CHAPTER 1

General Introduction
VENOUS THROMBOSIS

Venous thrombosis is a condition in which an obstructive blood clot (thrombus) forms in a vein. Most commonly, venous thrombosis occurs in the deep veins of the leg. The thrombus limits blood flow through the vein, causing swelling and pain of the affected leg. A part of the thrombus may break off and travel through the bloodstream (embolize). The traveling blood clot can lodge in the lungs causing a pulmonary embolism. In the 19th century Rudolf Virchow postulated a theory, Virchow’s Triad, which proposes that venous thrombosis is caused by alterations in blood flow (i.e. stasis), vascular endothelial injury or alterations in the constitution of the blood (figure 1). The triad remains clinically relevant over 150 years later.

The average annual incidence of venous thrombosis is around 2 per 1000 individuals1,2. The incidence rises exponentially with age, from 0.001% in childhood to nearly 1% per year in the very old3. Among venous thrombosis patients approximately two-third manifests deep venous thrombosis of the leg and one-third pulmonary embolism with or without deep venous thrombosis of the leg4,5. A common consequence of deep venous thrombosis is the post-thrombotic syndrome, which develops in 20 to 50% of patients6. It is characterized by pain, heaviness, swelling and cramps in the affected leg. The disease may be fatal when complicated by pulmonary embolism5.

Figure 1. Proposed causes of venous thrombosis by Virchow

RISK FACTORS

Venous thrombosis is caused by both acquired and genetic risk factors7. Known acquired risk factors include immobilization, surgery, trauma, lupus anticoagulant, malignant disease, pregnancy, puerperium, and female hormones. Genetic risk factors are inherited abnormalities affecting blood coagulation such as deficiencies of the anticoagulants protein S, protein C and antithrombin. The factor V Leiden and the prothrombin 20210A mutation are the two most common prothrom-
botic mutations\textsuperscript{7}. Recently the contribution of lifestyle factors to the risk of venous thrombosis has gained interest.

An increasing number of studies indicate that obesity increases the risk of venous thrombosis\textsuperscript{8-15}. Biological support for the relationship between obesity and the risk of venous thrombosis arises from studies showing an increase of prothrombotic factors, such as factor VII, factor VIII, factor XII and fibrinogen, with increasing body mass index\textsuperscript{16-18}. Viewed together with the association of obesity with venous stasis\textsuperscript{12} a relation between obesity and an increased risk of venous thrombosis becomes plausible. The multicausal nature of venous thrombosis dictates that risk factors have to be present simultaneously to lead to disease. Because obesity and oral contraceptive use are common in the general population, and because factor V Leiden and the prothrombin 20210A mutation are the two most frequent prothrombotic mutations, these are good candidates to investigate gene-environment interaction.

Like obesity, smoking is a well-established risk factor for arterial disease. However, the results of studies investigating the relationship between smoking and venous thrombosis are inconsistent\textsuperscript{9,10,12,19,20}. Results vary from an adverse to a protective effect of smoking. A possible risk increasing effect may be mediated through an increase in coagulation factors in smokers compared to non smokers. It is well-known that smokers have higher fibrinogen levels\textsuperscript{21-25} and smoking cessation causes a rapid fall in plasma fibrinogen\textsuperscript{22}. Supporting data for an association between fibrinogen and the risk of venous thrombosis arises from ‘The Leiden Thrombophilia Study’ (LETS)\textsuperscript{26} and a study among African-Americans\textsuperscript{27}. Given that smoking is still common worldwide\textsuperscript{28} it is important to address the controversy between study results and elucidate if there is an effect of smoking on the risk of venous thrombosis. In addition the joint effect of smoking and oral contraceptive use on venous thrombotic risk is of interest, since for arterial disease smoking has been shown to act synergistically with oral contraceptive use\textsuperscript{29}.

Unlike obesity and smoking, moderate alcohol consumption is known for its protective effect on arterial cardiovascular disease\textsuperscript{30}. A beneficial effect of moderate alcohol consumption on the risk of venous thrombosis is also not unlikely considering the effect of alcohol consumption on several coagulation factors. Reduced levels of fibrinogen, factor VII and von Willebrand factor are reported to be associated with moderate alcohol consumption. In contrast heavy and binge alcohol drinking is associated with increased levels of fibrinogen and factor VII\textsuperscript{31}. The effect of alcohol on venous thrombotic risk has only been investigated in a few studies with varying outcomes\textsuperscript{10,12,32}.

In this thesis the association of obesity, smoking and alcohol consumption with the risk of venous thrombosis is investigated. The joint effect of overweight and
smoking with important other risk factors for venous thrombosis such as oral contraceptive use and the factor V Leiden mutation is assessed to identify possible high-risk groups, which could be of importance in medical practice.

There are important acquired risk factors for venous thrombosis that are limited to women e.g., oral contraceptive use, hormone replacement therapy, pregnancy and puerperium. During pregnancy, the risk of venous thrombosis is about 5-fold increased with an even higher risk in the postpartum period. About 15% of maternal deaths in developed countries result from pulmonary embolism, which makes pulmonary embolism the most common cause of maternal mortality in these countries. In women with thrombophilia the pregnancy related risk is further increased, with varying risk estimates from studies of different designs. We evaluated pregnancy and the postpartum period as risk factors for venous thrombosis and the joint effect of pregnancy with the factor V Leiden and the prothrombin 20210A mutation.

Genetic factors also contribute to the thrombotic risk as indicated above. The two most common prothrombotic mutations, factor V Leiden and the prothrombin 20210A mutation, are present in respectively five and two percent of the Caucasian population. In addition there are various genetic variants with a lower prevalence and a smaller contribution to the risk of venous thrombosis than these mutations. A previous analysis within the LETS study found a genetic variant associated with reduced levels, but no deficiency, of the crucial anticoagulant protein C which was also associated with an increased risk of deep venous thrombosis of the leg. Individuals with the homozygous CGT genotype were found to have a 50% to 100% greater risk of venous thrombosis than individuals who were homozygous for the common genotype. Two of the three polymorphisms tested in the LETS were considered as functionally different and were tested again in a French study with 394 healthy individuals aged 20 to 60 years. This study confirmed the link between the protein C gene polymorphisms and circulating protein C levels, and suggested a complex effect on the risk of venous thrombosis. In this thesis we investigated these two polymorphisms within the protein C gene and different combinations of these polymorphisms as risk factors for venous thrombosis.

When designing a case-control study the choice of an appropriate control group is very important. The various sources of control subjects in the numerous case-control studies performed over the years show that several options exist. We included two separate control groups in our study on the etiology of venous thrombosis and explore the consequences of the choice for a particular control group.
MEGA STUDY

All research questions addressed in this thesis were studied in a large population-based case-control study, The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study). From March 1999 till September 2004, the MEGA study included consecutive patients with a first diagnosis of venous thrombosis. Patients were selected from the files of the anticoagulation clinics in Amsterdam, Amersfoort, The Hague, Leiden, Rotterdam and Utrecht. Patients with deep venous thrombosis of the leg, pulmonary embolism or a combination of these diagnoses were included in the study. Patients with deep venous thrombosis of the arm were also included, but are not part of the analyses presented in this thesis. As control subjects partners of patients were asked to participate. An additional control group was recruited from the general population using a random digit dialing method.

Patients and control subjects filled in a questionnaire about putative risk factors for venous thrombosis. Most questions referred to a period of 12 months prior to the index date, i.e. the date of diagnosis of the thrombosis for patients and their partners and the date of filling in the questionnaire for the random control subjects. At least three months after withdrawal of anticoagulation the patients and their partners were asked to visit the anticoagulation clinic where after an overnight fast a blood sample was drawn. From June 2002 onwards, blood draws were no longer performed in patients and their partners, and sampling was restricted to DNA collection by buccal swabs sent by mail. The random controls were invited for a blood draw within a few weeks after the questionnaire was sent. Within this group buccal swabs were sent when someone refused the blood draw.

OUTLINE OF THIS THESIS

Chapter 2: We describe overweight and obesity as risk factors for venous thrombosis and the joint effect of overweight and obesity together with Factor V Leiden, the prothrombin 20210A mutation and oral contraceptive use. In addition, body weight and height were also evaluated as separate risk factors for venous thrombosis.

Chapter 3: The relative risk of venous thrombosis associated with smoking is presented. We investigated smoking status, the amount of smoking, smoking duration and the number of pack-years as risk factors for venous thrombosis. By adjusting the smoking status analyses for fibrinogen levels we examined if fibrinogen levels were part of the mechanism behind the relationship between smoking and venous
thrombosis. Also the joint effect of smoking with two major risk factors for venous thrombosis, oral contraceptive use and the factor V Leiden mutation, was investigated.

**Chapter 4:** We report the association of a third lifestyle factor with the risk of venous thrombosis. Relative risks for different amounts of alcohol consumption were calculated.

**Chapter 5:** The risk of venous thrombosis in pregnant and post-partum women is presented. We studied different stages of pregnancy and the postpartum period and the risk in carriers of the factor V Leiden or the prothrombin 20210A mutation.

**Chapter 6:** We investigated the effect of two polymorphisms within the promoter region of the protein C gene (C/T at -2405 and A/G at -2418) on risk of venous thrombosis and on plasma protein C levels. In addition the combined effect of the two polymorphisms with factor V Leiden and oral contraceptive use was investigated.

**Chapter 7:** By addressing different hypotheses within the MEGA study we describe our considerations concerning control group choice and the importance of adaptation of statistical analyses to the source of controls.
REFERENCES


