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COMMENTARY

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Assessing plasminogen activation potential with global fibrinolytic assays

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1 | INTRODUCTION

The fibrinolytic system is composed of 2 essential steps, namely, plasminogen activation and fibrin degradation.[1,2] The first step is frequently modified in pathological conditions and triggers serious disorders, including uncontrollable bleeding and multiple organ failure due to microthrombi formation. However, despite clinical demand, there is no suitable global assay to instantly evaluate either whole fibrinolytic activity or the plasminogen activation step. The plasminogen activation step is largely modified by the existence of fibrin as well as the processes of clot formation and lysis. In other words, efficient plasminogen activation takes place only in the presence of fibrin.[2] Thus, the fibrin clot lysis assay is most suitable and has been used to accurately assess this step. The process of fibrin clot lysis, however, is strongly regulated by α2-antiplasmin (α2AP),[1,3] the main regulator of the second step of fibrinolysis, which makes evaluation of the first step by the fibrin clot lysis assay difficult.

2 | FIBRINOLYSIS IS A 2-STEP CASCADE, EACH OF WHICH IS REGULATED BY PHYSIOLOGICAL REGULATORS

The first step is initiated by plasminogen activators of either the tissue type (t-PA) or the urokinase type and is regulated by plasminogen activator inhibitor type 1 (PAI-1).[4] The second step is initiated by plasmin generated in the first step and is mainly regulated by α2AP. Thrombin activatable fibrinolysis inhibitor (TAFI) is another important regulator of fibrinolysis, and it regulates both steps after being activated by thrombomodulin-bound thrombin.[5] As t-PA–catalyzed plasminogen activation efficiently takes place only when fibrin is formed, the ability of plasma to generate plasmin after fibrin formation could be considered as the "plasminogen activation potential."

3 | GLOBAL ASSAY OF FIBRINOLYSIS

Although both the activities and the antigen levels of each factor involved in the 2 steps of the fibrinolytic system are easily assayed, no practical global assay is clinically available to evaluate fibrinolytic activity. This is in contrast to the coagulation system, wherein the prothrombin time and the activated partial thromboplastin time can be assayed.

As total lysis of spontaneous plasma clot takes many days, several attempts have been made at the laboratory level to shorten the clot lysis time for clinical testing. Supplementation with t-PA to overcome PAI-1 activity is one of these strategies and seems to be beneficial in evaluating the role of TAFI and α2AP. This method, however, is associated with difficulty in evaluating the potential to trigger the plasminogen activation step, which is regulated by the balance between t-PA and PAI-1[4] and is easily disrupted by t-PA supplementation.

Correlation between ECLT and fibrinolysis parameters
Cited from Ref #11

		ECLT	
t-PA			
	mass	0.604	*
	activity	−0.905	* *
	free t-PA#	−0.637	*
PAI-1			
	total	0.868	* *
	complex	NS	
	free	0.863	* *

*: $P < .05$, **: $P < .01$,

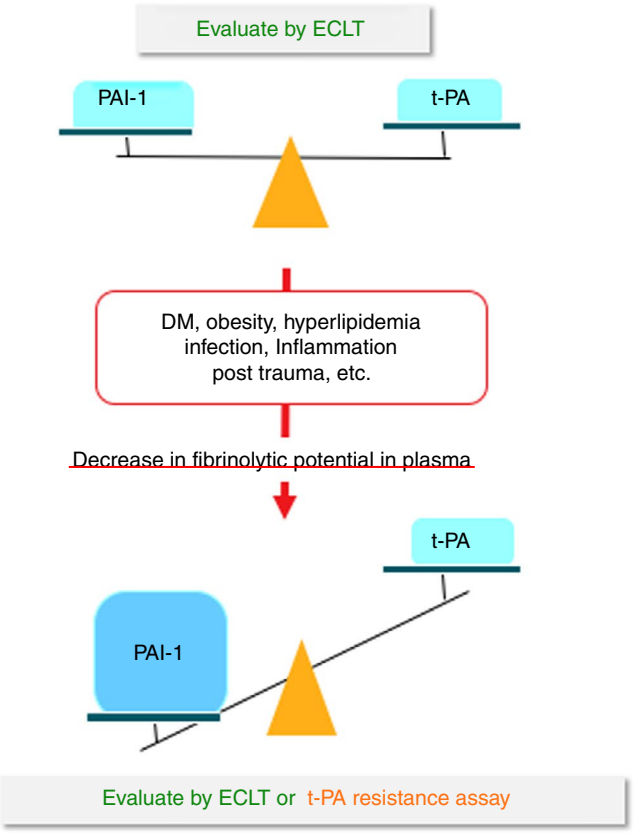

FIGURE 1 Regulation of fibrinolytic potential. ECLT correlates well with parameters involved in the plasminogen activation step of fibrinolysis. There is a good negative correlation with t-PA activity in plasma, and good positive correlations with either total or free PAI-1 in plasma. Free t-PA# concentration, calculated based on the assumption that t-PA forms a high-molecular-weight inactive complex with PAI-1 at a certain association constant as in a purified system, also showed good negative correlation with ECLT.[11] Thus, fibrinolytic potential could be appropriately assessed by ECLT. Under certain pathological condition where plasma PAI-1 increases, a t-PA resistance assay may prove useful. DM, diabetes mellitus; ECLT, euglobulin clot lysis time; PAI-1, euglobulin clot lysis time; t-PA, tissue-type plasminogen activator

Elimination of α2AP also successfully shortens the clot lysis time by abolishing the regulation at the second step and makes it possible to assess the plasminogen activation potential. The euglobulin clot lysis time (ECLT) assay is one of the approaches in which α2AP and α2-macroglobulin are eliminated from plasma by isoelectric precipitation at a pH of around 5.2 to 5.9. ECLT has a strong positive correlation with t-PA activity and a negative correlation with either free or total PAI-1 in plasma (Figure 1).[4] The free t-PA concentration, calculated on the basis of the assumption that PAI-1 inactivates t-PA by forming a high-molecular-weight complex even in plasma, showed a strong positive correlation with t-PA activity and a negative correlation with ECLT. This suggests that the initial step of fibrinolysis is simply regulated by the balance between t-PA and PAI-1, and ECLT is able to effectively assess this balance. However, when the PAI-1 concentration is high, the ECLT is too long (>6 hours) to be used as a clinical test.

4 | MODIFIED ECLT AND PLASMA CLOT LYSIS TO EVALUATE "t-PA RESISTANCE"

In their article in *Research and Practice in Thrombosis and Haemostasis*, Ilich et al[6] proposed a modified ECLT as a useful global fibrinolysis assay. Ovalbumin and human fibrinogen were supplemented to the euglobulin fraction of plasma to increase the turbidity of the clot, and the ECLT was measured. They also used the plasma clot lysis assay initiated by t-PA at a low concentration, which well reflected elevated PAI-1 levels in the plasma and was considered a "t-PA resistance test." The concentration of t-PA seemed high enough to make the clot lysis time short and low enough so as not to entirely disrupt the balance between t-PA and PAI-1 in plasma. As fibrinolytic activity or plasminogen activation potential could be severely attenuated by the increased PAI-1 level under many pathological conditions, this global assay may prove useful.

PAI-1 is known as an acute-phase reacting protein and increases in plasma under a variety of pathological conditions, including infection, inflammation, obesity, and a stage of fibrinolytic shutdown after trauma.[4,7,8] In trauma, fibrinolytic activity is highly enhanced at the beginning, and tranexamic acid (TXA) effectively attenuates bleeding.[8] At a later phase, however, PAI-1 increases in plasma and the potential to trigger the initial step of fibrinolysis declines quickly, in which case the use of TXA is not preferable.[8] Correct and quick assessment of modulated fibrinolysis status in trauma is crucial for appropriate and timely treatment. The tPA-supplemented ECLT or plasma clot lysis assay makes it possible to promptly assess such

quickly fluctuating PAI-1 levels. Both the ECLT and the t-PA resistance test are suitable to evaluate plasminogen activation potential, wherein the former is sensitive to enhanced potential including PAI-1 deficiency[9,10] and the latter is sensitive to fibrinolytic shut down due to increased PAI-1.[8]

5 | PROSPECTS

ECLT cannot assess the main regulatory function of the second step (fibrin degradation) of fibrinolysis in which α2AP and TAFI play essential roles. Though trace amounts of α2AP remain in the euglobulin fraction and show negative correlation with ECLT, ECLT does not show meaningful correlation with plasma α2AP level.[6] TAFI's effect is also not detected by ECLT when soluble thrombomodulin is not supplemented.[6] Decreased α2AP level or impaired function are also important clinical issues that can be induced by either congenital abnormality, by the use of fibrinolytic therapy or by disseminated intravascular coagulation. For a quick and correct assessment, other global assays such as t-PA– or t-PA and thrombomodulin–supplemented plasma clot lysis assays, might be helpful. Proper tailored usage of global fibrinolytic assays applying in-depth understanding of each assay is necessary to adequately understand disorders of the fibrinolytic system in patients.

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REFERENCES

1. Collen D. The plasminogen (fibrinolytic) system. Thromb Haemost. 1999;82(2):259–70.
2. Urano T, Castellino FJ, Suzuki Y. Regulation of plasminogen activation on cell surfaces and fibrin. J Thromb Haemost. 2018;16(8):1487–97.
3. Aoki N. Discovery of alpha2-plasmin inhibitor and its congenital deficiency. J Thromb Haemost. 2005;3(4):623–31.
4. Urano T, Suzuki Y, Iwaki T, Sano H, Honkura N, Castellino FJ. Recognition of plasminogen activator inhibitor type 1 as the primary regulator of fibrinolysis. Curr Drug Targets. 2019;20(16):1695–701.
5. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haemost. 2009;7(1):4–13.
6. Ilich A, Noubouossie DF, Henderson M, Ellsworth P, Betbadal KF, Campello E, et al. Development and application of global assays of hyper- and hypofibrinolysis. Research and Practice in Thromb Haemost (RPTH). 2020;4(1):46–53.
7. Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost. 2003;1(7):1575–9.
8. Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. Nat Rev Dis Primers. 2016;2:16037.
9. Iwaki T, Nagahashi K, Takano K, Suzuki-Inoue K, Kanayama N, Umemura K, et al. Mutation in a highly conserved glycine residue in strand 5B of plasminogen activator inhibitor 1 causes polymerisation. Thromb Haemost. 2017;117(5):860–9.
10. Iwaki T, Tanaka A, Miyawaki Y, Suzuki A, Kobayashi T, Takamatsu J, et al. Life-threatening hemorrhage and prolonged wound healing are remarkable phenotypes manifested by complete plasminogen activator inhibitor-1 deficiency in humans. J Thromb Haemost. 2011;9(6):1200–6.
11. Urano T, Suzuki Y, Arakida M, Kanamori M, Takada A. The expression of exercise-induced tPA activity in blood is regulated by the basal level of PAI-1. Thromb Haemost. 2001;85(4):751–2.