen levels between patients who suffered thrombosis and controls [18, 51, 52], whereas a recent large retrospective study has found PAI-1 to be a risk factor for first

venous thrombosis and to be the most important determinant in Clot Lysis Time (CLT), a global test for hypofibrinolysis described later in this chapter [27].

#### *Hyperfibrinolytic states*

PAI-1 and tPA levels have been investigated in various hyperfibrinolytic states. PAI-1 has been suggested as a potential therapeutic target in DIC since raised levels correlate with multi-organ failure and outcome [53], whereas in acute traumatic coagulopathy, no association between PAI-1 and severity of injury has been found [17]. In liver cirrhosis, raised tPA levels are proportional to the severity of cirrhosis and risk of variceal bleeding, and may be mediated by a relative deficiency of PAI-1 [discussed in 54].

#### *Summary*

Despite a large amount of data on tPA and PAI-1 levels in the literature, no current clinical applications have emerged. The methodological problems and confounding factors discussed above have played a major role in the lack of consistent clinical correlations with disease and outcome.

### **2.3. Thrombin activatable fibrinolysis inhibitor (TAFI)**

TAFI circulates in plasma as a proenzyme and is converted into its active form TAFIa by thrombin or the thrombin-thrombomodulin complex. It then removes specific lysine or arginine residues from partially degraded fibrin, thereby preventing tPA and plasminogen binding which is required for efficient activation of fibrinolysis [13]. It is unstable in plasma, spontaneously degrading to a latent form (TAFIai) with a half life of approximately 10 mins (Table 1).

TAFI can be quantified by ELISA or functional assay. The ELISAs have the advantage of being easy to perform. However there are important differences in the specificities of the antibodies used; they variably measure total antigen, be specific to certain TAFI genotypes, or measure activated TAFI only by being specific for the TAFIa-TAFIai complex [2, 7, 21]. The functional assays measure the ability to cleave residues from small synthetic substrates and have the advantage of measuring all active TAFI, although there may be interference by other carboxypeptidases in plasma. A practical disadvantage is that they are affected by the variable thermal instability of TAFIa, so samples have to be placed on ice immediately and centrifuged at 4°C, and they also must be collected in tubes containing thrombin and plasmin inhibitors to prevent in-vitro activation [21].

#### *Venous and arterial thrombosis*

Raised TAFI levels appear to be consistently associated with venous thromboembolism. In a large case-control study, the Leiden Thrombophilia Study (LETS), TAFI antigen levels above the 90<sup>th</sup> percentile of controls were found to be associated with an almost two-fold increased risk of first deep vein thrombosis [55], a finding replicated in a smaller case control study using an ELISA specifically designed to be insensitive to different polymorphisms of TAFI [56]. There is some evidence that recurrent venous thromboemboli may be predicted by high TAFI levels; a prospective study of 600 patients with unprovoked venous thrombosis found a two-fold risk

of recurrence if their TAFI levels were above the 75<sup>th</sup> percentile [57]. Disappointingly, this association could not be confirmed in a follow-up to the LETS study, although this may be because patients with provoked thrombotic events were included [58].

The association between TAFI levels and arterial disease are unclear. Some studies have found a link between high TAFIa (but not total TAFI antigen) and coronary artery disease or myocardial infarction [59, 60], but others have found no association [61, 62] and some have found low total TAFI antigen levels to be associated with increased risk [63, 64].

#### *Other diseases*

Associations have been investigated between TAFI and a variety of other disease states such as renal failure, hepatic disease, endocrine disorders, cancer, DIC and pregnancy complications [reviewed in 21] without useful clinical applications arising. One recent study has shown an interesting correlation that suggests the variable bleeding phenotype seen in severe Haemophilia A may be associated with TAFI activation levels [65].

#### *Summary*

Once again, the problems with TAFI assays and the lack of consistent significant associations with disease mean that there are no current clinical applications. However the possible association of raised TAFI with recurrent unprovoked venous thrombosis warrants further study.

### **2.4. Other factors affecting fibrinolysis**

*Lipoprotein(a)* or Lp(a) is a homologue of plasminogen that circulates in plasma and is able to inhibit t-PA mediated plasminogen activation at the fibrin surface [7]. It has been investigated as a risk factor for arterial thrombosis in several prospective studies, and a consistent but weak association has been seen with cardiovascular events in both young and elderly patients [reviewed in 7]. A meta-analysis of prospective studies over the last 40 years, involving approximately 127 000 subjects, found that Lp(a) concentrations were an independent risk factor for both coronary disease and stroke but not clinical outcome. The effect was weak, with a hazard ratio of just 1.13 [66]. Therefore on an individual patient basis, assays of Lp(a) are unlikely to be useful.

Annexin A2 receptors on the endothelial cell surface bind both plasminogen and tPA, and accelerate plasminogen activation 60-fold [13]. Recent publications have shown that autoantibodies to annexin A2 are associated with thrombosis in patients with antiphospholipid syndrome [67]. Another interesting finding has been that autoantibodies to annexin A2 occur in a subset of patients with cerebral sinus thrombosis with or without antiphospholipid antibodies; testing for anti-annexin antibodies may have a role in establishing aetiology in these patients whose thrombosis is otherwise often thought to be idiopathic [68].

### **2.5. D-dimers**

D-dimers are a specific cross-linked fibrin degradation product. Their formation depends on thrombin converting fibrin to fibrinogen and activating factor XIII (FXIIIa), which then cross-



links the D-domains of adjacent fibrin strands prior to cleavage by plasmin. Thus D-dimers can be seen as an indirect marker of both coagulation and fibrinolysis, although low levels can be seen under normal physiological conditions, and raised levels may occur in pregnancy, advancing age and a wide range of inflammatory and malignant conditions as well as thrombosis [reviewed in 69]. For these reasons specific, evaluation of the fibrinolytic system with D-dimer results alone is problematic, and we will only briefly summarise the assays and their established clinical applications below.

D-dimers can be measured using monoclonal antibodies that have variable specificity for an antigen found in the FXIIIa cross-linked fragments of fibrin compared to other fibrin degradation products. Multiple assays have been developed using three techniques: quantitative ELISAs, whole blood agglutination (qualitative), and latex bead agglutination assays that can be performed on routine laboratory coagulation analysers and may be quantitative or qualitative [69]. It is important to note that results are not comparable between different assays, even between those of similar formats. For this reason it is important that clinicians and researchers are aware of the performance characteristics of the individual assay they are using, and the clinical setting for which it has been validated [70]. In addition, many of the commonly used assays may give false positive results due to lipaemia, hyperbilirubinaemia, intravascular haemolysis or high levels of rheumatoid factor [69], and anticoagulation reduces D-dimer levels even within 24 hours of commencement [71].

#### *Venous thromboembolism*

D-dimers are typically elevated in acute VTE. However, because they are increased in a variety of other non-thrombotic disorders, raised D-dimers are a sensitive but not specific marker. As mentioned above, D-dimer assays vary; the latex agglutination and ELISA methods are the most sensitive (93-96%) but least specific (43-53%) [72], and consequently a 'negative' or normal range result in these has a high negative predictive value. Multiple studies have shown the D-dimer assay can be successfully combined with clinical probability scoring systems in the diagnosis of suspected lower extremity DVT or PE [reviewed in 69] and their use has been recommended in recent guidelines [73]. In patients with a low or moderate pre-test probability of DVT, a negative D-dimer test of high sensitivity can exclude a DVT without the need for further investigations. Similar strategies can be used in patients with suspected PE [69], but not in upper limb venous thrombosis [73], and the test is less useful in the elderly, hospital inpatients or those with malignancy due to the high prevalence of elevated D-dimers in these groups reducing assay specificity [74].

There is an increased risk of recurrent VTE in patients with evidence of ongoing activation of the coagulation and fibrinolytic systems, as indicated by a raised D-dimer after the cessation of anticoagulant therapy [69, 75]. This may be used to inform treatment decisions regarding the duration of anticoagulation; a major study (PROLONG) randomised patients to restarting anticoagulation or observation if their D-dimer was raised a month after treatment had ceased. The recurrent VTE rate was demonstrated to be significantly less in those who had restarted anticoagulation [76].

### *Disseminated Intravascular Coagulation*

DIC can be characterised as a syndrome with uncontrolled activation of the both coagulation and fibrinolytic systems, and consequently raised D-dimer levels are a sensitive but non-specific marker [69]. As no single test can establish the diagnosis, D-dimers have been used in scoring systems to establish the likelihood of DIC and monitor the effect of interventions, such as the one developed by the International Society on Thrombosis and Haemostasis (ISTH) [12].

## **3. Global fibrinolytic assays**

The difficulties described above in using individual fibrinolytic markers to measure the fibrinolytic potential of a patient's plasma has lead to increasing interest in global tests that provide an overview of the entire process. Below we discuss thromboelastography, and fibrin generation and lysis assays which have current or potential clinical applications.

### **3.1. Thromboelastography**

Thromboelastography uses viscoelastic changes during coagulation to produce a graphical representation of the fibrin polymerisation process and subsequent fibrinolysis, and as such it has the potential to provide a global evaluation of clot initiation, formation and lysis. First described in 1948 in Germany, two commercial analysers are currently available: the Thromboelastograph or 'TEG' (Haemonetics, MA, USA) and the Rotational Thromboelastogram or 'ROTEM' (Tem International GmbH, Munich, Germany). Both of these are suitable for use as 'point of care' (POC) devices, and this setting is where thromboelastography has developed its main clinical applications.

The principle of the thromboelastogram is as follows [8, 9]; whole blood is added to an incubated cup at 37°C, into which a pin is suspended connected to a detector system. As cup and pin are oscillated relative to each other, the increasing viscosity from the developing fibrin polymerisation affects the magnitude of the movement of the pin, which is converted by a mechanical-electrical transducer to a signal which is displayed as a trace (Figure 1a). As fibrinolysis occurs, the viscosity falls and is also recorded on the trace. As well as the graphical output, values are calculated for various parameters which include: time until initial fibrin formation, clot formation time, rate of polymerisation of fibrin, maximum clot firmness, and clot lysis. Table 2 lists these and the haemostatic variables they are proposed to measure.

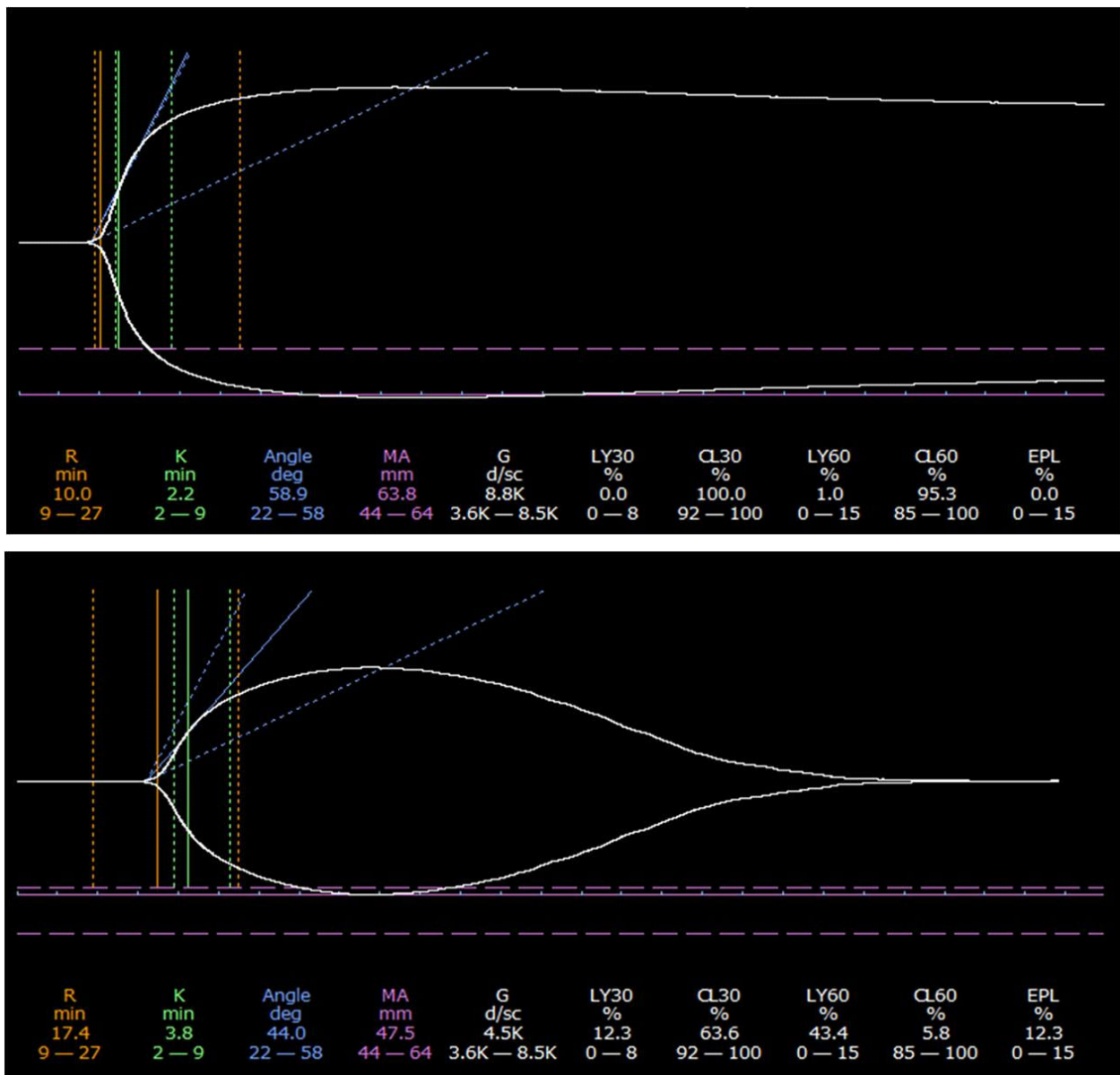
Although the TEG and ROTEM are similar in their technologies, there are some important differences. Firstly, in the TEG, the movement is initiated in the cup and a torsion wire monitors the changes in the sample, whereas in the ROTEM, the pin moves on a ball bearing and there is an optical detection system. Some authors claim this modification has led to the ROTEM being more robust in busy clinical settings such as emergency departments and operating theatres [78, 79]. The ROTEM system also has an electronic pipette to help standardise the method. Both systems use proprietary initiators or modifiers of haemostasis, and the TEG can run two assays in series, whereas the ROTEM can run four. The TEG initiates coagulation with

| TEG Parameter                      | ROTEM Parameter                    | Description   | Proposed measured variables                    |
|------------------------------------|------------------------------------|---|--|
| <b>R (Reaction)</b>                | <b>CT</b>                          | Clot Time – period to 2mm amplitude   | Coagulation factors, platelets, anticoagulants |
| <b>K</b>                           | <b>CFT</b>                         | Clot Formation Time – period between 2 – 20mm amplitude                               | Coagulation factors, fibrinogen, platelets     |
| <b><math>\alpha</math> - angle</b> | <b><math>\alpha</math> - angle</b> | Rate of clot formation – slope of trace   | Coagulation factors, fibrinogen, platelets     |
| <b>MA</b>                          | <b>MCF</b>                         | Maximum amplitude – maximum clot firmness on trace                                    | Fibrinogen, platelets, fibrinolysis            |
| <b>G</b>                           | <b>MCE</b>                         | Shear elastic modulus strength or clot elasticity – a representation of clot strength | Platelet function, fibrinogen                  |
| <b>LY30, LY60</b>                  | <b>LI 30</b>                       | Percent decrease in amplitude 30 or 60 minutes after MA is reached                    | Fibrinolytic factors, fibrinogen               |
| <b>EPL</b>                         | -                                  | Estimated Percentage Lysis - rate of change of amplitude after MA is reached          | Fibrinolytic factors, fibrinogen               |
| -                                  | <b>ML</b>                          | Maximum Lysis at the end of ROTEM test (20 - 40 mins)                                 | Fibrinolytic factors, fibrinogen               |

**Table 2.** TEG and ROTEM nomenclature for measured parameters [8, 9, 77]

kaolin or a combination of tissue factor and kaolin ( $\pm$  heparinase), and in the ROTEM there are multiple reagent options that may help to distinguish between the causes for abnormal traces, described in table 3 [8, 9]. Relevant to measuring fibrinolysis, one of these (APTEM) contains the fibrinolytic inhibitor aprotinin; correction of an abnormal trace by adding this reagent has been used to suggest the presence of hyperfibrinolysis. It is important to note also that reference ranges vary considerably between the two technologies, and hence results are not directly comparable between the instruments [80].

A major advantage of the thromboelastogram and one that has led to its principle uses during major surgery, is that it can be used as a bedside measure of global haemostasis, the graphical output appearing as a real-time representation of the patient's clot formation and lysis over a 20-30 minute assay time, avoiding delays inherent in sending samples for laboratory analysis. Furthermore, non-anticoagulated whole blood can be used, and therefore many of the interactions between coagulation, platelets and fibrinolytic factors are preserved. A limitation is that the coagulation is being measured under static (no shear) conditions in a plastic cuvette rather than an endothelialised blood vessel [9], although of course this disadvantage is shared with almost all other *in vitro* assays.



**Figure 1. (a) Normal TEG trace.** The patient's thromboelastographic trace is shown in white and is produced in real time. The x-axis is time; the y-axis is millimetres of deviation representing increasing visco-elasticity of the sample as clot forms. Derived parameters are listed below the trace with their proposed normal ranges. R = Clot time; K = Clot formation time; Angle deg =  $\alpha$  – angle or Rate of Clot formation; MA = Maximal Amplitude; G = Shear elastic modulus strength; LY30/60 = Percent Lysis at 30/60 minutes; CL30/60 = Inverse of LY30/60 parameter; EPL = Estimated Percentage Lysis; **(b) TEG trace showing hyperfibrinolysis.** The patient's trace in white shows early convergence representing a reduction in visco-elasticity caused by complete fibrinolysis. The key to the derived parameters displayed below the trace is given in Figure 1a above

A concern with thromboelastography has been in the standardisation of methods and reproducibility of results. Pre-analytical variables such as site of blood sampling, time from sampling to analysis, type of reagent and instrument used can alter results significantly [reviewed in 8]. In addition to native whole blood, re-calcified citrate samples may be used when thromboelastography is performed in the haemostasis laboratory, or when a delay

| Assay             | Reagents                            | Proposed Use   |
|-------------------|-------------------------------------|--|
| <b>TEG</b>        |                                     |  |
| <b>Kaolin</b>     | Kaolin                              | Overall coagulation assessment                         |
| <b>Rapid TEG</b>  | Kaolin + Tissue Factor              | Shorter test time / faster results                     |
| <b>Heparinase</b> | Kaolin + Heparinase                 | Detection of heparin                                   |
| <b>ROTEM</b>      |                                     |  |
| <b>In-TEM</b>     | Contact activator                   | Assessment of intrinsic pathway                        |
| <b>Ex-TEM</b>     | Tissue Factor                       | Assessment of extrinsic pathway                        |
| <b>Hep-TEM</b>    | Contact activator + Heparinase      | Detection of heparin                                   |
| <b>Ap-TEM</b>     | Tissue Factor + Aprotinin           | Detection of fibrinolytic effect when used with EX-TEM |
| <b>Fib-TEM</b>    | Tissue Factor + Platelet antagonist | Qualitative assessment of fibrinogen                   |

**Table 3.** Selected TEG and ROTEM reagents [9]

between sampling and analysis is expected in a POC setting. However it has been reported that stable results are not produced until after 30 minutes of collection in citrate, which defeats the purpose of the technique as a 'real-time' assessment of haemostasis, and values from citrate samples cannot be correlated to those from non-anticoagulated samples [81].

Operating the TEG or ROTEM in a POC setting means that non-laboratory staff must run these moderately complex tests and hence sufficient numbers need to be properly trained to operate and maintain the equipment to avoid further errors. Quality assessment (QA) must be carried out on a regular basis, the TEG for example requiring quality control (QC) samples to be run each time the machine is switched on, every 8 hours or if the analyser moved [10]. Commercial samples for internal quality control are available and external QA schemes exist, but the results from the latter have been disappointing [82]. Coefficient of variation (CV) results between centres have been reported between 7.6 – 39.9% in the TEG and 3.6 - 83.6% in the ROTEM [82, 83] for coagulation variables on plasma samples, although much better results in both devices can be produced in a single centre using whole blood if the manufacturer's method is followed exactly [9, 84].

No data on the reproducibility on fibrinolytic measures between centres has been published. The effect of fibrinolysis on TEG or ROTEM instruments can be detected using the parameters that are listed in Table 2. However, operators can also use a qualitative difference in the shape of the traces to diagnose hyperfibrinolysis, an example of which is shown in Figure 1b. Although subjective, experienced operators have used this strategy successfully in complex surgery to guide treatment of hyperfibrinolysis as described below, and the effect of giving an anti-fibrinolytic agent *in vivo* or adding aprotinin *in vitro* in the APTEM is clearly demonstrated in the traces of these patients [77, 78, 85-87]. However, the validity and sensitivity of this approach is unclear and in some reviews it has been suggested that only marked hyperfibrinolysis can be identified this way [33, 88]. Some investigators using the TEG have attempted

to standardise the definition of hypofibrinolysis as an 'Estimated Percent Lysis' (EPL) of greater than 15% [88]; this is calculated by the TEG software comparing the area under the curve at the MA amplitude and 30 minutes later. Validation of this definition as a measure of hyperfibrinolysis is lacking, but a recent publication has shown that increasing concentrations of tPA at pathophysiological levels added *in vitro* lead to higher EPL levels in a dose dependent way (but not in the less sensitive RapidTEG) [89]. Also, important clinical correlations with outcome have been found with this EPL threshold (see the studies reviewed below). In the ROTEM there is some data to suggest the maximum lysis measure at 1 hour correlates with tPA, PAPs and PAI-1 [79], and the clot lysis index at 30 mins has 71% sensitivity for hyperfibrinolysis when compared to the less quantitative euglobulin clot lysis test (described in a later section) [78].

In discussing the literature regarding the use of thromboelastography as an assay of fibrinolysis, first the established uses as a POC device in surgery and trauma will be summarised, although detection of hyperfibrinolysis in these situations is only a component of the data utilised. Following this, the research concerning the use of the technique in pregnancy, haemophilia and hypercoagulable states will be discussed.

#### *Hepatic surgery*

One of the first clinical applications described was in orthotopic liver transplantation where the TEG can be used to monitor coagulation and guide blood component and anti-fibrinolytic therapy [90]. The haemostatic defect in these patients is complex; they often start with a thrombocytopenia and depletion of clotting factors due to end stage liver disease, but also have low levels of the natural anticoagulant proteins C, S and antithrombin. During surgery they may have a rapidly changing picture due to consumption of factors and dilution. Patients may also develop hyperfibrinolysis caused by tPA build up during the anhepatic stage, followed by a surge of tPA release from the reperfused transplant liver [91, 92]. However, unnecessary use of anti-fibrinolytics should be avoided due to the risk of hepatic artery thrombosis. POC thromboelastography can produce timely information at intervals throughout the surgery on the nature of the defect and requirements for coagulation factor replacement or anti-fibrinolytic therapy, and has been shown to decrease the need for red cell transfusion and plasma products [90, 93-95].

#### *Cardiac surgery*

Cardiac surgery requiring cardiopulmonary bypass is a complex balance between anticoagulation with heparin, the effect of anti-platelet agents and the need for haemostasis at the end of surgery [10]. Hyperfibrinolysis may also be seen, particularly post-bypass. There is good evidence from randomised controlled trials and retrospective analysis that the use of thromboelastography-guided algorithms for heparin reversal, blood component and anti-fibrinolytic support reduce the need for blood product transfusion and the rates of surgical re-exploration due to post-operative bleeding [96-100]. For example, a randomised prospective trial of 105 patients undergoing complex cardiac surgery compared TEG-guided transfusion therapy with standard care and found significantly fewer transfusions in the TEG group due to less postoperative requirements [97]. This trial also showed a 75% drop in the number of

patients having FFP infusion and a 50% fall in platelet transfusion. Because the use of TEG and ROTEM has been shown to reduce the potential risk to the patient and to reduce costs [96, 101], their use has been recommended in both US and European guidelines [102, 103].

### *Trauma*

Increased understanding of trauma associated coagulopathy has shown that fibrinolysis is a key component which is associated with increased mortality and can be reliably detected by thromboelastography in the emergency department, combat settings and operating theatres [for recent reviews see 10, 33]. Interest in this area has grown further since the publication of the large multi-centre CRASH-2 study which showed decreased mortality if trauma patients were given tranexamic acid to inhibit fibrinolysis within three hours [11]. However the inclusion criteria were very broad in this study, and POC testing with TEG may be able to target anti-fibrinolytic therapy to those who would benefit most. For example, a recently published prospective study used TEG to evaluate a severely injured cohort of patients for hyperfibrinolysis, defined as an EPL of 15% or more [104]. The incidence was 11% overall, but these patients had 25 times the risk of early mortality. The TEG assay was reported to rapidly identify hyperfibrinolysis within the first hour in all patients, allowing potential early use of anti-fibrinolytics. The results replicate a smaller study using the ROTEM [105]. These developments warrant further prospective randomised trials to demonstrate a reduced morbidity or survival benefit in trauma patients monitored by thromboelastography and treated accordingly.

### *Haemophilia*

Little has been published on the use of thromboelastography in inherited bleeding disorders. However, the TEG has been used to demonstrate hyperfibrinolysis in Haemophilia A, and this was correctable with either recombinant FVIIa or an anti-fibrinolytic or both [86, 106], and the TEG is also able to monitor the effect of rFVIIa used to treat haemophilia patients with acquired inhibitors [107].

### *Hypercoagulable or hypofibrinolytic states*

Thromboelastography has been less successful in demonstrating hypofibrinolysis. This may be because normal subjects only show a minor degree of fibrinolysis in unmodified TEG or ROTEM assays, for example one study showing the normal range of the ROTEM Maximum Lysis at 60 minutes to be 0–12% (mean 3%) [84]. Modifications have been suggested to increase the sensitivity to hypofibrinolysis by adding exogenous tPA to the cuvette prior to clot initiation, but there has been little published on the success of this approach; one group described a standard method for measuring tPA induced fibrinolysis using the TEG and subsequently used it to show that children with idiopathic venous thrombosis have significantly reduced fibrinolysis compared to controls [108, 109]. The data concerning the use of thromboelastography in hypercoagulable states has otherwise concentrated on the ability of the technique to show shortened clotting times, rapid fibrin polymerisation or increased clot strength [e.g. 110], and is outside the scope of this chapter.

### *Pregnancy*

Pregnancy is a hypercoagulable state in which many of the components of the coagulation and fibrinolytic system are altered, but conventional assays fail to provide an overall picture of the patient's haemostatic potential. Thromboelastography has been used in this regard, and has been successful in guiding therapeutic anticoagulation with LMWH and assessing the risk of neuraxial anaesthesia [reviewed in 8, 10]. A minor degree of hypofibrinolysis can be detected by TEG at the end of the third trimester compared to 8 weeks post partum [111], and an algorithm for TEG-guided treatment of post partum haemorrhage has been suggested, similar to those used in trauma [112].

### *Conclusion*

Thromboelastography has an established clinical role in the detection of hyperfibrinolysis in major hepatic and cardiac surgery, and its use in the management of trauma victims is growing. As further awareness and understanding of the assay occurs, further applications in bleeding patients [112], haemophilia and pregnancy are likely [113]. However, an expansion of its role into detection of hypofibrinolysis is unlikely given the insensitivity of current methodologies.

## **4. Fibrin generation and lysis assays**

Attempts had been made to time the dissolution of plasma clots as an overall measure of fibrinolysis even before the development of molecular markers and their immunoassays. However, spontaneous fibrinolysis of plasma clots is an extremely slow process, for example taking as long as 20 hours to achieve just 10% lysis [114]. Laboratory assays were subsequently developed that could measure fibrinolysis over a shorter period, principally the euglobulin clot lysis time (ECLT) [115] and the dilute whole blood clot lysis time (DWBCLT) [116]. However they have not been widely adopted because of several drawbacks. The ECLT measures lysis in only a fraction of plasma precipitated by utilising a low pH and ionic strength; the main determinants are fibrinogen, tPA and plasminogen only, the natural inhibitors being absent. DWBCLT is performed in the absence of calcium and hence excludes the interplay of the coagulation and fibrinolytic systems, and both of these tests were also labour intensive and unsuited to modern clinical laboratories.

### **4.1. Clot lysis time (CLT)**

More recently an automated assay called the clot lysis time (CLT) has been developed to assess the dissolution of a tissue factor induced fibrin clot by exogenous tPA [117]. Citrated platelet-poor plasma (PPP) is added to a microtitre plate at 37°C with tissue factor, calcium, phospholipid to initiate clot formation and exogenous tPA to trigger fibrinolysis. The turbidity at 405nm is measured over time. The CLT is defined as the time from the midpoint of clot formation (between clear and maximum turbidity) to the midpoint of clot lysis (between maximum turbidity and clear). It has a mean ( $\pm$  SD) in normal individuals of 83.8 ( $\pm$  11.1) minutes [118].



The CLT has several advantages. Firstly, it is relatively simple and easy to run. It can be done on previously frozen samples and is insensitive to method of PPP preparation [118]. It provides an assessment of the overall fibrinolytic capacity of the plasma, being affected by most of the individual factors. Plasminogen,  $\alpha$ 2-plasmin inhibitor, PAI-1 and TAFI all have been shown to be important variables in the CLT, whilst prothrombin, fibrinogen and factors VII, X and XI have a progressively more minor effect [27]. However, the concentration of the exogenous tPA is above physiological levels, and important interactions with platelets are not accounted for.

The CLT has been poorly standardised between groups, but a recent publication has sought to address this and investigate the biological variation within healthy individuals [118]. It was identified that the assay has a total analytical CV of 13.4%, and there is substantial biological variance over time within normal individuals. Sequential samples are therefore required to establish the true fibrinolytic potential of an individual; the authors suggest a single result may differentiate up to 20% from the true value. These should be done at the same time of day because the assay is affected by the diurnal variation in fibrinolysis that was previously noted in plasma levels of tPA and PAI-1, but no seasonal variation was detected. As it is a turbidometric assay, results may also be affected by marked lipaemia or paraproteinaemia.

#### *Venous thromboembolism*

There is definitive evidence that reduced fibrinolytic potential as shown by prolonged CLT is a risk factor for first venous thromboembolism (VTE). The LETS case control study determined the CLTs for 421 patients following their first DVT selected consecutively from anticoagulation clinics and excluded only if aged over 70 or if they had a malignancy [117]. Samples were taken at least 6 months after the diagnosis and at least 3 months after the cessation of anticoagulant and were matched with 469 control samples from patient's partners. A dose-dependent correlation was seen between increased CLT and DVT, with those with CLTs above the 90<sup>th</sup> percentile having twice the risk of controls, even when corrected for age, sex, clotting factors, antithrombin and TAFI levels. A second smaller study investigated lysis times in a group of 100 patients with a first idiopathic VTE using a similar turbidometric assay [119]. Patients were excluded if they had any known congenital or acquired risk factors for thrombosis or evidence of an underlying inflammatory state; the included VTE patients showed mean clot lysis times that were a 31.9% longer than controls. Findings were replicated in the Multiple Environmental and Genetic Assessment (MEGA) study of 2090 patients with first DVT or PE and 2564 controls [120]. Patients were between 18-70 years and were excluded if death or end-stage disease prevented follow-up samples being taken, whilst the control group was comprised of the patient's partners and random healthy individuals. Again, a dose dependent increase in risk of DVT was seen for increasing CLTs. The authors defined hypofibrinolysis as those with a CLT above the 75<sup>th</sup> percentile, and these patients had a two-fold risk compared to those in the first quartile. Interestingly, a synergistic effect was seen when CLT was combined with other known risk factors. Immobilisation increased the risk of VTE 4.3-fold in this study, but combined with hypofibrinolysis the risk was over 10 times that of controls. The overall risk with Factor V Leiden heterozygosity increased from 3.5 to 8.1-fold but the combination with prothrombin 20210A mutation did not show the same effect. Most markedly, for women under 50 years on the oral contraceptive, the risk of first VTE went up from 2.6-fold to over 20-times

the risk if they also had hypofibrinolysis. Previously it had been shown that OCP use had either a minor [121] or no effect [122, 123] on prolonging the clot lysis time by itself.

#### *Recurrent VTE*

The establishment of hypofibrinolysis defined by CLT as a risk factor for first VTE has led to interest in whether it could predict recurrent events, and therefore be used in clinical decision-making on the duration of anti-coagulation in patients. The LETS population was prospectively studied to see if prolonged CLTs detected after the first DVT predicted recurrent VTE [58]. In a group of 447 patients with a mean follow-up of 7.3 years, no significant association was seen in the 90 patients with recurrence. In a second study, 704 patients with a first unprovoked VTE and no genetic risk factor, malignancy, lupus anticoagulant or other requirement for long-term anti-coagulation, were studied [124]. In the female population (n=378), there was a 3.28-fold risk of recurrence for those with a CLT in the fourth quartile, but no association with recurrence was found in men or the overall population. Whilst these results are disappointing, there may be reasons why some effect was not detected; it should be noted that in the LETS study, patients with temporary risk factors such as immobility were included in the study although they had a low risk of recurrence, and in both studies patients who remained on anti-coagulation for any reason were excluded, including those continuing because they were thought to be high risk for recurrence. In neither of these studies were CLTs combined with other known predictors of recurrence, such as elevated D-dimer or Factor VIII levels, to see if their predictive power could be increased.

#### *Uncommon thrombotic disorders*

Some recent interesting research suggests using global methods such as the CLT may provide insight into the pathogenesis of some poorly understood hypercoagulable diseases. For example, CLTs were significantly longer (p=0.001) in 81 patients with retinal vein occlusion compared to a matched control group, even when multivariate analysis adjusted for cardiovascular risk factors [125]. Another study showed CLTs above the 95<sup>th</sup> percentile gave a 3.4-fold increase risk of Budd-Chiari syndrome [28], and 92 patients with pre-eclampsia were found to have significantly prolonged clot lysis times compared to controls, independent of the presence of antiphospholipid antibodies [126].

#### *Arterial thrombosis*

Clot lysis times have also been studied as a risk factor for arterial disease in 3 recent studies. The first used samples from the large case control study population from the Study of Myocardial Infarction Leiden (SMILE) to test CLT in 421 men and 642 controls [127]. Samples in the study group were taken a median 2.6 years after first MI. In men under 50 years the overall risk of MI was 3.2 times (95% CI 1.5-6.7) increased for CLTs in the highest quartile; however, once cardiovascular risk factors were adjusted for the risk was attenuated to just 1.8 (95% CI 0.7-4.8), and no relationship was seen in the over 50s. Another case-control study (from the 'ATTAC' study) examined the association between CLTs and ischaemic stroke, peripheral vascular disease as well as MI in both sexes [123]. Even after adjusting for cardiovascular risk factors there was an approximately 2-fold increase in risk of arterial event associated with CLTs above the 80<sup>th</sup> percentile (compared to the risk associated with diabetes which was 2.5), and

the association was similar in the 3 categories of disease. Finally, a third case-control group (the RATIO study) found a significant association between CLT and arterial thrombosis in young women aged 18-50 [128]. The risk of MI was increased for those with CLT in the third tertile, but surprisingly the risk of ischaemic stroke was only increased by shortened CLTs, i.e. with hyperfibrinolysis, a finding which is yet to be validated or explained.

#### *Anticoagulants and hypocoagulable states*

Given the evidence that some patients with thrombosis have impaired fibrinolysis, one interesting area of investigation concerns the effect of different anticoagulants on fibrinolysis measured by the CLT. Data on their effect could help to interpret CLT studies on patients with VTE without the need to take them off their anticoagulation, which was a methodological problem in the studies on recurrent VTE risk described above. Another possible benefit might be the ability to individualise anticoagulant choices for patients with hypofibrinolysis. Three studies have addressed the effect of anticoagulants. CLTs were measured in an in-vitro study where varying concentrations of anticoagulants including a low molecular weight heparin (LMWH), a selective anti-Xa drug (fondaparinux) and thrombin inhibitors (hirudin and PPACK) [129]. The LMWH and fondaparinux both had a significant effect in shortening CLTs, whereas no effect was seen with the thrombin inhibitors. A second study looked at the in-vivo use of unfractionated heparin (UFH) and LMWH in the treatment of acute PE, and found that fibrinolysis was enhanced significantly in the UFH group versus those on LMWH [130]. A third study showed that fondaparinux shortened CLTs in vitro and in healthy subjects, and this could be partially reversed with rFVIIa [131].

There has been comparatively little research on the utility of CLT in measuring hypocoagulable or hyperfibrinolytic conditions. In haemophilia A, hyperfibrinolysis has been demonstrated by shortened CLTs, and can be abolished by adding FVIII, TAFI or recombinant activated Factor VII (rFVIIa) [132, 133]. Another study used shortened CLTs to demonstrate hyperfibrinolysis in liver cirrhosis [134].

#### **4.2. Overall Haemostatic Potential (OHP)**

A limitation of the CLT is that only a single timed variable representing fibrin degradation is produced from the process of fibrin generation and subsequent lysis. A more comprehensive method for investigating this process is provided by the *Overall Haemostatic Potential* assay which was first developed in Sweden by Blombäck and colleagues [135] and modified in subsequent publications [136-138]. The principle of the OHP is the generation of fibrin formation and lysis curves to represent the shifting balance between fibrin generation and lysis using serial spectrophotometric measurements plotted against time. Two other assays with very similar methods have been described and will be also considered in this section. These are the *Clot Formation and Lysis* (CloFAL) assay [139] and the *Coagulation Inhibitor Potential* (CIP) assay [140, 141].

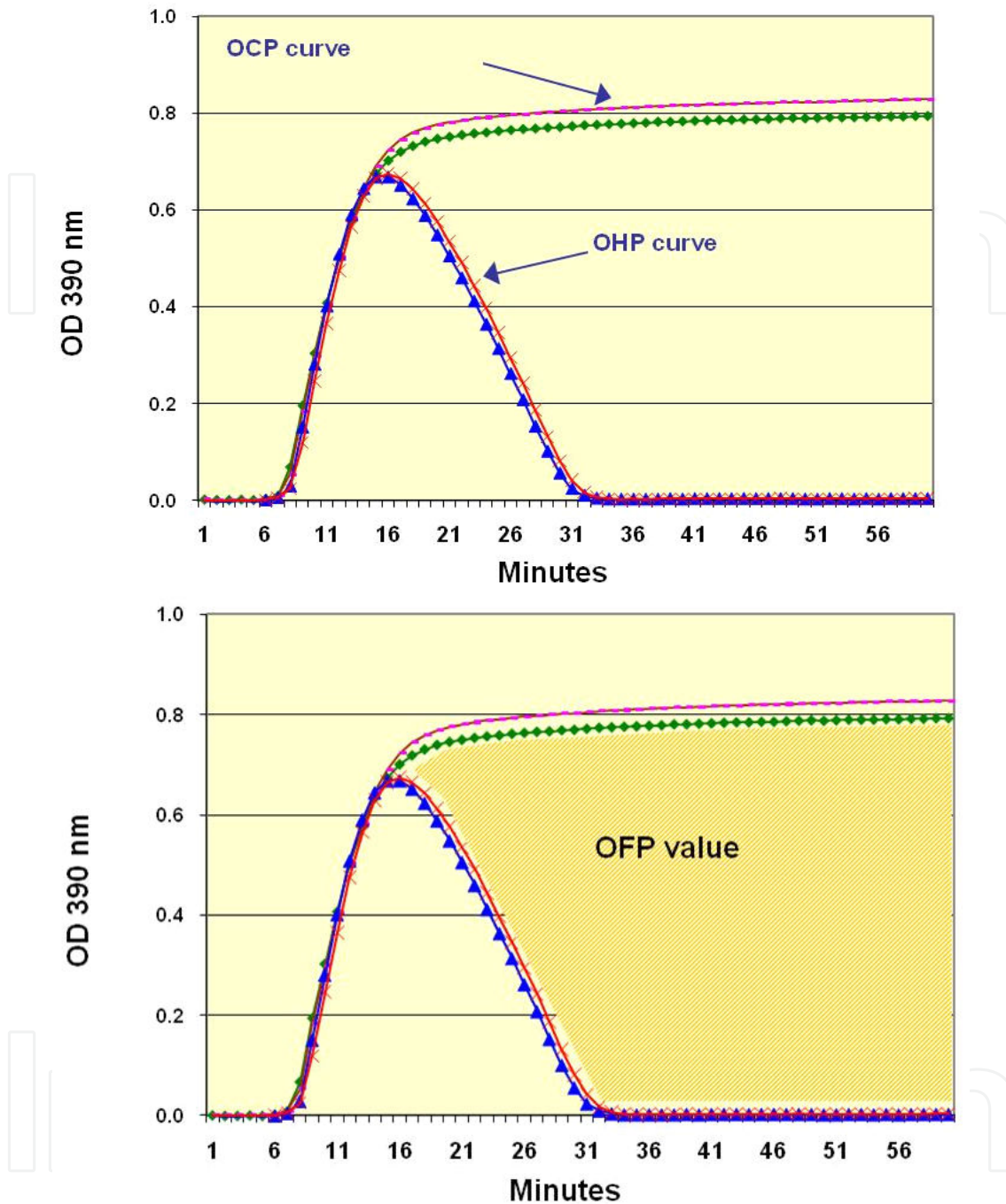
The modified OHP [136, 138] uses a small amount of thrombin to trigger coagulation. Fresh or thawed citrated PPP is added to a microtitre plate well at 37°C containing buffer with calcium chloride and thrombin at a final concentration of 0.03 – 0.04 IU/ml. Light absorbance at 390 or

405nm is recorded every minute for 40-60 mins. As the fibrinogen is gradually converted to fibrin by generated thrombin, the absorbance increases and is recorded at each time point, with the area under the curve (AUC) reflecting the total fibrin generated. The measurements from this well are termed the Overall Coagulation Potential (*OCP*). Each sample is run in parallel with another well containing the same reagents but with added tPA at 330 – 350ng/mL. In this analysis, called the Overall Haemostatic Potential (*OHP*), complete fibrinolysis occurs after initial fibrin generation, recorded by a fall in absorbance over time. The difference between the AUCs of the *OCP* (without tPA) and the *OHP* (with tPA) represents the overall fibrinolytic potential (*OFFP*) and is expressed as a percentage of the *OCP*. Examples of these curves are shown in Figures 2a and b. Other derived measures include the *Delay*, which is the time from start of the analysis to onset of fibrin generation and correlates with the APTT, the maximum optical density (*Max OD*) representing the maximal amount of fibrin generated (correlating to plasma fibrinogen levels), and the velocity (*Max Slope*) to describe the rate of fibrin generation [138]. In addition, the *OFFP* may be corrected for variations in *Delay* by standardising the time period over which it is calculated to the 45 minutes starting from the onset of fibrin generation (*OFFP 45*).

Tests are performed in duplicates in a microtitre plate. In a single laboratory the intra-assay CVs were approximately 3.1 – 8.7% and the inter-assay CVs 4.2 – 5.1% for the *OCP* and *OHP* values respectively [136]. Experiments in our laboratory have shown that standard precautions may be applied regarding pre-analytical variables; fresh samples are stable if processed within 2 hours or may be frozen and analysed later, and different methods of PPP preparation did not alter assay results either (unpublished data).

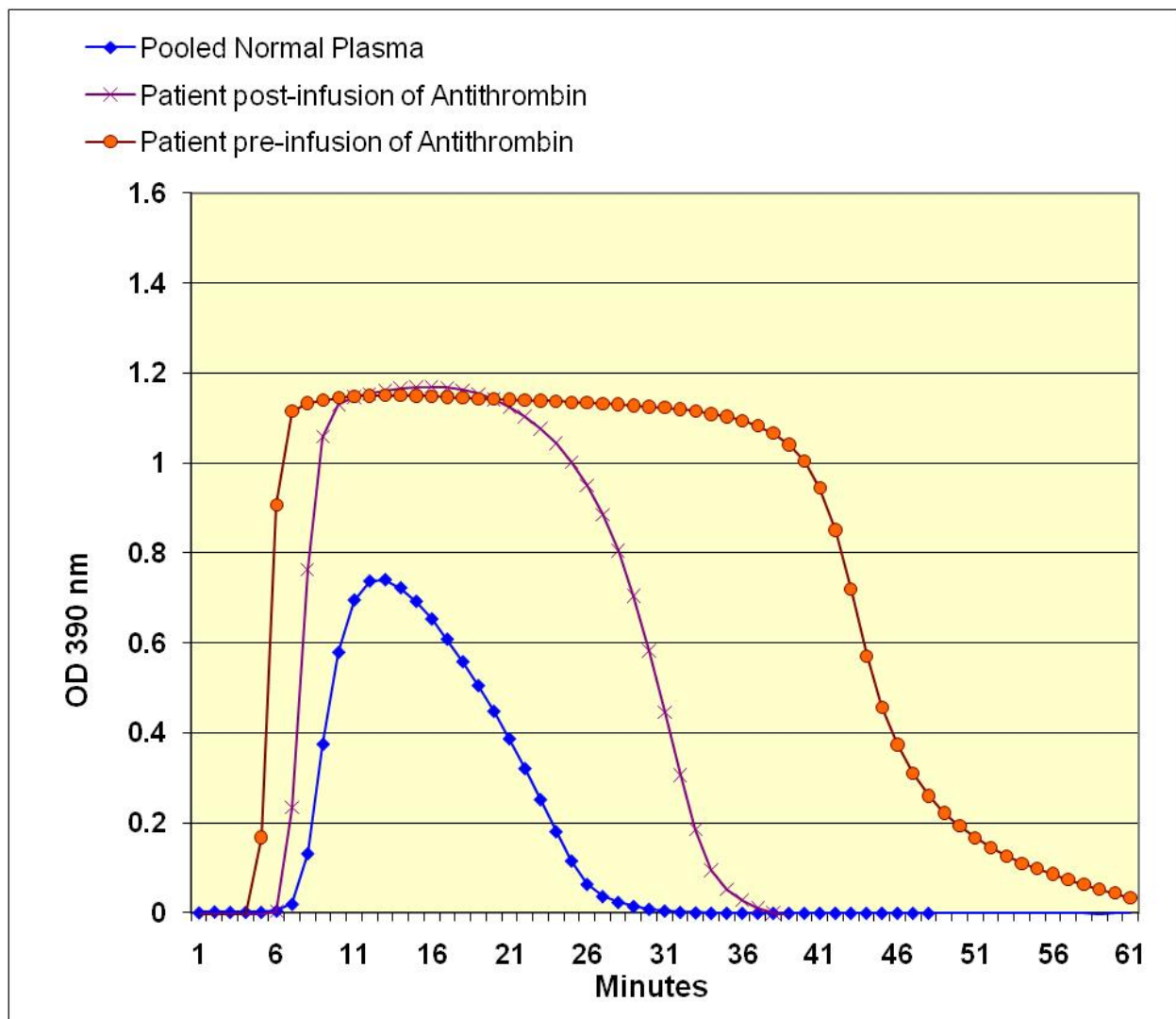
The *OHP* has demonstrated sensitivity to changes in both coagulation and fibrinolytic factors. In vitro experiments using factor deficient plasma have demonstrated significant correlations between *OHP* parameters and concentrations of factors II, V, VII, VIII, IX and X and XI [142]. These results were replicated in our laboratory, and we also have shown using in-vitro experiments that the *OFFP* parameter is sensitive to varying concentrations of plasminogen,  $\alpha$ 2-plasmin inhibitor, PAI-1, TAFI and the fibrinolytic inhibitor tranexamic acid [unpublished data and 143]. In vitro spiking experiments have shown fibrinogen concentration outside the normal range also has a negative correlation with fibrinolysis parameters; however, whereas fibrinogen levels can have a marked effect on the CLT assay because of the increase in the clot's peak light absorbance, the nature of the *OFFP* parameter as a ratio of the *OCP*:*OHP*:*OCP* controls for the maximum OD and is a reasonable expression of clot lysis.

The *OHP* can also be used as a screening assay for heritable thrombophilias; previously unpublished evidence from our laboratory shows sensitivity to antithrombin deficiency with both hypercoagulable and hypofibrinolytic changes (figure 3), the latter defect likely related to increased TAFIa levels. The *Coagulation Inhibitor Potential* (*CIP*) assay is a newer modification of the *OHP* specifically designed for thrombophilia screening which uses heparin pentasaccharide to potentiate antithrombin and the snake venom Protac to activate Protein C [140, 141]. It has shown excellent sensitivity of 100% and reasonable specificity of 70-80% in two small series of patients with FV Leiden, or Protein C, S or antithrombin deficiency [140, 144].



**Figure 2.** (a) Examples of Overall Coagulation Potential (OCP) and Overall Haemostatic Potential (OHP) curves. (b) The Overall Fibrinolytic Potential (OFP) is the difference of the area under the curves of the Overall Coagulation Potential (OCP) and Overall Haemostatic Potential (OHP)

Whilst there are clear advantages of the OHP as an assay for fibrinolysis, such as its simplicity, inexpensiveness and reproducibility, there have been several criticisms of the method [145]. The first is that thrombin is used to initiate coagulation. In earlier versions of the assay, higher thrombin concentrations were used and there was evidence of direct fibrinogen activation



**Figure 3.** Overall Haemostatic Potential (OHP) curves of a patient with congenital antithrombin deficiency before and after antithrombin concentrate infusion compared to a pooled normal plasma control. The pre-infusion OHP curves show decreased *Delay*, increased *Max Slope* and *Max OD*, and impaired fibrinolysis. *Delay*, *Max Slope* and fibrinolysis are partially corrected by antithrombin infusion.

occurring from the exogenous thrombin. However the very low concentrations used in the modified version produce no detectable clot in thrombin deficient plasma, suggesting the exogenous thrombin is only enough to trigger coagulation via a feedback reaction and not directly converting fibrinogen [145]. Even so, a further refinement of the method has been published which replaces the thrombin with a low concentration of tissue factor and phospholipid to more accurately mirror in-vivo coagulation and this method has also been shown to be sensitive to changes in coagulation factor and PAI-1 concentrations [137]. The *CloFAL* assay has also been independently developed with a very similar method to the OHP using tissue factor as an initiator, and a different algorithm to evaluate fibrinolysis [139].

A second criticism of the OHP is that plasminogen activation depends almost exclusively on the exogenous tPA, a modification of the method which is essential to produce measurable

fibrinolysis within a reasonable time frame but which makes the assay insensitive to changes in endogenous plasminogen activators [145]. Thirdly, the effects of the cellular components are excluded by the use of PPP, unlike in the TEG for example. Finally, the OHP and related assays currently lack standardisation and are not available commercially, and variations in type of initiator, concentrations of reagents and derived parameters making results between groups difficult to compare. As yet, no inter-laboratory CVs have been published. OFP results are also likely to show a diurnal variation due to their sensitivity to higher PAI-1 levels in the morning, and this has been demonstrated in patients with obstructive sleep apnoea [146].

#### *Arterial thrombosis*

Impaired fibrinolysis demonstrated by the OHP assay has been found in patients with coronary heart disease. A large retrospective study compared 800 patients three months after their first MI with 1123 normal controls from the local population [147]. Fibrinolytic parameters below the 10<sup>th</sup> percentile of the control group's values conferred an odds ratio of first MI of 1.66 (95% CI 1.22-2.27) after correction for cardiovascular risk factors in multivariate analysis. In another study, serial samples from patients admitted to hospital with acute coronary syndrome showed decreased fibrinolysis that improved on treatment with LMWH [148]. Patients with stable coronary artery disease also have reduced fibrinolysis; 56 patients with angiography proven coronary atherosclerosis had significantly reduced OFP values compared to controls [149]. The lowest tertile of OFP results conferred an odds ratio of 16.1 for coronary artery disease compared to the highest tertile. This major increase in risk was partly accounted for by higher PAI-1 levels although the global OFP measure remained an independent risk factor when adjusted for this.

The OHP has also demonstrated hypofibrinolysis in patients with cerebrovascular events. In one study, samples were taken from a group of 44 young patients a median of 5 years after they had experienced an episode of acute cerebral ischaemia and compared to healthy age matched controls. Little difference in the incidence of traditional thrombophilic measures was seen between the groups, but increased OCP parameters and decreased OFP results were demonstrated [150]. Another study examined TAFI levels and the OHP in patients at the time of acute ischaemic stroke and 60 days later [151]. Patients had impaired global fibrinolysis compared to controls which was partly, but not fully, explained by raised TAFI levels.

#### *Venous thrombosis*

Currently there is less published evidence regarding the use of the OHP to detect hypofibrinolysis in patients with venous thrombosis, although the assay does show promise in this area. The Swedish group first published data on a group of 88 women who had had a pregnancy related VTE, with the majority having evidence of activated protein C resistance with or without the Factor V Leiden mutation [152]. Blood samples were taken for OHP analysis from the patients 8 months to 13 years after the last VTE and compared to samples from a control group of healthy women. They were able to demonstrate a significant persisting hypercoagulability and hypofibrinolysis in the group which was most marked in those who had Factor V Leiden. Subsequently another publication studied 161 patients referred to haematology clinic with clinically defined hypercoagulable states [138]. The study group was heterogeneous

and included patients with VTE (n=73), arterial thrombi, antiphospholipid antibodies or lupus anticoagulant, and pregnancy complications. Despite this, significant differences in fibrin generation and lysis were found in the study population compared to a control group of blood donors, giving the OHP assay an estimated sensitivity of 96% for detection of clinically defined hypercoagulable states. The results of this study complement the excellent sensitivity the OHP has demonstrated in detecting inherited thrombophilic states that was described previously [140], and data showing that the OHP can discriminate factor XII deficient patients with a prothrombotic phenotype [153].

These promising results merit further prospective study to investigate whether the OHP assay can predict first or recurrent VTE. It may be particularly useful in identifying hypercoagulability in patients with otherwise unprovoked idiopathic VTE; in our laboratory we have noted impaired fibrinolysis in this group (unpublished), and similar results have been published using the CloFAL assay in paediatric VTE [154].

### *Pregnancy*

The OHP assay demonstrates both significantly increased fibrin generation and decreased hypofibrinolysis in normal pregnancy [J Curnow et al, personal communication, 136]. In high risk pregnancy, these changes have also been described [155] and unpublished data from our laboratory shows a significant worsening in these parameters compared to patients with uncomplicated pregnancy, which may indicate a role for the OHP in prospective studies of thrombosis risk.

### *Anticoagulation monitoring*

The OHP shows potential applications in the monitoring of overall coagulation and fibrinolytic changes with anticoagulation. As described above, improvement of fibrinolysis was seen in acute coronary syndrome patients once LMWH was started [148], and similar improvement may be seen with warfarin and the new oral anticoagulants (unpublished data). However, the response appears to be variable even when conventional assays show anticoagulation to be within the therapeutic window; for example, in a study of pregnant women with previous thromboembolism, dalteparin prophylaxis was shown to improve hypercoagulable OHP variables in the majority, but some patients were seen to remain hypercoagulable even with therapeutic anti-Xa results [155]. Further study is required to investigate if this translates to an increased thrombosis risk and whether changing treatment based on OHP results can result in improved patient care.

### *Hypocoagulable states*

As mentioned previously, the OHP is sensitive to factor deficiencies in vitro. Results from Haemophilia A patients indicate the OHP may even be a better predictor of clinical phenotype than APTT levels or FVIII assays [145], and in patients with inhibitors, the OHP may be used to monitor the fibrin generation response to recombinant FVIIa treatment [143]. Studies evaluating the OHP's ability to monitor FVIII dosing are ongoing [145].



### *Summary*

Fibrin generation and lysis assays show sensitivity to both hypo- and hyperfibrinolytic states, unlike the thromboelastography. Although they have not entered clinical practice currently, they show great promise in identifying global changes in coagulation and fibrinolytic tendency. In this regard, the OHP has an advantage over the CLT because both aspects of haemostasis are derived. Clearly, further study is required, but the OHP and related assays have the potential to better characterise global coagulation responses than conventional assays, possibly leading to individualised treatment for patients with thrombotic and bleeding conditions.

## **5. Conclusions**

Currently, fibrinolysis is rarely measured in clinical practice. This has been mainly due to the failure of assays of the specific factors to show consistent relationships with disease. In part, this failure has been related to methodological flaws in the assays, for example in the variable specificities of the antibodies used to detect TAFI or PAI-1. However, the principle problem is that the validity of one-off sampling of individual factors as a representation of the complex fibrinolytic pathway is questionable, especially when the sampling has occurred at a time distant to the pathological event being investigated.

D-dimer assays have been one notable success in clinical practice where they have established applications in the diagnosis of VTE and DIC. However, as a non-specific marker of thrombosis and fibrinolysis, they provide little information of the fibrinolytic potential of an individual. Global assays have the ability to improve clinical fibrinolytic testing in this regard. Thromboelastography is increasingly establishing itself as a tool to detect hyperfibrinolysis, particular in point-of-care settings such as cardiothoracic and liver transplant surgery. Fibrin generation and lysis assays such as the CLT and OHP have the disadvantage of requiring platelet poor plasma, but in contrast to thromboelastography, appear to be sensitive to both hyper- and hypofibrinolysis. Results in studies of arterial and venous thrombosis have been encouraging, but further research needs to be undertaken to find out whether the data has clinical applications, for example in predicting an individual patient's risk of recurrent thrombosis. In the meantime, the development of new whole blood tests is ongoing [156, 157], with the hope of achieving a better simulation of in-vivo fibrinolytic conditions.

## **Author details**

Dominic Pepperell, Marie-Christine Morel-Kopp and Chris Ward\*

\*Address all correspondence to: cward@med.usyd.edu.au

Northern Blood Research Centre, Kolling Institute of Medical Research, The University of Sydney and Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, NSW, Australia

## References

- [1] Prins MH, Hirsh J. A critical review of the evidence supporting a relationship between impaired fibrinolytic activity and venous thromboembolism. *Arch Intern Med.* 1991 Sep;151(9):1721-31.
- [2] Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR, Lisman T. The impact of the fibrinolytic system on the risk of venous and arterial thrombosis. *Semin Thromb Hemost.* 2009 Jul;35(5):468-77.
- [3] Spencer FA, Emery C, Joffe SW, Pacifico L, Lessard D, Reed G, et al. Incidence rates, clinical profile, and outcomes of patients with venous thromboembolism. The Worcester VTE study. *J Thromb Thrombolysis.* 2009 Nov;28(4):401-9.
- [4] Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. *Haematologica.* 2007 Feb;92(2):199-205.
- [5] Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. *Lancet.* 2003 Aug 16;362(9383):523-6.
- [6] Hansson PO, Sorbo J, Eriksson H. Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. *Arch Intern Med.* 2000 Mar 27;160(6):769-74.
- [7] Gorog DA. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. *J Am Coll Cardiol.* 2010 Jun 15;55(24):2701-9.
- [8] Luddington RJ. Thrombelastography/thromboelastometry. *Clin Lab Haematol.* 2005 Apr;27(2):81-90.
- [9] Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. *Anesth Analg.* 2008 May;106(5):1366-75.
- [10] MacIvor D, Rebel A, Hassan ZU. How do we integrate thromboelastography with perioperative transfusion management? *Transfusion.* 2013 Jul;53(7):1386-92.
- [11] Shakur H, Roberts I, Bautista R, Caballero J, Coats T, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet.* 2010 Jul 3;376(9734):23-32.
- [12] Taylor FB, Jr., Toh CH, Hoots WK, Wada H, Levi M, Scientific Subcommittee on Disseminated Intravascular Coagulation of the International Society on T, et al. Towards

definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost.* 2001 Nov;86(5):1327-30.

- [13] Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost.* 2009 Jan;7(1):4-13.
- [14] Bachmann F. Plasminogen-Plasmin Enzyme Systems. In: Colman R, Hirsh J, Marder V, Clowes A, George J, editors. *Haemostasis and Thrombosis, Basic Principles and Clinical Practice.* Fourth ed. Philadelphia, PA: Lippincott, Williams and Wilkins; 2001.
- [15] Brandt JT. Plasminogen and tissue-type plasminogen activator deficiency as risk factors for thromboembolic disease. *Arch Pathol Lab Med.* 2002 Nov;126(11):1376-81.
- [16] Tefs K, Gueorguieva M, Klammt J, Allen CM, Aktas D, Anlar FY, et al. Molecular and clinical spectrum of type I plasminogen deficiency: A series of 50 patients. *Blood.* 2006 Nov 1;108(9):3021-6.
- [17] Ostrowski SR, Sorensen AM, Larsen CF, Johansson PI. Thrombelastography and biomarker profiles in acute coagulopathy of trauma: a prospective study. *Scand J Trauma Resusc Emerg Med.* 2011;19:64.
- [18] Folsom AR, Cushman M, Heckbert SR, Rosamond WD, Aleksic N. Prospective study of fibrinolytic markers and venous thromboembolism. *J Clin Epidemiol.* 2003 Jun; 56(6):598-603.
- [19] Clason SB, Meijer P, Kluft C, Ersdal E. Specific determination of plasmin inhibitor activity in plasma: documentation of specificity of manual and automated procedures. *Blood Coagul Fibrinolysis.* 1999 Dec;10(8):487-94
- [20] Wiman B. The fibrinolytic enzyme system. Basic principles and links to venous and arterial thrombosis. *Hematol Oncol Clin North Am.* 2000 Apr;14(2):325-38, vii.
- [21] Heylen E, Willemsse J, Hendriks D. An update on the role of carboxypeptidase U (TA-FIa) in fibrinolysis. *Front Biosci (Landmark Ed).* 2011;16:2427-50.
- [22] Tait RC, Walker ID, Conkie JA, Islam SIAM, McCall F. Isolated familial plasminogen deficiency may not be a risk factor for thrombosis. *Thrombosis and Haemostasis.* 1996 December;76(6):1004-8.
- [23] Okamoto A, Sakata T, Mannami T, Baba S, Katayama Y, Matsuo H, et al. Population-based distribution of plasminogen activity and estimated prevalence and relevance to thrombotic diseases of plasminogen deficiency in the Japanese: the Suita Study. *J Thromb Haemost.* 2003 Nov;1(11):2397-403.
- [24] Bugge TH, Flick MJ, Daugherty CC, Degen JL. Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. *Genes and Development.* 1995 01 Apr;9(7):794-807.

- [25] Bugge TH, Kombrinck KW, Flick MJ, Daugherty CC, Danton MJS, Degen JL. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell*. 1996 15 Jan;87(4):709-19.
- [26] Zeng B, Bruce D, Kril J, Ploplis V, Freedman B, Brieger D. Influence of plasminogen deficiency on the contribution of polymorphonuclear leucocytes to fibrin/ogenolysis: studies in plasminogen knock-out mice. *Thrombosis and Haemostasis*. 2002 Nov; 88(5):805-10.
- [27] Meltzer ME, Lisman T, de Groot PG, Meijers JC, le Cessie S, Doggen CJ, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood*. 2010 Jul 8;116(1):113-21.
- [28] Hoekstra J, Guimaraes AH, Leebeek FW, Darwish Murad S, Malfliet JJ, Plessier A, et al. Impaired fibrinolysis as a risk factor for Budd-Chiari syndrome. *Blood*. 2010 Jan 14;115(2):388-95.
- [29] Juhan-Vague I, Pyke SD, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. *Circulation*. 1996 Nov 1;94(9):2057-63.
- [30] Folsom AR, Aleksic N, Park E, Salomaa V, Juneja H, Wu KK. Prospective study of fibrinolytic factors and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol*. 2001 Apr;21(4):611-7.
- [31] Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR, Lisman T. Plasma levels of fibrinolytic proteins and the risk of myocardial infarction in men. *Blood*. 2010 Jul 29;116(4):529-36.
- [32] Lijnen HR. Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost*. 2001 Jul;86(1):324-33.
- [33] Davenport R. Pathogenesis of acute traumatic coagulopathy. *Transfusion*. 2013 Jan;53 Suppl 1:23S-7S.
- [34] Favaloro EJ. Laboratory testing in disseminated intravascular coagulation. *Semin Thromb Hemost*. 2010 Jun;36(4):458-67.
- [35] Breen KA, Grimwade D, Hunt BJ. The pathogenesis and management of the coagulopathy of acute promyelocytic leukaemia. *Br J Haematol*. 2012 Jan;156(1):24-36.
- [36] Carpenter SL, Mathew P. Alpha2-antiplasmin and its deficiency: fibrinolysis out of balance. *Haemophilia*. 2008 Nov;14(6):1250-4.
- [37] Torr-Brown SR, Sobel BE. Attenuation of thrombolysis by release of plasminogen activator inhibitor type-1 from platelets. *Thromb Res*. 1993 Dec 1;72(5):413-21.

- [38] Declerck PJ, Collen D. Measurement of plasminogen activator inhibitor 1 (PAI-1) in plasma with various monoclonal antibody-based enzyme-linked immunosorbent assays. *Thromb Res Suppl.* 1990;10:3-9.
- [39] MacCallum PK, Cooper JA, Howarth DJ, Meade TW, Miller GJ. Sex differences in the determinants of fibrinolytic activity. *Thromb Haemost.* 1998 Mar;79(3):587-90.
- [40] van der Bom JG, de Knijff P, Haverkate F, Bots ML, Meijer P, de Jong PT, et al. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. *Circulation.* 1997 Jun 17;95(12):2623-7.
- [41] Katsaros KM, Kastl SP, Huber K, Zorn G, Maurer G, Glogar D, et al. Clopidogrel pretreatment abolishes increase of PAI-1 after coronary stent implantation. *Thromb Res.* 2008;123(1):79-84.
- [42] Simpson AJ, Booth NA, Moore NR, Bennett B. The platelet and plasma pools of plasminogen activator inhibitor (PAI-1) vary independently in disease. *Br J Haematol.* 1990 Aug;75(4):543-8.
- [43] Grimaudo V, Hauert J, Bachmann F, Kruithof EK. Diurnal variation of the fibrinolytic system. *Thromb Haemost.* 1988 Jun 16;59(3):495-9.
- [44] Smith A, Patterson C, Yarnell J, Rumley A, Ben-Shlomo Y, Lowe G. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly Study. *Circulation.* 2005 Nov 15;112(20):3080-7.
- [45] Kinlay S, Schwartz GG, Olsson AG, Rifai N, Bao W, Libby P, et al. Endogenous tissue plasminogen activator and risk of recurrent cardiac events after an acute coronary syndrome in the MIRACL study. *Atherosclerosis.* 2009 Oct;206(2):551-5.
- [46] Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med.* 2006 Dec 21;355(25):2631-9.
- [47] Cushman M, Lemaitre RN, Kuller LH, Psaty BM, Macy EM, Sharrett AR, et al. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.* 1999 Mar;19(3):493-8.
- [48] Itakura H, Sobel BE, Boothroyd D, Leung LL, Iribarren C, Go AS, et al. Do plasma biomarkers of coagulation and fibrinolysis differ between patients who have experienced an acute myocardial infarction versus stable exertional angina? *Am Heart J.* 2007 Dec;154(6):1059-64.
- [49] Cortellaro M, Cofrancesco E, Boschetti C, Mussoni L, Donati MB, Cardillo M, et al. Increased fibrin turnover and high PAI-1 activity as predictors of ischemic events in atherosclerotic patients. A case-control study. The PLAT Group. *Arterioscler Thromb.* 1993 Oct;13(10):1412-7.

- [50] Raiko JR, Oikonen M, Wendelin-Saarenhovi M, Siitonen N, Kahonen M, Lehtimäki T, et al. Plasminogen activator inhibitor-1 associates with cardiovascular risk factors in healthy young adults in the Cardiovascular Risk in Young Finns Study. *Atherosclerosis*. 2012 Sep;224(1):208-12.
- [51] Crowther MA, Roberts J, Roberts R, Johnston M, Stevens P, Skingley P, et al. Fibrinolytic Variables in Patients with Recurrent Venous Thrombosis: a Prospective Cohort Study. *Thromb Haemost*. 2001;85:390-4.
- [52] Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Shen C, Newcomer LM, et al. Baseline fibrinolytic state and the risk of future venous thrombosis. A prospective study of endogenous tissue-type plasminogen activator and plasminogen activator inhibitor. *Circulation*. 1992 May;85(5):1822-7.
- [53] Hack CE. Fibrinolysis in disseminated intravascular coagulation. *Semin Thromb Hemost*. 2001 Dec;27(6):633-8.
- [54] Ferguson JW, Helmy A, Ludlam C, Webb DJ, Hayes PC, Newby DC. Hyperfibrinolysis in alcoholic cirrhosis: relative plasminogen activator inhibitor type 1 deficiency. *Thromb Res*. 2008;121(5):675-80.
- [55] van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. *Blood*. 2000 May 1;95(9):2855-9.
- [56] Verdu J, Marco P, Benlloch S, Sanchez J, Lucas J. Thrombin activatable fibrinolysis inhibitor (TAFI) polymorphisms and plasma TAFI levels measured with an ELISA insensitive to isoforms in patients with venous thromboembolic disease (VTD). *Thromb Haemost*. 2006 Mar;95(3):585-6.
- [57] Eichinger S, Schonauer V, Weltermann A, Minar E, Bialonczyk C, Hirschl M, et al. Thrombin-activatable fibrinolysis inhibitor and the risk for recurrent venous thromboembolism. *Blood*. 2004 May 15;103(10):3773-6.
- [58] Meltzer ME, Bol L, Rosendaal FR, Lisman T, Cannegieter SC. Hypofibrinolysis as a risk factor for recurrent venous thrombosis; results of the LETS follow-up study. *J Thromb Haemost*. 2010 Mar;8(3):605-7.
- [59] Zorio E, Castello R, Falco C, Espana F, Osa A, Almenar L, et al. Thrombin-activatable fibrinolysis inhibitor in young patients with myocardial infarction and its relationship with the fibrinolytic function and the protein C system. *Br J Haematol*. 2003 Sep;122(6):958-65.
- [60] Tregouet DA, Schnabel R, Alessi MC, Godefroy T, Declercq PJ, Nicaud V, et al. Activated thrombin activatable fibrinolysis inhibitor levels are associated with the risk of cardiovascular death in patients with coronary artery disease: the AtheroGene study. *J Thromb Haemost*. 2009 Jan;7(1):49-57.
- [61] Folkeringa N, Coppens M, Veeger NJ, Bom VJ, Middeldorp S, Hamulyak K, et al. Absolute risk of venous and arterial thromboembolism in thrombophilic families is not

- increased by high thrombin-activatable fibrinolysis inhibitor (TAFI) levels. *Thromb Haemost.* 2008 Jul;100(1):38-44.
- [62] Morange PE, Tregouet DA, Frere C, Luc G, Arveiler D, Ferrieres J, et al. TAFI gene haplotypes, TAFI plasma levels and future risk of coronary heart disease: the PRIME Study. *J Thromb Haemost.* 2005 Jul;3(7):1503-10
- [63] de Bruijne EL, Gils A, Guimaraes AH, Dippel DW, Deckers JW, van den Meiracker AH, et al. The role of thrombin activatable fibrinolysis inhibitor in arterial thrombosis at a young age: the ATTAC study. *J Thromb Haemost.* 2009 Jun;7(6):919-27.
- [64] Meltzer ME, Doggen CJ, de Groot PG, Meijers JC, Rosendaal FR, Lisman T. Low thrombin activatable fibrinolysis inhibitor activity levels are associated with an increased risk of a first myocardial infarction in men. *Haematologica.* 2009 Jun;94(6):811-8.
- [65] Foley JH, Nesheim ME, Rivard GE, Brummel-Ziedins KE. Thrombin activatable fibrinolysis inhibitor activation and bleeding in haemophilia A. *Haemophilia.* 2012 May;18(3):e316-22.
- [66] Emerging Risk Factors C, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *Jama.* 2009 Jul 22;302(4):412-23.
- [67] Cesarman-Maus G, Rios-Luna NP, Deora AB, Huang B, Villa R, Cravioto Mdel C, et al. Autoantibodies against the fibrinolytic receptor, annexin 2, in antiphospholipid syndrome. *Blood.* 2006 Jun 1;107(11):4375-82.
- [68] Cesarman-Maus G, Cantu-Brito C, Barinagarrementeria F, Villa R, Reyes E, Sanchez-Guerrero J, et al. Autoantibodies against the fibrinolytic receptor, annexin A2, in cerebral venous thrombosis. *Stroke.* 2011 Feb;42(2):501-3.
- [69] Bates SM. D-dimer assays in diagnosis and management of thrombotic and bleeding disorders. *Semin Thromb Hemost.* 2012 Oct;38(7):673-82.
- [70] Dempfle CE. Validation, calibration, and specificity of quantitative D-dimer assays. *Semin.* 2005 Nov;5(4):315-20.
- [71] Couturaud F, Kearon C, Bates SM, Ginsberg JS. Decrease in sensitivity of D-dimer for acute venous thromboembolism after starting anticoagulant therapy. *Blood Coagul Fibrinolysis.* 2002 Apr;13(3):241-6.
- [72] Di Nisio M, Squizzato A, Rutjes AW, Buller HR, Zwinderman AH, Bossuyt PM. Diagnostic accuracy of D-dimer test for exclusion of venous thromboembolism: a systematic review. *J Thromb Haemost.* 2007 Feb;5(2):296-304.
- [73] Bates SM, Jaeschke R, Stevens SM, Goodacre S, Wells PS, Stevenson MD, et al. *Diagnosis of DVT: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: Ameri-*

can College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012 Feb;141(2 Suppl):e351S-418S.

- [74] Schouten HJ, Geersing GJ, Koek HL, Zuithoff NP, Janssen KJ, Douma RA, et al. Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. *Br Med J*. 2013;346:f2492.
- [75] Heit JA. Predicting the risk of venous thromboembolism recurrence. *Am J Hematol*. 2012 May;87 Suppl 1:S63-7.
- [76] Palareti G, Cosmi B, Legnani C, Tosetto A, Brusi C, Iorio A, et al. D-dimer testing to determine the duration of anticoagulation therapy.[Erratum appears in *N Engl J Med*. 2006 Dec 28;355(26):2797], [Reprint in *J Vasc Nurs*. 2007 Jun;25(2):39; PMID: 17531938]. *N Engl J Med*. 2006 Oct 26;355(17):1780-9.
- [77] De Souza RL, Short T, Warman GR, MacLennan N, Young Y. Anaphylaxis with associated fibrinolysis, reversed with tranexamic acid and demonstrated by thrombelastography. *Anaesth Intensive Care*. 2004 Aug;32(4):580-7.
- [78] Levrat A, Gros A, Rugeri L, Inaba K, Floccard B, Negrier C, et al. Evaluation of rotation thrombelastography for the diagnosis of hyperfibrinolysis in trauma patients. *Br J Anaesth*. 2008 Jun;100(6):792-7.
- [79] Spiel AO, Mayr FB, Firbas C, Quehenberger P, Jilma B. Validation of rotation thrombelastography in a model of systemic activation of fibrinolysis and coagulation in humans. *J Thromb Haemost*. 2006 Feb;4(2):411-6.
- [80] Nielsen VG. A comparison of the Thrombelastograph and the ROTEM. *Blood Coagul Fibrinolysis*. 2007 Apr;18(3):247-52.
- [81] Zambruni A, Thalheimer U, Leandro G, Perry D, Burroughs AK. Thromboelastography with citrated blood: comparability with native blood, stability of citrate storage and effect of repeated sampling. *Blood Coagul Fibrinolysis*. 2004 Jan;15(1):103-7.
- [82] Kitchen DP, Kitchen S, Jennings I, Woods T, Walker I. Quality assurance and quality control of thrombelastography and rotational Thromboelastometry: the UK NEQAS for blood coagulation experience. *Semin Thromb Hemost*. 2010 Oct;36(7):757-63.
- [83] Chitlur M, Sorensen B, Rivard GE, Young G, Ingerslev J, Othman M, et al. Standardization of thromboelastography: a report from the TEG-ROTEM working group. *Hæmophilia*. 2011 May;17(3):532-7.
- [84] Lang T, Bauters A, Braun SL, Potzsch B, von Pape KW, Kolde HJ, et al. Multi-centre investigation on reference ranges for ROTEM thromboelastometry. *Blood Coagul Fibrinolysis*. 2005 Jun;16(4):301-10.



- [85] Brenni M, Worn M, Bruesch M, Spahn DR, Ganter MT. Successful rotational thromboelastometry-guided treatment of traumatic haemorrhage, hyperfibrinolysis and coagulopathy. *Acta Anaesthesiol Scand*. 2010 Jan;54(1):111-7.
- [86] Ghosh K, Shetty S, Kulkarni B. Correlation of thromboelastographic patterns with clinical presentation and rationale for use of antifibrinolytics in severe haemophilia patients. *Haemophilia*. 2007 Nov;13(6):734-9.
- [87] Dirkmann D, Radu-Berlemann J, Gorlinger K, Peters J. Recombinant tissue-type plasminogen activator-evoked hyperfibrinolysis is enhanced by acidosis and inhibited by hypothermia but still can be blocked by tranexamic acid. *J Trauma Acute Care Surg*. 2013 Feb;74(2):482-8.
- [88] Kashuk JL, Moore EE, Sawyer M, Wohlauser M, Pezold M, Barnett C, et al. Primary fibrinolysis is integral in the pathogenesis of the acute coagulopathy of trauma. *Ann Surg*. 2010 Sep;252(3):434-42; discussion 43-4.
- [89] Genet GF, Ostrowski SR, Sorensen AM, Johansson PI. Detection of tPA-induced hyperfibrinolysis in whole blood by RapidTEG, KaolinTEG, and functional fibrinogen-TEG in healthy individuals. *Clin Appl Thromb Hemost*. 2012 Nov;18(6):638-44.
- [90] Kang YG, Martin DJ, Marquez J, Lewis JH, Bontempo FA, Shaw BW, Jr., et al. Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. *Anesth Analg*. 1985 Sep;64(9):888-96.
- [91] Porte RJ, Bontempo FA, Knot EA, Lewis JH, Kang YG, Starzl TE. Systemic effects of tissue plasminogen activator-associated fibrinolysis and its relation to thrombin generation in orthotopic liver transplantation. *Transplantation*. 1989 Jun;47(6):978-84.
- [92] Steib A, Gengenwin N, Freys G, Boudjema K, Levy S, Otteni JC. Predictive factors of hyperfibrinolytic activity during liver transplantation in cirrhotic patients. *Br J Anaesth*. 1994 Nov;73(5):645-8.
- [93] Coakley M, Reddy K, Mackie I, Mallett S. Transfusion triggers in orthotopic liver transplantation: a comparison of the thromboelastometry analyzer, the thromboelastogram, and conventional coagulation tests. *J Cardiothorac Vasc Anesth*. 2006 Aug;20(4):548-53.
- [94] Trzebicki J, Flakiewicz E, Kosieradzki M, Blaszczyk B, Kolacz M, Jureczko L, et al. The use of thromboelastometry in the assessment of hemostasis during orthotopic liver transplantation reduces the demand for blood products. *Ann Transplant*. 2010 Jul-Sep;15(3):19-24.
- [95] Wang SC, Shieh JF, Chang KY, Chu YC, Liu CS, Loong CC, et al. Thromboelastography-Guided Transfusion Decreases Intraoperative Blood Transfusion During Orthotopic Liver Transplantation: Randomized Clinical Trial. *Transplantation proceedings*. 2010;42(7):2590-3.

- [96] Spiess BD, Gillies BSA, Chandler W, Verrier E. Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. *Journal of Cardiothoracic and Vascular Anesthesia*. 1995;9(2):168-73.
- [97] Shore-Lesserson L, Manspeizer HE, DePerio M, Francis S, Vela-Cantos F, Ergin MA. Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. *Anesthesia and Analgesia*. 1999 February;88(2):312-9.
- [98] Royston D, von Kier S. Reduced haemostatic factor transfusion using heparinase-modified thromboelastography during cardiopulmonary bypass. *Br J Anaesth*. 2001;86(4):575-8.
- [99] Anderson L, Quasim I, Soutar R, Steven M, Macfie A, Korte W. An audit of red cell and blood product use after the institution of thromboelastometry in a cardiac intensive care unit. *Transfusion Medicine*. 2006 February;16(1):31-9.
- [100] Avidan MS, Alcock EL, Da Fonseca J, Ponte J, Desai JB, Despotis GJ, et al. Comparison of structured use of routine laboratory tests or near-patient assessment with clinical judgement in the management of bleeding after cardiac surgery. *Br J Anaesth*. 2004 February;92(2):178-86.
- [101] Spalding GJ, Hartrumpf M, Sierig T, Oesberg N, Kirschke CG, Albes JM. Cost reduction of perioperative coagulation management in cardiac surgery: value of 'bedside' thromboelastography (ROTEM). *European Journal of Cardio-thoracic Surgery*. 2007 June;31(6):1052-7.
- [102] Ferraris VA, Ferraris SP, Saha SP, Hessel IEA, Haan CK, Royston BD, et al. Perioperative Blood Transfusion and Blood Conservation in Cardiac Surgery: The Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists Clinical Practice Guideline. *Annals of Thoracic Surgery*. 2007 May;83(5 SUPPL.):S27-S86.
- [103] Dunning J, Versteegh M, Fabbri A, Pavie A, Kolh P, Lockowandt U, et al. Guideline on antiplatelet and anticoagulation management in cardiac surgery. *European Journal of Cardio-thoracic Surgery*. 2008 July;34(1):73-92.
- [104] Ives C, Inaba K, Branco BC, Okoye O, Schochl H, Talving P, et al. Hyperfibrinolysis elicited via thromboelastography predicts mortality in trauma. *J Am Coll Surg*. 2012 Oct;215(4):496-502.
- [105] Theusinger OM, Wanner GA, Emmert MY, Billeter A, Eismon J, Seifert B, et al. Hyperfibrinolysis diagnosed by rotational thromboelastometry (ROTEM) is associated with higher mortality in patients with severe trauma. *Anesth Analg*. 2011 Nov;113(5):1003-12.
- [106] Hvas AM, Sorensen HT, Norengaard L, Christiansen K, Ingerslev J, Sorensen B. Tranexamic acid combined with recombinant factor VIII increases clot resistance to accelerated fibrinolysis in severe hemophilia A. *J Thromb Haemost*. 2007 Dec;5(12):2408-14.

- [107] Sorensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. *J Thromb Haemost.* 2004 Jan;2(1):102-10.
- [108] Kupesiz A, Rajpurkar M, Warriar I, Hollon W, Tosun O, Lusher J, et al. Tissue plasminogen activator induced fibrinolysis: standardization of method using thromboelastography. *Blood Coagul Fibrinolysis.* 2010 Jun;21(4):320-4.
- [109] Kupesiz OA, Chitlur MB, Hollon W, Tosun O, Thomas R, Warriar I, et al. Fibrinolytic parameters in children with noncatheter thrombosis: a pilot study. *Blood Coagul Fibrinolysis.* 2010 Jun;21(4):313-9.
- [110] O'Donnell J, Riddell A, Owens D, Handa A, Pasi J, Hamilton G, et al. Role of the Thrombelastograph as an adjunctive test in thrombophilia screening. *Blood Coagul Fibrinolysis.* 2004 Apr;15(3):207-11.
- [111] Karlsson O, Sporrang T, Hillarp A, Jeppsson A, Hellgren M. Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy. *Anesth Analg.* 2012 Oct;115(4):890-8.
- [112] Hill JS, Devenie G, Powell M. Point-of-care testing of coagulation and fibrinolytic status during postpartum haemorrhage: developing a thrombelastography-guided transfusion algorithm. *Anaesth Intensive Care.* 2012 Nov;40(6):1007-15.
- [113] Othman M, Falcon BJ, Kadir R. Global hemostasis in pregnancy: are we using thromboelastography to its full potential? *Semin Thromb Hemost.* 2010 Oct;36(7):738-46.
- [114] Taylor FB, Jr., Lockhart MS. Whole blood clot lysis: in vitro modulation by activated protein C. *Thromb Res.* 1985 Mar 15;37(6):639-49.
- [115] Comp PC, Jacocks RM, Rubenstein C, Radcliffe R. A lysine-absorbable plasminogen activator is elevated in conditions associated with increased fibrinolytic activity. *J Lab Clin Med.* 1981 May;97(5):637-45.
- [116] Hersch SL, Kunelis T, Francis RB, Jr. The pathogenesis of accelerated fibrinolysis in liver cirrhosis: a critical role for tissue plasminogen activator inhibitor. *Blood.* 1987 May;69(5):1315-9.
- [117] Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood.* 2005 Feb 1;105(3):1102-5.
- [118] Talens S, Malfliet JJ, Rudez G, Spronk HM, Janssen NA, Meijer P, et al. Biological variation in tPA-induced plasma clot lysis time. *Thromb Haemost.* 2012 Oct;108(4):640-6.
- [119] Undas A, Zawilska K, Ciesla-Dul M, Lehmann-Kopydlowska A, Skubiszak A, Ciepluch K, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. *Blood.* 2009 Nov 5;114(19):4272-8.

- [120] Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008 May 6;5(5):e97.
- [121] Krzek M, Ciesla-Dul M, Zabczyk M, Undas A. Fibrin clot properties in women heterozygous for factor V Leiden mutation: effects of oral contraceptives. *Thromb Res.* 2012 Oct;130(4):e216-21.
- [122] Meijers JC, Middeldorp S, Tekelenburg W, van den Ende AE, Tans G, Prins MH, et al. Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives. *Thromb Haemost.* 2000 Jul;84(1):9-14.
- [123] Guimaraes AH, de Bruijne EL, Lisman T, Dippel DW, Deckers JW, Poldermans D, et al. Hypofibrinolysis is a risk factor for arterial thrombosis at young age. *Br J Haematol.* 2009 Apr;145(1):115-20.
- [124] Traby L, Kollars M, Eischer L, Eichinger S, Kyrle PA. Prediction of recurrent venous thromboembolism by clot lysis time: a prospective cohort study. *PLoS ONE.* 2012;7(12):e51447.
- [125] Cellai AP, Lami D, Fedi S, Marcucci R, Mannini L, Cenci C, et al. A hypercoagulable and hypofibrinolytic state is detectable by global methods in patients with retinal vein occlusion. *Atherosclerosis.* 2012 Sep;224(1):97-101.
- [126] Martinez-Zamora MA, Tassies D, Carmona F, Espinosa G, Cervera R, Reverter JC, et al. Clot lysis time and thrombin activatable fibrinolysis inhibitor in severe preeclampsia with or without associated antiphospholipid antibodies. *J Reprod Immunol.* 2010 Nov;86(2):133-40.
- [127] Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR, Lisman T. Reduced plasma fibrinolytic capacity as a potential risk factor for a first myocardial infarction in young men. *Br J Haematol.* 2009 Apr;145(1):121-7.
- [128] Siegerink B, Meltzer ME, de Groot PG, Algra A, Lisman T, Rosendaal FR. Clot lysis time and the risk of myocardial infarction and ischaemic stroke in young women; results from the RATIO case-control study. *Br J Haematol.* 2012 Jan;156(2):252-8.
- [129] Lisman T, Adelmeijer J, Nieuwenhuis HK, de Groot PG. Enhancement of fibrinolytic potential in vitro by anticoagulant drugs targeting activated factor X, but not by those inhibiting thrombin or tissue factor. *Blood Coagul Fibrinolysis.* 2003 Sep;14(6):557-62.
- [130] Schutgens RE, Esseboom EU, Snijder RJ, Haas FJ, Verzijlbergen F, Nieuwenhuis HK, et al. Low molecular weight heparin (dalteparin) is equally effective as unfractionated heparin in reducing coagulation activity and perfusion abnormalities during the early treatment of pulmonary embolism. *J Lab Clin Med.* 2004 Aug;144(2):100-7.

- [131] Lisman T, Bijsterveld NR, Adelmeijer J, Meijers JC, Levi M, Nieuwenhuis HK, et al. Recombinant factor VIIa reverses the in vitro and ex vivo anticoagulant and profibrinolytic effects of fondaparinux. *J Thromb Haemost.* 2003 Nov;1(11):2368-73.
- [132] Mosnier LO, Lisman T, van den Berg HM, Nieuwenhuis HK, Meijers JC, Bouma BN. The defective down regulation of fibrinolysis in haemophilia A can be restored by increasing the TAFI plasma concentration. *Thromb Haemost.* 2001 Oct;86(4):1035-9.
- [133] Lisman T, Mosnier LO, Lambert T, Mauser-Bunschoten EP, Meijers JC, Nieuwenhuis HK, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe hemophilia A. *Blood.* 2002 Jan 1;99(1):175-9.
- [134] Rijken DC, Kock EL, Guimaraes AH, Talens S, Darwish Murad S, Janssen HL, et al. Evidence for an enhanced fibrinolytic capacity in cirrhosis as measured with two different global fibrinolysis tests. *J Thromb Haemost.* 2012 Oct;10(10):2116-22.
- [135] He S, Bremme K, Blomback M. A laboratory method for determination of overall haemostatic potential in plasma. I. Method design and preliminary results. *Thromb Res.* 1999 Oct 15;96(2):145-56.
- [136] He S, Antovic A, Blomback M. A simple and rapid laboratory method for determination of haemostasis potential in plasma. II. Modifications for use in routine laboratories and research work. *Thromb Res.* 2001 Sep 1;103(5):355-61.
- [137] He S, Zhu K, Skeppholm M, Vedin J, Svensson J, Egberg N, et al. A global assay of haemostasis which uses recombinant tissue factor and tissue-type plasminogen activator to measure the rate of fibrin formation and fibrin degradation in plasma. *Thromb Haemost.* 2007 Oct;98(4):871-82.
- [138] Curnow JL, Morel-Kopp MC, Roddie C, Aboud M, Ward CM. Reduced fibrinolysis and increased fibrin generation can be detected in hypercoagulable patients using the overall hemostatic potential assay. *J Thromb Haemost.* 2007 Mar;5(3):528-34.
- [139] Goldenberg NA, Hathaway WE, Jacobson L, Manco-Johnson MJ. A new global assay of coagulation and fibrinolysis.[Erratum appears in *Thromb Res.* 2006;118(6):771]. *Thromb Res.* 2005;116(4):345-56.
- [140] Andresen MS, Abildgaard U. Coagulation Inhibitor Potential: a study of assay variables. *Thromb Res.* 2005;115(6):519-26.
- [141] Andresen MS, Iversen N, Abildgaard U. Overall haemostasis potential assays performed in thrombophilic plasma: the effect of preactivating protein C and antithrombin. *Thromb Res.* 2002 Dec 15;108(5-6):323-8.
- [142] Antovic A, Blomback M, Sten-Linder M, Petrini P, Holmstrom M, He S. Identifying hypocoagulable states with a modified global assay of overall haemostasis potential in plasma. *Blood Coagul Fibrinolysis.* 2005 Nov;16(8):585-96.
- [143] Antovic JP, Antovic A, He S, Tengborn L, Blomback M. Overall haemostatic potential can be used for estimation of thrombin-activatable fibrinolysis inhibitor-dependent

fibrinolysis in vivo and for possible follow-up of recombinant factor VIIa treatment in patients with inhibitors to factor VIII. *Haemophilia*. 2002 Nov;8(6):781-6.

- [144] Andresen MS, Abildgaard U, Liestol S, Sandset PM, Mowinckel MC, Odegaard OR, et al. The ability of three global plasma assays to recognize thrombophilia. *Thromb Res*. 2004;113(6):411-7.
- [145] Antovic A. The overall hemostasis potential: a laboratory tool for the investigation of global hemostasis. *Semin Thromb Hemost*. 2010 Oct;36(7):772-9.
- [146] McEwen B, Morel-Kopp MC, Phillips C, Sullivan D, Ward CM, Grunstein R, et al. Circadian changes in thrombotic potential in obstructive sleep apnea (OSA): A randomized, placebo-controlled crossover study. *Journal of Thrombosis and Haemostasis*. 2011 July;9:841.
- [147] Leander K, Blomback M, Wallen H, He S. Impaired fibrinolytic capacity and increased fibrin formation associate with myocardial infarction. *Thromb Haemost*. 2012 Jun;107(6):1092-9.
- [148] Skeppholm M, Kallner A, Malmqvist K, Blomback M, Wallen H. Is fibrin formation and thrombin generation increased during and after an acute coronary syndrome? *Thromb Res*. 2011 Nov;128(5):483-9.
- [149] Reddel CJ, Curnow JL, Voithl J, Rosenov A, Pennings GJ, Morel-Kopp MC, et al. Detection of hypofibrinolysis in stable coronary artery disease using the overall haemostatic potential assay. *Thromb Res*. 2013 May;131(5):457-62.
- [150] Anzej S, Bozic M, Antovic A, Peternel P, Gaspersic N, Rot U, et al. Evidence of hypercoagulability and inflammation in young patients long after acute cerebral ischaemia. *Thromb Res*. 2007;120(1):39-46.
- [151] Rooth E, Wallen H, Antovic A, Von Arbin M, Kaponides G, Wahlgren N, et al. Thrombin activatable fibrinolysis inhibitor and its relationship to fibrinolysis and inflammation during the acute and convalescent phase of ischemic stroke. *Blood Coagulation and Fibrinolysis*. 2007 June;18(4):365-70.
- [152] Antovic A, Blomback M, Bremme K, Van Rooijen M, He S. Increased hemostasis potential persists in women with previous thromboembolism with or without APC resistance. *J Thromb Haemost*. 2003 Dec;1(12):2531-5.
- [153] Antovic JP, Antovic A, Sten-Linder M, Wramsby M, Blomback M. Overall hemostatic potential (OHP) assay-a possible tool for determination of prothrombotic pattern in FXII deficiency. *J Thromb Haemost*. 2004 Nov;2(11):2058-60.
- [154] Bombardier C, Villalobos-Menuey E, Ruegg K, Hathaway WE, Manco-Johnson MJ, Goldenberg NA. Monitoring hypercoagulability and hypofibrinolysis following acute venous Thromboembolism in children: application of the CloFAL assay in a prospective inception cohort study. *Thromb Res*. 2012 Sep;130(3):343-9.

- [155] Antovic A, Blomback M, Bremme K, He S. The assay of overall haemostasis potential used to monitor the low molecular mass (weight) heparin, dalteparin, treatment in pregnant women with previous thromboembolism. *Blood Coagul Fibrinolysis*. 2002 Apr;13(3):181-6.
- [156] Yamamoto J, Yamashita T, Ikarugi H, et al. Gorog Thrombosis Test: a global in-vitro test of platelet function and thrombolysis. *Blood Coagul Fibrinolysis*. 2003;14:31-9.
- [157] Rijken DC, Hoegge-de NE, Jie AF, Atsma DE, Schlij MJ, Nieuwenhuizen W. Development of a new test for the global fibrinolytic capacity in whole blood. *J Thromb Haemost*. 2008;6:151-7.