Thrombin generation and venous thromboembolism

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Keywords
Venous thromboembolism, recurrence, thrombin generation

Summary
Venous thromboembolism is a chronic and potential fatal disease. Determination of recurrence risk is time-consuming and costly, and sometimes not feasible: many patients carry more than one risk factor, the relevance of some factors with regard to risk of recurrence is unknown, and existence of thus far unknown risk factors must be considered. A laboratory assay that measures multifactorial thrombophilia would be useful to identify patients at risk of thrombosis. The process of thrombin generation is the central event of the hemostatic process. Thrombin generation is increased in patients at risk of thrombosis including those with antithrombin deficiency or those who are taking hormonal contraceptives. Risk of first and recurrent venous thrombosis is higher in patients with increased thrombin generation. Thus, by use of a simple global marker of coagulation stratification of patients according to their risk of thrombosis is possible. Future studies are needed to improve the management of patients with VTE and increased thrombin generation.

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Hämostaseologie 2008; 28: 37–39

- activated partial thromboplastin time,
- prothrombin time,
- fibrinogen,
- platelet count.

Hypercoagulability is seen when procoagulant forces increase and/or anticoagulant forces decrease, and may clinically manifest as thrombosis. Over recent years, numerous hereditary and acquired thrombotic risk factors were identified. Our current understanding of venous thromboembolism (VTE) is that of a multifactorial disease, which occurs when two or more risk factors are present at the same time. The likelihood of VTE varies between individuals and is strongly influenced by the presence of persistent and/or acquired risk factors. Whether the knowledge of laboratory risk factors in an individual patient translates into a clinical benefit remains uncertain.

The limited usefulness of laboratory thrombophilia screening can be easily explained: Many patients with VTE have more than one thrombophilic condition and important risk factors for VTE are hitherto unidentified. In addition, determination of recurrence risk by measuring a panel of single thrombophilic factors is time-consuming and costly. Ideally, a single laboratory test suited for detecting multifactorial thrombophilia would determine overall risk of (recurrent) VTE. However, such a laboratory assay is not available.

Haemostatic system activation

Activation of the haemostatic system is initiated by the interaction of TF (tissue factor) and factor (F) VIIa (2). The TF-FVIIa complex catalyzes the activation of factors IX and X, the latter being initially the more effective substrate. Activated factor X generates small amounts of thrombin, which are essential to accelerate the coagulation process by serving as the activator of platelets, factor V and factor VIII. The factor IXa generated by TF-FVIIa combines with factor VIIIa on the platelet membrane to form the intrinsic tenase, which is the major activating complex of factor X. Finally, FXa-induced generation leads to large amounts of thrombin (prothrombinase) that are sufficient to activate fibrinogen to fibrin which ultimately results in clot formation.

These processes of coagulation activation are under tight control of natural anticoagulants. As soon as FXa is produced by TF-FVIIa, this initial step of coagulation is shut down by tissue factor pathway inhibitor

Slüsselwörter
Venöse Thromboembolie, Rezidivrisiko, Thrombinbildung

Zusammenfassung

The human haemostatic system keeps a balance between pro- and anticoagulant forces to insure maintenance of blood fluidity as well as to limit and localize thrombus formation to the site of vascular injury. Any disturbance in the balance of these forces may lead to a state of
- hypocoagulability or
- hypercoagulability.

Hypocoagulability may occur when anticoagulant forces increase and/or procoagulant forces decrease, and may clinically manifest as bleeding. Severe states of hypocoagulability can be detected in the majority of affected patients by routine laboratory assays, i. e.
(TFPI), which forms a quaternary complex with the other three components. Antithrombin is an effective neutralizer of all of the procoagulant serine proteases. The procoagulant protein C system is initiated by thrombin binding to thrombomodulin, which leads to activation of protein C. Activated protein C in turn cleaves the cofactors Va and VIIIa and thereby eliminates the activation complexes tenase and prothrombinase. Thus, thrombin generation occurs in two phases.

- Initiation: Small amounts of thrombin are generated upon the (extrinsic) activation of FX by TF-FVIIa.
- Propagation: The major portion of thrombin (>96%) is subsequently produced.

The end point of routine clotting assays is the generation of a fibrin clot, which occurs already after the formation of small amounts (~4% of total) of thrombin. Thus, the vast majority of thrombin is ignored by routine laboratory tests.

**Measurement of thrombin generation**

Since thrombin is a protease, a common way to determine thrombin activity is by measuring the rate of cleavage of a respective substrate (3, 4). Cleavage of fibrinopeptide A from fibrinogen by thrombin is rapid and can be used to document the presence of thrombin in purified systems. Since thrombin is bound to and inactivated by antithrombin, forming irreversible thrombin-antithrombin complexes, their appearance serves as marker for the generation and presence of thrombin in whole blood or plasma. If measured repetitively as a function of time, fibrinopeptide A and thrombin-antithrombin complexes document the extent and rate of thrombin production in a given sample.

Thrombin activity can also be assessed by slow reaction with chromogenic or fluorescent substrate. The rate and extent of colour or fluorescence development correlate with the presence and activity of thrombin. Each method has its potential and limitations.

- Repeated measurement of thrombin substrates by subsampling is labour-intensive and time consuming.
- Methods applied in platelet poor plasma are more applicable for routine use, but miss the effects of platelets. Moreover, the influences of whole blood and of the endothelium are missed as well.

When thrombin generation is continuously registered by measuring cleavage of a chromogenic or fluorescent substrate, a thrombin generation curve is obtained (Fig. 1). From this curve five important parameters can be deferred:

- the lag-phase (the time until the thrombin burst occurs),
- the peak height of thrombin generation,
- the time to peak,
- the velocity of thrombin decay, and
- the area under the thrombin generation curve, which is called the endogenous thrombin potential (ETP).

Measurement of thrombin generation has been used to investigate platelet-plasma interactions, for pharmacologic research, to monitor procoagulant therapy, e.g. DDAVP or inhibitor bypassing therapies (5), to study the effects of anticoagulant drugs (e.g. heparin) (6), and to detect and quantify bleeding or thrombotic tendencies.

**Thrombin generation**

**Hypercoagulable disorders**

The ETP is higher in women taking hormonal contraceptives than in those without (7). It is also high in patients with natural inhibitor deficiencies particularly in those with antithrombin deficiency (8). Heterozygous carriers of the prothrombin mutation G20210A have significantly higher ETP values than carriers of wildtype factor II. The ETP is exceedingly higher in those who are homozygous for this mutation (9).

**Venous thromboembolism**

By computer simulation of thrombin generation and the data from the Leiden Thrombophilia study, Brummel-Ziedins et al. demonstrated an increased risk of first venous thrombosis associated with an elevated ETP (10). These findings were confirmed in the same study by measuring ETP by use of a fluorogenic substrate. Patients with an ETP above the 90th percentile had a 1.7-fold (95% CI 01.0–2.8) increased risk of first idiopathic deep venous thrombosis (11).

**AUREC**

We thought that by measuring thrombin generation patients with VTE could be stratified into high- and low risk categories for recurrence. To test this hypothesis, we determined thrombin generation in patients included in the Austrian Study on Recurrent Venous Thromboembolism (AUREC). AUREC is an ongoing prospective cohort study aimed at the identification of risk factors of recurrent VTE (12).

Patients older than 18 years who had been treated with vitamin K antagonists for at least three months for an objectively documented VTE were eligible. Patients
with previous VTE, VTE secondary to surgery, trauma or pregnancy, with antithrombin-, protein C-, or protein S deficiency, with cancer or need for long term antithrombotic treatment were excluded. Study endpoint is objectively documented, recurrent symptomatic deep vein thrombosis and/or symptomatic pulmonary embolism.

We showed that patients with a first spontaneous VTE and peak thrombin generation of less than 400 nmol/l after withdrawal of vitamin K antagonists have a low risk of recurrence (13). According to Kaplan-Meier analysis, likelihood of recurrent VTE was as low as 7% after four years with an upper 95% confidence interval of 9%. Compared to patients with higher levels, those with peak thrombin generation of less than 400 nmol/l had an almost 60% lower risk of recurrence. Most importantly, the group of patients with low peak thrombin generation represented two thirds of the total patient population.

These findings are of potential major clinical relevance. Using a simple commercially available laboratory method developed to measure thrombin generation, identification of patients was possible in whom the long-term risk of recurrent VTE is almost negligible. Considering the incidence rates of severe or fatal haemorrhage related to anticoagulant therapy and the cases-to-fatality rate of recurrent VTE, patients with a low peak thrombin generation (<400 nmol/l) would almost certainly not benefit from indefinite anticoagulation. Consequently, extensive thrombophilia screening appears to be unnecessary in this large low-risk group.

In this respect, it is of interest that important thrombotic risk factors including high plasma levels of factor VIII and factor IX, or factor II G20210A were less prevalent in patients with peak thrombin generation of less than 400 nmol/l compared to those with higher thrombin levels.

Stratification of patients with VTE according to their risk of recurrence is possible by use of a chromogenic thrombin generation assay and the ETP as read out variable (14). Patients included in AUREC with

- ETP > 100% had an almost two-fold higher relative risk (RR) of recurrence than patients with lower levels (RR 1.6, 95% CI 1.0–2.5).
- At four years, the cumulative probability of recurrence was 14.6% in patients with ETP > 100% and 6.1% in those with lower levels (p = 0.05).
- Patients with ETP ≥ 100% also had higher clotting factor levels.
- ETP was significantly increased in heterozygous carriers of factor II G20210A as compared to patients with wild type factor II (128% ± 18% versus 100 ± 12%, p < 0.001).

In the follow-up study of the original Leiden Thrombophilia study, no significant association between ETP as measured by a fluorogenic assay and risk of recurrent VTE was found (11). However, the study included patients with initial secondary thrombosis. It is well known that the risk of recurrence is low in these patients, and the subgroup of patients with initial idiopathic thrombosis was small.

Conclusions

Venous thromboembolism is a multifactorial disease. The process of thrombin generation is the central event of the haemostatic process. Determination of thrombin generation has been shown to be useful for detecting states of hypo- and hypercoagulability. Limited data indicate that risk of first deep vein thrombosis is associated with increased thrombin generation. We showed that simple and inexpensive laboratory assay systems to measure thrombin can serve to stratify patients according to their risk of recurrent VTE.

Further studies concerning thrombin generation are needed to provide new insights in the pathophysiology of hypo- and hypercoagulable states and to improve the management of patients with a bleeding or thrombotic tendency.

References


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