Elevated Clotting Factor Levels and Venous Thrombosis

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Key Words

Elevated clotting factor levels · Venous thrombosis · Factor VIII · Factor IX · Factor XI · Prothrombin · Fibrinogen

Abstract

Increased plasma levels of fibrinogen, factor VIII (FVIII), factor IX (FIX), factor XI (FXI) and prothrombin all were reported to be independent risk factors of venous thromboembolism (VTE). However there is only limited information on the molecular basis of these plasma phenotypes. In addition some of these plasma phenotypes may form clusters, which may point to defects in common pathways affecting biosynthesis or clearance. Uncertainties as to what should be considered the cut-off for an elevated plasma level and in some cases (FIX, FXI) the lack of published confirmatory and/or prospective studies, have delayed the incorporation of these parameters in thrombophilia screening procedures.

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Introduction

Venous thrombosis is a multicausal disease. Multiple genetic and environmental factors contribute to the development of the disease. Most of these factors relate to changes in blood flow and changes in the composition of the blood. In combination with a damaged endothelium they create locally a hypercoagulable state. When the hypercoagulability surpasses a certain threshold, excessive thrombus formation will occur which may present as thrombosis of the large veins of legs and arms (DVT), thrombosis of the superficial veins (STP) and pulmonary embolism (PE) [1]. For the management of patients with thrombophilia (tendency to develop venous thrombi) it would be helpful to be able to assess their hypercoagulability. At present this is not possible. We do know of many risk factors for venous thrombosis; genetic defects, environmental/acquired conditions and abnormal plasma phenotypes which all contribute to this hypercoagulability [1,2]. From the study of familial thrombophilia we also know that there are still other genetic risk factors that we have not identified yet [3]. Also we lack reliable quantitative information on the interaction between all these risk factors. Most importantly, however, we lack a relatively simple (set of) blood test(s) which quantifies hypercoagulability.

During the past 10 years many novel risk factors for venous thrombosis have been reported. An increasing number of these concern abnormal plasma phenotypes. The most

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important ones are the presence of lupus anticoagulant (LAC) and/or anticardiolipin antibodies [4], APC resistance not associated with FV Leiden [5,6], elevated plasma homocysteine levels [7] and elevated clotting factor levels [8,9]. The molecular basis of most of these plasma phenotypes is incompletely known. Some of these plasma phenotypes might form clusters [10,11], which may point to defects in common pathways affecting biosynthesis and clearance. Uncertainties as to what should be considered the cut-off for an elevated clotting factor level and in some cases the lack of confirmatory and prospective studies have so far prevented that these novel risk parameters have been included in routine thrombophilia screening procedures. The next paragraphs will briefly summarize what we presently have learnt about plasma phenotypes defined by elevated clotting factor levels.

Elevated Plasma Clotting Factor Levels

Since the first report that elevated plasma FVIII levels were found to be associated with an increased risk of venous thrombosis [12], studies have been published on the effect on venous thrombotic risk of almost every procoagulant and anticoagulant protein; prothrombin [13], factor V (FV) [14], factor VII (FVII) [15], factor IX (FIX) [16], factor X (FX) [17], factor XI (FXI) [18], factor XII (FXII) [19], factor XIII (FXIII) [20], fibrinogen [15], TAFI [21], protein C and protein S [22], TFPI [23]. For some of the plasma phenotypes the effect on venous thrombotic risk seemed to be continuous; the risk of thrombosis increased linearly with the plasma factor level (FVIII, FIX, FXII, FII). For others, thrombosis risk was only associated with levels above the 90th percentile (FVII, FV, FX, TAFI) or below the 10th percentile (TFPI) of the distribution in the normal population.

Factor VIII

In 1996 Koster et al reported that non-O blood group, VWF and FVIII levels all influence the risk of a first deep vein thrombosis (DVT) and that the effects of blood group and VWF were mediated through FVIII [10]. The FVIII level seems to be the final effector in promoting venous thrombosis. Subjects with plasma FVIII above 150 IU/dl had a 4.8-fold higher risk of DVT than those with plasma FVIII below 100 IU/dl. Furthermore each increase in FVIII level with 10 IU/dl is associated with a 10% increase in the risk of a first thrombotic event [10,24]. These findings have been essentially confirmed now in several independent studies, also after adjustment for CRP levels [24-27]. Measurement of FVIII levels by one stage clotting assay,

chromogenic assay and antigen assay all gave very similar results [28]. More recently it was reported that in families with thrombophilia and FV Leiden, FVIII levels >150 IU/dl contribute to the risk of venous thrombosis of FV Leiden carriers [29,30].

So far there is one study that prospectively investigated the effect of FVIII and VWF on risk of venous thromboembolism [31]. In this study both FVIII and VWF were found to be linearly associated with risk for VTE. The multivariate adjusted hazard ratio of venous thrombosis was 2.6 for those with FVIII levels in the upper quartile when compared with those in the lowest quartile.

Three studies report on the effect of FVIII levels on the recurrence of venous thrombosis [24,32,33]. They all seem to indicate that VTE patients with high FVIII levels (as measured several months after the first/last VTE) have an increased risk on a next event when compared to those with lower FVIII levels. If this is true this might have implications for the duration of anticoagulant treatment. On the other hand, the prospective follow-up of the Leiden thrombophilia study (LETS) did not find an increase in the relative risk of recurrence for elevated levels of clotting factors FVIII, FIX or FXI [34]. More data are needed before we can consider elevated FVIII levels as an established risk factor for a recurrent event.

The mechanism by which FVIII levels influence thrombotic risk has not yet been identified. Most likely it is the increase in factor IXa cofactor activity, which results in an increase of factor Xa and thrombin formation. This will not only lead to formation of more fibrin but also to increased TAFI activation (impaired fibrinolysis). In addition it might result in an increased resistance of factor Va to activated protein C (APC) [5,35].

There are many factors, which might influence the plasma FVIII level. FVIII circulates in the blood in a complex with VWF [36]. Free FVIII has a much shorter half-life than the VWF-FVIII complex [37], suggesting that the half-life of plasma FVIII is mainly determined by the clearance of VWF. Although the heritability of plasma FVIII levels is high (0.4-0.6) [38-40] and familial aggregation of elevated FVIII levels remained significant even after correction for blood group [41] there are no indications that a common variation in the gene coding for FVIII influences plasma FVIII levels via enhanced FVIII expression or an increased affinity for binding to VWF (28,42). Interestingly Soria et al reported recently that in the GAIT families a locus on chromosome 18 importantly influences FVIII levels and APC-sensitivity [43].

It is well known that non-O blood group is associated with higher levels of VWF (which carries the blood group antigens) and FVIII [44,45]. Most likely the presence of A/B antigens on VWF prolongs the half-life of VWF [46].

Orstavik et al [40] reported that blood group explains about 30% of the total variance in VWF. Apart from the blood group locus the VWF locus itself also contributes to the plasma concentration of VWF [47]. So far there are no sequence variations in the VWF gene that persistently are found to be associated with increased VWF levels [48,49].

At present we only have started to explore all the mechanisms that might contribute to an elevated plasma FVIII/VWF level. Changes in the rate of biosynthesis and/or secretion (release of Weibel Palade bodies, endothelial damage) and in the clearance rate of VWF/FVIII [50-52], all can influence plasma FVIII levels.

Factor IX

There are two recent case control studies that report that high FIX levels increase the risk of venous thrombosis [16,53]. Lowe et al included 66 women with idiopathic thrombosis and 163 controls, and found that plasma levels of FIX activity >150 IU/dl were associated with a 2.3 fold increased risk of VTE after adjustment for HRT use [53]. Van Hylckama Vlieg et al used 426 patients and 473 controls from the Leiden Thrombophilia Study to investigate the effect of FIX antigen levels on the risk of a first DVT. They used the 90th percentile in control subjects as cut off value (129 U/dl). Individuals who had FIX levels above 129 U/dl had a 2.8 fold increased risk of DVT after adjustment for age, sex and oral contraceptive use [16]. They found a slightly higher relative risk in women than in men and an even higher risk in premenopausal women not using oral contraceptives. Interestingly, the effect of FIX levels on thrombosis risk is continuous; the risk increases with increasing FIX levels. Elevated FIX levels (>P90) further increased the risk associated with elevated FVIII levels (OR= 8 when compared with individuals with both FVIII and FIX ≤ P90).

There is so far only one study that reports that high levels of FIX confer an increased risk of recurrent VTE and enhance the risk of recurrence among patients with high FVIII [54]. Obviously this result needs to be confirmed by additional independent studies.

The molecular basis of elevated plasma FIX levels has been poorly investigated so far. It is known that FIX levels increase with age [55] and Kurachi and coworkers have identified regions in the 5'- and 3'untranslated regions of the FIX gene which are involved in stable age-associated expression of FIX [56]. Blood lipids and use of oral contraceptives also influence FIX levels. The heritability of plasma FIX levels is relatively high [39,40]. There are no reports on variations in the FIX gene that are associated with FIX levels. Sequencing of the FIX genes of 19 men with an iso-

lated elevated FIX did not reveal common sequence variations that were associated with FIX levels (Poort SR, unpublished data)

Factor XI

There is only one study that reports on the effect of plasma FXI antigen levels on the risk of a first DVT [18]. Subjects with FXI levels above 110 U/dl (upper quartile of the distribution in controls) have a 2-fold increased risk of venous thrombosis when compared with subjects with FXI levels in the lowest quartile (<83.3 U/dl). Like elevated plasma FVIII also elevated plasma FXI may contribute to the thrombotic risk of FV Leiden carriers [57]. Clearly confirmation of these results is needed. Preliminary data indicate that elevated plasma FXI level is not associated with an increased rate of recurrences.

Recently, Gerdes et al reported that sequencing of the FXI genes of 22 probands from thrombophilic families and of nine patients with FXI levels above 140 U/dl did not yield any novel candidate risk factor for venous thrombosis [58].

Prothrombin

In 1996 Poort et al reported that subjects with a plasma prothrombin level above 115 U/dl (upper quartile of the distribution) had a 2.1-fold increased risk of deep vein thrombosis compared to those with a prothrombin level below 95 U/dl (lower quartile of the distribution) [13]. This observation has not been confirmed yet in independent patient control studies or in prospective follow-up studies. The effect of elevated plasma prothrombin on the risk of recurrence has not been investigated so far.

About 18% of patients with plasma prothrombin above 115 U/dl carry the G20210A mutation in the 3'UT of the prothrombin gene [13]. Heterozygous carriers of the 20210A allele have about 30% higher plasma prothrombin levels [13]. This has been confirmed in a series of independent studies. Carriers of the 20210A allele have an 2-7 fold increased risk of a first deep vein thrombosis [59], but a similar risk of recurrences as non-carriers [60]. In an attempt to find other genetic causes of an elevated plasma prothrombin level, Ceelie et al sequenced the prothrombin genes of selected individuals with the 20210GG genotype and a prothrombin level above 130 U/dl. However, no novel sequence variations were observed. Comparison of the allele frequencies of several known polymorphisms in the prothrombin gene in controls and subjects with an elevated prothrombin level (>130 U/dl) resulted in the identification of the A19911G variation in intron 13 of the prothrombin

gene as an additional determinant of plasma prothrombin levels [61]. Subjects with the 19911GG genotype had about 7% higher prothrombin levels than those with the 19911AA genotype [61]. Initial data indicate that the 19911G allele might increase the risk of venous thrombosis of subjects carrying the 20210A allele on the other chromosome [61].

In vitro studies have demonstrated that the G20210A variation influences prothrombin levels by a more efficient processing of prothrombin transcripts containing the A in position 20210 [62-64]. In addition there also might be an effect of G20210A variation on the stability of the prothrombin mRNA, but this needs further confirmation [63]. It is not clear how the G19911A variation influences prothrombin levels. It has been suggested that it may result in a more efficient splicing of intron 13. There are several other polymorphisms in the region of the prothrombin gene surrounding position 20210 (A20207C, C20209T, C20209A, A20218G, C20221T). The frequencies of the rare alleles of these polymorphisms are so low, that it has been not possible to study their effects on thrombosis risk and prothrombin levels.

Fibrinogen

In 1994 Koster et al reported a positive level related association between plasma fibrinogen level (as measured with the Claus assay) and risk of venous thrombosis [15]. This conclusion was based on the analysis of the first 199 patients and 199 controls of the Leiden thrombophilia study (LETS). Later the analysis was extended to all the 474 patients and controls of the LETS [65]. Subjects with plasma fibrinogen above the 95th percentile of the distribution measured in the control subjects, had a 2.8-fold increased risk of a first deep vein thrombosis. This risk was found to be equal in men and women, and even higher (OR 4.2) in subjects older than 45 years. Adjustment for other risk factors (prothrombin, FVIII, FIX, FXI, FV Leiden, PT20210A) and C-reactive protein reduced the OR to 1.5 (95%CI 0.8-3.0) [65]. This finding indicates that the effect of elevated

fibrinogen levels on thrombotic risk might be very small, even in subjects older than 45 years. In the LITE study elevated fibrinogen levels did not predict an increased risk of venous thromboembolism [31].

The heritability of plasma fibrinogen levels is high [39]. Several polymorphisms in the fibrinogen genes (FGA, FGB, FGC) have been reported which might be associated with fibrinogen levels. However, results are not consistent and none of these polymorphisms has been found to be associated with an increased risk of venous thrombosis [66]

TAFI

TAFI (thrombin-activatable fibrinolysis inhibitor, pro-carboxypeptidase U) is the precursor of a plasma carboxypeptidase, which cleaves end-terminal lysins from partially degraded fibrin and thus inhibits fibrinolysis by preventing the assembly of plasminogen and t-PA on the fibrin surface [67]. In 2000 van Tilburg et al reported that plasma TAFI levels above the 90th percentile of the distribution in control subjects were associated with a two-fold increased risk of venous thrombosis [21]. They measured TAFI antigen with an electroimmunoassay which measures both non-activated and activated TAFI. The choice of the TAFI antigen assay is important because the specificity and sensitivity of these assays for the different (iso)forms of TAFI can differ considerably [68]. Initial observations indicated that elevated TAFI levels interact with elevated FVIII levels but not with FVLeiden [21]. Also, elevated plasma TAFI might increase the risk of recurrent venous thrombosis in patients with elevated FVIII [69]. More information is needed to decide whether or not there is a significant contribution of TAFI to the risk of venous thrombosis.

There are several polymorphisms in the TAFI promoter, which influence TAFI levels. Some of these polymorphisms are strongly linked to polymorphisms in the coding and 3'-UT region of the gene [70,71]. So far there is no strong evidence that TAFI genotypes importantly influence the risk of venous thrombosis [72-74].

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