Recurrent venous thrombosis: a disorder of thrombin generation stimulus-response coupling?

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A B S T R A C T

One-quarter of patients who suffer an initial episode of venous thrombosis have no identifiable provocation. The risk of recurrent venous thrombosis in these patients is much greater than in patients who have an identifiable trigger. However, it is uncertain if patients with unprovoked thrombosis genuinely have suffered from a 'spontaneous' event or whether the episode was triggered by a factor that was not readily identifiable, that is, this was venous thrombosis due to a 'silent' provocation. Characterization of hypercoagulability may help to elucidate the relative contributions of an underlying prothrombotic state and silent provocation to venous thrombosis risk. In this paper, a model of recurrent venous thrombosis as a disorder of exaggerated thrombin generation due to abnormal stimulus-response coupling is proposed.

Introduction

Venous thrombosis (VT), also referred to as venous thromboembolism (VTE), describes deep vein thrombosis (DVT) with or without symptomatic pulmonary embolus (PE). Superficial vein thrombosis might also be considered within the spectrum of disease. After a first episode of VTE, patients are 40 times more likely to suffer a further event compared with previously unaffected individuals.[1] The post thrombotic syndrome is more likely to occur after recurrent ipsilateral DVT, and chronic thromboembolic pulmonary hypertension is more than ten times as likely after recurrent PE as after a first event.[2] Therefore, preventing recurrent VTE prevents fatal recurrence and reduces the burden of disease in survivors.

Treatment with an oral vitamin K antagonist (VKA), such as warfarin, or a direct thrombin or factor Xa inhibitor will prevent more than 95% of recurrent episodes of VTE.[3,4] However, VTE is only prevented for as long as the anticoagulant therapy is continued. Therefore, anticoagulation must be continued indefinitely in patients at high risk to prevent recurrence. The risk of anticoagulant therapy-related bleeding precludes routine continued treatment for all patients, and long-term (lifelong) treatment should ideally be given only to patients considered to have a risk of recurrent VTE that exceeds the risk of clinically significant bleeding associated with continued treatment.

Clinical risk factors and likelihood of recurrent venous thrombosis

Patients with cancer are known to be at high risk of recurrent VTE, and treatment of cancer-associated VTE is usually continued for as long as treatment with chemotherapy and/or radiotherapy is being given and for as long as cancer is considered to be present. Treatment with a low molecular weight heparin is considered superior to a vitamin K antagonist for these patients, at least for the first 6 months[5,6] and is now the preferred treatment.[7]

Patients with detectable antiphospholipid antibodies are often thought to be at continued risk of venous thrombosis and are considered for continued anticoagulation. However, what constitutes an abnormal test result that predicts a high risk of recurrence remains uncertain. Furthermore, whilst criteria for Antiphospholipid Syndrome (APS) have been defined for the purposes of clinical study recruitment and reporting, the diagnosis of APS in an individual in a routine clinical setting can be difficult, as can the decision regarding duration of anticoagulant therapy.

Patients with other persisting risk factors are also typically treated for as long as the risk factor persists. For example, a woman who suffers thrombosis in pregnancy is usually treated until at least 6 weeks after delivery. The risk of recurrence in the absence of a further pregnancy is low.

In a study of unselected patients with a first episode of VTE, 15% of patients had cancer-associated venous thrombosis, 6% were diagnosed with APS, and 1.5% were women with pregnancy-associated thrombosis.[8] The remaining patients were all treated for 6 months with oral anticoagulant therapy. Since then, randomized trials have indicated that continuing treatment for 6 months is no more beneficial than treating for only 3 months.[9,10] This has resulted in the recent recommendation that after a period of venous thrombosis, initial treatment with anticoagulant therapy should be for 3 months.[11]

Since the early 1990s, it was recognized in clinical trials and observational cohort studies that patients who suffered a provoked episode of venous thrombosis, for example, after surgery, were at lower risk of recurrence than patients whose first episode was unprovoked.[12–16] This relationship between the likelihood of recurrence and the clinical circumstances at the time of the first event was demonstrated prospectively in a study, which determined recurrence rates after a first episode of venous thrombosis in relation to clinical risk factors and thrombophilia testing.[8] It is now accepted that the relationship between clinical factors at the time of venous thrombosis and likelihood of recurrence is sufficiently strong and robust as to be used as the basis for determining which patients should be considered for continued anticoagulant therapy.[11] However, as VTE is only prevented for as long as the anticoagulant therapy is continued, this equates to potential lifelong anticoagulation after a first episode of venous thrombosis. The recommendation for consideration of continued (lifelong) anticoagulation after a first episode of unprovoked venous thrombosis is considered contentious by some experts, and further clinical investigation into refining individual risk, both of recurrent thrombosis and anticoagulant therapy-related bleeding is necessary.

A further consideration when determining duration of anticoagulation for a patient is the likely consequence of recurrence if it were to occur. The risk of fatal PE is two to four times more likely in patients with symptomatic PE as compared with patients with symptomatic DVT alone,[17,18] and chronic pulmonary hypertension is at least ten times more likely after recurrence.[2] Therefore, if recurrence is more likely to be PE than DVT then the consequences of recurrence are potentially greater in patients with a first event manifesting as symptomatic PE. Previous studies suggest that 75% of recurrences are PE in patients initially presenting with PE, compared with 20% in patients presenting with DVT.[18–20] A patient level meta-analysis of seven recent prospective studies showed that patients presenting with a first episode of PE are at the same risk of recurrent VTE as patients presenting with a first episode of DVT alone but they are three times more likely to suffer PE than DVT as a recurrence.[21]

Unprovoked venous thrombosis and risk of recurrence

Within the group of patients who have suffered an unprovoked episode of venous thrombosis, there is a heterogeneous mix of individual risk. This is appreciated by observation of the distribution of D-dimer levels in these patients,[22–25] with approximately 50% having a D-dimer level below a predefined threshold.[26] The definition of unprovoked VTE is clinical and is dependent on an absence of identifiable risk in temporal association with the episode of VTE. A number of strong and moderate clinical risk factors have been used to distinguish provoked and unprovoked VTE in clinical studies (Table 1). In the absence of these recognizable factors, it is possible that some cases of unprovoked VTE are misclassified as unprovoked. Our understanding of the totality of environmental factors and how these interact at a moment in time is still limited. It is possible that a proportion of unprovoked cases are actually provoked by an unknown combination of temporary environmental risks. Patients with a low D-dimer following a finite period of anticoagulation may be representative of this group of patients, that is, patients with venous thrombosis due to 'silent provocation'. However, there appears to be a continuous accrual of recurrent events over time even in patients with a low D-dimer after unprovoked VTE, albeit at a lower rate than that observed in patients with a high D-dimer. This suggests either that silent provocation is a recurring theme or that these patients are at increased risk of genuine unprovoked VTE despite a relatively low D-dimer. The issue that now has to be addressed in clinical studies is whether measurement of hypercoagulability can identify a group of patients that exists with venous thrombosis due to 'silent provocation' who are not at risk of recurrence in the future, or alternatively if measurement of hypercoagulability is quite simply a measure of the rate of recurrence over time (for all patients). Studies with prolonged follow-up without intervention would be required to answer this question. There is a proposed illustration of alternative outcomes of recurrent venous thrombosis in relation to hypercoagulability (Figure 1).

Table 1. Examples of risk factors (and exclusions) used in clinical studies to distinguish provoked from unprovoked VTE.

Unprovoked
No identifiable provoking factors
Provoked
Surgery within previous 6 weeks (possibly within previous 90 days)
Trauma including fracture
Plaster cast immobilisation
Oestrogen exposure (contraceptive or hormone replacement)
Immobilisation for 3 days or more (including hospitalisation)
Travel (more than 6 hours continuous travel within 1 week of onset of symptoms of VTE)
Exclusions
Cancer
Myeloproliferative disorder
Antiphospholipid syndrome
Pregnancy

Definition of hypercoagulability and the prothrombotic state

Global tests that measure the composite effect of variation in procoagulant and anticoagulant factors can be used to quantify 'coagulability' as a parameter. Hence it might be possible to define 'hypercoagulability'. Two approaches have been used in clinical studies so far: measurement of biomarkers and determination of the thrombin generating potential. Biomarkers of thrombin generation reflect thrombin generation that has taken place *in vivo*, and D-dimer measurement after completion of a finite period of anticoagulation has been shown to stratify patient risk.[23,26] Measurement of the thrombin generating potential quantifies the ability to generate thrombin *in vitro* (typically in a plasma sample)

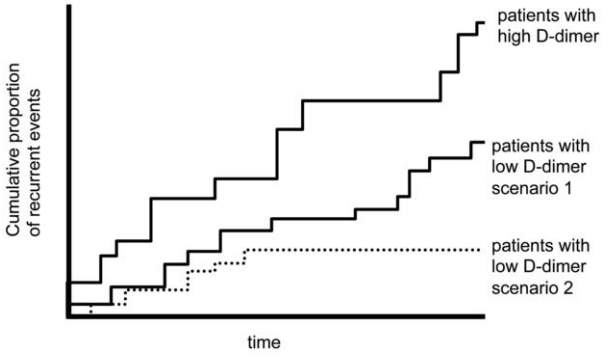

Figure 1. Illustration of alternative outcomes of recurrent venous thrombosis in relation to coagulability. Patients with elevated D-dimer have a cumulative risk of recurrence greater than those with a low D-dimer. For patients with a low D-dimer two survival curves are shown. The first (solid line, scenario 1) shows a continuous accrual of recurrent events and is in keeping with the hypothesis that measurement of coagulability (as measured by D-dimer in this example) is quite simply a measure of the rate of recurrence over time for all patients. The rate of recurrence is simply lower in patients with a low D-dimer but given sufficient time all patients will eventually suffer a recurrence. The second (broken line, scenario 2) shows a plateau implying that within the cohort of patients with a low D-dimer some are not at risk and do not suffer a recurrence. The distinction between patients with a low D-dimer who are and who are not at risk of recurrence might be determined prospectively by measurement of thrombin generating potentials or characterisation of the genetic architecture of an individual's thrombin generating potential.

in response to a pre-defined stimulus, usually a low concentration of tissue factor.[27] These complimentary approaches might be used to define hypercoagulabilty and the prothrombotic state as distinct entities.[28] Hypercoagulability might be defined as a predetermined 'exaggerated' response of thrombin generation to a stimulus. This might be identified by measurement of thrombin generating potentials or characterization of the genetic architecture of an individual's thrombin generating potential. The prothrombotic state might be defined as active (ongoing) exaggerated thrombin generation. This might be identified by measurement of D-dimer in the absence of an identifiable reason for increased thrombin generation, that is, in the absence of infection, pregnancy, recent surgery, and so on. This model of recurrent venous thrombosis is proposed as a disorder of exaggerated thrombin generation due to abnormal stimulus-response coupling (Figure 2).

It is not inconceivable that patients with extreme hypercoagulability are in a constant prothrombotic state as the normal environment in those individuals is a sufficient trigger for increased thrombin generation. Patients with a persistently elevated D-dimer after an episode of VTE may be representative of such patients who are at high risk of recurrence, either of unprovoked events or in response to 'silent' provoking factors.

The thrombophilia paradox

Heritable thrombophilia describes an inherited tendency for venous thrombosis. So far, only deficiencies of antithrombin, protein C, and protein S due to mutations in the corresponding genes *SERPINC1*, *PROC*, *PROS*, and the two common mutations *F5*G1691A and *F2*G20210A have been shown to be unequivocally associated with venous thrombosis, as defined by at least a two-fold increased risk.[29] Deficiencies of antithrombin, protein C, and protein S might be considered 'high risk' thrombophilias compared with the 'low risk' *F5*G1691A and *F2*G20210A mutations.[30–33]

At a patient-group level, it has been demonstrated in prospective cohort studies of consecutive unselected patients that testing does not usefully predict likelihood of recurrence after a first episode of venous thrombosis.[8,34,35] This also holds true for the group of patients who suffer an unprovoked first episode of venous thrombosis. A review of the clinical utility of thrombophilia testing, published in 2008, concluded that testing for heritable thrombophilia serves a limited purpose and should not be performed on a routine basis.[36] An analysis of the Multiple Environmental and Genetic Assessment (MEGA) study showed that testing for inherited thrombophilia did not reduce recurrence of venous thrombosis.[37] Guidelines now recommend thrombophilia testing in a minority of patients with venous thrombosis.[38]

A paradox seemingly exists, namely that these five thrombophilias are associated with an increased risk of a first venous thrombosis but not, apparently, of a high risk of recurrence.[28] This is likely the result of limitations imposed by testing for only a minority of heritable thrombophilic defects and adopting a dichotomous testing strategy, whereby a defect is defined as present or absent rather than quantified in terms of risk. The complete genetic contribution to thrombosis risk in patients is not known. Multiple other genetic factors will be present, which may be associated with a low risk in isolation[39] but result in a significant risk when clustered in an individual[40] or present in addition to one of the five 'usual suspects'. Therefore, only a fraction of an individual's genetic framework is appreciated with a limited dichotomous testing strategy. Consequently, the material contribution of an individual's genetic framework is not accurately estimated by current thrombophilia testing strategies. These limitations are likely compounded in practice by test inaccuracy and imprecision, such that the intermediate phenotype of anticoagulant deficiency (defined by a low plasma level of antithrombin, protein C or S) is not fully concordant with the heritable genotype.[41] As rapid inexpensive genotyping becomes a reality in the next 5 years, the identification of the totality of low risk mutations combined with characterization of the structure-function consequences of high risk mutations may improve the estimate of genetically determined thrombosis risk in an individual. Such analysis may equate into a useful predictor of recurrent thrombosis risk, such that individual genomic analysis will be considered to have clinical utility. There is already proof of principle that multiple testing for common mutations (single nucleotide polymorphisms) quantifies the risk of recurrent VTE,[40] and the number of common gene variants shown to be possibly related to risk of VTE is increasing.[39,42] Ultimately, the potential application of genomic DNA analysis to individualized risk assessment remains to be determined as the interaction of complex

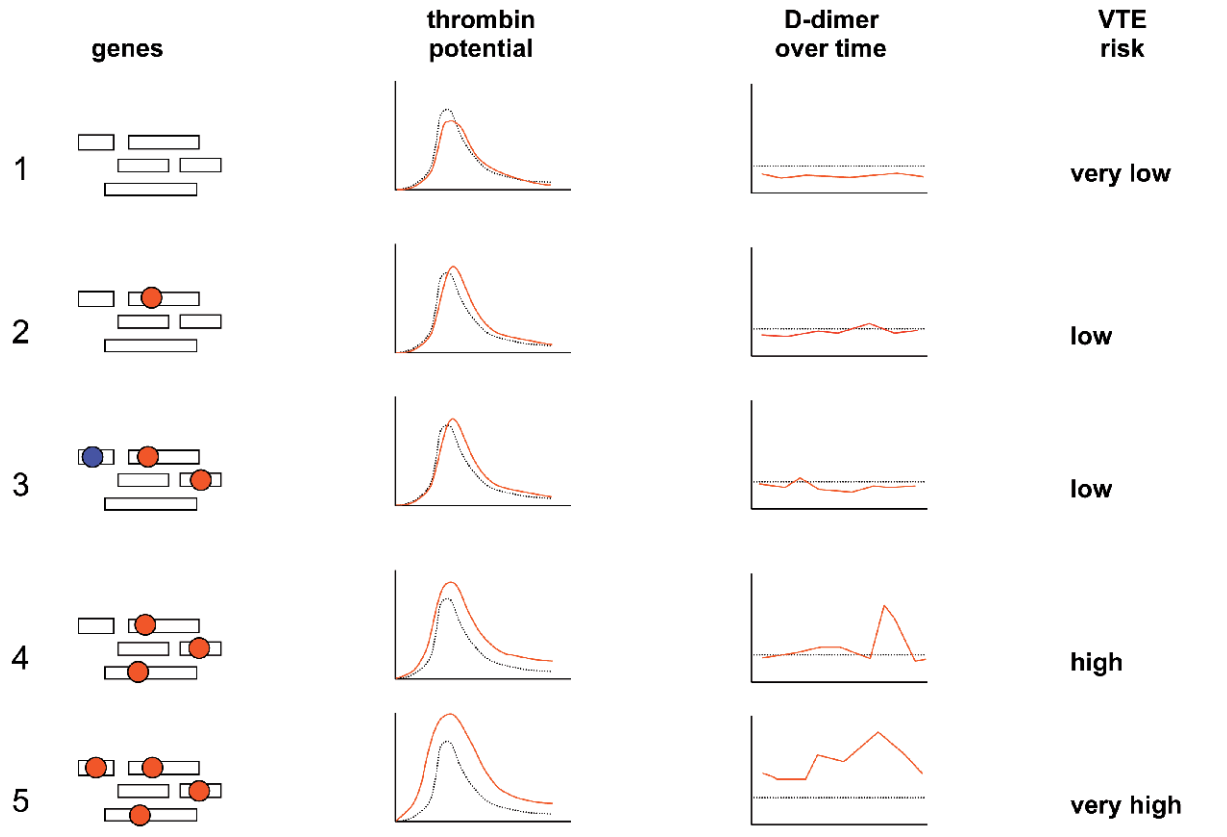

Figure 2. Proposed model of recurrent venous thrombosis as a disorder of exaggerated thrombin generation due to abnormal stimulus-response coupling. An exaggerated thrombin generation response would be genetically determined and might be identified by measurement of thrombin generating potentials or characterisation of the genetic architecture of an individual's thrombin generating potential. Some patients with extreme hypercoagulability are in a constant prothrombotic state as the normal environment in those individuals is a sufficient trigger for increased thrombin generation (individual 5). Others require a trigger to produce a prothrombotic state but depending on the degree of hypercoagulability a trigger may be slow slight as to not be readily identifiable; the 'silent trigger' (individual 4). In the model 5 individuals are presented: 1) normal with no thrombophilic mutations, 2) normal with minimal thrombophilic mutations, 3) normal with balanced thrombophilic and haemophilic mutations, 4) mild hypercoagulablity due to more thrombophilic mutations, 5) severe hypercoagulability due to most thrombophilic mutations. Thrombophilic mutations are shown in red and haemophilic mutations in blue. An average normal thrombin generation curve and the upper limit of normal (threshold) for D-dimer are shown as dashed lines.

combinations of gene variants with environmental factors may still prove to be relatively unpredictable at an individual level. On the other hand, if it is shown to have useful predictive power, it may also help our understanding of hypercoagulability and of how this translates into a prothrombotic state. In other words, does an individual's genotype result in a constant hypercoagulable state and hence constant relatively increased risk or venous thrombosis, or alternatively does it predispose to hypercoagulability in response to environmental stimuli and hence, only an increased risk of venous thrombosis on occasion. Current evidence in patients with type 1 antithrombin deficiency suggests the latter relationship.[43] In a study from Leiden, the annual incidence of venous thrombosis in antithrombin-deficient intervals who were not exposed to an environmental risk (for example, as in Table 1) was 0.3%. In patients who had surgery, the annual risk in the year that surgery was performed was 20%. Clearly, these individuals were at very high risk of provoked venous thrombosis. This suggests that measuring both thrombin generating capacity and D-dimer may be helpful in identifying patients at high risk of recurrence and whether recurrence is likely to be provoked (which would indicate the need for prophylaxis at times of identifiable risk, for example, patient 4 in Figure 2) or unprovoked (which would indicate a need for lifelong prophylaxis, for example, patient 5 in Figure 2).

Clinical utility of measures of hypercoagulability and the prothrombotic state

D-dimer is a marker of fibrin degradation formed by the sequential action of three enzymes: thrombin, factor XIIIa, and plasmin.[44] Therefore, increased thrombin generation is associated with increased D-dimer formation. In a series of studies from observation through to a patient management study (PROLONG), Palareti and colleagues showed that measurement of D-dimer levels following cessation of anticoagulant therapy predicts likelihood of recurrent thrombosis.[23–25] A meta-analysis of cohort studies indicates that the annualized risk of recurrence is 9% in patients with an elevated D-dimer

compared with 3.5% in patients with a low D-dimer after completion of a finite period of anticoagulation after a first venous thrombosis (relative risk 2.4, 95% CI 1.9 to 3.1).[26] In the PROLONG II study, D-dimer measurements were repeated at 2 monthly intervals for 1 year after an initial normal D-dimer following completion of initial therapy.[45] D-dimer was normal in 68% of patients 1 month after stopping treatment. Fourteen percent of patients developed an abnormal D-dimer 2 months after an initial normal result. The rate of VTE recurrence over a mean follow up of 10.6 months was 22.6% in these patients compared with 4.6% in patients whose D-dimer remained negative. This is an important finding that needs to be replicated in further studies. The predictive value of D-dimer may be influenced by interacting or confounded factors, such as sex and age. Furthermore, the predictive value of D-dimer measurement has typically been evaluated in patients with unprovoked venous thrombosis and is different in patients after provoked events.[46] A secondary analysis of the PROLONG study indicated that in patients with a normal D-dimer after completion of anticoagulant therapy after unprovoked venous thrombosis, recurrence rates were higher in males than females (7.4% vs. 4.3% patient-years) and in patients aged 65 years or more (8.4% vs. 3.6%). However, in a meta-analysis of cohort studies, only male sex had a significant effect on risk for recurrent VTE independent of D-dimer status; age, hormone therapy use at the time of the index event, body mass index, and timing of post-anticoagulation testing did not influence the predictive value of the D-dimer test result.[47]

Measurement of the thrombin generating potential is an alternative 'global testing' strategy that is possibly complimentary to measurement of D-dimer. This measurement has been shown in independent cohort studies to predict likelihood of recurrence with hazard ratios from 2.5 to 4.0.[48–50] Measurement of thrombin generation is technically difficult, and results are more influenced by pre-analytical variables than D-dimer measurements.[27,51–53] Thrombin generation assays measure the thrombin-time curve, which is the enzymatic work potential of thrombin. The Calibrated Automated Thrombogram ® (Thrombinoscope BV) and Technothrombin ® TGA (Technoclone) utilize a fluorogenic substrate, and the Endogenous Thrombin Potential Assay ® (Siemens healthcare Diagnostic Inc.) and Pefakit Thrombin Dynamics Test ® (Pentapharm) employ a chromogenic substrate. Activation of thrombin generation and interpretation of the thrombin-time curve varies between assays. Various parameters of the thrombin-time curve can be reported, including lag time, peak thrombin, time to peak, and the area under the curve (AUC, Endogenous thrombin Potential).

A recently developed alternative to these optical detection methods is continuous registration of the thrombin-time curve using an electrochemical biosensor.[54] Sensor strips with an amperometric substrate are electrically connected to a measuring unit. Thrombin cleaves the substrate producing an electric current. As electrochemical detection of thrombin activity is not affected by color or turbidity, the measurement can be performed on a whole blood sample. Clinical studies utilizing this technology have not yet been reported.

More studies are required to examine the clinical utility of measurement of D-dimer and thrombin generating potential and to determine:

- the performance characteristics of different assays;
- the value of quantitative (continuous variable) versus qualitative (dichotomized positive/negative or high/low) measurement;
- the influence of the clinical profile of the patient on the predictive value of the test result;
- the value of serial measurement, including measurements during and after completion of an initial period of anticoagulant therapy.

Conclusion

Prevention of recurrent venous thrombosis prevents fatal recurrence and reduces the burden of disease in survivors. Distinguishing patients at high and low risk of recurrence will permit continued anticoagulation in those patients in whom it is beneficial and avoid anticoagulant therapy-related bleeding in those who do not require continued treatment. In the last 10 years, measurement of 'coagulability' has been shown to be able to stratify patient risk and when used in conjunction with assessment of clinical risk factors, can increase the objectivity of clinical decision making. It is still not fully understood why some patients are at risk of recurrent unprovoked recurrent VTE. As well as validating measures of global coagulability for clinical use, ongoing studies may help to explain the mechanisms leading to thrombosis and clarify the relationship between thrombophilia, hypercoagulability, and the prothrombotic state.

As the 'silent' environmental trigger factors are currently unknown, it is not feasible to measure D-dimer at the time of an 'unknown risk event' to determine a patient's thrombin generating response. Therefore, all that can be suggested at present is measurement of D-dimer 'randomly', which in practice means repeated measurements. The PROLONG II study has paved the way for this approach. From a pragmatic clinical perspective, it may not be necessary to perform more than a few measurements to identify patients who readily 'slip into a prothrombotic state'.

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