Global coagulation assays versus differentiated testing: will endogenous thrombin potential replace established thrombophilia screening? Pro.1)

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Venous thrombosis is a common and chronic condition. In Europe about 500,000 people develop venous thrombosis and 300,000 pulmonary embolism per year [1]. The risk of recurrence is about 7–10% per year, and 5% of relapsed patients die of pulmonary embolism [2]. Over time the risk of a recurrence decreases somewhat, but after eight years the proportion of patients with recurrent thrombosis goes up to 30% [3].

The 1990s saw a major boost to thrombosis research. A variety of hitherto-unknown risk factors affecting venous thrombosis were discovered. Some of these changes are not only frequent in thrombosis patients but also in the general population. For example, a third of patients with venous thrombosis and five to seven percent of the general population are carriers of the factor V-Leiden mutation. Other risk factors commonly detected in lab tests for venous thrombosis are the G20210A V-Leiden mutation. Other risk factors commonly detected in lab tests for venous thrombosis are the G20210A mutation in the prothrombin gene, hyperhomocysteinemia or high activity levels in various coagulation factors [4]. The “Leiden Thrombophilia Study”, a Dutch case control study, has been instrumental in defining the increase in the risk for a first venous thrombosis according to each of these risk factors [5]. The “Austrian Study on Recurrent Venous Thromboembolism” (AUREC), a prospective cohort study initiated in 1992, described many important risk factors for recurrent thromboses. Following the findings of other study groups, it has been meanwhile confirmed that patients with antithrombin deficiency, a lupus anticoagulant, high factor VIII or factor IX activity levels, hyperhomocysteinemia, increased TAFI (thrombin activatable fibrinolysis inhibitor) or reduced TFPI (tissue factor pathway inhibitor), or patients with combined defects show a higher risk of recurrence.

It is unclear whether the risk of recurrent venous thromboembolism is increased for heterozygous carriers of factor V-Leiden or those with factor II G20210A. Based on current evidence, it can be assumed that the risk of recurrence is only slightly higher, if at all, for these patients [6]. Why is it important to know the risk of recurrence for a patient? The incidence of recurrent thrombosis can be effectively prevented by adequate anticoagulant therapy. Vitamin K antagonists, however, increase the risk of bleeding. The optimal duration of secondary prophylaxis following a venous thromboembolism is thus derived from balancing the risk of recurrence and the risk of bleeding. The risk of bleeding during treatment with vitamin K antagonists is well known. Each year three to four percent of patients develop severe bleeding, and two to three out of 1000 patients die [7]. However, assessing the risk of recurrence in a single patient in order to determine the optimal duration of secondary prophylaxis is often impossible. Venous thromboembolism has multifactorial causes. The significance of many risk factors (e.g., protein C or S deficiency, homozygous FV-Leiden) or combined defects with regard to the risk of recurrence is still unknown. The risk of thrombosis is also increased by acquired and most often temporally limited factors including surgery, pregnancy, immobilisation, hormone treatment. In addition, the existence of hitherto unknown risk factors must be assumed.

Patients can be stratified according to their thrombotic risk and their risk of recurrence by use of global coagulation tests. These tests should be simple, easy to standardise, and cost-efficient. First results have been obtained by determining the activated partial thromboplastin time (aPTT). The aPTT is a global coagulation test that is affected by alterations in the intrinsic coagulation factors (FVII, FIX, FXI, FXII), the thrombin and fibrinogen. An association between a shorter aPTT and an increased risk of venous thrombosis has been found in post-operative patients and patients with first venous thrombosis [8, 9]. Our hypothesis that patients with a shorter aPTT also have an increased risk of recurrence, has been confirmed. Patients with a longer aPTT showed a significantly lower risk of recurrence than patients with a shorter aPTT [10]. Routine application is limited, however, because a ratio of patient and normal plasma has
to be calculated for reasons of standardisation and because the absolute differences in coagulation times between groups with a high risk of recurrence and those with a low risk amount to no more than one to two seconds.

D-dimer, a fibrin degradation product, has long been established for excluding acute venous thromboembolism. Some patients with venous thrombosis have very high D-dimer levels over several months [11]. We have been able to demonstrate that patients who have suffered a venous thrombosis can be stratified in groups of high and low risk of recurrence on the basis of their D-dimer levels [12]. Patients whose D-dimer concentration was <250 ng/mL after discontinuation of anticoagulant had a very low risk of recurrence. Two years after discontinuation of anticoagulation the risk of recurrence was only 3.9% in patients with low D-dimer concentrations (upper limit of the 95% confidence interval 6.5%) and was 60% lower than that of patients with higher D-dimer concentrations. The findings of an Italian study have shown that patients whose D-dimer concentrations were >500 ng/mL after withdrawal of a six-month treatment with vitamin K antagonists have a higher risk of recurrence than those with a lower level [13].

Promising results for optimising the risk stratification of patients with venous thrombosis have been achieved by means of quantification of thrombin generation. The end point of routine coagulation tests is the determination of the time of clot formation. This occurs at a time when only few amounts of thrombin have been formed. The bulk of thrombin (>95%), however, is not formed until after feedback processes have been activated, during which the activation of factor X by factor IX and factor VIII is of crucial importance. The formation of thrombin over time follows a characteristic curve. After a delay (lag phase), the end of which is marked by the formation of small quantities of thrombin, large amounts of thrombin are rapidly generated reaching their maximum (“peak thrombin”) after a certain period of time. The area under the thrombin generation curve is called endogenous thrombin potential (ETP). Thrombin generation can be assessed with several assay systems, which are now commercially available and differ with regard to the type of coagulation activation, the substrate and its detection (chromogenic, fluorogenic) as well as the software.

Using a computer simulation and the data set of the “Leiden Thrombophilia Study”, it has been shown that increased thrombin generation is associated with an increased risk of first venous thrombosis [14]. These results were subsequently confirmed within the same study, with ETP being measured by use of a fluorogenic substrate. Patients with ETP levels above the 90th percentile had a 1.7 times (95% CI 1.0–2.8) higher risk of first idiopathic venous thrombosis [15]. Within the frame of AUREC we tested the hypothesis that patients with initial venous thromboembolism can be stratified into those with a high risk of recurrence and those with a low risk based on determination of thrombin generation. The study included patients older than 18 years who had suffered an objectively diagnosed deep vein thrombosis with or without pulmonary embolism and who had been treated with vitamin K antagonists for at least three months. Patients with recurrent or secondary venous thrombosis, with an antithrombin, protein C or protein S deficiency or with malignancy as well as patients requiring an ongoing treatment with antithrombotics were excluded. The end point of the study was an objectively diagnosed recurrence of venous thrombosis or pulmonary embolism. We showed that patients with a first, spontaneous venous thromboembolism and a “peak thrombin” of <400 nM after discontinuation of vitamin K antagonists had a very low risk of recurrence [16]. The probability of recurrence four years after the discontinuation of anticoagulation was 7% (upper 95% confidence interval 9%). Patients with a “peak thrombin” of <400 nM had an almost 60% lower risk of recurrence than those with higher levels. The group of patients with low “peak thrombin” levels comprised almost two-thirds of the entire patient cohort. Stratification of thrombosis patients according to their risk of recurrence is also feasible by use of chromogenic assays and ETP analysis [17]. Patients in the AUREC study whose ETP was ≥100% had almost twice as high a relative risk (RR) of recurrence as those with lower levels (RR 1.6; 95% CI 1.0–2.5). Four years after the completion of anticoagulation the recurrence probability of patients with ETP ≥100% amounted to 14.6%, compared to 6.1% in patients with lower values (p = 0.05). A follow-up of the “Leiden Thrombophilia Study” has not detected any significant relationship between ETP (fluorogenic method) and risk of recurrence [15]. This difference in the study findings can be explained by the inclusion of patients with secondary thrombosis in the Dutch study, who have a significantly lower risk of recurrence. The percentage of patients with idiopathic venous thrombosis in this study was low.

Several studies have shown that simple, commercially available global tests can be used for the stratification of patients according to their risk of recurrence. As for daily practice, this raises the question whether such tests should be used to identify patients with a high or low risk or recurrence (or both). The Italian PROLONG study used D-dimer analyses in order to stratify patients in groups with a high or low risk of recurrence [18]. Patients with normal D-dimer after discontinuation of a six-month treatment with vitamin K antagonists did not receive any further anticoagulation therapy. These patients had a low risk of recurrence (4.4 recurrences/100 patient years). For patients with an abnormal D-dimer at that time, anticoagulation was either discontinued or recommenced. Patients with an abnormal D-dimer who had not undergone anticoagulation had a fivefold higher risk of recurrence (10.9 recurrences/100 patient years) than patients with a high D-dimer concentration who had received anticoagulation (2.0/100 patient years). It was in this group that a single serious bleeding incident occurred; the other patients did not experience any bleeding. PROLONG is the first interventional study to use a global marker to
stratify risk and determine the duration of secondary thromboprophylaxis. On the basis of a single intervention-al study it is, however, impossible to recommend D-dimer as the sole parameter in assessing the risk of recurrence and determining the duration of anticoagulation therapy, because some aspects of the study need to be discussed and addressed in more detail. The duration of anticoagulation in patients with normal D-dimer concentration levels was six months. However, it is not known whether this is the optimal duration in this patient population. The average observation period was only 1.4 years. The effectiveness and safety of a prolonged anticoagulation therapy for patients with high D-dimer levels is therefore unknown. Any decision on the necessity of a long-term thrombosis prophylaxis, must therefore be made on an individual basis for each single patient. The D-dimer analyses were done about four weeks after the completion of anticoagulation. Since some patients suffer recurrence very shortly after discontinuation of anticoagulation, a marker should be available that can be used for decision making already during anticoagulant treatment. PROLONG employed a qualitative test to analyse D-dimer. It is not known, however, whether the results of the study can be extrapolated to other D-dimer tests.

We have been able to demonstrate that patients with low D-dimer levels or low thrombin generation (measured as “peak thrombin”) have a very low risk of recurrence. Long-term anticoagulation would probably not be reasonable in these patients because the bleeding risk would outweigh the risk of recurrence. The number of patients that can be identified as low risk of recurrence is relatively large. A large number of thrombosis patients would therefore not require a complex thrombophilia screening.

The risk of recurrence for a patient with venous thromboembolism cannot be assessed by analysing a panel of thrombophilia markers. Currently clinical parameters (e.g., localisation of thrombosis or the patient’s gender) substantially influence the therapeutic management. Risk stratification of patients with initial idiopathic venous thromboembolism is feasible on the basis of simple and well standardised global coagulation tests. The ability to identify a large number of patients with a low risk of recurrence and to determine the duration of anticoagulation therapy on the basis of a single test seems particularly relevant. Global coagulation assays can be helpful in situations in which decisions on optimal duration of anticoagulation based on other parameters (clinic, bleeding risk) are difficult.

References


