CHAPTER 12

SUMMARY AND DISCUSSION
12.1 SUMMARY

There is growing evidence that venous thromboembolism often results from an interaction between various genetic and/or exogenous risk factors. An increasing number of thrombophilic disorders has been identified, like inherited resistance to activated protein C (APC) due to factor V Leiden, and the prothrombin G20210A gene (factor II) mutation. Factor V Leiden is found in about 20% of consecutive patients with venous thromboembolism and in 50% of selected patients, the factor II mutation in 3% and 18% of these patients, respectively. This thesis mainly deals with the clinical expression of factor V Leiden and the factor II mutation, either in the presence or absence of concomitant genetic or exogenous risk factors. It further focuses on acquired APC resistance in conditions that are known as risk factors for venous thromboembolism, particularly the use of oral contraceptives, pregnancy and major surgery.

Chapter 1 reviews the epidemiology of the currently known thrombophilic disorders and their clinical relevance. It further discusses the possible clinical implications of screening for thrombophilic disorders in patients with venous thromboembolism and their relatives if an inherited disorder is demonstrated.

A prospective family cohort study to assess the incidence of venous thromboembolism was performed in asymptomatic carriers of factor V Leiden, identified by screening of first degree relatives above the age of 15 years of probands who showed to be carriers of this mutation and had had an episode of venous thromboembolism (chapter 2). A total of 1564 observation years in 470 asymptomatic carriers (234 men, mean age 43 years [range 15-88], 12 homozygous) were recorded. Nine events were observed, all in heterozygous carriers, resulting in an annual incidence of 0.58% (95%CI 0.26-1.10). The incidence of spontaneous venous thromboembolism was 0.26% (0.07-0.65) per year. The incidence per episode of surgery, trauma or immobilization was 3.5% (0.1-17.8), per pregnancy 0.0% (0.0-19.5), per year of oral contraceptive use 1.8% (0.4-5.2), and per year of use of hormone replacement therapy 2.9% (0.8-15.3).

The low absolute annual incidence of spontaneous venous thromboembolism in asymptomatic carriers of factor V Leiden does not justify routine family screening of symptomatic patients. Whether screening may be beneficial in order to adjust prophylactic strategies during high risk situations for venous thromboembolism, or to discourage estrogen use, in all identified asymptomatic carriers of the factor V Leiden mutation remains to be determined.

The results of an interim analysis of an ongoing retrospective family cohort study in first degree relatives of symptomatic carriers of the factor II mutation are described in chapter 3. The annual incidence of venous thromboembolism in 155 relatives of 42 heterozygous
Summary and discussion

The absolute incidence of venous thromboembolism in carriers of the factor II mutation seems to be comparable to that found in carriers of factor V Leiden. A limitation of this study is the small number of observation years and exposures to exogenous risk factors at time of analysis. More families need to be included and the findings should be validated in prospective cohort studies.

Chapter 4 describes an unique patient with a first episode of spontaneous venous thromboembolism at an age of 34 years who showed to be a double-homozygous carrier of factor V Leiden and the factor II mutation. Heterozygosity for one or both mutations were demonstrated in several relatives, who remarkably had not experienced venous thromboembolism, despite exposure to numerous exogenous thrombotic risk factors.

These family findings stress the need for further studies to assess the clinical implications of these common mutations in compound carriers.

In chapter 5 the results of a retrospective study are reported, that was performed in 226 patients with factor V Leiden and documented venous thromboembolism (probands) and 400 first degree relatives with this mutation to assess the contribution of concomitant genetic risk factors to the occurrence of venous thromboembolism. The factor II mutation was found in 8.3%, homozygosity for factor V Leiden in 7.2%, and inherited deficiencies of antithrombin, protein C or protein S in 4.7% of symptomatic carriers (probands and relatives), as compared with 6.0%, 3.4% and 0.9% of asymptomatic carriers, respectively. Annual incidences of venous thromboembolism in relatives were 0.57%, 1.41% and 4.76%, respectively, as compared with 0.39% in single heterozygous carriers of factor V Leiden. Multivariate analysis showed a small and non-significant additional effect of the factor II mutation on the risk of venous thromboembolism in factor V Leiden carriers (adjusted hazard ratio 1.3, 95% CI 0.5-3.8). This effect was more pronounced for homozygosity of factor V Leiden (3.9, 1.7-9.0), and inherited protein C or protein S deficiencies (17.5, 3.8-81.2).

Our data provides evidence of clustering of the evaluated genetic thrombophilic defects in symptomatic factor V Leiden carriers and supports the assumption that the clinical expression of factor V Leiden carriers depends on clustering in a part of carriers.

A retrospective cohort study was performed in 329 factor V Leiden carriers with a history of venous thromboembolism (262 probands, 67 relatives) to assess the risk of recurrence and the influence of concomitant thrombophilic disorders on the recurrence rate (chapter 6). The annual incidence of a first recurrence was estimated in relatives. The contribution of concomitant thrombophilic disorders to the recurrence rate was evaluated by a nested case-control analysis in 105 matched pairs of carriers (probands and relatives) either with or without recurrence. The overall annual recurrence rate was 2.3 per 100 patient-years. The
adjusted risk of recurrence for concomitant thrombophilic disorders was 9.1 (95% 1.3-62.8) for the factor II mutation, 1.0 (0.2-4.9) for homozygosity for factor V Leiden, 1.5 (0.2-9.5) for inherited deficiencies of protein C or S, 1.8 (0.7-4.9) for factor VIII:C levels >122%, 5.4 (1.6-18.6) for fasting homocysteine levels >15.2 μmol/L and 4.4 (1.0-18.7) for loading homocysteine levels > 45.8 μmol/L. The estimated recurrence rate ranged from 0.45 per 100 patient-years after a secondary first event in the absence of concomitant thrombophilic disorders to 4.8 per 100 patient-years when a spontaneous first event was combined with concomitant disorders.

This study provides evidence that the incidence of recurrent venous thromboembolism in heterozygous factor V Leiden carriers depends on the concomitance of other thrombophilic disorders, as well as to whether the first thrombotic event occurred spontaneously. A possible clinical implication of these findings is the need for risk stratification in heterozygous factor V Leiden carriers who experience a first episode of venous thromboembolism since their risk of recurrence may range widely. As a consequence of individual risk assessment, especially heterozygous carriers of factor V Leiden with a first spontaneous episode of venous thromboembolism may have to be tested for all known thrombophilic disorders.

The risk of fetal loss in carriers of factor V Leiden was assessed in 228 carriers of factor V Leiden (77 probands, 151 relatives) and 121 non-carrier relatives who had been pregnant at least once (Chapter 7). Fetal loss occurred in 31.6% of carriers and 22.3% of noncarriers, miscarriage (fetal loss within 20 weeks of gestation) in 29.4% of carriers and in 17.4% of noncarriers, and stillbirth (fetal loss after 20 weeks of gestation) in 5.7% of carriers and 5.0% of noncarriers. Fetal loss recurred in 10.1% of carriers and 4.1% of noncarriers (odds ratio 2.60, [95% CI, 0.96-7.03]). Adjusted odds ratios were 2.12 (1.35-3.33) for fetal loss, 2.08 (1.33-3.25) for miscarriage, and 1.60 (0.58-4.43) for stillbirth, when pregnancies in carriers and noncarriers were compared. Homozygous carriers had a greater risk of fetal loss (adjusted odds ratio 2.01, 0.94-4.32) and stillbirth (4.85, 0.82-25.58) than heterozygous carriers.

Carriers of the factor V Leiden mutation have a greater risk for fetal loss than noncarriers. These data further suggest a greater risk of recurrence of fetal loss in carriers than in noncarriers and a greater risk for fetal loss and stillbirth in homozygous carriers than in heterozygous carriers. Assuming fetal loss is due to placental thrombosis, carriers with recurrent fetal loss could benefit from anticoagulant treatment. Properly designed clinical trials are needed to assess the supposed benefit and safety of anticoagulant treatment.

Chapter 8 describes the results of a large case-control study that was performed to evaluate the effect of factor V Leiden, the factor II mutation, and inherited deficiencies of antithrombin, protein C and protein S on the risk of Budd-Chiari syndrome (BCS) or portal vein thrombosis (PVT). We compared 43 BCS patients and 92 PVT patients with 474 population-based controls. Due to the presence of liver failure and use of anticoagulants in BCS and PVT
patients, the prevalence of inherited deficiencies of antithrombin, protein C, and protein S could only be assessed by modified criteria as compared to controls. The relative risk of BCS was 11.3 (95%CI 4.8-26.5) for individuals with factor V Leiden, 2.1 (0.4-9.6) for those with the factor II mutation, and 6.8 (1.9-24.4) for those with protein C deficiency. The relative risk of PVT was 2.7 (1.1-6.9) for factor V Leiden carriers, 1.4 (0.4-5.2) for carriers of the factor II mutation, and 4.6 (1.5-14.1) for those with protein C deficiency. Antithrombin or protein S deficiency were not associated with an increased risk of BCS or PVT. The presence of any inherited thrombophilic factor was higher in BCS (33%) than PVT (19%) patients. Concurrence of either acquired or inherited thrombotic risk factors were found in 26% and 37% of patients with BCS and PVT, respectively.

These findings show that factor V Leiden and hereditary protein C deficiency appear to increase the risk of BCS and PVT. Coexistence of thrombogenic risk factors in many patients indicates that BCS and PVT can result from a combined effect of different pathogenetic mechanisms.

Chapter 9 is addressed to acquired APC resistance probably due to hormonal effects. The normalised APC sensitivity ratio (n-APC-SR) was measured in 42 women not using oral contraceptives (median age 36 years, range 24-53 years), 38 women using oral contraceptives (median age 27 years, range 19-47 years), and 38 pregnant women at 36 weeks’ gestation (median age 31 years, range 19-40 years) and compared to 43 men (median age 35 years, range 22-63 years). In men and pregnant women, n-APC-SR was also measured with a modified assay, prediluting test plasma 1:5 with factor V deficient plasma. The standard n-APC-SR showed values below the lower limit of the normal range (0.82-1.22) in 10% of women not using oral contraceptives, 32% of women using oral contraceptives, and 82% of pregnant women, as compared to 5% of men. The modified test showed no significant difference between pregnant women and men. Lowered values were found in 5% of each of these groups, corresponding with the prevalence of factor V Leiden in the general population. DNA analysis was not performed.

This study shows that oral contraceptives and particularly pregnancy are associated with acquired APC resistance, probably due to hormonal effects. The modified test seems more appropriate to identify carriers of factor V Leiden.

The thrombogenic potential of acquired APC resistance was retrospectively studied in 41 neurosurgical patients who were enrolled in the placebo group of a thromboprophylaxis trial (chapter 10). N-APC-SR, clotting activity of factors V and VIII and levels of protein C antigen were measured prior to and at days 3 and 7 after surgery. Bilateral venography was done in all patients at days 8-10 to demonstrate deep vein thrombosis. A lowered n-APC-SR was found in 76% (baseline), 80% (day 3) and 88% (day 7) of patients. It was inversely related to clotting activity of factor VIII (p=0.0003) and protein C antigen (p=0.02). Deep vein thrombosis was demonstrated in 30% of patients with a normal n-APC-SR and in 23% of patients with a lowered n-APC-SR. Pulmonary embolism was not observed. Multivariate
analysis did not identify a lowered n-APC-SR as a thrombotic risk factor, in contrast with female gender (p=0.02) and Quetelet index ($25 \text{ kg/m}^2$, p=0.006).

These findings provide no evidence that acquired APC resistance, frequently found in neurosurgical patients, contributes to their high risk of postoperative deep vein thrombosis.

In chapter 11 a study to evaluate a new screening test of the Protein C anticoagulant Pathway, the PCP™ test, is described. This test is based on activation of factor X (common pathway) by phospholipid rich Russell viper venom reagent (PRVV). The ratio of two phospholipid rich PRVV-initiated clotting times, measured with and without a protein C (PC) activator, respectively, is calculated. We measured the normalised PCP ratio (n-PCP) in 91 patients known with inherited thrombophilic defects, of whom 31 were carriers of factor V Leiden (20 heterozygotes, 11 homozygotes), 12 were carriers of the factor II mutation (10 heterozygotes, 2 homozygotes), 41 had hereditary deficiencies of protein C (n=20), protein S (n=17) or antithrombin (n=4), and 7 patients had a combination of these defects. Furthermore, 37 women using oral contraceptives and 21 pregnant women at 36 weeks’ gestation were tested. In both groups of women acquired APC resistance had previously been demonstrated (see chapter 9). Sensitivity of the PCP test test (percentage of n-PCP-SR values below the lower limit of the normal range) for detection of factor V Leiden, protein C deficiency, protein S deficiency, factor II mutation, or combined defects, was 100%, 95%, 65%, 50%, and 100%, respectively. In 62% of women using oral contraceptives and in 100% of pregnant women a lowered n-PCP-SR was found.

The PCP test showed a high sensitivity for factor V Leiden, protein C deficiency, and combined genetic defects, but was less sensitive for protein S deficiency and the factor II mutation. The lowered n-PCP-SR values, frequently found during oral contraception and in pregnancy, indicate that the test results should be interpreted cautiously in these conditions.

12.2 DISCUSSION

Although our studies show that both factor V Leiden and the factor II mutation are risk factors for venous thromboembolism, they also provide evidence that the contribution of these disorders strongly depends on the concomitance of other inherited thrombophilic defects and exogenous risk factors. However, the high prevalence of these mutations and other thrombophilic disorders, all associated with a mild risk of venous thromboembolism, increases the chance to find combined defects in the population. Therefore, it may be worthwhile to test patients with venous thromboembolism in order to assess the risk of recurrence, provided that all known thrombophilic disorders are included.

Screening of relatives seems not to be justified, unless concomitant thrombophilic disorders cosegregate with factor V Leiden or the factor II mutation within one family. Familial cosegregation is evident if it is demonstrated in the proband. It may be suspected by
thrombosis at unusual sites, like Budd Chiari syndrome and portal vein thrombosis. It should also be considered when the proportion of relatives with a history of venous thromboembolism exceeds the expected rate for factor V Leiden or the factor II mutation. In families with factor V Leiden, less than 15% of relatives will be symptomatic, since half of the relatives is carrier and the lifetime risk of thrombosis in carriers is less than 30%. Thus, a higher rate of venous thromboembolism suggests (a) concomitant disorder(s), even when not demonstrated in the proband.

Risk stratification is valuable if it has practical implications. Prolonged or even lifelong anticoagulant treatment should be considered in patients with venous thromboembolism if they are at persistently high risk of recurrence. Our data show that thromboprophylaxis for periods of exposure to exogenous risk factors is not sufficient, because a majority of recurrences were found to be spontaneous. Although the risk of major bleeding is an important limitation of anticoagulant thromboprophylaxis, it should be noticed that the bleeding risk is not necessarily the same in patients with symptomatic thrombophilic disorders and in patients who are treated with anticoagulants for other reasons. Thusfar, neither the optimal duration nor the optimal level of anticoagulant thromboprophylaxis have been assessed in symptomatic carriers of thrombophilic disorders. Actually, thromboprophylaxis is still mainly empiric and irrespective of demonstrated thrombophilic disorders.

Acquired thrombophilic disorders, separately or in combinations, may also be contributive to the development of venous thromboembolism. We showed that acquired APC resistance is frequently found in conditions, that are known exogenous risk factors for venous thromboembolism, including use of oral contraceptives, pregnancy, and neurosurgery. Although there is some evidence that acquired APC resistance is an independent risk factor, further studies are warranted to establish the contribution of this and other acquired disorders to the risk of venous thromboembolism and to assess their clinical implications.

Extensive screening for inherited (and acquired) thrombophilic disorders in all patients who have experienced venous thromboembolism and their relatives if one or more inherited disorders are demonstrated, is premature. For the time being it should preferentially be restricted to specialized centres in order to enable further studies, that are required to define selection criteria in clinical practice and to assess the clinical implications of separate thrombophilic disorders and various combinations.