

# Thrombin generation assays

## Citation for published version (APA):

Hemker, H. C., Al Dieri, R., & Beguin, S. (2004). Thrombin generation assays: Accruing clinical relevance. *Current opinion in hematology*, 11(3), 170-175. <https://doi.org/10.1097/01.moh.0000130314.33410.d7>

## Document status and date:

Published: 01/01/2004

## DOI:

[10.1097/01.moh.0000130314.33410.d7](https://doi.org/10.1097/01.moh.0000130314.33410.d7)

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

# Thrombin generation assays: accruing clinical relevance

H. Coenraad Hemker, Raed Al Dieri and Suzette Béguin

## Purpose of review

After decades of near oblivion, thrombin generation is being revived as an overall function test of the plasmatic coagulation system in platelet-poor plasma (PPP). In platelet-rich plasma (PRP) it assesses platelet procoagulant functions as well.

## Recent findings

The recently developed use of special fluorogenic thrombin substrates allows monitoring of thrombin concentration in clotting PPP and PRP on line in up to 24 parallel samples. Studies in model systems stress the importance of cell-bound thrombin generation such as measured in PRP.

## Summary

The method can be profitably applied to various hitherto unyielding problems such as the control of (low-molecular-weight) heparin therapy, the detection of lupus anticoagulant, and various forms of thrombomodulin and activated protein C resistance (including the use of oral contraceptives) as well as monitoring the treatment of hemophiliacs by factor VIII bypassing therapy. In PRP it reflects the abnormalities encountered in von Willebrand disease and Glanzmann and Bernard-Soulier thrombopathy as well as the action of antiplatelet drugs.

## Keywords

thrombin generation, platelet-poor and platelet-rich plasma, monitoring antithrombotics, lupus anticoagulant, hemophilia and von Willebrand disease

Curr Opin Hematol 11:170–175. © 2004 Lippincott Williams & Wilkins.

Synapse BV, Cardiovascular Research Institute, Maastricht, The Netherlands

Correspondence to H. C. Hemker, Synapse BV, Cardiovascular Research Institute, PO Box 616, 6200MD Maastricht, The Netherlands  
E-mail: HC.Hemker@Thrombin.com

Current Opinion in Hematology 2004, 11:170–175

## Abbreviations

APC	activated protein C
aPTT	activated partial prothrombin time
CAT	calibrated automated thrombogram
ETP	endogenous thrombin potential
$\alpha_2M$	$\alpha_2$ -macroglobulin
PAR	protease activatable receptor
PPP	platelet-poor plasma
PRP	platelet-rich plasma
TF	tissue factor
TG	thrombin generation
TM	thrombomodulin
vWF	von Willebrand factor

© 2004 Lippincott Williams & Wilkins  
1065–6251

## Introduction

Because of its numerous positive and negative feedback controls, the hemostatic-thrombotic system is so complicated that it is practically impossible to judge the overall hemostatic function of the blood from the concentration or structure of its components. Information on details of the system therefore is not an alternative for an overall function test. Clotting times (prothrombin time, activated partial prothrombin time [aPTT], activated whole blood clotting time) do not indicate hypercoagulability and are insensitive to mild bleeding disorders. For over a century (*eg*, Hayem [1]), the generation of thrombin in clotting blood or plasma has been used to assess the coagulation system, but only recently have technical developments brought it into reach of the nonspecialized laboratory.

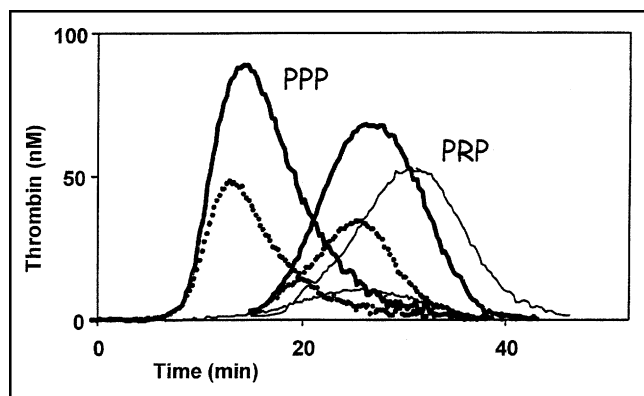
*Ex vivo* thrombin generation (TG) should be distinguished from *in vivo* TG, revealed by products of an ongoing clotting process in the body (prothrombin fragment 1-2, D-dimer etc.). Increased *in vivo* TG indicates an ongoing pathologic process. Increased or decreased *ex vivo* TG means that the function of the coagulation process is abnormal (*eg*, hyperprothrombinemia [2], hemophilia [3••], use of anticoagulants [4]). It does not necessarily mean ongoing pathology but indicates an increased risk of thrombosis or bleeding [5]. *In vivo* TG is a smoke detector signaling ongoing evil; *ex vivo* TG is like the smell of gasoline indicating an increased risk.

## The first law of hemostasis and thrombosis

“The more thrombin the less bleeding but the more thrombosis, the less thrombin the less thrombosis but the more bleeding” may be called the “first law of hemostasis and thrombosis.” The converse is not true. The cause of thrombosis or bleeding can be in the vessel wall, with the hemostatic function of the blood being perfectly normal. The circumstantial evidence for the first law is overwhelming; we know of no refutation. Bleeding may be caused by a lack of any known clotting factor or by an excess of antithrombin activity (antithrombin Baltimore, heparin); thrombosis by an excess of clotting factor (*eg*, prothrombin); or a shortage of coagulation delimiters (antithrombin, proteins C and S).

The time course of TG (the “thrombogram”) is shown in Figure 1. After a lag time, a burst of thrombin is observed. Clotting occurs at the end of the lag time, when more than 95% of all thrombin is still to be formed. We

**Figure 1. Thrombin generation curves as obtained with the calibrated automated thrombogram (CAT) technique**



Platelet-poor plasma (PPP) was triggered with low (5 pM) tissue factor (TF); platelet-rich plasma (PRP) with traces (0.5 pM) of TF to mask variations in endogenous TF. Bold lines: no addition; dotted lines: 10 nM thrombomodulin (TM) added; thin lines: 6 nM APC added (lower curve: PPP; upper curve PRP).

may well ask what the purpose is of all the thrombin that is formed after clotting [6]. We see two main functions: *In vivo* thrombin diffuses out, around the primary focus of its formation (*eg*, wound, ruptured plaque). Above a certain threshold concentration, it will autocatalytically promote more thrombin formation and thus thrombus growth. Under that threshold it will be washed away or neutralized. The amount of thrombin formed in a focus will thus determine the extent of a thrombus/hemostatic plug. Thrombin in a clot also prevents subsequent lysis via the activation of thrombin-activatable fibrinolysis inhibitor [7,8]. This explains why hemophilic bleeding often develops after a bleeding-free interval, as if a formed hemostatic plug is precociously dissolved (*eg*, Verstraete [9]). Thrombin also acts on a number of different cells in the neighborhood of a focus and has a function in tissue repair and proliferation of surrounding cells [10,11].

### Models of *ex vivo* thrombin generation

The course of thrombin concentration in a hemostatic plug or thrombus is technically impossible to measure. Measuring thrombin (-products) in samples from the blood in a wound comes close [12,13], as does subsampling from clotting blood [14,15]. Both require heavy experimentation. Two essential different types of model are of more practical use: reconstituted systems and plasma.

Reconstituted systems (for reviews see Monroe *et al.* [16] and Mann [17•]) use purified clotting factors to represent the physiologic situation. Reaction conditions are under tight control and can be varied at will. To investigate TG at cell surfaces, notably platelets with or without cells bearing tissue factor (TF) or thrombomodulin (TM) (monocytes, endothelial cells) they proved very useful [18]. However, purified factors will not necessarily retain

their native activity (*eg*, Hemker [19]). Reconstituted systems are as realistic as our insight into the clotting mechanism allows; extrapolation to physiology should therefore be regarded with due suspicion. Minor players (*eg*,  $\beta$ 1glycoprotein Ib,  $\alpha_2$ macroglobulin [ $\alpha_2$ M]) and *a fortiori* unrecognized proteins/functions escape notice; fibrin(-ogen) and von Willebrand factor (vWF) are often absent but do play a role in rendering platelets procoagulant [20]; see below).

Thrombin generation in plasma (in platelet-poor or platelet-rich plasma, PPP or PRP [21,22]) represents the function of a relevant slice of the *in vivo* system with all the plasma proteins present, unmodified, near their physiologic concentrations and independent of *a priori* hypotheses. It represents a function test of the “isolated organ” PPP or PRP. The vessel wall is lacking, however. To simulate its presence, the two most important known elements, TF and TM, may be added to the plasma (Fig.1).

### Cell-bound thrombin generation: the role of platelets

The arm-to-tongue circulation time of the blood (~30 seconds) is short compared with a whole blood clotting time, so thrombin formed in flowing blood *in vivo* is rapidly diluted and inactivated before clotting can occur. Thrombin will only build up in unstirred boundary layers at cell surfaces and in the unstirred plasma caught in a clot or an aggregate. Transport by diffusion will therefore tend to govern reaction rates. According to our interpretation [23] diffusion limitation, for instance, explains the kinetics observed in a cell-bound model of TG by Allen *et al.* [24•].

Cell-bound TG is dependent on TF-bearing cells (monocytes, perivascular cells) and platelets [18,16]. The role of the platelet in physiologic thrombin generation is twofold. By adhesion and aggregation it forms a maze in which plasma can clot without the thrombin being washed away; conversely, activated platelets provide the surface on which TG can take place. Upon activation of the platelet [25], procoagulant phospholipids appear at its outside. Thrombin (PAR 1) and collagen (GPVI) bring about this process, especially in combination. GPIIb/IIIa plays a role as well and GPIIb/IIIa antagonists inhibit [26]. vWF adsorbs onto polymerizing fibrin and this probably brings about a molecular change (like ristocetin), which makes it interact with GPIb-V. This enhances the formation of a procoagulant surface [27,28•].

### Techniques of thrombin generation measurement

The thrombogram can be obtained through subsampling [29] or through monitoring the conversion of a suitable substrate directly added to the clotting plasma [30]. The former method is straightforward and time consuming;

the latter allows automatic continuous measurement of many samples in parallel. Via subsampling, the thrombin (~30% of total) that adsorbs onto the formed clot [31•,32,33] escapes notice. Such thrombin can activate factors V, VIII, and XI or platelets and thus probably is essential in thrombin growth [34].

Added thrombin substrate occupies part of the thrombin formed. Enough free thrombin should remain to allow for natural feedback reactions and for adequate removal of thrombin by antithrombins; therefore binding should be relatively loose (low  $K_m$ ). Suitable substrates should also be converted slowly (low  $k_{cat}$ ) so as not to be consumed during the experiment [35].

The fluorescent signal has the drawback of not being linear with product concentration. To compensate for this and for the effects of substrate consumption, the calibrated automated thrombogram (CAT) method has been developed that continuously compares the signal from the experimental sample to that of a fixed known thrombin activity [36•]. This method allows visualizing the thrombin concentration in clotting PPP or PRP in 24 parallel experiments.

### The thrombogram

Typical thrombograms as obtained with the CAT are shown in Figure 1. The three most important parameters are the lag time, the peak value, and the area under the curve or endogenous thrombin potential (ETP), which quantifies the enzymatic “work” that thrombin can do during its lifetime (“person-hours” of thrombin) [37]. Plasma clots at the end of the lag phase so the clotting time can be read from the thrombogram. During the lag phase the reaction mechanisms are essentially different from those during the thrombin burst [15]); this is one of the reasons that the clotting time does not represent TG. The normal values and coefficients of variation as obtained with the CAT-method are given in Table 1.

### Partial techniques

Both the lag time and the ETP can be obtained by alternative techniques without monitoring the complete thrombogram. The clotting time represents the lag time. The ETP can be assessed by measuring the product from any natural or added substrate that is not exhausted during the clotting process. One natural substrate is  $\alpha_2M$ ,

which, in defibrinated plasma, will bind ~30% of the thrombin formed (~5% with fibrinogen). The final concentration of the (amidolytically active)  $\alpha_2M$ -thrombin complex is proportional to the ETP [30]. Rosing *et al.* [38] used this approach to demonstrate acquired activated protein C (APC) resistance through the use of oral contraceptives. The ETP can also be assessed by measuring the end level of conversion of a slow-reacting artificial substrate, provided that it does not react with  $\alpha_2M$ -thrombin.

### Surrogate techniques of thrombin generation

Several techniques have been published that depend on fibrinogen polymerization. Apart from the clotting time, they give little information on thrombin generation because fibrinogen is exhausted before 5% of all thrombin is formed. In so far as the properties of the clot are determined by the velocity or the amount of thrombin formed, some information can be retained, however. Indeed tensile strength [39•], clot retraction [40•], turbidity [41], and fibrinolysis [42•] are derived variables that to some extent are determined by the amount or velocity of thrombin formation. They recently have been (re-)used for the assessment of overall hemostatic function. Some of these methods have the advantage of using full blood. A disadvantage is that the indicating substance (fibrinogen) may increase when TG decreases, *eg*, in active thrombosis under anticoagulant treatment. It may be extremely confusing that curves are obtained that resemble real TG curves but are not.

### Applications of thrombin generation measurement

Thrombin generation measurement has been shown to be a useful tool in several different domains.

#### Platelet-plasma interactions

Thrombin generation has been instrumental in unveiling the role of platelet receptors in the production of a procoagulant surface by platelets. In short, a role of GPIIb/IIIa, of GPIb/V, and vWF [28•] and of GPI (collagen), as well as of the PAR receptors, has been demonstrated (see Hemker and Lindhout [43] for a review).

#### Detection and quantification of thrombotic tendency

Deficiencies of proteins S or C are readily recognized when TM is added to the plasma, factor  $V_{Leiden}$  as well [44,45]. APC resistance, either acquired (oral contracep-

**Table 1. Normal values and variability**

		n	Average (pop.)	StDev (pop.)	CV (pop.)	CV (ind.)	CV (exp.)
PPP	ETP (nM.min)	36	1879	284	15	4.5	2.5
	Peak (nM)		458	60	13	5.5	4.5
	Lag (min)		3.1	1.4	47	8.1	5
PRP	ETP (nM.min)	24	1678	371	22	7.8	3
	Peak (nM)		117	42	35	8.3	3.5
	Lag (min)		8.1	1.9	23	9.1	7

CV, ; ETP, endogenous thrombin potential; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

tive treatment) or congenital, can also be detected with a TG-based endpoint technique [45].

Thrombin generation also solves the long-standing enigma of the prothrombotic anticoagulant in lupus erythematoses. It has been shown that this antibody prolongs the lag phase (*ie*, is anticoagulant in clotting tests) but induces TM and APC resistance [46••].

#### Detection and quantification of bleeding tendency

In deficiencies of factors II, V, VII, VIII, IX, X, and XI, it has been demonstrated that TG is diminished in PPP and that clinical bleeding is observed at ETP values less than 30% [3••,47].

The thrombasthenias of Glanzmann [48] and Bernard-Soulier [28•], as well as severe thrombopenia, show a moderately diminished TG in PRP. Von Willebrand disease, unless accompanied by severe factor VIII deficiency, shows normal TG in PPP but decreased TG in PRP [20].

#### Control of procoagulant therapy

As expected, restoration of the factor VIII level of hemophilic plasma restores TG, as does DDAVP treatment in mild hemophilia and vWD [49]. More interesting: inhibitor bypassing therapy with either factor VIIa, or this factor in combination with other factors (Feiba), can be monitored with TG [50•,51••].

#### Control of antithrombotic therapy

Under oral anticoagulation the incidence of bleeding increases as soon as the international normalized ratio drops below 3 [52], which is equivalent to ETP = 20% [36•]. Heparins, including the low-molecular-weight types, inhibit TG primarily by increasing thrombin breakdown [53•]. Twofold prolongation of the aPTT corresponds to ~80% inhibition of the ETP [54•]. TG is the only available method to quantify the combined effect of heparin and vitamin K antagonists or other anticoagulants.

Platelet “aggregation” inhibitors in general such as abciximab [48], clopidogrel [55], and aspirin [56] also inhibit TG to a certain degree. This is not to say that inhibition of aggregation as such would not have an—or even be the main—antithrombotic action. It is an interesting possibility that through decreasing the size of the platelet aggregate, the volume in which thrombin can form undisturbed by flow is also diminished.

#### Pharmacologic research

As yet we have not encountered any antithrombotic, either anticoagulant or antiaggregant, that did not inhibit TG [5]. We can assume that any drug that inhibits TG to ~50% of normal will show an antithrombotic effect at an acceptable bleeding risk. By introducing TG as an inter-

mediate step between the biochemical experiments and thrombosis models in experimental animals, we can significantly diminish the latter, especially in dose-finding experiments. The test can also be used to assess the effects of a candidate molecule in volunteers.

#### Conclusion

To understand thrombin generation, we have to measure thrombin generation, the whole thrombin generation, and nothing but thrombin generation—under conditions as close as possible to those *in vivo*. This offers a wealth of information that is not otherwise available. Measurement in an undisturbed fibrin clot in which activated platelets are fixed, as is possible with fluorogenic substrates, probably resembles the situation in a hemostatic plug or thrombus more closely than stirred systems do. The calibrated automated technique makes it possible to obtain a graph of thrombin concentration against time in real time in up to 24 parallel experiments. Whole blood measurement is as yet technically impossible.

#### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
  - Of outstanding interest
- 1 Hayem: Du Sang et de Ses Altérations Anatomiques. G. Masson Editeur; 1889:266–274.
  - 2 Kyrle PA, Mannhalter C, Béguin S, et al.: Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene. *Arterioscler Thromb Vasc Biol* 1998, 18:1287–1291.
  - 3 Siegemund T, Petros S, Siegemund A, et al.: Thrombin generation in severe haemophilia A and B: the endogenous thrombin potential in platelet-rich plasma. *Thromb Haemost* 2003, 90:781–786.
  - Thrombin generation was measured in PRP from hemophilia A and hemophilia B patients. Platelets increase ETP in the hemophilias. There was an almost linear relationship between increasing platelet count and thrombin generation, reaching a limit at  $10^{11}$  platelets/L. The influence of platelets diminishes with increasing concentration of either FVIII or FIX.
  - 4 Kakkar VV, Hoppenstead DA, Fareed J, et al.: Randomized trial of different regimens of heparins and *in vivo* thrombin generation in acute deep vein thrombosis. *Blood* 2002, 99:1965–1970.
  - 5 Hemker HC, Béguin S: Phenotyping the clotting system. *Thromb Haemost* 2000, 84:747–751.
  - 6 Mann KG, Brummel K, Butenas S: What is all that thrombin for? *J Thromb Haemost* 2003, 1:1504–1514.
  - 7 Broze GJ Jr: Thrombin-dependent inhibition of fibrinolysis. *Curr Opin Hematol* 1996, 3:390–394.
  - 8 Mattsson C, Bjorkman JA, Abrahamsson T, et al.: Local proCPU (TAFI) activation during thrombolytic treatment in a dog model of coronary artery thrombosis can be inhibited with a direct, small molecule thrombin inhibitor (melagatran). *Thromb Haemost* 2002, 87:557–562.
  - 9 Verstraete M: Clinical application of inhibitors of fibrinolysis. *Drugs* 1985, 29:236–261.
  - 10 Ruf W, Dorfleutner A, Riewald M: Specificity of coagulation factor signaling. *J Thromb Haemost* 2003, 1:1495–1503.
  - 11 Major CD, Santulli RJ, Derian CK, Andrade-Gordon P: Extracellular mediators in atherosclerosis and thrombosis: lessons from thrombin receptor knockout mice. *Arterioscler Thromb Vasc Biol* 2003, 23:931–939.
  - 12 Jensen AH, Béguin S, Jossen F: Factor V and VIII activation “*in vivo*” during bleeding: evidence of thrombin formation at the early stage of hemostasis. *Pathol Biol (Paris)* 1976, 24(suppl):6–10.
  - 13 Undas A, Brummel K, Musial J, et al.: Blood coagulation at the site of microvascular injury: effects of low-dose aspirin. *Blood* 2001, 98:2423–2431.
  - 14 Kessels H, Béguin S, Andree H, Hemker HC: Measurement of thrombin generation in whole blood: the effect of heparin and aspirin. *Thromb Haemost* 1994, 72:78–83.

- 15 Brummel KE, Paradis SG, Butenas S, Mann KG: Thrombin functions during tissue factor-induced blood coagulation. *Blood* 2002, 100:148–152.
- 16 Monroe DM, Hoffman M, Roberts HR: Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* 2002, 22:1381–1389.
- 17 Mann KG: Thrombin formation. *Chest* 2003, 124:4S–10S.
- This review provides a summary of the evolution of knowledge with respect to present-day concepts of TG via the TF pathway and its regulation, seen from a personalized standpoint.
- 18 Hoffman M, Monroe DM 3rd: A cell-based model of hemostasis. *Thromb Haemost* 2001, 85:958–965.
- 19 Hemker HC: Thrombin generation in a reconstituted system: a comment. *Thromb Haemost* 2002, 87:551–554.
- 20 Béguin S, Kumar R, Keularts I, et al.: Fibrin-dependent platelet procoagulant activity requires GPIb receptors and von Willebrand factor. *Blood* 1999, 93:564–570.
- 21 Béguin S, Lindhout T, Hemker HC: The effect of trace amounts of tissue factor on thrombin generation in platelet rich plasma: its inhibition by heparin. *Thromb Haemost* 1989, 61:25–29.
- 22 Hemker HC, Béguin S: Thrombin generation in plasma: its assessment via the endogenous thrombin potential. *Thromb Haemost* 1995, 74:134–138. Published erratum appears in *Thromb Haemost* 1995, 74:1388.
- 23 Hemker HC, Béguin S: The love of the artist for his model: of thrombin generation. *J Thromb Haemost* 2004, 2:400–401.
- 24 Allen GA, Hoffman M, Roberts HR, Monroe DM 3rd: 2004.
- The dose-effect relations are determined between the concentration of a number of clotting factors and TG in a system consisting of platelets and TF-bearing monocytes and purified clotting factors. TG increases linearly with prothrombin concentration but reaches an upper limit at minimal concentrations of the other clotting factors. The authors explain this in terms of enzyme kinetics of the clotting process. We consider it to demonstrate diffusion limitation of cell-bound TG.
- 25 Heemskerk JW, Bevers EM, Lindhout T: Platelet activation and blood coagulation. *Thromb Haemost* 2002, 88:186–193.
- 26 Reverter JC: Fondaparinux sodium. *Drugs Today (Barc)* 2002, 38:185–194.
- 27 Béguin S, Kumar R, Keularts I, et al.: Fibrin-dependent platelet procoagulant activity requires GPIb receptors and von Willebrand factor. *Blood* 1999, 93:564–570.
- 28 Béguin S, Keularts I, Al Dieri R, et al.: Fibrin polymerization is crucial for thrombin generation in platelet-rich plasma in a VWF-GPIb-dependent process, defective in Bernard-Soulier syndrome. *J Thromb Haemost* 2004, 2:170–176.
- Induction of fibrin polymerization during the lag phase of TG by a snake venom enzyme induces an immediate burst of TG that is inhibited by a monoclonal antibody against GPIb. Inhibition of polymerization decreases TG. So polymerizing fibrin interacts with VWF so as to activate GPIb and produce platelet procoagulant activity.
- 29 Hemker HC, Willems GM, Béguin S: A computer assisted method to obtain the prothrombin activation velocity in whole plasma independent of thrombin decay processes. *Thromb Haemost* 1986, 56:9–17.
- 30 Hemker HC, Wielders S, Kessels H, Béguin S: Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thromb Haemost* 1993, 70:617–624.
- 31 Mosesson MW: Antithrombin I: inhibition of thrombin generation in plasma by fibrin formation. *Thromb Haemost* 2003, 89:9–12.
- Thrombin substrate binding to fibrinogen is mediated through exosite 1. Nonsubstrate binding of thrombin to fibrin occurs at low affinity in the fibrin E domain and at high affinity to a variant gamma chain, found in ~15% of the fibrinogen molecules (fibrinogen 2). Fibrin formation (antithrombin I) thus inhibits the appearance of thrombin in the fluid phase and "high-affinity" thrombin-binding plays a dominant role in this process.
- 32 Kumar R, Béguin S, Hemker HC: The influence of fibrinogen and fibrin on thrombin generation: evidence for feedback activation of the clotting system by clot bound thrombin. *Thromb Haemost* 1994, 72:713–721.
- 33 de Bosch NB, Mosesson MW, Ruiz-Saez A, et al.: Inhibition of thrombin generation in plasma by fibrin formation (Antithrombin I). *Thromb Haemost* 2002, 88:253–258.
- 34 Kumar R, Béguin S, Hemker HC: The effect of fibrin clots and clot-bound thrombin on the development of platelet procoagulant activity. *Thromb Haemost* 1995, 74:962–968.
- 35 Rijkers DT, Hemker HC, Tesser GI: Synthesis of peptide p-nitroanilides mimicking fibrinogen- and hirudin-binding to thrombin: design of slow reacting thrombin substrates. *Int J Pept Protein Res* 1996, 48:182–193.
- 36 Hemker HC, Giesen P, Al Dieri R, et al.: Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003, 33:4–15.
- By using a "slow" fluorogenic thrombin substrate and continuous comparison to a simultaneously run calibrator, TG can be monitored automatically, on line, in clotting PPP or PRP. The resulting thrombogram in PPP measures hypocoagulability (hemophilias, oral anticoagulants, heparins, and heparin-likes), direct inhibitors and hypercoagulabilities (AT deficiency, prothrombin hyperexpression, protein C and S deficiency, factor V Leiden, oral contraceptives). In PRP it is diminished in thrombopathies, in von Willebrand disease, by antibodies blocking GPIIb/IIIa or GPIb, or by antiplatelet drugs like aspirin and clopidogrel.
- 37 Hemker HC, Béguin S: Thrombin generation in plasma: its assessment via the endogenous thrombin potential. *Thromb Haemost* 1995, 74:134–138.
- 38 Rosing J, Tans G, Nicolaes GA, et al.: Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol* 1997, 97:233–238.
- 39 Sorensen B, Johansen P, Christiansen K, et al.: Whole blood coagulation thrombelastographic profiles employing minimal tissue factor activation. *J Thromb Haemost* 2003, 1:551–558.
- Whole blood thrombelastography was used according to the traditional technique but the data were mathematically treated to resemble TG curves. Changes of these curves appeared to be dependent on the nature and severity of the hemostatic deficit in hemophiliacs and could be normalized with recombinant factor VIIa.
- 40 Carr ME, Martin EJ, Kuhn JG, Spiess BD: Onset of force development as a marker of thrombin generation in whole blood: the thrombin generation time (TGT). *J Thromb Haemost* 2003, 1:1977–1983.
- Reports an assay that measures platelet contractile force as a surrogate marker of TG.
- 41 Shima M: Understanding the hemostatic effects of recombinant factor VIIa by clot wave form analysis. *Semin Hematol* 2004, 41:125–131.
- 42 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, 14:31–39.
- Reports a technique for testing *ex vivo* blood flow. Aggregation and the explosive TG result in occlusion. Occlusion time was dose-dependently inhibited by monoclonal antibody against GPIb, auroic tricarboxylic acid, monoclonal antibody against GPIIb/IIIa, a GPIIb/IIIa antagonist, argatroban, and anti-vWF, but not by antifibrinogen. The test also measures thrombolysis.
- 43 Hemker HC, Lindhout T: Interaction of platelet activation and coagulation. Fuster et al. 2004; to be published
- 44 Duchemin J, Pittet JL, Tartary M, et al.: A new assay based on thrombin generation inhibition to detect both protein C and protein S deficiencies in plasma. *Thromb Haemost* 1994, 71:331–338.
- 45 Curvers J, Thomassen MC, Rimmer J, et al.: Effects of hereditary and acquired risk factors of venous thrombosis on a thrombin generation-based APC resistance test. *Thromb Haemost* 2002, 88:5–11.
- 46 Regnault V, Béguin S, Wahl D, et al.: Thrombinography shows acquired resistance to activated protein C in patients with lupus anticoagulants. *Thromb Haemost* 2003, 89:208–212.
- Using thrombinography, APC resistance can be demonstrated in patients with lupus anticoagulants. A long time lag is observed before the thrombin burst (lupus anticoagulant effect) together with a marked inability of APC to diminish the thrombin activity. The effects were more outspoken in the presence of phospholipids from patients' platelets than with added phospholipids.
- 47 Al Dieri R, Peyvandi F, Santagostino E, et al.: The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. *Thromb Haemost* 2002, 88:576–582.
- The relation between clotting factor concentration, the ETP, and the severity of bleeding was investigated in patients with congenital deficiency of factors II, V, VII, XI, and XII. In all the patients with severe bleeding, the ETP was less than 20% of normal. Bleeding tendency was absent or mild in patients with an ETP of 30% or higher.
- 48 Reverter JC, Béguin S, Kessels H, et al.: Inhibition of platelet-mediated, tissue factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody: potential implications for the effect of c7E3 Fab treatment on acute thrombosis and "clinical restenosis." *J Clin Invest* 1996, 98:863–874.
- 49 Keularts IM, Hamulyak K, Hemker HC, Béguin S: The effect of DDAVP infusion on thrombin generation in platelet-rich plasma of von Willebrand type 1 and in mild haemophilia A patients. *Thromb Haemost* 2000, 84:638–642.
- 50 Turecek PL, Varadi K, Keil B, et al.: Factor VIII inhibitor-bypassing agents act by inducing thrombin generation and can be monitored by a thrombin generation assay. *Pathophysiol Haemost Thromb* 2003, 33:16–22.
- A TG assay seems suitable for monitoring the pharmacokinetics of inhibitor bypassing agents during treatment and possibly for predicting responses to treatment.

51 Varadi K, Negrier C, Berntorp E, et al.: Monitoring the bioavailability of FEIBA with a thrombin generation assay. *J Thromb Haemost* 2003, 1:2374–2380. A TG assay enables the pharmacodynamic and pharmacokinetic properties of factor VIII-inhibitor bypassing therapies to be monitored, thus helping to optimize treatment.

52 Azar AJ, Cannegieter SC, Deckers JW, et al.: Optimal intensity of oral anticoagulant therapy after myocardial infarction. *J Am Coll Cardiol* 1996, 27:1349–1355.

53 Al Dieri R, Wagenvoord R, van Dedem GW, et al.: The inhibition of blood coagulation by heparins of different molecular weight is caused by a common functional motif: the C-domain. *J Thromb Haemost* 2003, 1:907–914.

For any type of heparin, the capacity to inhibit the coagulation process in plasma is primarily determined by the concentration of the AT-binding pentasaccharide with 12 or more sugar units at its nonreducing end, *ie*, the structure that induces anti-thrombin activity. Antifactor Xa activity hardly influences either ETP or APTT.

54 Al Dieri R, Alban S, Béguin S, Hemker H. Thrombin generation for the control of heparin treatment: comparison to the activated partial thromboplastin time. *J Thromb Haemost* 2003;2:yy.

In 12 volunteers, 9000 IU of four heparins of different molecular weight distribution was injected. The aPTT showed the effect of heparin in 34[ref] of the samples; the ETP in 80[ref]. Relative to the baseline value of the individual, the heparin effect was recognized by the aPTT in 55[ref] of the cases and by the ETP in 98[ref]. There were no large differences between the different types of heparin.

55 Herault JP, Dol F, Gaich C, et al.: Effect of clopidogrel on thrombin generation in platelet-rich plasma in the rat. *Thromb Haemost* 1999, 81:957–960.

56 Kessels H, Béguin S, Andree H, Hemker HC: Measurement of thrombin generation in whole blood: the effect of heparin and aspirin. *Thromb Haemost* 1994, 72:78–83.