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## VENOUS THROMBOEMBOLISM IN SOUTHERN SWEDEN EPIDEMIOLOGY AND RISK FACTORS

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VENOUS THROMBOEMBOLISM IN  
SOUTHERN SWEDEN  
EPIDEMIOLOGY AND RISK FACTORS



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av

Nazim Isma

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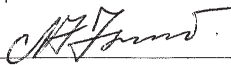
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Title and subtitle VENOUS THROMBOEMBOLISM IN SOUTHERN SWEDEN EPIDEMIOLOGY AND RISK FACTORS			
Abstract  <p>Despite the fact that venous thromboembolism (VTE) is an important cause of morbidity and mortality there is still insufficient information on VTE risk factors and patient characteristics in the general population. Previously published incidences of VTE (deep venous thrombosis [DVT] and pulmonary embolism [PE]) vary from 71 to 192 per 100,000 individuals per year. Incidences of DVT and PE have been estimated to 48-160 and 23-60 cases per 100,000 inhabitants per year, respectively.</p> <p>The aims of this thesis were to evaluate VTE epidemiology and risk factors in a population based total material from southern Sweden, to investigate potential relationships between the factor V Leiden (FVL)-mutation and haemoglobin (Hb) levels in VTE patients, to evaluate how socioeconomic status (SES) and concomitant diseases influence VTE risk during long time follow-up, and to investigate effects of low molecular weight heparin (LMWH) upon time of labor and bleeding in pregnant women with increased VTE risk.</p> <p>Incidences of VTE (lower extremity DVT, PE, and upper extremity DVT [UEDVT]) were all lower than in previous epidemiological studies. Hormone therapy, immobilization, previous surgery and concomitant malignancy were the most common acquired risk factors for VTE. Concomitant malignancy and thrombophilia were especially common in UEDVT, and mortality was high among UEDVT patients with malignancy at diagnosis. Female VTE patients &lt; 50 years of age with the FVL-mutation had higher median Hb compared to female patients without the mutation. SES as reflected by lower income, single marital status, and lower level of education were all independently related to an increased VTE risk during prospective long time follow-up. Foreign ancestry, on the other hand, was related to lower VTE risk. LMWH treatment reduced the first stage of labour by 1 h and results in a significantly lower risk of prolonged first stage of labour among nulliparous pregnant women, but is also associated with an increased risk of prematurity and blood loss complications.</p>			
Key words Venous thromboembolism (VTE), epidemiology, risk factors, factor V leiden (FVL), haemoglobin (Hb), upper extremity deep venous thrombosis (UEDVT), socioeconomic status (SES), low molecular weight heparin (LMWH), labour			
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VENOUS THROMBOEMBOLISM  
IN SOUTHERN SWEDEN  
EPIDEMIOLOGY AND RISK  
FACTORS



**LUND**  
UNIVERSITY

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Cover: 3D rendered close up of a blood clot. With permission from Dreamstime.com

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*“Do not go where the path may lead, go instead where there is  
no path and leave a trail”.*

*Ralph Waldo Emerson*

To Gabriella, Benjamin and Anton



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## LIST OF ABBREVIATIONS

aCL	anti-cardiolipin antibodies
ADP	adenosine diphosphate
anti- $\beta$ 2-GP1	anti- $\beta$ 2-glycoprotein-1
APC	activated protein C
aPL	anti-phospholipid
APS	anti-phospholipid antibody syndrome
APTT	activated partial thromboplastin time
AT	antithrombin
C4BP	complement regulator C4b-binding protein
CI	confidence interval
COC	combined oral contraceptives
CT	computer tomography
DVT	deep vein thrombosis
ECs	endothelia cells
EPCR	endothelial protein C receptor
ET	endothelin
F	factor
FVL	factor V Leiden
FI	fibrinogen
GP	glycoprotein
Hb	haemoglobin
HHcy	hyperhomocysteinemia
HMWK	high-molecular weight kininogen
HR	hazard ratio
HRT	hormone replacement therapy

HSP	heparin sulphate proteoglycans
LAC	lupus anticoagulant
LMWH	low-molecular weight heparin
MPs	microparticles
MRI	magnetic resonance imaging
MTHFR	methylene tetrahydrofolate reductase
NO	nitric oxide
NS	not significant
OAC	oral anticoagulants
OR	odds ratio
PAC	port-a-cath
PAF	platelet-activating factor
PAI-1	plasminogen activator inhibitor-1
PAI-2	plasminogen activator inhibitor-2
PAR-1	protease activated receptor-1
PC	protein C
PE	pulmonary embolism
PICC	peripherally inserted central catheter
PS	protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	prothrombin
RR	risk ratio
SD	standard deviation
SES	socioeconomic status
SPSS	statistical package for the social sciences
SUS	Skåne University Hospital
TAFI	thrombin activatable fibrinolysis inhibitor
TAT	thrombin-antithrombin complex

TF	tissue factor
TFPI	tissue factor pathway inhibitor
TM	thrombomodulin
t-PA	tissue plasminogen activator
TXA <sub>2</sub>	thromboxane A <sub>2</sub>
UEDVT	upper extremity deep vein thrombosis
UFH	unfractionated heparin
UK	United Kingdom
UMAS	Malmö University Hospital (presently termed SUS)
uPA	urokinase
VTE	venous thromboembolism
vWF	von Willebrand factor



# LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals

- I. Isma N, Svensson PJ, Gottsäter A, Lindblad B. Prospective analysis of risk factors and distribution of venous thromboembolism in the population-based Malmö Thrombophilia Study (MATS). *Thromb Res* 2009;124:663-666.
- II. Isma N, Breslin T, Lindblad B, Svensson PJ. The Factor V Leiden mutation is associated with a higher blood haemoglobin concentration in women below 50 of the Malmö Thrombophilia Study (MATS). *J Thromb Thrombolysis* 2009;28:255-258.
- III. Isma N, Svensson PJ, Gottsäter A, Lindblad B. Upper extremity deep venous thrombosis in the population-based Malmö thrombophilia study (MATS). Epidemiology, risk factors, recurrence risk, and mortality. *Thromb Res* 2010;125:e335–e338.
- IV. Isma N, Svensson PJ, Lindblad B, Merlo J, Ohlsson H, Gottsäter A. Socioeconomic factors and concomitant diseases are related to the risk for venous thromboembolism during long time follow-up. Manuscript.
- V. Isma N, Svensson PJ, Lindblad B, Lindqvist PG. The effect of low molecular weight heparin (dalteparin) on duration and initiation of labour. *J Thromb Thrombolysis* 2010; 30:149-153.



# INTRODUCTION

## Historical background

Despite extensive search by medical historians, no descriptions of patients with symptoms compatible with venous thromboembolism (VTE) have been found in the Bible or in the writings of Hippocrates, Galenus, Celius Aurelianus, Ibn an-Nafiz, or Avicenna [1-3].

The first well-documented case of a thrombotic event found in the literature is a manuscript written in the 13<sup>th</sup> century, preserved at *Bibliothèque National* in Paris (MS Fr 2829, Folio 87), regarding a man aged twenty years. According to interpretation by Dexter et al [4], the manuscript text is as follows: “A man named, Raoul, a knight native to Normandy, who, when he was about the age of twenty years, was overtaken in the ankle of his foot on the right side by a swelling of the part which became abscessed and gave him pain, and there came three two holes, and this said illness rose from the foot up to leg....”.

The exotic, dramatic and possibly first description of subclavian vein thrombosis refers to Henry IV of Navarra (1553-1610) King of France [5, 6]. After he led his forces in the battle of Ivry 1590 it is recorded that he had used his sword hand so much that it swelled and was intensely painful and unusable for many weeks. This is strikingly similar to what we see today in patients with subclavian vein thrombosis.

The knowledge on the pathogenesis of VTE has developed further during the following centuries. In the 19<sup>th</sup> century, Armand Trousseau documented the first case of an association between VTE and cancer. His observation was confirmed and extended nearly 70 years later by Sproul 1938, [7] who described a high frequency of VTE during post-mortem examinations of patients with various malignancies.

The modern era of understanding of VTE pathogenesis started in 1856, when the pathologist Rudolf Virchow on the basis of observations made in fatal cases of post-partum thrombosis postulated the major causes of thrombosis [8]: alterations in the blood flow (stasis), alterations in the blood composition (hypercoagulability) and alterations in the vessel wall. According to Virchow thrombosis was a result of at least 1 of the 3 above mentioned factors. This triad, also known as “Virchow’s triad”, of risk factors is still valid and considered as the most important causes for VTE development. Since then, the knowledge about both environmental and genetic risk factors affecting these underlying pathophysiologic mechanisms of VTE has profoundly increased, however.



# Haemostasis

Hemostasis or haemostasis, from the Ancient Greek: αἱμόστασις *haimóstasis* "styptic drug" is a dynamic process constituting a protective mechanism for impediment of life-threatening bleeding from injured blood vessels. In this process, the blood changes from a liquid to a solid state and is hereby kept within an injured blood vessel through generation of a protective haemostatic plug. This process is achieved by a careful teamwork and balance between various systems like platelet, procoagulant, anticoagulant and fibrinolytic pathways [9, 10]. An imbalance in this well-regulated interaction may result in pathologic conditions, such as thrombosis and bleeding.

Traditionally, haemostasis includes three different stages:

- **Primary haemostasis**

through an intricate interaction between vasoconstriction, sub-endothelial tissues, exposure, platelets activation and adhesive proteins the primary haemostatic plug is formed.

- **Secondary haemostasis**

plasma coagulation, a sequence of proteolytic steps, the cascade/waterfall model, and the TF-pathway result in formation and stabilization of fibrin network and thrombus.

Anticoagulation mechanisms ensure that platelet clotting and thrombus propagation restrict themselves around the area of injury only.

- **Fibrinolysis**

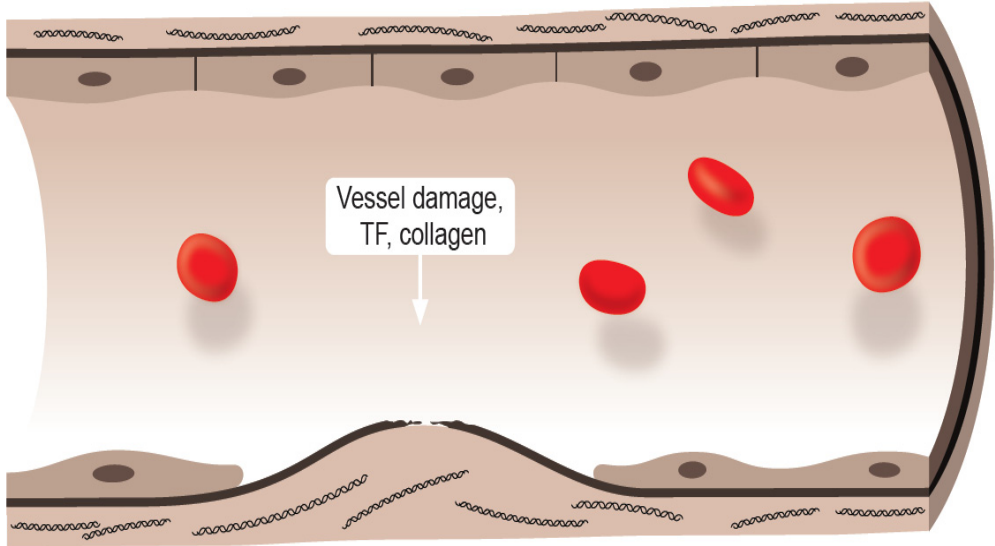
lysis of the thrombus.

## *Primary haemostasis*

Under normal conditions blood vessel walls are covered by a negatively charged layer of healthy endothelial cells (ECs). These healthy ECs not only provide a physical barrier between circulation and surrounding tissues, but also prevent haemostasis by release of endogenous heparin sulphate proteoglycans (HSP), vasodilators such as nitric oxide (NO) and prostaglandin I<sub>2</sub> (prostacyclin), as well as vasoconstrictors, including endothelin (ET) and platelet-activating factor (PAF). Since both the platelets and the healthy ECs are negatively charged they repel each other. In the presence of vascular injury this balance between ECs and platelets will disrupt at the damaged area of the blood vessel wall. A transient locally-induced phenomenon, so called local vasoconstriction of vascular smooth muscle, will occur by the release of endothelium-derived factors such as ET. The healthy ECs are now damaged, platelets are less repellent and local blood flow will slow in the vasoconstricted area (**Fig.1**).

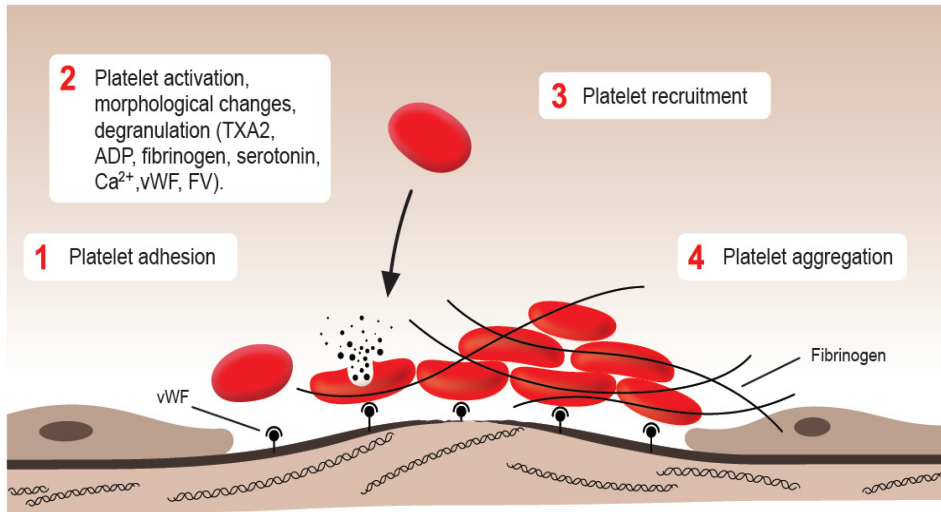
**Figure 1. Blood vessel damage.**

Expression of TF = tissue factor and collagen. With permission from Casper Asmussen and Studentlitteratur.



This is enough to enhance adherence of platelets through the endothelium to exposed sub-endothelial thrombogenic components. This platelet adhesion will be implemented by a platelet membrane receptor glycoprotein (GPIb-V-IX) when circulating von Willebrand factor (vWF) attaches to the sub-endothelium collagen and serve as a bridge between the tissue and platelets [11]. The collagen-activated platelets will now undergo morphological changes from a smooth, discoid form to a more irregular shape, forming pseudo-pods which stretch out to cover the injured surface of the vessel wall and finally they will release  $\alpha$ - and dense granule contents (**Fig.2**). The  $\alpha$ -granules contain vWF, factor (F) V, FXIII and fibrinogen, whereas the dense bodies contain adenosine diphosphate (ADP),  $\text{Ca}^{2+}$ , and serotonin. Since ADP and serotonin are platelet agonists, further activation and recruitment of additional platelets will occur through ADP-receptors, whereas  $\text{Ca}^{2+}$  ions are needed for both activation of coagulation factors and binding of fibrinogen to platelets.

**Figure 2. Primary Hemostasis.** TXA<sub>2</sub> = thromboxane A<sub>2</sub>, ADP = adenosine diphosphate, vWF = von Willebrand factor, FV = factor V. With permission from Casper Asmussen and Studentlitteratur.



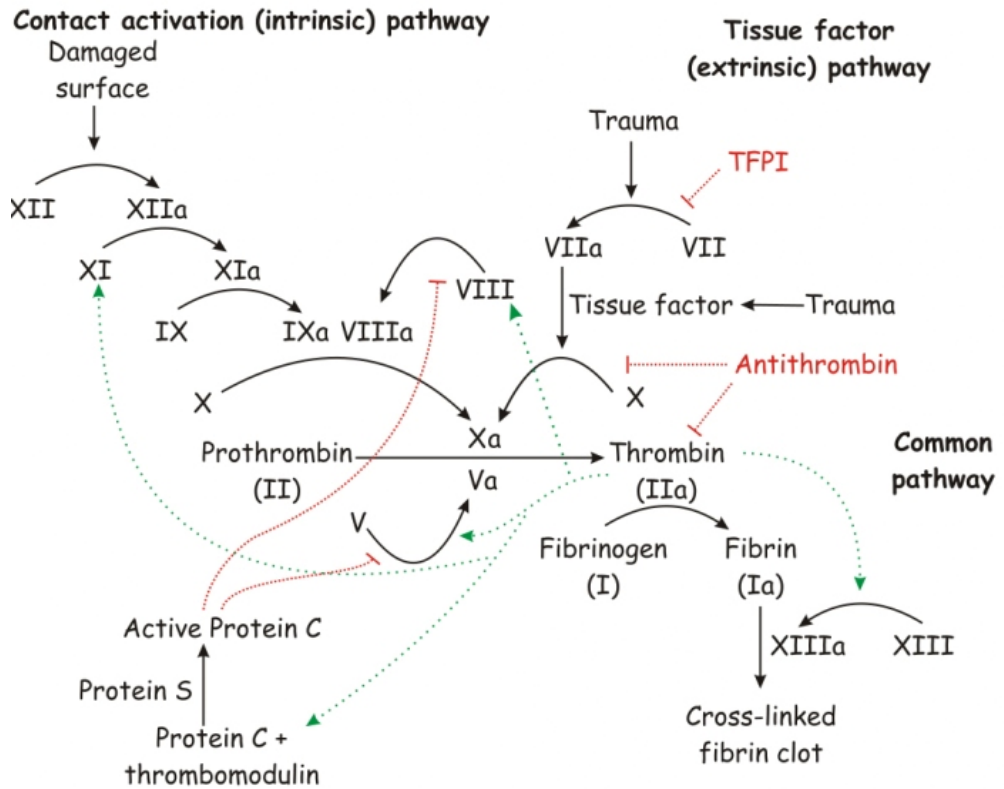
These activated platelets will further synthesize and release thromboxane A<sub>2</sub> (TXA<sub>2</sub>), promoting expression of GPIIb/IIIa and PAF which are important platelet aggregating agonists and vasoconstrictors for further platelet aggregation and finally formation of a platelet plug. In addition, the platelet membrane integrin receptor called GPIIb/IIIa will be activated, permitting fibrinogen binding to its receptor and formation of bridges between the platelets. Moreover, phosphatidyl-serine (a phospholipid) with platelet procoagulant activity will provide an essential binding site for the activated coagulation factors (such as *tenase-complex* [FVIIIa/FIXa] and *prothrombinase-complex* [FXa/FVa]), optimizing activation of the coagulation cascade and the formation of fibrin [12].

### *Secondary haemostasis*

In 1964 the so called cascade/waterfall model was introduced by two different groups of biochemists [13, 14]. According to this model, coagulation is a sequence of proteolytic steps where one clotting factor induces activation of another. Coagulation can be initiated by either the *intrinsic* or the *extrinsic pathway*, which both converge in the so called *common pathway* [15] leading to thrombin generation, which in turn converts fibrinogen (FI) to fibrin. In addition, thrombin also activates FV and FVIII leading to stimulation of further thrombin generation (**Fig.3**).

**Figure 3. The cascade/waterfall model.**

Coagulation can be initiated by either the *intrinsic* or the *extrinsic pathway*, which both converge in the so called *common pathway*.



The cascade/waterfall model has served as a basis for understanding of coagulation enzymatic steps *in vitro*, and also for development of screening tests for prediction of clinical bleeding tendency, such as the prothrombin (PT)-test for the *extrinsic* and activated partial thromboplastin time (APTT) test for the *intrinsic pathway* [16].

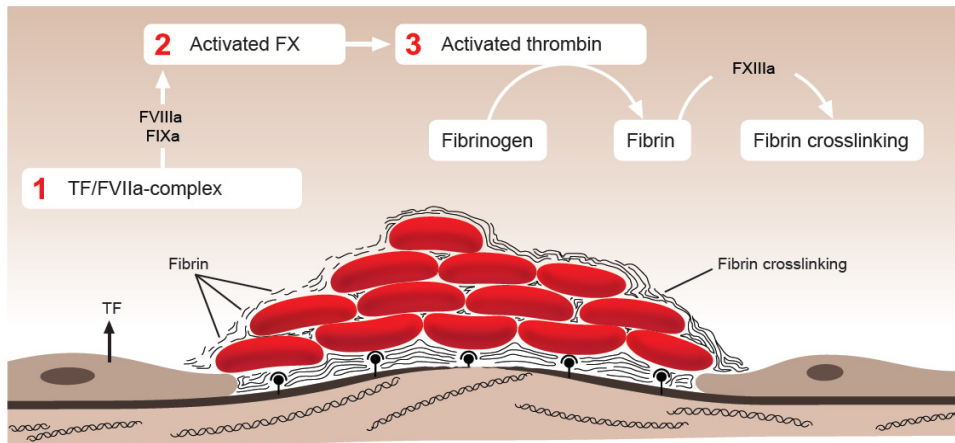
However, the cascade/waterfall model failed to explain coagulation mechanisms *in vivo* and the limitation of the cascade/waterfall model as model of the haemostatic process was highlighted by diverse clinical observations such as that patients with deficiencies of FXII, high-molecular weight kininogen (HMWK) and prekallikrein in the *intrinsic pathway* all have prolonged APTT without bleeding tendency, whereas patients with known deficiencies of FVIII (thrombophilia A) and FIX (thrombophilia B) have a serious bleeding tendency, in spite of an intact *extrinsic pathway* [16]. Furthermore, serious bleeding tendency is also common in patients with deficiency of FVII, an *extrinsic pathway* factor, even if the *intrinsic pathway* is intact [15]. These

phenomena suggest that the *extrinsic* and *intrinsic pathways* are interdependent *in vivo*, instead of being separate and functionally independent [17] as suggested by *in vitro* studies [18, 19].

The new understanding of haemostasis began with Hoffman and Monroe's [17] presentation of a "Cell-Based Model of Haemostasis" divided in three overlapping steps. This process, however, is dependent on the participation of 2 different cell types: tissue factor (TF) bearing cells (ECs, sub-intimal cells, monocytes) and platelets (**Fig.1**). According to this model initiation of coagulation *in vivo* in response to trauma occurs on a TF-bearing cell and its binding to freely circulating FVII in presence of  $Ca^{2+}$  ions, creating a FVIIa/TF-complex activating FX (FX/FXa) and FIX (FIX/FIXa), (**Fig.4**).

**Figure 4. Secondary hemostasis (Plasma coagulation).** TF = tissue factor.

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This in turn amplifies the system by feedback activation of FVII on the TF-bearing cell. The FVIIa/TF-complex further results in formation of an *extrinsic* FX-activated complex where the activated FXa binds to activated FVa on the TF-bearing cell at the site of vessel wall injury. This leads to a formation of a prothrombinase-complex (FXa/FVa), and finally to conversion of PT to a small amount of thrombin. This small amount of thrombin is a critical effector enzyme of coagulation, fulfilling many biologically important functions as well as ensuring that initiation of coagulation is successful. It feedback amplifies coagulation through activation of FVa, FVIIIa, FXIa, FXIIIa and activates platelets by cleaving protease activated receptor-1 (PAR-1) [12], resulting in a procoagulant membrane surface, and accumulation of thrombin-activated FVIIIa and FVa on the activated platelet surface. This activated FVIIIa on the platelet surface is liberated from vWF during the thrombin-mediated activation process [20].

In addition, FIXa originating from TF-bearing cells activated during the initiation of coagulation will now migrate to the activated platelet phospholipid membrane and bind to its cofactor FVIIIa. Finally in the presence of  $\text{Ca}^{2+}$  ion a complex also called *intrinsic tenase-complex* (FVIIIa/FIXa) is formed, in turn converting FX to its active form FXa. This activated FXa will subsequently bind to its cofactor FVa on the activated platelet phospholipid membrane in the presence of  $\text{Ca}^{2+}$  ions as described above, leading to formation of a *prothrombinase-complex* (FXa/FVa). Furthermore, a *prothrombinase-complex* (FXa/FVa) on the activated platelets will convert PT to a burst of its active form thrombin which in turn converts FI to fibrin. This thrombin is also important for activation of thrombin activatable fibrinolysis inhibitor (TAFI) and FXIIIa, transglutaminases participating in the formation of a stable fibrin clot by catalyzing cross-linkage of fibrinogen [21].

The alternative *intrinsic pathway* (Fig.3) of coagulation initiated by diverse factors present in circulating blood is initiated through interaction between FXII (Hagemann factor) and HMWK produced from platelets. This induces the transformation of FXII to its active form FXIIa and prekallikrein, subsequently leading to cleavage of FXI to its active form FXIa. In addition, active FXIa activates FIX which in turn interacts with its cofactor, FVIIIa. However, it is quite clear that *intrinsic pathway* is not important for trauma related coagulation because individuals with inherited deficiency of the *intrinsic pathway* protein FXII as explained above do not experience increased bleeding tendency.

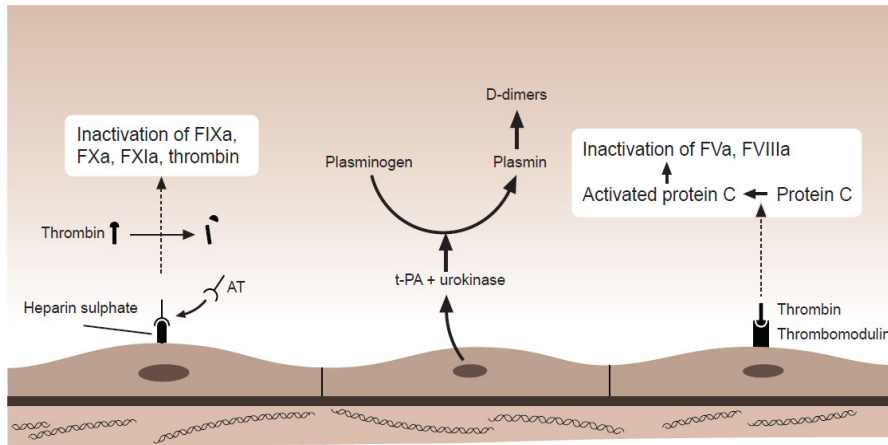
### *Anticoagulation*

In order to ensure that platelet clotting restricts itself only around the area of injury and simultaneously minimizing the risk of continued platelet clotting throughout the entire vascular tree, there is a need for an appropriately controlled system of coagulation (Fig.5). This occurs on negatively charged phospholipid surfaces by three different anticoagulant mechanisms at all levels of the system [22]. Firstly, the tissue factor pathway inhibitor (TFPI) is a single-chain polypeptide, a protein secreted by the endothelium regulates clotting by reversibly inhibition of FXa and thrombin. While FXa is inhibited, the FXa-TFPI complex can subsequently also downregulate the TF/FVIIa complex.

A second crucial anticoagulant is antithrombin (AT), a serine protease inhibitor (serpin) inhibiting thrombin, FIXa, FXa, FXIa, and FXIIa (Fig.5). AT adhesion to FIXa, FXa, FXIa, and FXIIa is increased by the presence of HSP presented on endothelial cell surfaces or the administration of heparin [23]. By itself, however AT is an inefficient inhibitor of coagulation.

**Figure 5. Mechanisms of anticoagulation.**

AT = antithrombin. With permission from Casper Asmussen and Studentlitteratur.



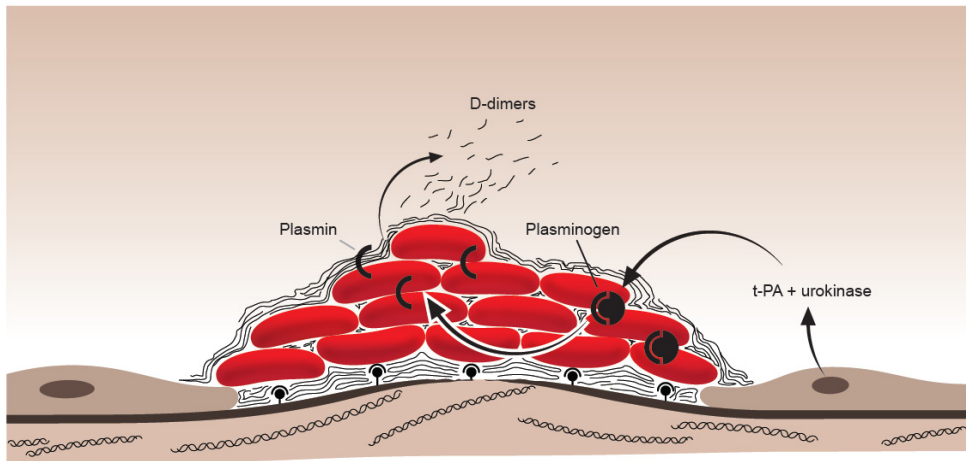
The third important mechanism of anticoagulation involves thrombomodulin (TM), a cell surface-expressed glycoprotein, predominantly synthesized by healthy vascular ECs (Fig.5). TM is a critical cofactor for thrombin-mediated activation of protein C (PC), a vitamin K-dependent proenzyme to an anticoagulant serine protease [24]. In addition, the endothelial protein C receptor (EPCR) [22, 24, 25] will further amplify activation of PC by its binding to and presentation of PC for thrombin-TM complex. Once activated Protein C (APC) has been generated, APC in presence of the cofactor protein S (PS) acts as a major anticoagulant through its ability to inactivate FVa, and FVIIIa on the surface of negatively charged phospholipid membranes [25]. This demonstrates that thrombin have both pro- and anticoagulant properties, depending on whether the vessel wall is damaged or intact.

Approximately 30% of PS in human plasma is present as free protein, while the remaining 70% of PS is bound to the complement regulator C4b-binding protein (C4BP) [26]. It is the free PS that serves as APC-cofactor [27]. As FVIII is bound to vWF in the blood circulation, it cannot interact with the phospholipid membrane or be cleaved by APC [28], unlike FV which can bind to phospholipid membrane both as FV and FVa and shortly thereafter be cleaved by APC. As result of this reaction FV will now convert to an anticoagulant cofactor of APC, and together with PS participate in the degradation of FVIIIa in the *tenase-complex* (FVIIIa/FIXa). The above-mentioned mechanisms highlight the fact that both FV and thrombin may exert procoagulant effects, when FVa is generated by thrombin or FXa, and anticoagulant properties when FV is cleaved by APC [29]. Eventually, the PC anticoagulant system is the anticoagulant system most exposed to genetic risk factors, such APC resistance, caused by a point mutation

involving the FV-gene (FV-Leiden) [30], heterozygous deficiencies of PC [31], PS [32], AT [33], and a single point mutation of PT [34] which until today are the most known underlying genetic causes of thrombophilia leading to increased risk of VTE.

**Figure 6. Mechanisms of fibrinolysis.**

t-PA = tissue plasminogen activator. With permission from Casper Asmussen and Studentlitteratur.



### *Fibrinolysis*

After a certain time, the injured vessel wall is healed and the stabilized, covalent cross-linked fibrin clot made by FXIII is therefore no longer needed. Dissolution of the stabilized covalent cross-linked fibrin clot occurs by fibrinolytic systems including tissue plasminogen activator (t-PA) [35], a serine protease, synthesized in the ECs surrounding the injured vessel wall, a factor converting plasminogen into plasmin (**Fig 6**). Converted plasmin will now initiate fibrin degradation by removal of the carboxy-terminal part of  $\alpha$ -chains and the amino-terminal part of  $\beta$ -chains from fibrin, leading to fragment generation of cross-linked fibrin-D-dimers [36] which can be measured in plasma. D-dimers are unspecific markers for VTE, however, since they are formed during many different pathological conditions with increased fibrinogen or fibrinolytic activity. Therefore measurements of D-dimers can only contribute to exclusion and not confirmation of VTE.

In order to ensure that fibrinolysis restricts itself only around the stabilized/covalent cross-linked fibrin clot area and simultaneously minimizing the risk of severe bleeding or destruction of diverse proteins, there is a need for an appropriately controlled system of fibrinolysis. This occurs by several inhibitors of fibrinolytic mechanisms: plasminogen activator inhibitor-1 (PAI-1) [37] is a key inhibitor of fibrinolysis *in vivo* by fast inhibition of t-PA and urokinase (uPA), factors that convert plasminogen into plasmin. Another critical inhibitor of fibrinolysis is TAFI, a carboxypeptidase synthesized by



hepatocytes which down-regulates fibrinolysis by removing carboxy-terminal lysine residues from partially degraded fibrin [38, 39]. Elimination of these lysines by TAFI interrupt the fibrin cofactor function of t-PA-mediated plasminogen activation, resulting in a decreased rate of plasmin generation and thus down-regulation of fibrinolysis [40]. Additionally, fibrinolysis is also prevented by both  $\alpha$ 2-antiplasmin [37] and  $\alpha$ 2-microglobulin [41].

# Venous thromboembolism (VTE)

## Definition and pathophysiology

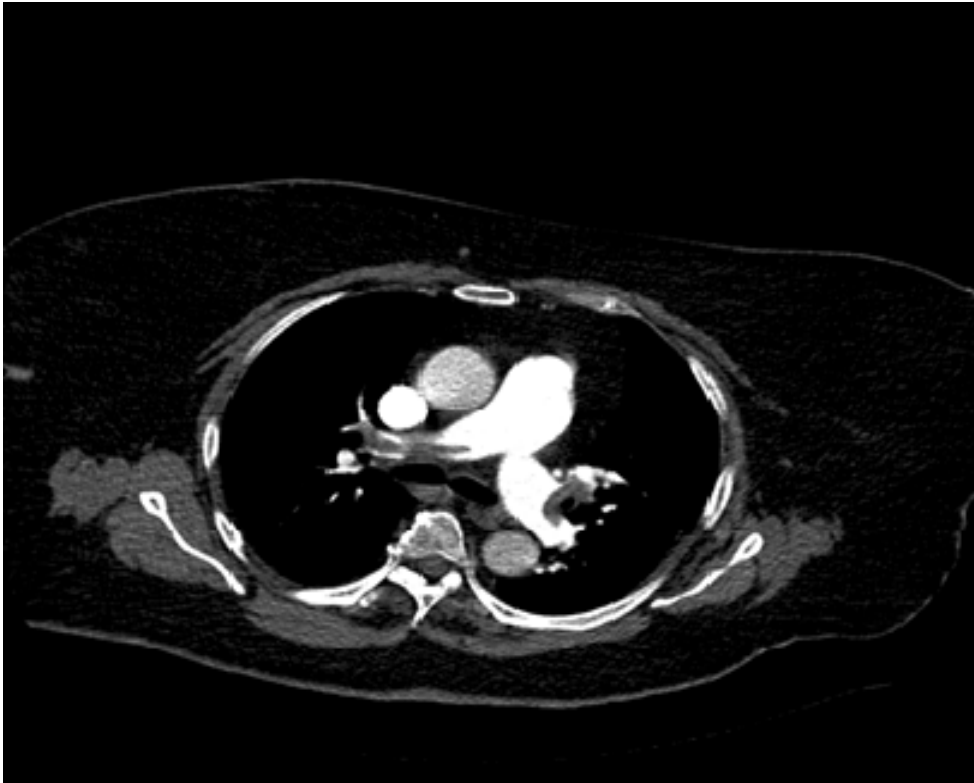
A deep venous thrombosis (DVT), (**Picture 1**) is a fibrin-rich clot [42] that usually develops in the large venous valves of the leg. The main task of these valves is to maintain blood circulation in the legs by assisting return of blood to the right atrium of the heart through compression of the deep veins by muscular contractions. A thrombosis might compromise function of these valves, or might organize in the vessel wall, or grow further causing partial or total occlusion of blood flow in the vein.



**Picture 1.**

Phlebography of the left leg deep veins of a patient from our clinic with left femoropopliteal deep vein thrombosis.

Another possible complication of the VTE is pulmonary embolism (PE), (**Picture 2**), which might occur when a part of the thrombus travels through the right heart to the lung, resulting in a partial or complete cessation of blood flow in the pulmonary artery or its branches.



**Picture 2.**

Computer tomography of the pulmonary arteries of a patient from our clinic with bilateral pulmonary emboli.

As opposed to arterial thrombosis, which occurs due to arterial injury and TF derived from the arterial wall or within a ruptured plaque, VTE mainly occurs on endothelial surfaces in the absence of previous vein wall injury [42]. Moreover, during normal conditions the endothelial surface of vein walls exerts antithrombotic and anticoagulant effects due to its high levels of TFPI, TM, and EPCR [43].

The mechanisms leading to VTE are less known compared to the pathogenesis of arterial thrombosis. However, both a meta-analysis and several other studies have demonstrated increased risk of VTE in both immobilized patients and in the paralyzed limb of hemiplegic patients [44, 45]. The above-mentioned results highlight the

significant role of venous stasis in the pathogenesis of VTE. When flow is reduced, and stasis of blood has persisted for some time, the endothelial antithrombotic effect decreases by two possible mechanisms, resulting in:

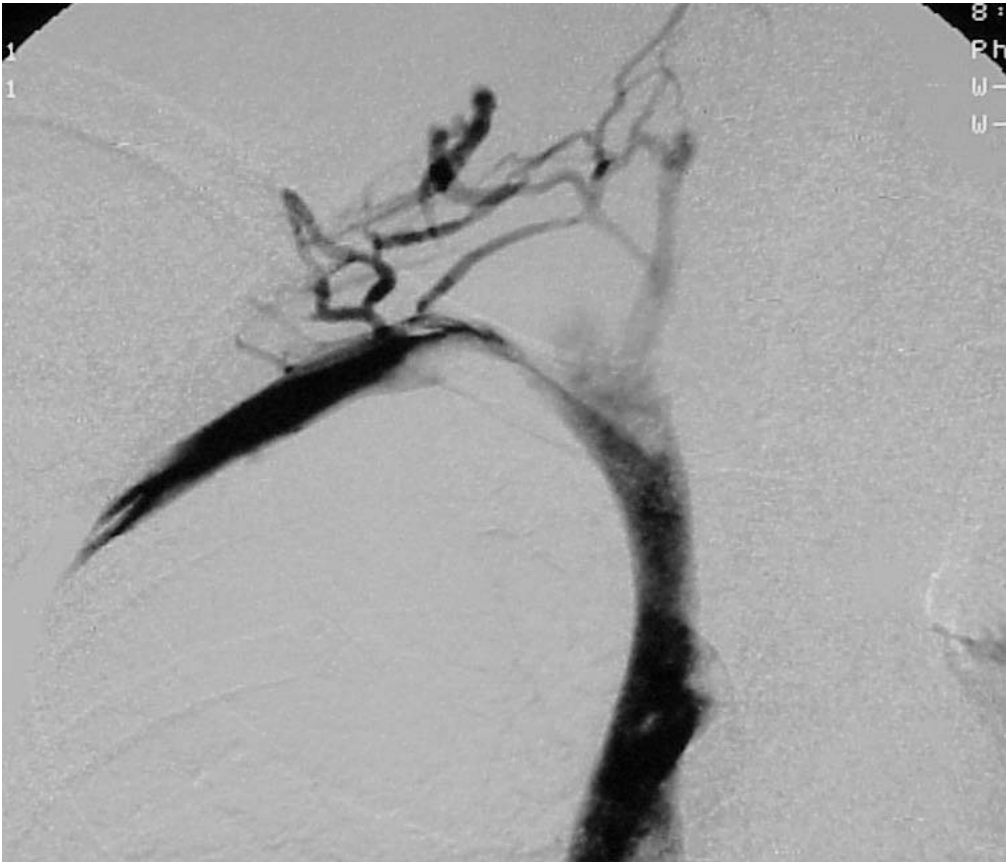
- 1 Increased accumulation in vein valve pockets of prothrombotic substances such as thrombin and TF-positive microparticles (MPs). This thrombin should under normal circumstances instead have been washed downstream in the capillary beds of the lungs, which are covered with anti-thrombotic substances such as TM as integral membrane proteins on ECs, and heparin HSP by converting thrombin from procoagulant to an anticoagulant condition [46].
- 2 Hypoxic responses from leukocytes, ECs and platelets due to rapid hemoglobin desaturation in valve pockets under static conditions [47]. Hypoxia is a pathological condition which will also stimulate TF expression from monocytes, neutrophils, ECs and platelets, in turn leading to increased levels of TF-positive MPs [48, 49]. Moreover, activation of ECs by hypoxia or local inflammation results in increased levels of vWF and expression of membrane-bound P-selectin, providing a possible receptor for TF-positive MPs as well as for platelets and leukocytes [50-52].

According to several reviews [46, 53], TF-positive MPs may enhance propagation of VTE in a manner similar to in arterial thrombosis by activated platelets. During pathological circumstances TF-positive MPs expressing P-selectin glycoprotein ligand-1 (PSGL-1) will associate and fuse with activated ECs expressing P-selectin and phosphatidylserine on their surfaces. When the MPs transfer TF to membranes of ECs the endothelium-associated anticoagulants such as TFPI, TM, and HSP will be neutralized. An enzymatic cascade of coagulation is hereby initiated on the endothelial surface leading to enhanced propagation of VTE, thrombin generation and fibrin deposition [46, 53]. The importance of TF for VTE development has also been confirmed in various animal models [54, 55]. Moreover, increased levels of TF-positive MPs are also found in human tumors such as pancreatic and colorectal cancer. Such effects on coagulation by TF-positive MPs may help explain the increased incidence of VTE in cancer patients [56, 57].

## Epidemiology

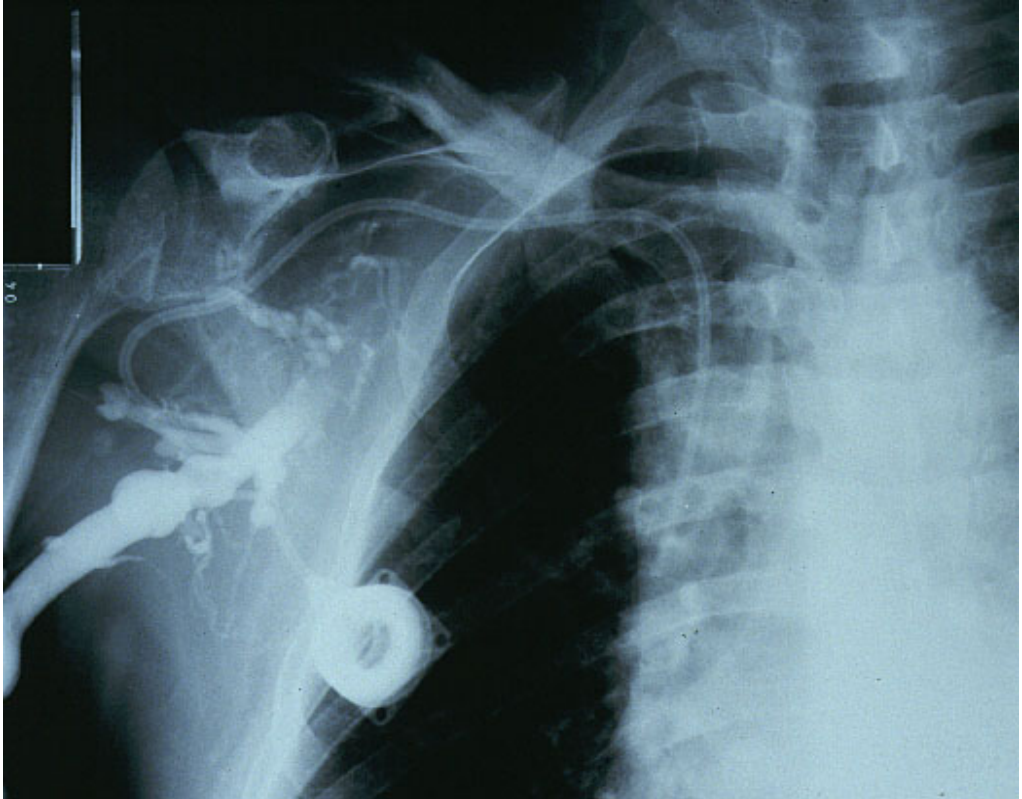
VTE, manifesting itself as DVT or PE is a major cause of mortality and morbidity worldwide. The most frequent clinical manifestation is thrombosis in the deep veins of the legs. The annual incidence of VTE is 100-200 per 100,000 individuals per year [58-63]. DVT affecting the upper extremities (UEDVT) is much more uncommon, however, as only approximately 2 % - 11 % of all DVTs involve upper extremity veins [62-64]. Furthermore, thrombosis of the axillary and subclavian veins are subdivided into primary UEDVT (**Picture 3**); cases in which no certain explanation for the development of

thrombosis is found, and secondary UEDVT (**Picture 4**); cases with an obvious causal factor for thrombosis development [64].



**Picture 3.**

Spontaneous subclavian vein thrombosis with near occlusion of the subclavian vein and with developed collaterals.



**Picture 4.**

A secondary subclavian vein thrombosis due to a Port-a-Cath inserted for chemotherapy. The catheter worked without problem at infusion and was not removed and allowed further chemotherapy.

Current data on VTE incidence is mainly based on large community-based epidemiological studies, and therefore reflect symptomatic rather than asymptomatic disease [65]. It is thus possible that the incidence of VTE is under-estimated. The disorder is exceptionally rare (<5 cases / 100,000 / year) among children below 15 years of age. VTE incidence, however, increases steeply with age, the annual incidence rate being 450-600 cases / 100,000 / year) among individuals > 80 years of age [58, 66]. Moreover, Anderson et al observed yearly incidences of first-time or recurrent VTE of 62/100,000 among individuals between 50 and 59 years, and 316/100,000 among those between 70 and 79 years of age [58].

There is disagreement between scientists regarding the effect of gender upon the incidence rate of VTE. In a prospective study by Nordström et al, all positive phlebographies within the well-defined population of the city of Malmö, Sweden, during

1987 were studied in order to determine DVT incidence. The overall incidence of DVT was found to be equal in men and women, 160 per 100,000 inhabitants / year [59].

VTE risk, however, is higher in women during child bearing years compared to men in the same age group [59, 60, 66]. Moreover, in a community-based study in Western France, annual incidence rates of VTE were lower in men (40 / 100,000) compared to women (58 / 100,000) in the age group 20–39 years, whereas the opposite relationship was seen in older subjects between 40 and 59 years (150 / 100,000 in men and 105 / 100,000 in women) and between 60 and 74 years (533 / 100,000 in men and 433 / 100,000 in women) [60].

Furthermore, in a population-based epidemiological study carried out in Olmsted County, Minnesota the overall age-adjusted yearly incidence rate of VTE was higher in men (130 / 100,000) compared to women (110 / 100,000) [67]. And in addition, another longitudinal investigation of the causes of VTE reported that male gender appeared as a significant risk factor for the development of a first episode of VTE (hazard ratio [HR]:1.44, 95% confidence interval [CI]:1.10–1.89) [68].

In the Austrian Study on recurrent VTE, the recurrence rate of VTE after an average follow-up of 36 months after withdrawal of oral anticoagulants in 826 patients with a first episode of spontaneous VTE was 74 (20%) among 373 men compared to 28 (6%) among 453 women (risk ratio [RR]:3.6, 95% CI, 2.3–5.5;  $p < 0.001$ ) [69]. The above-mentioned findings were also confirmed by a large prospective single-centre cohort study from the UK which reported a 2.7-fold increased risk of recurrent VTE (95% CI, 1.49-4.77;  $p < 0.0006$ ) in men. The 2-year cumulative rate of recurrent VTE in this study was 19% among men compared to 8% in women [70].

Consequently, a review by Kyrle et al [71] concluded that male gender is not only a risk factor for a first episode of VTE but also confers a higher risk for recurrence of VTE. In conflict with the conclusion drawn in the above-mentioned review, however, Roemro and his coworker in their qualitative systematic review of available studies claimed that gender per se is not an independent risk factor of VTE, and that the absolute VTE risk frequently instead is related to specific circumstances related to female gender, such as pregnancy (123 cases / 100,000 women-year), puerperium (320 cases / 100,000 woman-years), pregnancy in thrombophilic women (400 cases / 100,000 pregnancy-years), pregnancy and previous VTE (11000 cases / 100,000 pregnancy-years), hormone replacement therapy (HRT) (20-59 cases / 100,000 woman-years), tamoxifen (360-1200 cases / 100,000 treatment-years) and raloxifene (950 cases / 100,000 treatment-years) [72].

It has been suggested that ethnicity is a major determinant of VTE incidence, since it varies widely among diverse ethnic cohorts [73]. According to White et al [74], directly standardized yearly incidence (number of cases if the entire population would have been comprised of one single ethnic group) of all VTE events was significantly increased

among African-Americans (141 / 100,000 adults) compared to Caucasians (103 / 100,000;  $p < 0.001$ ), and significantly decreased among Hispanic individuals (61.5 / 100,000;  $p < 0.001$ ) and strikingly lower among Asians and Pacific Islanders compared to Caucasians (29 / 100,000;  $p < 0.001$ ). Moreover, African-Americans seem to have the highest standardized incidence of both idiopathic and secondary VTE when compared to all other included ethnic group. Furthermore, 36% of all VTE events in subjects of African-Americans ethnicity were diagnosed as PE compared to 32% in Caucasian and 26% in Hispanic subjects.

A review by Keenan et al [75] reported a higher risk of recurrent VTE among African-Americans and Hispanics compared to Caucasians. However, both the type of index VTE and gender seemed to play a role for this risk, and therefore no definitive conclusions could be drawn on the biological plausibility of this finding. A part of this ethnic difference regarding incidence rates of VTE could be explained by differences in genetic predisposition. For example, the Factor V Leiden (FVL) and PT polymorphism (G20210A) gene mutations are very common in Caucasians (3–7%), whereas the mutations appear very rarely in Asians, particularly in populations from Japan [76], Taiwan [77, 78], China [79] and Korea [80]. On the other hand, the prevalence of lupus anticoagulant (LAC) in the general population is higher in Caucasians (54%) compared to Asians (8–27%), whereas its occurrence is nearly unknown in Blacks [81]. However, a possible hypothesis that might explain the increased incidence rates of VTE among African-American is the overrepresentation of high FVIII levels in this minority group compared to in Caucasians (34% vs 25%, respectively) [82]. According to several studies both the ABO-blood group and the vWF are important determinants of FVIII levels in plasma [83, 84], and higher levels of FVIII are often associated with non-O blood. Moreover, the alpha fibrinogen 312 Ala mutation which is overrepresented in black subjects, is a known risk factor for VTE conferring increasing clot rigidity [85].

Like many other common diseases, VTE is believed to be a multicausal disease and its occurrence is a result of interaction between several both environmental and genetic risk factors [86, 87]. Some of the environmental risk factors are: old age, surgery, trauma, prolonged immobilization, cancer, prior VTE, long distance travel, oral contraceptives, hormonal replacement therapy, pregnancy, the postpartum period, and occurrence of antiphospholipid antibodies [88, 89]. Genetic risk factors include the factor V-Leiden mutation, PT 20210GA mutation, methylene tetrahydrofolate reductase (MTHFR) 677T mutation, ABO blood group, and deficiencies of AT, PC and PS [62, 88-90].

It is, however difficult to predict the risk of VTE, its recurrence and VTE mortality rate for patients with different environmental and genetic risk factors, since VTE is such a multicausal disease. It is shown in several studies [91-95] that a prior history of VTE is a strong predictor for VTE, with recurrence rates in consecutive patients of 6%-13% after 1 year and between 13%-28% after 5 years. The overall mortality rate within one month



of diagnosis is approximately 6% for DVT and 12% for PE [73]. These figures increase in elderly patients up to 21% for DVT, 39% for PE after 1 year [96].

## Acquired and environmental risk factors for VTE

### *Age*

VTE is a rare condition in children and young subjects [97, 98]. Even if the disease does not occur only in older age, age is one of the strongest risk factors for VTE [58-60, 62, 66, 99, 100], however. Furthermore, the incidences of both DVT and PE increase exponentially with age in both genders [62, 66]. For example, in a recent population based study by Spencer et al [101] VTE incidence increased from 71 per 100,000 per year in subjects <65 years to 885 per 100,000 per year in subjects 85 years of age and older. Recurrence rates of VTE, however, were similar in elderly and younger patients. Hypothetically, the higher incidence of VTE in older age might possibly not only be related to age itself, but also to other underlying risk factors or diseases more common in old age, such as cancer, decreased mobility and muscular tone.

### *Surgery*

In general, major surgical interventions are associated with increased incidence of VTE, especially in patients undergoing surgical interventions at ages 65 years or higher [102]. Some surgical procedures are associated with especially high risk: orthopaedic surgery, neurosurgery, and major vascular and gynaecologic surgery [102]. According to several studies, the risk of venous thrombosis reaches 30% to 50% in patients undergoing orthopaedic surgical interventions in the lower extremities without use of antithrombotic prophylaxis [103, 104]. Symptomatic VTE, however, occurs in only 5% of such patients with the use of appropriate prophylaxis [105, 106]. Increased risk for VTE also occurs in patients undergoing other surgical interventions such as abdominal, urological, and gynaecologic surgery [107]. The overall incidence rate of DVT in patients undergoing general surgery is approximately 20%, compared to in cancer patients undergoing similar surgical interventions where the overall incidence is nearly doubled, approximately 37% [108].

### *Multitrauma*

Multitrauma has frequently been associated with increased risk for VTE [109, 110]. Some of the independent risk factors for VTE in multitrauma patients are: injury severity score, number of operative procedures, pelvic injury, concomitant diseases (i.e. renal failure, diabetes, cancer, congenital/acquired coagulation disorders) [109, 111]. A meta-analysis performed by Rogers et al [110] showed that patients with spinal cord injury or spinal fracture run the highest risk for VTE manifestations. According to a study by Paffrath et al [111], the occurrence of PE in patients with severe trauma was associated with a

mortality rate of 25.7%. Data on the incidence of VTE in different study populations of trauma cases varies widely, however, ranging from 1%-60% in patients with different types of injuries (head trauma, spinal injury, pelvic fractures and fractures in the lower extremity) [109, 111-116]. Another potential reason for this discrepancy regarding VTE incidence in multitrauma patients could be different policies about administration of antithrombotic prophylaxis worldwide.

### *Immobilization*

Although current literature highlights the importance of immobilization when assessing the risk for VTE and the benefit of prophylactic strategies, the impact of immobilization on the incidence rate of VTE still remains largely unknown. This is due to the fact that the definition of immobilization may vary, and there is a lack of data regarding the association between bed rest and VTE. Most of the different epidemiological studies that have analyzed risk factors for VTE were not designed to evaluate the impact of immobilization. However, several recently published studies have reported that conditions such as neurological disorders and lower extremity fractures are often associated with both immobilization and increased VTE risk [117-119]. A meta-analysis performed by Pottier et al [44] showed that immobilized patients run an approximately two times higher risk of VTE compared to ambulant patients.

### *Long-distance travel*

The first reported case of death due to PE after air travel occurred in a young passenger who died after a flight from Australia to the United Kingdom in 1954 [120]. During the last two decades, increasing attention has been paid to the risks of VTE associated with long-distance voyages, in particular air travel.

In a randomized controlled study performed by Scurr et al [121], 89 male and 142 female passengers over 50 years of age without prior history of VTE embarking on flights with duration over eight hours were randomized to either use or non-use of compression stockings. Twelve of the 116 passengers (10%; 95% CI 4.8-16.0%) in the control group without compression stockings developed asymptomatic DVT, whereas in the group treated with compression stockings no case of DVT could be demonstrated on subsequent duplex ultrasonography [121]. The clinical relevance of asymptomatic DVT for later development of symptoms or PE is unclear, however. Moreover, Lapostolle et al [122] highlighted the association between PE risk and flight duration by reporting a 50-fold difference in risk between flights of less than 2500 km, and those over 10,000 km.

In a recently published study by Cannegieter et al [123], a 3-fold increased risk of VTE after long-distance travel was shown, without apparent difference caused by various modes of travel such as air, train, car etc. This large “Mega-study”, which also analyzed data from 1851 matched controls, showed that subjects with factor V Leiden, obesity, or

oral contraceptive use ran a particularly increased risk for VTE. Despite these facts, general consensus is still lacking regarding potential benefits of providing VTE prophylaxis before long distance flights [122].

### *Cancer*

Cancer is one of the well-known acquired risk factors for VTE. According to several population-based epidemiological studies, approximately 20% of all VTE cases are associated with cancer [124, 125]. Furthermore, cancer patients have four- to seven-fold increased risk for VTE compared to patients without known malignancy [126, 127]. A multivariate analysis by Heit et al [128] showed that use of chemotherapy as a therapeutic strategy further increases VTE risk (up to 6.5-fold, odds ratio [OR] 6.5; 95% CI 2.1-20.2). This is probably caused by damage on the vascular endothelium and by reduction of the naturally occurring coagulation inhibitors [129]. Similar data have been reported by Khorana et al [130] in a prospective observational study, and by Blom et al [131] in a large retrospective cohort study of 66329 cancer patients.

As discussed above, surgery is a well-recognized risk factor of VTE. This relationship is of particular relevance in patients with malignant disease. Geerts et al [132] showed that cancer patients undergoing major surgical treatment ran at least a two-fold increased risk for DVT, and a more than three-fold increased risk for PE compared to patients with benign conditions, undergoing similar procedures. This was particularly highlighted in a retrospective study where patients with glioma undergoing major neurosurgery were 70% more likely to develop postoperative VTE during the first 3 month [133] than those operated for non-malignant diseases. In contrast, however, there are several other studies where surgery has not been found to be a major risk factor for VTE in patients with cancer [131, 134, 135].

Furthermore, VTE incidence is strongly dependent on the type and stage of spreading of malignant disease [131, 134, 136]. The above-mentioned studies also confirmed that metastatic cancer already at the time of diagnosis is one of the strongest independent risk factor for VTE. Malignant brain tumours and adenocarcinomas of the pancreas, stomach, ovary, kidneys, lungs and colon are associated with highest incidences of VTE [126, 131, 136]. However, according to a population-based case-control study by Blom et al [127], patients with haematological malignancies ran the highest risk of VTE after adjustment for age and gender, (OR 28.0; CI 4.0- 199.7), followed by those with lung (OR 22.2; CI 3.6-136.1) and gastrointestinal (OR 20.3; CI 4.9-83.0) cancers.

In several studies, VTE incidence seems to be highest during the first few months after the initial diagnosis of cancer, and thereafter decreases over time [127, 137]. Similar results have also been demonstrated in patients with colon, lung and ovarian cancer [134, 135, 138].

Cancer patients with a previous episode of VTE have reduced life expectancy. A large population-based study by Sorenson et al [139] was the first to report reduced survival in

cancer patients with VTE compared to patients with cancer but without VTE. Furthermore, the 1-year survival rate for cancer patients with VTE was 12% compared to 36% in patients with cancer without VTE, ( $p < 0.001$ ). In the same study mortality rate associated with VTE was 2.2 during 1-year follow-up period. Six years later a study from the California Cancer Registry database showed that VTE was a significant predictor of decreased survival during the first year for all cancer types diagnosed between 1993 and 1995 after adjustment for age, ethnicity, and tumour stage (HR 1.6-4.2;  $P < .01$ ) [136].

### *Pregnancy and the postpartum period*

Pregnancy is a hypercoagulable state featuring activation of coagulation and hypofibrinolysis by increased concentrations of coagulation factors V, VII, VIII, IX, X, XII, VWF and fibrinogen [140, 141]. Endogenous anticoagulants such as PS [141], and t-PA activity decreases during pregnancy due to a five-fold increase in PAI-1 [141], and enormously increased levels of plasminogen activator inhibitor-2 (PAI-2) produced by the placenta during the third trimester [142]. Moreover, PT and thrombin-antithrombin (TAT) complex levels are also increased during pregnancy [143]. These phenomena, probably occurring to protect the pregnant woman from fatal hemorrhage during delivery and the postpartum stage also increase the risk for VTE, however. VTE occurs with an incidence of 100-200 per 100,000 births or woman-years [144, 145], and remains a major cause of maternal mortality [146] with death rates of 1.4 per 100,000 pregnancies [147], constituting 10% of all maternal deaths. Additionally, Heit et al [145] confirmed a 5 fold increased annual VTE-incidence among postpartal women compared to pregnant women (511.2 vs. 95.8 per 100,000). Furthermore, VTE more often manifested itself as DVT than PE (151.8 vs. 47.9 per 100,000). The above mentioned study also showed a 4- to 5-fold increased risk of VTE among pregnant compared to non-pregnant women. This risk of VTE continued to increase further up to approximately 20-fold during the postpartal stage [145].

According to a study by James et al [148], cases of DVT constitute approximately 80% of all VTE events during pregnancy whereas 20% are due to PE. DVT during pregnancy affects the left leg in up to 90% of cases, and is usually localized proximally in the leg [149]. A possible explanation for this left-side DVT predominance could be compression of the left common iliac vein caused by the right common iliac artery and the pregnant uterus [149], the so called May-Thurner syndrome (**Picture 5**).



**Picture 5.**  
May-Thurner syndrome

Pregnant women with a previous history of VTE run a 3- to 4-fold increased risk of recurrent VTE compared to non-pregnant women (risk ratio [RR] 3.5; 95% CI 1.6 – 7.8;  $p=.002$ )[150]. Furthermore, in pregnant women with a previous history of VTE not receiving antithrombotic prophylaxis during pregnancy and postpartum VTE recurrence rate was 12.2% (19/155 pregnancies) [151], whereas in a retrospective study by Lepercq

et al [152] the VTE recurrence rate in pregnant women with appropriate antithrombotic prophylaxis was only 2.4% (3/125 pregnancies) [152]. The above-mentioned findings strongly suggest a benefit of prophylactic antithrombotic treatment during pregnancy and postpartum in this group of women.

Except previous history of thrombosis, genetic thrombophilia is one of the most important risk factor for VTE during pregnancy [148]. In a prospective study from Malmö, the overall prevalence of APC resistance among pregnant women was found to be 11% (270/2480), and seven pregnant women (0.3%) were carriers of the FV:Q506 allele in its homozygous form [153]. Pregnant women in the APC-resistant subgroup ran an 8-fold higher risk of VTE (3/270 vs. 3/2210) [153]. Additionally, between 20 to 50% of VTE cases during pregnancy were associated with inherited or acquired thrombophilia [154, 155]. According to a systematic review by Robertson et al [156], the absolute risk of VTE during pregnancy is variable depending on the type of thrombophilia as follows: FVL-homozygosity (OR 34.40; 95% CI 9.86-120.05), FVL-heterozygosity (OR 8.32; 95% CI 5.44-12.70), PT gene mutation-homozygosity (OR 26.36; 95% CI 1.24-559.29), PT gene mutation-heterozygosity (OR 6.80; 95% CI 2.46-18.77), PC deficiency (OR 4.76; 95% CI 2.15-10.57), PS deficiency (OR 2.19; 95% CI 1.48-6.00), and AT deficiency (OR 4.69; 95% CI 1.30-16.96).

Other risk factors for VTE during pregnancy are heart disease, sickle cell disease, lupus anticoagulant, obesity, anemia, diabetes, black ethnicity and age >35years [148]. Moreover, multiple gestation, hyperemesis, electrolyte and acid-base imbalance, antepartum hemorrhage, cesarean delivery, postpartum infection and bleeding are other pregnancy-related risk factors according to James et al [148].

The present drug of choice in the prevention and treatment of VTE during pregnancy is low molecular weight heparin (LMWH) due to its better side effect profile, good safety, and practical advantages over unfractionated heparin (UFH) [157]. Labour is a complex process involving several different mechanisms such as synchronized uterine muscle contraction and cell to cell communication acquired by the action potential propagation. Knowledge of such mechanisms is of highest importance [158] when assessing VTE risk and the need for prophylactic treatment. Studies by Hjelm et al [159, 160] have shown lower mRNA expression of syndecan 3 (which belong to the heparan sulphate proteoglycan group) in women with post term labour, as compared to normal labour. This suggests that this protein may be involved in the coordination of myometrial contractility. LMWH is a highly sulphated proteoglycan, well-known modulators of intracellular communication and also involved in the establishment of cell-cell and cell-matrix contacts [161]. In spite of the common use of LMWH, little is known about the effects on labour. Recently, primiparous women treated with LMWH have been reported to have 30% shorter time in labour [162].

### *Oral Contraceptives and hormone replacement therapy*

Combined oral contraceptives (COC) containing high dose of both estrogen and progestagen were first launched in the American market in 1960. The first case of VTE due to oral contraceptives use occurred in a nurse treated for endometriosis, and was reported already in 1961 by Jordan [163]. Over the subsequent years, several large studies have confirmed a two-fold to six-fold increased risk of DVT with use of COC [164-168].

Another group of patients treated with estrogen and progestagen as hormone replacement therapy (HRT) are women in post-menopausal age, affected by endogenous climacteric disorders due to the lack of estrogen. There is also a body of evidence for an association between such HRT and increased risk of VTE. This risk varies depending on the dose of estrogen, the type of concomitant progestagen, and the way in which estrogen is administered [169]. According to a recent meta-analysis, users of oral estrogen among post-menopausal patients run an increased risk of VTE (OR 2.1 [95% CI, 1.7-2.6] and 1.4 [95% CI, 1.3-1.5] in case/control and cohort studies respectively [170].

The underlying mechanisms explaining the increased risk of VTE among users of oral contraceptives or HRT are still not fully known. However, several studies indicate that oral contraceptives of the second and third-generations may have several prothrombotic effects by increasing procoagulants such as PT, FVII, FVIII,FX, fibrinogen, PT-fragment 1+2 and decreasing both FV [171] and PS, the cofactor of PC [172], and also by causing acquired resistance to APC [173]. Furthermore, oral contraceptives affect fibrinolysis by increasing levels of TAFI [174]. Similar effects on coagulation and fibrinolysis have been attributed to HRT [175, 176]

Since the estrogen doses in COC were shown to be associated with increased risk for VTE, the doses of estrogen in COC have been stepwise reduced over the years [177, 178]. Both a population-based case-control study from the Netherlands including 1524 patients and 1760 controls [166], and a cohort study from a population-based Danish national registry with 4213 patients with VTE events out of which 2045 occurred among current COC users [167] showed a risk reduction for VTE with 20µg reduced estrogen dose in the COC used. In addition, the risk of VTE in current users of COC decreases with prolonged use of COC. The RR was 4.17 [95% CI, 3.73-4.66] after <1 year; 2.98 [95% CI, 2.73-3.26] after 1-4 years, and 2.76 [95% CI, 2.53-3.02] after >4 years, compared to in non-users [167]. Similar results were also shown in a population-based case-control study from the Netherlands, where the risk of VTE was highest during the first 3 months of oral contraceptive use (OR 12.6 [95% CI, 7.1-22.4]) [166].

There are different types of progestagens with different chemical compositions, and in the mid-1990s they came in focus of the discussion due to evidence that progestagens counteract prothrombotic effects of estrogens in different ways. A large meta-analysis of cohort and case-control studies demonstrated a two-fold increased risk of VTE for users of third-generation COC (containing desogestrel or gestodene) compared to users of

second-generation COC (containing levonorgestrel) [165]. The above-mentioned findings are also supported by both a population-based case-control study from the Netherlands [166] and a cohort study using the population-based Danish national registry [167].

However, from a security point of view regarding the risk for VTE there is scientific data demonstrating that the lowest excess risk of VTE in users of COC is conferred by so-called mini-pills (progestagen-only preparations) [179]. More recently, a study from the above mentioned population-based Danish national registry failed to show any excess risk of VTE at all during use of oral contraceptive progestagen-only preparations, regardless of the type of progestagen in the mini-pill: desogestrel-containing progestagen-only mini-pill (RR 1.1 [95% CI, 0.4-3.4]), norethisterone-containing or levonogestrel-containing preparations (RR 0.6[95% CI, 0.3-1.0]) [167].

### *Socioeconomic status (SES)*

SES is a complex multifactorial variable influencing many aspects of human health [180-182] such as coronary heart disease (CHD) [183-185], diabetes mellitus [186-188], obstructive sleep apnea syndrome (OSAS) [189], cardiovascular mortality [190-192], and cancer [190, 193]. Information on possible associations between SES and VTE are scarce, however. In 1981, a study by Samkoff et al [194] demonstrated an increased rate of PE in subjects with less than 8 years of education compared to in subjects with longer education. In another study by Rosengren et al [195] it was shown that SES variables such as persistent stress and low occupational class were independently related to future PE, while no such significant relationship could be demonstrated between DVT and SES [195]. This study [195] which to the best of our knowledge is the only that has evaluated possible associations of this kind, was performed in male subjects only.

## Hereditary Thrombophilia

The term thrombophilia has been used for description of different both hereditary and acquired changes of biochemical state in blood, all resulting in a pre-disposition for thromboembolism (**Table 1**, page 41). During the last four decades several hereditary thrombophilic disorders have been identified. In 1965, Egeberg [33] described the first case of inherited thrombophilia caused by AT deficiency in a Norwegian family. In 1976, Stenflo [196] purified a new vitamin K-dependent protein called PC, and one year hereafter PS was described by DisCipio [197].

Subsequently, in the 1980s deficiencies of both PC [31] and PS [32] respectively were found to increase the risk of VTE. The interest in inherited causes of thrombophilia increased further after the first description of APC resistance by Dahlbäck et al in 1993 [30]. One year hereafter in 1994, Bertina et al from Leiden [198] showed that APC resistance is caused by a point mutation involving the FV-gene, namely the replacement



of arginine (R) at position 506 by glutamine (Q), and named this mutation FVL with reference to the geographical location of their institution [198].

According to a haplotype analysis by Zivelin et al [199], the FVL mutation is thought to have occurred in one individual some 21,000-34,000 years ago. The FVL mutation is the most common form of hereditary thrombophilia in healthy individuals of Caucasian origin. Its prevalence varies widely, however, from being practically absent to occurring in up to 15% of the population [200, 201]. The highest frequencies of the FVL-mutation have been reported from European countries, such as southern Sweden, Germany, and Cyprus [200, 202], but similarly high prevalences occur in several Middle East countries such as Lebanon and Jordan [203, 204]. Among patients with VTE, the FVL-mutation occurs in 20% of all cases, and in up to 50% of selected patients with thrombophilia [205]. The mutation confers a life-long hypercoagulable state, and heterozygous carriers have an approximately fivefold increased risk of VTE. This risk is even more pronounced in homozygote carriers of the mutation [206, 207], however, up to approximately 50-fold.

Furthermore, carriers of the FVL mutation also run increased risk for recurrence of VTE after their first episode. According to a systematic review by Ho et al [208], the FVL mutation was present in 21.4% among 3104 patients with first-ever VTE, and the odds ratio for recurrence of VTE in this group was 1.41(95% CI, 1.14-1.75;  $p=0.08$ ). One year later Marchiori et al [209] published a systematic review and meta-analysis of 10 different studies, involving 3203 patients with their first provoked or unprovoked VTE episode. The relative risk for recurrence of VTE in patients with heterozygous FVL mutation compared to VTE patients without the mutation was 1.39 (95% CI, 1.15-1.67).

Poort et al in 1996 [34] discovered a PT G20210A mutation occurring in the 3'-untranslated region of PT at position 20210 (G to A, PT20210A) associated with increased levels of PT. After the FVL mutation, the PT20210A mutation is the second most common hereditary form of thrombophilia in healthy individuals of Caucasian origin with prevalences from 1.7% to 3% [34]. The prevalence of this mutation is substantially higher, up to 18% in patients with personal or family history of VTE, however. Moreover 6.2% of unselected patients with VTE in this study were heterozygous for the PT20210A mutation, corresponding to a RR of 2.8 (95% CI, 1.4-5.6) [34].

A study from Malmö by Hillarp et al [210] in 99 unselected outpatients with phlebography verified DVT and 282 healthy controls showed prevalences of the PT20210A allele of 7.1% (7/99) in the patient group, and 1.8% (5/282) in the healthy control group ( $p = 0.0095$ ). The RR for VTE was calculated to be 4.2 (95% CI, 1.3 to 13.6), and the increased risk conferred by the mutation was still significant when adjustment was made for age, sex and the factor V:R506Q mutation causing APC resistance (OR3.8 [95% CI, 1.1 to 13.2]). The literature in this field is partly conflicting,

however, as a meta-analysis of 10 different studies by Marchiori et al [209] showed a non-significantly increased risk for recurrence of VTE of 1.20 (95% CI, 0.89-1.61).

According to several studies [211, 212] the PT20210A mutation is entirely absent in indigenous populations and rare in other ethnic groups of Asia, Africa, America, Australia, and in Middle Eastern Arabs. However, in the recently published study from the Iranian population the prevalence of the PT20210A mutation was 1.6% (95% CI, 0.5-2.7) [213], whereas the mutation has yet not been detected in black populations in the UK according to Patel et al [82].

In subjects of Caucasian origin deficiencies of AT, PC and PS are all rarer compared to the FVL and PT20210A mutations, with prevalences of 0.02-0.15% [214], 0.2-0.4% and 0.03-0.13 respectively [215]. The prevalence of these conditions are higher in patients with VTE, however, up to 1-3% for AT-deficiency, 3-5% for PC deficiency and 1-5% for PS deficiency [215].

Unfortunately, there are only a few studies investigating AT, PC and PS deficiencies in other different ethnic groups. In the thrombophilia screening study of 142 cases of VTE in black subjects from the UK prevalences were 0.7% for AT deficiency, 4.2% for PC deficiency, and 2.8%, for PS deficiency [82]. Moreover, a large Japanese study [216] among blood donors showed prevalences of 0.15% for AT deficiency and 0.13% for PC deficiency in the background population, which is in accordance with results presented in the above mentioned study by Franco et al [215]. However, the prevalence of PS deficiency in the healthy population was higher 1.12% [217].

Increased levels of procoagulants such as FVIII, FIX or FXI are also recognised as risk factors for VTE according to several studies [218-220]. FVIII was also shown to be an independent risk factor for VTE among black patients with VTE in the UK compared to black control subjects, with an OR of 4.64 (95% CI, 2.02-10.85) when FVIII:C was above the 90<sup>th</sup> centile (228 IU/dL) [82].

Antiphospholipid antibodies (aPL) constitute an inhomogeneous group of autoantibodies directed against phospholipidbinding proteins [221]. aPL antibodies include lupus anticoagulant (LAC), anti-cardiolipin antibodies (aCL), and anti- $\beta$ -glycoprotein-1 (anti- $\beta$ -GP1) [222]. Presence of these antibodies, the so called antiphospholipid antibody syndrome (APS), is a prothrombotic state associated with increased risk for arterial and venous thrombosis, and recurrent miscarriages.

DVT is a common condition among patients with APS, with reported prevalences of up to 38.9%, in the Euro-Phospholipid project of multicenter, consecutive and prospective design, derived from 13 countries [223]. The precise cause of the high prevalence of DVT in patients with aPL and the underlying mechanisms are still unknown, however. Several possible physiological phenomena have been proposed such as: inhibition of PC and AT, activation of platelets enabling increased expression of adhesion molecules and tissue factor from endothelial cells, and finally complement cascade activation [222]. A

prospective study by Kearon et al [224] showed increased risk for recurrence of VTE, (HR 6.8 [95% CI, 1.5-3.1]) in VTE patients with LAC after their anticoagulants have been discontinued. In another study the risk for recurrent VTE was 29% in patients with aCL compared to 14% in those without antibodies ( $P = 0.0013$ ) [225].

Homocysteine is an amino acid formed by methionine. Its synthesis is dependent on several vitamin cofactors such as folate, vitamin B12, vitamin B6 and flavin adenine dinucleotide. Hyperhomocysteinemia (HHcy) may occur as the result of several different acquired conditions such as deficiencies of folate, vitamin B6, and vitamin B12, or by mutations such as the C677T polymorphism in the MTHFR gene [226], and is associated with increased risk for VTE. In which way HHcy affects the risk of thrombosis is not yet fully understood. However, HHcy may trigger endothelial dysfunction through oxidative damage, thereby inducing increased oxidation of low density lipoprotein and subsequently a hypercoagulable state. The toxic effects of high HHcy concentrations on endothelial cells also result in increased platelet adhesion, impaired regulation of endothelium-derived relaxing factor, induction of tissue factor, suppression of heparan sulfate expression, and stimulation of smooth muscle cell proliferation.

**Table 1.**

Prevalence of different forms of hereditary thrombophilia, and their relevance for the occurrence and recurrence of VTE. Modified with permission from treatment guidelines for VTE in Southern Sweden [227].

Trombophilic factor (acquired/hereditary)	Prevalence in the population (%)	Prevalence in patients with VTE (%)	Risk increase for VTE (x)	Estimated risk of VTE recurrence
Antithrombin deficiency	0,02	0,8	10-20	+++
Protein C deficiency	0,2	1	5-10	++
Protein S deficiency	0,1	1	5-10	++
Faktor V* mutation, heterozygosity	5-10	20-25	3-5	+
FV mutation, homozygosity	0,1	3-4	30-40	+++
PT* mutation, heterozygosity	2	6-7	3-5	+
PT mutation, homozygosity	0,01	unknown	?	+(+)
Hyperhomocysteinemia	5	10-15	3	(+)
Lupus anticoagulant	1	10	10	++
Anti-cardiolipin antibodies	2	10	5	+

\*Factor V = APC-resistence, factor V Leiden, PT= prothrombin mutation

## Diagnosis and treatment

As reflected in its title the focus of this thesis is upon epidemiology of and risk factors for VTE, and no major emphasis has been put on diagnosis and treatment of VTE. Both diagnosis and treatment is in the majority of VTE cases conducted in accordance with international [228, 229], national [230, 231], and local [227] guidelines. The diagnosis of DVT is established by phlebography or ultrasonography, and the diagnosis of PE by computer tomography (CT) of the pulmonary arteries or by lung scintigraphy. Guidelines all advocate anticoagulant drugs such as oral anticoagulants (OAC), mainly warfarin, low molecular and unfractionated heparins, or thrombolytic substances such as alteplase in selected cases. Of particular interest in this thesis is, however, VTE treatment in pregnancy which is conducted with LMWH or UFH [227, 228, 230, 231], as warfarin is unsuitable during pregnancy due to both risk for fetal bleeding and teratogenic effects. During 2012-2013, several new oral anticoagulant drugs for VTE treatment will be available on the market [232]

# AIMS

The aims of the studies presented in this thesis were the following:

- I. To evaluate distribution, epidemiology and risk factor patterns in a population based total material of 1140 consecutive adult patients with DVT and PE diagnosed during 8 years at Skåne (previously called Malmö) University Hospital, (Paper I).
- II. To investigate potential relationships between the FVL-mutation and Hb levels in 927 patients with VTE, in particular women of pre- and postmenopausal age in the above cohort (Paper II).
- III. To investigate the epidemiology of UEDVT, and risk factors, recurrence risk and mortality in a population based total material of 63 consecutive patients with UEDVT in the above cohort (Paper III).
- IV. To prospectively assess whether variables reflecting SES and concomitant diseases influences the risk for VTE during long time follow-up in a total population (Paper IV).
- V. To investigate effects of LMWH upon time of labour and bleeding in 216 pregnant women with increased risk for VTE (Paper V).



# SUBJECTS

## Paper I.

All VTE patients aged >18 years diagnosed at Malmö University Hospital (UMAS) during 1998-2006 who were able to communicate in Swedish were invited to the Malmö Thrombophilia study (MATS). Of a total of 1203 patients in the study, 1140 (age range 61±18, 581 women) had lower extremity DVT or PE and were studied in paper I. An objective diagnosis of DVT or PE was made by methods such as phlebography, duplex ultrasound, computed tomography (CT), lung scintigraphy or magnetic resonance imaging (MRI). All patients were treated in accordance with the standard treatment protocol [227] of UMAS (later termed Skåne University Hospital [SUS]).

## Paper II.

One thousand fifty six of the above mentioned VTE patients included in MATS during 1998-2005 were studied in paper II. In this cohort, 927 (473 women) underwent analysis of carriership of the FVL-mutation. Diagnosis and treatment of VTE was conducted as stated for paper I.

## Paper III.

Of all 1203 patients in MATS with VTE diagnosed during 1998-2006, 63 (5%, 33 men [52%, age 54±17 years], and 30 women [48%, age 55±22 years]) had UEDVT and were evaluated concerning risk factors, treatment, recurrent VTE, and mortality in paper III. Diagnosis and treatment of VTE was conducted as stated for paper I.

## Paper IV.

The study reported in paper IV was performed within the research project “Longitudinal Multilevel Analysis in Scania” conducted at the Unit for Social Epidemiology at Lund University. The project has been approved by the Data Safety Committees at Statistics Sweden, the Swedish Centre for Epidemiology, and by the Regional Ethical Review Board in Lund. The personal identification number assigned to each person in Sweden was used by the Swedish authorities to link information on socioeconomic, demographic and health care variables from different registers. Once the record linkage was done the original personal identification number was encrypted to strength the anonymity of the individuals.



In 1990, all 730050 inhabitants (379465 women and 350585 men) above 25 years of age in the County of Skåne in Sweden were evaluated with regard to age, household income, marital status, and country of birth, number of years of residence in Sweden, educational level, and concomitant diseases. The cohort was hereafter prospectively investigated regarding diagnosis of, or death from VTE (DVT or PE), during the period 1991-2003.

## Paper V.

In paper V, we retrospectively studied 217 consecutive gravidae who had been treated with LMWH (dalteparin subcutaneously) daily during pregnancy, and delivered at the maternity ward of UMAS during 1996–2005. The included women had all been treated due to high VTE risk caused by risk factors such as: prior VTE, FVL or PT gene mutations, overweight, heredity of thrombosis, maternal age >40, or hyperhomocysteinemia. PS or PC deficiencies were counted as two such risk factors, and homozygous FVL or homozygous PT-mutation as three. These gravidae were compared with 1,499 pregnant women without thromboprophylaxis, who gave birth during the same time period.

## METHODS

The Ethics Committee of Lund University approved all studies in paper I-V.

### Paper I

To evaluate the participation rate in MATS, computerized inpatient records at UMAS during the study period were screened for VTE-diagnoses (DVT or PE) by a research nurse, and the rate of consensual study participation was estimated to 70%. The remaining 30% of VTE patients had not been included due to language problems, dementia or other severe disease, and in a few cases direct unwillingness to participate in MATS.

In included patients, the following risk factors for VTE were recorded: surgical intervention, immobilization or cast therapy within the last month, tobacco use before VTE diagnosis, and travel more than three hours by bus, air, car or train within the last month. We also recorded use of COC and HRT, pregnancy, and ongoing DVT prophylaxis at diagnosis. The postpartum period was defined as the first 6 weeks after delivery. Malignancies diagnosed prior to or at diagnosis of VTE were also recorded. Heredity was defined as a history of VTE in first-degree relatives.

Cases of DVT were defined as iliacal DVT if involving the iliac veins or the inferior vena cava, as femoropopliteal DVT in the absence of more proximal thrombosis than in the popliteal or superficial femoral veins, and as calf (distal) DVT if involving only the anterior tibial vein or more distal venous segments. Patients with PE were analyzed as one group without respect to anatomical localization of the embolic material in the pulmonary arterial tree.

### Paper II

In this article, we state that the rate of consensual participation in MATS was estimated to about 90%, and that the remaining 10% of patients were excluded due to language problems, and unwillingness to participate in MATS. However, paper II was written and this statement was made prior to the above mentioned screening of patient files made by our research nurse, and therefore probably represents an overestimation of the participation rate. In addition, it has to be taken into consideration that we in paper II only studied patients included in MATS up to 2005, and that participation rates might have differed during different parts of the study. To each of the patients in paper II, a characteristic haemoglobin (Hb) was assigned by computing the median of all Hb values available for that patient in UMAS computerized records. The median number of Hb

values per patients was 7. This characteristic Hb was henceforth referred to as the patients Hb. Since data for menopausal onset were not readily available, we used an age limit of 50 year or lower to characterize the group of pre-menopausal women. VTE was diagnosed and defined as in Paper I.

## Paper III

All 63 patients in MATS with UEDVT diagnosed during 1998-2006 underwent screening for hereditary thrombophilia: (FVL-mutation, PT-mutation, antiphospholipid antibodies and FVIII, PS and PC, AT, and lupus anticoagulant). The following risk factors for UEDVT were recorded: malignancies diagnosed prior to or at diagnosis of UEDVT, central vein catheter (CVC), COC or HRT use, and pregnancy. Primary UEDVT was defined as UEDVT with uncertain etiology, and secondary UEDVT as UEDVT due to iatrogenic causes or malignancy. The postpartum period was defined as the first 6 weeks after delivery. Heredity was defined as a history of VTE in first-degree relatives.

## Paper IV

All 730050 inhabitants above 25 years of age in the County of Skåne in 1990 were evaluated with regard to age, household income, marital status, and country of birth, number of years of residence in Sweden, educational level, and concomitant diseases. The cohort was prospectively investigated regarding diagnosis of, or death from VTE (DVT or PE), during 1991-2003.

Information on discharge diagnoses was obtained from the National Patient Register, and on causes of death from the National Mortality Register at the Swedish national Board of Health and Welfare (<http://www.sos.se/sose/sos/omsos/statist.htm>).

We defined VTE/DVT/PE – the outcome in this study – as an in hospital diagnosis of or death due to VTE according the International Classification of Diseases and Causes of Death, versions 9<sup>th</sup> and 10<sup>th</sup> (ICD9, ICD10) codes (ICD9: 451-453 & ICD10: I80-I82 or ICD9: 415 & ICD10: I26), during 1991 to 2003.

Statistics Sweden (<http://www.scb.se/>) provided information on socioeconomic and demographical variables for 1990, according to the Swedish census and the Population Register.

Age was categorized into six groups (60-64, 65-69, 70-74, 75-79, 80-84 and 85 years or more), and the youngest age group was used as reference in the comparisons. Annual equalized family disposable income in 1990 was divided into three categories (i.e., low, medium and high) using the tertiles of distribution. The highest tertile was used as reference in the analysis. Formal education was categorized into less than 9 years, 10 – 12

years and 13 years or more and the last category was used as reference in the comparisons. Since information on educational achievement was lacking in 16% (n = 60136) of the women and 11% (n = 37937) of the men lack we created a category with missing values. Marital status was dichotomised into married/cohabiting and living alone (i.e., single, divorced or widowed). Married/cohabiting was used as reference in the analysis. Country of origin was categorized into two groups, Sweden or other. Using information from the Population Register we calculated the number of years every individual had been registered as resident in Sweden and categorized this variable into 0-4, 5-9, 10-14 and 15 years or more. We applied this last category as reference in the comparisons.

## Paper V

In this retrospective study 217 consecutive gravidae were treated with LMWH (dalteparin 5000 U daily) during pregnancy, and hereafter followed regarding VTE complications and by analysis of labour charts. No LMWH was given during active labour or before Caesarean delivery, however. The first injection of LMWH after delivery was scheduled to occur 4 h postpartum and epidural anesthesia was not given within 10 h after the last LMWH injection. The LMWH treated gravidae were compared with 1499, unselected pregnant women.

The first stage of labour was defined as the period from the active phase of contraction and cervical dilatation from 4 to 10 cm. The second stage of labor was defined as the period from complete cervical dilatation (10 cm) to delivery. A prolonged first stage of labour was defined as duration exceeding 6 h and a prolonged second stage of labour as duration exceeding 60 minutes.

Intrapartum blood loss was estimated by the delivering midwife, by measuring the volume of free blood, and in addition, approximating the amount of blood in swabs and cloths, and subtracting the amount of amniotic fluid mixed with it. Postpartum anaemia was defined as an Hb-value  $\leq 100$  g/l on the second day postpartum. Profuse haemorrhage during delivery was defined as blood loss  $\leq 1,000$  ml (according to the International Classification of Diseases, tenth revision). Pre-eclampsia was defined as pregnancy-induced hypertension and proteinuria  $>0.3$  g/1 (Albustix\_ Boehringer Mannheim  $\geq 1+$ ). Pregnancy-induced hypertension was defined as resting diastolic blood pressure  $>90$  mmHg measured on two occasions at an interval of at least 5 h, and developing after 20 weeks of gestation in a previously normotensive gravidity. Preterm delivery was defined as delivery at less than 37 completed weeks of gestation. Gestational age was estimated by ultrasonographic measurements of biparietal diameter and femur length in 98% of cases, and from the date of the last menstrual period in the remaining 2%.

## Laboratory analysis (Papers II and III)

All patients included in papers II-III were tested for APC resistance by the COATEST®, APC™ RESISTANCE V (Chromogenix) according to the manufacturers' instructions. All patients with APC resistance ratios < 2.0 were analysed for the FVL-mutation as previously described [198, 233]. Carriership of FVL was defined as either heterozygous or homozygous. Blood haemoglobin was automatically analyzed on a Coulter CH 750 System analyzer (Beckman Coulter Inc; CA USA). Presence or absence of the prothrombin gene 20210 G to A transition was determined as described previously (Poort SW 1196).

## Statistical analyses (Papers I-V)

Data are expressed as mean  $\pm$  SD, or median, unless otherwise stated. P-values <0.05 were considered statistically significant. Statistical analyses were carried out using StatView 5.0 (SAS Institute, Cary, NC, USA) (**papers I and III**), Macintosh powerbook G3, running Debian GNU/Linux version 3.1 (**paper II**), SPSS software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, USA) (**papers IV and V**).

In **papers I and III** only descriptive analysis was performed. In **Paper II** we employed medians and Mann-Witney U tests for statistical characterization and testing to avoid the effects of outliers, small samples, and non-normality of distributions. In **paper IV** we performed a sex stratified Cox regression and obtained hazard ratios and 95% confidence intervals. We developed two different models. In the first set of analysis we included only demographical and socioeconomical variables. In the 2<sup>nd</sup> set we expanded the previous model and added previous diseases and risk factors for VTE.

In **Paper V** the statistical analysis includes Mann-Whitney U tests and Chi-squared or Fishers exact test for categorical variables, since the duration of delivery and blood loss were non-normally distributed variables.

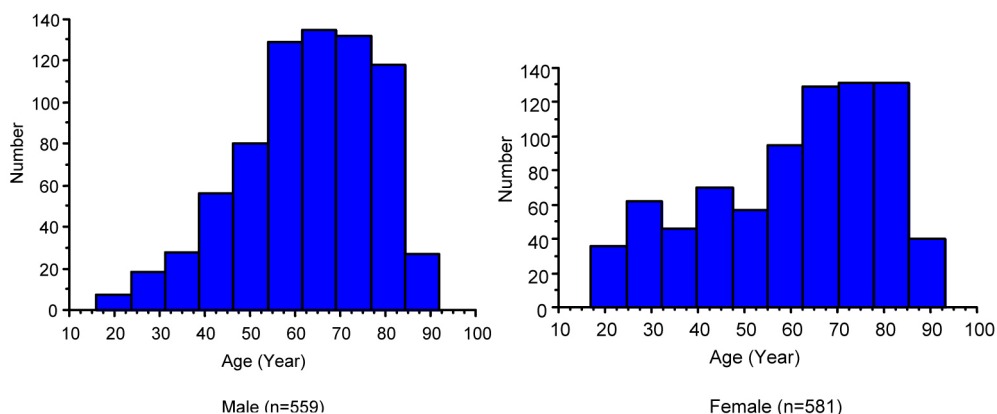
# RESULTS

## Paper I

Of the 1140 consecutive VTE patients, (559 men [49%, age range 62±16 years], and 581 women [51%, age range 61±20 years]), 882 (77%) patients had DVT, 330 (29%) patients had PE and 72 (6%) patients had both DVT and PE. Of 882 DVT patients, 499 (56%) patients had left leg DVT, 116 (13%) had iliacal DVT, 465 (53%) femoropopliteal DVT, 246 (28%) calf DVT and 55 (6%) had unknown DVT location. The most common acquired risk factors were COC use or HRT (24% of female DVT patients and 19% of female PE patients), previous surgery (13% of DVT patients and 19% of PE patients), and concomitant malignant disease (12% of DVT patients and 11% of PE patients). A family history for VTE existed in 25% of DVT patients and 22% of PE patients. Malignancy was more prevalent in ages 50–69 years, (59 [15%]) and >70 years (67 [14%]). **Fig. 7** shows the age distribution in all 1140 patients with DVT and PE.

**Figure 7.**

Age distribution in male (n=559) and female (n=581) patients with VTE (lower extremity DVT or PE).



For DVT diagnosis, phlebography had been used in 739 (84%), duplex ultrasound in 214 (24%) patients, other methods in 13(1.4%) patients, and 142 (16%) patients had undergone both phlaebography and duplex ultrasound. In 58 (7%) DVT cases the diagnosis was based on clinical symptoms in patients with concomitant PE. Of 330 PE patients, 313 (95%) had been diagnosed with CT, 19 (6%) with lung scintigraphy, 2 (1%) with other methods, and in 6 (2%) with both CT and scintigraphy. Analysis of treatment showed that 864 (98%) of DVT patients received LMWH, 851 (96%) OAC, 41 (0.5%)

UFH, and 20 (0.2%) thrombolysis. Of PE patients, 319 (97%) received LMWH, 314 (95%) OAC, 97 (29%) UFH, and 61 (18%) thrombolysis. Incidences of VTE, DVT, and PE were 66, 51, and 19 / 100,000 / year, respectively.

## Paper II

The FVL-mutation occurred in 288 (31%) patients, of which 261 were heterozygous, and 27 homozygous. Female patients below age 50 years with FVL-mutation had significantly ( $P < 0.001$ ) higher median-Hb compared to female patients below age 50 years without FVL mutation (**Table 2**). No significant difference was found between women with and without the FVL-mutation and Hb above the age of 50 years. There were no significant differences in Hb value between men with and without FVL-mutation below or above age 50 years. Patients with FVL-mutation had significantly lower median age compared to patients without FVL-mutation ( $P = 0.024$ ). Male carriers of the FVL-mutation had significantly lower median age compared to non-carriers ( $P = 0.004$ ), whereas the median ages did not differ ( $P = 0.687$ ) between the female groups. The overall prevalence of the prothrombin gene mutation was 46 of 916 (5%). Analysis of Hb-values in relation to the prothrombin gene mutation and age over and below 50 in men and women revealed no significant differences.

**Table 2.**

Population characteristics of the MATS cohort with respect to FVL-mutation and blood haemoglobin value. The numbers in each cell denote median blood Hb value and the numbers within parenthesis denote the number of patients in each category. The last column contains p-values from comparison between the FVL-mutation and non FVL-mutation populations.

	Median Hb (g/l)			P-value
	All	FVL	Non-FVL	
All	136 (n = 927)	137 (n = 288)	135 (n = 639)	0.054
Male	144 (n = 454)	144 (n = 143)	143 (n = 311)	0.394
Female	131 (n = 473)	132 (n = 145)	130 (n = 328)	0.182
Female <50	128 (n = 106)	133 (n = 37)	126 (n = 69)	0.001

## Paper III

Of the 63 UEDVT patients, 33 were men (52%, age range 54±17 years), and 30 women (48%, age range 55±22 years) (**Table 3**). Five (8%) had previous VTE. At diagnosis, 19 (30%) had known malignancy and 6 (10%) VTE heredity. Among the 30 female patients, 1(3%) was pregnant, 4(13%) used COC or HRT, whereas none was in the postpartum period. Of the 63 patients, 33(52%) had primary and 30(48%) secondary UEDVT. Of 30 (48%) patients with secondary UEDVT, 11 (17%) were iatrogenic, 13 (21%) had malignancy only, and 6 (9%) had both malignancy and peripherally inserted central catheter (PICC) or port-a-cath (PAC).

**Table 3.**

Patient characteristics and risk factors for thrombosis in 63 patients with UEDVT. Mean±SD or n (%).

	All UEDVT patients n=63	Men n=33(52)	Women n=30(48)
Age (Years)	54±20	54±17	55±22
Body mass index (kg/ m <sup>2</sup> )	25±5	26±4	25±6
Malignancy at diagnosis	19(30)	7(21)	12(40)
Hereditary thrombophilia	18(29)	10(30)	8(27)
Pregnancy*			1(3)
Hormone therapy*			4(13)

\* Percentage of female patients only.

For diagnosis of UEDVT, phlebography had been used in 48(76%), ultrasound in 16(25%), and CT in 9(14%) patients. Of the 63 patients with UEDVT, 22(35%) were treated in hospital, and 41(65%) as out-patients. Sixty-two (98%) patients received LMH, 60(95%) OAC, 3(5%) UFH, and 3(5%) thrombolysis. Yearly UEDVT incidence was 3.6 / 100,000, (95% CI, 3.3-4.0). VTE recurrence rate in all UEDVT patients was 8/63(13%) during median 62 (range 31-117) months of follow-up (2.6% per year). Recurrences occurred in 5/35(14%) patients with known malignancy or hereditary thrombophilia (2.8% per year), and in 3/28(11%) among other patients (2.2% per year, NS; p=0.712). Time to first recurrence was median 11 (range 1-56) months. Mortality during median 62 (range 31-117) month of follow-up was 15/63(24%), 9/19(47%) among patients with known malignancy at diagnosis, and 6/44(14%) among the other patients. These figures correspond to yearly mortality rates of 4.6%, 9.1%, and 2.7%, respectively in these three groups. No deaths in our material were caused by VTE.



## Paper IV

During the 13 years prospective follow-up, 10212 women and 7922 men were diagnosed with VTE. Characteristics of the population are shown in **Table 4**. In both genders, age above 64 years at baseline, low income, single status, and a lower level of education were associated with an increased risk of VTE. Both men and women born outside of Sweden experienced lower risk for VTE during follow-up, however.

**Table 4.**

Characteristics of the population with and without venous thromboembolism (VTE) during 1991-2003. Socioeconomic and demographical variables are from 1990, and data on previous diseases and risk factors refer to 1987-1990.

	Women (n = 379456)		Men (n = 350585)	
	Without VTE	With VTE (2.7%)	Without VTE	With VTE (2.3%)
<b>Socioeconomical / demographical variables</b>				
Mean age (years)	52.51	67.15	49.79	63.35
Income level				
- Low (%)	37	52	29	34
- Medium (%)	33	28	34	34
- High (%)	31	20	37	32
Living alone (%)	43	53	38	33
Education				
- Missing data (%)	15	36	11	22
- 0-9 years (%)	34	39	34	41
- 0-12 years (%)	34	19	37	27
- >13 years (%)	17	6	19	10
Born outside Sweden (%)	11	7	11	7
Number of years in Sweden				
- 0-4 years (%)	2.4	0.6	2.8	0.6
- 5-9 years (%)	1.5	0.6	1.5	0.6
- 10-14 years (%)	1.4	0.5	1.3	0.5
- > 15 years or always (%)	95	98	94	98
<b>Previous diseases/risk factors* (%)</b>				
Chronic obstructive pulmonary disease	0.3	0.6	0.4	1.3
Diabetes mellitus	1.1	2.2	1.2	2.4
Congestive heart disease	0.9	1.6	0.9	2.1
Ischemic heart disease	1.6	3.4	2.7	5.3
Cancer	1.7	3.8	1.5	3.3
Inflammatory bowel disease	0.2	0.3	0.2	0.3
Sepsis	0.2	0.3	0.2	0.5
Pneumonia	0.9	1.9	1.0	2.4
Fracture or trauma	3.5	5.8	3.2	4.0
Surgery	19	23	12	20

\*Chronic obstructive pulmonary disease (ICD9: 490-492, 496 & ICD10: J40-J44), diabetes mellitus (ICD9: 250 & ICD10: E10-E11), Ischemic Heart Disease (ICD9: 410-414 & ICD10: I20-I25), congestive heart disease (ICD9: 428 & ICD10: I50), Cancer (ICD9: 140-208 & ICD10: C00-C97), inflammatory bowel disease (ICD9: 555-556 & ICD10: K50-K51), sepsis (ICD9: 036C, 038 & ICD10: A327, A392-394, A40-41, A483), pneumonia (ICD9: 480-486, 510-11, 513 & ICD10: A481, B012, B015, J12-J18, J20-22, J85-86), fractures and trauma (ICD9: 8-, 91,92,95, 900-904 & ICD10:S00-T14).

## Paper V

Women on thromboprophylaxis were older and had higher weight than the control group. They also had an increased likelihood of being delivered by Caesarean Section. The deliveries in this group also occurred at a shorter gestational age, and with an increased incidence of preterm delivery compared to the control group. In addition, the risk of profuse blood loss and postpartum anaemia was doubled (10.6% vs. 5.9%,  $P = 0.009$ , 12.9% vs. 8.7%,  $P = 0.048$ , respectively) in the LMWH treated women (**Table 5**). Among primiparous women the first stage of labour in the study group was found to be 1 h shorter in comparison with the control group (5.2 vs. 6.2 h;  $P = 0.06$ ). On the other hand, there was no difference in the duration of the second stage of labour. The risk of a prolonged first stage of labour was significantly lower with LMWH treatment among primiparous women (4.1% vs. 8.5%;  $P = 0.047$ ), whereas no such differences could be demonstrated among multiparous women (first stage 4.0 vs. 3.8 h, second stage 0.33 vs. 0.38 h).

**Table 5.**

Clinical characteristics of subgroups with and without LMWH of parturients.

n	LMWH (n = 217)		Control (n = 1499)		P-value
<b>Maternal characteristics</b>					
Age (years)	30.7	4.7	29.3	5.3	<0.001
Nulliparac (n)	104	48.0%	787	52.5%	0.2
Preeclampsia (n)	5	2.3%	26	1.7%	0.6
Weight (kg)	72.2	15	65.1	14.9	<0.001
<b>Characteristics of delivery</b>					
Vaginal, spontaneous (n)	163	74.7%	1189	79.3%	0.2
Vaginal, operative (n)	12	5.5%	104	6.9%	0.4
Cesarean section (n)	42	19.4%	205	13.3%	0.03
Use of epidural anesthesia (n)	26	12,00%	235	15.7%	0.2
<b>Neonates</b>					
Male gender	114	52.5%	762	50.8%	0.6
Gestational age at birth (days)	273.9	18.3	277,2	13.4	0.012
Preterm delivery (< 37 weeks)	25	11.5%	89	5.9%	0.002
Postterm delivery (> 42 weeks)	12	5.5%	80	5.3%	0.9
Birthweight (g)	3389	676	3454	570	0.2
Birthweight deviation (%)*	-0.79	13.7	-1.3%	12.6	0.6
Small-for-gestational age* (n)	10	4.6%	73	4.9%	0.9
5-min Apgar score <7	7	2.2%	24	1.7%	0.09
pH umbilical artery **	7.23 (n = 148)	±0.08	7.22 (n = 1065)	±0.09	0.4
pH umbilical vein **	7.31 (n = 197)	±0.08	7.31 (n = 1372)	±0.08	0.8

Means ± standard deviations, or numbers and percentages are given.

\* Birthweight as compared to a gestational age adjusted reference population (Marsal 1996).

\*\* Not investigated in all cases.

## GENERAL DISCUSSION

Our present knowledge about the incidence of VTE is based upon studies performed either in Northern or Western Europe [59, 60, 63], or in the United States [58, 62, 66]. These results show that VTE including both PE and DVT is a major health problem with incidences that vary widely, however, ranging from 71 to 192 per 100,000 individuals per year [58-60, 62, 66]. The specific incidences of DVT and PE also vary widely, and have been estimated to 48 – 160 and 23 – 60 cases per 100,000 inhabitants per year, respectively [58-60, 63, 66]. Indeed, this wide variation in reported VTE incidence rates probably reflects differences in study design, different inclusion and exclusion criteria, but also the use of data from a variety of sources. Previous studies have been based on records from inpatients only [58], hospital records for both in- and outpatients [66], patients from different geographic areas [62], patients with a specific concomitant disease such as malignancy, vasculitis, lung transplant, surgery, hamatopoietic stem cell transplantation [234-239], postoperative patients, or upon all DVT patients at a specific hospital [59]. Furthermore, some of these studies did not separate first-time VTE cases from recurrent events [58-60], whereas the studies by Silverstein et al [66] and by Cushman et al [62] both have separated first-time VTE from recurrent events and also included VTE cases diagnosed by autopsy. All these factors evaluated together severely complicate comparisons between these different studies regarding the true incidence of VTE.

All the three first studies (**paper I-III**) in this thesis, on the other hand, were performed in a cohort from a well-defined geographic area, and included both out-patients presenting at our hospital because of suspected VTE symptoms, and in-patients from all hospital departments, including surgical patients with a VTE diagnosis obtained pre- or postoperatively. Both all patients with their first episode of VTE during 1998-2006, and those with a prior event occurring before 1998 were included in MATS. Furthermore, UMAS (later termed SUS) is the only hospital in the Malmö region treating VTE patients, and our results are therefore hopefully representative for the true VTE epidemiology and risk factor panorama in the Malmö area.

Yearly incidences of VTE, DVT, and PE in our study (**Paper I**) including both first-time VTE and recurrent events as above defined were 66, 51, 19 per 100,000 inhabitants per year, respectively, representing a lower incidence than the one reported in several earlier epidemiological studies [59, 60, 62, 63]. Our results may reflect an underestimation of the true incidence of VTE, however, since we did not exclude children (newborn to 18 years of age) from the Malmö population, when calculating VTE incidence. On the other hand, a paediatric retrospective study by Rask et al [98] of 128 children with a first episode of DVT (newborn to 20 years of age) from our hospital with the same catchment area showed an annual incidence of only 5 per 100,000 children per year. Due to this low VTE incidence in this young age group, we believe that the estimated

incidence of VTE in our study (**paper I**) would not have been effected to a great extent even if we had excluded the number of children from the population figure when the incidence was calculated.

Moreover, another concern regarding our estimation of a comparably low VTE incidence (**paper I**) was that in patients with both a first and a second episode of VTE during 1998-2006, the second episode was counted as a recurrence and not as an incident event. Furthermore, in the calculations of incidence in **paper I** we did not include UEDVT cases, or autopsy diagnoses that might have revealed previously asymptomatic cases of PE. Such cases might have contributed to the higher incidences in previous studies [59, 66] in which post mortem diagnoses were included. Instead, we chose to evaluate UEDVT incidence separately in an independent study on the 63 patients diagnosed with UEDVT (**Paper III**) among the consecutively enrolled 1203 patients with VTE at our hospital during the same time period between 1998 and 2006. An addition of the UEDVT figures to the lower extremity DVT incidence in our population would have increased this figure from 51 to 55 / 100,000 individuals / year. The yearly incidence of 3.6 per 100.000 inhabitants per year for UEDVT in our study was, also lower compared to earlier retrospective epidemiological study by Spencer et al [240] in UEDVT patients. A possible explanation for the lower incidence of UEDVT in our study (**paper III**) could be the comparably low number of patients with central line placement. In our population, 52 % of all UEDVT cases were instead considered as being primary UEDVT, corresponding to an incidence of 2 / 100,000 / year for primary UEDVT. This indicates that primary UEDVT is a rare disorder (**paper III**) compared to secondary axillary or subclavian vein thrombosis, which today accounts for a majority of UEDVT cases [64]. In an earlier series (1971-1986) from UMAS representing a total population of about 350,000 inhabitants, 61 % of patients with UEDVT were considered to have a primary thrombosis [241], and the majority of these patients had no well-defined factors contributing to the development of thrombosis. A thrombosis caused by effort or physical activity was probable in only 6 %. A proven hypercoagulable state was seen in 9 % of these patients, and another 17 % used COC or had experienced previous thromboembolic episodes. In a more recent analysis of the time period 1987-2001 from the same catchment area only 3 % out of the total 155 cases of UEDVT were effort-induced, 30 % had probable spontaneous UEDVT, whereas the majority of patients had secondary thrombosis due to for example central venous catheters or malignancy [242].

Many reports have shown different coagulation and fibrinolytic defects in patients with VTE. Unfortunately, at the time our first study (**paper I**) was conducted we did not have the resources to carry out a complete analysis of all genetic risk factors in such a large patient material as 1140 patients. Complete coagulation analysis was possible only in a smaller material such as the 63 UEDVT patients reported on in **paper III**, and analysis restricted to the factor V Leiden and prothrombin mutations in a part of the MATS material in **paper II**. After completion of MATS, however, our research group has further investigated the same data base with a complete array of coagulation analyses,

and data recently submitted by Sveinsdottir et al [243] showed a prevalence of 23.3% for the heterozygous form of the FVL mutation, and 2.4% for the homozygous form. The PT mutation in its heterozygous form was seen in 3.1% of patients, whereas no homozygous carriers of the mutation were found. In 0.8% of patients we found both the FVL and PT mutations.

Genetic thrombophilia was found in 29 % in our material of UEDVT patients (**paper III**); 19 % had heterozygous and 5 % homozygous FVL, 3 % had the PT mutation and 2 % had combined APS and heterozygous FVL mutation. In our series, no cases of AT, PC- or S- deficiency were found. Heron and co-workers analyzed 51 patients with primary UEDVT revealing a higher frequency of a familiar history of DVT (42% vs. 15%) and at least one coagulation abnormality (42% vs. 15%) in patients with spontaneous UEDVT (n=31) compared with effort-induced UEDVT (n=20) [244]. Interestingly, Leebeek and associates reported a high frequency of antiphospholipid antibodies, 27% in UEDVT [245].

About 20 % of thrombosed veins in the upper extremity will recanalize after anticoagulant treatment [242]. In our study (**paper III**) 8/63 patients experienced recurrent VTE during a median follow-up of 62 months. Recurrences occurred in 5 cases that were considered as having secondary thrombosis with known malignancy or thrombophilia, and in 3 cases with primary UEDVT. Four of the patients with recurrences experienced a new episode in the upper extremity, one had a PE and the remaining three had lower extremity DVT. None of the recurrences occurred during ongoing anticoagulant treatment. In a review Sajid et al [246] reported from compiled data that the same risks for recurrences were seen in spite of different initial treatment strategies. In their report 21 of 379 patients treated with standard anticoagulation had recurrent thrombosis compared to 18 of 262 patients that underwent thrombolysis with or without adjunctive thoracic outlet decompression.

VTE is predominantly a disease of older age with incidences rising from < 5 cases per 100,000 children below 15 years of age to 450 to 600 cases per 100,000 individuals over the age of 80 years [66]. The same study also showed declining overall VTE incidence by 35% between 1977 and 1979. This decrease in incidence over time was most evident for PE for both genders and all age groups, whereas the incidence of DVT remained relatively constant [66]. That age is an important risk factor for VTE was also confirmed in our studies with mean age at diagnosis  $61 \pm 18$  years in **paper I**, and median ages 67 and 70 years in patients with or without the FVL-mutation, respectively in **paper II**. Furthermore, age above 64 years at baseline was associated with an increased risk of VTE in **paper IV**. On the other hand, patients with UEDVT in **paper III** were comparably younger ( $54 \pm 20$ ) and had clearly lower BMI ( $25 \pm 5$ ), in accordance with previously reported figures reported in studies by Joffe et al [64] and Spencer et al [240].

In **paper I** we showed that besides higher age hormone therapy, immobilization, previous surgical intervention and concomitant malignancy were most common risk

factors for VTE. Also in **paper IV**, concomitant disease such as malignancies, inflammatory bowel disease, congestive heart disease, recent fractures, trauma or surgery were shown to be associated with increased VTE risk during follow-up. Our findings thus confirm those from previous studies in which immobilization [117-120], surgical intervention [102-104, 108], malignancy [124-126, 131] and hormone therapy [164-167, 170] have all emerged as very important VTE risk factors. Furthermore, concomitant malignancy was even more prevalent in patients with UEDVT (**paper III**), as 30% of our UEDVT patients had malignancy at diagnosis. In the majority of cases associated with malignant disease the cause is direct pressure or ingrowth by tumor mass [242], and cases with concomitant malignancy account for 10-30% of UEDVT [64, 247, 248]. Less frequently the cause of thrombosis can be ascribable to a hypercoagulable state adding to other risk factors for the development of axillary or subclavian thrombosis. Local radiotherapy may also be a contributory factor for thrombosis development, and in patients with malignant disease the use of central venous lines and chemotherapy may further contribute to development of UEDVT [247, 248].

There is a wealth of reports relating thrombosis to genetic thrombophilia. However, there are only a small number of studies and reviews in which the single gene FVL-mutation has been suggested have confer an evolutionary selection advantage [153, 202, 249]. Since the FVL-mutation is one of the most common genetic risk factors for VTE [198, 200-202], is associated with a procoagulant state, and is thought to have occurred in one individual some 21,000-34,000 years ago [199], one may speculate that it in ancient times might have constituted an evolutionary selection advantage by helping its carriers avoid anemia or profuse blood loss during childbirth. According to studies by Högberg et al [250] and Chi et al [251], females with anemia have highly increased case fatality rates from sepsis, preeclampsia, and profuse blood loss. The combination of anemia and obstetric hemorrhage has been estimated to be responsible for up to 50% of maternal deaths. In female VTE patients below the age of 50 years in our study, we observed relationships between the FVL-mutation and Hb levels which might be beneficial in this context, as the FVL-mutation was associated with significantly higher median Hb (**paper II**). We therefore speculate that the FVL-mutation may serve as protection against profuse menstrual blood loss since there were no differences between the groups of women below 50 years of age with the FVL-mutation and women above 50 years of age irrespective of FVL-mutation or not (**paper II**). These findings are in accordance with a retrospective study of Lindqvist et al [249] in which pregnant women with the FVL-mutation suffered lower blood loss, lower incidence of profuse blood loss, lower prepartum Hb difference, lower rates of postpartum anemia, and had higher postpartum Hb values compared to pregnant control women without the mutation. Furthermore, lower incidence of profuse postpartum blood loss and lower blood loss were also confirmed in pregnant FVL-carriers in a prospective study by Lindqvist et al [153]. The main limitation of **paper II** was that women in our cohort were selected for laboratory investigation of thrombophilia because of incident VTE and that the cohort is

therefore not entirely representative of the general female population. Nor can we safely exclude other risk factors which may have had impact on Hb. Another limitation of the study was the low number of patients with FVL-mutation below age of 50 years, necessitating caution when interpreting the results.

Relationships between socioeconomic status (SES) and increased risk for arterial vascular disease, in particular coronary atherosclerosis are well established [183-185] . Potential relationships between SES and VTE have previously been insufficiently studied, however. The few existing studies have demonstrated an increased rate of PE in subjects with less than 8 years of formal education [194], and in male subjects of low occupational class subjected to persistent stress [195]. No such relationship could be demonstrated between SES and VTE manifesting itself as DVT, however. Our study (**paper IV**) is to the best of our knowledge the only confirmation of the fact that low socioeconomic status as measured by lower income, single marital status, and lower level of education is of relevance for increased both DVT and PE risk in both genders, whereas foreign ancestry, on the other hand, was related to a lower risk for VTE. As these data were adjusted for age, this could not only be a result of a younger mean age in the immigrant group. Furthermore, as the risk for VTE in immigrants increased with longer duration of inhabitation in Sweden, the results cannot be entirely explained by genetic factors. Our results thus clarify that both environmental and genetic factors interact in VTE pathogenesis. As shown in previous studies [102, 127, 195, 252, 253], our study (**paper IV**) also confirmed that several important concomitant diseases such as for example malignancies, inflammatory bowel disease, congestive heart disease, recent fractures, trauma or surgery were associated with an increased risk for VTE in both genders.

The unique key strength of **paper IV** is the large sample size, explained by the fact that we did include the whole population above 25 years of age in the County of Skåne in southern Sweden in 1990. Our cohort is larger than previous materials as it includes over 700000 subjects. However, the present study (**paper IV**) has some important limitations. Since this database includes data on the entire population in the County of Skåne we could not objectively verify diagnosis in all included cases. Therefore, there is a possible risk for errors in diagnostic. Another limitation is that we only assessed VTE necessitating hospital care, and therefore base our conclusion upon only inpatients. Outpatient care of VTE was rare in 1990, but gained popularity during the study period that lasted until 2003. Milder cases of VTE during the latter part of the study period might therefore well have passed unnoticed. Furthermore, since data on hereditary thrombophilia, body mass index, history of smoking [254] and alcohol abuse are not recorded by the Swedish authorities and therefore not available in the official registries, it was not feasible to separate possible contributions of these variables from socioeconomic factors.

Until recently, unfractionated heparin (UFH) was considered essential for prevention and treatment of VTE during pregnancy and the postpartum period. This treatment strategy



for VTE during pregnancy was subsequently questioned, however, and LMWH emerged as the present drug of choice for both prevention and treatment of VTE during pregnancy due to its both better side effect profile, practical advantages such as for example once daily dosing, and good safety [157, 255-257]. In spite of the present common use of LMWH, little is known about its effects on labour, however. Since LMWH is a highly sulphated proteoglycan, Swedish scientists started to ponder over the potential capability of LMWH for modulation of the myometrial contractility. In an in vitro study by Ekman-Orderberg et al [258], increased contractile activity in myometrial smooth muscle strips pretreated with LMWH compared to untreated smooth muscle strips was demonstrated for the first time. Furthermore, secretion of IL-8 from cultured cervical fibroblasts was significantly increased after treatment with LMWH. The following year, the same research group reported 30% shorter time of labour in primiparous women treated with LMWH compared to matched controls without LMWH treatment [162].

In accordance with the above mentioned study, we shortly thereafter in **paper V** confirmed that duration of the first stage of labour tended to be 1 h shorter among nulliparous pregnant women treated with LMWH compared to non-treated pregnant women. Our results fell just outside of the range of significance (5.2 vs. 6.2 h,  $P = 0.06$ ), however. The risk of prolonged first stage of labour, on the other hand, was significantly lower with LMWH treatment in our study (4.1% vs. 8.5%,  $P = 0.047$ ). Our findings (**paper V**) thus support the effects of LMWH on the duration and initiation of labour previously reported by Ekman-Orderberg and co-workers [162]. Unfortunately, in accordance with a study by Andersen et al [259], our study (**paper V**) also confirmed that pregnant women on LMWH treatment had an increased incidence of Caesarean section, and a higher incidence of preterm delivery. We therefore speculate that administration of LMWH may influence stimulation of myometrial contractility and cervical remodelling as showed in the in vitro study by Ekman-Orderberg and her co-workers [258]. A possible explanation for the shorter time of labour and higher incidence of preterm delivery in our study (**paper V**) could be pro-inflammatory properties of LMWH as reported in a clinical study by Holst et al [260]. This is also in accordance with several other both clinical and experimental studies where anti-inflammatory properties of LMWH have been described, thought to occur through various potential mechanisms [261-264]. Furthermore, the risk of profuse blood loss and postpartum anaemia in pregnant women treated with LMWH was doubled compared to the control group (10.6% vs. 5.9%,  $P = 0.009$ , 12.9% vs. 8.7%,  $P = 0.048$ , respectively) in **paper V**.

Labour is a complex process which sometimes can be protracted increasing the risk of bleeding (**paper V**) and other complications during delivery. The risk for complications of childbirth is already not negligible in pregnant women treated with LMWH, due to the underlying increased risk for VTE that has necessitated treatment. In fact, protracted labour is one of the most well-known causes of delivery complications which could have drastic negative consequences for both mother and child. This scientific knowledge has

led to the introduction of a derivate of LMWH, tafoxiparin, as a pharmacological agent directed against protracted labour. This drug is currently undergoing clinical evaluation in the field of obstetrics. Tafoxiparin is presently being developed by Gunvor Ekman-Ordeberg, one of the founders of Dilafor and professor at Karolinska Hospital, and her co-workers. The compound Tafoxiparin shares many similarities with LMWH, the only essential difference is that its anticoagulant properties have been removed.

The strength of **paper V** is the quite large population based sample size of 217 pregnant women. Furthermore, all consecutive pregnant women were delivered at the same maternity ward at UMAS. However, **paper V** also has several important limitations. Since the study was of retrospective design we could not objectively verify if any important data was missed. Nor can we safely exclude other risk factors which might have had impact on the rates of preterm delivery, blood loss complications, or explain the increased frequency of Caesarean sections. Furthermore, the delivering midwives had access to information about which of the pregnant women that had ongoing treatment with LMWH, and measurement of blood loss might therefore have been subjected to bias.



## CONCLUSIONS

In the population-based **paper I**, VTE-incidence was lower than in earlier epidemiological studies. This could reflect truly receding VTE-incidence, more strict diagnostic criteria, early mobilization or improved thromboprophylaxis in risk groups for VTE. Hormone therapy, immobilisation, previous surgery and concomitant malignancy were the most common acquired risk factors.

In **paper II**, we found that VTE female patients below age 50 with FVL-mutation had higher median Hb compared to female patients without this form of hereditary thrombophilia.

This finding supports an earlier hypothesis that the FVLmutation may have conferred an evolutionary selection advantage over time by avoidance of bleeding.

The incidence of UEDVT in the population based **paper III** was lower than in earlier epidemiological studies. Concomitant malignancy and thrombophilia were commonly occurring risk factors. Mortality was high among UEDVT patients with known malignancy at diagnosis.

**Paper IV** demonstrated that higher age, and socioeconomic status as reflected by lower income, single marital status, and lower level of education were independently related to an increased risk of VTE during prospective long time follow-up. Foreign ancestry, on the other hand, was related to a lower VTE risk.

In **paper V** we showed that LMWH treatment reduces the first stage of labour by 1 h and results in a significantly lower risk of prolonged first stage of labour among nulliparous pregnant women. However, there was also a significantly increased risk of prematurity and blood loss complications.

## FUTURE CONSIDERATIONS

Improved routines concerning prophylaxis against VTE might well already have contributed to the comparably low incidences of VTE in **papers I and III**. Our data presented in **paper IV** demonstrate that not only the classical risk factors, but also socioepidemiologic factors influence the risk of VTE and highlight the need to decrease the VTE incidence particularly in specific high risk groups as defined in **papers I, III and IV**. Improved routines concerning prophylaxis against VTE are of particular interest in this context and will hopefully also help us in this direction.

The high rates of concomitant malignancy in the UEDVT patients in **paper III** highlights the need to consider an extended laboratory and radiological investigation of such patients.

Further examination of our material with respect to genetic risk factors is important, and in fact already ongoing. As many VTE patients still have normal results on coagulation testing, future research in this area might perhaps also help us to identify more genetic causes for VTE susceptibility.

As also demonstrated in **paper IV**, arterial and venous thrombosis share many common features. An interesting observation in this context is the fact that statin treatment, known for its many beneficial effects in arterial vascular disease [265-268], has also been shown to reduce the risk for venous thrombosis [269].

The data presented in **paper V** has as mentioned above already been confirmed by other scientists and have contributed to their introduction of a LMWH derivate directed against protracted labour. The clinical evaluation of this compound is awaited with interest.

# POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

(Comprehensive summary in Swedish)

## Bakgrund

Trots att venös blodpropp (venös tromboembolism [VTE]) är en viktig orsak till sjuklighet och dödlighet är data ofullständiga gällande dess förekomst och bakomliggande riskfaktorer hos oselekerade patienter. I begreppet VTE inkluderas blodproppar i benets djupa ådror (djup ventrombos [DVT]) och i lungorna (lungemboli [LE]). Insjuknandefrekvens i VTE varierar mellan 71 till 192/100 000 invånare och år. Andelen invånare som årligen insjuknar specifikt i DVT och LE varierar också alltifrån 48 till 160 respektive 23 till 60/100 000 invånare och år. Blodproppar i övre extremiteter (ÖEDVT) är mindre vanliga och utgör cirka 2% - 11% av all insjuknande i DVT.

VTE har många orsaker, och både ärftliga och förvärvade faktorer samt miljöfaktorer bidrar till sjukdomens utveckling i samspel med varandra. Faktor V Leiden är den vanligaste ärftliga riskfaktorn. Hög ålder, kirurgi, svåra kroppsskador, nedsatt rörlighet (immobilisering), cancer, graviditet och perioden omedelbart härefter samt hormonterapi utgör i sin tur de mest kända bidragande riskfaktorerna till utvecklingen av VTE.

## Målsättning

De övergripande målen med denna avhandling har varit att:

- Kartlägga insjuknandefrekvensen av VTE i Malmö, samt beskriva de första 1140 patienterna i Malmö Thrombophilia Study (MATS) avseende förvärvade riskfaktorer, diagnostik och behandling (**delarbete I**).
- Beskriva relationen mellan den genetiska riskfaktorn faktor V (FVL)-mutation och hemoglobin (Hb) -nivåer hos 927 VTE-patienter från MATS (**delarbete II**).
- Kartlägga 63 patienter med VTE i övre extremiteten avseende riskfaktorer, behandling, VTE-återinsjuknande och dödlighet (**delarbete III**).
- Klarlägga betydelsen av socioekonomiska faktorer för utveckling av VTE under 13 års uppföljning (**delarbete IV**).
- Utvärdera effekter på förlossningstid av den blodproppsförebyggande medicinen lågmolekylärt heparin (LMH) under graviditet (**delarbete V**).

Material och metoder.

**Delarbetena I-III** utgår från Malmö Thrombophilia Study (MATS) till vilken patienter rekryterades år 1998-2006. DVT och LE diagnostiserades hos 1140 patienter medan övre extremitets DVT diagnostiserades hos 63 patienter på Universitetssjukhuset i Malmö, (UMAS), (numera Skånes universitetssjukhus [SUS]), som då servade en befolkning på 280 000 invånare. Deltagandefrekvensen beräknas vara upp till 70% av patienter med VTE, resterande 30% kunde inte ingå i studien pga. språkproblem, demens, andra svåra sjukdomar eller att de avböjde deltagande i studien.

I **delarbete IV**, ingår alla invånare i Skåne 1990 över 25 år utan VTE under de sista 3 åren (379465 kvinnor och 350585 män). Mellan åren 1991-2003 följdes dessa avseende VTE eller dödsfall orsakad av VTE. De med och utan VTE jämfördes avseende data från 1990 gällande hushållsinkomst, civilstånd, födelseland, antal år i Sverige, utbildningsnivå samt föregående slutenvårdsdiagnoser.

I **delarbete V** undersöktes effekterna av LMH på förlossningstid hos 217 gravida med ökad VTE-risk under åren 1996-2005, dessa jämfördes med en oselektad kontrollgrupp (n=1499).

Resultat

I **delarbete I**, redovisas 1140 patienter med VTE, varav 882 (77%) hade DVT, 330 (29%) hade LE samt 72 (6%) hade både DVT och LE. Av patienterna var 559 män (49%, ålder  $62 \pm 16$ ) och 581 kvinnor (51%, ålder  $61 \pm 20$ ). Den årliga frekvensen av insjuknande med VTE var 66/100 000 vilket är lägre än i tidigare epidemiologiska studier. Hormonbehandling, nedsatt rörlighet, kirurgi och cancersjukdom var de mest vanligt förekommande förvärvade riskfaktorerna hos dessa patienter.

I **delarbete II** visades att kvinnor under 50 år med FVL-mutation hade högre blodvärde, Hb än kvinnor utan FVL (133 jämfört med 126 g/l). Inga skillnader sågs däremot bland män eller kvinnor över 50 år. Detta stödjer en tidigare hypotes att FVL-mutationen kan ha utgjort en fördelaktig urvalsmekanism under evolutionen genom att minska risken för blödning.

Av 63 patienter med ÖEDVT i **delarbete III** var 33 män (52%, ålder  $54 \pm 17$  år), och 30 kvinnor (48%, ålder  $55 \pm 22$  år). Den årliga frekvensen av insjuknande i ÖEDVT var 3.6/100 000 innevånare. Bakomliggande cancersjukdom (30%) och FVL-mutation (29%) var vanligt förekommande hos dessa patienter, vilka även hade hög dödlighet, 24% under 62 månaders uppföljning.

I **delarbete IV** visades att socioekonomiska faktorer såsom låg inkomst, ensamboende och låg utbildningsnivå, liksom förekomst av flera andra samtidiga sjukdomar innebar en ökad risk för insjuknande eller död i VTE under 13 års uppföljning, medan utländsk härkomst innebar minskad risk.

I **delarbete V** gav LMH hos förstföderskor ett 1 timme kortare första förlossningsskede i jämförelse med kontrollgruppen (5.2 jämfört med 6.2 timmar). Inga skillnader sågs däremot vid jämförelse mellan grupperna av andra förlossningsskedet. Förstföderskor som behandlats med LMH hade lägre risk för förlängt första förlossningsskede (4.1% jämfört med 8.5%) i jämförelse med kontrollgruppen. Inga skillnader sågs däremot hos omföderskor. Riskerna för förtidig födsel, blodförlust och lågt blodvärde efter förlossningen var däremot högre hos LMH-behandlade.

### Sammanfattning

Sammanfattningsvis är avhandlingens nyhetsvärde att den utgör en uppdaterad kartläggning av riskfaktorer för VTE i ett modernt oselekerat patientmaterial. Vi fann även en lägre årlig frekvens av insjuknande i VTE (DVT, LE, ÖEDVT) än vad som rapporterats tidigare. Detta reflekterar antingen en äkta sjunkande förekomst av sjukdomen eller striktare diagnostiska kriterier. Vidare påvisar denna avhandling för första gången att socioekonomiska faktorer som låg inkomst, ensamboende och låg utbildningsnivå innebar ökad risk för insjuknande eller död i VTE hos individer av båda kön, medan utlandsfödda svenskar löper lägre VTE-risk. Resultaten i delarbete V har även legat till grund för fortsatt läkemedelsutveckling i syfte att medikamentellt påverka förlossningstid.



# PËRMBLEDHJE SHKENCORE NË GJUHËN SHQIPE

(Comprehensive summary in Albanian)

## Hyrja

Edhe pse trombembolizmi i venave është një shkaktarë i rëndësishëm i morbiditetit dhe mortalitetit, në përgjithësi studimet shkencore në lidhje me përcaktimin e incidencës së saj dhe faktorëve të rrezikut nuk janë të plota sidomosë kur janë në pyetje pacientë të pazgjedhur. Termi trombembolizëm i venave përfshin trombozën venoze të thellë dhe embolinë pulmonare. Incidenca e trombembolizmit të venave shtrihet nga 71 deri 192 raste të reja në 100,000 banorë në vit. Incidenca e banorëve me trombozën venoze të thellë është nga 48 deri 160 ndërsa me embolinë pulmonare është nga 23 deri 60 raste të reja në 100,000 banorë në vit.

Etiologjia (shkaqet) e trombembolizmit të venave është mjaft e ndërlikuar duke përfshinë jo vetëm faktorët gjenetikë por dhe ata të fituar (mënura e jetesës). Këta faktorë sëbashku ndihmojnë në zhvillimin e sëmundjes. Mutacioni i Factor V Leiden (FVL) është faktori gjenetik (i lindur) më i zakonshëm që lidhet me zhvillimin e sëmundjes. Ndërsa pleqëria, ndërhyrja kirurgjike (operacionet), trauma (aksidentet), palëvizshmëria, tumoret (kanceri), shtatzania, kontraceptivët oral dhe terapia hormonale janë faktorët më të zakonshëm të fituar.

## Qëllimi

Qëllimet e këtij punimi janë:

- Gjetja/Llogaritja e incidencës së trombembolizmit të venave (tromboza venoze e thellë dhe embolia pulmonare) në Malmö dhe përshkrimi/gjetja e faktorëve të fituar që ndikonin në zhvillimin e sëmundjes bazuar në 1140 pacientë nga Malmö Thrombophilia Study (MATS). **(punimi I)**
- Përshkrimi i mardhënieve ndërmjet faktorëve gjenetikë (factor V Leiden [FVL]) dhe nivelit të hemoglobinës në 927 pacientë nga MATS. **(punimi II)**
- Përshkrimi i 63 pacientëve me trombozën venoze të thelle në gjymtyrët e sipërme (trombozë e venës subclavia ose ndryshe e venës nënkularthore), faktorët gjenetikë dhe të fituar, mjekimi i trombozës, përsëritja e trombozës dhe përqindja e vdekshmërisë. **(punimi III)**
- Identifikimi, përshkrimi i faktorëve socialo-ekonomik të rëndësishëm për zhvillimin e trombembolizmit të venave gjatë një monitorimi 13 vjeçar. **(punimi IV).**

- Vlerësimi/kontrolli i efektit të heparinës me peshë të vogël molekulare (LMWH) në kohëzgjatjen e lindjes tek gratë shtatzane (**punimi V**).

## Materiali dhe metodat

Punimet I-III janë marrë nga Malmö Thrombophilia Study (MATS) në të cilën pacientët janë përfshirë dhe studiuar gjatë viteve 1998-2006. Diagnoza e trombozës venoze të thellë dhe embolia pulmonare është bërë në 1140 pacienta kurse diagnoza e trombozës venoze të thellë në gjymtyrët e sipërme është bërë në 63 pacientë në spitalin universitar të Malmös. Në Malmö banojnë 280,000 banorë. Pjesëmarrja e pacientëve në këtë studim, llogaritet deri në 70% ndërsa 30% e pacientëve nuk u përfshinë në MATS për shkak të problemeve gjuhësore, smundjeve të vështira të sklerozës mendore ose nuk donin të merrnin pjesë në MATS.

Punimi IV, përfshin të dhëna për të gjithë banorët e pjesës jugore të Suedisë që ishin mbi 25 vjeç në vitin 1990 kur studimi filloi dhe që nuk kishin tromboembolizmin e venave të paktën 3 vitet e fundit para se studimi të fillonte. (379465 femra dhe 350585 meshkuj). Këta banorë u studiuan gjatë viteve 1991-2003 për incidencën e tromboembolizmit të venave (trombozë venoze të thellë dhe embolinë pulmonare) si dhe vdekshmërinë nga kjo sëmundje. Banorët u ndanë në 2 grupe; ku në grupin e parë ishin ata banorë që patën tromboembolizmin e venave ndërsa në grupin e dytë ishin banorët pa këte sëmundje. Këto 2 grupe u krahasuan për të ardhurat ekonomike, statusin martesor, shtetin e lindjes, numrin e viteve me banim në Suedi, nivelin arsimor, dhe praninë e sëmundjeve të tjera. Ky informacion u mor nga kartela mjeksore.

Punimi V, studion efektet e heparinës me peshë të vogël molekulare (LMWH) në kohën e lindjes tek 217 gra shtatzene gjatë vitit 1996-2005, që kanë një shtim të rrezikshmërisë për t'u sëmurur me tromboembolizmin e venave. Këto gra janë krahasuar me një grup të pazelektuar grash shtatzene (n=1499).

## Rezultati

Punimin I, nga 1140 pacientë me tromboembolizmin e venave, 882 (77%) janë diagnostikuar me trombozën venoze të thellë, 330 (29%) janë diagnostikuar me emboli pulmonare dhe 72 (6%) janë diagnostikuar me trombozën venoze të thellë dhe embolinë pulmonare njëkohësisht. Pacientët ishin 559 meshkuj (49%, moshë 62±16) dhe 581 femra (51%, moshë 61±20). Incidenca e tromboembolizmit të venave (trombozë venoze të thellë dhe embolinë pulmonare) ishte 66 raste të reja në 100,000 banorë në vit. Me fjale të tjera kjo incidence e tromboembolizmit të venave është më e ulët se në studimet e mëparshme epidemiologjike. Terapia hormonale, imobilizimi, ndërhyrjet kirurgjike, dhe tumorët janë faktorët më të zakonshëm që ndikojnë në shfaqjen e sëmundjes tek 1140 pacientët e studiuar.

Punimi II, tregoi se gratë nën 50 vjec me mutacionin e faktorit V (V Leiden (FVL) kanë nivel më të lartë të hemoglobinës në krahasim me gratë pa këtë mutacion (133g/l në krahasim me 126 g/l). Analizat statistikore nuk treguan ndryshim midis meshkujve dhe grave mbi 50 vjec. Rezultatet e këtij punimi përkrasin hipotezën e mëparëshme që mutacioni në faktorin V Leiden mund të ketë qenë një mekanizëm i dobishëm gjatë evolucionit njerzor duke zvogëluar rrezikun e gjakderdhjes.

Nga 63 pacientë me trombozën venoze të thelle në gjymtyrët e sipërme, në punimin e III, 33 ishin meshkuj (52%, mosha  $54 \pm 17$  vjeq) dhe 30 femra (48%, mosha  $55 \pm 22$  vjeq). Incidenca e trombozës venoze të thellë në gjymtyrët e sipërme ishte 3.6 raste të reja në 100,000 banorë në vit. Tumoret (30%) dhe mutacioni në factor V Leiden (29%) janë diagnostikuar në këta 63 pacientë, të cilët gjithëashtu kishin mortalitetin më të lartë, 24% gjatë një periudhe 62 muajorsh.

Punimi IV tregoi për herë të parë rëndësinë e faktorëve socialo-ekonomike. Banorët me të ardhura të ulëta ekonomike, ata që jetojnë vetëm dhe ata që kanë shkollim të ulët, rrezikojnë më shumë të sëmurën ose të vdesin nga tromboembolizmi i venave. Me gjithë atë banorët me prejardhje jo suedeze rrezikonin më pak për t'u sëmurur me tromboembolizmin e veneve.

Punimi V tregoi që heparini me peshë të vogël molekulare e ulte kohën e lindjes (pjesës së parë të procesit; atë të hapjes) tek gratë që ishin shtatzanë për herë të parë (5.2 në krahasim me 6.2 ore). Po këto gra kishin rrezikonin më pak për zgjatjen e pjesës së parë të procesit të lindjes (4.1% në krahasim me 8.5%) në krahasim me grupin e kontrolles. Këto dallime nuk u gjetën te gratë që kanë qënë shtatzanë më parë. Rreziku për lindje të parakohshme, gjakëderdhje dhe hemoglobin të ulët mbas lindjes ishte më i lartë në grupin e grave që mjekoheshin me heparin me peshë të vogël molekulare.

#### Përmbledhje

Vlera e këtij punimi shkencor qëndron në përshkrimin e faktorëve që ndikojnë në zhvillimin e tromboembolizmit të venave në kohët tona tek pacientët e pa seleksionuar (dmth tek popullsia në përgjithësi). Në gjithëashtu kemi përshkruar një incidencë më të ulët të tromboembolizmit të venave (trombozën venoze të thellë, embolinë pulmonare dhe trombozën venoze të thelle në gjymtyrët e sipërme). Ky rezultat pasqyron një rënie të vërtetë të incidencës së kesaj sëmundje, ose kritere më të rrepta diagnostikale. Më tej ky punim dëshmon për herë të parë që faktorët socialo-ekonomik ndikojnë njëkohësisht në zhvillimin e sëmundjes si dhe në mortalitetin nga tromboembolizmi i venave. Interesant ishte dhe fakti që banorët me prejardhje jo suedeze janë në rrezik më të ulët për zhvillimin e kesaj sëmundje. Tek rezultati nga punimi i V është bazuar dhe zhvillimi i barnave të reja që ndikojnë kohëzgjatjen e lindjes.

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