Regulation and Dysregulation of Fibrinolysis

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Introduction

Claude Bernard (1813–1878), a French physiologist, first coined the term *milieu intérieur* (the environment within), now known as homeostasis. Hemostasis is one such homeostatic process. Hemostasis could be defined as a state of physiologic homeostasis resulting from a dynamic equilibrium between coagulation and fibrinolysis. The endothelium, a single layer of cells, which line the intimal surface of the entire cardiovascular system (estimated to be 1000–5000 square meters), is by far the largest endocrine, paracrine, and autocrine gland ever known to man and plays a central role in maintaining blood fluidity. The coordinated interplay of primary hemostasis (platelet-vessel wall interactions), secondary hemostasis (fibrin formation), and fibrinolysis ensures arrest of blood flow at sites of injury and restoration of vascular patency during wound healing by local activation of plasminogen to plasmin. This finely tuned balance enables healing of a vascular lesion without compromising the stability of the clot and to contain the fibrinolytic activity to the injured area. This can be achieved by the intricate fine balance between coagulation, fibrinolysis, and proteolytic and inhibitory proteins [1].

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Regulation of Fibrinolysis

The fibrinolytic system is an important endothelium-dependent mechanism for limiting fibrin accumulation [2]. Plasmin plays a central role in fibrinolysis and is formed from plasma zymogen, plasminogen, by the action of plasminogen activators derived from the endothelium. The endogenous plasminogen activators are, namely, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) which themselves exist in zymogenic forms, tPA as single-chain tPA and uPA as prourokinase [3]. The tPA is fibrin selective since its catalytic activity is enhanced significantly following binding to fibrin. Specific cell surface membrane receptors also allow plasminogen activation by tPA and uPA and thereby promote dissolution of fibrin clot from the microenvironment of the endothelial cell, macrophages, or platelet surface [3].

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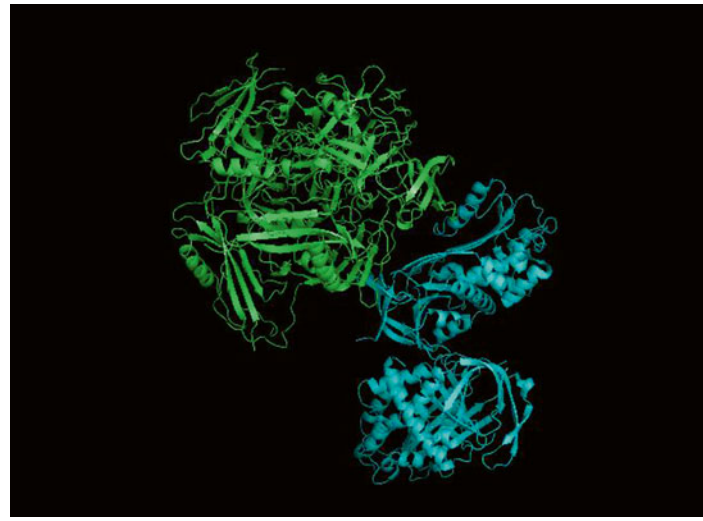

Fig. 9.1 Plasmin shown in *green* is regulated via plasminogen activator inhibitor-1 (PAI-1) in *blue*

Once plasmin is formed it degrades the fibrin and forms fibrin degradation products (FDPs) and by virtue of their competition with fibrin monomer for fibrin binding sites and with fibrinogen for platelet surface glycoprotein IIb/IIIa (GPIIb/IIIa) binding sites, they have anticoagulant properties [3]. Plasmin besides lysing the fibrinogen is also known to split prothrombin, thereby triggering thrombin generation and causing a procoagulant effect. Plasminogen activation is regulated by α2-antiplasmin, and plasmin activity is regulated by plasminogen activator inhibitors (PAI-1 and PAI-II) by forming one-to-one stoichiometric complexes with the active enzymes (see Fig. 9.1) [3]. Another non-plasmin fibrinolytic pathway described involves binding of fibrin to the pluripotent macrophage integrin Mac-1 (DC11b/CD18), and then the fibrinogen-Mac-1 complex is then internalized in the macrophage, and then the fibrin is degraded by the action of cathepsin D into FDPs [4]. This macrophage-mediated, non-plasmin fibrinolytic pathway takes place during the process of recanalization of the vessel [3].

The proteases involved in fibrinolysis include plasmin and tissue-type plasminogen activator (tPA) and urinary-type plasminogen activator (uPA) or urokinase which like the serine proteases exist as zymogens. The plasmin cleaves the α-chain of fibrinogen to release the αC Fragments, thereby producing Fragment X of about 260 kDa, and upon further plasmin action produces Fragment Y and Fragments D and E. However, through enzymatic actions of thrombin and FXIIIa, cross-linked fibrin is formed which upon action by plasmin forms D-dimer and E complex. The human plasminogen is the zymogenic form of plasmin. It is a single-chain glycoprotein of 92 kDa. All plasminogen activators cleave the Arg561-Val562 bond in plasminogen. There are several variants of plasminogen which could result from glycosylation and genetic polymorphism besides limited proteolysis and activation processes. The physiologic plasminogen activators include tPA and uPA. Another activation system, the contact plasminogen activator pathway, is dependent on the FXII, prekallikrein, and high molecular weight kininogen. There are some plasminogen activators from bacteria and vampire bat such as staphylokinase and vampire bat plasminogen activator.

Tissue-type plasminogen activator: It is a serine protease of 68 kDa, which, having secreted by the endothelial cells, exerts its effects in the vessels. Besides the circulating amount of approximately 5 μg/L (70 pM), the rest complexes with its primary inhibitor plasminogen activator inhibitor (PAI-1). The tPA cleaves the Arg561-Val562 bond in plasminogen but is ineffective activator of plasminogen in the absence of fibrin. Mutations engineered in tPA alter its characteristics which may make tPA resistant to

the action of PAI-1 or make more effective agents such as new mutant tenecteplase (TNK-tPA).

Urinary-type plasminogen activator: Although found in the urine at 40–80 μg/L, it is synthesized by cells with a fibroblast-like morphology and also epithelial cells, monocytes, and macrophages. It activates plasminogen by cleaving Arg561-Val562 bond, but does not require fibrin. While tPA functions in the vasculature, uPA has a role in degradation of extracellular matrix and cell migration which may have effects on wound healing, inflammation, and embryogenesis and invasion of tumor cells and metastases. However, this does not discount its role in fibrin degradation.

Contact pathway system: The contact system comprising of FXII, prekallikrein, FXI, and high molecular weight kininogen. The negatively charged surfaces such as kaolin, ellagic acid, sulfatides, and dextran sulfate activate FXII to FXIIa and prekallikrein to kallikrein. Factor XIIa further activates the FXI to FXIa, triggering the coagulation cascade. The contact factor, FXII, although a clotting factor, is structurally similar to tPA, uPA, and plasminogen and causes fibrinolysis at least by three mechanisms such as (1) direct activation of plasminogen by FXIIa, (2) generation of kallikrein by FXII-dependent and FXII-independent pathways triggering activation of scuPA, and (3) indirect stimulation of fibrinolysis caused by kallikrein cleavage of high molecular weight kininogen, generating bradykinin, which is a potent stimulator of tPA release from the endothelial cells. In plasma, the presence of fourfold higher concentration of FXII than tPA and uPA is suggestive of its important role in activation of plasminogen as effective as urokinase. The contact pathway regulates fibrinolysis by these mechanisms.

Other Plasminogen Activators

Streptokinase: Streptokinase is not an enzyme but an extracellular protein produced by streptococci and is capable of activating the human fibrinolytic system, by forming a 1:1 stoichiometric complex with plasminogen. The activated streptokinase-plasminogen complex binds with another molecule of plasminogen and cleaves it at Arg541-Val562 that forms plasmin. The activated streptokinase-plasminogen complex retains its ability to bind to the fibrin and is protected by inhibitory effects of α2-antiplasmin. Streptokinase causes systemic conversion of plasminogen to plasmin and depletion of circulating fibrinogen, plasminogen, and FV and FVIII. Streptokinase is antigenic.

Staphylokinase is also not an enzyme but a protein produced by *Staphylococcus aureus*, which forms a stoichiometric complex with plasminogen. Staphylokinase is fibrin-dependent and hence in the absence of fibrin does not activate plasminogen. Trace amount of plasmin on the surface of fibrin cleaves ten residues from the amino terminus of staphylokinase, allowing staphylokinase to bind with plasminogen and interacting with fibrin-bound plasminogen forming free plasmin. The free plasmin converts the staphylokinase-plasminogen complex to staphylokinase-plasmin complex which is not vulnerable to inactivation by α2-antiplasmin. Staphylokinase is antigenic.

Vampire bat plasminogen activator: Four plasminogen activators in the salivary glands of vampire bat (*Desmodus rotundus*) were identified, namely, DSPAα1 and DSPAα2 and DSPAβ and DSPAγ. DSPA unlike other plasminogen activators is very fibrin specific and the activity of DSPAα1 is in fact 105,000-fold higher in the presence of fibrin than in its absence. Enhanced fibrin specificity and long clearance time are some of its important properties.

Inhibitors of the fibrinolytic system: While plasminogen activator inhibitor-1 is the main inhibitor of tPA and uPA, α2-antiplasmin inhibits plasmin. **PAI-1 and α2-antiplasmin are members of the serpin family which includes antithrombin and heparin cofactor II**.

Plasminogen activator inhibitor-1 (PAI-1): There are several sources of production of PAI-1, such as platelets, megakaryocytes, endothelial cells, hepatocytes, and adipocytes; however, the

source of resting plasma PAI-1 is not known. The source of increased PAI-1 levels as an acute phase response is hepatocytes. Half of the circulating PAI-1 is known to come from the platelets and its large amounts accumulate in the thrombi. While PAI-1 inhibits tPA and uPA, it does not inhibit scuPA which is largely inactive. Once bound to uPAR on the surface of monocytes, the activity of scuPA is profoundly increased. The receptor-bound scuPA initiates proteolytic activity. The conversion of receptor-bound scuPA to uPA and the vulnerability of uPA to the inhibitory effects of PAI-1 are considered to be ways of regulating fibrinolysis. PAI-1 deficiency is quite rare and leads to bleeding.

PAI-1 inhibitors: Inhibition of PAI-1, which is a major inhibitor of tPA and uPA, results in increased endogenous fibrinolytic activity. PAI-1 synthesis is decreased in vitro by lipid lowering drugs such as niacin and fibrates [5, 6]. Similarly, peptides that block PAI-1 activities are also identified which prevent insertion of the reactive center loop upon cleavage by the target protease [7] or by converting PAI-1 into a latent conformation [8]. Development of small molecule PAI-1 inhibitors, some of which may have antithrombotic properties in vivo, may be quite interesting [9].

α2-antiplasmin: It is a fast-acting inhibitor of plasmin. The circulating concentration of α2-antiplasmin is 70 mg/L. It is synthesized in the liver and obviously is decreased in patients with hepatic insufficiency. It exists in several forms in the plasma and its inhibitory capability resides in the core of the protein. **α2-antiplasmin causes interference in the interaction of plasminogen, plasmin, tPA, and fibrin**. Plasminogen and tPA may interact directly with fibrin, by acting as a cofactor in plasminogen activation resulting in fibrin degradation due to action of plasmin. α2-antiplasmin can bind with plasminogen and prevent binding of plasminogen to fibrin. α2-antiplasmin can also cross-link to fibrin preventing plasmin binding to fibrin and by binding to plasmin prevents plasmin's action, thereby inhibiting fibrin degradation. Deficiency of α2-antiplasmin results in delayed bleeding and normal initial hemostasis characteristic of fibrinolytic defects.

TAFI: As the name implies, thrombin activatable fibrinolytic inhibitor is a fibrinolytic inhibitor, activated by thrombin generated by the coagulation cascade. As an intermediate between coagulation and fibrinolysis, it cross-regulates coagulation and fibrinolysis. TAFI is a procarboxypeptidase U (pro-CPU) and is a zymogen of the active enzyme TAFIa, a plasma carboxypeptidase B (pCPB). The generation of C-terminal lysyl residues serves as an important feedback mechanism and enables the plasminogen to bind to fibrin in enhancing fibrinolysis. TAFIa removes these C-terminal lysyl residues, thereby regulating the fibrinolytic process. Thus TAFI is activated by thrombin-thrombomodulin complex and by plasmin in a reaction that is enhanced by glycosaminoglycans (see Fig. 9.2). TAFI is produced in the liver and circulates in the blood at approximately 75 nM concentration, and only a small proportion is necessary for activation to have complete function. Due to polymorphisms in TAFI gene, there could be wide ranges of its concentration in healthy population and does not necessarily correlate with disease. There is no known endogenous inhibitor of TAFIa. As an acute phase reactant, the TAFI levels are increased in inflammation. Elevated levels of TAFI and PAI-1 and associated hypofibrinolysis may pose a risk for venous thrombosis. Deficiency of TAFI in mice has shown to increase the risk of cerebral thrombosis and ischemic stroke in mice. Hemophilic patients show defective activation of TAFI that leads to increased fibrinolysis and increased bleeding. Most of the clot is formed after the formation of a clot by the intrinsic pathway activation of FXI by thrombin. Clots formed in vitro in the presence of FIX deficient plasma lysed prematurely and could be reversed by the addition of FIX and thrombomodulin to the plasma. Normal fibrinolysis could be achieved, if TAFI, thrombomodulin, and FVIII are added to the plasma from hemophilic patient. Administration of Factor XIa antibodies and inhibition of TAFI in rabbit models is reported to have shown twofold increased endogenous fibrinolysis.

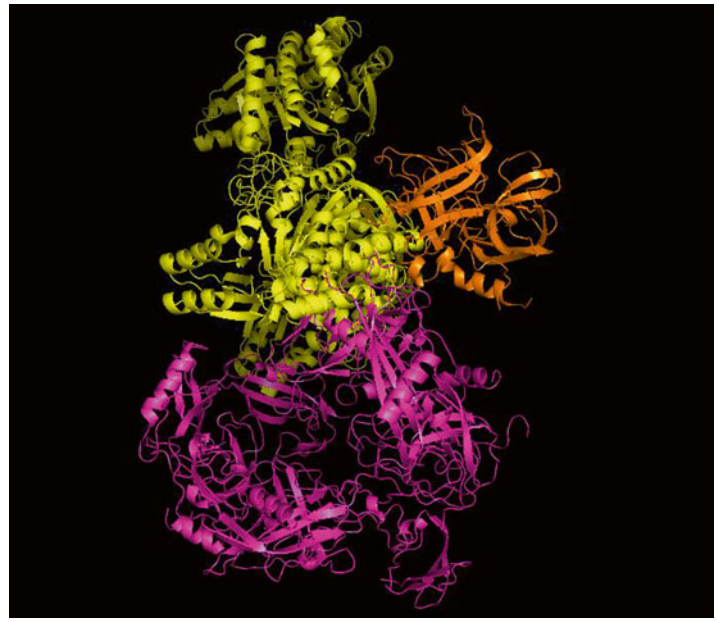

Fig. 9.2 TAFI seen in *yellow* is activated by the thrombin-thrombomodulin complex in addition with plasmin seen in *pink*. This reaction is also enhanced by glycosaminoglycans. The *orange* protein seen above is thrombin

TAFI cross-regulates coagulation and fibrinolysis. Hemophilia A is caused by the deficiency of FVIIIa and the bleeding, as a result of defective clot formation. The defective role of TAFI in hemophilia plays a role in less protection of the clot. It is interesting to note the degree of bleeding in hemophilic patients due to defective clot formation or defective clot protection as a result of defective TAFI activation. It is also interesting to know as to how much does activation of TAFI contribute to the increased risk of venous thrombosis associated with high levels of coagulation factors II, VIII, IX, and XI.

Interactive role of thrombin-thrombomodulin complex, TFPI, protein C, APC, TAFI, and fibrinolytic pathways in severe sepsis: Tissue factor-induced thrombin generation is downregulated by TFPI and the functional protein C pathway [10]. Thrombin-TM complex links coagulation with the fibrinolysis by TAFI [11, 12]. In a severe septic state, increased thrombin and reduced APC inhibition of thrombin generation, leading to increased thrombin levels, promote TAFI activity, thereby inhibiting fibrinolysis. When the TM levels are increased, TAFI activity is promoted and there is more fibrinolytic deficit.

Protein C also combines with PAI-1 to prevent inhibition of fibrinolysis. In sepsis there is reduced protein C/APC activity. This formed the basis of administering protein C to patients with meningococcal septicemia where changes in TM and EPCR result in purpura fulminans [13–15]. Maruyama demonstrated in rodent and primate models of TF-induced DIC that recombinant soluble TM may prevent DIC even when the AT levels are low [16, 17].

Other Inhibitors

PAI-2: PAI-2 is a urokinase inhibitor in placenta. Monocytes being the important source of PAI-2, it gets into the clot and causes fibrin stability by cross-linking with fibrin. PAI-2 is known to inhibit two-chain tPA. By its intracellular presence, it is led to believe that, besides inhibition of plasminogen activator, it may have protective effects on the cells from cytopathic effects of alpha virus infection.

Alpha2-macroglobulin: It is a glycoprotein circulating in the blood in the amounts of 2.5 g/L. It inhibits several proteases including plasmin but initiates its activity when the activity

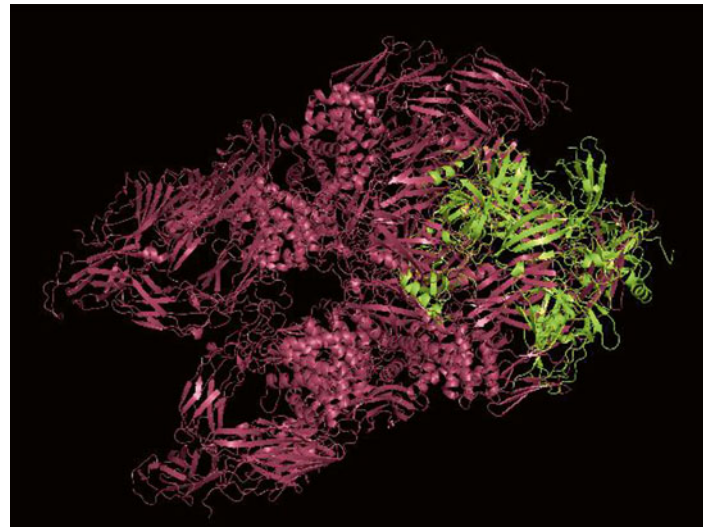

Fig. 9.3 Alpha2-macroglobulin seen in *red* interacting with plasmin in *yellow*. Alpha2-macroglobulin inhibits several proteases, including plasmin when the activity of α2-antiplasmin is diminished. In addition it can form complexes with tPA and uPA

of α2-antiplasmin is worn off. It forms complexes with tPA and uPA (see Fig. 9.3).

C1-inhibitor: It is a highly glycosylated serpin, circulating in the blood at a concentration of 1.7 μM. Besides inhibiting the activated complements of Cl, namely, Clr and Cls, it also inhibits the FXIIa, FXIa, plasma kallikrein, plasmin, tPA, and uPA. Although the precise function of C1-inhibitor is not known, it may regulate fibrinolysis by virtue of its inhibition of contact proteases and the conversion of scuPA to uPA.

Lipoprotein (a): Circulating in the blood at 50–1000 mg/L, lipoprotein (a) binds to fibrin, extracellular matrix, platelets, and cells. It competes with plasminogen and tPA for binding sites on fibrin, thereby exerting an antifibrinolytic effect.

Histidine-rich glycoprotein: Histidine-rich glycoproteins are synthesized in the liver and are taken up by the platelets and megakaryocytes and released upon thrombin activation. HRG may combine with plasminogen and circulate as a complex, thereby decreasing the plasminogen available for fibrin. Although rare, congenital heterozygous HRG deficiency may lead to thrombotic complications.

Balance of plasminogen activation and regulation of fibrinolysis: Conversion of plasminogen to active plasmin only takes place on the fibrin and cell surfaces; otherwise, the plasmin activity may cause generalized fibrinolysis, proteolysis, and degradation of clotting factors FV, FVIII, vWF, and platelet glycoproteins. Once fibrin is formed, the plasminogen is activated to plasmin which acts on the fibrin converting it into very early FDP and FDP. The very early FDP converts the plasminogen to plasmin in the presence of fibrin. However, the thrombin-thrombomodulin complex converts TAFI into TAFIa which removes the formed CT lysines from the FDP, which does not allow it to activate plasminogen to plasmin.

Dysregulation of Fibrinolysis

In disseminated intravascular coagulation (DIC), both the processes of coagulation and fibrinolysis are dysregulated. The activation of coagulation is evident in DIC, by the presence of intravascular thrombi, manifested by increased levels of activated coagulation factors, tissue factor (TF), D-dimer, and decreased fibrinogen [18, 19]. The lipopolysaccharide can initiate clotting by triggering the contact activation of the intrinsic pathway of clotting process and TF activating the extrinsic pathway of coagulation activation, leading to the generation of thrombin. Thrombin once formed can combine with thrombomodulin

to activate the protein C to activated protein C. Thrombin will also directly activate the TAFI to TAFIa which can result in fibrinolytic deficit (see Fig. 9.2). In severe sepsis, activation of the coagulation system can activate the endothelial cells, resulting in the potentiation of the pro-inflammatory responses and the production of cytokines such as TNF-α and IL-1. Anticoagulants in the form of antithrombin, TFPI, APC, anti-Xa inhibitors, anti-IIa inhibitors, TM, TAFI inhibitors, etc. control the activation of the coagulation. The dysregulated fibrinolytic process, manifested by an increased level of plasminogen activator inhibitor-1, can be targeted for therapeutic intervention and by supplementing the decreased endogenous anticoagulant agents such as APC, AT, and TFPI in sepsis [17]. Activation of coagulation and dysregulated fibrinolytic process can lead to intravascular deposition of soluble fibrin, platelet, and leukocyte activation, leading to multiple organ failure in severe sepsis. Leukocytes are found in high numbers in venous thrombi. The leukocytes and activated platelets can form rosettes mediated by P-selectin expression on the activated platelets [20, 21]. Prevention of this interaction between inflammatory cells and platelets resulted in inhibition of both arterial and venous thrombi in animal models [22, 23]. Activation of the endothelium due to thrombin results in increased leukocyte adhesion due to P- and E-selectin expression [20, 24]. Thrombin is an agonist for the formation of platelet-activating factor (PAF), and the adherent neutrophils on the endothelium are vulnerable to the action of PAF, resulting in the release of proteases and oxidants which might increase damage to the endothelium [25]. Furthermore, the factor VIIa-TF complex and FXa are known to activate cells through protease-activated receptors, thereby generating cellular responses similar to those initiated by thrombin activation of protease receptor 1 [25]. Procalcitonin is reported to be as a marker of systemic inflammatory response and as a potential biomarker of sepsis [26, 27]. A recent randomized, double-blind, placebo-controlled phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin (ART-123), in patients with sepsis and DIC, reported that there was an increase in procalcitonin, PAI-1, C5a, IL-6, IL-10, and myeloperoxidase (MPO) and a decrease in protein C [28]. Furthermore, the levels of procalcitonin, PAI-1, IL-6, and IL-10 were reported to be much higher than that of both overt and nonovert and normal controls, and the protein C antigen and functional levels were decreased in a larger extent in the overt group [28].

Diabetes mellitus is known to cause increased cardiovascular morbidity and mortality. Hyperglycemia stimulating the coagulation process and hyperinsulinemia impairing fibrinolysis in healthy humans has been reported earlier [29]. A recent study, in which whether elevated antigen levels of tPA, PAI-1, and tPA/PAI-complex, or von Willebrand factor (vWF) precede the diagnosis of type II diabetes, was evaluated and concluded that elevated levels of fibrinolytic variables in fact precede the manifestation of type II diabetes after adjusting for metabolic and cardiovascular risk factors and can be detected several years before the changes in glucose tolerance occur [30]. Association of obesity with chronic inflammation [31] and activation of endothelial cells that produce vWF by inflammatory markers [32] have been reported earlier. Similarly, CRP causing upregulation of PAI-1 gene expression in human aortic endothelial cells has also been shown [33]. Obesity-induced development of diabetes manifested by resident macrophages in the adipose tissue producing cytokines such as transforming growth factor (TGF)-β and TNF-α causing increase in PAI-1 is reported [34, 35]. The upregulation of PAI-1 early in the development of type II diabetes may be as a result of metabolic syndrome [30].

The important implication of the intricacies of regulation and dysregulation of fibrinolysis is in the cautious use of fibrinolytic drugs especially in neurosurgical patients. There has been a paradigm shift in the management of patients with acute ischemic stroke very recently. While the results of studies, such as the International Management of Stroke (IMS III) [36], Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE) [37], and SYNTHESIS Expansion [38], presented and

published 2 years ago provided evidence that mechanical thrombectomy was ineffective in treating acute stroke secondary to emergent large vessel occlusion (ELVO), there has been a dramatic significant paradigm shift. The current evidence based on large studies such as the Multicenter Randomized Clinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands (MR CLEAN) [39], the Endovascular treatment for Small Core and Anterior circulation Proximal occlusion with Emphasis on minimizing CT to recanalization times (ESCAPE) [40], the Extending the Time for Thrombolysis in Emergency Neurological Deficits-Intra-Arterial (EXTEND-IA) [41], and the Solitaire FR With the Intention For Thrombectomy as Primary Endovascular Treatment for Acute Ischemic Stroke (SWIFT PRIME) [42] provides evidence on the contrary that thrombectomy is profoundly beneficial in patients with ELVO. Thus, based on the results of these new trials, the focus in the management of patients with acute ischemic stroke will shift from intravenous thrombolysis to thrombectomy. While currently 24 % of patients with stroke are transferred to inpatient rehabilitation services and 31 % transferred to skilled nursing facilities and of those returning home directly, 32 % use home healthcare services [43]. The projected total direct medical stroke-related costs between 2012 and 2030 are expected to triple from 71.6 billion to 184.1 billion [44] with 70 % of the first year poststroke costs due to inpatient hospital costs [45]. Due to decreased length of stay for patients undergoing thrombectomy, the overall costs are anticipated to significantly decrease [46].

References

1. Mutch NJ, Booth NA. Plasminogen activation and regulation of fibrinolysis. In: Marder VJ, Aird WC, Bennett JS, Schulman S, White II GC, editors. Hemostasis and thrombosis. 6th ed. Philadelphia, PA: Walters Kluwer/Lippincott Williams & Wilkins; 2013.
2. Collen D, Lijnen HR. Basic and clinical aspects of fibrinolysis and thrombosis. Blood. 1991;78: 3114–24.
3. Loscalzo J, Schafer AI. Overview of hemostasis and fibrinolysis. In: Sasahara AA, Loscalzo J, editors. New therapeutic agents in thrombosis and thrombolysis. 2nd ed. New York: Marcel Decker Inc.; 2003.
4. Simon DI, Ezratty AM, Francis SA, et al. Fibrin(ogen) is internalized and degraded by activated human monocytoid cells via Mac-1 (DC11b/DC18): a non-plasmin fibrinolytic pathway. Blood. 1993;82: 2414–22.
5. Brown SL, Sobel BE. Attenuation of the synthesis of plasminogen activator inhibitor type 1 by niacin: a potential link between lipid lowering and fibrinolysis. Circulation. 1995;92:767–72.
6. Fuji S, Sawa H, Sobel BE. Inhibition of endothelial cell expression of plasminogen activator inhibitor type-1 by gemfibrozil. Thromb Haemost. 1993;70: 642–7.
7. Kvassman J, Lawrence D, Shore J. The acid stabilization of plasminogen activator inhibitor-1 depends on the protonation of a single group that affects loop insertion into b-sheet A. J Biol Chem. 1995;270: 27942–7.
8. Eitzman DT, Fay WP, Lawrence DA, et al. Peptide-mediated inactivation of recombinant and platelet plasminogen activator inhibitor-1 in vitro. J Clin Invest. 1995;95:2416–20.
9. Frederich PM, Levi M, Biemond B, et al. Novel low molecular weight inhibitor of PAI-1 (XR5118) promotes endogenous fibrinolysis and reduces post-thrombolysis thrombus growth in rabbits. Circulation. 1997;96:916–21.
10. Iba T, Yagi Y, Kidoworo A, et al. Increased plasma levels of soluble thrombomodulin in patients with sepsis and organ failure. Jpn J Surg. 1995;25:585–90.
11. Lopez-Aguirre Y, Paramo JA. Endothelial cell and homeostatic activation in relation to cytokines in patients with sepsis. Thromb Res. 1999;94:95–101.
12. Drake TA, Cheng J, Chang A, et al. Expression of tissue factor, thrombomodulin and E-selectin in baboons with lethal E. coli sepsis. Am J Pathol. 1993;142: 1458–70.
13. Laszik Z, Carson CW, Nadasdy T, et al. Lack of suppressed renal thrombomodulin expression in a septic rat model with glomerular thrombotic microangiopathy. Lab Invest. 1994;70:862–7.
14. Rivard GE, David M, Farrell C, et al. Treatment of purpura fulminans in meningococcemia with protein C concentrate. J Pediatr. 1995;126:646–52.
15. Smith OP, White B. Infectious purpura fulminans. Diagnosis and treatment. Br J Haematol. 1999;104: 202–7.
16. Maruyama I. Recombinant thrombomodulin and activated protein C in the treatment of disseminated intravascular coagulation. Thromb Haemost. 1999; 104:202–7.
17. Iqbal O, Messmore H, Fareed J, et al. Antithrombotic agents in the treatment of severe sepsis. Expert Opin Emerg Drugs. 2002;7(1):111–39.

18. Camerota A, Creasey A, Patla V, Larkin V, Fink M. Delayed treatment with recombinant human tissue factor pathway inhibitor improves survival in rabbits with Gram-negative peritonitis. J Infect Dis. 1988; 177:668–76.
19. Park T, Creasey A, Wright S. Tissue factor pathway inhibitor blocks cellular effects of endotoxin by binding to endotoxin and interfering with transfer to CD14. Blood. 1997;89:4268–74.
20. Schaub RG, Simmons CA, Koets MH, Romano PJ, Stewart GJ. Early events in the formation of a venous thrombus following local trauma and stasis. Lab Invest. 1984;51:218–24.
21. Yang J, Furie BC, Furie B. The biology of P-selectin glycoprotein ligand-1: its role as a selectin counter receptor in leukocyte endothelial and leukocyte platelet interaction. Thromb Haemost. 1999;81:1–7.
22. Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature. 1992;359:848–51.
23. Wakefield TW, Strieter RM, Schaub R, et al. Venous thrombosis prophylaxis by inflammatory inhibition without anticoagulant therapy. J Vasc Surg. 2000;31: 309–24.
24. Coughlin SR. Thrombin receptor function and cardiovascular disease. Trends Cardiovasc Med. 1994;4: 77–83.
25. Lorante DE, Patel KD, McIntire TM, McEver RP, Prescott SM, Zimmerman GA. Co-expression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol. 1991; 115:223–34.
26. Reinhart K, Karzai W, Meisner M. Procalcitonin as a marker of the systemic inflammatory response in infection. Intensive Care Med. 2000;26(9):1193–200.
27. Erickson Y, Samia N, Bedell B, Friedman K, Atkinson B, Raife T. Elevated procalcitonin and C-reactive protein as potential biomarkers of sepsis in a subpopulation of thrombotic microangiopathy patients. J Clin Apher. 2009;24(4):150–4.
28. Hoppensteadt D, Tsuruta K, Hirman J, Kaul I, Osawa Y, Fareed J. Dysregulation and inflammatory hemostatic markers in sepsis and suspected disseminated intravascular coagulation. Clin Appl Thromb Hemost. 2015;21(2):120–7.
29. Stegenga MB, van der Crabben SN, Levi M, de Voss AF, Tanck MW, Sauerwein HP, van der Poll T. Hyperglycemia stimulates coagulation and hyperinsulinemia impairs fibrinolysis in healthy humans. Diabetes. 2006;55:1807–12.
30. Hernestal-Boman J, Norberg M, Jansson J-K, Eliasson M, Erickson JW, Lindahl B, Johansson L. Signs of dysregulated fibrinolysis precede the development of type 2 diabetes mellitus in a population-based study. Cardiovasc Diabetol. 2012;11:152. http://www.cardiab.com/content/11/1/52.
31. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest. 2003;112:1785–8.
32. Ker R, Stirling D, Ludlam CA. Interleukin-6 and haemostasis. Br J Haematol. 2001;115:3012.
33. Devraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation. 2003;107:398–404.
34. Cigonoli M, Tonoli M, Borgato L, Frigotto L, et al. Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role of TNF-α? Atherosclerosis. 1999;143:81–90.
35. Alessi MC, Bastelica D, Morange P, et al. Plasminogen activator inhibitor-1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes. 2000;49:1374–80.
36. Broderick JP, Palesch YY, Demchuk AM, et al. Endovascular therapy after intravenous t-PA versus t-PA alone for stroke. N Engl J Med. 2013;368:893–903.
37. Kidwell CS, Jahan R, Gombein J, et al. A trial of imaging selection and endovascular treatment for ischemic stroke. N Engl J Med. 2013;368:914–23.
38. Ciccone A, Valvassori L, Nichellati M, et al. Endovascular treatment for acute ischemic stroke. N Engl J Med. 2013;368:904–13.
39. Berkhemer OA, Fransen PS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med. 2015;372:11–20.
40. Goyal M, Demchuk AM, Menon BK, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke—for the ESCAPE Trial Investigators. N Engl J Med. 2015;372:1019–30.
41. Campbell BCV, Mitchell PJ, Kleinig TJ, et al. for the EXTEND-IA Investigators. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 2015;372:1009–1018.
42. Saver J. SWIFT PRIME Results. Paper presented at International Stroke Conference Annual Meeting; 2015 Feb 11; Nashville, TN, USA.
43. Buntin MB, Garten AD, Paddock S, et al. How much is postacute care use affected by its availability? Health Serv Res. 2005;40:413–34.
44. Ovblagele B, Goldstein LB, Higashida RT, et al.; on behalf of the American Heart Association Advocacy Coordinating Committee and Stroke Council. Forecasting the future of stroke in United States: a policy statement from the American Heart Association and American Stroke Association. Stroke 2013;44:2361–75.
45. Taylor TN, Davis PH, Tomer JC, et al. Lifetime cost of stroke in the United States. Stroke. 1996;27: 1459–66.
46. Fiorella DJ, Fargen KM, Mocco J, et al. Thrombectomy for acute ischemic stroke: an evidence-based treatment. J NeuroInterv Surg. 2015. doi:10.1136/neurintsurg-2015-011707.