Emerging risk factors for venous thromboembolism: The role of commonly prescribed drugs for cardiovascular disease and inflammatory disorders

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Summary and Discussion

SUMMARY AND GENERAL DISCUSSION

The aim of this thesis was to investigate the association of commonly prescribed drugs, such as statins and glucocorticoids, with changes in hemostasis and VTE risk. In this chapter we provide an overview of our main findings. Furthermore, we considere the clinical implications and discuss directions for future research.

STATINS TO MODULATE HYPERCOAGULABILITY AND THROMBOTIC RISK

In **Chapter 2**, we performed a narrative review on the effect of statins on hemostasis and VTE risk. We observed that previous data have suggested that statins might decrease VTE risk. The JUPITER trial, which is thus far the only statin trial performed with VTE as the outcome of interest, showed that rosuvastatin may reduce the risk of VTE in 40% in apparently healthy men over the age of 50 years and women over 60 years old [1]. The authors called "apparently healthy persons" those individuals with no history of cardiovascular disease, low levels of low-density lipoprotein (LDL) cholesterol and increased levels of high-sensitivity C-reactive protein level [1]. We also described that meta-analyses of observational studies reported a VTE risk reduction of 14 - 54% and meta-analyses of randomized controlled trials (RCT) reported a 11 – 25% reduction in VTE risk among statin users [2-4]. Furthermore, a meta-analysis of observational studies showed that statins may reduce the overall risk of recurrent VTE by 27% [5].

Although these findings might encourage the use of statins for VTE prevention, the results must be interpreted with caution for several reasons. First, data from the JUPITER trial may be questioned because the results on VTE came from a secondary analysis of the original trial, in which the primary outcome was the occurrence of a first major cardiovascular event (myocardial infarction or unstable angina, stroke, arterial revascularization procedure, or confirmed death from cardiovascular causes); moreover, the results may have run into statistical problem of small numbers, since in the statin treatment arm only 34 participants developed a venous thrombotic event, and chance, or a type I error, may therefore have influenced this result. Second, meta-analysis of observational studies has limitations, related to the studies included, that cannot be fully addressed, such as confounding and particularly survivor bias [6, 7]. As examples of potential bias in observational studies, the underlying disease severity in patients selected

for statin therapy may be different, even milder than that in patients for whom statin was not indicated (healthy user effect) [8], the inclusion of prevalent statin users results in missing the events that occurred in statin users before the inclusion, early after starting treatment (survivor bias), and in addition, observational studies are not able to control for those patients who do or do not adhere to statin treatment (adherence bias) [6]. Third, meta-analyses of RCTs included non-published data, which could have led to biased estimates, and were influenced by the results of the JUPITER trial. Fourth, the meta-analysis that pointed to a potential role of statin therapy in secondary VTE prophylaxis relied on a small number of observational studies, and a biased association between statin use and prevention of VTE recurrence cannot be ruled out. So far, the use of statins for secondary VTE prophylaxis has not been evaluated in well-designed intervention studies, such as RCTs.

More high quality evidence is needed to establish whether statin therapy is effective to prevent incident or recurrent VTE. However, the only trial currently being conducted for evaluating the effect of statins use on venous events, the SAVER trial (NCT02679664), aims to determine the feasibility of recruitment of VTE patients and to assess whether rosuvastatin can reduce post-thrombotic syndrome in VTE patients. The risk of recurrent VTE may be evaluate as a secondary endpoint if the recruitment is feasible. Therefore, the question on the role of statin therapy in VTE prevention will probably remain unanswered for the following years.

A possible explanation for the lack of conducting such costly studies is the fact that a clear biological mechanism behind the supposed effect of statins on the pathophysiology of VTE is not known. For this reason, the biological effect of statins on hemostasis was reviewed in **Chapter 2**, in which we observed that basic research and clinical studies suggest that statins are capable of decreasing platelet activity [9-21], downregulate both initiation and amplification phases of coagulation [12, 14, 22-32] and stimulate fibrinolysis [33-37]. However, the mechanisms underlying the statins effect on several phases of hemostasis are not explained. Thus, the reported findings raise suspicions of a problem similar to that encountered in the parable of "the blind men and the elephant", in which blind men reported different opinions about what was an elephant after touching only one part of the animal. This means that the inability of observing a problem as a whole may lead to opposing opinions on the same issue. As a result of such

imprecise findings, the potential antithrombotic property of statins is regarded with skepticism.

The best known mechanism by which statins may impair hemostasis is the direct downregulation of TF expression on endothelium cells and monocytes [38-42]. Several studies consistently demonstrated that statins affect hemostasis particularly by downregulating TF expression on endothelium cells and monocytes through a direct inhibitory effect on transcriptional activation of TF gene [38-42]. Although this mechanism can explain, in part, the downregulation of coagulation pathways, it cannot explain the impairment in platelet function and fibrinolysis. Furthermore, TF is not associated with the risk of VTE [43] and, therefore, a decrease in TF antigen or activity could not explain the supposed effect of statins on VTE prophylaxis. In this regard, the use of standard methods to measure the individual hemostatic potential, such as global assays of coagulation or established biomarkers of VTE risk, could be helpful to provide more accurate data on the capacity of statins to revert a hypercoagulability state or even cause hypocoagulability.

Therefore, in **Chapter 3** we investigated whether rosuvastatin use decreases thrombin generation potential in patients with a prior VTE in the STAtins Reduce Thrombophilia (START) trial. Thrombin generation potential is a global coagulation test that reproduces the kinetics of thrombin formation [44, 45], reflects the coagulation potential of an individual [46-48] and also predicts the risk of a first and recurrent VTE [49-51]. The START trial is a RCT aimed to investigate if statin improves the coagulation profile in patients with prior VTE. After anticoagulation withdrawal, patients with VTE were randomized to rosuvastatin 20mg/day for 4 weeks or no intervention. The thrombin generation potential was assessed at baseline and at end of study by means of the thrombin generation assay (TGA), which is a global coagulation test using the Calibrated Automated Thrombogram® (Diagnostica Stago, France).

The primary endpoint was the difference in change in endogenous thrombin potential (ETP) and peak between rosuvastatin users and non-users. Analyses were done by intention to treat and regression models were adjusted for age and sex. The study comprised 245 patients, 126 rosuvastatin users and 119 non-users. Mean age was 58 years, 61% were men, 49% had unprovoked VTE and 75% had cardiovascular (CV) risk factors. Endogenous thrombin potential (ETP) increased from baseline to end of study in non-statin users (mean change: 97.22nM*min; 95%CI 40.92 to 153.53) and decreased in

rosuvastatin users (mean change: -24.94nM*min; 95%CI -71.81 to 21.93). The mean difference in ETP change between treatments was -120.24nM*min (95%CI -192.97 to -47.51), yielding a 10.4% ETP reduction by rosuvastatin. Thrombin peak increased in both non-statin (mean change: 20.69nM; 95%CI 9.80 to 31.58) and rosuvastatin users (mean change: 8.41nM; 95%CI -0.86 to 17.69). The mean difference in peak change between treatments was -11.88nM (95%CI -26.11 to 2.35), yielding a 5% peak reduction by rosuvastatin. Other thrombin generation parameters, such as time to peak, lag time and velocity index, did not change substantially. All these results were not materially affected when we restricted the analyses to patients who did not develop an acute infection during follow-up, and when we adjusted our findings for age and sex. Predefined subgroup analyses with regard to sex and VTE cause (provoked or unprovoked) revealed similar results as in the main analysis, while the reduction in ETP by rosuvastatin appeared more pronounced in participants with CV risk factors and with pulmonary embolism than in those without CV risk factors and with deep vein thrombosis, respectively.

We concluded that rosuvastatin 20mg/day improves the coagulation profile in patients who had a VTE by reducing thrombin generation potential after anticoagulation withdrawal. Given that thrombin generation potential is associated with a first VTE [16, 28, 29, 31] and can predict the risk of recurrent VTE [25-27, 30], our finding could be interpreted as statins having the potential to decrease the risk of recurrence. Although our results do not answer the question whether this effect of rosuvastatin on changing the coagulation profile has clinical significance in terms of reducing VTE risk, they provide a basis for interventional studies necessary to establish the efficacy of rosuvastatin on VTE prophylaxis.

Another open question is whether the effect of statins on hemostasis is dependent of the drug-related lipid-lowering effect. Studies *in vitro* have shown that statins are capable of inhibiting TF expression and platelet activity by different mechanisms from that involved in the lipid-lowering process [11, 20, 52]. From a clinical perspective, it is not possible, however, to rule out that the effect of statin use on hemostasis is due to a decrease in the serum levels of lipids, since many lipoproteins have been related to coagulation activity [16, 53, 54]. Recently, apolipoproteins (apos) A and B, which are the major functional components of high and low density lipoprotein respectively, have been associated with altered coagulation profile [53]. In **Chapter 4**, we further evaluated the association of apos C-I,-II,-III and E with hemostasis and with VTE risk. Apos C-I, C-II,

C-III and E have been associated with risk of arterial thrombotic diseases and whether these apos have prothrombotic properties and are also associated with risk of VTE is not known. The study population comprised a total of 127 VTE patients and 299 controls randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis study (1999–2004), in the Netherlands. The apos were quantified using mass spectrometry (LC/MS/MS) and their levels were analysed as continuous variable (per SD increase). In controls, increases in levels of apolipoproteins were associated with increases in levels of vitamin K dependent factors, factor XI, antithrombin and clot lysis time. Additionally, increasing apos C-III and E levels were associated with higher factor VIII and von Willebrand factor levels. Levels of C-reactive protein were not associated with any apolipoprotein. The age and sex adjusted odds ratios of apos E, C-III, CII and CI to the risk of venous thrombosis were 1.21 (95% CI, 0.98-1.49), 1.19 (0.99-1.44), 1.24 (0.95-1.61) and 1.06 (95% CI, 0.87-1.30) per SD increase, respectively. These odds ratios did not attenuate after adjustments for statin use, estrogen use, BMI, alcohol use, and self-reported diabetes. We concluded that apos C-I, C-II, C-III and E are associated with several coagulation factors. However, whether these apos are also associated with an increased risk of VTE remains to be established as numbers on VTE outcomes were small in our study. These findings are, however, in line with previous population studies that showed that apo A and B are associated with a mild increase in the risk of VTE (roughly 30-50%) despite being consistently associated with levels of coagulation markers [53]. Furthermore, only apos C-III and E were associated with levels of FVIII and VWF, which coagulation proteins are well known risk factors for VTE [55-57]. A potential direct effect of statins on coagulation factors release by the liver or by the endothelium may play a role in the association of statins use and hemostasis. Future clinical studies to investigate/ confirm the effects of statins and apos to modulate hypercoagulability and thrombotic risk are warranted.

ROLE OF GLUCOCORTICOIDS IN THROMBOTIC RISK: PLAYERS OR VIEWERS?

Systemic glucocorticoids are steroid hormones prescribed to decrease inflammation in diseases and conditions such as arthritis (rheumatoid arthritis), systemic lupus erythematosus, inflammatory bowel diseases, asthma, bronchitis, allergy and neoplasia [58-62]. These drugs are potent anti-inflammatory drugs widely used in patients with

different diseases of various severity, from autoimmune to neoplastic disorders and pulmonary diseases that, in contrast, can cause severe adverse events. Experimental studies show that glucocorticoids increase coagulation factor levels and possibly the risk of VTE. Although the risk of VTE has been reported to be two- to three-fold increased with the use of glucocorticoids [63-65], several confounders may account for the reported association, such as chronic diseases [58-62], periods of disease exacerbation or flares [59, 66, 67], comorbidities [68] and toxicity of concomitant medications [69]. The effect of the underlying disease on the risk of VTE is of particular concern. Firstly, because asthma, rheumatoid arthritis, inflammatory bowel diseases, lupus erythematosus, Sjögren syndrom, polymyositis, multiple sclerosis, immune thrombocytopenia and malignancy, which are widely treated with oral glucocorticoids, have been associated with a 3 to 9-fold increased risk of VTE [59-62, 66, 67, 70, 71]. Second, because the suppression of the underlying inflammatory process by glucocorticoid use might otherwise contribute to prevent thrombotic events [68]. The latter is in line with the observations that glucocorticoid use are associated with decreased VWF and platelet activities and increased natural anticoagulants in the context of inflammatory states [72, 73]. Such effects of glucocorticoids on coagulation markers are consistent with a downregulation in hemostasis.

Therefore, in **Chapter 5** we evaluated the risk of first and recurrent VTE associated with the use of oral glucocorticoids, addressing the issue of potential confounders by employing the self-controlled case-series (SCCS) method. The periods of exposure to oral glucocorticoids were divided into: 1) the seven day-period immediately before a prescription was given, 2) the first 7 days with glucocorticoid treatment, 3) 8-30 days with oral glucocorticoid treatment, 4) 31-180 days with oral glucocorticoid treatment and 5) >180 days with oral glucocorticoid treatment. In addition, we evaluated the effect of oral glucocorticoids on the risk of recurrent VTE in a cohort design. Patients with VTE from the MEGA study were linked to the Dutch Foundation for Pharmaceutical Statistics (SFK) register based on a combination of age, sex, digit postal code and vitamin K antagonist use within the first month after the initial VTE. Prescriptions of oral glucocorticoids in the period of the MEGA study were identified. The risk for the first VTE was estimated using SCCS method and comparing the within-patients rate of events during periods of exposure and periods of non-exposure to oral glucocorticoids. The association between oral glucocorticoids and recurrent VTE was examined using Cox regression models and two different approaches: 1) comparing the recurrence rates of

VTE in patients whose first event was associated, or not, with an oral glucocorticoid treatment and 2) comparing the recurrence rates of VTE in patients with and without current use of oral glucocorticoids.

A total of 2547 patients were linked to the SFK data register, from those 363 received at least one outpatient prescription of oral glucocorticoids. The risk for a first VTE event was 3.5-fold higher in the aggregated period of oral glucocorticoid treatment as compared with baseline periods (incident rate ratio [IRR] 3.5, 95%confidence interval [CI] 2.6-4.8). IRR of a first VTE event was 2.5 (95% CI, 1.1– 5.7) in the week before treatment started, 5.3 (95% CI, 2.9 – 9.5) during the first 7 days with treatment, 3.7 (95% CI, 2.6 – 5.2) until six months with treatment and 1.6 (95% CI, 0.8 – 3.1) after 6 months with oral glucocorticoids, as compared with baseline period. A dose-dependent relationship between oral glucocorticoid treatment and VTE risk was observed as the IRR increased from 3.4 (95% CI, 2.3 – 5.0) with 30-day cumulative doses below 300mg to 4.9 (95% CI, 1.7 – 14.0) with 30-day cumulative doses above 2000mg, as compared with baseline periods. IRRs for DVT (3.9; 95% CI, 2.9 – 9.5) and PE (3.1; 95% CI, 2.0 – 4.9) were similar and IRR for unprovoked VTE (2.4; 95% CI, 1.3 – 4.7) was lower than the IRR for provoked VTE (4.2; 95% CI, 2.9 – 6.0). The rates of recurrent VTE were elevated in patients with unprovoked VTE and in those who had their first VTE during a period of oral glucocorticoid treatment, either if the first event was otherwise classified as provoked or unprovoked. The adjusted HRs for recurrent VTE were 1.6 (95% CI, 1.2 – 2.0) in patients with unprovoked first VTE not using oral glucocorticoids at the time of their first event, 2.1 (95% CI, 1.2 – 3.8) in those who had a provoked first VTE while using oral glucocorticoids and 2.3 (95% CI, 1.2 – 7.0) in those with an unprovoked first VTE while using the drug, as compared with patients with a provoked first event not using oral glucocorticoids at the time of their first event. The risk for recurrent VTE was 2.7-fold increased (95% CI, 1.6 – 4.8) during treatment periods as compared to baseline periods and did not vary substantially according to the length of the treatment (below or above 180 days). We concluded that patients receiving oral glucocorticoids had a more than three-fold increase in the risk for first VTE event. The observed risk for VTE was associated with the underlying disease (pre-exposure period) but increased further after oral glucocorticoids were prescribed. The risk for a VTE recurrence was 2-fold increased during a period of oral glucocorticoid treatment. Additionally, patients using oral glucocorticoids at the time of their first VTE event had higher risk for subsequent events than those without glucocorticoids at that time. The results underscore that treatment

strategies to prevent VTE in patients treated with oral glucocorticoids are needed. As oral glucocorticoids are commonly prescribed for a wide range of conditions, awareness of the drug associated risk for VTE may improve treatment strategies to prevent this complication. Although there is not enough evidence to support changes in current indication for oral glucocorticoid treatment, patients with higher risk for VTE, including those with a prior VTE event, should be treated and followed with caution. Given the risk for incident and recurrent VTE associated with oral glucocorticoids, future clinical trials are warranted to examine whether prophylactic anticoagulation is beneficial for patients starting oral glucocorticoid treatment, in particular for those at higher risk for VTE. As an example, the incidence rate of VTE in patients with active inflammatory bowel disease during non-hospitalized periods is 9 per 1000 person-years (59). Assuming that the relative risk of VTE increases 3-fold when a glucocorticoid treatment is started and anticoagulation decreases the risk of VTE by 80% (74), a trial with 470 participants receiving anticoagulation and 470 receiving placebo would be statistically powered (2 sided alpha=5%, power=80%) to determine whether anticoagulants reduce the risk of VTE in these patients.

Conclusions

Statins and systemic glucocorticoids are commonly prescribed drugs that interfere with the risk of VTE in different ways. Systemic glucocorticoid use increases the relative risk of first VTE by more than three-fold and *confers* an additional 5% *absolute risk of* recurrent VTE per year. These data underscore that prior use of systemic glucocorticoids should be considered when identifying provoking factors of VTE and that treatment strategies to prevent glucocorticoid-associated VTE are needed, especially in patients with higher risk for VTE. Since there is no evidence on which is the best treatment strategy to prevent glucocorticoid-associated VTE, and given the high risk of incident and recurrent VTE associated with oral glucocorticoids, clinical trials are warranted to examine whether prophylactic anticoagulation is beneficial for patients starting an oral glucocorticoid treatment.

On the other hand, rosuvastatin use may reduce the risk of first VTE by 40%. Although the mechanisms behind this association are not fully elucidated, this thesis has shown that rosuvastatin is capable of decreasing the thrombin generation potential by 10% in patients with a prior VTE. The finding that rosuvastatin modulates the coagulation

profile supports the hypothesis that rosuvastatin may be capable of reducing the risk of recurrent VTE, while not increasing the risk of bleeding. However, clinical evidence on the effect of statins on recurrent VTE risk is lacking and the role of statin use in recurrent VTE prophylaxis needs careful evaluation/confirmation in well-defined trials.

In conclusion, this thesis has shown that both statins and systemic glucocorticoids are capable of affecting the risk of VTE, improving the knowledge on the influence of this two commonly prescribed drugs on VTE pathophysiology. These findings have the potential to further refine the assessment of VTE risk since they highlight that the use of these drugs should be considered when evaluating the risk of VTE. Finally, this thesis provides insight into new therapeutic approaches since the results underscore that treatment strategies on VTE prevention in patients already taken statins, which may be sufficient for VTE prevention, are lacking. Treatment strategies to prevent glucocorticoid-associated VTE are also needed.

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