Venous and arterial thrombosis : associations and risk factors

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Summary and discussion

This chapter summarizes the results of the investigations presented in this thesis.

Part I

In part I, the aim was to search for new risk factors of venous thrombosis by analyzing the genetic linkage signals for venous thrombosis and for intermediate phenotypes that were observed in the GENES study (**chapters 2, 3 and 4**). Furthermore, we aimed to evaluate the implications of the findings for diagnosis and prevention of venous thrombosis (**chapters 5 and 6**).

Novel risk factors of venous thrombosis

Venous thrombosis is a multifactorial disease that demands individually tailored prognostic and diagnostic procedures. The tailoring is impeded by the fact that almost half of the genetic risk factors are unknown. Progress in DNA technology in the last decades is enabling the identification of new genetic risk factors by genome-wide linkage studies and genome-wide association studies, methods that each has their own limitations. Genome-wide linkage studies are hampered by the heterogeneity in genetic variations predisposing to a single disease, meaning that findings in one family might not have validity for other families or for the general population. Furthermore, the statistical power of this type of study is dictated by the number of affected individuals in a family which is not always large. Population-based genome-wide association studies do not have this limitation, as individuals of the study group are not related to each other. However, confounding by population admixture, false positive associations and inability to study rare genetic variations are among its disadvantages.

Following the result of a previously conducted genome-wide linkage study, in **chapter 2** we evaluated the effect of genetic variations in three genes (*PROCR, THBD* and *FOXA2*) on the levels of protein C (PC) in a large pedigree and also in the control population of LETS (Leiden Thrombophilia Study). Haplotype 3 (H3) of *PROCR* was associated with higher levels of PC in the pedigree and also in the LETS controls. This finding is in line with the result of a recently published genome-wide association study [1]. Increased levels of sEPCR, as reported before in H3 carriers [2], could not explain the higher levels of PC because, although the affinity of PC to bind to circulating EPCR is the same as the affinity for

endothelial EPCR, the PC levels in H3 carriers are much higher than that of sEPCR. We hypothesized that the amount of endothelial EPCR in H3 haplotype carriers was decreased and consequently the pool of PC bound to this fraction decreased, leaving more PC to circulate in the blood. An analysis of blood-originated endothelial cells of *PROCR* H3 carriers and non-carriers with flow cytometry did not confirm this hypothesis as we observed no difference in the expression of EPCR between cells from H3 carriers and non-carriers. Hence, higher levels of PC in H3 carriers remain to be explained from a biochemical viewpoint. Interestingly, opposite to our expectation, H3 carriership with its inherent high PC levels does not protect against venous thrombosis [2].

In **chapter 3**, the effect of different haplotypes of *NQO1* on the levels of factor (F) V and FII was investigated. We observed a negative association between FV and H4 in the pedigree, but we did not find such an association in the controls of the LETS, indicating the presence of other genetic variations on chr16 to be responsible for the linkage peaks that were first observed in the GENES study [3]. In the LETS controls, H4 carriers had lower levels of vitamine K dependent coagulation factors, especially FII and total protein S, which is plausible knowing that H4 carriers have lower or undetectable activity of the NQO1 enzyme.

We studied the risk of venous thrombosis and the levels of vitamin K dependent coagulation factors for different haplotypes of the enzymes (*VKORC1*, *GGCX and NQO1*) involved in the vitamine K cycle in **chapter 4**. Similar to other studies, we noticed no association between haplotypes of *VKORC1* and the risk of venous thrombosis in LETS [4-6]. No association existed either with haplotypes of *GGCX* and *NQO1*. The levels of a panel of coagulation factors (protein C, protein S, protein Z, FII, FVII, FIX and FX) were reduced in carriers of the VKORC1*2B haplotype which is probably due to lower expression of VKORC1 in the liver of the carriers. The strongest effect was on FIX levels; each copy of VKORC1*2B haplotype was associated with a reduction of 3.26 U/dl.

Clinical aspects of thrombophilia

In **chapter 5**, we reviewed hereditary and acquired thrombophilia and the indications for thrombophilia testing. These were discussed in relation to the impact of test results on primary and secondary prevention settings or as a family

screening tool. Routine thrombophilia screening does not seem to be justified as most individuals with thrombophilia will not develop venous thrombosis. The usefulness of thrombophilia testing for secondary prevention of venous thrombosis depends on its impact on clinical management regarding dosis or duration of anticoagulation treatment and the risk-benefit balance of prophylaxis in high-risk situations. Although some authors suggest thrombophilia testing for patients with thrombosis before 50 years of age, recurrent events, family history of venous thrombosis and thrombosis in unusual sites [7;8] a recent evidence-based guideline recommends against thrombophilia testing [9]. Family screening remains also questionable because, although the risk of thrombosis in first degree relatives of patients with thrombophilia is two- to ten-fold increased, the absolute risk of venous thrombosis is low, even in high-risk situations [10-12].

Pregnancy is associated with a 5-fold increased risk of venous thrombosis, and the risk is even higher postpartum (±20-fold) [13]; a quarter of these events is a recurrence. Since a significant decrease in recurrence rate is observed with prophylaxis, [14;15] pregnant women with a history of thrombosis are generally advised to use of anticoagulation. Thrombophilia is not an indication for prophylaxis during pregnancy or postpartum in women without a history of venous thrombosis with the possible exception of antithrombin deficiency, homozygosity for factor V Leiden or prothrombin G20210A mutations, or combined heterozygosity for both mutations [12;16]. The optimal doses of LMWH in pregnancy with respect to thrombosis recurrence risk and the risk of postpartum bleeding is not clear. The increasing number of reports of the failure of low dose prophylaxis [17-19] indicates the need for randomized clinical trials to demonstrate the safety of high doses of LMWH for prophylactic measures in pregnancy.

In **chapter 6**, we observed that postpartum hemorrhage (PPH) did not occur more often in women who were given therapeutic doses of LMWH (RR: 0.8, 95% CI: 0.5-1.4). For women who delivered vaginally, this risk estimate of no increase was firm (RR: 0.5, 95%CI: 0.3-1.1), whereas for those women who delivered by cesarean section the risk of PPH (for cesarion section a priori defined as more than 1000 mL blood loss) appeared increased but due to the low number of women the confidence interval is very wide (RR: 2.5, 95% CI: 0.3-18.9). The median blood loss was found to be similar in treated and untreated women, except for the subgroup of normal vaginal deliveries where it was lower in the LMWH users

(median difference: -100, 95% CI: -156 to -44). A likely explanation for this observation is differential use of oxcytocics in LMWH users.

Part II

In part II we addressed the relationship between venous and arterial thrombosis. We aimed to test the hypothesis that the two conditions are related by the presence of shared risk (**chapters 7, 9 and 10**). In **chapter 8**, inflammatory markers were studied as risk factors for recurrence of venous thrombosis, since several lines of evidence have indicated that inflammation promotes the development of atherosclerosis and cardiovascular disease [20;21].

Several previous studies have questioned the distinction between venous and arterial thrombosis. An increased risk of arterial thrombosis [22-25] among individuals who have had previous venous thrombosis was established in three cohort studies [26-28]. Whether this association was based on "shared risk factors" is unlikely since the corresponding risk did not differ by adjusting for age and established cardiovascular risk factors [26]. In an analysis of the Beethoven study, a large cohort study of thrombophilic families in **chapter 7**, the same modestly increased risk of arterial thrombosis in individuals with previous venous thrombosis was observed, although it did not reach statistical significance. The risk did not change by adjusting for "shared risk factors" separately and simultaneously by using a propensity score considering age, cardiovascular risk factors and presence of one ore more thrombophilic defects conditional to venous thrombosis history. Therefore we concluded that "shared risk factors" alone can not explain this association.

Generally speaking, conventional cardiovascular risk factors have, if any, a mild effect on the development of venous thrombosis [29]. It should be noted that atherosclerosis also does not raise the risk of future venous thrombotic events [30;31]. Likewise, the role of thrombophilia in pathogenesis of arterial thrombosis remains obscure, especially for the rare thrombophilic defects such as antithrombin deficiency [32;33], protein C deficiency [34;35] or protein S deficiency [34;35]. A borderline effect on myocardial infarction though has been attributed to FVL and prothrombin G20210A [36]. The effect of double heterozygosity or homozygosity for FVL or prothrombin mutations was not studied because of the low prevalence of these mutations. In a post-hoc analysis of the Beethoven study (**chapter 8**), we

observed that relatives who were double heterozygous or homozygous for FVL or the prothrombin mutation were at a nonstatistically significant 1.6 times higher risk of arterial thrombosis as compared to heterozygotes for either mutation. The risk after excluding relatives with concomitant thrombophilia was 5.1 (95% CI: 1.2-22.9). In conclusion, double heterozygosity or homozygosity for FVL or the prothrombin mutation seemed to increase the risk of arterial thrombosis.

In the context of the relation of venous and arterial thrombosis, retinal vein occlusion (RVO) is an interesting disease, since risk factors for this venous occlusion mainly are established arterial risk factors, such as hypertension, hyperlipidemia and diabetes, whereas the relationship with thrombophilia is controversial [37]. In **chapter 10**, we evaluated the role of established thrombophilic defects, of assays indicating procoagulant state as well as of platelet receptor polymorphisms that are known to increase the thrombosis tendency [38-40], in patients with idiopathic RVO. The only suggestive association was found for platelet receptor polymorphism rs5918 with a dose-dependent effect on the risk of idiopathic RVO (OR for heterozygotes: 1.7, 95% CI: 0.8-3.3 and for homozygotes: 2.8, 0.5-15.9). No association was observed for established thrombophilia and clot lysis time.

Inflammation initiated by thrombosis in the veins can possibly contribute to higher risk of arterial thrombosis after venous thrombotic events. The formed thrombin triggers inflammation in the endothelium by activating neutrophils and inducing the production of selectins, cytokines and cellular adhesion molecules [41;42]. Dysfunctional endothelium not only lacks its normal antithrombotic and fibrinolytic activity but also becomes more thrombogenic by expressing higher amounts of von Willebrand factor, tissue factor, plasminogen activator inhibitor and factor V [43]. Chronically increased CRP and IL6, however, do not seem to influence the development of new venous thrombosis [44;45]. On the contrary, acute inflammatory diseases are known to increase the risk of venous and arterial thrombosis for a short period [46;47].

In **chapter 9**, the association between high levels of inflammatory biomarkers and D-dimer and the risk of recurrent venous thrombosis was evaluated in the case population of the LETS. The risk of recurrence, adjusted for age, sex and BMI was about 2.2 times higher during ongoing inflammation, indicated by CRP levels above 3 mg/L (95% CI: 1.3-3.8). No association was noted between

cytokine levels and the risk of recurrence, probably because of the higher detection limit of beads assays compared to ELISA assays which could have led to fewer individuals with detectable levels. As previously shown, higher D-dimer levels were associated with a higher risk of recurrence (HR: 1.7, 95% CI: 0.9-3.4). Furthermore, we observed an additive effect between D-dimer and CRP. Therefore, individuals with either elevated D-dimer or CRP and those with both elevated CRP and D-dimer had a higher recurrence risk compared to patients with low CRP and D-dimer levels (HR 1.9; 95% CI 1.1-3.5 and 3.1; 1.4-7.2 respectively).

Future perspectives

Despite advances in prediction of venous thrombosis, its incidence has not been changed which indicates the need to identify new risk factors for first and recurrent venous thrombosis. In this context, genome wide association studies seem promising in finding new candidate genetic risk factors especially for particular types of thrombosis such as retinal vein thrombosis where there are still ambiguities surrounding risk factors. Interestingly, sometimes the results of these studies are in line with genome wide linkage studies, like the one mentioned about the role of variations in chromosome 20 and the levels of protein C.

The risk of arterial thrombosis rises modestly after an episode of venous thrombosis. Whether shared risk factors or chronic inflammation triggered by venous thrombosis explains this risk remains unclear. There are indications, though not strong enough, that traditional cardiovascular risk factors can not solely justify this association.

At last, as mentioned above, a randomized clinical trial study between high and prophylactic doses of LMWH will answer which dose in pregnancy is optimal with respect to efficacy and bleeding risk during pregnancy and postpartum.

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