Chapter 10

Summary and Discussion
SUMMARY

Chapter 1 describes the outline of this thesis and the rationale to subdivide this thesis into two parts: risk factors for first venous thrombosis, and risk factors for recurrent venous thrombosis.

Part I: Risk factors for first venous thrombosis

Chapter 2 describes the association between pneumonia and risk of venous thrombosis in a large population-based case-control study (MEGA study), and whether this risk was influenced by immobility or unhealthy lifestyle factors. Gene-environment interactions were also assessed (i.e., the combined effect of pneumonia and prothrombotic genes), as this association could be clinically relevant for targeted thromboprophylaxis. Odds ratios were calculated as estimates of the relative risk, and were adjusted for age and sex by logistic regression methods to take the matching into account. Subgroup analysis involved stratification of patients with deep vein thrombosis of the leg only, or with pulmonary embolism with or without diagnosed deep vein thrombosis. We adjusted our findings for classical venous thrombosis risk factors, for unhealthy life style and for immobilization to determine whether the associations could be explained by confounding through these factors. The combined effect of prothrombotic genes and pneumonia was evaluated through a stratification analysis.

Pneumonia was associated with an increase in risk for venous thrombosis (5 times more likely to have venous thrombosis, odds ratio 5.0; 95% CI, 3.9-6.3). This risk was higher when pulmonary embolism was considered (odds ratio 7.9; 95% CI, 6.1-10.3, for pulmonary embolism with or without deep vein thrombosis) than when deep vein thrombosis only was considered (odds ratio 3.0; 95% CI, 2.2-4.0). None of the adjustments substantially changed the results. A joint positive effect was observed for factor V Leiden /prothrombin G20210A and pneumonia to venous thrombosis risk (odds ratios > 17).

This study shows that pneumonia is a moderate to strong risk factor for venous thrombosis. This study also provides evidence that the association between infectious or inflammatory disease and venous thrombosis cannot be explained by concurrently unhealthy life style.
In Chapter 3, we had the opportunity to study circannual variability of venous thrombosis in three regions (i.e. Milan/Italy, Leiden/the Netherlands and Tromsø/Norway). These three regions have a similar climate type (Köppen classification Cfa, Cfb, Cfb/Dfc, respectively), and are similarly situated around sea level, but differ in the annual temperature, which is highest in Milan, lower in Leiden and lowest in Tromsø. The regions are therefore matched on demographic and climate variables, but differ in terms of temperature, sunlight and air pollution. Three different data set were used in the analysis, the Multiple Environmental and Genetic Assessment (MEGA) study (Leiden), the Tromsø study (Tromsø) and from the Lombardy region (Milan). We evaluated the absolute risk differences between seasons of prevalent venous thrombosis.

Approximately half of the patients were men, and the mean age at time of thrombosis was 49 years (interquartile range 39-61). In all three studies, the prevalence of venous thrombosis was lowest in Spring as compared with the other seasons (absolute risk difference - 2.3%, 95% CI, -4.0 to -0.7). The absolute risk difference (taking Spring as a reference group) was -2.6% (95% CI, -4.3 to -1.0) to Winter, -2.2% (95% CI -3.8 to -0.6) to Summer and -2.2% (95% CI, -3.8 to -0.5) to Autumn, respectively. When unprovoked events, or incident pulmonary embolism, or men only were analyzed separately, a similar pattern was observed, that is: a lower prevalence of thrombosis in Spring than in the other seasons.

We observed a difference in the distribution of venous thrombosis over the seasons in all three regions, wherein the prevalence was lowest in Spring. Though the absolute difference of venous thrombosis between Spring and the other seasons was statistically significant, the clinical relevance is negligible. The causal relation between weather and venous thrombosis incidence could be real, although a causal interpretation of this finding is hard to provide. To proof causality, one would like to know an intervention strategy, and the intervention “Spring” seems too ambiguous to use as an intervention tool. Nevertheless, we inferred some things from this study. First, our findings cannot be explained by a sole linear increase of temperature. Second, transient infections are also not very likely to explain our results. Third, the results are also probably not explained by sunlight exposure. Fourth, air pollution, seemed also not to explain the difference in risk over the seasons. Finally, a referral bias seemed also unlikely. To conclude, in our study the risk of venous thrombosis was different between the seasons. We were unable to provide a reason for the difference in venous thrombosis risk between the seasons.
In **Chapter 4** we investigated whether the presence of factor (F)V Leiden with blood group non-O modifies the risk for venous thrombosis in individuals with different body mass index strata in a case-control study (N=11253). We observed that a progressive increase in body mass index was associated with an ever increasing risk for venous thrombosis, odds ratio 1.9 (95%CI, 1.6-2.3) for those in the upper tertile, as compared with individuals in the first body mass index tertile, blood group O, and no FV Leiden (the reference group). The addition of FV Leiden and blood group non-O in the model increased the risk in all body mass index tertiles; odds ratios were 3.8 (95%CI, 3.2-4.6) in the third body mass index tertile of individuals with blood group non-O, and 5.4 (95% CI, 3.5-8.5) in the third body mass index tertile of individuals with FV Leiden, respectively. When both FV Leiden and blood group non-O were present, odds ratios were 9.1 (95% CI, 5.9-14.0) in the first body mass index tertile, 9.4 (95% CI, 6.6-13.5) in the second body mass index tertile, and 12.5 (95% CI, 8.9-17.6) in the third body mass index tertile. In conclusion, individuals with a high body mass index, blood group non-O or factor V Leiden are at higher thrombosis risk than expected when these prothrombotic factors are analyzed separately. The high risks of venous thrombosis in some subgroups may justify targeted screening and thromboprophylaxis decisions.

In **Chapter 5** we aimed to determine whether protein S deficiency is associated with venous thrombosis in a population-based case-control study (n=5317) as it is in thrombophilic family studies. Subjects were regarded protein S deficient when protein S levels were <2.5th percentile of the controls. Free- and total protein S deficiency was not associated with venous thrombosis: free protein S <53 U/dL, odds ratio [OR] 0.82 (95%CI, 0.56-1.21) and total protein S <68 U/dL, OR 0.90 (95%CI, 0.62-1.31). Restriction, stratifications and adjustments for possible confounders did not chance findings. When lower cut-off values were applied, it appeared that individuals at risk of venous thrombosis could be identified at levels <0.10th percentile of free protein S (<33 U/dL, OR 5.4; 95% CI, 0.61-48.8). In contrast, even extremely low total protein S levels were not associated with venous thrombosis. *PROS1* was sequenced in 48 subjects with free protein S level <1st percentile (<46 U/dL), and copy number variations were investigated in 2718 subjects, including all subjects with protein S (free or total) <2.5th percentile. Mutations in *PROS1* were detected in five patients and five controls reinforcing the observation that inherited protein S deficiency is rare in the general population. Our results show that protein S testing and subsequent testing on *PROS1* mutations should not be considered in unselected patients with venous thrombosis.
Part II: Risk factors for recurrent venous thrombosis

Chapter 6 is a review which outlines what is currently known about the epidemiology of recurrent venous thrombosis, and focuses in more detail on potential new risk factors for venous recurrence. The general implications of these findings in patient management are discussed. Venous thrombosis, including deep vein thrombosis and pulmonary embolism, is a common disease that frequently recurs. Recurrence can be prevented by anticoagulants, but this comes at the risk of bleeding. Therefore, prevention of recurrent venous thrombosis will be more profitable if it becomes possible both to identify more precisely those individuals who are at risk of recurrent venous thrombosis and to quantify the risk to which they are exposed. Consideration of the discussed new risk factors for recurrence may allow us a more optimal use of prophylactic strategies against recurrent venous thrombosis.

In Chapter 7, we evaluated the possibility of new strategies to classify the risk for recurrent venous thrombosis. Some patients may have experienced many venous thrombosis risk situations during their life time without developing venous thrombosis, while others may have experienced few of such risk factors and then develop venous thrombosis idiopathically or after a single provoked risk factor. We hypothesized that those who had 'survived' many risk situations without developing an event would, after a first venous thrombosis, have a low recurrence risk. Patients who were included in a Brazilian tertiary hospital were followed after anticoagulation withdrawal for a first venous thrombosis. Patients with indication for indefinite anticoagulation were not included. The primary end point was objective recurrent venous thrombosis.

Recurrent venous thrombosis was recorded in 7% of 378 eligible patients. Patients with a provoked first event and positive past risk situations for venous thrombosis had an incidence rate of recurrence of 1.16 (95% confidence interval [CI], 0.47-2.39) per 100 patient-years. The incidence rate ratio of this subgroup compared with patients with a provoked event without other past risk situations for venous thrombosis was 1.1 (95% CI, 0.3-4.4). This incidence rate ratio was 3.3 (95% CI, 1.3-8.7) in patients with an unprovoked event and positive past risk situations and 5.1 (95% CI, 1.6-16.1) in patients with an unprovoked event and no past risk situations.

In this study the risk for recurrence was related with the nature of a first provoked or unprovoked event and only slightly with the presence of other past risk situations for venous thrombosis. Asking a patient about past exposure of venous thrombosis risk factors long before the occurrence of a first venous thrombosis occurred, does not provide information to classify patients at lower risk for recurrence of venous thrombosis.
In Chapter 8, we reevaluated the same cohort described in chapter 7, but now with a longer follow up. The objective was the same, i.e., to try and find a variable that could help us in the decision about to keep or not the anticoagulation treatment.

Patients, after a first confirmed venous thrombosis event, were followed for an average of 43 months after suspension of anticoagulation and the primary end point was objective recurrent venous thrombosis. Patients with a provoked first event and positive past risk situations had an incidence rate of recurrence of 1.26 (95% CI, 0.60-2.31) per 100 patient-years and this was our “reference group”. The incidence rate ratio of patients with a provoked event without other past risk situations for venous thrombosis compared with the reference group was 0.8 (95% CI 0.2-2.9). This incidence rate ratio was 2.8 (95% CI, 1.2-6.5) in patients with an unprovoked event and positive past risk situations and 7.1 (95% CI, 3.0-17.1) in patients with an unprovoked event and no past risk situations versus our control group. The incidence rate ratio was 2.5 (95% CI, 1.1-5.9) for patients without past risk situation compared with those with these history when only idiopathic first events were evaluated.

We conclude that previous risk situations for venous thrombosis affected recurrence risk only in patients with an unprovoked first event.

In Chapter 9, we performed a prospective cohort study to assess the risk of recurrence in patients with provoked and unprovoked first venous thrombosis, related to the presence or absence of prothrombotic alterations. Since prothrombotic alterations can be demonstrated in at least 50% of patients with venous thrombosis, testing patients with a first venous thrombosis has gained great interest. Subjects were patients with one previous venous thrombotic event followed from April 2000 to July 2011 at the University Hospital of Universidade Federal de Minas Gerais. The study cohort included 378 patients and the total follow-up was 1573 person-years, with a median follow-up of 43 months.

Laboratory test information was available for factor VIII activity, homocysteine, factor V Leiden, (rs6025), prothrombin G20210A (rs1799963) and blood group. We analysed these risk factors individually and grouped. We considered high factor VIII as levels > 150 IU/dL in two occasions after at least three months apart. Similarly a high level of homocysteine (> 20 µmol/L) was only considered when confirmed in a subsequent testing.
Recurrent venous thrombosis occurred in 35 (9%) patients, for an incidence of 22 per 1000 person-years. The relative risk estimates were approximately 1 for all comparisons showing no association between venous thrombosis recurrence risk and prothrombotic laboratory abnormalities. In those with abnormal prothrombotic tests and an unprovoked first event there was a 4-fold increase in the recurrence risk. From our results, we concluded that thrombophilia testing in all patients with a first venous thrombosis is not useful. Future studies are required to further elucidate whether patients with a first unprovoked event and a negative result for prothrombotic abnormalities are at reduced risk for venous thrombosis recurrence.
DISCUSSION

Venous thrombosis is a common disorder that can affect apparently healthy individuals as well as hospitalized patients. One estimate is that there are worldwide about 10 million people suffering from venous thrombosis per year with admission to hospital, with an equal incidence in high-income and low-income countries, but with more deaths in the latter (ISTH Steering Committee for World Thrombosis Day; J Thromb Haemost. 2014;12:1580–1590). Venous thrombosis is probably best understood as a multicausal disease in which more than one genetic or environmental condition coincides to produce clinically apparent thrombosis.

Venous thrombosis can easily be prevented by anticoagulants, but at the risk of side-effects, most notably hemorrhage. Anticoagulant-associated bleeding is the number one cause of iatrogenic admission to hospital. Hence, prevention should be tailored to those individuals who have the highest risk to develop venous thrombosis.

Searching for risk factors or a combination of concurrently present risk factors can improve our capacity of identification the risk situations in specific individuals, therefore reaching a personalized relation between risk and benefits for thromboprophylaxis applications. The aim of this thesis was to understand better and to search for such risk situations.

In this thesis, it was shown that pneumonia increases the risk for venous thrombosis. The positive interaction between pneumonia and factor V Leiden or prothrombin G20210A besides assets our findings, and should be taken into consideration for thromboprophylaxis decisions that should be studied in clinical trials. In our seasons study (chapter 3) we observed a difference in the distribution of venous thrombosis over the year, wherein the prevalence was lowest in Spring, and somewhat similar over the other seasons. The difference of venous thrombosis between spring and the other seasons was (2%). Although this difference may seem relevant, we concluded from this study that the clinical applicability is negligible, as we also explained in a rebuttal (see J Thromb Haemost. 2013;11:570-2) to a comment (in J Thromb Haemost. 2013;11:568-70). First, we explained that when we considered spring as our reference season (23.2% of all events occurred in spring), and compared this finding with winter (25.9% of all events occurred in winter), we would have found a 25.9/23.2 × 100 = 12% higher rate of venous thrombosis in winter than in spring. This relative increase was in the same range as the authors in the rebuttal found for risk of venous thrombosis in winter as compared with spring (19% higher rate). Next, we explained that a relative increase of a first venous thrombosis of even 19% on a bad winter day as compared with a good summer day can ever be clinically useful. As the absolute risk of venous thrombosis is 1 in
1000 persons per year or 1/365.25 in 1000 persons per day, a relative increase of 19% of a bad winter day as compared with a good summer day would mean that about 5000 persons (1000/0.19) would need to be treated with a good summer day annually to prevent 1 venous thrombotic event which is about 1.8 million per day to prevent one venous thrombosis event.

If we could give one person a summer season instead of a winter season, we would need to give this to 400 000 people to prevent one thrombotic event, and likewise for other interventions. Even if we were able to completely replace 3 months of Dutch winter by summer, for 3 months, we would need to maintain this for hundreds of years to prevent one case of thrombosis. Furthermore, the intervention ‘good summer day’, however desirable, seems a difficult tool to introduce into clinical practice. Though we tried, we were unable to provide a reason for the difference in venous thrombosis risk between the seasons. The latter is somewhat disappointing, as according to the principle of sufficient reason everything must have a reason or a cause. Others may be more successful than we were here. By courtesy of professor le Cessie, another issue about the calculated “2%” should be mentioned here, that is that spring was not prespecified as being the season in which venous thrombosis would have occurred less as compared with the other seasons and that for this reason it would have been better to compare the four observed percentages in the four different seasons to the expected percentages (25; 25; 25; 25), yielding a p-value of 0.03.

That venous thrombosis is a multicausal disease was (again) shown in chapter 4, where it was shown that individuals with a high body mass index, blood group non-O or factor V Leiden are at a 12.5 fold higher venous thrombosis risk than when these prothrombotic factors are absent. When other transient risk situations such as hormone use, immobilization and recent travel were analyzed in subgroups, the same pattern was observed. As the daily life situations described above are frequent, even a small increase in relative risks can be clinically relevant.

Changing paradigms, these two words help to discuss Chapter 5. Although individuals with very low levels of free protein S appeared to be at increased risk of unprovoked venous thrombosis (odds ratio 2.31, 95%CI, 1.06-5.05), the prevalence of venous thrombosis patients with such levels was very low (0.4%), making it impractical to use free protein S levels to help identify patients at risk in a clinical setting. Total protein S levels were not able to identify subjects at risk of venous thrombosis even when these levels are very low. Therefore, a relevant question, about the utility of searching for protein S deficiency in any patient with venous thrombosis is answered with “No”. Maybe only patients with venous thrombosis who originate form thrombophilic families should be screened for protein S deficiency.
The optimal duration of anticoagulant therapy in patients with a first episode of venous thrombosis, occurring in the absence of antecedent major risk factors, is unknown. Based on a 5-year cumulative risk of disease recurrence of 25 to 30% in these patients, current American College of Chest Physicians guidelines recommend at least 3 months of treatment with a vitamin K antagonist, and an option for life-long anticoagulation in patients at low risk of bleeding. While preventing most recurrences, this approach has some drawbacks. Whereas the risk of recurrence appears to diminish with time, the risk of anticoagulant related hemorrhage increases with ongoing anticoagulation and advancing age. Hence, the net clinical benefit of indefinite anticoagulation may vary considerably over the long term. The risk of recurrent venous thrombosis may be lower in certain patients. Therefore, at least in some patient subgroups, the risk of recurrence approximates the annual risk of anticoagulant-related major hemorrhage. Another challenge that was faced in this thesis was to identify venous thrombosis patient groups that may have a low risk of recurrence and could for this reason receive anticoagulants for only a short time.

We hypothesized (in chapters 6-8) that patients at risk for recurrent venous thrombosis, who had experienced transient provoked risk factors previously, yet not developed thrombosis at that time, would have a lower risk of recurrence than those whose first (provoked or unprovoked) event was not preceded by provoked risk factors. This hypothesis was based on the thrombosis potential model, first described by Frits Rosendaal in 1999, where all individuals have a thrombosis threshold and a thrombosis potential; when this limit is crossed venous thrombosis occurs. Therefore, if someone has already been pushed through a risk situation for venous thrombosis in the past and an event did not happen, it suggests that this person has a high thrombosis threshold (or a low thrombosis potential) and, therefore, probably a lower risk for venous thrombosis recurrence. From chapters 7 and 8 we concluded that the absence of past exposure to risk factors for venous thrombosis long before the occurrence of a first venous thrombosis occurred, may be used to classify patients with a first unprovoked venous thrombosis at higher risk for recurrence of venous thrombosis. Although the cohort in which this result originated from was validated through stratified analyses using risk factors for recurrence already described, the finding needs to be validated by other groups and in different populations before it can be incorporated in clinical practice.

We finally assessed whether identification of prothrombotic abnormalities could lead to reduced venous thrombosis recurrence due to changes in clinical management, such as prolongation of initial anticoagulant treatment or intensified prophylaxis during high-risk situations. However, as also shown by previous studies, no different recurrence risk was found. Although
we observed that patients with a first unprovoked event and prothrombotic abnormalities had an increased risk of recurrence, numbers were small and the confidence interval was wide. Another point is that we evaluated thrombophilias as one group, “present or not”, and that we could not simultaneously test for all concurrent known laboratory abnormalities related with an increased risk for first venous thrombosis. It has recently been shown in the MEGA follow-up study that such a strategy, may, after all, be useful in the prediction of recurrent thrombosis. Therefore, the finding in chapter 9 must be observed with restrictions.

In conclusion, in this thesis, the thrombosis potential model was successfully applied to several known risk factors for venous thrombosis to better understand why first and recurrent venous thrombosis can develop in an individual patient. Thanks to the old concept of the thrombosis potential model, which supposes that patients with past provoking risk factors should have a lower risk of recurrent venous thrombosis than those whose first (provoked or unprovoked) event was not preceded by provoked risk factors, has led to the new insight for clinicians that these past provoking risk factors might be of use to stratify patients at low or high risk of recurrent venous thrombosis to which they can adapt thrombosis prevention strategies.

**Future directions:**

The most important risk factor for recurrence is the presence of any triggering factor during or close to the venous thrombotic event. Therefore, the discovery of unknown/new risk factors in patients with unprovoked venous thrombosis will add to our ability to predict recurrence. An easy, costless, anamnestic test for physicians is to ask patients with venous thrombosis about preceding triggering factors, yet not developed thrombosis at that time. These patients had a lower risk of recurrence than those whose first (provoked or unprovoked) event was not preceded by provoked risk factors in this thesis and may, for this reason, be candidates who do not need to be treated with anticoagulants for a prolonged (indefinite) time. Nevertheless, one cannot be confident that this finding, as well as all other findings that are reported in this thesis, will prove true in the long run. For this reason one needs replication studies, and it is to be hoped that the results from this thesis will lead to such replication studies. That replication studies are vital to science and progress is not only true for the studies that are reported in this thesis: it is true for all studies, including clinical trials from pharmaceutical companies. The reason to specifically mention these clinical trials here, is because we are now entering an era in which the direct oral anticoagulants (DOACS) are introduced as anticoagulant treatment to patients with venous thrombosis. These trials
showed (for the specific DOAC classes) that treatment with DOAC was non-inferior to vitamin K antagonist treatment. They are now given to patients with venous thrombosis on a global scale, but none of these studies have been replicated thus far, for which the reason is unknown. It could be due to costs. Trials are expensive and are very time consuming enterprises. It could also be because patients, researchers, clinicians and/or politicians think that replication studies are unnecessary: a waste of time, effort and money. If the latter is the reason to not replicate trials (and other studies), then we cannot be sure that medical science is built on a solid foundation. Indeed, researchers showed in Nature (Begley et al, Nature 2012) that in hemato-oncology 47 out of 53 highly promising results from pharmaceutical trials could not be replicated. And the epidemiologist Ioannidis has argued that failure or non-willingness to replicate study results in medical science is a reason why most published research findings are false (Ioannidis, PLoS Med 2005). It is therefore not with modesty, but with urgency to draw the attention to the necessity to replicate research findings from all theses, including this thesis. Following such a strategy will lead to new insights from old concepts, as this thesis has shown.
Addendum

Nederlandse Samenvatting
List of publications
Curriculum Vitae
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