Venous Thromboembolism: Mechanisms, Treatment, and Public Awareness

Biomarkers and Venous Thromboembolism

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Abstract—Venous thromboembolism (VTE) represents a significant health concern because of its high morbidity and mortality and is moreover characterized by high rates of recurrence. It would be useful to know biomarkers that enable early identification of patients at high or low risk of primary and recurrent VTE. Various established and novel biomarkers associated with VTE have been investigated with regard to their potential for predicting primary or recurrent VTE, for facilitating the diagnosis and for optimizing the clinical management of VTE. In this review, data on selected biomarkers (D-Dimer, soluble P-selectin, coagulation factor VIII, inflammatory markers and thrombin generation) having procoagulant properties or reflecting a prothrombotic state are summarized, and their role in clinical application is discussed. (Arterioscler Thromb Vasc Biol. 2009;29:332-336.)

Key Words: venous thromboembolism ■ recurrence ■ biomarker ■ D-Dimer ■ P-selectin

Venous thromboembolism is a frequent disease with an age-adjusted incidence of 1 to 2 events per 1000 of the general population and constitutes a major health care problem because of its high morbidity and mortality.1 About one-third of patients suffering from a first episode of deep venous thrombosis (DVT) or pulmonary embolism (PE) develops a recurrence of VTE within 10 years.2 From a clinical perspective, it would be extremely helpful to have biomarkers that enable early identification of patients at high or low risk of primary and recurrent VTE and allow prompt diagnosis and therapy assessment.

In this brief overview only some selected biomarkers associated with a first or recurrent event of VTE are highlighted that are either well-investigated (D-Dimer and coagulation factor VIII), novel and promising (P-selectin and endogenous thrombin potential), or controversially discussed (inflammatory cytokines).

D-Dimer

D-Dimer is a degradation product of cross-linked fibrin that is formed immediately after thrombin-generated fibrin clots are degraded by plasmin and reflects a global activation of blood coagulation and fibrinolysis. As D-Dimer levels rise during an acute event of VTE, testing for D-Dimer was explored as a tool for the diagnosis of VTE and has been integrated into diagnostic algorithms in the management of patients with suspected VTE.3-5 Being the best-recognized biomarker for the initial assessment of suspected VTE, a negative value of D-Dimer may safely rule out both DVT and PE with a high sensitivity of up to 95% and a negative predictive value of nearly 100%, using the various commercially available D-Dimer assays. However, because of its poor specificity to prove VTE, D-Dimer testing has to be included in comprehensive sequential diagnostic strategies that incorporate clinical probability assessment and imaging techniques.

Elevated D-Dimer levels may also indicate the presence of hypercoagulability. In a case–control study high D-Dimer levels (>70th percentile of levels in healthy controls) measured at least 6 months after a first event of DVT in 474 patients and 474 age- and sex-matched control individuals have been shown to be associated with a 2.2-fold increase in the risk of thrombosis.6 In a population-based cohort study D-Dimer was investigated as a risk factor for the occurrence of a future first event of VTE and was associated with a 3-fold increased risk.7 Moreover, D-Dimer levels are a well-investigated biomarker for the prediction of the risk of VTE recurrence after discontinuing oral anticoagulant therapy in prospective cohort studies.8 Palareti et al measured D-Dimer levels 1 month after withdrawal of oral anticoagulation in subjects with previous unprovoked VTE and demonstrated normal levels (≤500 ng/mL) to have a high negative predictive value for recurrence of VTE.9 Similarly, Eichinger et al reported that patients with a first unprovoked VTE and D-Dimer levels of less than 250 ng/mL measured 3 weeks after discontinuation of oral anticoagulation had a low risk of VTE recurrence.10 The cumulative probability of VTE recurrence after 2 years was 3.7% in patients with a D-Dimer less than 250 ng/mL compared to 11.5% in those with higher levels. Interestingly, it was also demonstrated that elevated D-Dimer levels were associated with an even higher risk of

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reCURRENT VTE especially in patients with congenital thrombophilia such as a factor V Leiden or prothrombin variation (hazard ratio [95% CI] for VTE recurrence: 8.3 [2.7 to 17.4]).11 Accordingly, in a prospective interventional study in patients with unprovoked VTE, who had received vitamin K antagonists, D-Dimer tests were performed 1 month after discontinuation of anticoagulation.12 Patients with normal D-Dimer levels did not resume anticoagulant treatment, whereas those with elevated D-Dimer were randomly assigned to either resume or permanently discontinue secondary prophylaxis. During an average follow-up period of 1.4 years the authors found that patients with elevated D-Dimer levels 1 month after discontinuation of anticoagulation had a significantly increased incidence of recurrent VTE, which was reduced by the resumption of anticoagulation. These data suggest that D-Dimer might be a useful biomarker to determine the duration of anticoagulation, and that patients with an elevated D-Dimer benefit from a longer duration of anticoagulation therapy.

In conclusion, the measurement of D-Dimer has become a cornerstone in the diagnostic work-up of patients with suspected VTE and plays a pivotal role in the detection of hypercoagulable states and, thus, may guide the decision on the duration of oral anticoagulation for secondary VTE prophylaxis.

**Soluble P-Selectin**

P-selectin, a member of the selectin family of cell adhesion molecules, is primarily stored in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells. After activation of platelets and endothelial cells, P-selectin is translocated to the cell surface and in part released into the plasma in soluble form. P-selectin acts through binding to its main counter-receptor, the P-selectin glycoprotein ligand-1 (PSGL-1), located on leukocytes.13 Mounting evidence suggests P-selectin to be an important molecule in hemostasis and thrombosis (reviewed in14–16). The P-selectin receptor, PSGL-1, is also expressed on platelets and mediates platelet-endothelium interaction and supports fibrin formation and thrombus growth.17–19 Furthermore, the interaction of P-selectin and its receptor leads to several mechanisms that induce a procoagulant state by triggering the generation of procoagulant microparticles from leukocytes,20–22 upregulating the expression of tissue factor on monocytes,23 and inducing phosphatidylserine exposure and an increased surface-dependent thrombin generation on monocytes.24

Recent studies have demonstrated the clinical significance of P-selectin for thrombosis, and elevated soluble P-selectin (sP-selectin) has been implicated as a risk factor for venous thromboembolism. sP-selectin levels have been observed to increase during an acute event of VTE.25,26 In a pilot study, Rentsenwald et al measured sP-selectin in patients with acute DVT confirmed by duplex ultrasound and compared values to a group of healthy and a group of symptomatic individuals that were negative on duplex ultrasound for DVT.26 They found sP-selectin levels to be significantly elevated in patients with acute DVT and of predictive value for confirming DVT with a sensitivity, specificity, and accuracy comparable to D-Dimer. Larger studies are expected to establish the role of sP-selectin as a novel biomarker for the assessment of suspected VTE.

In the Leiden Thrombophilia Study (LETS), measurements of sP-selectin were performed at least 6 months after DVT occurrence in a subgroup of 89 patients and revealed significantly higher plasma levels in patients than in control individuals.27 The odds ratio of elevated sP-selectin for DVT was 2.1 (95% CI: 1.2 to 3.6). Our group reported the association of elevated sP-selectin (>95th percentile for healthy controls) with VTE to be even stronger in a case-control study of 116 patients with objectively confirmed recurrent VTE and 129 age- and sex-matched healthy individuals with an OR of 10.6 (95% CI: 3.7 to 23.3), adjusted for established VTE risk factors.28 These results were subsequently supported by a large prospective cohort study that investigated circulating sP-selectin as a predictive biomarker for recurrence of VTE in patients with a first episode of unprovoked VTE, in the absence of cancer or other known risk factors.29 sP-selectin levels were also found to be elevated in lupus anticoagulant patients with a previous event of VTE,30 and preliminary results indicate that sP-selectin plasma levels are partially modulated by genotype status.28,31 Furthermore, also variations and haplotypes of the P-selectin gene (SELP) might contribute to the risk of VTE.31 Recently, a prospective cohort study of 687 cancer patients demonstrated a relationship between sP-selectin plasma levels and the risk of VTE.32 According to this study, sP-selectin levels were higher in cancer patients with VTE compared to those without and allowed to predict the occurrence of cancer-associated VTE with a hazard ratio of 2.6 (95% CI: 1.4 to 4.9).

Summarizing the evidence from basic and clinical studies, P-selectin is a biomarker that clearly has procoagulant properties and reflects a prothrombotic state in human subjects. However, the clinical applicability of sP-selectin measurements to assess the risk of VTE needs to be standardized and investigated in interventional trials.

**Inflammatory Cytokines**

A clear link between atherosclerosis, arterial thrombosis, and inflammation has been established.33 There is ongoing discussion as to whether VTE and arterial thrombosis share the same risk factors.34–36 Advanced age, obesity, pregnancy, malignancy, or surgery are common risk factors for VTE and are accompanied by elevation of inflammatory markers, especially C-reactive protein (CRP). A mechanism through which CRP promotes a prothrombotic state is, for example, the induction of tissue factor synthesis by monocytes, leading to activation of the extrinsic coagulation pathway.37 A number of studies have been carried out to evaluate markers of inflammation, such as CRP, levels of interleukins (IL) 1β, 6, 8, 10, 12p70, and tumor necrosis factor (TNF) as risk markers for VTE. Data on the relationship between markers of inflammation and VTE have been previously summarized in a review by Fox and Kahn.38 Four studies evaluated the sensitivity and specificity of CRP levels in the diagnosis of VTE. Pooled weighted analysis revealed a sensitivity of 77% with a wide range (60% to 100%) between the studies and a specificity of 66%.38 Thus, determination of CRP is not
superior to the predictive value of D-Dimer for the diagnosis of VTE. Furthermore, several case–control studies investigated the association of CRP levels with VTE: In the LETS a positive association was seen with CRP-levels.\textsuperscript{39} IL-6, IL-8, and TNF-\textalpha.\textsuperscript{40,41} Values were not corrected for body mass index (BMI) in this study. However, in more recent studies there was no statistically significant association of VTE with levels of CRP and IL-6 and polymorphisms of the CRP and IL-6 gene after adjustment for the BMI.\textsuperscript{42–44} In line with these observations, no increased risk for development of new VTE events was found in two large prospective studies, the Physicians Health\textsuperscript{33} and the LITE Study.\textsuperscript{45} Presumably, increased inflammatory cytokines do not constitute an independent risk marker for future VTE.

**Clotting Factor VIII**

Various clotting factors have been investigated with regard to their potential for increasing the risk of VTE, elevated levels of factor VIII have consistently been found to be a risk factor for primary VTE in case-control\textsuperscript{46} and prospective\textsuperscript{45} studies. Prospective observational studies\textsuperscript{47–49} and one interventional trial\textsuperscript{50} revealed that elevated factor VIII is also a strong risk factor for recurrent VTE. It seems that the increase of risk is not linear, but is present only when factor VIII exceeds a certain level of approximately 230% to 250%. The risk for recurrence can be expected to be close to 30% 2 years after the discontinuation of anticoagulation. When factor VIII levels are considered for individual counseling on the duration of anticoagulation in patients with previous VTE, it is presumably important to exclude an acute phase reaction, which leads to elevation of factor VIII. Basal levels of factor VIII are currently thought to be genetically determined.\textsuperscript{51} A major determinant is the ABO blood group system. For instance, individuals with blood group non-O have higher levels in comparison to those with blood group O.\textsuperscript{52} Recently a polymorphism in the low-density lipoprotein receptor–related protein 1 gene (663 C>T) was described, which is clearly associated not only with factor VIII levels, but also with the risk of VTE, independent of the blood group and von Willebrand factor.\textsuperscript{53}

Conclusive data indicate that factor VIII is a risk predictor for primary and recurrent VTE. However, appropriate interventional trials designed to establish its relevance for guidance on duration of anticoagulation have not yet been carried out, and therefore its usefulness for VTE-related decisions still has to be questioned.

**Thrombin Generation**

A very promising approach to detect an individual’s coagulation potential is the measurement of the thrombin generation (TG), which may quantify the composite effect of multiple risk factors for VTE and predict a prothrombotic state. Thrombin is a key enzyme in the coagulation process and leads to conversion of fibrinogen to fibrin resulting in clot formation. The generation of thrombin—although it cannot be called a classical biomarker—is measured in plasma using a chromogenic or fluorescent substrate and can be registered in a TG curve. From this curve various parameters describing thrombin activity can be deduced, including the lag time (time until thrombin burst occurs), the peak value of thrombin, and the area under the curve (or endogenous thrombin potential [ETP], which represents the total amount of thrombin generated).

Increased TG was reported in patients at risk for VTE, such as patients with elevated coagulation factors VIII, IX or XI, patients with protein C and S deficiencies, lupus anticoagulant and factor V R506Q mutation, or in women taking oral contraceptives or hormone replacement therapy (reviewed in\textsuperscript{54,55}).

Several recent studies have also investigated the association of increased TG with the risk of a first and recurrent event of VTE. ETP and peak thrombin were found to be increased in patients with a history of VTE compared to healthy individuals and were related to the risk of a first event of VTE.\textsuperscript{56,57} Hron et al reported on the relationship between recurrent VTE and peak thrombin generation and concluded that patients with a peak thrombin less than 300 nM (lower tertile) had a significantly lower risk of recurrence compared to patients with peak thrombin generation above the upper tertile of 400 nmol/L (relative risk [95% CI]: 0.37 [0.20 to 0.67]).\textsuperscript{58} Data on the ETP and other parameters of the TG assay are not given in this report. The relationship of ETP and peak thrombin with the risk for recurrent VTE is supported through other prospective studies on patients with a first episode of VTE.\textsuperscript{59–61} Interestingly, although ETP measured in the LETS patient cohort was associated with a first event of idiopathic DVT, this study failed to demonstrate an association of high ETP with a risk of recurrent thrombotic events.\textsuperscript{57}

To conclude, TG assays, evaluating an overall hemostatic status of patients at risk of VTE and measuring the cumulative effect of prothrombotic tendencies, may be potentially valuable for prediction of VTE recurrence and for the clinical management of VTE patients when trying to identify those who might be considered for long-term anticoagulation. However, currently, TG testing is limited to being a research tool and further large prospective and interventional studies and standardization of test conditions are needed for application of TG in clinical practice.

**Conclusions**

A number of biomarkers have been evaluated with regard to their potential for predicting primary or recurrent VTE, but results are currently far from being satisfactory. The positive and negative predictive values of potential VTE-related biomarkers allow either no (inflammatory markers) or just a limited prediction for an individual patient (D-Dimer, factor VIII, ETP, sP-selectin). Interventional studies of relevant size on risk markers for recurrent VTE are available just for D-Dimer, a number of studies investigating the predictive value of D-Dimer for duration of anticoagulation are underway. However, in individual cases, in which the decision on the duration of anticoagulation is to be tailored individually because of various reasons (eg, increased risk of bleeding or patients’ negative attitude toward long term anticoagulation after DVT or PE), biomarkers with proven predictive value may help in reaching a decision.
Disclosures

None.

References


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