Determinants of plasma levels of von Willebrand factor and coagulation factor VIII

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Chapter 2

Plasma Coagulation Factor Levels in Venous Thrombosis

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Summary

High plasma levels of several coagulation factors have been described to be associated with an increased risk of venous thrombosis. The mechanisms underlying these associations as well as the mechanisms involved in the regulation of plasma levels of coagulation factors however, are mostly unknown. Whether or not these factors should be included in the workup of patients with venous thrombosis remains to be determined.
In this review article we discuss the present knowledge on the effects of plasma levels of coagulation factors on the development of venous thrombosis. Furthermore, we review recent findings and ideas on the mechanisms through which elevated plasma coagulation factor levels may influence thrombosis. And finally, we enter into the matter of the possible determinants of elevated plasma levels of coagulation factors.

Introduction

Venous thrombosis is a common disease with an incidence of one to two per 1000 individuals per year[1]. Even though a venous thrombosis is rarely fatal, it is a disease with considerable morbidity. Deep venous thrombosis can lead to pulmonary embolization, which may have very severe consequences[2]. A more common consequence of thrombosis is the development of post-thrombotic syndrome, of which the symptoms can vary from a mere discoloration of the skin to severe ulceration of the affected area[3].
Thrombosis is a multicausal disease[4]. Many factors can contribute to shift the balance between coagulation and anticoagulation towards a state of hypercoagulability. Such 'risk factors' can be genetically determined, acquired or even a combination of both. Because of the complex pathophysiology of the disease, it is still not possible to predict whether or not an individual will develop venous thrombosis or not. To improve our ability to predict thrombotic disease, it is important to extend our understanding of the pathophysiological processes that lead to thrombosis and to identify new risk factors for the disease. In recent years, many risk factors for thrombosis have been discovered. An important group of risk factors is formed by elevated plasma levels of various

coagulation factors. In this review article, we will discuss the present knowledge on the association between plasma levels of coagulation factors and the risk of venous thrombosis, the mechanisms through which elevated coagulation factor levels influence risk and the determinants of elevated coagulation factor levels.

Fibrinogen

Fibrinogen is the precursor of the end-product of secondary hemostasis, fibrin. The soluble fibrinogen is cleaved by thrombin to form fibrin monomers which subsequently polymerize to form an insoluble fibrin clot. Factor XIII (FXIII) covalently links fibrin chains to each other to stabilize the fibrous network. Fibrinogen is comprised of dimers of three subunits, Aα, Bβ and γ, which are encoded by three genes clustered together on chromosome 4.
In 1994, Koster et al[5] first described the association between high levels of fibrinogen and the risk of a first venous thrombosis. In 199 patients and 199 healthy control subjects from the Leiden Thrombophilia Study (LETS), individuals with fibrinogen levels over 5 g/L had a nearly fourfold increase in risk of venous thrombosis. Furthermore, there was a dose-response relationship between levels of fibrinogen and the risk of thrombosis. In 2003, Van Hylckama Vlieg et al[6] reanalyzed the LETS, now including 474 patients and 474 healthy controls. Again, an increase in the risk of thrombosis was observed for high levels of fibrinogen. However, after stratification on age, the risk remained only in the older participants of the LETS. In people younger than 45 years, no increase in risk was observed. After adjustment for other known risk factors, the increase in risk largely disappeared even in the elderly. The authors suggested that high levels of fibrinogen are not causal to the risk of thrombosis, but merely reflect other age-related risk factors. In 2000, Austin et al reported[7] only a weak association between fibrinogen levels and the risk of thrombosis in a population of 91 African-American patients with a first or a recurrent thrombosis and 185 African-American controls. The risk observed was small. In individuals with fibrinogen levels over 5 g/L, the odds ratio was 1.5 and this finding was not statistically significant. Austin et al reported the absence of a

dose response relationship between fibrinogen levels and thrombosis risk. In addition, according to Tsai et al in 2002[8] high levels of fibrinogen were not associated with venous thrombosis in the prospective Longitudinal Investigation of Thromboembolism Etiology (LITE). To our knowledge, the association between high fibrinogen levels and the risk of recurrent thrombosis has not been studied extensively.

Levels of fibrinogen are determined both environmentally and genetically[9]. Fibrinogen is an acute phase protein which levels strongly increase after strenuous exercise, trauma and inflammation. It is very likely that a thrombotic event can increase levels of fibrinogen. However, Kamphuisen et al have shown that elevated levels of fibrinogen amongst cases of the LETS were not the consequence of an acute phase reaction induced by the thrombotic event[10]. Gene polymorphisms in the fibrinogen β gene have been reported to be associated with levels of fibrinogen, however they appear not to be associated with the risk of venous thrombosis[5,7,11]. In contrast, Uitte de Willige et al[12] recently reported a gene polymorphism in the fibrinogen γ gene which was not associated with levels of total fibrinogen but with reduced levels of fibrinogen γ' and with an increased risk of venous thrombosis in the LETS. They hypothesized that the single nucleotide polymorphism (SNP) c10034t, which is linked to several other variations in the fibrinogen α and β and γ genes, influences the splicing of fibrinogen γ pre-mRNAs, resulting in altered levels of the splicing variant γ'. Uitte de Willige et al reported that decreased levels of fibrinogen γ' and particularly decreased fibrinogen γ'/total fibrinogen ratios increase the risk of venous thrombosis in the LETS.

High levels of fibrinogen are associated with venous thrombosis, but it seems unlikely that they lie in the causal pathway. It is more likely that high levels of fibrinogen reflect the presence of other risk factors that lead to the development of thrombosis. However, the study performed by Uitte de Willige et al did show that changes in the γ'/total fibrinogen ratio do affect the risk of thrombosis[12]. It is possible that changes in this ratio influence the functioning of fibrinogen or of the fibrin clots derived from it.

Prothrombin

Prothrombin is the precursor of thrombin which is the key player in secondary hemostasis. Thrombin can cleave fibrinogen to enable fibrin clot formation. Furthermore, thrombin functions as a positive feedback in the activation of secondary hemostasis and it activates platelets via interaction with and cleavage of protease-activated receptors (PARs). However, thrombin also exhibits anticoagulant and antifibrinolytic activities[13,14].

In 1996 Poort et al reported that elevated levels of prothrombin increase the risk of venous thrombosis in the LETS [15]. Levels in the highest quartile (>1.15 U/ml) increased the risk more than twofold compared to levels in the lowest quartile (<0.95 U/ml). The authors showed that there is a dose-response effect between levels and risk. These results have been confirmed in one other study[16]. Legnani et al reported in 2003 that in 152 pre-menopausal women with venous thromboembolism and 296 healthy female controls, prothrombin levels in the upper quartile (>1.11 IU/ml) increased the risk of thrombosis at least twofold. In combination with either the use of oral contraceptives or with a common SNP in the prothrombin gene, G20210A, which we will discuss below, the risk increased even further. In contrast, Folsom et al reported that in the prospective LITE study, high prothrombin antigen levels of prothrombin were not a risk factor of venous thrombosis[17].

Poort et al also described the influence of a common SNP in the 3'UTR of the prothrombin gene, G20210A, on prothrombin levels and the risk of thrombosis[15]. Heterozygous carriers of the rare 20210A allele have increased levels of prothrombin compared to homozygous wild type carriers. Also, heterozygous carriers had a nearly threefold increase in the risk of venous thrombosis. These associations have been confirmed in several independent studies[18-22]. Also, the risk of thrombosis was increased in carriers of the prothrombin 20210A allele in the prospective LITE study[17]. The G20210A variation did not increase the risk of recurrent thrombosis[23,24].

Another SNP, A19911G, associated with slightly elevated prothrombin levels in the LETS was described in 2001 by Ceelie et al[25]. Homozygous carriers of the G allele had 8 U/dl higher levels of prothrombin than homozygous carriers of the

A allele. A19911G alone did not affect the risk of venous thrombosis. However, in heterozygous carriers of the G20210A mutation, the odds ratio increased from 1.6 in 19911AA carriers to 4.7 in those with 19911G on the other allele (since 20210A is always linked to 19911A, 19911G must lie on the other allele). Several groups studied the mechanism through which these two prothrombin mutations could influence levels of prothrombin. It has been shown repeatedly that the 20210A variation leads to increased mRNA processing and an increased mRNA stability[26-28]. In 2004, Danckwardt et al showed that the 3'UTR of the prothrombin gene is highly susceptible to function-altering variations[29]. The authors also described the functionality of another 3'UTR polymorphism, C20221T, which enhances mRNA processing. It is believed that 19911G increases pre-mRNA processing, by an increase in splicing efficiency[30]. Soria et al showed in the GAIT study in 2000, that there was strong linkage between G20210A and prothrombin levels, indicating that 20210A is indeed directly responsible for increased prothrombin levels[21].

Mechanisms through which high levels of prothrombin influence thrombosis risk have been studied, but not extensively. In 2001, Castaman et al [31] studied resistance to activated protein C (APC) in 27 thrombosis families with the G20210A mutation. APC resistance increased with levels of prothrombin and with the presence of the 20210A allele. The increase in APC resistance in individuals heterozygous for G20210A and with high levels of prothrombin may help explain the increase in the risk of thrombosis. Furthermore, Kyrle et al[32] reported that the endogenous thrombin potential, a measure of thrombin-forming capacity, was strongly increased in heterozygous carriers of 20210A and even more so in homozygous carriers of 20210A compared to controls. This was confirmed by Curvers et al in 2002[33]. In addition, Koenen et al found that the APC- independent anticoagulant activity of protein S was reduced in carriers of the prothrombin 20210A allele and that this was due to the elevated prothrombin levels in these individuals[34]. Finally, it was shown by Wolberg et al in 2003[35] that increasing levels of prothrombin can alter the structure of the fibrin clot formed. The authors speculated that this alteration in fibrin clot structure may contribute to the risk of thrombosis.

The literature has provided convincing evidence for the role of prothrombin gene variations associated with elevated plasma prothrombin levels in the development of thrombosis, even though additional data from prospective studies are needed. However, it remains unclear what the precise mechanism behind these effects is.

Factor VIII

Activated factor VIII (FVIII) is the cofactor for activated factor IX (FIX) in the activation of factor X (FX). The gene encoding FVIII is located on the X chromosome and mutations in the FVIII gene can lead to the bleeding disorder, hemophilia A. For stability in the circulation and protection from proteolytic cleavage by APC, FVIII depends strongly on its carrier protein von Willebrand Factor (VWF). FVIII levels in plasma are mainly determined by VWF levels, which in turn strongly depend on ABO blood group. Levels of VWF, and therefore of FVIII are higher in individuals with non-O blood groups[36].
In 1995, Koster et al studied the influence of ABO blood group, levels of VWF and levels of FVIII on the risk of a first venous thrombosis in the first 301 patients and 301 controls of the LETS[37]. In univariate analyses, all three parameters were associated with the risk of venous thrombosis. However in a multivariate analysis, FVIII proved to be the main determinant of risk. In individuals with FVIII levels over 150 IU/dl the risk of thrombosis increased nearly five-fold compared to individuals with levels lower than 100 IU/dl and a clear dose-response relationship was observed between risk and levels of FVIII. The risk increase caused by high levels of VWF disappeared completely in the multivariate analysis, for ABO blood group pheno- and genotype however, a small effect remained[37,38]. The results from the LETS have been confirmed in a number of other independent studies [39-46]. Furthermore, in the prospective LITE study, high levels of FVIII proved to be a risk factor of thrombosis [8]. Besides increasing the risk of a first venous thrombosis, high levels of FVIII may also increase the risk of recurrences, even though the literature is not entirely consistent[24,47,48].

As mentioned above, VWF is the main determinant of levels of FVIII. However, other than ABO blood group, which explains up to 30 % of variability in VWF and FVIII levels, little is known about the determinants of high levels of VWF and FVIII. Since VWF and FVIII are acute phase proteins, it might be that the association observed between high levels of both proteins and the risk of thrombosis is merely a reflection of a post-thrombotic acute phase response. However, it has been shown in at least two studies that this is not the case[10,49] and also, high levels of FVIII are predictive to a thrombosis and not only observed to be a risk factor after a thrombotic event[8].

Several studies have indicated a genetic role in the regulation of FVIII levels [36,50-52], but no association between high levels of VWF and FVIII and variations in the VWF and FVIII genes has been found[53-56]. Recently, a haplotype in the FVIII gene was reported to be associated with a small decrease in FVIII levels and, correspondingly, with a decrease in risk[57,58]. Surprisingly, the risk decrease associated with this haplotype could only partially be explained by the decrease in FVIII levels[58].

To identify the genetic determinants of increased levels of FVIII, proteins that influence secretion and clearance of FVIII and VWF are being studied. Adrenalin infusion causes a rise in levels of VWF and FVIII. Since the rise in levels can be blocked by β blockers, this effect is believed to be mediated through the β adrenergic receptors[59]. Therefore, it was hypothesized that variations in the genes encoding the β1 adrenergic receptor (ADBR1) or the β2 adrenergic receptor (ADBR2) could influence levels of VWF and FVIII. However, even though the results are not completely consistent, it was shown that functional variations in these genes are not associated with levels of VWF and FVIII[60,61]. Another gene that was studied for its association with FVIII levels encodes the LDL receptor related protein (LRP). It was shown in both in vitro and in vivo studies that LRP is involved in the plasma clearance of FVIII [62,63]. However, again, no association was observed between LRP gene variations and FVIII levels[64].

The mechanism through which high FVIII levels could influence thrombosis risk has not been fully clarified. It has been reported that high levels of FVIII

associate with decreased APC sensitivity[65,66]. In 2001 O'Donnell showed that high levels of FVIII can drastically increase thrombin generation [67]. Besides the effects of high levels of FVIII, the functionality of the protein may also play a role, since a reduction in thrombosis risk associated with a common FVIII gene haplotype could only partially be explained by a reduction in protein levels[58]. Both in retrospective and in prospective studies, high levels of FVIII are a strong risk factor of thrombosis. Even though it cannot be excluded that FVIII levels are not causal to thrombosis but reflect for instance the amount of endothelial damage, high FVIII is one of the strongest predictors of venous thrombosis. The mechanisms that underlie high FVIII levels remain to be determined.

Factor IX

FIX is a vitamin K dependent glycoprotein. It is activated by activated factor XI (FXI) or activated factor VII (FVII) and together with its cofactor activated FVIII, activated FIX activates FX. Just like the FVIII gene, the gene encoding FIX is located on the X chromosome and mutations in the FIX gene can lead to the bleeding disorder hemophilia B. High levels of FIX however may increase the risk of venous thrombosis.

In 2000, Van Hylckama Vlieg et al reported that individuals with FIX levels over the 90th percentile (>129 U/dl) have a nearly threefold increase in thrombosis risk when compared to individuals with levels below this cutoff value in the LETS[68]. The risk remained after adjustment for possible confounders. The risk of thrombosis appeared to increase linearly with FIX levels. The authors reported that the risk was slightly higher in women than in men and that the risk was highest in pre-menopausal women not using oral contraceptives. In the same year Lowe et al reported similar findings in a study consisting of 66 women with venous thromboembolism (VTE) and 163 controls between the ages of 45 and 64[69]. After adjustment for hormone replacement therapy, women with high FIX levels (>150 IU/dl) had a 2.3 fold increase in the risk of VTE. In an Austrian study, it was found that high levels of FIX also increase the risk of recurrence after a previous thrombosis[70], however, in the

LETS, high levels of FIX were not associated with recurrent thrombosis[24]. Weltermann et al followed 546 patients after finishing anticoagulant treatment for a first spontaneous thrombosis. After three years of follow-up, the relative risk of recurrence for patients with levels of FIX in the highest quartile (>138 IU/dl) was 2.2. After adjustment for other risk factors, the relative risk was smaller, 1.6, but remained significant. Furthermore, high levels of FIX increased the risk of recurrence caused by high levels of FVIII from 2.7 to 6.6. To our knowledge, the effect of high levels of FIX on the risk of thrombosis has not yet been examined in a prospective study.

Little is known about the causes of elevated levels of FIX. It has been shown that FIX levels depend on age, increasing strongly during adolescence [71]. The age-dependent regulation of FIX levels has been linked to two age regulatory elements in the 5' upstream region and the 3' untranslated region (UTR) of the FIX gene. No common genetic variations associated with high levels of FIX have been reported. Very recently, Khachidze et al[72] reported the results of a genome-wide scan in the GAIT study. No quantitative-trait loci were identified that were linked to levels of FIX.

Besides genetics, environmental factors affect levels of FIX as well. In 2001 Lowe et al showed that levels of FIX are increased in postmenopausal women on hormone replacement therapy[69]. This finding is in agreement with earlier reports of elevated factor IX levels in oral contraceptive users[73].

Like FVIII, literature seems consistent on the role of FIX in the development of thrombosis. No evidence for direct causality has been provided, but in retrospective studies, high levels of FIX are associated with venous thrombosis. Again, like in the case of FVIII, the mechanisms that underlie high FIX levels remain to be determined.

Factor XI

FXI is a component of the contact system which is activated by FXII after contact with a thrombogenic surface, but can also be activated by thrombin[74,75]. FXI plays a role in the formation of fibrin, but is indirectly also involved in the

inhibition of fibrinolysis, via a stimulatory effect on the activation of thrombin activatable fibrinolysis inhibitor (TAFI)[76,77].

There is only one report on the association between levels of FXI and the risk of venous thrombosis. In 2000, Meijers et al reported that high levels of FXI are a risk factor for thrombosis in the LETS[78]. The risk increased twofold in individuals with FXI levels over the 90th percentile (>120.8%). The risk remained after adjustment for other known risk factors of thrombosis and after exclusion of known genetic risk factors. A dose response relationship was observed between FXI levels and thrombosis risk. In addition, Souto et al showed in the GAIT study that there is a large phenotypic correlation between FXI and thrombosis[39]. To our knowledge, there are no data on FXI levels and thrombosis risk in prospective studies. Furthermore, it appears as if high FXI levels have no effect on the risk of recurrent thrombosis, even though Eichinger et al have reported that high FXI levels may facilitate an increase in this risk caused by high levels of TAFI[24,79].

It is unclear what factors influence levels of FXI. Gerdes et al sequenced the FXI gene in 22 patients from thrombophilic families lacking an established risk factor for thrombosis and nine patients with FXI levels higher than 140%[80]. No variations were found that associated with increased levels of FXI.

The effect of high FXI levels on the risk of thrombosis has to be confirmed in other studies.

TAFI

TAFI is a procarboxypeptidase that can be activated by enzymes as trypsin, plasmin and thrombin/thrombomodulin. Activated TAFI can cleave off carboxy-terminal lysine residues of fibrin polymers that are important for the binding of plasminogen to fibrin and thus delays the fibrinolytic process[81,82].

In 2000, Van Tilburg et al studied the effects of TAFI levels on the risk of venous thrombosis in the LETS[83]. The risk of thrombosis increased nearly 2 fold for TAFI levels over the 90th percentile (>122 U/dl). The risk remained after adjustment for possible confounders. However, there was no dose-response relation between risk and TAFI levels. High levels of TAFI also influence the

risk of recurrent thrombosis[79]. The risk of recurrence was twice as high in patients with high TAFI levels compared to patients with lower levels. Patients with high TAFI levels also had higher levels of FVIII, FIX and FXI. Patients with both high levels of TAFI and high levels of FVIII had the highest risk of recurrence. Juhan-Vague et al studied the effect of known cardiovascular risk factors on the inter-individual variability of TAFI levels[84]. The authors concluded that there is a high inter-individual variation in levels, but that this variation is poorly explained by lifestyle characteristics. In 2001, Henry et al identified seven new SNPs in the TAFI gene, five in the promoter and two in the 3' UTR. These SNPs explained up to 60% of the variability in TAFI levels[85]. Several other studies have reported on the association between TAFI gene variations and protein levels[86-88]. However, the association between TAFI SNPs and the risk of thrombosis remains inconclusive[89-93]. A haplotype that is associated with reduced TAFI levels also encodes a protein which is more stable after activation[87]. This complicates the analysis of association studies, where the result will depend on the actual TAFI assay used[87].
In contrast to other known risk factors of venous thrombosis, high levels of TAFI did not interact with Factor V Leiden[83,90]. However, in the LETS[83] high TAFI and high FVIII levels appeared to interact in enhancing the risk of thrombosis. Besides this possible interaction with FVIII, it has been shown in a rabbit thrombosis model that TAFI interacts with FXI[76].
The effects of TAFI on thrombosis need further confirmation by other independent studies. The lack of a dose-response effect between TAFI levels and thrombosis risk and the lack of strong associations between TAFI gene variations and thrombosis risk indicates that TAFI may not be causally related to venous thrombosis.

Factors V, VII, X and XII

The associations between the risk of venous thrombosis and high levels of several coagulation factors, other than the factors discussed above, have been studied. However the results of these studies were either negative or inconclusive and further studies have not been performed.

Factor V (FV), which, when activated, can accelerate the conversion of prothrombin to thrombin by activated FX, is one of the most extensively studied coagulation factors with respect to the risk of thrombosis. A mutation in the FV gene, FV Leiden, causes APC resistance and is a strong risk factor for venous thrombosis[94]. However, this is a functional change in the FV molecule, whereas neither high nor low levels of FV are associated with the risk of venous thrombosis[95].
Inconclusive results were observed for the protein involved in the initiation of the coagulation cascade, FVII. In the prospective LITE study, high levels of FVII were a possible determinant of venous thrombosis[8], but in the LETS, no association between FVII and thrombosis risk was observed[5].
High levels of FX are associated with risk of thrombosis, as shown by de Visser et al in the LETS, however, the association disappeared completely after adjustment for other vitamin K dependent factors[96].
Finally, levels of FXII, a component of the contact system proved not to associate with the risk of venous thrombosis in at least two studies[97,98].

Conclusions

Elevated levels of some, but not all coagulation factors are important risk factors of venous thrombosis. High levels of factors VIII and IX showed to be strong predictors of thrombosis on their own. Clustering of high levels of these factors contributes to an even higher risk of venous thrombosis than either factor alone[70,99]. Intermediate dose dependent effects on thrombosis risk were observed for elevated prothrombin and factor XI levels. However more data, especially from prospective studies are needed to confirm these results. High levels of other factors, such as fibrinogen and FX, are associated with venous thrombosis, but it appears as if they merely reflect the presence of others risk factors and are not predictors of thrombosis on their own. For some factors, like FVII and TAFI more research is required to determine the true association between plasma levels and the risk of thrombosis. Finally, plasma levels of for instance FV and FXII do not associate with the risk of thrombosis at all.

Despite the extensive literature on the thrombotic risk of increased levels of several coagulation factors, it still remains uncertain whether these factors should be determined in thrombophilia screening. Current data do not support screening, as the presence or absence of increased factor levels do not determine the duration of anticoagulant treatment nor do they clearly determine the risk of recurrent venous thrombosis. Furthermore, the risk associated with increased factor levels is generally continuous and any cutoff value is arbitrary. Finally, the measurement of these factors is also greatly dependent on several clinical and (pre-)analytical conditions. At the time we think measurement of these factors is not to be recommended in routine thrombophilia screening. For all coagulation factors, however, it is important that the mechanisms that underlie elevated plasma levels and the mechanisms through which high levels may influence the development of thrombosis are studied further.

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