Lifestyle and venous thrombosis

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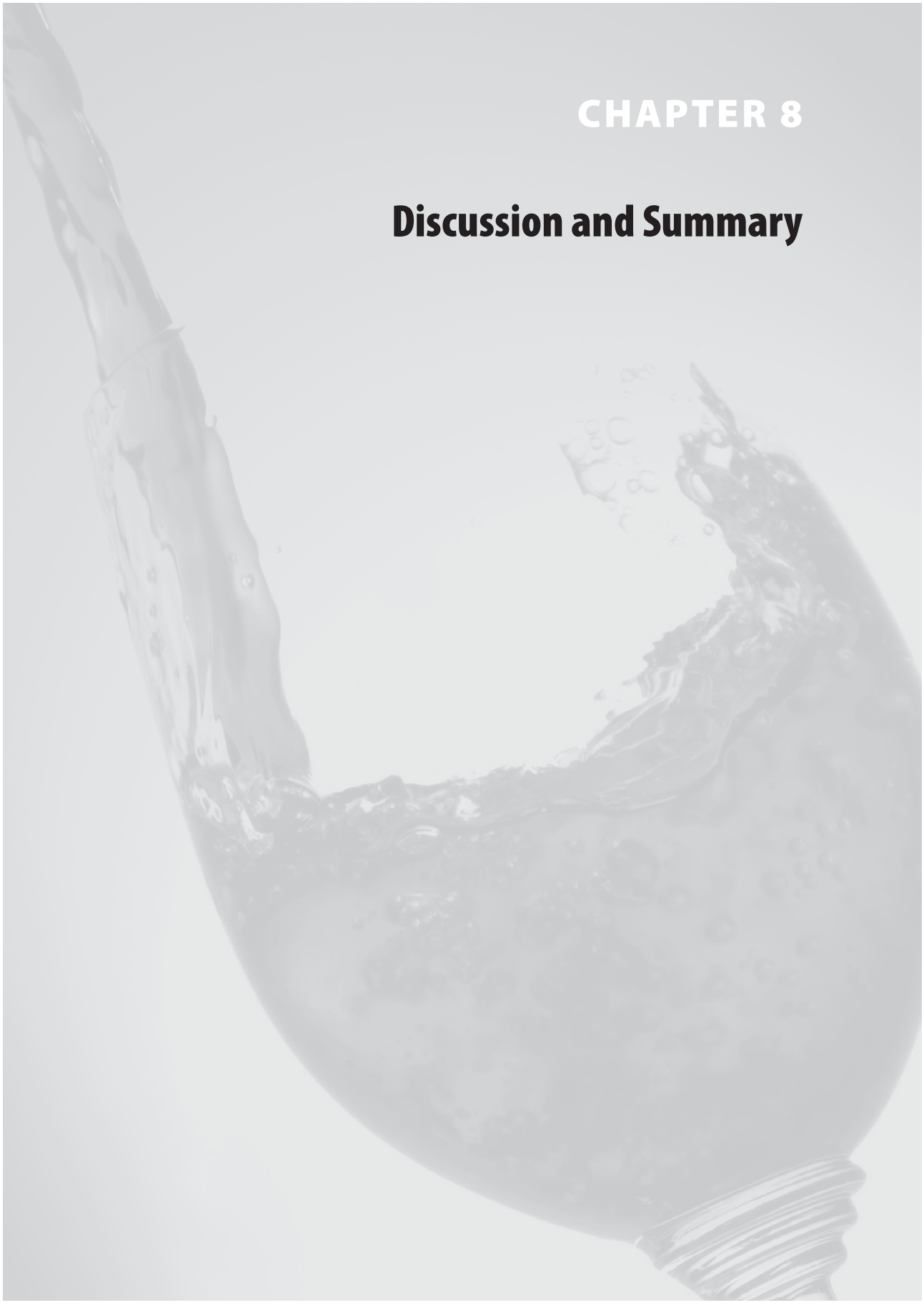

CHAPTER 8

Discussion and Summary

In the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study), a large population-based case-control study, we investigated lifestyle factors as risk factors for venous thrombosis. Overweight, smoking and alcohol consumption were addressed and pregnancy and the postpartum period were evaluated in women. Due to the large sample size of the study it was possible to investigate the joint effect of these risk factors with important genetic risk factors for venous thrombosis such as the factor V Leiden and the prothrombin 20210A mutation. In addition to these lifestyle related risk factors, two polymorphisms within the promoter region of the protein C gene were studied as risk factors for venous thrombosis and the influence of genotypic variation on plasma protein C levels was assessed. Finally, we described our experience with the inclusion of two different control groups in the MEGA study.

This discussion evaluates the main findings of this thesis and includes brief summaries of all chapters.

Recent studies indicate that obesity increases the risk of venous thrombosis[1-8]. In accordance with these studies we found that relative to those with a normal body mass index (BMI<25 kg/m^2), overweight (BMI≥25 and BMI<30 kg/m^2) increased the risk of venous thrombosis 1.7-fold and obesity (BMI≥30 kg/m^2) 2.4-fold. Body weight as a separate risk factor for venous thrombosis was also positively associated with thrombotic risk. Tall men had an increased risk of venous thrombosis, in short men a protective effect was found. This latter is remarkable, since body height is not associated with the relative amount of fat, in contrast body weight and body mass index both are. Biological support for the observed relationship between obesity and the risk of venous thrombosis arises from studies showing an increase of procoagulant factors, such as factor VII, factor VIII, factor XII and fibrinogen, with increasing body mass index[9-12]. Together with the fact that the association between body mass index and venous thrombosis is consistent over studies and shows a dose response relationship, the association is likely to be causal. The effect of obesity was more pronounced in women than men, with a 24-fold increased risk for women using oral contraceptives compared to normal weight women who did not use oral contraceptives. The joint effect of obesity with the factor V Leiden mutation or the prothrombin mutation appeared both slightly higher than the sum of the separate effects. The synergistic effect of both oral contraceptive use and factor V Leiden with obesity may be explained by the fact they all lead to APC-resistance[13;14] which is associated with a higher risk of venous thrombosis **(chapter 2)**.

The results of studies investigating the relationship of smoking with venous thrombosis are inconsistent[2;4;6;15;16]. In our study, smoking was associated with a moderately increased risk of venous thrombosis; in current smokers the risk was

1.4-fold increased and former smokers had a 1.2-fold increased risk compared to individuals who had never smoked. In current smokers the risk increased with the amount of smoking. No dose response relation was found for the number of smoking-years in either current or former smokers. In the youngest age category (<37.8 yrs) the risk of thrombosis increased with pack-years smoked, with a 4.3-fold increased risk for smokers with 20 or more pack-years. In those aged over 38, no association between pack-years and the risk of venous thrombosis was found. The presence of a dose response relationship for the amount but not the duration of smoking, the higher risk in current compared to former smokers and the finding of a dose response relationship with pack-years in young individuals only, suggests that the effect of smoking on venous thrombosis is largely an acute effect. The effect of smoking was more pronounced in women than men, which may be explained by our finding of a synergistic effect of smoking with oral contraceptive use; smoking together with oral contraceptive use resulted in an 8.8-fold increased risk compared to non-smokers who did not use oral contraceptives. This interaction between smoking and oral contraceptive use is in accordance with the results of studies on myocardial infarction[17]. To investigate a mechanism for the association between smoking and venous thrombosis we adjusted our analyses for fibrinogen levels, hypothesizing that the risk was mediated via elevated fibrinogen levels. This adjustment, however, resulted only in slightly decreased risk estimates for current smoking, and therefore fibrinogen levels are not a crucial part of the mechanism. Besides coagulation factors, inflammatory factors may be involved. Interleukin-6 has been shown to be elevated in smokers[18] and is also associated with the risk of recurrent venous thrombosis[19]. The involvement of inflammatory factors in the etiology of venous thrombosis would be an interesting topic for future research **(chapter 3)**.

Moderate alcohol consumption is an established protective factor for cardiovascular disease[20], however the effect on venous thrombosis is unknown. In the MEGA study, alcohol consumption was associated with a decreased risk of venous thrombosis, with two to four glasses per day resulting in the strongest effect compared to abstainers. The effect appeared to be more pronounced in women than men and for pulmonary embolism than for deep venous thrombosis of the leg. In the literature, an association between moderate alcohol intake and reduced levels of fibrinogen, factor VII and von Willebrand factor has been reported[21] which may explain the relationship between alcohol consumption and the reduced risk of venous thrombosis. In our study, fibrinogen levels were decreased in individuals who consumed alcohol compared to abstainers. Factor VII and von Willebrand levels were mildly decreased in these individuals but not consistently over the categories of alcohol consumption. Therefore, the effect of alcohol seems to be mainly medi-

ated by a decrease in fibrinogen. The difference between men and women in the alcohol-related risk of venous thrombosis may be explained by the differential effects of wine and beer[22], the latter of which is consumed more by men than women. The inverse relationship between alcohol consumption and fibrinogen was most marked with wine drinking. In our study we had no information about the kind of alcoholic drinks the participants consumed. It was striking that the protective effect of alcohol was still present at high alcohol intake for pulmonary embolism but not for deep venous thrombosis of the leg. We do not have an explanation for this finding **(chapter 4)**.

In addition to these common lifestyle factors we also studied a women-specific risk factor. In women of reproductive age, over half of all venous thrombotic events are related to pregnancy[23]. During pregnancy, we found a 4.6-fold increased risk of venous thrombosis, which is in accordance with the results of other studies[24;25]. While previous reports were conflicting about the risk per trimester of pregnancy[24;26-29], we found the highest risk during the third trimester, namely an 8.8-fold increased risk. The risk of venous thrombosis during the first six weeks after delivery was very high compared to the overall pregnancy-associated risk. Our finding of an 84.0-fold higher risk during this period is however within the range of findings from the majority of other studies[29-31]. During pregnancy venous thrombosis occurred far more often in the left than in the right leg. In factor V Leiden carriers the risk of pregnancy-associated venous thrombosis was 52.2-fold increased and 30.7-fold increased in carriers of the prothrombin 20210A mutation compared to non-pregnant women without the mutation.

A consideration with the pregnancy analysis is the inclusion of patients through anticoagulation clinics. Some women with venous thrombosis during pregnancy are initially treated without involvement of the anticoagulation clinic and receive low molecular weight heparin (LMWH). Women who had their venous thrombosis during the first or second trimester are more likely to be treated with LMWH only than women with a venous thrombosis during the third trimester, the latter who are referred to the anticoagulation clinic for additional treatment after child delivery. This might have led to an underestimate of the risk of thrombosis during early stages of pregnancy in our study **(chapter 5)**.

Besides acquired risk factors, genetic factors play an important role in the etiology of venous thrombosis. The factor V Leiden and the prothrombin 20210A mutation are important risk factors, but there are many polymorphisms with a relatively small contribution to the risk of venous thrombosis. Two polymorphisms within the protein C gene (2405C/T and 2418A/G) were investigated as risk factors for venous thrombosis. Out of the various combinations of these two polymorphisms, the CC/GG genotype was associated with lowest mean protein C levels and high-

est risk of venous thrombosis. Compared to carriers of the TT/AA genotype - a genotype associated with high protein C levels - the relative risk of venous thrombosis was 1.3-fold increased in CC/GG carriers. The effect of the CC/GG genotype was mainly mediated by the 2418A/G polymorphism; the effect of the 2405C/T polymorphisms disappeared after adjustment for the 2418A/G polymorphism. The finding of low protein C levels and an elevated risk of venous thrombosis in carriers of the homozygous CC/GG genotype is in agreement with other studies[32;33]. To verify if the effect of the CC/GG genotype on the risk of venous thrombosis was truly mediated via protein C levels, low protein C levels themselves had to be associated with an increased risk of venous thrombosis. Previous studies did not investigate this relationship. We found a small increase in thrombotic risk, only for protein C levels below 81%. It seems that the risk of venous thrombosis is only influenced by protein C levels in the very low range **(chapter 6)**.

In the MEGA study we included two different control groups; partners of patients were asked to participate as control subjects and a control group was recruited using a random digit dialing (RDD) method. Asking partners as control subjects was very practical. They could be approached together with the patient, which was very efficient. Another advantage was their high participation rate (81%). They were aware of the importance of the study since they had seen the consequences of the disease in the patient. Since not all patients had a partner an additional control group was recruited with the RDD method. This method has proved to be a constructive method to collect a nearly random sample of all individuals in the population, but it is expensive and time-consuming. In the MEGA study, sixty-nine percent of eligible RDD controls participated.

In **chapter 7** we evaluated the analytic possibilities of these two different control groups and described the association of a general lifestyle risk factor (body mass index), a lifestyle risk factor in women (pregnancy) and an example of a genetic risk factor (factor V Leiden mutation) with the risk of venous thrombosis.

When evaluating body mass index as risk factor for venous thrombosis, partners and patients have more similar body mass indices than patients compared to random digit dialing controls (chapter 2). This matching between patients and partners has to be considered in the statistical analysis since ignoring matching generally introduces bias. A conditional logistic regression analysis (i.e. matched analysis) takes these similarities into account. It is important to realize that in a matched analysis only patient-partner pairs can be included, resulting in less power than an ordinary unconditional logistic regression analysis. Besides this, both patient and partner of a pair must have valid data for the required variables, otherwise the complete pair cannot be included in the analysis. Finally, the matched analysis itself only uses pairs who are discordant for the variable of interest, resulting in further reduced

power. For the analysis of body mass index, both matched analyses with partners and unmatched analyses with RDD controls showed consistent results in terms of clearly increased risks. We performed a combined analysis to obtain the most powerful estimate. A simple approach was used in which the estimates of the odds ratios of the matched and unmatched analyses were pooled[34]. In this combined analysis we accounted for the correlation between the estimated odds ratios since most patients were included both in the matched and unmatched analysis.

We assumed that asking partners would make it easier to recruit control subjects with pregnancies, malignancies or chronic diseases, which was a prerequisite if we wanted to study these diseases in relation to the risk of venous thrombosis. However, our pregnancy analysis showed that the opposite was true (chapter 5); partner controls group had fewer pregnancies than the RDD group. These findings could indicate that being pregnant for RDD controls was an extra motivation to participate, which is plausible considering the common knowledge that pregnancy is a risk factor for venous thrombosis. Because only women were included in the pregnancy analysis, simple pooling of both control groups was possible. In the combined control group pregnancy frequencies were comparable with data from the general Dutch population. These analyses illustrate that the inclusion of multiple control groups appeared to be very useful. A priori assumptions about control group characteristics were not in line with the collected data. If only a partner control group or only the RDD control group was collected, pregnancy associated risks were either over- or underestimated.

In the analysis of a genetic risk factor, frequencies of the factor V Leiden mutation were identical in both control groups and in line with published data. For the analyses of factor V Leiden as risk factor for venous thrombosis both control groups were thus equally suitable and could be simply combined.

Concluding remark

In the past, lifestyle factors as obesity, smoking and alcohol use were only considered to be associated with the risk of arterial disease. In this thesis we show that these factors are also related to the risk of venous thrombosis. Nowadays an increasing number of 'arterial risk factors' are linked with venous thrombosis[35;36]. This sharing of common risk factors between arterial and venous thrombosis suggests that the link between these two diseases is stronger than previously thought.

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