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

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Chapter 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Venous thromboembolism (VTE) is a common thrombotic condition that causes a major disease burden worldwide. VTE comprises deep vein thrombosis (DVT), pulmonary embolism (PE) or both. Although the disease presentation is acute, long-term sequelae may occur, such as post-thrombotic syndrome and chronic pulmonary hypertension, which can be associated with severe disability and reduced life expectancy [1]. Fatal PE also accounts for the disease-associated mortality [2].

The reported annual incidence rates of the disease ranges from 0.5 to 55.9 per 10000 individuals in the population, and increases exponentially with age in both men and women [1, 3] as illustrated in Figure 2. In addition, VTE mortality rates range from 0.94 to 3.23 per 10000 individuals per year [4] and the rate of disability-adjusted life years, which is the sum of the years of life lost and the years lived with disability, is estimated to be 0.12 per 10000 individuals per year [5]. Given the disease burden, the recognition of risk factors and the development of preventive measures and adequate treatment are warranted [1].

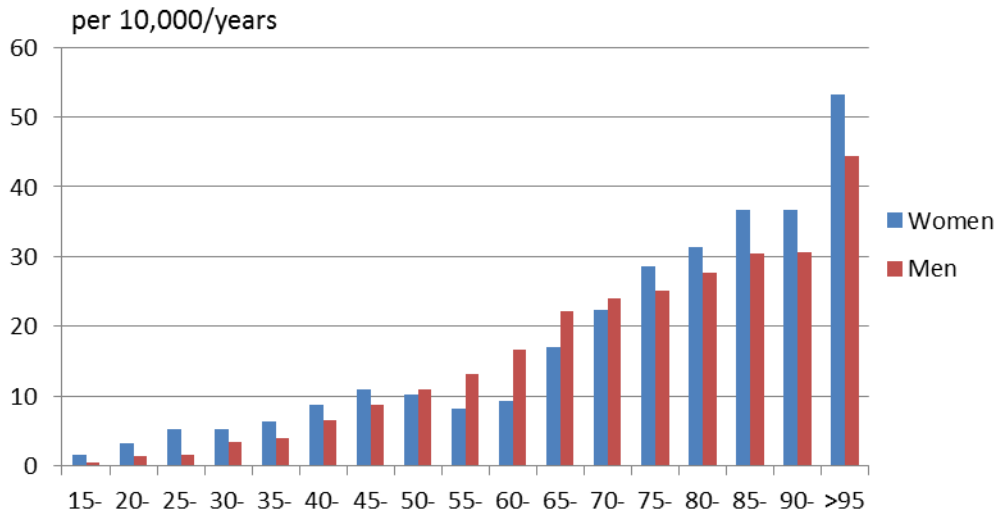

Figure 1. Incidence rates of first venous thromboembolism for men and women. Adapted from Kort et al. [3].

THE ROLE OF HEMOSTASIS IN THE PATHOGENESIS OF VTE

Hemostasis is a physiological dynamic process necessary to maintain the blood flow in a damaged vessel, which process is highly regulated to avoid either blood losses or thrombosis [6]. Hemostasis can be divided in three phases: primary hemostasis, coagulation and fibrinolysis. Primary hemostasis requires the participation of the vessel wall (mainly the media layer and the endothelium), platelet activation and von Willebrand factor (VWF), and is responsible for the formation of platelet thrombi [6].

Coagulation (or secondary hemostasis) is a cascade of enzymatic reactions that culminates in a burst of thrombin generation, and is responsible for thrombus consolidation. The coagulation pathways can be divided in initiation and amplification phases. The initiation phase starts when a damaged vascular endothelium allows plasma factor (F)VII to come into contact with tissue factor (TF)-bearing cells, the complex of TF and activated FVII activates FX and generates small amounts of thrombin [7]. In the amplification phase of coagulation, small amounts of thrombin activate FVIII and FV. Activated FVIII promotes the formation of the tenase complex (FIXa-FVIIIa) which activates FX. Activated FX moves to a complex with FVa (prothrombinase complex) that activates prothrombin (FII) and produces a large-scale thrombin burst, which is necessary for fibrinogen conversion to fibrin and thrombus consolidation [7].

Thrombin is also responsible for the activation of the anticoagulant system that, in turn, downregulates both initiation and amplification phases. Thrombin is responsible for the activation of tissue factor pathway inhibitor (TFPI), which is a protein that inhibits TF, FXa and FVIIa and downregulates the initiation phase of coagulation. Furthermore, thrombin binds to thrombomodulin (TMP) and to the endothelium protein C receptor (EPCR) to form a complex that promotes the protein C conversion to activated protein C. Activated protein C moves to a complex with protein S that inactivates factors Va and VIIIa [8].

Finally, fibrinolysis is responsible for the dissolution of the fibrin clot. The primary protein in fibrinolysis in plasmin, which is activated from plasminogen by either tissue plasminogen activator (t-PA), released by endothelial cells, and urokinase plasminogen activator (u-PA), released by monocytes. Both activators are inhibited by plasminogen activator inhibitor – 1 (PAI-1), which downregulates the fibrinolytic process [9].

The mechanisms that lead to thrombus formation was first described by Rudolf Virchow and comprise:1) reduction of blood flow, causing venous stasis; 2) vessel wall injury,

causing loss of vascular wall integrity; and 3) alteration of hemostasis in favor of a procoagulant state (hypercoagulability) [10]. Figure 2 illustrates the the Virchow triad. Interestingly, most of the risk factors for venous thrombosis are involved in pathological mechanisms that lead to hypercoagulability. Several genetic and acquired factors associated with imbalance in hemostasis have been consistently shown to increase the risk of VTE, such as increased levels of coagulation factors (FVIII, FIX, FXI, von Willebrand factor), deficiency of natural anticoagulants (protein S, protein C, antithrombin), gain of function mutations in factors V and II (FV Leiden and FII20210A), hypofibrinolysis, lupus anticoagulant, nephrotic syndrome, inflammatory diseases, estrogen use, pregnancy and malignancy [10-12]. That observation is consistent with the hypothesis that hypercoagulability is the most important component implicated in thrombus formation [6].

EMERGING RISK FACTORS FOR VTE

Despite the list of risk factors for VTE being extensive, only 50 to 60% of VTE episodes occur in the presence of major provoking factors [4]. Additionally, individuals with several risk factors may never develop VTE [11]. These observations underscore that the current knowledge on risk factors associated with VTE is not sufficient to identify all causes of the disease and potential risk factors are not being considered in the diagnostic workup [13].

Recently, inflammation has emerged as a mechanism potentially associated with thrombus formation via a process called immunothrombosis [14]. Thrombosis under certain circumstances is initiated by the innate immune system and has a major physiological role in immune defense, however when uncontrolled, immunothrombosis contributes to venous and arterial thrombosis [14]. In addition, inflammatory response leads to hypercoagulability, dyslipidemia, atherosclerosis and, in turn, increases the risk of thrombosis [15].

As hypercoagulability is the most important pathological determinant of VTE risk, theoretically any newly detected factor capable of causing impairment in the hemostatic process may cause or prevent VTE. However, determining a causal relationship between these emerging risk factors and VTE risk is challenging, as many other factors can influence or confound that association.

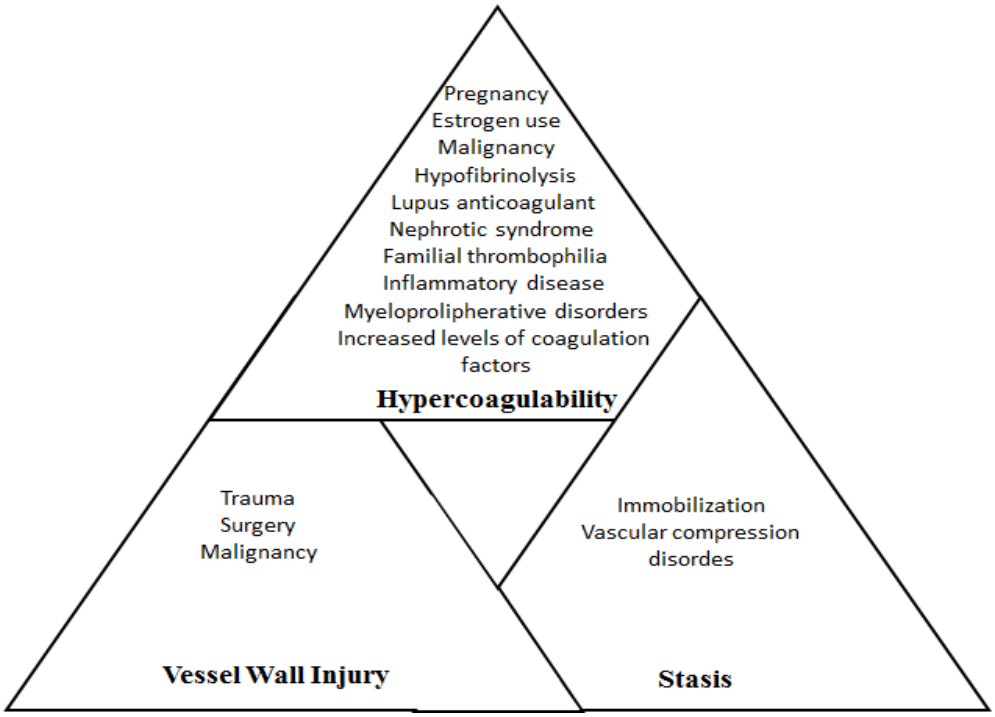

Figure 2. Virchow's triad and the pathological mechanisms by each factor affects the risk of thrombosis.

Therefore, the aim of this thesis is to identify emerging risk factors for VTE. To achieve this goal, we will describe the supposedly causal role of statin and glucocorticoid use (*i.e.* two drugs that can influence inflammation) with may lead to changes in hemostasis and VTE risk.

Statins to modulate hypercoagulability and thrombotic risk

The effect of statins on hemostasis and VTE risk confers particular therapeutic interest. Although anticoagulation therapy is indicated to prevent VTE episodes, it is not always prescribed because of the associated risk of bleeding [16]. The search for new medications capable of decreasing VTE risk without increasing the risk of bleeding is warranted. As statins do not increase the risk of bleeding, [17-19], the drug could become an alternative preventive treatment for patients who are at high risk of VTE and bleeding [20]. However, the effect of statins on reducing VTE risk is regarded with some skepticism, since the underlying mechanisms by which statins may control the hemostatic pathways are not clear.

Table 2 summarizes possible effects of statin treatment on hemostasis. Most of these mechanisms were reported “in vitro” and in animal models [20, 21] and controlled clinical

studies are scarce [21]. Furthermore, results from observational studies showing reduction on VTE risk by statins could be subject to several types of bias, while only one randomized trial was conducted to evaluate this issue.

For this reason, in **chapter 2** we performed a literature review to describe the epidemiology and potential hemostatic mechanisms behind the finding that statin use is related with a decreased risk of VTE. The review summarizes several etiological insights behind the supposed causal association, and includes recent trial evidence. It also combines and integrates different types of knowledge, *i.e.* from very fundamental to purely clinical, putting previous findings in a methodological context. The review summarizes recent data from randomized trials showing that statin therapy, in particular the high-potency statins atorvastatin and rosuvastatin, is associated with a decrease in levels of coagulation factors and D-dimer and an increase in levels of PAI-1 [22-25], even in patients with prior VTE [22]. It also highlights that, although increasing evidence indicates that statins, in particular rosuvastatin, may reduce the risk of first VTE, the estimated risk reduction varies from 11 to 54% [26, 27] and the strongest evidence is limited to a single randomized clinical trial (RCT) [28]. Clinical trials are still needed to determine if statins can play a role in recurrent VTE prophylaxis, although no randomized trial is being performed for that purpose.

In **chapter 3** we studied the effect of rosuvastatin on individual prothrombotic profiles. This was a secondary analysis of the STAtins Reduce Thrombophilia (START) trial, in which patients with prior VTE (n=247), who recently discontinued anticoagulant therapy, were randomized to rosuvastatin 20 mg/day for 4 weeks or no intervention. The primary analysis showed that rosuvastatin decreases plasma levels of FVIII, FXI and VWF [22]. In the current study, we evaluated whether rosuvastatin could affect thrombin generation potential, a coagulation marker that not only reflects the individual coagulation potential but also predicts the risk of incident and recurrent VTE. The primary outcome was the change in the endogenous thrombin potential and in the thrombin peak by rosuvastatin.

A possible explanation for the effect of statins on hemostasis is that lowering of lipids may affect hemostasis. Although this seems a reasonable pathophysiological hypothesis, no association between lipids and hypercoagulability or VTE risk has been confirmed so far [29-31]. However, only few lipids within established lipoprotein classes, such as low density lipoprotein (LDL-c), and very low-density lipoprotein (VLDL) and triglycerides, have been tested and other lipid species, in particular apolipoproteins (apos), have not been completely evaluated. Only recently, common apos A-I and B have been associated with levels of

hemostatic factors and VTE risk in population studies [30, 32]. Therefore, the effect of statins on hemostasis may be associated with the serum levels of apos. To test our hypothesis, we first evaluated whether levels of newly described apos C-II, C-III and E were associated with hemostatic factors and VTE risk. Since the development of high-sensitivity liquid chromatography–tandem mass spectrometry method for the absolute quantification of small molecules, progress has been made towards the quantification of these small apos. Since then, apos C-II, C-III and E have emerged as risk factors for arterial cardiovascular diseases. It was also demonstrated that apos C-III and E levels are reduced by statins [33]. Using this quantification method, in **chapter 4** we investigated whether these apos have prothrombotic properties and are associated with risk of VTE.

Role of glucocorticoids in thrombotic risk: players or viewers?

As with statins, glucocorticoids are frequently prescribed drugs with a potential effect on hemostasis and VTE risk. The potential effects of glucocorticoids on hemostasis are summarized in Table 3 [34-36]. However, evaluating the effect of glucocorticoids on VTE risk is challenging as the underlying condition that motivated the therapy may also be associated with thrombotic risk [37-39]. It has been shown that the risk of VTE increases two to three times in patients with chronic inflammatory diseases, such as chronic obstructive pulmonary disease [40], autoimmune diseases [41] and inflammatory bowel diseases [42, 43]. Whether the thrombotic risk associated with these diseases may also be affected by the use of glucocorticoids is not yet determined [39].

In **chapter 5** we evaluated the association of oral glucocorticoids use and the risk of incident and recurrent VTE. Confounding by indication is a major bias in any population study aiming to evaluate the effects of a drug. In **chapter 3** we overcame this potential bias by performing a randomized controlled trial (RCT). In **chapter 5**, as we aimed to evaluate an adverse effect of oral glucocorticoids, as performing a RCT is not ethically possible. Therefore, we determined the frequency of a first VTE event associated with the use of oral glucocorticoids employing the self-controlled case-series (SCCS) method. By using the SCCS method, we controlled for fixed confounding such as chronic disease and socioeconomic status [44, 45]. In order to distinguish the effects of the underlying disease from that of oral glucocorticoids on the risk of VTE, we separated the total period of exposure to oral glucocorticoids into five periods: one period immediately before the exposure, when we

expected to observe only the effect of the exacerbation of the disease on the risk of VTE and four periods with glucocorticoid treatment, which represented short and long-term glucocorticoid use [35, 46, 47]. In addition, we evaluated the effect of oral glucocorticoids on the risk of recurrent VTE in a cohort design. Studies on the association between oral glucocorticoids and recurrent VTE are unprecedented.

Finally, this thesis comprises studies that are aimed to improve the understanding of the pathophysiology of VTE, to further refine the assessment of VTE risk and to provide insight into new therapeutic approaches.

Table 2. Potential effects of statin therapy on hemostasis.

Phases of the process of hemostasis	
Primary hemostasis	
Von Willebrand levels	Decreased [48]
Platelet aggregation	Decreased [49]
Nitric oxide release	Increased [50]
P-selectin expression	Decreased [51]
Coagulation	
Tissue factor expression and activity	Decreased [52, 53]
TFPI levels	Decreased [54]
Factors Va, VII, VIII, XIII	Decreased [22, 48]
Thrombin	Decreased [53, 55]
Fibrinolysis	
Plasminogen activator inhibitor-1 (PAI-1)	Decreased [56, 57]
Tissue plasminogen activator (tPA)	Decreased [56, 57]

Table 3. Potential effects of glucocorticoid therapy on hemostasis [34, 58].

Phases of the process of hemostasis	**Absence of potentially influencing factors**	**Increased inflammatory activity setting**
Primary hemostasis		
Von Willebrand	Not changed	Decreased
Platelet aggregation	Not changed	Increased
P-selectin	Not changed	-x-
Coagulation		
Natural anticoagulants (proteins C, S and antithrombin)	-x-	Increased
Tissue factor	-x-	Increased
Prothrombin fragment 1+2	Decreased	Not changed
Antithrombin - Thrombin	Increased	Not changed
Fibrinolysis		
Plasminogen activator inhibitor-1 (PAI-1)	Increased	Increased

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