Venous and arterial thrombosis : associations and risk factors

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General introduction and outline of the thesis

Venous thromboembolism, risk factors and prophylaxis

Venous thrombosis, encompassing the clinical spectrum of deep vein thrombosis (DVT) and pulmonary embolism (PE), poses a significant clinical and economic burden on Western societies. It occurs annually in 1 to 2 per 1000 inhabitants, and has a steep age gradient, with an incidence of up to 1 per 100 in individuals older than 80 years. [1]. Individuals with venous thrombosis constitute 0.64% of all hospital admissions [2], and two-thirds have DVT as their primary manifestation, while the remaining one-third has PE. The overall mortality rate within 30 days of an event is nearly 6%. [3] A recurrence occurs in 7 to 14% of patients within the first year after the initial event. [4] The recurrence rate is even as high as 30% in the first 10 years, and remains high lifelong. [4] Other long-term complications include post-thrombotic syndrome in nearly one-third to half of DVT patients [5], and pulmonary hypertension, which has been reported to occur in nearly 4% of patients within the first two years after the first PE.[6] These epidemiological characteristics of DVT and PE underline the importance of understanding the etiology of these events, as such understanding would promote our ability to predict and prevent risk.

Venous thrombosis is a multi-factorial disease that is influenced by genetic determinants as well as acquired risk factors such as major trauma, prolonged immobilization, surgery, oral contraceptive use, hormone replacement therapy, pregnancy and puerperium. Thrombophilia, a term coined by Nygaard and Brown[7], is used to describe the inherited tendency toward venous thrombosis. Mutations underlying thrombophilia vary from rare 'loss-of-function' mutations in natural anticoagulants to common 'gain-of-function' mutations such as factor V Leiden and prothrombin G20210A. [8] Twenty-five to 35% of individuals experiencing a first episode of DVT or PE are heterozygous or homozygous for at least one of these mutations. Classifying the risk factors as 'genetic' or 'acquired' is not always straightforward. Examples are elevated factor VIII levels and hyperhomocysteinemia that are known to increase the risk of both venous and arterial thrombosis, and that are genetically and environmentally determined. [9]

The risk factors underlying thrombophilia have a varying clinical penetrance, and the presence of a thrombophilic defect *per se* does not always result in a thrombosis. Moreover, it is well documented that individuals carrying more than

one thrombophilic defect are at higher risk than those with a single inherited risk factor. [10] However, approximately one-third of familial venous thromboembolic events remain unexplained.[11]

Appropriate prophylaxis can significantly reduce venous thrombosis related mortality and morbidity.[12] Without prophylaxis, the incidence of hospital-acquired DVT is 10 to 40% among medical or general surgical patients and 40 to 60% following major orthopedic surgery.[13;14] Certain issues remain unresolved surrounding appropriate prophylaxis, including optimal dosing in specific patient populations with respect to efficacy, safety and patient compliance. [15] For instance, the presumed lower risk of significant bleeding by low dose prophylaxis with low-molecular-weight heparin during pregnancy may be outweighed by an unacceptable high risk of venous thrombosis recurrence. [16;17]

Arterial and venous thrombosis association

Arterial thrombotic events, i.e. myocardial infarction, stroke and peripheral artery disease have long been considered an entity separate from venous thrombosis. This distinction was supported by differences in the blood clot composition, underlying risk factors, and prophylactic as well as therapeutic measures. More recent evidence indicates that these two types of thrombosis might share at least some common risk factors [18;19], and experiencing one type of thrombotic event appears to predispose to the development of the other.[20] For example, a consistent finding in several cohort studies [21-23] was that patients with a previous venous thrombotic episode had an about 50% higher risk to develop arterial thrombotic events in subsequent years than individuals without prior venous thrombosis. However, the underlying mechanisms, particularly the role of multiple thrombophilic defects and classical cardiovascular risk factors in this association have not been elucidated.

In this thesis, we address several unresolved questions. First, can we identify new hereditary thrombophilic defects in a large family with an unexplained thrombotic tendency? What are the potential clinical implications of thrombophilia testing? Is our strategy of thrombosis prophylaxis in pregnant women with thrombophilia adequate? Second, are there common risk factors that may explain the association of arterial and venous thrombosis?

Study populations

The investigations described in this thesis were performed in three previously described studies with exception of those in chapter 5 and 8. The earlier studies were the Beethoven study (chapter 6 and 9), the GENES study (chapter 2 and 3) and the Leiden Thrombophilia Study (LETS) (chapter 7, 2 and 3).

The Beethoven study

The Beethoven study consists of three prospective cohorts of thrombophilic families which were identified by probands with documented DVT, PE, or premature arterial cardiovascular diseases (any arterial thrombotic event before 50 years of age), and either hyperhomocysteinemia, prothrombin G20210A, or persistently elevated levels of factor VIII. Subjects were recruited between August 1997 and May 2004 from three academic hospitals: Academic Medical Center, Amsterdam, University Medical Center, Groningen and Academic Hospital Maastricht. Details of these studies have been published previously. [24-26] Various other thrombophilic defects were tested in all participants. Information on previous episodes of venous thrombosis, arterial cardiovascular disease, exposure to exogenous risk factors for thrombosis, anticoagulant treatment, and the presence of cardiovascular risk factors was collected by validated questionnaire and by reviewing medical records at baseline. Also, every 6 months until April 2006, all participants provided a detailed questionnaire focusing on new episodes of venous thrombosis, arterial cardiovascular diseases, exposure to risk factors, and medication use.

The GENES study

The pedigree studied for this thesis was drawn from the GENES study [27] in which Dutch thrombophilic families were included with the purpose of discovering new genetic risk factors of venous thrombosis. The probands had at least one first-degree or two second-degree family members with the same diagnosis and did not carry one of the known thrombophilic defects, i.e. factor V Leiden, prothrombin G20210A and deficiencies of antithrombin, protein C and protein S. One pedigree was selected for further investigation because it showed the highest heritability of 'endogenous thrombin potential' (ETP) levels (68%). Subsequently,

a genome wide linkage analysis was performed in the selected pedigree for several coagulation factor levels and global coagulation determinants using the 'Sequential Oligogenic Linkage Analysis Routines' (SOLAR) program.

The Leiden Thrombophilia Study (LETS)

LETS is a population based case-control study, originally meant to investigate new risk factors of venous thrombosis. Between January 1988 and December 1992, 474 patients younger than 70 years from anticoagulation clinics in Leiden, Amsterdam and Rotterdam with a first DVT of the leg or arm were included in LETS. An unrelated control for each case was selected matched on age and sex. Participants did not have overt malignancy. All participants filled out a standard questionnaire regarding risk factors of venous thrombosis.

The follow-up part of the LETS was performed to investigate the risk factors for recurrent venous thrombosis. Cases were followed as described previously [28] after anticoagulation cessation until January 2000. Information during follow-up on the occurrence of risk situations, use of anticoagulation treatment, and recurrent events was collected by repeated mailed questionnaires. Patients were interviewed by telephone when they responded positively to any item of the questionnaire or when they did not return it.

Outline of the thesis

The studies presented in this thesis follow two objectives, each presented in a separate section. The first section focuses on investigations performed in a selected pedigree from GENES that were aimed at discovering a genetic explanation for the significant quantitative trait loci that were found in a genome wide linkage study. In the other section, we examined the implications of thrombophilia testing, and safety of high doses of low-molecular-weight heparins as prophylaxis during pregnancy.

Part I

Chapter 2: We studied the linkage observed in the selected pedigree on chromosome 20 for the levels of protein C by evaluating the association between protein C levels and polymorphisms of three candidate genes encoding

thrombomodulin, endothelial protein C receptor and forkhead-box A2. In addition we assessed the association of the levels of protein C with the levels of soluble endothelial protein C receptor. To investigate the external validity of our observations, we confirmed the results in the control population of LETS.

Chapter 3: We scrutinized the linkage signals for the levels of factor (F) V and prothrombin on chromosome 16 by studying the association of haplotypes of *NQO1* (candidate gene) with the levels of FV and prothrombin in the pedigree from GENES. We performed similar analyses in the control population of the LETS. Furthermore, the associated risk of venous thrombosis with each *NQO1* haplotype was calculated in the LETS. In **chapter 4,** 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.

Chapter 5: The current knowledge of hereditary and acquired thrombophilic defects and the associated risk of venous thrombosis are reviewed in this chapter and the clinical implications for thrombophilia testing are addressed.

Chapter 6: We discussed the risk of major bleeding around the delivery, known as post-partum hemorrhage, in women who received high doses of low-molecular-weight heparin to prevent venous thrombosis.

Part II of this thesis deals with the association between arterial and venous thrombosis.

Chapter 7: We intended to confirm the increased risk of arterial cardiovascular diseases after an episode of venous thrombosis in the Beethoven study. More importantly, we investigated whether the presence of multiple cardiovascular risk factors and thrombophilic defects could explain this increased risk.

Chapter 8: In this chapter the risk of venous thrombosis recurrence, in the LETS follow-up study, in patients with low and elevated levels of cytokines as compared with patients with undetectable levels of cytokines, are presented. In addition, we investigated the influence of high D-dimer (>250 ng/ml) and CRP (>3 mg/L) on recurrence risk of venous thrombosis.

Chapter 9: Here we report the role of established thrombophilic defects, fibrinolysis markers, and polymorphisms in genes encoding platelet receptors in the pathogenesis of idiopathic retinal vein thrombosis (RVO) by studying a

large number of patients without known risk factors for RVO and a sex and age matched control group.

Chapter 10: The risk of arterial thrombosis in double heterozygous or homozygous carriers of factor V Leiden or prothrombin G20210A is compared with single heterozygous carriers of one of these mutations. This analysis was done in the relatives on the Beethoven study and was recalculated by excluding all relatives who had another co-inherited thrombophilic defect.

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