Measuring Fibrinolysis

Colin Longstaff¹

¹ Department of Biotherapeutics, National Institute for Biological Standards and Control, South Mimms, Herts, United Kingdom

Hämostaseologie 2021;41:69–75.

Address for correspondence Colin Longstaff, PhD, Department of Biotherapeutics, National Institute for Biological Standards and Control, South Mimms, Herts, United Kingdom EN6 3QG (e-mail: Colin.longstaff@nibsc.org).

Abstract

Physiological fibrinolysis under normal conditions progresses slowly, in contrast to coagulation which is triggered rapidly to stop bleeding and defend against microbial invasion. Methods to detect fibrinolysis abnormalities are less simple and poorly standardized compared with common coagulation tests. Fibrinolysis can be accelerated by preparing euglobulin from plasma to reduce endogenous inhibitors, or by adding plasminogen activators to normal plasma. However, these manipulations complicate interpretation of results and diagnosis of a “fibrinolysis deficit.” Many observational studies on antigen levels of fibrinolysis inhibitors, plasminogen activator inhibitor 1 or thrombin-activatable fibrinolysis inhibitor, zymogen or active enzyme have been published. However, conclusions are mixed and there are clear problems with harmonization of results. Viscoelastic methods have the advantage of being rapid and are used as point-of-care tests. They also work with whole blood, allowing the contribution of platelets to be explored. However, there are no agreed protocols for applying viscoelastic methods in acute care for the diagnosis of hyperfibrinolysis or to direct therapy. The emergence of SARS-CoV-2 and the dangers of associated coagulopathy provide new challenges. A common finding in hospitalized patients is high levels of D-dimer fibrin breakdown products, indicative of ongoing fibrinolysis. Well-established problems with D-dimer testing standardization signal that we should be cautious in using results from such tests as prognostic indicators or to target therapies.

Keywords

- fibrinolysis
- biomarkers
- standardization
- thrombolytics
- antifibrinolytics

Introduction

A simplified outline of fibrinolysis is presented in ► **Fig. 1** and more detailed reviews can be found elsewhere.^{1,2} By necessity, in response to vascular damage, coagulation must be rapid to reduce the dangers of bleeding and prevent entry of pathogenic microorganisms. Subsequently, fibrinolysis takes place slowly under normal circumstances, over hours and days as vessels are repaired. These considerations explain why there are many relatively simple, rapid, well-standardized tests to measure blood clotting, which can identify defects in coagulation pathways. However, fibrinolysis is more difficult to measure for diagnostic purposes and methods are more cumbersome, so the role of fibrinolysis in the hemostatic balance may be underestimated.

Fibrinolysis assays are needed to study antifibrinolytic therapy and also in the development and quality control of thrombolytic drugs. Diverse approaches are available to assess fibrinolysis in healthy or sick populations to identify factors that may be involved in regulation or dysregulation, and hyperfibrinolysis or fibrinolysis resistance.

The National Institute for Biological Standards and Control (NIBSC) is a World Health Organization (WHO) collaborating center with the responsibility to generate, store and distribute biological standards. A major part of our portfolio covers diagnostic and drug-related standards in hemostasis, including fibrinolysis³ and reviews on the ways in which our biological standards can be used have been published elsewhere.^{4,5}

received
September 28, 2021
accepted after revision
November 26, 2020

© 2021. Thieme. All rights reserved.
Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI <https://doi.org/10.1055/a-1325-0268>.
ISSN 0720-9355.

Fig. 1 Outline of the fibrinolysis system. The pathway of fibrin formation and degradation is shown by *heavy arrows*. Fibrin is a substrate (surface) for reactions and a substrate (target for enzymes) for plasmin.¹ Fibrin degradation products (FDP) are heterogeneous in size⁸⁸ and expose binding sites for D-dimer antibodies. Enzymes are in ovals and include the plasminogen activators, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). tPA activity is stimulated by binding to fibrin, where the finger domain is dominant,^{11,89} whereas uPA is generated from an inactive zymogen scuPA (not shown) by the action of plasmin. Serpin inhibitors, PAI-1 and α₂-antiplasmin (α₂-AP) form inactive complexes to reduce fibrinolysis. Thrombin-activatable fibrinolysis inhibitor (TAFI, also known as procarboxypeptidase U, or CBP2 gene product) is activated by thrombin (and plasmin, not shown), to the active form (TAFIa). This enzyme modifies fibrin (shown as Fibrin') to remove C-terminal lysines, which is less effective at binding plasminogen and plasmin and more resistant to lysis. TAFIa is thermally unstable and degrades to an inactive form, shown as TAFIa'. Other components that are involved include α₂-macroglobulin, a broad specificity inhibitor and thrombomodulin which has a role in regulating the activation of TAFI.⁹⁰ Additional factors that can impair fibrinolysis include variants of fibrinogen such as γ'-fibrinogen which affects clot architecture and fibrinogen-binding sites to make more resistant clots⁹¹ and FXIII which creates a more resistant clot by cross-linking fibrin chains and α₂-AP to fibrin.^{92,93} The incorporation of cells into clots can also delay fibrinolysis. Platelets cause clot retraction and release PAI-1⁹⁴; and red blood cells can interact with fibrin,⁹⁵ and also become compressed during clot retraction to form an impermeable barrier which delays clot lysis.⁹⁶ PAI-1, plasminogen activator inhibitor 1.

Assays for Thrombolytic Proteins

Thrombolytics such as tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) are serine proteases that transform plasminogen into plasmin. Microbial plasminogen binding and activating proteins such as streptokinase and staphylokinase have no intrinsic protease activity but in practice, experimentally their reaction kinetics look like other plasminogen activators and they can be analyzed in the same way (although the details of the kinetic mechanisms may be complicated⁶). In practice, investigations on enzyme mechanism and regulation, or determination of specific activity or concentration will be performed *in vitro* in purified systems of proteins, or in plasma-based systems and can be optimized over a chosen thrombolytic enzyme range to give the most robust results. Activities of fibrinolytic proteins are often determined relative to the WHO International Standard as a primary calibrator.³ Examples are available in publications of the development of fibrinolysis as per WHO International Standards (e.g., Locke et al⁷).

The simplest methods for following proteolytic activity involve optical monitoring of amidolytic substrates made of peptides linked to a chromophore or fluorophore. These types of substrates are not so useful when used directly on

an enzyme of interest, but are valuable to study linked reactions that generate plasmin, for example. Active-site titration of serine proteases is a subgroup of chromogenic/fluorogenic assays and is useful to establish molar concentrations of active enzymes, including thrombolytic enzymes and thrombin.⁸ Early fibrin-based methods for measuring plasminogen activator activity used fibrin plates, but have been superseded by microtiter plate-based methods.⁹ This approach can be adapted to internal lysis (in which plasminogen activator is mixed with fibrinogen, plasminogen and thrombin to form a clot that is subsequently lysed evenly throughout), or external lysis (where activator is added to the top of a preformed clot). Internal lysis is related to normal hemostasis, while superficially added activator more closely reflects the situation during thrombolytic therapy. It is possible to combine fibrin-based methods and chromogenic substrate-linked measurement of plasminogen activation to precisely follow plasmin generation in the presence of fibrin.^{10,11} In this way, rates of plasmin generation in SI units (e.g., pM/s) for tPA, uPA and streptokinase can be compared in the same format. Comparison of WHO assigned international units (IU) for these activators is not so useful as the units are unrelated. The long-established European Pharmacopoeia method for assaying alteplase

(tPA) activity,¹² in which clot lysis time is determined by passage of a ball through the clot in a tube or release of trapped bubbles from the fibrin network, is simple and reliable. More recently, the “Halo” method has been published that uses a small volume of whole blood, clotted in a ring or halo around the edge of a microtiter-plate well.¹³ Lysis is followed by monitoring increasing absorbance as the clot breaks down and products released into solution.

Although it is relatively simple to generate reproducible time courses of data to study the activity of thrombolytic enzymes using these methods, the analysis of the resulting data is the next challenge. It is common in fibrinolysis assays to report lysis times, usually as time to 50% lysis, but how this is calculated is not always explained. Alternatively, zymogen activation rates determined with chromogenic substrates require the use of time squared plots, which can be tedious to generate and analyze. To standardize calculations of lysis times and zymogen activation rates and improve reproducibility, several online apps have been developed and published in association with ISTH/SSC (International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee) Subcommittee on Fibrinolysis.^{14,15} These apps are freely available and run in a computer browser without downloading any software (see also Longstaff¹⁶ for summaries, links to apps and detailed instructions).

Diagnostic Methods

Functional Tests

Functional tests begin with the problem mentioned above that fibrinolysis without any stimulation is slow. To speed up the process, it is common to either add tPA to plasma to stimulate the generation of plasmin, or to remove inhibitors, by for example preparing the euglobulin fraction from plasma. A long-established functional method for measuring fibrinolysis in subject samples is to determine euglobulin clot lysis times (ECLTs), and this approach has been reviewed previously.^{5,17,18} The general approach is time-consuming and difficult to automate. The euglobulin fraction is reported to have a greater than 90% reduction in α_2 -antiplasmin, but there is also significant depletion of plasminogen activator inhibitor 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI).¹⁹ It is observed that the ECLT is strongly influenced by PAI-1 (and free tPA) concentrations. Recently an updated method has been published²⁰ where samples received added fibrinogen and ovalbumin to increase the clot turbidity. These authors also explored a fibrinolysis resistance test using added tPA which identified samples with high free active PAI-1, but also showed some sensitivity of lysis times to TAFI levels.

When normal plasma is used to investigate fibrinolysis, it is common to add significant amounts of extraneous tPA (from 200 to 700 ng/mL, see Table 2 in Longstaff¹⁸). To generate a clot, CaCl_2 is added as a minimum, and often thrombin and/or tissue factor (TF) with phospholipids may also be used. Attempts have been made to establish a standardized method for this procedure to improve reproducibility.²¹ A commonly used output is 50% lysis time where

lysis is followed optically, and analysis can be facilitated by online apps.¹⁴ A thorough review of data from clot lysis studies has been presented to explore the relationship of fibrinolytic potential and risk for arterial and venous thrombosis.²² Conclusions from many studies are not always strong or consistent in identifying molecular risk factors in different populations. However, an important observation is that hypofibrinolysis, especially in combination with hypercoagulability, can constitute an increased thrombosis risk.

An interesting development in this area is methods to simultaneously measure generation of both thrombin and plasmin during plasma clotting and lysis.²³ However, the methods proposed so far (reviewed in Longstaff¹⁸) have not become established, possibly because they are technically difficult to perform and analyze and no commercial equipment or software is available, in contrast to popular thrombin-generation platforms.

Antigen Assays

Antigen tests for plasma proteins involved in fibrinolysis are common and relatively simple to perform, even in large population studies. Many studies have been organized to investigate variations in circulating tPA, PAI-1 and TAFI, including free-active/inactive/latent and inhibited tPA–PAI-1 complex forms, to look for associations with arterial or venous thrombosis.^{24,25}

Reduced levels of fibrinolysis inhibitors are not diagnosed as often as coagulation deficiencies, but when found often lead to increased bleeding risk.¹⁷ Alternatively, high circulating PAI-1 and TAFI may indicate a “fibrinolysis deficit” and thrombosis risk. There are many large-scale population studies involving PAI-1 and TAFI, but results are not consistent.²⁶ Standardization of assay methods is poor in this area and it is not possible to directly compare absolute values of analytes from studies using different methods for PAI-1 antigen or activity or tPA antigen.^{27,28} Generally, elevated PAI-1 is associated with cardiovascular disease, metabolic syndrome, diabetes, obesity, senescence and as a prognostic marker for several cancers.²⁹ It is likely that harmonization of results from different methods could be improved with common standards.^{30,31} However, although there are WHO International Standards for tPA antigen in plasma and PAI-1 activity, they are not so popular because they are labeled in IU, while commercial methods report results in ng/mL. Unfortunately, the origin of the commercial kit standards is not consistent, and each “ng” is different. To establish a firm basis for reported ng, we at NIBSC are pursuing isotope dilution mass spectrometry using ¹³C-labeled recombinant proteins as a way of establishing real gravimetric concentrations of plasma analytes. Work on TAFI and PAI-1 antigens is underway. In addition to these considerations, pre-test processing issues further complicate PAI-1 measurements and make tPA activity measurements unreliable. Factors such as diurnal and seasonal variations,³² and release of PAI-1 from platelets during venepuncture must be considered.³³ Historically, a source of variation in TAFI assays has been the Thr325Ile polymorphism, which affects TAFI activation and stability.^{34,35} It has been proposed that TAFIa is a

more important biomarker than zymogen,³⁶ but measurements require very sensitive methods.^{37–39}

Interest in PAI-1 and TAFI as drug targets to modulate hemostasis has raised awareness of the importance of robust assay methods.^{40–42} The idea of inhibiting PAI-1 activity is interesting in the context of aging as a mutation in the SERPINE1 gene resulting in around 50% reduced circulating PAI-1 is associated with longevity in animal models and a population of Amish in the United States.⁴³

Hyperfibrinolysis and Hypofibrinolysis

Moore and colleagues⁴⁴ have attempted to define or clarify different types of pathological fibrinolysis observed clinically, for instance in trauma but also in surgery and disseminated intravascular coagulation (DIC). In addition to hyperfibrinolysis, there are varieties of fibrinolysis resistance (classically identified in ECLT assays) including hypofibrinolysis (a failure to trigger fibrinolysis after clotting) and fibrinolysis shutdown (where there is a rebound of increased PAI-1 activity and antigen after the triggering of coagulation and early release of tPA). The timing of fibrinolysis resistance and concepts such as occult fibrinolysis provide further complications.⁴⁴

Hyperfibrinolysis in acute situations such as trauma or surgery is a life-threatening situation requiring rapid tests to direct treatment such as plasma or clotting factor replacements, or possibly with antifibrinolytics such as tranexamic acid (TXA). Instances where markers of fibrinolysis are elevated include trauma, DIC (where there is a fibrinolytic phenotype),⁴⁵ acute promyelocytic leukemia, liver damage, congenital abnormalities and surgical procedures (reviewed in Kolev and Longstaff¹⁷). Common markers for ongoing fibrinolysis would be elevated D-dimer,⁴⁶ raised tPA or decreased PAI-1, reduced plasminogen, reduced α_2 -antiplasmin and elevated plasmin- α_2 -antiplasmin (P-AP) complexes. Assays for these proteins are time-consuming, with the possible exception of point-of-care tests for D-dimer. However, D-dimer tests are approved for excluding thrombosis and the accurate measurement of high D-dimer levels is complicated by the low specificity of these tests and poor standardization.^{47,48} Where hyperfibrinolysis is detected there is a high risk of death. For instance, in trauma only a minority of patients display a hyperfibrinolysis phenotype but mortality in this group is very high.^{49,50} The underlying fibrinolysis imbalance is likely dominated by an increase in tPA resulting in plasmin generation with concomitant consumption of inhibitors PAI-1 and α_2 -antiplasmin.^{51,52} The potential for hyperfibrinolysis to be associated with thrombotic complications has been highlighted previously.²²

Viscoelastic methods potentially have a role in diagnosing hyperfibrinolysis and fibrinolysis resistance as they are capable of generating results more rapidly than other available methods.⁵³ In particular, rapid thrombelastography (r-TEG) has been developed to speed up clotting by stimulating both intrinsic and extrinsic coagulation using kaolin and TF activators, and making tests available in cartridge form with potential for improved reliability.⁵⁴ The fundamentals of

viscoelastic methods have been reviewed elsewhere^{55,56} and involve the analysis of clot formation and lysis by physical measurement of blood viscosity and clot strength. In practice, the common platforms rotational thromboelastometry (ROTEM) and TEG provide an array of parameters from multiple variations of clotting tests and there is no agreed way of implementing results from these tests, although attempts are being made to develop optimized algorithms.^{57,58} While the speed of testing is attractive, sensitivity and specificity may be an issue. A study by Raza and colleagues tested samples from trauma patients and found high P-AP complexes and D-dimer in samples where ROTEM did not detect ongoing fibrinolysis.⁴⁹ It has been proposed that occult (local) fibrinolysis or the earlier production of long-lived D-dimer or P-AP complexes could be responsible for this discrepancy between fibrinolysis biomarkers and viscoelastic methods.⁴⁴

An interesting and controversial aspect of these discussions is how to use antifibrinolytic therapies, particularly TXA to reduce bleeding in surgery⁵⁹ or trauma. Several large-scale clinical trials have demonstrated that TXA given early in trauma is safe and effective⁶⁰ and pre-hospital treatment is recommended in Europe. In some quarters there are concerns that adding antifibrinolytics to a situation where there may be fibrinolysis resistance is potentially dangerous and could lead to thrombotic complications, including widespread vascular microthrombosis, organ failure and death. Thus, it is proposed that rapid testing, by viscoelastic methods, particularly rapid TEG, should be used to target only those patients who would benefit from antifibrinolytic treatment. However, several large clinical trials of TXA in trauma,⁶¹ postpartum hemorrhage⁶² and traumatic brain injury⁶³ observed no increases in thromboembolic complications,⁶⁰ and there is a lack of evidence of disperse microvascular thrombi.^{64,65} On the other hand, the HALT-IT trial, which failed to show benefit of TXA in the treatment of gastrointestinal bleeding, did observe an increased risk of venous thromboembolic events (deep vein thrombosis plus pulmonary embolism) in the TXA treatment group.⁶⁶ The authors speculated that the increased risk may be due to disturbed hemostasis in the liver cirrhosis and variceal bleeding patients that made up half the subjects in the study, and/or the high dose of TXA used (4 g over 24 hours), which may also explain an observed increase in seizures. The conclusions from many studies and systematic reviews seem to be that more research is needed before there is sufficient confidence in viscoelastic methods for routine diagnostic testing, although there are promising signals of benefit in situations to reduce blood component use.^{67–69} Hard evidence from randomized controlled trials to support the application of viscoelastic methods in targeting antifibrinolytic therapy is lacking.^{50,70} It is argued that without this evidence the established risks of TXA treatment delay should outweigh theoretical but unproven risks of non-targeted treatment.

As TXA treatment is delayed, it becomes progressively less effective and after 3 hours benefit is lost⁶⁰ and the dangers appear to be excess bleeding, not thrombosis. There are few

studies of uPA in pathological fibrinolysis, but Hijazi and colleagues⁷¹ have identified the slow development of a peak of uPA after several hours in a mouse model of brain injury, following the earlier rise and decay of tPA. We have shown in vitro that TXA plus urokinase stimulates plasmin generation to aggravate consumption of α_2 -antiplasmin, which is often reduced in trauma patients, to allow the fibrinolytic system to go unchecked. The consequences are destruction of fibrinogen and clotting factors, which could contribute to a bleeding pathology.⁷² There are little data available on changes in uPA or uPA-PAI complexes in trauma patients and currently tests for these analytes are poorly standardized so that estimates of gravimetric or molar concentrations in patient plasma are unreliable.

Covid-19

Coagulopathy was soon observed to be a life-threatening complication of infection with SARS-CoV-2 in hospitalized patients.^{73,74} Venous thromboembolic events are noted in many patients in intensive care and a high proportion of patients are diagnosed with pulmonary embolism.^{75,76} Post-mortem investigations have identified widely distributed microvascular thrombi in the lungs, heart, kidney, liver, skin and fat.⁷⁷ Biomarkers in severely ill patients include raised fibrinogen and D-dimer, which can reach very high levels,^{77,78} often $>2 \mu\text{g/mL}$, many times over the routine cut-off used to exclude a diagnosis of thrombosis ($0.5 \mu\text{g/mL}$). D-dimer has been investigated as a marker to predict mortality and manage patient care and direct anticoagulant treatment.^{74,79} Reporting results and standardization of D-dimer testing protocols is an area of concern in Covid-19 patients and is particularly important if it is to be linked to patient care.⁸⁰ Given the prevalence of thrombotic complications in Covid-19 patients in intensive care, it is not surprising that thrombolytic therapy with tPA is being considered for seriously ill patients via the intravenous route⁸¹ or in nebulizer form.⁸² Other factors that may influence coagulation and fibrinolysis in Covid-19 patients may include neutrophil extracellular trap (NET) formation, which stimulates coagulation and retards fibrinolysis^{83,84} and NETs have been observed in some early studies with samples from Covid-19 patients.^{77,85,86} There is interest in changes in PAI-1 levels during Covid-19 infection, and there are early reports of fibrinolysis resistance assessed by antigen studies and viscoelastic methods.^{85,87}

Conclusions

There is a long history of fibrinolysis research and assay method development, but no simple direct methods to establish something like a fibrinolysis capacity or a measurement of fibrinolysis resistance on par with prothrombin time or activated partial thromboplastin time, for example. Many problems associated with poor standardization and assay variability remain unresolved. Rapid universal methods would be useful in situations such as trauma, DIC and surgery, but there is a need for large-scale randomized trials to establish safety and efficacy. A better understanding is

needed of changes in fibrinolysis following infection with agents causing hemorrhagic diseases, and the emergence of SARS-CoV-2 coagulopathy highlights the need for ongoing research to improve the measurement of fibrinolysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Longstaff C, Kolev K. Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost* 2015;13(Suppl 1):S98–S105
- Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost* 2009;7(01):4–13
- Thelwell C. Fibrinolysis standards: a review of the current status. *Biologicals* 2010;38(04):437–448
- Coxon CH, Longstaff C, Burns C. Applying the science of measurement to biology: Why bother? *PLoS Biol* 2019;17(06):e3000338
- Longstaff C, Whitton CM, Stebbings R, Gray E. How do we assure the quality of biological medicines? *Drug Discov Today* 2009;14(1–2):50–55
- Huish S, Thelwell C, Longstaff C. Activity regulation by fibrinogen and fibrin of streptokinase from *Streptococcus pyogenes*. *PLoS One* 2017;12(01):e0170936
- Locke M, Rigsby P, Longstaff C SSC Communication on behalf of the Fibrinolysis Subcommittee. An international collaborative study to establish the WHO 4th International Standard for Streptokinase: communication from the SSC of the ISTD. *J Thromb Haemost* 2020;18(06):1501–1505
- Thelwell C. Biological standards for potency assignment to fibrinolytic agents used in thrombolytic therapy. *Semin Thromb Hemost* 2014;40(02):205–213
- Beebe DP, Aronson DL. An automated fibrinolytic assay performed in microtiter plates. *Thromb Res* 1987;47(01):123–128
- Longstaff C, Whitton CM. A proposed reference method for plasminogen activators that enables calculation of enzyme activities in SI units. *J Thromb Haemost* 2004;2(08):1416–1421
- Silva MM, Thelwell C, Williams SC, Longstaff C. Regulation of fibrinolysis by C-terminal lysines operates through plasminogen and plasmin but not tissue-type plasminogen activator. *J Thromb Haemost* 2012;10(11):2354–2360
- European Commission. Alteplase for injection. Monograph, European Pharmacopoeia, 2007. European Pharmacopoeia; Strasbourg 2013:1170
- Bonnard T, Law LS, Tennant Z, Hagemeyer CE. Development and validation of a high throughput whole blood thrombolysis plate assay. *Sci Rep* 2017;7(01):2346
- Longstaff C Subcommittee on Fibrinolysis. Development of Shiny app tools to simplify and standardize the analysis of hemostasis assay data: communication from the SSC of the ISTD. *J Thromb Haemost* 2017;15(05):1044–1046
- Rosendaal FR, Reitsma PH. Reproducibility. *J Thromb Haemost* 2017;15(05):833
- Longstaff C. Shiny-Clots. Updated 2019. Accessed November, 2020 at: <https://drclongstaff.github.io/shiny-clots/>
- Kolev K, Longstaff C. Bleeding related to disturbed fibrinolysis. *Br J Haematol* 2016;175(01):12–23
- Longstaff C. Measuring fibrinolysis: from research to routine diagnostic assays. *J Thromb Haemost* 2018;16(04):652–662
- Smith AA, Jacobson LJ, Miller BI, Hathaway WE, Manco-Johnson MJ. A new euglobulin clot lysis assay for global fibrinolysis. *Thromb Res* 2003;112(5–6):329–337
- Ilich A, Noubouossie DF, Henderson M, et al. Development and application of global assays of hyper- and hypofibrinolysis. *Res Pract Thromb Haemost* 2019;4(01):46–53

- 21 Pieters M, Philippou H, Undas A, de Lange Z, Rijken DC, Mutch NJ Subcommittee on Factor XIII and Fibrinogen, and the Subcommittee on Fibrinolysis. An international study on the feasibility of a standardized combined plasma clot turbidity and lysis assay: communication from the SSC of the ISTH. *J Thromb Haemost* 2018;16(05):1007–1012
- 22 Lisman T. Decreased plasma fibrinolytic potential as a risk for venous and arterial thrombosis. *Semin Thromb Hemost* 2017;43(02):178–184
- 23 van Geffen M, van Heerde WL. Global haemostasis assays, from bench to bedside. *Thromb Res* 2012;129(06):681–687
- 24 Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. *Lancet* 2010;376(9757):2032–2039
- 25 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;112(20):3080–3087
- 26 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;35(05):468–477
- 27 Declerck PJ, Moreau H, Jespersen J, Gram J, Kluft C. Multicenter evaluation of commercially available methods for the immunological determination of plasminogen activator inhibitor-1 (PAI-1). *Thromb Haemost* 1993;70(05):858–863
- 28 Gram J, Declerck PJ, Sidelmann J, Jespersen J, Kluft C. Multicentre evaluation of commercial kit methods: plasminogen activator inhibitor activity. *Thromb Haemost* 1993;70(05):852–857
- 29 Song C, Burgess S, Eicher JD, O'Donnell CJ, Johnson AD. Causal effect of plasminogen activator inhibitor type 1 on coronary heart disease. *J Am Heart Assoc* 2017;6(06):e004918
- 30 Dempfle CE, Zips S, Ergül H, Heene DL Fibrin Assay Comparative Trial study group. The Fibrin Assay Comparison Trial (FACT): evaluation of 23 quantitative D-dimer assays as basis for the development of D-dimer calibrators. FACT study group. *Thromb Haemost* 2001;85(04):671–678
- 31 Meijer P, Haverkate F, Kluft C, de Moerloose P, Verbruggen B, Spannagl M. A model for the harmonisation of test results of different quantitative D-dimer methods. *Thromb Haemost* 2006;95(03):567–572
- 32 Rudnicka AR, Rumley A, Lowe GD, Strachan DP. Diurnal, seasonal, and blood-processing patterns in levels of circulating fibrinogen, fibrin D-dimer, C-reactive protein, tissue plasminogen activator, and von Willebrand factor in a 45-year-old population. *Circulation* 2007;115(08):996–1003
- 33 Macy EM, Meilahn EN, Declerck PJ, Tracy RP. Sample preparation for plasma measurement of plasminogen activator inhibitor-1 antigen in large population studies. *Arch Pathol Lab Med* 1993;117(01):67–70
- 34 Schneider M, Boffa M, Stewart R, Rahman M, Koschinsky M, Nesheim M. Two naturally occurring variants of TAFI (Thr-325 and Ile-325) differ substantially with respect to thermal stability and antifibrinolytic activity of the enzyme. *J Biol Chem* 2002;277(02):1021–1030
- 35 Frère C, Morange PE, Saut N, et al. Quantification of thrombin activatable fibrinolysis inhibitor (TAFI) gene polymorphism effects on plasma levels of TAFI measured with assays insensitive to isoform-dependent artefact. *Thromb Haemost* 2005;94(02):373–379
- 36 Tregouet DA, Schnabel R, Alessi MC, et al; AtheroGene Investigators. 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;7(01):49–57
- 37 Neill EK, Stewart RJ, Schneider MM, Nesheim ME. A functional assay for measuring activated thrombin-activatable fibrinolysis inhibitor in plasma. *Anal Biochem* 2004;330(02):332–341
- 38 Heylen E, Van Goethem S, Augustyns K, Hendriks D. Measurement of carboxypeptidase U (active thrombin-activatable fibrinolysis inhibitor) in plasma: Challenges overcome by a novel selective assay. *Anal Biochem* 2010;403(1–2):114–116
- 39 Ceresa E, Brouwers E, Peeters M, Jern C, Declerck PJ, Gils A. Development of ELISAs measuring the extent of TAFI activation. *Arterioscler Thromb Vasc Biol* 2006;26(02):423–428
- 40 Hendrickx ML, Zatloukalova M, Hassanzadeh-Ghassabeh G, Muyl-dermans S, Gils A, Declerck PJ. Identification of a novel, nanobody-induced, mechanism of TAFI inactivation and its in vivo application. *J Thromb Haemost* 2014;12(02):229–236
- 41 Wyseure T, Rubio M, Denorme F, et al. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood* 2015;125(08):1325–1332
- 42 Willemse JL, Heylen E, Nesheim ME, Hendriks DF. Carboxypeptidase U (TAFIa): a new drug target for fibrinolytic therapy? *J Thromb Haemost* 2009;7(12):1962–1971
- 43 Khan SS, Shah SJ, Klyachko E, et al. A null mutation in *SERPINE1* protects against biological aging in humans. *Sci Adv* 2017;3(11):o1617
- 44 Moore HB, Moore EE, Neal MD, et al. Fibrinolysis shutdown in trauma: historical review and clinical implications. *Anesth Analg* 2019;129(03):762–773
- 45 Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. *Nat Rev Dis Primers* 2016;2:16037
- 46 Weitz JI, Fredenburgh JC, Eikelboom JW. A test in context: D-dimer. *J Am Coll Cardiol* 2017;70(19):2411–2420
- 47 Longstaff C, Adcock D, Olson JD, et al. Harmonisation of D-dimer – a call for action. *Thromb Res* 2016;137:219–220
- 48 Jennings I, Woods TA, Kitchen DP, Kitchen S, Walker ID. Laboratory D-dimer measurement: improved agreement between methods through calibration. *Thromb Haemost* 2007;98(05):1127–1135
- 49 Raza I, Davenport R, Rourke C, et al. The incidence and magnitude of fibrinolytic activation in trauma patients. *J Thromb Haemost* 2013;11(02):307–314
- 50 Gall LS, Brohi K, Davenport RA. Diagnosis and treatment of hyperfibrinolysis in trauma (A European Perspective). *Semin Thromb Hemost* 2017;43(02):224–234
- 51 Cardenas JC, Matijevic N, Baer LA, Holcomb JB, Cotton BA, Wade CE. Elevated tissue plasminogen activator and reduced plasminogen activator inhibitor promote hyperfibrinolysis in trauma patients. *Shock* 2014;41(06):514–521
- 52 Chapman MP, Moore EE, Moore HB, et al. Overwhelming tPA release, not PAI-1 degradation, is responsible for hyperfibrinolysis in severely injured trauma patients. *J Trauma Acute Care Surg* 2016;80(01):16–23; discussion 23–25
- 53 Cotton BA, Faz G, Hatch QM, et al. Rapid thrombelastography delivers real-time results that predict transfusion within 1 hour of admission. *J Trauma* 2011;71(02):407–414, discussion 414–417
- 54 Neal MD, Moore EE, Walsh M, et al. A comparison between the TEG 6s and TEG 5000 analyzers to assess coagulation in trauma patients. *J Trauma Acute Care Surg* 2020;88(02):279–285
- 55 Ilich A, Bokarev I, Key NS. Global assays of fibrinolysis. *Int J Lab Hematol* 2017;39(05):441–447
- 56 Whiting D, DiNardo JA. TEG and ROTEM: technology and clinical applications. *Am J Hematol* 2014;89(02):228–232
- 57 Baksaas-Aasen K, Van Dieren S, Balvers K, et al; TACTIC/INTRN Collaborators. Data-driven development of ROTEM and TEG algorithms for the management of trauma hemorrhage: a prospective observational multicenter study. *Ann Surg* 2019;270(06):1178–1185
- 58 Juffermans NP, Wirtz MR, Balvers K, et al; TACTIC Partners. Towards patient-specific management of trauma hemorrhage: the effect of resuscitation therapy on parameters of thromboelastometry. *J Thromb Haemost* 2019;17(03):441–448
- 59 Ker K, Prieto-Merino D, Roberts I. Systematic review, meta-analysis and meta-regression of the effect of tranexamic acid on surgical blood loss. *Br J Surg* 2013;100(10):1271–1279
- 60 Gayet-Ageron A, Prieto-Merino D, Ker K, Shakur H, Ageron FX, Roberts I Antifibrinolytic Trials Collaboration. Effect of treatment

- delay on the effectiveness and safety of antifibrinolytics in acute severe haemorrhage: a meta-analysis of individual patient-level data from 40 138 bleeding patients. *Lancet* 2018;391(10116):125–132
- 61 Roberts I, Shakur H, Afolabi A, et al; CRASH-2 Collaborators. The importance of early treatment with tranexamic acid in bleeding trauma patients: an exploratory analysis of the CRASH-2 randomised controlled trial. *Lancet* 2011;377(9771):1096–1101, 1101.e1–1101.e2
 - 62 Shakur H, Roberts I, Fawole B, et al; WOMAN Trial Collaborators. Effect of early tranexamic acid administration on mortality, hysterectomy, and other morbidities in women with post-partum haemorrhage (WOMAN): an international, randomised, double-blind, placebo-controlled trial. *Lancet* 2017;389(10084):2105–2116
 - 63 CRASH-3 Trial Collaborators. Effects of tranexamic acid on death, disability, vascular occlusive events and other morbidities in patients with acute traumatic brain injury (CRASH-3): a randomised, placebo-controlled trial. *Lancet* 2019;394(10210):1713–1723
 - 64 Cole E, Davenport R, Willett K, Brohi K. Tranexamic acid use in severely injured civilian patients and the effects on outcomes: a prospective cohort study. *Ann Surg* 2015;261(02):390–394
 - 65 Van Haren RM, Valle EJ, Thorson CM, et al. Hypercoagulability and other risk factors in trauma intensive care unit patients with venous thromboembolism. *J Trauma Acute Care Surg* 2014;76(02):443–449
 - 66 Collaborators HALT-IT/HAEM-IT Trial Collaborators. Effects of a high-dose 24-h infusion of tranexamic acid on death and thromboembolic events in patients with acute gastrointestinal bleeding (HALT-IT): an international randomised, double-blind, placebo-controlled trial. *Lancet* 2020;395(10241):1927–1936
 - 67 Hunt H, Stanworth S, Curry N, et al. Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) for trauma induced coagulopathy in adult trauma patients with bleeding. *Cochrane Database Syst Rev* 2015;2(02):CD010438
 - 68 Wikkelsø A, Wetterslev J, Møller AM, Afshari A. Thromboelastography (TEG) or thromboelastometry (ROTEM) to monitor haemostatic treatment versus usual care in adults or children with bleeding. *Cochrane Database Syst Rev* 2016;(08):CD007871
 - 69 Rugeri L, Levrat A, David JS, et al. Diagnosis of early coagulation abnormalities in trauma patients by rotation thrombelastography. *J Thromb Haemost* 2007;5(02):289–295
 - 70 Roberts I. Fibrinolytic shutdown: fascinating theory but randomized controlled trial data are needed. *Transfusion* 2016;56(Suppl 2):S115–S118
 - 71 Hijazi N, Abu Fanne R, Abramovitch R, et al. Endogenous plasminogen activators mediate progressive intracerebral hemorrhage after traumatic brain injury in mice. *Blood* 2015;125(16):2558–2567
 - 72 Longstaff C, Locke M. Increased urokinase and consumption of α_2 -antiplasmin as an explanation for the loss of benefit of tranexamic acid after treatment delay. *J Thromb Haemost* 2019;17(01):195–205
 - 73 Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of coronavirus disease 2019. *Crit Care Med* 2020;48(09):1358–1364
 - 74 Langer F, Kluge S, Klamroth R, Oldenburg J. Coagulopathy in COVID-19 and its implication for safe and efficacious thromboprophylaxis. *Hamostaseologie* 2020;40(03):264–269
 - 75 Klok FA, Kruip MJHA, van der Meer NJM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. *Thromb Res* 2020;191:148–150
 - 76 Middeldorp S, Coppens M, van Haaps TF, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost* 2020;18(08):1995–2002
 - 77 Becker RC. COVID-19 update: Covid-19-associated coagulopathy. *J Thromb Thrombolysis* 2020;50(01):54–67
 - 78 Giusti B, Gori AM, Alessi M, et al. Sars-CoV-2 induced coagulopathy and prognosis in hospitalized patients: a snapshot from Italy. *Thromb Haemost* 2020;120(08):1233–1236
 - 79 Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J Thromb Haemost* 2020;18(06):1324–1329
 - 80 Thachil J, Longstaff C, Favaloro EJ, Lippi G, Urano T, Kim PYSSC Subcommittee on Fibrinolysis of the International Society on Thrombosis and Haemostasis. The need for accurate D-dimer reporting in COVID-19: communication from the ISTH SSC on fibrinolysis. *J Thromb Haemost* 2020;18(09):2408–2411
 - 81 Barrett CD, Moore HB, Moore EE, et al. Fibrinolytic therapy for refractory COVID-19 acute respiratory distress syndrome: Scientific rationale and review. *Res Pract Thromb Haemost* 2020;4(04):524–531
 - 82 Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. *J Thromb Haemost* 2020;18(07):1548–1555
 - 83 Longstaff C, Varjú I, Sótönyi P, et al. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones. *J Biol Chem* 2013;288(10):6946–6956
 - 84 Varjú I, Longstaff C, Szabó L, et al. DNA, histones and neutrophil extracellular traps exert anti-fibrinolytic effects in a plasma environment. *Thromb Haemost* 2015;113(06):1289–1298
 - 85 Weiss E, Roux O, Moyer JD, et al. Fibrinolysis resistance: a potential mechanism underlying COVID-19 coagulopathy. *Thromb Haemost* 2020;120(09):1343–1345
 - 86 Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps in COVID-19. *JCI Insight* 2020;5(11):138999
 - 87 Nougier C, Benoit R, Simon M, et al. Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. *J Thromb Haemost* 2020;18(09):2215–2219
 - 88 Walker JB, Nesheim ME. The molecular weights, mass distribution, chain composition, and structure of soluble fibrin degradation products released from a fibrin clot perfused with plasmin. *J Biol Chem* 1999;274(08):5201–5212
 - 89 Longstaff C, Thelwell C, Williams SC, Silva MM, Szabó L, Kolev K. The interplay between tissue plasminogen activator domains and fibrin structures in the regulation of fibrinolysis: kinetic and microscopic studies. *Blood* 2011;117(02):661–668
 - 90 Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *J Biol Chem* 1996;271(28):16603–16608
 - 91 Uitte de Willige S, Standeven KF, Philippou H, Ariëns RA. The pleiotropic role of the fibrinogen gamma' chain in hemostasis. *Blood* 2009;114(19):3994–4001
 - 92 Duval C, Allan P, Connell SDA, Ridger VC, Philippou H, Ariëns RAS. Roles of fibrin α - and γ -chain specific cross-linking by FXIIIa in fibrin structure and function. *Thromb Haemost* 2014;111(05):842–850
 - 93 Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through α_2 -antiplasmin cross-linking. *Blood* 2011;117(23):6371–6374
 - 94 Whyte CS, Mitchell JL, Mutch NJ. Platelet-mediated modulation of fibrinolysis. *Semin Thromb Hemost* 2017;43(02):115–128
 - 95 Wohner N, Sótönyi P, Machovich R, et al. Lytic resistance of fibrin containing red blood cells. *Arterioscler Thromb Vasc Biol* 2011;31(10):2306–2313
 - 96 Cines DB, Lebedeva T, Nagaswami C, et al. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood* 2014;123(10):1596–1603