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Conditions known to be associated with an increased risk of thrombosis often are referred to as hypercoagulable or prothrombotic states. This reflects the general idea that these conditions are associated with subtle changes in the balance between (anti)coagulation and (anti)fibrinolytic pathways which may result in increased fibrin formation. Local conditions (vessel wall, flow and composition of the blood) will define whether a thrombus will be formed (initiation clot formation), whether a thrombus will obstruct the circulation (extension of the clot) and whether a thrombus will dislodge emboli (embolisation). Many different factors—alone or in combination—may influence the kinetics of fibrin formation and dissolution. These may be environmental or acquired factors, but also genetic factors. Thrombosis may occur in superficial or deep veins of the legs but also in arteries on the surface of a disrupted plaque. It is evident that we can obtain valuable information on the pathogenesis of thrombosis by identifying these risk factors.

Much effort has been invested in finding the changes in the coagulation and fibrinolytic pathways which turn the hemostatic mechanism into an thrombotic one. These changes can be caused by genetic factors or may be acquired as a result of environmental factors or disease. Further it is obvious that different mechanisms can be involved in the initial formation, the extension and the embolisation of venous and arterial thrombi. In the next paragraphs I will briefly summarize what we have learnt so far from the study of risk factors for venous thrombosis.

Venous thrombosis is a good example of a multifactorial or complex disease\(^1,2\). Both environmental and genetic factors are known to contribute to the development of the disease. Present models propose that an individual will develop thrombosis only when the cumulative effect (in terms of hypercoagulability) of environmental and genetic factors (and their interactions) exceeds a certain threshold value. Knowing the risk factors and understanding their interactions is crucial to come to individualised risk profiles and treatment or prevention.

Several environmental factors are known to be associated with an increased risk of venous thrombosis (secondary hypercoagulability states). For some of these their relation with thrombosis is obvious. For instance immobilization will result in reduced blood flow or stasis, which will favor leukocyte adherence and fibrin formation (slow removal of activated coagulation factors). Trauma and surgery generally will be accompanied by vessel wall damage, activation of the coagulation system and acute phase reactions. On the other hand pregnancy and puerperium are well-established risk factors for venous thrombosis, but the underlying mechanism is largely unknown. The same is true for the use of female hormones (oral contraceptive use, hormone replacement therapy): OC use introduces so many changes in coagulation and fibrinolytic parameters that it is difficult to decide whether the thrombotic risk is related to the sum of all changes or to one or more specific effects (e.g. the introduction of APC resistance). Also diseases like malignancies and myeloproliferative disorders are known to be associated with increased risk of venous thrombosis. It is difficult to establish the molecular basis of this association, although the observation that many tumor cells express procoagulant activity is very suggestive. So, for most of the environmental risk factors it is obvious that they may influence the local kinetics of fibrin formation in multiple ways. Studying their interaction with other risk factors (e.g. genetic risk factors) might help to identify the level at which these factors act in the coagulation system when promoting thrombus formation. For instance the strong interaction between OC use and the Factor V Leiden mutation suggests that both act at the same level in the coagulation system (prothrombinase?)

The primary hypercoagulable states include the genetic risk factors for venous thrombosis\(^1,3,4\). Apart from blood group non-O we know of at least five different genetic defects that are associated with an increased risk of venous thrombosis. It concerns defects (loss of function mutations) in the genes coding for three anticoagulant proteins (protein C, protein S and antithrombin). These deficiency states are relatively rare (prevalence of heterozygotes in the population is \(< 0.3\%\)) and genetically heterogeneous (\(> 100\) different mutations in each of these genes). Heterozygous protein C deficiency is obser-
ved in 3 % of unselected patients with a first DVT (RR = 7) and in 6 % of selected thrombophilia families. Antithrombin deficiency is found in 1 % of unselected patients (RR = 5) and in 6 % of selected thrombophilia families. The prevalence of protein S deficiency among unselected DVT patients is difficult to estimate5,6 mainly due to uncertainties in the laboratory diagnosis (free PS, total PS, age and gender specific normal ranges). The prevalence of heterozygous PS deficiency among thrombophilia families is again about 6 %. In these families carriers of a defective protein S gene have an increased risk of venous thrombosis.

The laboratory diagnosis of these deficiency states completely relies on phenotypic measurements and is severely hampered (at least in case of PC and PS deficiency) by the large overlap in values between heterozygous carriers and normals. Confirmation of the diagnosis by mutation analysis would be useful, but is not feasible in the setting of a diagnostic laboratory.

The situation is completely different for the other two genetic factors for VT: the factor V Leiden mutation and the prothrombin 20210 G/A mutation7,8. Both are gain of function mutations that concern unique mutational events (single founder haplotype). They are relatively common in the general population (population prevalence of heterozygotes is 3-16 % and 0.7-4 %, respectively). The 1691 G→A transition in exon 10 of the factor V gene causes the replacement of Arg506 by Gln (FV Leiden), which is responsible for the APC resistant phenotype of FV Leiden carriers. The G→A transition in position 20210 in the 3’ UT region of the prothrombin gene is responsible for more efficient polyadenylation of prothrombin transcripts and thus may explain the increased plasma prothrombin levels in carriers of this mutation. Both APC resistance (as measured by APTT or ETP based tests) and elevated prothrombin levels are associated with increased risk of venous thrombosis. FV Leiden explains the large majority of cases of APC resistance, while the PT 20210 A mutation is found only in a minority of the cases with elevated plasma prothrombin levels.

Familial thrombophilia is the result of the clustering of risk factors in a family2,9,10. We and others have demonstrated that familial thrombophilia is an oligogenic disease (epistatic interactions of at least two genes). It is interesting that those genes that show such an epistatic interaction do also interact in the biochemical pathways of the coagulation system (e.g. substrate, enzyme and cofactor). For example FV Leiden (substrate) interacts with protein C defects (enzyme) and with protein S defects (cofactor). FV Leiden (cofactor) also interacts with the prothrombin mutation (substrate). There is no support for the existence of interaction between a protein C gene defect and the prothrombin mutation.

We can find ≥ 2 of the known genetic risk factors in about 15 % of the thrombophilia families. In 65 % we find only one genetic defect and in 30 % none of the known genetic risk factors is segregating10. This illustrates that there still must be thrombosis susceptibility genes that we don’t know. Finding these genes is one of the major challenges for the next years.

An increasing number of risk factors for VT take a position between primary and secondary hypercoagulable states. Generally it concerns laboratory phenotypes of which the molecular basis still is not known; lupus anticoagulant, mild hyperhomocysteinemia11, elevated levels (> P90) of prothrombin8, factor VIII12, factor IX13, factor XI14, fibrinogen15 and possibly TAFI16; but also APC resistance not associated with FV Leiden17. Most of these risk phenotypes are common (per definition) in the general population (≥ 10 %) and associated with a mildly increased risk of VT (OR 2-3). Sometimes there is evidence that the OR increases with the actual level of the risk factor (e.g. F IX and F VIII). However, in most cases such a relationship is not found.

Mild hyperhomocysteinemia (> 18.5 μmol/L). Many studies have investigated the relationship between the 677 C/T polymorphism in the MTHFR gene, plasma homocysteine levels and thrombotic risk. So far there is no convincing evidence that this polymorphism is associated with an increased risk of venous thrombosis15, although under certain conditions it is associated with plasma homocysteine levels. Also the intake of vitamins (B6, B12, folic acid) seems to be an important determinant of this phenotype. It seems unlikely that the 677 C/T polymorphism is interacting with the factor V Leiden mutation18.

Elevated prothrombin (> P90; OR 1.9). SNP analysis of the prothrombin genes of individuals with an elevated plasma prothrombin level (≥ 130 U/dl) has resulted in the identification of a second polymorphism (19911 A/G) which is associated with plasma prothrombin levels19. Interestingly we found some support for an interaction between the 20210A and the 19911G alleles. However in the majority of cases an elevated prothrombin level is not explained by variations in the prothrombin gene.

Elevated factor VIII (> P90; OR 2.8) (for review see ref 20). The risk associated with elevated factor VIII levels remains after adjustment for WVF and blood group, indicating that factor VIII itself is responsible for this risk. In general elevated levels of factor VIII are a persistent finding and not caused by acute phase reactions. In the large majority of cases (75 %) an elevated factor VIII level is associated with a high WVF level (WVF is the carrier protein of factor VIII in plasma). Determinants of plasma WVF factor are blood group, polymorphisms in the WVF gene (?) and conditions associated with endothelial damage/stimulation. Individuals with non-O blood group have higher WVF levels than those carrying blood group O. This is probably related to the longer half-
life of VWF molecules, which carry non-O oligosaccharides. More recently it has been suggested that there is signaling via the \( \beta_2 \)-adrenoceptor and V2 receptor might be involved in the up-regulation of plasma factor VIII levels. For review see reference 17

Other risk phenotypes. So far there is no information on genetic determinants of elevated factor IX and factor XI levels. Although there are a few SNPs in the fibrinogen gene cluster which are associated with plasma fibrinogen levels none of these appear to be associated with increased risk for venous thrombosis. In fact it still is uncertain to what extent phenotypes like high factor IX, high factor XI and high fibrinogen are determined by genetic factors (no data on heritability). Elevated TAFI levels (> P90) are found to be associated with a very minor increase in the risk of VT. Interestingly low TAFI levels and especially SNPs/ haplotypes associated with reduced TAFI levels seem to be protective against VT in certain subgroups of patients.

In the past, the identification of deficiency states associated with bleeding has led to our understanding of the hemostatic process. Similarly identification of so called primary hypercoagulability states (genotypes and laboratory phenotypes) will further our understanding of the thrombotic process. The next step will be to find out how primary and secondary hypercoagulability states interact in increasing the risk of thrombosis. Hopefully such studies will give us clues on how these secondary hypercoagulability states may influence the kinetics of fibrin formation.

References