Understanding hypercoagulability: determinants and impact on cardiometabolic disease

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Chapter 1

General introduction and thesis outline

Hypercoagulability is a pathophysiological condition characterized by an increased tendency to form blood clots. Since hypercoagulability contributes to an increased risk of venous thromboembolism (VTE), research has focused on elucidating the underlying mechanisms that lead to hypercoagulability and on identifying preventive and therapeutic targets for VTE. Changes in the coagulation system, which includes procoagulants, anticoagulants, and pro- and anti-fibrinolytic factors, may lead to a hypercoagulable state. Often the cause of these changes is unknown. For example, many genetic variants associated with these factors have been identified that affect coagulation factor levels; however, there is a need for further investigations to explain the remaining unexplained heritability (1-14). Furthermore, growing evidence suggests that an integrated approach is necessary to fully understand the coagulation system in the context of vascular health (15-17). This approach highlights a need to investigate the link between coagulation and inflammation, immune response, endothelial dysfunction, and metabolic stress, which may explain how hypercoagulability contributes to the development of VTE but in particular also other cardiometabolic disorders. This thesis aims to provide insight into mechanisms leading to hypercoagulability and its role in related disorders by 1) exploring in depth the genetic determinants of coagulation parameters and VTE risk, and 2) examining the complex interplay between coagulation parameters, endothelial function and metabolic markers (i.e., high-density lipoprotein; HDL), and their impact on the risk of type 2 diabetes. This introduction chapter presents background information on VTE, the coagulation system, and hypercoagulability, and describes previous research related to our research goals. It also outlines the thesis structure and describes the main study population.

Venous thromboembolism

VTE is characterized by abnormal blood clot formation in veins, and the third most common cardiovascular disease, affecting 1 or 2 per 1000 individuals annually (18-21). Multiple factors, including advanced age, pregnancy, oral contraceptive use, hormone replacement therapy, surgery, hospitalization, and genetic factors increase the risk of VTE (22). Increased knowledge regarding VTE risk factors improved the identification of high-risk individuals and contributed to developing preventive and therapeutic strategies for VTE. However, despite advancements, VTE incidence has not decreased over the last decades (23) and the biological mechanism underlying an increased VTE risk remains only partially understood due to the complex pathophysiology of the disease. All identified risk factors can be grouped into three mechanistic categories, already postulated in the 19th century by Virchow (24, 25), i.e., changes in the composition of the blood (hypercoagulability), stasis of the blood, and vessel wall injury. Investigating these subclinical conditions, particularly hypercoagulability and the interaction with the vessel wall, may help elucidate the underlying mechanism of VTE and further the development of better prevention and treatment of VTE.

Coagulation system and hypercoagulability

Blood clot formation is an essential process to stop bleeding in damaged blood vessels. When vessels are damaged, coagulation factors are activated, ultimately leading to the formation

of fibrin clots (blood clots). After the bleeding stops, degradation of fibrin clots (fibrinolysis) occurs to return the blood flow to normal. This sequential process has been described as a cascade and researched in various models. described via coagulation cascade or cell-based models (26-29). In summary, when vessel wall injury leads to a release of tissue factor from endothelial cells, these factors form a complex with coagulation factor (F) VII to activate FX to FXa and FIX to FIXa (30-32). FXa converts prothrombin to thrombin, which amplifies the coagulation process by activating platelets, FV, FVIII, and FXI (33-36). Also, the activation of FXII, when FXII, high molecular weight kininogen, and prekallikrein bind to a negatively charged surface, results in activation of FXI. FXIa activates FIX, which forms a complex with FVIIIa to activate FX. A complex of FXa, FVa, and calcium ions produces a large amount of thrombin, which converts fibrinogen to fibrin. Thrombin further plays a role in maintaining the balance of the coagulation system by activating FXIII involved in stabilizing fibrin clots and thrombin activable fibrinolysis inhibitor involved in inhibiting the activation of fibrinolytic factor, plasminogen (37), while inducing the activation of the anticoagulant protein C (38). In the presence of protein S, activated protein C inactivates FVa and FVIIIa (39, 40). Antithrombin also regulates the coagulation system by inhibiting thrombin and other procoagulants. In the fibrinolytic system, plasmin, which is an activated form of plasminogen by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), degrades fibrin into fibrin degradation products such as d-dimer (41). These activators are inhibited by plasminogen activator inhibitor-1.

In healthy conditions, clot formation and breakdown are balanced. However, the balance is shifted towards hypercoagulability when increased levels of procoagulants or decreased levels of anticoagulants cause excessive blood clot formation or when the blood clots fail to dissolve properly, due to an improperly functioning fibrinolytic system. Since all these factors intricately interact with each other to regulate blood coagulation, it is important not only to examine the role of individual factors but also to understand the global coagulation system. To assess the dynamic changes in the global coagulation system, a thrombin generation assay was developed (42), measuring thrombin generation potential with parameters including endogenous thrombin potential, peak height, velocity, lag time, and time-to-peak. Investigating determinants of abnormal procoagulant, anticoagulant, fibrinolytic factors, and thrombin generation potential could help to understand the mechanism leading to hypercoagulability. Of these parameters, this thesis focuses on the following coagulation parameters as these were most consistently associated with VTE risks: procoagulants including FVIII, FIX, FXI, and fibrinogen as well as thrombin generation potential.

Genetic determinants of coagulation parameters

The levels of coagulation factors are determined by both genetic components and environmental factors. With advancements in next-generation sequencing technology over the past decades, there has been a shift from a target gene approach that investigates variants located in genes related to phenotypes to a genome-wide association study (GWAS) that explores the genome without a prior assumption, allowing for the discovery of novel genes

related to the phenotypes. The latter therefore helps to more comprehensively capture the genetic basis of coagulation parameters. Previous GWAS studies have identified genetic variants associated with thrombin generation potential (1, 2), FVIII (3-8), FXI (9, 10), and fibrinogen (6, 11-14). However, as the heritability of these coagulation parameters remained insufficiently explained, further research with increased sample size and genome coverage is required to identify additional genetic determinants affecting thrombin generation potential and other procoagulant levels. Furthermore, despite evidence that increased levels of FIX are associated with an increased risk of VTE (43), a GWAS for FIX remains absent. Identifying additional genetic markers associated with thrombin generation potential and underexplored FIX could enhance our understanding of biological mechanisms underlying hypercoagulability and increased risks of VTE, which could subsequently provide insight into developing therapeutic targets to control hypercoagulability and treat the disease.

Coagulation parameters, vascular health, and cardiometabolic disease

The term "cardiometabolic disease" describes a cluster of subclinical disorders that are shared by cardiovascular disease and type 2 diabetes, including abdominal adiposity, hypertension, dyslipidaemia, hyperinsulinaemia and glucose intolerance. In addition to VTE, hypercoagulability is also observed in patients with other cardiometabolic disease, such as arterial thrombosis, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (44-51). Since inflammation and metabolic stress are well-established risk factors for cardiometabolic disease, research has explored associations between inflammation, glucose levels, hepatic triglyceride levels, and coagulation parameters (45, 52-56). However, the complex pathophysiology of cardiometabolic disease requires further research to identify additional factors associated with coagulation parameters and to determine the role of coagulation parameters in cardiometabolic disease beyond VTE.

Focusing studies on endothelial function may help to further elucidate the underlying mechanism of hypercoagulability, due to its close relationship with the coagulation system and its crucial role in cardiometabolic disease (57-59). Endothelial dysfunction is a pathological condition in which endothelial cells show procoagulant and proinflammatory states. As mentioned above, coagulation is initiated when tissue factor forms a complex with FVII. Under normal conditions, endothelial cells retain tissue factor to prevent unnecessary activation of the coagulation cascade. Furthermore, to maintain an anticoagulant and anti-inflammatory state, endothelial cells express anticoagulants, fibrinolytic factors, and vasodilatory factors. Meanwhile, when activated and dysfunctional, endothelial cells express tissue factors, von Willebrand factor, adhesive proteins, and cytokines, thereby activating coagulation and inflammation (60). The endothelial glycocalyx covering endothelial cells plays a role in maintaining endothelial cell integrity and homeostasis (61). Degradation of this layer contributes to endothelial dysfunction and a pathological condition which is suggested as one of the earliest markers of vascular disease (62-64). However, whether the degradation of this layer has an impact on circulating coagulation parameters is undetermined. An association between endothelial glycocalyx degradation and coagulation may have a functional impact on cardiometabolic disease.

High-density lipoprotein (HDL) is another interesting marker related to coagulation parameters and cardiometabolic disease. Although HDL has been associated with an atheroprotective role, accumulating evidence indicates that elevated HDL cholesterol levels per se are not causally linked to cardiovascular disease (65-72). The inconsistent effects of HDL on disease pathogenesis may be due to the heterogeneity of HDL composition and sizes (72, 73). Different HDL composition and particle size reflect distinct functionalities including mediating cholesterol efflux, anti-oxidation, anti-inflammation, and anti-thrombotic processes. In addition to lipid molecules, proteomic analyses demonstrated that HDL carries factors in the coagulation system including FXII, fibrinogen, prothrombin, antithrombin, and plasminogen (15). Also, HDL inhibits the self-association of von Willebrand factors, leading to reduced platelet adherence and activation, which is crucial for activating the coagulation cascade (74). Despite some hints that HDL plays a role in the coagulation system, questions remain unanswered, such as whether HDL particles are associated with coagulation parameter levels, whether heterogeneity of HDL particles has different effects on coagulation system, and whether these associations impact cardiometabolic disease.

Outline of this thesis

The aim of the research described in this thesis is to understand the mechanism leading to hypercoagulability and its role in cardiometabolic disease with a focus on coagulation parameters, with two parts. **Part I** focuses on the genetic determinants of coagulation factor levels, thrombin generation potential, and VTE risk. Before we dive into the genetic architecture of coagulation parameters, we first explore the literature on genetics in VTE. **Chapter 2** summarizes the current knowledge of genetics in the field of VTE with clinical implications and challenges of genetic prediction models. In **Chapter 3** we aim to elucidate how specific genetic factors contribute to an increased risk of VTE. We investigate the effect of 61 recently identified VTE-associated genetic variants on coagulation parameters. In **Chapter 4** and **Chapter 5**, we present a meta-analysis of GWASs to identify novel genetic loci associated with coagulation parameters. In **Chapter 4**, we show results of the first GWAS for plasma FIX activity. Identified genetic loci are used as instrumental variables to provide insights into the causal effect of FIX activity on cardiovascular disease. **Chapter 5** describes novel genetic loci influencing the thrombin generation potential, exploring other OMICS datasets to understand the function of identified genetic loci. **Part II** focuses on the complex interplay between hypercoagulability, endothelial function, and HDL parameters, and the role of coagulation parameters in cardiometabolic disease. In **Chapter 6**, we investigate whether perfused boundary regions indicating endothelial glycocalyx health status are associated with coagulation parameters. **Chapter 7** addresses the effects of HDL compositions and sizes on the levels of coagulation parameters. In **Chapter 8**, we examine the role of hypercoagulability in cardiometabolic disease beyond VTE, especially studying how coagulation parameters relate to the incidence of type 2 diabetes.

Main study population

The Netherlands Epidemiology of Obesity (NEO) study

The main study population for this thesis is sampled from the Netherlands Epidemiology of Obesity (NEO) study, an ongoing population-based cohort study initiated in 2008 to explore pathways leading to obesity-related disorders. The NEO study comprises 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited by letters sent by general practitioners and municipalities and by local advertisements. They were invited to respond when they were aged between 45 and 65 years and had a self-reported body mass index of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate, irrespective of their body mass index. The study design is described in detail elsewhere (75). The Medical Ethical Committee of the Leiden University Medical Center approved the study design. All participants gave their written informed consent.

CROSSLINK consortium

Chapter 4 and Chapter 5 are part of a collaborative effort within the Coagulation Research using multi-Omics data to aSseSs pathomechanisms LINKed to thrombosis (CROSSLINK) consortium. The CROSSLINK consortium includes eight studies: the Gutenberg Health Study (GHS) (76), the NEO study (75), the Netherlands Twin Register (NTR) (77), the MARseille THrombosis Association VTE Study (MARTHA) (78, 79), the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study (MEGA) (80, 81), and MyoVasc study (82). The first three are population-based cohorts, while the MARTHA study and the MEGA study are case-control studies for VTE, and the MyoVasc study focuses on heart failure. A detailed description of each study is provided in previous literature (75-82). All studies were approved by the Institutional Review Board of its respective institutions. All participants provided informed consent.

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