Cardiometabolic risk factors and venous thrombosis

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Chapter 1

General Introduction and Outline of this Thesis

GENERAL INTRODUCTION

Thrombosis

The obstructive clot formation that defines thrombosis is the end product of an imbalance between procoagulant, anticoagulant and fibrinolytic factors [1]. The obstructive clot formation in arteries most commonly leads to myocardial infarction and ischemic stroke. In the venous system, the blood clot is most often formed in the deep veins of the leg, i.e. deep vein thrombosis. Pulmonary embolism occurs when the blood clot dislodges from its site and embolizes to the arterial blood supply of the lungs.

The current understanding of the pathophysiology of thrombosis dates back to 1856 when the pathologist Rudolf Virchow explained thrombosis as the result of changes in blood flow, damage to the vessel wall, and changes in blood composition [2]. This broad concept is still valid. However, the mechanisms that are at the basis of venous versus arterial thrombosis are different. As depicted in Fig. 1, an arterial thrombus is typically formed after rupture of an atherosclerotic plaque (Fig. 1A), whereas venous thrombi assemble on the surface of a largely intact vessel wall (Fig. 1B) [3].

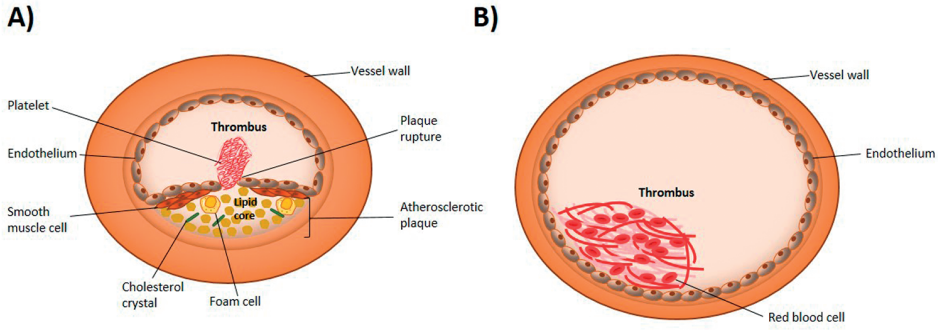

Figure 1. Triggers of arterial and venous thrombosis. A) Artery. The primary trigger of arterial thrombosis is rupture of an atherosclerotic plaque. This involves disruption of the endothelium and release of constituents of the plaque into the lumen of the blood vessel [3]. B) Vein. By contrast, in venous thrombosis, the endothelium remains intact but can be converted from a surface with anticoagulant properties to one with procoagulant properties. Venous thrombosis can be triggered by several factors: abnormal blood flow (stasis), altered properties of the blood itself with increased coagulability and alterations in the endothelium [3].

Venous thrombosis

Venous thrombosis, encompassing deep vein thrombosis and pulmonary embolism, is a common disease, with an overall incidence of 1-2 per 1000 persons each year [4]. Incidence rates rise exponentially with age, from < 0.005% per year in children to nearly 0.5% per year in the elderly [5]. Venous thrombosis is the third most common cardiovascular disease (CVD) after myocardial infarction and stroke, and has become a major challenge to health care systems due to frequent hospitalizations, severe co-morbidities, and a high mortality rate [6-8]. The economic burden caused by long-term complications of venous thrombosis is also a major concern to public health. In individuals who survive a first event of venous thrombosis, the risk of recurrence is high, with a 5-year cumulative incidence ranging from 12% to up to 30% [8-11]. On the basis of contemporary prospective studies with 12 months or longer follow-up, one-third to one-half of deep vein thrombosis patients can expect to develop post-thrombotic syndrome, a potentially debilitating condition for which patients frequently seek medical advice [12]. Even though the incidence of chronic thromboembolic pulmonary hypertension is low in survivors of acute pulmonary embolism (~3%), this complication is associated with poor prognosis when surgical removal of the chronic thrombi is not feasible [13].

Venous thrombosis is a multicausal disease occurring as the result of interacting genetic, environmental and behavioral risk factors [1]. Despite an extensive list on risk factors, there are continuing efforts to identify novel and clinically relevant risk factors for a first and recurrent venous thrombosis. Knowledge of risk factors is crucial, as it may guide strategies to prevent and treat venous thrombosis, thereby allowing improvement of patient care.

Several studies, mainly in the past decade, have shown a relationship between venous thrombosis and arterial CVD [14-19], and suggested that venous thrombosis and arterial CVD may be two different phenotypes of the same disease [20]. Indeed, cardiometabolic risk factors, such as dyslipidemia, inflammation and obesity, are well known to increase the risk of arterial CVD but may also be associated with venous thrombosis [20,21]. Next the rationale behind the association between venous thrombosis and arterial CVD is addressed in more detail.

Association between venous thrombosis and arterial CVD

Arterial CVD (i.e. myocardial infarction and ischemic stroke) and venous thrombosis have traditionally been regarded as two separate diseases due to different pathophysiology (Fig. 1), clinical presentation, and treatment [22]. In the past decade, however, this notion has been challenged. Several studies have shown that venous thrombosis patients have an increased risk of subsequent arterial CVD [15-19]. In a large population-based cohort study in Denmark, patients with venous thrombosis

had an about 2-fold increased risk of subsequent arterial cardiovascular events compared with population controls, and the risk, albeit attenuated, persisted over the long-term follow-up [15]. One of the hypotheses for the association between venous thrombosis and arterial CVD is that the two diseases may share common risk factors [20]. In line with this hypothesis, the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) follow-up study also showed that patients with venous thrombosis had a 2.2-fold increased risk of subsequent arterial cardiovascular events compared with controls from the community [17]. However, upon adjustment for potential common risk factors (confounders), i.e. factors that are related to both exposure (venous thrombosis) and outcome (arterial CVD), but do not lie on the causal pathway between exposure and outcome (see Fig. 2), the risk estimate was attenuated to 1.5. These results suggest that the increased risk of arterial CVD in venous thrombosis patients could be explained, at least in part, by common risk factors.

Among the traditional cardiometabolic risk factors, obesity has consistently been associated with venous thrombosis [23-26]. However, the relationship between other traditional cardiometabolic risk factors and venous thrombosis is less clear. For instance, dyslipidemia, high glucose levels, and renal dysfunction are established cardiometabolic risk factors [27-29]. Still, whether the above mentioned factors are associated with risk of a first or recurrent venous thrombosis is not known in detail due to controversial or scarce data in literature. In this thesis, the association between lipid levels and risk of a first and recurrent venous thrombosis is studied in detail in **chapters 2** and **3,** respectively. The associations of renal dysfunction and glucose levels with recurrent venous thrombosis are further addressed in **chapter 4**. Some

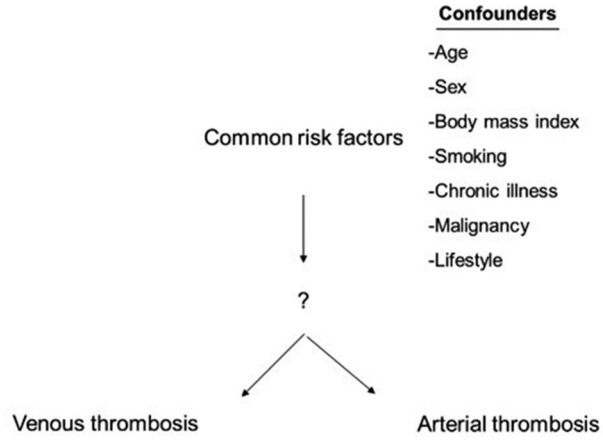

Figure 2. Proposed mechanism for the association between venous thrombosis and an increased risk of subsequent arterial cardiovascular disease [17,20].

hematologic variables, such as red cell distribution width, that are associated with risk of arterial CVD [30] or first venous thrombosis [31-35], are additionally studied in chapter 4 in relation to recurrence.

Knowledge on how cardiometabolic risk factors are related to components of the hemostatic system may provide insights on possible mechanisms underlying the associations of these factors with venous thrombosis. In this thesis, the relationship of lipids, particularly serum and hepatic triglycerides, with hemostatic factors is assessed in **chapters 5** and **6**.

The aim of this thesis is to investigate the associations of traditional cardiometabolic risk factors with risk of a first and recurrent venous thrombosis. Additionally, associations between lipids and levels of several hemostatic factors are assessed.

Study population

To answer the research questions addressed in the chapters of this thesis, data from different population-based studies are used: the MEGA study, the MEGA follow-up study, and the Netherlands Epidemiology of Obesity (NEO) study. These studies will be briefly described.

The MEGA study

The MEGA study is a large case-control study into risk factors for venous thrombosis [36]. Between March 1999 and September 2004, 4956 consecutive patients aged 18-70 years with a first objectively confirmed deep vein thrombosis of the leg or pulmonary embolism were enrolled from six anticoagulation clinics in the Netherlands. For this thesis, participants with active or a previous history of malignancy within 5 years before the index date are excluded. Among the 4956 patients, 4463 are eligible for the studies that are performed in this thesis. Control subjects, without a history of venous thrombosis, are partners of the patients (n= 3297) or individuals approached by random digit dialing [RDD] (n= 3000). The exclusion of control subjects with active or a previous history of malignancy results in 3222 partner and 2939 RDD controls.

All participants filled in a detailed questionnaire on their medical history and the presence of possible risk factors for venous thrombosis. Additionally, blood was collected from patients three months after discontinuation of anticoagulant treatment or one year after the event if they continued anticoagulant treatment for more than one year. Partner controls provided blood at the same time as the patient, and RDD controls provided blood within a few weeks after the questionnaire was sent. This study was approved by the Ethics Committee of the Leiden University Medical Center, and written informed consent was obtained from all participants.

The MEGA follow-up study

The aim of the MEGA follow-up study is to assess the incidence of recurrent events and to identify new risk factors and predictors of recurrences [37]. Of 4956 patients included in the MEGA study, 225 did not consent for follow-up, leaving 4731 patients for the MEGA follow-up study. As patients with active or previous history of malignancy within 5 years before the first event of venous thrombosis are excluded from this thesis, there are 4275 consenting patients eligible for follow-up.

Between June 2008 and July 2009, patients were asked whether they had developed a recurrent venous thrombotic event by means of a short answer form. Furthermore, between 2007 and 2009, the vital status of all MEGA follow-up patients was obtained from the Dutch population register and causes of death from the national registry of death certificates. Data from the answering forms, causes of death, anticoagulation clinics and discharge letters from treating physicians were combined to make a classification of certain and uncertain recurrences [37].

The NEO study

The NEO study is a population-based cohort study designed to investigate pathways that lead to obesity-related diseases [38]. The NEO study includes 6671 participants, with an oversampling of individuals with overweight or obesity. Between September 2008 and September 2012, men and women aged 45-65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged 45-65 years from one municipality (Leiderdorp) were invited to participate, irrespective of their BMI, in order to obtain a reference distribution of BMI.

All participants visited the NEO study centre after an overnight fast for baseline measurements, including blood sampling and anthropometry. Prior to the baseline visit, participants completed questionnaires on demographic, lifestyle and clinical data. In addition, among the eligible participants, 2580 were randomly selected to undergo localized hydrogen 1 (^{1}H) magnetic resonance spectroscopy to assess hepatic triglyceride content, and magnetic resonance imaging to assess abdominal subcutaneous and visceral fat. This study was approved by the Ethics Committee of the Leiden University Medical Center, and written informed consent was obtained from all participants.

OUTLINE OF THIS THESIS

In **chapter 2**, the association between lipid levels and risk of a first venous thrombosis is investigated in the MEGA study, and the possible underlying mechanism is evaluated in detail, considering confounding by common risk factors and mediation via hemostatic factors and C-reactive protein. An important rationale supporting the research question addressed in this chapter is the fact that several studies have shown that lipid-lowering drugs (statins, most notably rosuvastatin) are associated with a decreased risk of venous thrombosis [39-41], thereby indicating a possible role for lipids in the pathophysiology of venous thrombosis. Lipids are also interesting candidates to be investigated in relation to the risk of recurrent venous thrombosis, as statins have been associated with a decreased risk of recurrence [42-44]. Therefore, in **chapter 3,** the association between lipid levels and risk of recurrent venous thrombosis is assessed in the MEGA follow-up study.

Kidney function, measured as estimated glomerular filtration rate (eGFR), glucose levels, and hematologic variables (i.e., cell blood count) are easily obtainable tests, not influenced by anticoagulation on their measurements, that have been associated with risk of a first venous thrombosis in several studies [31-35,45-51]. However, data on the role of these tests in assessing risk of recurrent venous thrombosis are scarce for hematologic variables [33,52] and eGFR [53], or even unknown for glucose levels. **Chapter 4** describes the association of eGFR, glucose levels and hematologic variables with recurrent venous thrombosis.

Previous studies have shown that hemostatic factor levels are interrelated and clustered together [54-56]. However, results were not consistent, probably due to differences in samples sizes, the study population and the hemostatic factors studied. Furthermore, since venous thrombosis and arterial CVD have been shown to be associated [14-19], and may share some traditional cardiometabolic risk factors [20,21], in **chapter 5** we hypothesize that hemostatic factors cluster with lipids and C-reactive protein (i.e. an inflammatory risk marker of vascular disease) [57].

Excess fat accumulation in the liver, also referred to as non-alcoholic fatty liver disease (NAFLD), is strongly associated with obesity [58], and could be one of the mechanisms by which obesity increases the risk of venous thrombosis. In the past two decades, several small studies have investigated the association between NAFLD and hemostatic factors [59-67]. However, whether NAFLD contributes to levels of coagulation factors beyond total body and visceral fat is unclear, particularly in relation to those factors associated with an increased risk of venous thrombosis, i.e. factors VIII, IX and XI [1]. In **chapter 6**, the association between liver fat, quantified as hepatic triglyceride content by magnetic resonance spectroscopy, and levels of factors VIII, IX and XI, and fibrinogen is investigated in the NEO study while adjusting for total body and visceral fat.

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