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Venous thromboembolism with special focus on genetic and potential acquired risk factors

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PO Box 117
221 00 Lund
+46 46-222 00 00

Venous thromboembolism with special focus on genetic and potential acquired risk factors

Signý Vala Sveinsdóttir

LUND UNIVERSITY

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Abstract

Venous thromboembolism (VTE) is a relatively common cause of morbidity and mortality. It has an annual incidence of around 0.1- 0.3%. It is a multifactorial disease with many known risk factors, both transient and persistent. Among these, several genetic risk factors have been described. The most common genetic risk factor, factor V Leiden mutation in heterozygote form, is found in 5-8% of the Caucasian population. Although much is known about the VTE disease, evaluating the recurrence risk, duration and risk of the anticoagulation therapy remains a challenge and many questions are still unanswered.
The aims of this thesis are to evaluate the distribution and clinical impact of the most common inherited risk factors for VTE in a population based total material from southern Sweden as well as estimating heterozygous FVL mutation as a risk factor for VTE recurrence. Furthermore, to look into other potential acquired risk factors for VT, such as inflammation in a male cohort from a screening program and, finally, evaluate the risk for bleeding in relation to renal function within VTE patients on warfarin treatment in a cohort from a Swedish national quality registry for anticoagulation (AuriculA).
The prevalence of the FVL mutation in heterozygous form was found in approximately one fourth of the VTE patients and increased the risk for recurrence significantly, by 2-3 fold. The mutation in homozygous form is much less frequent but these patients had a higher average age at first thrombosis than many studies previously described. Furthermore, homozygous women were affected at an earlier age compared to men and female controls and it appeared that thrombi in homozygous FVL were more prone to be in the lower extremity. The odds ratio for thrombosis was lower than previously described.
The risk for VTE in relation to the number of raised inflammatory specific proteins (ISPs), i.e. fibrinogen, haptoglobin, ceruloplasmin, alfa-1-antitrypsin and orosomucoid, as well as individual ISPs was not significantly increased. However, age, BMI and diabetes mellitus type 2 were significant risk factors for developing a VTE. On the other hand, factors such as cholesterol, triglycerides, blood pressure and smoking were not. VTE patients on anticoagulation treatment with warfarin seemed to be younger, and hence had a better renal function, than patients with other indications for warfarin therapy. Among those VTE patients there was not significantly

Key words
Venous thromboembolism (VTE), epidemiology, risk factors, factor V Leiden (FVL), recurrence risk, iflammation, renal function, warfarin

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Front page: The “Eye” (Augað), a spring in Rangárvallasýsla, Iceland

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Faculty of Medicine
Department of Translational Medicine, Clinical Coagulation Research Unit
Skåne University Hospital, Malmö, Sweden

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One´s philosophy is not best expressed in words;
it is expressed in the choices one makes….and the choices
we make are ultimately our responsibility

-Elenor Roosevelt

To Þórir, Hrafnhildur, Sigrún and Skarphéðinn

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ABSTRACT

Venous thromboembolism (VTE) is a relatively common cause of morbidity and mortality. It has an annual incidence of around 0.1- 0.3%. It is a multifactorial disease with many known risk factors, both transient and persistent. Among these, several genetic risk factors have been described. The most common genetic risk factor, factor V Leiden mutation in heterozygote form, is found in 5-8% of the Caucasian population. Although much is known about the VTE disease, evaluating the recurrence risk, duration and risk of the anticoagulation therapy remains a challenge and many questions are still unanswered.

The aims of this thesis are to evaluate the distribution and clinical impact of the most common inherited risk factors for VTE in a population based total material from southern Sweden as well as estimating heterozygous FVL mutation as a risk factor for VTE recurrence. Furthermore, to look into other potential acquired risk factors for VT, such as inflammation in a male cohort from a screening program and, finally, evaluate the risk for bleeding in relation to renal function within VTE patients on warfarin treatment in a cohort from a Swedish national quality registry for anticoagulation (AuriculA).

The prevalence of the FVL mutation in heterozygous form was found in approximately one fourth of the VTE patients and increased the risk for recurrence significantly, by 2-3 fold. The mutation in homozygous form is much less frequent but these patients had a higher average age at first thrombosis than many studies previously described. Furthermore, homozygous women were affected at an earlier age compared to men and female controls and it appeared that thrombi in homozygous FVL were more prone to be in the lower extremity. The odds ratio for thrombosis was lower than previously described.

The risk for VTE in relation to the number of raised inflammatory specific proteins (ISPs), i.e. fibrinogen, haptoglobin, ceruloplasmin, alfa-1-antitrypsin and orosomucoid, as well as individual ISPs was not significantly increased. However, age, BMI and diabetes mellitus type 2 were significant risk factors for developing a VTE. On the other hand, factors such as cholesterol, triglycerides, blood pressure and smoking were not. VTE patients on anticoagulation treatment with warfarin seemed to be younger, and hence had a better renal function, than patients with other indications for warfarin therapy. Among those VTE patients there was not significantly increased bleeding with impaired renal function, although a trend could be seen.

LIST OF ABBREVIATIONS

aCL	Anti-cardiolipin antibodies
ADP	Adenosine diphosphate
Anti-β2-GP1	Anti-β2-glycoprotein-1
APC	Activated protein C
aPL	Anti-phospholipid
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time
AT	Antithrombin
BMI	Body mass index
C4BP	Complement regulator C4b-binding protein
CI	Confidence interval
CKD	Chronic kidney disease
COC	Combined oral contraceptives
CT	Computer tomography
CRP	C-reactive protein
DVT	Deep vein thrombosis
ECs	Endothelial cells
EPCR	Endothelial protein C receptor
eGFR	Estimated glomerular filtration rate
ET	Endothelial
F	Factor
FDP	Fibrin degradation products
FVL	Factor V Leiden
FI	Fibrinogen
GFR	Glomerular filtration rate
GP	Glycoprotein
Hb	Haemoglobin
HHey	Hyperhomocysteinemia
HMWK	High-molecular weight kininogen
HR	Hazard ratio
HRT	Hormone replacement therapy
HSP	Heparin sulphate proteoglycans
INR	International normalized ratio
ISI	International sensitivity index
ISP	Inflammatory sensitivity proteins
ISTH	International Society on thrombosis and Haemostasis

LAC	Lupus anticoagulant
LM	Lund-Malmö
LMWH	Low-molecular weight heparin
MDRD	Modification of diet in Renal Disease
MPs	Microparticles
MRI	Magnetic resonance imaging
MTHFR	Methyline tetrahydrofolate reductase
NO	Nitric oxide
NS	Not significant
OAC	Oral anticoagulation
OR	Odds ratio
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
p-Cr	Pulmonary embolism
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothrombin time
PTM	Prothrombin gene mutation
RR	Relative risk
SD	Standard deviation
SPSS	Statistical package for the social sciences
SUS	Skåne University Hospital
TAFI	Thrombin activatable fibrinolysis inhibitor
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TM	Thrombomodulin
t-PA	Tissue plasminogen activator
TTR	Time in treatment/therapeutic range
	iTTR-individual time in treatment/therapeutic range
cTTR	-centre time in treatment/therapeutic range
TXA_2	Thromboxane A_2
UEDVT	Upper extremity deep vein thrombosis
UFH	Unfractionated heparin
UMAS	Malmö University Hospital
u-PA	Urokinase-type plasminogen activator
VKA	Vitamin K antagonist
VKOR	Vitamin K epoxide reductase
VTE	Venous thromboembolism
VWF	Von Willebrand factor

LIST OF PAPERS

This thesis is based on the following papers:

I. **Sveinsdottir SV**, Saemundsson Y, Isma N, Gottsäter A, Svensson PJ**.** Evaluation of recurrent venous thromboembolism in patients with Factor V Leiden mutation in heterozygous form. Thrombosis Res 2012; 130(3): 467-71.

II. Saemundsson Y, **Sveinsdottir SV**, Svantesson H**,** Svensson PJ. Homozygous Factor V Leiden and Compound Heterozygosity for Factor V Leiden and Prothrombin Mutation. J Thrombosis Thrombolysis 2013; 36(3): 324-31

III. **Sveinsdottir SV**, Svensson PJ, Engström G. Inflammatory plasma markers and risk for venous thromboembolism; J Thromb Thrombolysis 2014; 38(2): 190-5

IV. **Signy V Sveinsdottir**, Mattias Wieloch, Sigrun H Lund, Peter J Svensson. Major bleedings and thromboembolic complications in relation to kidney function in warfarin treated venous thromboembolic patients. Submitted.

INTRODUCTION

Historical review

The first known description on the formation of a blood clot date back to as early as around 2650 B.C. Around that time, Chinese physician named Huan-Ti, wrote that "when it coagulates within the pulse, the blood ceases to circulate beneficially; when the blood coagulates within the food it causes pain and chills", indicating the idea of blood clot formation (1). Very few historical descriptions of patients with symptoms of venous thromboembolism (VTE) can be found. Hippocrates and Aristotle thought the formation of a solid blood clot from fluid blood was related to cooling of the blood after a cut. Descriptions of phenomena that can be compatible with VTE, can be found from the 13th and 16th centuries. There are historical accounts of a French knight that had DVT in his leg (2) and Henry IV of Navarra, King of France had with subclavian vein thrombosis (3). It was only centuries later that a understanding of VTE pathogenesis began to develop. Rudolf Virchow, professor at the University of Berlin, (1821 – 1902), was the first to explain the three main elements of the process of venous thrombosis, the "Virchow´s triad". These are alterations in blood flow, the wall of a blood vessel and in the constitution of the blood (4). His illustration initiated the development of modern understanding of VTE process and is still relevant. In the subsequent decades, the blood clotting theory evolved, based upon the work of many scientists, such as Johannes Müller (1802 – 1858) and Alexander Schmidt (1831 - 1894). They explained the involvement of thromboplastin, prothrombin, fibrinogen and calcium in haemostasis (5). Initiation of the coagulation cascade remained, however, a mystery until Paul Morawitz (1879 – 1930) identified the substance that takes part in the initiation of clot formation (thrombokinase or, later, tissue factor) (6). Blood platelets were identified as part of the coagulation process in 1865 and their function was described by Giulio Bizzozero in 1882 (7).

The knowledge of the coagulation cascade has expanded much further during the last 70 years with the identification of the various coagulation factors, mostly through studies on individuals with a clear hereditary bleeding tendency. In relation to some of these factors, Armand Quick (1894 – 1977) developed the one-stage prothrombin time (PT) (8). The influence of coumarin anticoagulant on the test became clear in the 1940s. Subsequently the most widely used anticoagulant,

warfarin was introduced, which still has a significant role in modern praxis of the treatment of VTE.

Haemostasis

Research on coagulation through the last decades, the pathogenesis of clot formation, and subsequently anticoagulation therapy, has led to a better understanding of the importance of the delicate balance between bleeding and coagulation. The haemostasis has developed as an important protective mechanism against life threatening bleedings, constituting various networks of platelets, procoagulant- and anticoagulant factors as well as fibrinolytic pathways. Any factors, genetic- or acquired, that disturb the balance between those well-regulated systems may result in either bleeding or thrombosis (9).

Haemostasis can be divided into three different stages:

- **Primary haemostasis**- formation of a platelet plug
- **Secondary haemostasis**- activation of the coagulation system leading to generation of fibrin to reinforce the plug
- **Fibrinolysis**- lysis of the clot

Primary haemostasis

Primary haemostasis is the early phase in preventing major bleeding, when coagulation has not yet started to play role in the haemostasis. It involves the interaction between endothelial cells of a blood vessel, subendothelium and platelets to result in the initial sealing of the damaged area by forming a platelet plug (10). Normally, the endothelial cells, with their negatively charged layer of glycocalyx and secretion of endogenous antithrombotic factors, prevent haemostasis by repelling platelets and inactivating the coagulation factors. When vascular injury occurs, local vasoconstriction slows down the rapidly circulating platelets. Platelets come into contact to collagen and other thrombogenic components in the subendothelium (**Fig.1**).

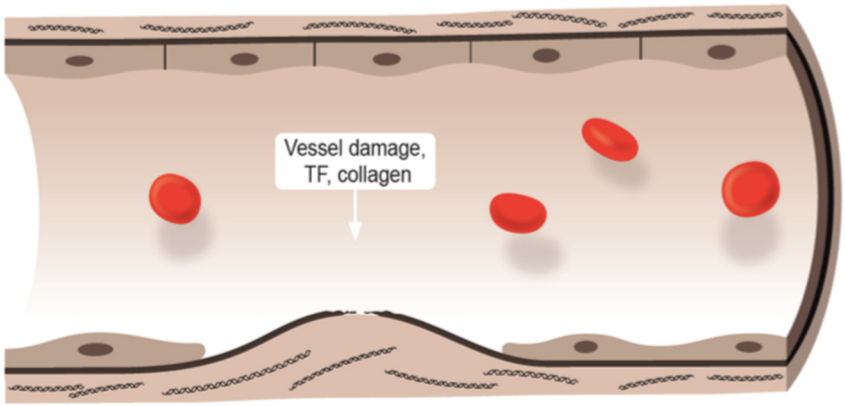

Figure 1.
Subendothelial components such as collagen are exposed and tissue factor (TF) expressed at vascular injury. With permission from Casper Asmussen.

The platelets adhere to the exposed subendothelium via proteins in the plasma and receptors on the platelets. Of these, von Willebrand factor (vWF) is the most important protein, while GPIb-V-X, is the most important receptor. Von Willebrand factor is a high molecular plasma protein made up of multimers of disulphide bonded monomers and in plasma it undergoes proteolytic processing mediated by a metalloprotease, ADAMTS13 (11). Under shear stress of the flowing blood, the vWF is stretched and then serves as a bridge between the exposed collagen and platelets via the platelet membrane receptor glycoprotein GPIb-V-X. Further binding of the platelets to the damaged surface occurs through the platelet receptor GPIa-IIa (**Fig.2**).

Now the platelets activate and undergo structural changes. These include rearrangement of their membrane and exposure of negatively charged phospholipids as well as transforming from a smooth, discoid shape to a more irregular form with pseudo-pods that cover the injured surface of the vessel wall. They release the content of α-and dense-granules (including adenosine diphosphate (ADP), Ca^{2+}, serotonin, vWF, factor V, factor XIII and fibrinogen) which enhance the platelet activation. Through ADP receptors on platelet surfaces, Ca^{2+} and serotonin, additional activation and aggregation of the platelets takes place. Activation of the platelet membranes provides receptors for other plasma coagulation factors such as prothrombin and factor V, X and XI. Furthermore, highly activated platelets release thromboxane A_2 (TXA_2) to enhance the formation of a bridge between the platelets via expression of GPIIb/IIIa fibrinogen receptors forming fibrinogen cross-linking (Fig. 2). The result is a platelet plug, which is coordinated with the activation of the blood coagulation system. Its goal is to generate thrombin and a fibrin net (12) (**Fig.2**).

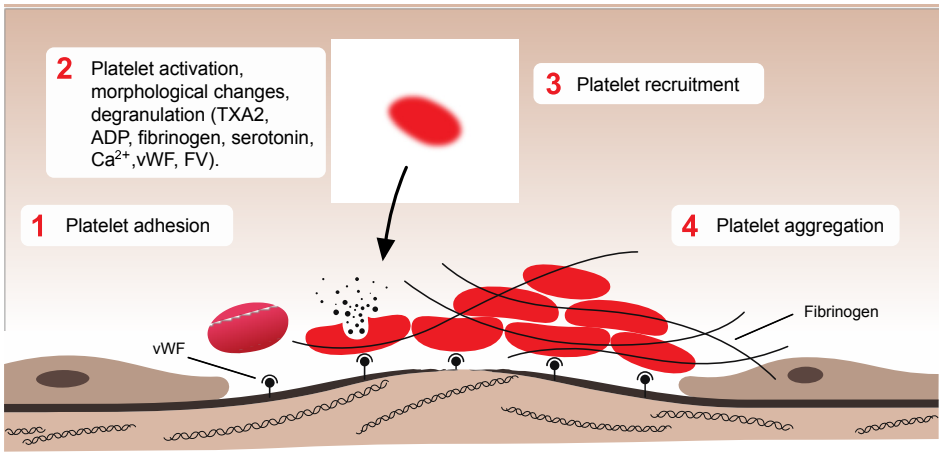

Figure 2.
Primary haemostasis showing the adhesion and aggregation of the platelets to form a platelet plug. TXA_2= thrombocane A_2, ADP= adenosine diphosphate, vWF= von Willebrand factor, FVa= activated factor V. With permission from Casper Asmussen.

Secondary haemostasis

For many years, biochemists have tried to understand the process of coagulation by introducing different models to mimic *in vivo* situations with experiments done in laboratory settings (*in vitro*) (13-15). The most persistent model is the cascade/waterfall model, where coagulation is thought to involve a sequence of proteolytic steps forming two pathways (contact activation, or *intrinsic pathway* and tissue factor, or *extrinsic pathway*) ultimately joined in one *common pathway*. This in turn leads to generation of thrombin to convert fibrinogen (FI) to fibrin (16, 17). Although we now have a new understanding of haemostasis, this former model has provided understanding of these coagulation steps in vitro as well as helping evolve screening tests to predict clinical bleeding tendency. The prothrombin (PT) test reflects the *extrinsic pathway* while the activated partial thromboplastin time (APTT) test represents the *intrinsic pathway* (17). However, this model does not fully explain the haemostatic process clinically and its correlation to the APTT screening test. Patients with prolonged APTT because of deficiencies of FXII, high-molecular weight kininogen (HMWK) and prekallikrein (all constituting the initiation of the *intrinsic pathway*) did not have a bleeding tendency. Patients deficient in other factors in the *intrinsic pathway*, such as factor VIII (haemophilia A) and factor IX (haemophilia B), can have serious bleeding disorders although the *extrinsic pathway* remains intact. The opposite is also seen, i.e. patients having an intact *intrinsic pathway* but lacking factors in the *extrinsic pathway*, such as factor VII, can lead to a serious bleeding tendency. Consequently, we now understand that

these two pathways are not a simple linear cascade but an interdependent network of reactions in vivo although in vitro studies imply the opposite (18-21) (**Fig. 3**).

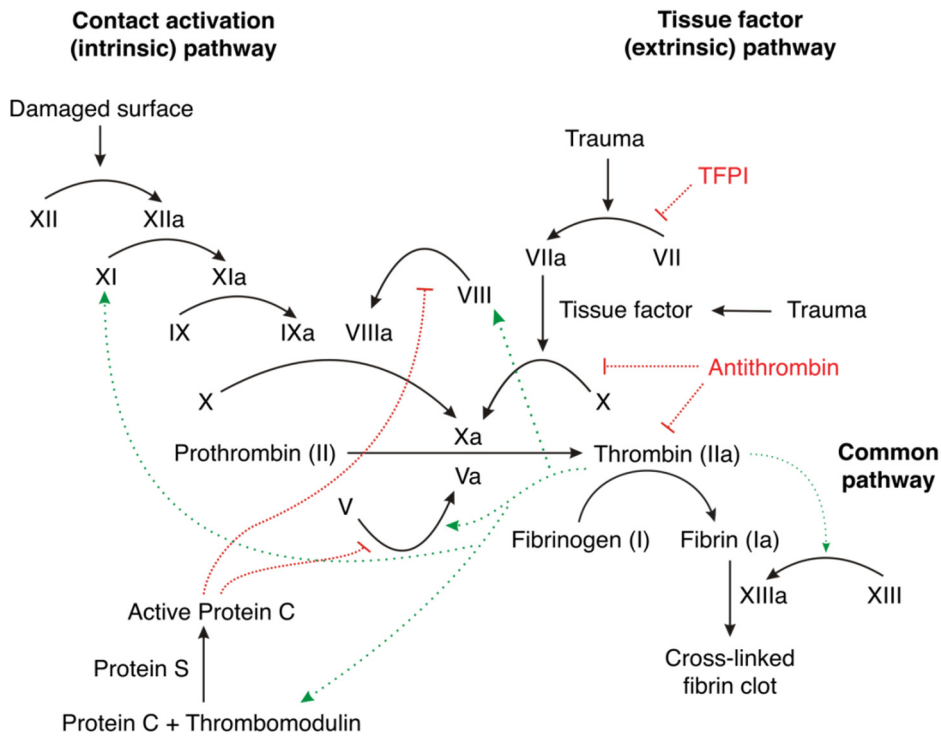

Figure 3.
The coagulation cascade. Inhibition of active coagulation is marked red. Positive feedback loops of throbin are marked green. TFPI= tissue factor pathway inhibitor.

Research has now shown that different cell surfaces have different properties regarding the coagulation process, where a cell-based model had been developed to reflect the pathways of haemostasis in vivo. This model divides the process into three overlapping steps involving platelets and tissue factor (TF) bearing cells (EC, sub-intimal cells or monocytes). The *initiation* is triggered when the exposed TF, in the presence of Ca^{2+}, binds to the small amount of freely circulating activated factor VII (FVIIa). This TF/FVII complex then activates factor X (FXa) and, to some extent, factor IX (FIXa) on the platelet surface. This amplifies (*amplification phase)* the system further by feedback activation of FVII on the TF-bearing cells. This complex results in the formation of FXa complex where it binds to activated FV on the TF-bearing cell. On the activated platelets, this leads to the form the "prothrombinase complex" (FXa/FVa) that activates thrombin (FIIa) from its precursor prothrombin (FII) (22-24) (**Fig.4**).

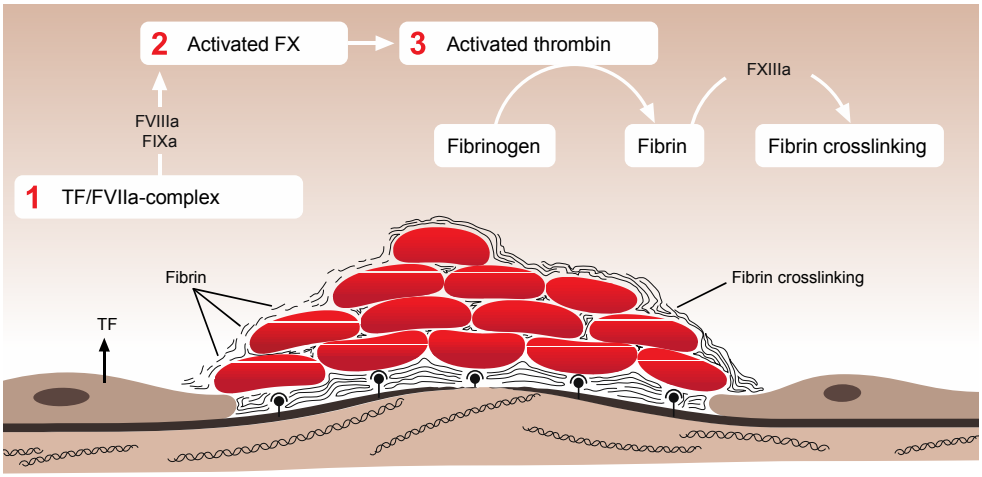

Figure 4.
Plasmacoagulation during secondary haemostasis. With permission from Casper Asmussen.

This small initial amount of thrombin, at the side of tissue injury, is critical for successful initiation of coagulation and initiates the *propagation phase* which includes positive feedback reactions that result in generation of much larger amounts of thrombin. The main role of thrombin is to attract and activate more platelets to further amplify the cascade by activating more FV as well as vWF-bound FVIII on the activated platelet surface. This leads to activation of FXI, which further activates FIX. Activated FVIII (FVIIIa) is a cofactor to FIXa in a "tenase complex" which converts FX to FXa (12, 17, 20). The explosive generation of thrombin, through the prothrombinase complex, converts large amounts of fibrinogen (FI) to fibrin. This fibrin network is stabilized by cross-linking by factor FXIIIa which has been activated from FXIII by thrombin which also activates thrombin activatable fibrinolysis inhibitor (TAFI) for further stabilisation (25, 26). These feedback loops amplify the coagulation cascade with thrombin and FXa being the most important amplifiers (Fig3) but, simultaneously, initiate mechanism of anticoagulation.

Anticoagulation

To prevent excessive intravascular coagulation and ensure that platelet clotting is restricted around the injured area, the plasma contains a series of proteins that inhibit activated procoagulant factors. This regulation is exerted on negatively charged phospholipid surfaces at any level of the coagulation cascade by three different mechanisms (27).

The *tissue factor pathway inhibitor (TFPI*) is a polypeptide secreted by endothelial cells and binds FXa and thrombin. The FXa- TFPI complex rapidly inhibits the TF/VIIa complex (28, 29).

Antithrombin (AT), a serine protease inhibitor (serpin), is a potent and crucial inhibitor of thrombin, FIXa, FXa, FXIa, FXIIa as well as the TF-FIIa complex, thereby limiting the overall activation of the coagulation mechanism. Circulating AT is relatively ineffective until heparin and heparin-like molecules present on the surface of endothelial cells stimulate it. This provides the base for using heparin as a therapeutic anticoagulant. Deficiency of AT is a known inherited thrombophilia (27, 30).

The third mechanism is the *protein C anticoagulation system* with one of the key regulatory proteins, the vitamin K-dependent proenzyme protein C. It inhibits the procoagulant functions of FVIIIa and FVa which are the cofactors in the tenase- and prothrombinase complexes, respectively. This pathway is initiated by thrombin when it binds to the membrane protein thrombomodulin (TM) on the endothelial surface, forming a T-TM complex, thereby activating protein C. The endothelium also contains the endothelial protein C receptor (EPCR), which binds to the GIa domain of protein C and helps present the protein C for the T-TM complex (31). Now, activated protein C (APC) is generated and, in the presence of its vitamin K-dependent cofactor, protein S, cleaves FVIIIa and FVa on the negatively charged phospholipid membranes (32-35) (**Fig.5**).

In human plasma, about 30% of protein S is a free circulating protein serving as a cofactor to APC. The remaining 70% bound to a complement regulatory protein C4b-binding protein (C4BP) which takes part in regulating the complement system (36).

In the circulation, FVIII is bound to vWF which stabilizes the otherwise lable FVIII and protects it from degradation of APC. However, FV can bind phospholipids both in its active and inactive form. Therefore, FV can be converted to an anticoagulant cofactor to APC which, together with protein C, can degrade FVIIIa in the tenase complex. This suggests that FV has both procoagulant (when activated by thrombin or FXa) and anticoagulant (cleaved by APC) properties (37, 38).

The protein C anticoagulant system is exposed to many inherited thrombotic risk factors, the most common of which is APC resistance, caused by a point mutation in the gene coding for FV (FV Leiden, FVL). Other thrombophilias are protein C, protein S and antithrombin deficiencies as well as point mutation of the gene coding for prothrombin, all predisposed to venous thromboembolism.

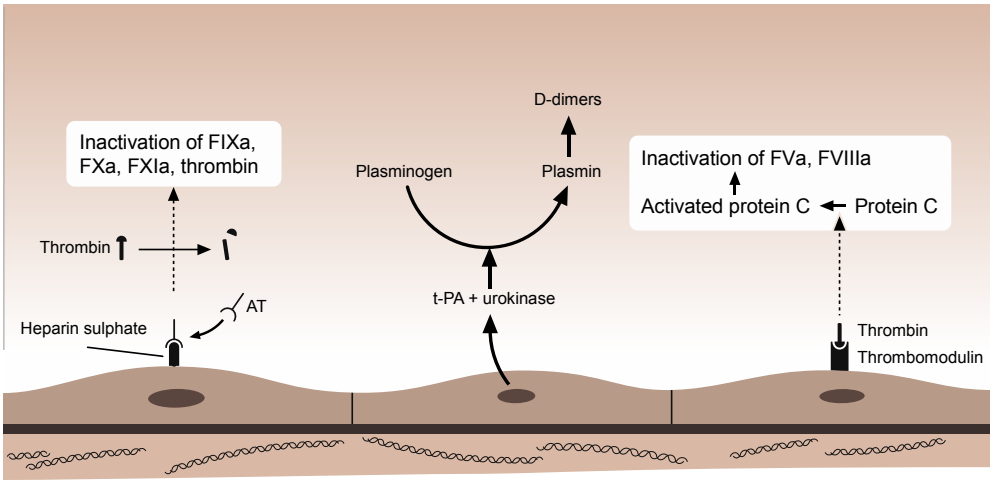

Figure 5.
Anticoagulation. t-PA= tissue plasminogen activatior. With permission from Casper Asmussen.

Fibrinolysis

When the clot is formed, with the fibrin network made of the cross-linked monomers by FXIII into polymers, and coagulation has stopped by the aforementioned anticoagulant systems, the thrombus must be dissolved to prevent further expansion. The initiator of this process, in the *fibrinolytic system*, is the tissue plasminogen activator (t-PA) that normal endothelial cells synthesize and secrete and urokinase-type plasminogen activator (u-PA) (39) (**Fig.6**).

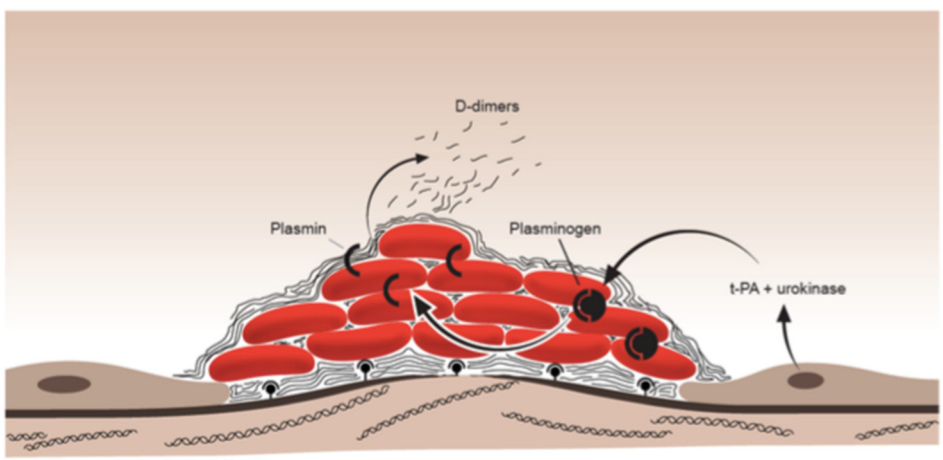

Figure 6.
Fibrinolysis. With permission from Casper Asmussen.

These proteases convert plasminogen into plasmin with the help of fibrin, which serves as a cofactor. Plasmin lyses intravascular fibrin, by enzymatic cleavage, to the soluble fibrin degradation products (FDP), such as cross-linked fibrin called D-dimers (40). These are sensitive but non-specific markers that can be measured in plasma and may indicate VTE but are more sensitive in excluding it. Normally, the *fibrinolytic system* is constantly active in removing the small amount of fibrin being formed in the vessels, thereby taking part in keeping the delicate balance in haemostasis.

Fibrinolysis must then be inhibited to minimize the risk for severe bleeding. There are several inhibitors serving this process, such as plasminogen activator inhibitor-1 (PAI-1) which is synthesized by the endothelial cells. It serves as the key inhibitor of fibrinolysis and effectively inhibits t-PA and u-PA (41).

Thrombin activatable fibrinolytic inhibitor (TAFI) is a plasma carboxypeptidase that is a more recently described fibrinolytic inhibitor and an important link between coagulation and fibrinolysis. It is slowly activated by thrombin but even more by thrombomodulin. It cleaves lysine from fibrin thereby preventing plasminogen binding to fibrin leading to decreased plasmin generation (42).

Disorders of the *fibrinolytic system* such as excess activation or impaired activation may lead to excess bleeding or thrombotic complications, respectively (43).

Venous thromboembolism (VTE)

Venous thromboembolism is a relatively common disease with a major morbidity and mortality rate, affecting approximately 1-3/1000 individuals annually (44, 45). It has been widely investigated for many decades to understand the pathophysiology behind the disease. In 1856, the German pathologist Rudolph Virchow described the connection between thrombosis and embolism and already then influenced the present´s day understanding in the VTE pathogenesis. He described the so called Virchow´s triad, which is made of three physiologic factors that need to be present for the development of a thrombosis, i.e. changes in blood composition, blood flow and alterations in the blood vessel wall (4, 27). This concept is still useful today, and what we now know about VTE risk factors, acquired or genetic, supports it even further. Later, clinical trials regarding therapeutic options such as heparin and vitamin K-antagonists and large epidemiologic studies added even more to our knowledge of VTE and the relationship between deep vein thrombosis (DVT) and pulmonary embolism (PE).

Definition and pathophysiology

The process that initiates the formation of venous thrombosis is not as well known as the one for arterial thrombosis, where blood vessel injury and the rupture of an atherosclerotic plaque plays a central role. (46, 47). In venous thrombosis the main substance is fibrin, which attaches the thrombus to the vessel wall, whereas arterial thrombus is bound to the injured wall mainly by platelets (47, 48). Moreover, the venous clot has another region, i.e. lines of platelet rich white thrombus further inside the clot that separate the regions of red (fibrin) thrombus (47, 49). Furthermore, venous thrombosis occurs mainly in the absence of vessel wall injury where other factors are needed to activate the endothelium. Normally, the endothelium is kept non-thrombogenic with high levels of antithrombotics and anticoagulants such as TM with subsequent activation of protein C, heparin sulfate, TFPI and local production of t-PA as well as various vasodilators (50). Conditions that lead to endothelial disturbances, such as vascular trauma or sepsis, trigger vasoconstriction and many procoagulant substances are released to augment thrombosis. Additionally, leucocytes are activated and initiate inflammation in the vessels and subsequently thrombosis (51). The relationship between inflammation and thrombosis has been studied for the last 50 years. Inflammation increases TF, platelet reactivity, fibrinogen and phosphotidylserines, as well as decreasing TM and inhibiting fibrinolysis. It is generally accepted that venous thrombosis involves TF as the initiator of the coagulation. However, the source of TF is not completely understood. Vessel injury is part of the source but microparticles (MPs) also seem to play a role. Microparticles (MPs) are small phospholipid vesicles secreted by platelets, leukocytes (mainly monocytes) and ECs. Many of them are rich in TF and express P-selectin glycoprotein ligand-1 (PSGL-1), which will help them to interact by associating with activated ECs expressing P-selectin and phosphatidylserine. Both TF and P-selectin appear to be necessary for thrombus formation and this blood borne TF on MPs contributes to the process.

Venous stasis is another mechanism that promotes the formation of a thrombus. Many studies have established this relationship, both in immobilized patients, especially in bedridden and hospitalized patients, and in the paralyzed limb of hemiplegic patients (52-54). The large veins of the legs contain valves that assist the blood returning to the right atrium of the heart when muscular contraction compresses the deep veins. When these valves are not working properly, the stasis of the blood allows prothrombotic factors to accumulate that normally are washed from the lower extremities in to the capillary bed of the lung that is covered with anti-thrombotic substances.

Stasis promotes hypoxic responses in leukocytes, platelets and ECs because of rapid desaturation of haemoglobin. Hypoxia is a pathological state that probably initiates thrombosis through endothelium activation and subsequent deposits of platelets,

leukocytes and fibrin within valve cusps. Moreover, expression of P-selectin is enhanced, accompanied by secretion of vWF, which binds platelets, leukocytes and erythrocytes and promotes venous thrombosis (55-57).

Hypoxia also stimulates TF synthesis from leucocytes, ECs and platelets, increasing MPs bearing TF and contributing to the VTE risk and, according to some reviews, do that mostly through the platelet activation (56, 58, 59). The TF-positive MPs levels are increased in some types of human tumours, which could partially explain the increased incidence of VTE in cancer patients (60).

The result of this pathogenesis is a thrombus that can either organize in the vein or grow further, partially or totally occluding the affected vein. This may lead to dysfunction of the valves in the veins, reduced blood flow with symptoms such as swelling, redness and soreness of the affected area. The thrombus occasionally causes embolism, where part of it travels to the right heart and then to the ung resulting in affected blood flow in the pulmonary artery or its branches. This is the life-threatening or even fatal aspect of the VTE disease.

Epidemiology

Venous thromboembolism is most commonly found in the legs but can also affect the arm or any of the various venous circulations, such as the cerebral, mesenterial, renal, hepatic and portal circulation. All of these can lead to the life threatening complication, pulmonary embolism. It is a major cause of morbidity and mortality, and represents an extensive worldwide health problem. It is a multifactorial disease where both acquired/environmental and inherited risk factors can predispose to the disease, leading to a provoked VTE, but it can also be unprovoked (idiopathic).

Compared to the related disease, arterial thromboembolism, population based studies on VTE have been inadequate. Many previous studies have focused on pre-defined VTE patients where the incidence can be expected to be higher than in the general population. The symptomatology is difficult and the studies are dependent on objective methods. Current data on the incidence of VTE (DVT or PE) in the general population is mostly based on large community-based epidemiological studies where the overall annual incidence of symptomatic VTE is 100-200 per 100.000 individuals (45, 61-65). However, the incidence is probably under-estimated due to many asymptomatic VTE patients. Isma et al demonstrated a lower VTE incidence in their population-based study on consecutive VTE patients in Malmö, Sweden (66), partly because they did not include autopsy data as in many other studies (45, 62, 63).

Age is a very well known and one of the strongest risk factors for VTE (45, 61, 62, 65). The disease is extremely rare among children and incidence is low under the

age of 40 years (67, 68). After that, the incidence rises steeply with as many as 450-600 VTE cases per 100.000 individuals per year within patients over 80 years old (61, 63, 64, 66).

So far there has not been consensus on whether the incidence varies between the two genders and data on the matter has been controversial. Many studies have indicated a generally slightly higher incidence of VTE in men, with the exception of women in their fertile age due to risk factors such as oral contraceptives and pregnancy (65). A population- based study by Heit et al from Minnesota showed an overall age-adjusted yearly incidence rate of VTE higher in men (130/100 0000) compared to women (110/100 000) (69). More studies have demonstrated that male gender is a risk factor for developing VTE (70, 71).

It has been indicated that there is a difference in the VTE risk among different racial and ethnic groups (72). However, the studies have been small and not conducted at geographic areas that are not racially diverse (61, 63, 73, 74). White et al showed in their incidence studies of different ethnic populations in California, that the VTE risk is significantly increased among African-Americans compared to Caucasians, being almost 30 % higher. These have also the highest standardized incidence of both idiopathic and secondary VTE compared to all other ethnical groups. The reason for this difference is not clear but Keenan et al, indicate in their study that both the type of index VTE and gender seem to be important (75). The risk however appears to be much lower among Hispanics and, particularly, Asians, that run a 40 % and 70 %, respectively, lower risk for VTE than Caucasians (76). Some possible explanations to the different VTE risk between ethnic groups are thought to be genetic differences. Some types of hereditary thrombophilia, such as Factor V Leiden (FVL)- and prothrombin G20210A (PT) gene mutation, are significantly more frequent within Caucasians and hardly detectible in Asians (77, 78). Ridker et al demonstrated in their large population based study, including 4047 US people, that FVL mutation was found in 5.3% (CI 4.4% to 6.2 %) of Caucasians, 2.2% of Hispanics, 1.2% of African-Americans and 0.5% of Asians (77). Moreover, lupus anticoagulant (LAC) is more common in Caucasians than all other ethnic groups (79). However, what is thought to explain the much higher VTE incidence in African-Americans is, amongst other factors, their higher levels of FVIII (80).

Risk factors for VTE

Thrombophilia is a term used to describe any, hereditary or acquired, disorder of the haemostatic system that is likely to predispose to thrombosis. The use of the term can have disadvantages when describing venous thromboembolic disease, because it is so multifactorial, with many interacting causes, either transient or persistent. Causes can both be acquired or inherited in addition to the aforementioned

demographic risk factors. What they all have in common is that they affect the delicate equilibrium in the haemostasis by shifting it into a hypercoagulable state and reflect the underlying pathophysiologic processes proposed by Virchow. Although much is known about the pathogenesis and various predisposing factors of VTE, the risk varies greatly among individuals and often the cause remains unknown. The most important acquired risk factors for VTE include high age, malignancy, trauma, major surgery, immobilisation, hormone therapy, obesity, pregnancy and the postpartum period (44, 45, 81-83). Several genetic risk factors for VTE have also previously been described as leading to a significantly increased risk of VTE (84, 85). The most common of these are the Factor V Leiden mutation (FVL) (28, 86), prothrombin (PT) G20210A mutation (87, 88) and the less frequent mutations leading to deficiencies of the natural anticoagulants, i.e. protein C, S and antithrombin. Several other genetic risk factors are known, such as methylene tetrahydrofolate reductase (MTHFR) 677T mutation and different ABO blood groups. Studies have shown that risk increases in proportion to the number of predisposing factors (89).

However, according to many studies the greatest risk for developing VTE is a prior history of VTE, where the recurrence rate has been shown to be up to 13% and 30% after 1 and 8 years, respectively (90).

Acquired risk factors

Surgery and trauma

Surgical interventions and trauma are an example of transient risk factors leading to a temporarily increased risk for VTE. The risk related to these factors has been extensively documented, both regarding major general and orthopaedic surgery. Studies have tried to define the incidence of symptomatic VTE after different surgical procedures for physicians to estimate the need for prophylactic anticoagulation (91). The surgical interventions with the highest VTE risk are orthopaedic procedures, especially hip and knee arthroplasty, major vascular surgery and neurosurgery (91-94). General surgery such as abdominal, thoracic, urological and gynaecological, especially those who require ≥30 minutes anaesthesia, also increase the VTE risk (95). Furthermore, when other risk factors , such as high age and/or cancer are present, the incidence of VTE increases extensively (91, 96).

Trauma, especially multitrauma, has been related to increased risk for developing a thrombotic disease. The incidence varies widely depending on the different types and number of injuries as well as concomitant risk factors but can be as high as 60% within this type of patients (97, 98).

Immobilization and long distance travel

Immobilization is a factor that has been difficult to define and it is difficult to estimate what effect it has on the risk for VTE. However, in several studies, the VTE risk regarding immobilization has particularly been associated with patients with neurological disorders, such as stroke, and lower extremity fractures (99-101). A study has shown 15% incidence of VTE in patients who had bed rest < 1 week but rising up to 80% when in bed for a longer period (52). On the other hand, immobility alone is not a major risk factor but in the presence of other ones the use of prophylactic anticoagulant therapy usually is motivated.

Long distance air travel had already been observed as a risk factor for VTE in the ´50 but only in the last couple of decades, studies on this so-called "economy class syndrome" have shown air travel as a VTE risk factor (102) where the duration of the travel is the most important part of it (103).

Obesity

Several studies have highlighted obesity, defined as BMI > 30 kg/m^2, as a VTE risk factor (64, 104) although many others have given controversial results (105), or not shown any relationship at all (106). If it is a risk factor, it is probably a weak one.

Malignancy

Malignancy has been one of the best known acquired risk factors for VTE. Already in 1865, Armand Trousseau demonstrated a relationship between VTE and cancer. Studies on patients undergoing surgery have shown that the frequency of VTE increases 2-3 fold in the group of patients with malignant disease, compared to those with benign conditions (93, 107), although this has not been confirmed in some other studies (108). The VTE incidence is highly dependent on which type of cancer the patient has and the stage of disease, where metastatic cancer disease is the strongest risk factor, especially during the first months after diagnosis (108-112). The types of cancer that have the highest VTE risk are malignant brain tumours, cancer in the pancreas, kidneys, lungs, breasts, ovaries, pelvis and gastrointestinal tract as well as some haematological malignancies (108, 109, 113, 114). Cancer patients, in general, have about a 4-7 fold increased risk of contracting VTE (112, 115) and chemotherapy treatment is an additional risk factor for these patients (105, 116). Several large population-based epidemiological studies demonstrate that approximately 20% of all VTE cases are associated with cancer. Patients with malignancies that will be diagnosed with VTE have a worse prognosis compared to those cancer patients with no VTE, with a shorter one-year survival (117).

Pregnancy and puerperium

The risk for pregnant women to contract VTE is 4-5 times higher than the risk for for women who are not. Postpartum women have an even have even higher risk for

VTE than pregnant women, with an additional 5 fold risk (118). During pregnancy there is a shift towards a hypercoagulable state because of increased levels of coagulation factors, decreased natural anticoagulants and hypofibrinolysis (45, 119). This is a physiologic phenomenon intended to decrease the risk for fatal haemorrhage during delivery and the postpartum period. However, this can lead to an increased risk for VTE, especially in the developed countries where fatal bleedings are better prevented and is now the leading cause of maternal death (120) with mortality rates of 1.4 per 100,000 pregnancies (121). VTE has an incidence of 100-200 per 100,000 births (118, 122). VTE during pregnancy and the postpartum period increases chronic morbidity such as post-thrombotic syndrome. Additional risk factors, such as inherited thrombophilia, increase the VTE risk even further.

Oral contraceptives and hormone replacement therapy

In the 1960s oral estrogen/progestagen compounds became available as oral contraceptives. Shortly after that, the first reports emerged suggesting that these combined oral contraceptives (COC) increase VTE risk (123). Women in their post-menopausal age are another group that faces an increased risk since they are often treated with estrogen/progestogen compounds as a part of a hormone replacement therapy (HRT). It is now known that VTE risk varies depending on the dose of estrogen, type of progestagens and route of administration (124), leading to a gradual reduction in the estrogen dose over the past years (125). With additional large studies the last decades, the risk for VTE within women on COC has been estimated about 2-6 fold depending on the composition of the compound. The risk is lower among those using low-dose second-generation COC (126-129) but with the use of so called mini-pills containing only progestogen, there is almost no increased risk (129).

Inn women on HRT the estrogen dose is generally lower than in the modern oral contraceptives (130) but despite that, they have a 2-4 fold increased risk of VTE (131-134).

Inherited risk factors

Only a limited number of genetic mutations have been shown to be risk factors for VTE, all of which involve genes encoding for the natural anticoagulants, thereby identifying some hereditary thrombophilic disorders. The first to be described was *antithrombin (AT) deficiency* in 1965, by Egeberg (135). Then, in the 1980s, two other thrombphilias were discovered, *protein C (136)* and *protein S* (137) *deficiencies*. Since the prevalence of these three types of hereditary thrombophilias is very low, the studies investigating them are limited. Studies have estimated a prevalence of 0.02-0.17% (138), 0.2- 0.3% (139) and 0.03-0.13% (140) in the

general population for AT, PC and PS deficiencies, respectively. These are thought to increase the VTE risk by 5-50 fold, with AT deficiency leading to the highest risk by far (83, 141).

Activated protein C resistance and FVL mutation

Since the above mentioned thrombophilias only account for less than 10% of the patients with VTE, despite positive family history within up to 40% of the cases (142, 143), further investigations led to the discovery of resistance to activated protein C (APC-resistance), a condition first described in 1993 by Dahlbäck et al. It is an impaired plasma anticoagulant response to APC added *in vitro*, initially done on plasma samples from Swedish families with severe recurrent venous thromboembolism (28, 144, 145). Subsequently, in 1994, Bertina et al, identified a single point arginine-for-glutamine mutation at position 506 involving the FV gene (86). The consequence is the loss of APC cleavage site in FV and FVa. This leads to both impaired degradation of the procoagulant form of FVa and decreased APC-mediated conversion of FV to anticoagulant form. This imbalance between the procoagulant and anticoagulant mechanisms pushes the haemostasis to the hypercoagulable state (146).

Shortly after the discovery of this mutation, it was named Factor V Leiden (FVL), referring to the institution in Leiden, where the mutation was first reported (86). Subsequent cohort studies demonstrated that APC resistance was found to have high prevalence (20-60%) among VTE patients. Furthermore, it is relatively common in the general population (3-15%) (147-152), being the most common hereditary thrombophilia in Caucasians. However, the prevalence varies widely though in different populations depending on their geographically distribution. The highest frequencies (up to 15%) of the FVL mutation have been reported within European countries such as Sweden, Germany and Cyprus (150-152) and in the Middle Eastern countries, such as Lebanon (153). Interestingly, the same gene haplotype is seen in all FVL alleles, indicating that only one mutation occurred. Zivelin et al (154) have estimated the age of the mutation to be approximately 20,000 to 30,000 years old, i.e. after the " Out of Africa Exodus" which separated the human races. This could explain the geographic distribution of the FVL mutation and why it is almost absent in certain populations, such as Far East Asia, African Americans and Australia.

Large cohort studies have been carried out to estimate the frequency of FVL mutation. In the Leiden thrombophilia Study, APC-resistance was detected in 21% of consecutive patients with DVT compared to 5% in the healthy controls, which gave an almost 7-fold increase in risk (OR 6.6, CI 3.6-12.0) for VTE in people with APC- resistance (148). In the Malmö Thrombosis Study (MATS), the same cohort we use in Paper I and II in this thesis demonstrates this relationship as well. Out of 1140 unselected consecutive patients with VTE, FVL mutation was found in 31%

of patients of which 91% were heterozygous and only 9% homozygotes (66). Heterozygosity for FVL mutation gives a lifelong hypercoagulable state with approximately 5-fold increased risk of VTE (155-157). However, less is known about those who are homozygous for the condition and only few studies describe them. These indicate that these persons suffer from their first thrombosis at a far younger age and have a 10-80 fold increased risk of VTE compared to controls (156, 158-160). Furthermore, individuals with homozygous FVL have been shown to have a higher rate of recurrence of VTE than controls (161). Earlier studies have debated whether the clinical course of VTE events in FVL patients differs from that of normal controls. For example some studies have indicated a lower frequency of PE in this group of patients, where DVT is common. It has been hypothesized that a different structure or location of thrombi in FVL patients leads to a decreased risk of embolic events (162-164). In Paper II of this thesis we further examine the clinical features associated with occurrence of VTE in homozygous FVL patients.

Although APC-resistance is not considered a strong risk factor for VTE, it increases significantly with age as shown by a study of Ridker et al (165) and it can greatly enhance the risk from other factors such as pregnancy and women on COC (166). Furthermore, FVL confers a lower risk of severe bleeding after delivery which has provided, during the history, a survival benefit (167).

Prothrombin G20210A mutation

The prothrombin G20210A (PT) mutation is a mutation occurring in the 3´-untranslated region of the prothrombin gene at position 20210 (G to A transition) leading to elevated plasma prothrombin levels and an increased risk of VTE (168). To detect the mutation, DNA-based procedures are used.

After the FVL, PT mutation is the second most common hereditary form of thrombophilia in healthy individuals of Caucasian origin. The prevalence is around 1.7% to 3% in healthy individuals with the higher prevalence in Southern Europe compared to the Northern part (168, 169). Data has been conflicting regarding the distribution of the PT mutation, although many studies show it is nearly absent or rare in Asia, Africa, America, Australia and Middle Eastern countries (170-172). The risk of VTE in heterozygous carriers of the PT G20210A allele is estimated to be almost 3-fold compared to non-carriers according to Poort et al in the Leiden Thrombophilia study, with a RR of 2.8 (95% CI, 1.4-5.6). The frequency of the mutation in unselected VTE patients was 6.2% and, furthermore, the frequency is substantially higher, or 18%, in patients with family history of VTE (168). At our hospital in Malmö, a study by Hillarp et al, showed prevalence of the PT 20210 mutation within unselected, sex, age and APC-resistance adjusted, DVT patients of 7.1% compared to 1.8% in the healthy control group (p=0.0095). This gave a RR 3.8 (95% CI, 1.1-13.2) (87).

The PT mutation is very rare in a homozygous form in the general population and prevalence has been difficult to demonstrate, with most of the numbers based on case reports (173-175). Large cohort studies have failed to describe any individuals with PT mutation in homozygous form, confirming its rarity. Rosendaal et al conducted a meta-analysis with a total of 5,527 individuals (mainly Caucasians) from 11 centres. None of these were homozygotes for the PT mutation (88). Because of this, the VTE risk is difficult to estimate. Moreover, some studies have reported that the risk is probably not as high as one would have predicted since many asymptomatic cases have been reported (174, 176, 177). In Paper I and II we demonstrate the frequency of the PT mutation among VTE patients.

Antiphospholipid antibodies (aPL)

Antiphospholipid antibodies (aPL) is an inhomogeneous group of autoantibodies directed against phospholipid binding proteins, including lupus anticoagulant (LAC), anti-cardiolipin antibodies (aCL) and anti-β2-glycoprotein-1 (anti-β2-GP1) (178). These have been associated with increased thrombotic risk, both venous and arterial as well as recurrent miscarriages and are a part of the antiphospholipid antibody syndrome (APS). The overall prevalence in the general population is not certain but a frequency of 1-5% has been estimated (179). The rates are higher among the elderly and individuals with comorbidities such as cancer, severe atherosclerosis and infections (180). VTE is common among APS patients where cohort studies have described the prevalence up to 39% (181) and the risk for recurrence is also higher with in these individuals, or HR=6.8 (95% CI, 1.5-3.1), as shown by a prospective study by Kearon et al with (182). The VTE risk has been reported to be around 5-fold over a 5-year period, according to the Physicians Health Study (183).

Hyperhomocysteinemia

Mildly and moderately elevated homocysteine plasma levels are considered a risk factor for VTE development. However, it is unknown how much it attributes to the risk. In several studies (184) , including the Leiden Thrombophilia Study (185), the OR for VTE compared with healthy controls has been estimated 2.5. The pathogenesis is not fully understood, but hyperhomocysteinemia is thought to involve endothelial dysfunction. The condition may result from deficiencies of vitamin B12, B6 and folate as well as the mutations in the MTHFR gene (186).

Diagnosis and treatment

Both diagnosis and treatment of VTE is conducted in accordance to international, national and/or local guidelines (187, 188). The diagnosis of DVT is verified by ultrasonography or phlebography. In symptomatic thrombosis in the proximal veins

of the lower extremity, ultrasonography has high sensitivity and specificity for the diagnosis but substantially less sensitive for asymptomatic patients or those with DVT in the calf (189-191). Phlebography or contrast venography, however, is considered the gold standard for diagnosing DVT (192).

Regarding PE, helical computer tomography (CT) of the pulmonary arteries is the diagnostic test of choice. Systematic reviews reported a wide range of summary sensitivities and specificities (193, 194) and the technique is probably not sufficiently sensitive to exclude PE in patients who have relatively high pre-test probability (195). In those cases, further imaging studies are likely needed. Lung scintigraphy is another option in the diagnosis of PE, especially for those who cannot receive contrast.

In the majority of VTE cases, guidelines recommend anticoagulant drugs such as oral anticoagulation (OAC). Coumarins are vitamin K antagonists (VKA), whereof warfarin has been the leading OAC. Other options have been low molecular and unfractionated heparins or, in selected cases, thrombolytic therapy is indicated. Heparins and coumarins have been the mainstay of anticoagulant therapy during the last decades but since 2012, a new era of anticoagulation has been introduced. The new oral anticoagulants (NOACs), which are direct thrombin inhibitors, have come to the market as a much- awaited addition to previous therapeutic options. However, in this thesis, all our VTE subjects in the different cohorts are treated with either heparins or, in the majority, with warfarin.

Heparins

Heparin is one of the oldest drugs still widely used. It is a negatively charged sulphated glycosaminoglycan which, through activation of antithrombin (AT), inactivates thrombin and Factor Xa. Its discovery, about a century ago, has been debated but in 1916, at John Hopkins Medical School, a medical student, Jay McLean, working under William Howell, extracted a fat-soluble anticoagulant from canine liver that appeared to demonstrate anticoagulant properties *in vitro* and then led to bleeding in experimental animals. Howell took over the work on the anticoagulants and named another fat- soluble anticoagulant he had isolated, *heparin* (from the Greek word “hepar” for liver). In 1918. After this discovery, it took many years for heparin to move from the laboratory to clinical use when the non-toxic product became available in 1936. That was the work of the Swedish scientist Erik Jorpes, who first used the drug intravenously (196). However, heparin, as we know it today in its unfractionated form (UFH), has limitations, both pharmacokinetic, biophysical and biological. These lead to unpredictable anticoagulant responses to UFH, resistance, its inability to inactivate surface-bound thrombin and FXa, bleeding complications and risks for thrombocytopenia and osteoporosis (197). Subsequently, so-called low molecular weight heparins were introduced around the year of 2000. These are derived from UFH by chemical or

enzymatic depolymerisation to yield fragments that are approximately one third the size of heparin. These products have overcome some of the of UFH (197) and are the heparins predominantly used in current clinical praxis.

Vitamin K-antagonists

The coumarins or vitamin K-antagonists (VKA) have been clinically used for over 60 years. Of these, warfarin is the most widely used anticoagulant in the world. Its discovery has a fascinating history beginning in the 1920s, in Canada and USA, when cattle began dying of internal bleedings which pathologist Frank Schofield, found to be due to infected damp hay with moulds such as Penicillum nigricans and jensi. Almost twenty years later, in 1940, Karl Link, at the University of Wisconsin, isolated the haemorrhagic agent in the spoiled sweet hay clove, where the anticoagulant was 3.3´-methylene-bis -(4 hydroxycoumarin), named dicoumarol. Coumarins need to be fermented by fungi to receive their anticoagulant properties. Subsequently, after Link´s laboratory's observations, patent rights for dicoumarol were given to a foundation called Wisconsin Alumni Research Foundation (WARF) which funded the work. Later on, Link and colleagues began working on variations of the naturally occurring coumarin, one of these, discovered in 1948, was used as rat poison. A few years later, this rat poison named "warfarin" (after the foundation WARF) was introduced as an anticoagulant agent that could be used in humans (198). Scientists knew already then that the effect of warfarin could be reversed by vitamin K, the fat-soluble vitamin which had been discovered by Henrik Dam and Edward Doisy in 1943 (eventually earning them the Nobel price). However, problem remained with the laboratory method used for dosage control, i.e. the prothrombin time (PT). PT is the time it takes plasma to clot *in vitro* by the addition of tissue factor and represents the extrinsic pathway of the coagulation cascade. The PT varied greatly depending on the thromboplastin that was used. This led to the World Health Organisation (WHO) adopting a model in 1982 to convert the PT to an International Normalised Ratio (INR). The INR is a standardized method and represents the ratio of the patient´s PT to a normal (control) sample, raised to the power of a ISI value, which is an international sensitivity index for the analytical system used, and gives an international standardized result. Higher INR ratio reflects more anticoagulation. The normal INR range is 0.8-1.2. The method used in Sweden and the other Nordic countries is the Owrens PT and reflects the total activation of the vitamin K dependent coagulation factors, i.e. PT(FII), FVII, FX. These factors, with the addition of the other vitamin K dependent factors, FIX, PC and PS, need a γ-carboxylation to become procoagulant. Vitamin K-antagonists like warfarin inhibit vitamin K-epoxide reductase (VKOR), which is an enzyme that reduces oxidized vitamin K after its participation in the carboxylation of the coagulation factors. Warfarin is metabolized by the enzyme cytochrome $P450_2C_9$, which is coded by the CYP_2C_9 gene. The warfarin dose that patients need varies greatly, mainly because there are many inherited variants of this gene, which lead

to a lower activity of the enzyme, hence the different rate of warfarin metabolism. There are other factors that interfere with its metabolism such as a long list of other drugs, diet and alcohol. This is one of the main problems associated with the use of warfarin, i.e. its many interactions. The anticoagulant effect of warfarin is not reached directly after administration because previously synthesized vitamin K-dependent factors need to be catabolized and then replaced by the insufficiently carboxylated ones. Moreover, the vitamin K-dependent factors have different half-time and full anticoagulation is thus not seen until significant reduction in FII has occurred, normally after three to five days. In the meantime, the levels of PC and PS, which have shorter half-life, have declined, leading to a temporary shift to the thrombotic side of haemostasis. As a consequence, patients must be treated with another anticoagulant agent during this thrombotic period, most often LMWH.

After its first introduction, warfarin continued to develop and already in the 1940s there were reports of its effect in the treatment of thrombotic events. The first randomised trial of warfarin was conducted in 1960, by Barritt and Jordan. They randomised patients with pulmonary embolism and divided them into two groups, one that received a placebo and another that received active therapy, i.e. heparin and warfarin. This study was stopped due to a strikingly higher death rates in the placebo group compared to the anticoagulation group (199). After this, anticoagulation, mainly with warfarin and/or LMWH has been the cornerstone of VTE treatment and, during the last decades, the main focus of studies has been on its duration and intensity.

Time in therapeutic/treatment range (TTR)

The laboratory test used to monitor the VKA treatment is PT or INR and the INR target, or therapeutic range, depends on the indication for the anticoagulation treatment. For the majority of indications, such as VTE and atrial fibrillation, the range is between 2.0 to 3.0. Since mechanical heart valves cause an extra high risk for thrombotic events, the treatment target has the higher level of 2.0-3.5. If the INR value <2.0 is associated with increased thrombotic risk and if it lies >4.0 there is a high risk for major bleeding complications. Consequently, the more time a warfarin treated person is out of its therapeutic range, the higher the risk is for complications. The definition of an individual´s time within the therapeutic range (iTTR) is the percentage of time within the target range, out of the total treatment time. Then the TTR is calculated with the assumption of a linear increase or decrease between two consecutive INR determinations according to Rosendaal´s method of linear interpolation (200).

TTR is accepted as an indicator of the quality of warfarin treatment (200, 201). Studies have shown that centres with high TTR (>70 %) have a reduced risk of both thromboembolic and severe bleeding complications (202).

Recurrence of VTE

Much is known about the different risk factors for VTE, both transient and permanent, which can be either acquired or inherited. These risk factors are used to decide on the duration of anticoagulation, both full dose therapy for VTE patients as well as prophylaxis. Since long-term anticoagulation can both be inconvenient and cause major bleedings (203, 204), it is desirable to give prolonged treatment only to patients at the highest risk and to limit treatment duration in patients with lower risk of recurrence.

Studies have shown that patients are at increased risk for recurrence after first episode of VTE, especially after an unprovoked thrombosis. Many cohort studies have demonstrated the yearly risk in recurrence between 3-8%, depending on the type of thrombophilia the patient, while without thrombophilia have around 2-5% risk of a new VTE episode (205-207). The cumulative incidence of recurrence of VTE after a first deep vein thrombosis has been shown to be about 17% and 30% at 2 years and 8 years of follow up, respectively (90).

Although much is known about the risk of recurrence after a first episode of VTE, it has been controversial whether the most common thrombophilic mutation, heterozygous Factor V Leiden, confers increased risk of VTE recurrence or not (205, 208-214). Previous studies have been of both prospective (203, 204, 208, 215-217) and retrospective (209-211) in design as well as a few meta-analyses (205, 212). Strong prospective studies are preferable, but are dependent on adequate cohorts of consecutive patients to generate reliable data.

A higher incidence of recurrent VTE events has been shown among patients with the rare thrombophilias, i.e. protein C, S and antithrombin deficiencies, homozygous mutations of FVL and PTM as well as multiple defects. However, the data mainly stem from small and/or retrospective studies (69, 218, 219).

Results concerning acquired risk factors and distribution of VTE from the the Malmö Thrombophilia Study (MATS) have been published (66). In Paper I, we evaluated MATS regarding heterozygous FVL mutation as a risk factor for recurrence of VTE.

Coagulation testing for thrombophilia

Familial thrombophilia is a concept that was first introduced in the 1956 by Jordan and Nandorff (220). They described 22 cases of VTE where clear inheritance was found. However, already in 1905, Briggs (221) reported that VTE aggregated within a family. In 1965, the first knowledge of specific inherited thrombophilia was described with antithrombin deficiency (135) and then subsequently, protein C and

S deficiencies were defined. Later, the FVL and prothrombin 20210A mutation were acknowledged. All of these thrombophilias can be associated with approximately 30-60 % of VTE patients. Family history is very important, especially in patients that have had unprovoked VTE and studies have shown that the relatives of those patients have a substantially higher risk of contracting VTE (222, 223). Moreover, studies have indicated that the number of affected relatives (two or more affected siblings) gave higher risk for VTE (224), especially when they are at younger age (<45 years) (224-227). It has been controversial whether family history is an independent risk factor for recurrence of VTE has been controversial. Some studies have only found a weak association or none at all (228, 229), whereas some recent data have indicated that there is a relationship even in the presence of genetic risk factors of VTE (230, 231). However, it seems that family history is a much weaker risk factor when it comes to estimating recurrence than risk for primary thrombosis, although this remains to be debated.

The main reason for doing a coagulation testing is that some thrombophilias can give higher risk for recurrence, both during anticoagulation treatment and after cessation of the therapy, which could influence a physician´s decision on the intensity and duration of treatment. Furthermore, since thrombophilia tend to run in families, this could influence prophylactic treatment among the relatives of the VTE patient.

Because of their substantially higher frequencies, factor V Leiden and PT mutations seem to have much higher impact on the development of VTE in the population than deficiencies of antithrombin, protein C and S, that are much stronger risk factors. When both family history and FVL or PTM mutations are present, the increased recurrence risk is approximately the same as for protein C, S and antithrombin deficiencies. However, since the most common mutation, heterozygous FVL, actually has a low VTE penetrance, the disease being multifactorial and clearly age dependent, it is not recommended to do a general screening for thrombophilia after a VTE episode. Thrombophilia testing, on the other hand, is most often considered within individuals with an unprovoked VTE, both as a first or repeated occurrence, individuals who have a strong family history for VTE, are young (aged <50 years), have VTE in conjunction to oral contraceptives or have VTE at uncommon sites without the presence of cancer. A first VTE episode occurring in relation to well known and transient risk factors have a very low risk of recurrence (90, 232), whereas the risk in the presence of inherited thrombophilia is much more controversial (233). A study (234) that tried to investigate if testing for thrombophilia reduced the risk of recurrent VTE in patients with a first VTE episode, compared 197 patients with VTE recurrence from the MEGA case control study (235) to 324 controls (i.e. without recurrence). Thrombophilia tests were performed in 35 % of cases and 30 % of the controls. The conclusion was that the OR for recurrence was only 1.2 (CI 0.9-1.8) for tested versus non-tested VTE

patients, hence doing a thrombophilia test does not reduce the risk of recurrence after a first VTE episode.

Although it can be justifiable to do a thrombophilia testing, it has to be determined if test is going to alter management. Studies within families with thrombophilia have indicated that VTE can be prevented by testing asymptomatic relatives. This is particularly regarding families with AT, PC or PS deficiency as well as homozygosity for FVL mutation where women are planning pregnancy or the use of oral contraceptives (236, 237). Hence, coagulation testing for thrombophilias can be useful in only limited situations and should and should only be done where it is thought to impact the clinical management.

Inflammatory markers and VTE

Venous thromboembolism (VTE) and arterial thrombosis have been thought to result from two different mechanisms (238, 239). During the last two decades, studies have shown the important role of inflammation in the pathophysiology of arterial atherothrombosis (238) and its cross linkage to the coagulation system (240). Moreover, studies have now shown that atherosclerosis is more prevalent in patients with VTE (241, 242) indicating that the two diseases may share some common mechanisms (239, 241, 243) and risk factors. In the both diseases endothelial dysfunction and vascular inflammation are part of the pathogenesis (244). Furthermore, arterial and venous thrombosis are thought to share risk factors such as hypertension, hyperlipidemia, chronic arterial disease of the legs, diabetes mellitus and obesity even though the evidence is controversial (71, 245-250).

Damage to the vein wall has been thought to be necessary for the initiation of a thrombotic event. However, data now indicate that the process is more complex and the haemostatic balance can be disturbed by inflammation with increased production of procoagulant factors activating the blood coagulation as well as inhibiting the fibrinolytic pathway (251, 252). The thrombus in turn increases the inflammatory process, thereby activating many factors such as platelets, leucocytes, various interleukins and cell-derived microparticles (253) (241, 243, 244, 254, 255). D-dimer is a biomarker that has been extensively analysed. When raised in plasma, it is a known risk factor for first event and recurrence of VTE (256, 257). During the recent years, FVIII has been the focus point of some studies, as a potential risk factor for VTE. These studies, such as case-control (258), and prospective observational studies (259), have indicated that FVIII is a risk factor, but its relevance for guidance on anticoagulation duration has though not been clear. C-reactive protein, the widely used inflammation marker, has been investigated in a few case-control studies (260) and has not been accepted as a risk factor for developing VTE. A large prospective, population based cohort study, the

Longitudinal Investigation of Thromboembolism Etiology (LITE) (261), has shown that elevated levels of the coagulation factor VIII as well as von Willebrand factor are positively and independently associated with the risk of VTE. The same study did not show a significant relationship between some other inflammatory markers (fibrinogen and C-reactive protein) and VTE.

Previous studies from the Malmö Preventive study have demonstrated that raised concentrations of five inflammatory proteins (fibrinogen, haptoglobin, ceruloplasmin, orosomucoid, and a1-antitrypsin) are major risk factors for myocardial infarction, stroke and other cardiovascular outcomes. It is however unclear whether these plasma proteins are associated with VTE (262). In Paper III we investigate if inflammatory plasma markers, in a large cohort of men followed for more than 20 years, are associated with VTE.

Renal function and VTE

Many studies have established that patients with chronic kidney disease (CKD) at any stage have increased risk of developing both arterial and venous thromboembolism (263-266). The exact mechanism is not fully understood but activation of procoagulant factors, decreased natural anticoagulants, hyperactive platelets and decreased activity of the fibrinolytic system (267) are thought to play a role. However, CKD also increases the risk for haemorrhage, both in end-stage renal disease (268, 269) and in patients with a moderate decrease in glomerular filtration rate (GFR) (270). Hence, patients with impaired renal function on anticoagulation treatment with warfarin have been suggested to have a higher risk for poor time in therapeutic range (TTR), especially for supra-therapeutic anticoagulation (271). Patients with CKD therefore need closer warfarin dose adjustment and tend to require lower doses of the drug (272, 273). Other studies on patients with impaired renal function have demonstrated that a significant bleeding risk persists in patients on warfarin in spite of excellent INR control (274).

Previously, real world data from a Swedish national quality register, established in 2006, for patients on warfarin treatment (AuriculA) has been presented (275). The authors showed that TTR in AuriculA was higher compared to TTR in a large randomized trials of warfarin treatment (276, 277) and complications were low (275). In the same material, Per Sandén et al, found no correlation between high TTR and rate of complications, where all centres displayed a high TTR over 70% (mean TTR >78.7%) in patients with atrial fibrillation, which supports the general notion that cTTR should be kept >70%. They imply that after reaching this level of quality of warfarin treatment, other factors, such as patient education, antihypertensive treatment and compliance could be more important for reducing the bleeding risk, at least in patients with atrial fibrillation. However, for patients

with venous thromboembolism (VTE), a lower mean TTR was observed and a correlation between increasing TTR and reduction in the rate of complications was demonstrated (201). TTR varies greatly between countries, as was demonstrated in the large randomized controlled trials RELY and RECOVER, where the mean TTR was 64% and 60% respectively (276-278).

As previously mentioned, results from the AuriculA registry on atrial fibrillation patients treated with warfarin have been published. These indicate an association between impaired renal function and risk for major bleeding events despite good anticoagulation control in patients with atrial fibrillation (274), similar to some previous studies (271, 273, 276, 278). However, since TTR was much higher (74.5 %) than in the other studies, it was assumed that even in centres with good anticoagulation control there is still a correlation with INR out of range and complications. Despite that, there were indications that other factors might impact the risk for bleeding, such as renal function.

In Paper IV we investigate the relationship between renal function assessed by eGFR, major bleeding and thromboembolic complications in patients with venous thromboembolism as an indication for warfarin treatment.

AIMS OF THE STUDIES

PAPER I

To evaluated the distribution of the two most common thrombophilias, i.e. FVL and PTM, in a total material of consecutive adult patients with VTE in the Malmö Thrombophilia Study (MATS) during a 10-year period. Additionally, and its main focus, is to study FVL as a risk factor for recurrence of VTE.

PAPER II

To examine the clinical features associated with the occurrence of VTE in patients with homozygous FVL and PTM as well as patients with double heterozygosity for both the mutations.

PAPER III

To investigate, in a large cohort of approximately 6,000 men from the "Malmö Preventive Study", collected during a 10-year period, whether raised levels of inflammation sensitive plasma markers (ISPs) are associated with VTE.

PAPER IV

To investigate, in a cohort from a Swedish national quality registry for anticoagulation (AuriculA), the relationship between renal function, assessed by eGFR, major bleeding and thromboembolic complications in patients with venous thromboembolism as an indication for anticoagulation treatment.

SUBJECTS

In this thesis, the four studies are based on three different cohorts. **Papers I and II** both describe the Malmö Thrombophilia Study (MATS) cohort, **paper III** contains subjects from a large screening program of men in Malmö and **paper IV** includes all patients on OAT with warfarin in the Anticoagulation Clinic at the former University Hospital in Malmö (UMAS), now known as Skåne University Hospital (SUS). Malmö is a city of 300,000 inhabitants in southern Sweden and its hospital, SUS is one of the largest in the country. It is the only hospital treating VTE patients in the area.

All the studies were approved by the Ethics Committee of Lund University and comply with the Declaration of Helsinki.

PAPER I + II

The MATS study at the University Hospital in Malmö (UMAS), later SUS, ran from March 1998 to able to communicate in Swedish) with VTE were invited to participate in. All subjects gave their informed written consent. The patients had to have objectively verified DVT and/or PE with phlebography, duplex ultrasound, computed tomography (CT), lung scintigraphy or magnetic resonance imaging (MRI). Out of the total 1,465 patients (721(49%) men and 744(51%) women) with the mean age 63 ± 17, we analysed, thrombophilia data we analysed, were available for 1267. All patients were treated in accordance to the standard treatment protocol of UMAS (later SUS) (279).

PAPER III

During the period between 1974-1984, a screening program was conducted to detect individuals with a high risk for cardiovascular diseases. Complete birth cohorts from the city of Malmö were used to invite men to take part in the program and the participation rate was 71%, yielding a total of 22,444 men. Out of those, 6,193 men were randomly selected from the birth cohorts examined between 1974 - 1982. Men with history of myocardial infarction, stroke or cancer (according to questionnaire)

and former VTE (according to hospital registers) were then excluded, leaving 6068 men in the study. Within those men five plasma proteins were determined, i.e. haptoglobin, fibrinogen, ceruloplasmin, orosomucoid and alfa1-antitrypsin, at the time of inclusion.

PAPER IV

The Auricula registry (AuriculA), a Swedish national quality registry for anticoagulation on various indications, was started in 2006. It includes all patients on OAT in the Anticoagulation clinic at the Skåne University Hospital (SUS). It keeps information on patient characteristics, treatment, concurrent illnesses, investigations and complications to atrial fibrillation and VTE as quality indicators. AuriculA has a part for dosing of anticoagulation, i.e. warfarin treatment and, later, the new oral anticoagulants.

Data from AuriculA were extracted for all patients on oral anticoagulation treatment (OAT) with warfarin from 1 January 2008 and 31 December 2008 in the Anticoagulation centre at SUS, Skåne University Hospital in Malmö. This includes 98% of all patients on warfarin in the catchment area (280), hence representing the ”real world” patients. A total of 3,536 patients (1,925 males (54%) and 1,611 females (46%)) had a mean age (SD) of 72 (13) years and the total number of patients with venous thromboembolism as an indication for warfarin treatment was 963 (27.2%) with a mean age (SD) of 67(16) years. The remaining patients (n=2,634) with other indications (mainly atrial fibrillation) had a mean age (SD) of 73 (11). In 2008, there was a follow-up of all registered patients in AuriculA and events of major bleeding and/or thrombotic complications were recorded as well as renal function.

METHODS

PAPER I and II

MATS is a prospective population-based study conducted at UMAS. Computerized inpatient records at the hospital during the study period were screened for VTE-diagnoses (DVT and/or PE) by a research nurse. The 1465 consecutive VTE patients collected, were estimated to represent 70% of all patients diagnosed at the Emergency Department with VTE. The remaining 30% were excluded due to language difficulties, dementia or other illnesses and, in a few cases, unwillingness to participate.

Included patients were required to leave blood samples, answer a questionnaire and were evaluated concerning risk factors for VTE. The DNA mutations for factor V and II were analysed using Taqman allele discrimination with gene specific assays for the two factors (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA). Age and gender was recorded, body mass index (BMI) in kg/m^2 as well as tobacco use before VTE diagnosis (defined as smoking ≥ ca 5 cigarettes/day or ≥ ca 25 g pipe tobacco/week,). We did not take into account if the patients stopped smoking under the follow-up period. We also recorded surgical intervention, immobilisation or cast therapy within the last month, travel more than three hours by bus, car, train or air within the last month. Other risk factors such as malignancies diagnosed prior to or at the diagnosis of VTE, heredity (defined as a history of VTE in first-degree relatives), and use of contraceptive pills, hormonal therapy, pregnancy and postpartum period (defined as first 6 weeks after delivery) among women were assessed. The location of VTE at inclusion, VTE events prior to study inclusion, and all VTE recurrences during follow-up were recorded. DVT was defined as proximal if involving the veins in and above poplitea and distal if involving only the anterior tibial vein or more distal venous segments. Patients with PE were considered as one group, independent of its size and localization in the pulmonary arterial tree.

All patients were treated with low molecular weight (LMH) or unfractionated (UFH) heparin during initiation and then warfarin as oral anticoagulation (OAC). The hospital treatment protocol suggests therapy for 3-6 months for first-time VTE with consideration of extended treatment in some cases, such as massive unprovoked or recurrent VTE. Thrombolysis was considered in specific cases

according to protocol. The treating physician had no initial knowledge of the patient´s thrombophilia status when determining the duration of anticoagulation treatment.

In paper I, when estimating the recurrence of VTE we excluded those subjects with prior VTE events before inclusion as well as those with active malignancy at the time of event.

PAPER III

Within the 6068 men in this study, five plasma proteins were measured at the time of inclusion, i.e. haptoglobin, fibrinogen, ceruloplasmin, orosomucoid and alfa1-antitrypsin.

Since cancer is a potential confounding factor, which could increase inflammation as well as increase the risk of VTE, we performed the analysis first by not taking into account history of cancer and then by excluding the VTE patients that had cancer before or up to 180 days after VTE episode.

Baseline characteristics included the age of the subjects at inclusion, smoking according to a questionnaire where the patients were categorised as current smokers or non-smokers and body max index (BMI), calculated as weight/height2 (kg/m2). Systolic blood pressure was measured twice in the right arm after a 10-minute rest and the average value of these two measurements was used as blood pressure. Blood samples were taken after an overnight fast. Diabetes mellitus was recorded when venous blood glucose was ≥ 6.1 mmol/L (according to The American Diabetes Association guidelines, ADA, 2007), measured in whole blood and in those using anti-diabetic mediation. Serum cholesterol and triglyceride concentrations were analysed with standard methods at the laboratory of the hospital and expressed as mmol/L.

Inflammation-sensitive plasma proteins (ISPs) were measured using electroimmunoassay (281) consecutively at the time of study entry. The coefficient of variation of this method is considered to be < 5% (282). For fibrinogen, haptoglobin and orosumucoid we used detection limits 350 mg/l, 50 mg/l alfa1-antitrypsin and 20 mg/l for ceruloplasmin. Median (interquartile range) levels for the ISPs were 3.46 (3.0-4.0) g/l for fibrinogen, 0.80 (0.67-0.93) g/l for orosomucoid, 1.28 (1.09-1.42) g/l for alfa1-antitrypsin, 1.30 (0.89-1.75) g/l for haptoglobin and 0.30 (0.26-0.35) g/l for ceruloplasmin.

Previously, all the five ISPs have been associated with different cardiovascular diseases and that the hazard ratio (HR) is approximately the same for all the five proteins (262). In accordance with several previous studies from this cohort, we

constructed a composite score (i.e. the number of ISPs in the fourth quartile) from these five proteins.

The, the Swedish hospital discharge registry was used to retrieve the VTE cases during the *follow-up* period This registry covers all hospitalizations in the south of Sweden during the entire follow-up period and the register became nation-wide in 1987. VTE was defined as ICD-8 codes 450-451, ICD-9 codes 415B or 451, and ICD-10 codes I26 and I80. All the men were followed from the baseline examination until VTE, death, emigration from Sweden or until the end of the follow-up time, 31 December 2008.

PAPER IV

As well as being a quality registry, AuriculaA has an additional role for warfarin dosing, where an algorithm suggests dosing based on the last two international normalized ratio (INR) results. Key outcome measures for patients on anticoagulation treatment are on the one hand major bleeding, according to the International Society on Thrombosis and Haemostasis definition (283) (fatal bleeding, and/or symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or bleeding causing a fall in hemoglobin level of 20 g/L or more, or leading to transfusion of two or more units of whole blood or red cells), and clinically verified arterial or venous thrombosis on the other (clinical suspicion of deep vein thrombosis, pulmonary embolism, myocardial infarction, ischemic stroke or peripheral arterial embolism, verified objectively using ultrasound or phlebography, computed tomography of the chest, electrocardiogram and troponin T or I, computed tomography of the brain, or angiography, respectively).

In the study, indications for OAT were grouped either as venous thromboembolism (VTE) or other (atrial fibrillation, mechanical valve replacement, biological valve replacement, mitral stenosis, left ventricular aneurysm, cardiomyopathy, nephrotic syndrome, transitory ischemic attack and pulmonary hypertension). All key outcome measures registered in AuriculA during 2008 were followed up and all hospital records for every patient were reviewed to make sure no complications were missed or incorrectly classified. Only the first event for each patient in each separate category (major bleeding/thrombosis) was used for statistical analysis. The age of the patients was defined as the age at first INR test in 2008 and iTTR was calculated according to the Rosendaal algorithm with linear interpolation (200). Mean iTTR was calculated as the mean of the individual iTTR values of each patient that had warfarin treatment >1 week and INR target interval of 2.0-3.0, excluding 642

patients (18.2%) that had different target intervals and 10 patients without enough INR results. Among patients with complications, only INR values before the event were used for statistical analysis.

For the measurement of renal function, we used a database containing all laboratory results of previous blood samples in our region. Plasma creatinine (p-Cr) was measured using a modified Jaffe colorimetric method on a Beckman LX20 analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) traceable to a common reference material including a zero-point calibrator and as such also traceable to isotope dilution mass spectrometry. Age (years), gender and the last p-Cr (mcmol/L) registered between 1 January 2008 and 31 December 2008 were used for estimating GFR, Subjects lacking p-Cr (n=187, 5.3% of all patients) from that time period were excluded from eGFR analysis.

For eGFR calculations in patients with complications, p-Cr levels at the time of the event were used. For potential effect of complication on p-Cr levels, p-Cr level at the time of event was compared to pCr measured at least one month before the event. There was no difference in mean or median p-Cr and median data is presented. Two patients did not have a p-Cr measured at the time of the event and were excluded in the eGFR analyses.

Two different formulas for eGFR estimation were used:

1. The IDMS-traceable four-variable Modification of Diet in Renal Disease (MDRD) Study equation (284):

   $175\ (\text{p-Cr}/88.4)^{-1.154} \times \text{age}^{-0.203} \times 0.742(\text{if female}) \times (\text{if Afro-American})$

2. The Lund-Malmö (LM) equation, derived and internally validated at the present University Hospital (285):

   $e^{x-0.0124 \times \text{age} + 0.339 \times \text{In (age)} - 0.226 (\text{if female})}$;

   $x = 4.62 - 0.0112 \times \text{p-Cr}$ if p-Cr<150 µmol/L

   $x = 8.17 + 0.0005 \times \text{p-Cr} - 1.07 \times \ln(\text{p-Cr})$ if p-Cr≥150 µmol/L

We did not take into account ethnicity when using the MDRD equation since AuriculA contained no information about race. Patients were divided into groups, with pre-specified eGFR cut offs at 30 ml/min/1.73m^2, corresponding to CKD stage 1-3 and 4-5 (286), 45 and 60 ml/min/1.73m^2, representing suggested boundaries between CKD stages (287).

STATISTICAL ANALYSES

In all papers the statistical analyses were carried out by using SPSS statistics software, version 17.0, 19.0 and 20.0 (SPSS, Inc, Chicago, IL) with the addition of the analysis software R in paper IV.

Results are expressed as mean or median ±SD, n (%), odds ratio (95% CI) and p-values < 0.05 were considered as statistically significant.

In **Papers I-II** descriptive analyses were performed. For calculating p values we used Fischer´s exact test, Chi-2 test and Student´s t-test when appropriate. Cox-regression and Kaplan-Meier tables were made for the risk analyses.

When we looked at baseline characteristics in relation to the number of raised ISPs in **Paper III**, we used one-way ANOVA for age and BMI and Pearson chi-square for smoking. As triglycerides showed a skewed distribution, Mann-Witney test was used for the analysis. When the VTE risk was assessed in relation to the number of elevated ISPs, the Cox proportional hazards model was performed. Age, BMI and cholesterol were modelled as continuous variables whereas current smoking and diabetes mellitus were used as dichotomous variables. The number of raised ISPs was used as an ordinal variable to get the p value for trend. The same statistical tests were used regarding the individual ISPs.

In **Paper IV**, we calculated skewness to assess normal distribution on continuous data. Differences in means were tested with Student´s t-test and Fisher´s exact test for differences in proportions. Log Rank was used to test the equality of cumulative incidence distribution for different levels of eGFR, and to calculate the adjusted hazard rations we used Cox regression. All statistical tests were two-sided with the afore mentioned p value of < 0.05.

RESULTS

PAPER I

A total of 1465 consecutive patients were included in the study, of which 721 (49%) were men with a mean age of 64±15 years and 744 (51%) were women with a mean age of 63±19 years. DVT was diagnosed in 1021 (70%) patients, PE in 352 (24%), whereas 91 (6%) had both DVT and PE. Data was missing in one patient. Mean age at first VTE was 62.4±15.1 years in men and 61.4±19 years in women (p=0.241). Out of those patients with DVT, 300 (27%) were distal thrombosis, 682 (61%) were proximal and other locations or unknown site was in 130 of DVT cases. PE was significantly more frequent among women than men (27% versus 21%; p=0.013) whereas the opposite was true for DVT (68% versus 72%; p=0.013).

The 1,267 VTE patients, from whom data regarding FVL and PTM status were available, were divided into a group with either or both the mutations (n=432) and a group without these mutations (n=835). Common acquired risk factors for VTE were compared between the two groups (**table 1**). Positive heredity (a first degree relative with VTE) existed in 145 (34%) patients with thrombophilia and 172 (21%) patients without (p<0.01). Previous surgical intervention, cast therapy and malignancy were all more prevalent in the group without the mutations. No significant differences were found between the two groups regarding other acquired risk factors.

Among the 1,267 VTE patients with information on mutation status, 835 (66%) had normal genotypes, whereas 339 (27%) patients were heterozygous and 36 (3%) homozygous for FVL. Forty-five (4%) were heterozygous and none homozygous for the PTM, whereas 12 (1%) were compound (FVL + PTM) heterozygous. No significant differences existed between genders (**table 1**).

Table 1.
Genetic thrombophilia among 1267 consecutive patients with venous thromboembolism. N (%). PTM, prothrombin gene mutation.

Type of thrombophilia	All patients (n=1,267)	Men (n=620)	Women (n=647)
Without thrombophilia	835 (66)	400 (64)	435 (67)
Heterozygous FVL	339 (27)	178 (29)	161 (25)
Homozygous FVL	36 (3)	15 (2)	21 (3)
Heterozygous PTM	45 (4)	21 (3)	24 (4)
Homozygous PTM	0 (0)	0 (0)	0 (0)
Compound PTM and FVL	12 (1)	6 (1)	6 (1)

Follow up for all the 1465 patients was 4.8±2.3 years and the total follow up time was 6133 patient years. When estimating VTE recurrence after inclusion, we excluded patients (n=25) with thrombotic events at any time before study inclusion or recurrence during anticoagulation therapy (n=281) and complete information on recurrences was missing in 51 subjects. Among the remaining 1108 patients, 131 (12%, CI 10–14%) suffered a VTE recurrence after study inclusion. In this group, 49 (42%) were heterozygous and 2 (2%) homozygous for the FVL mutation. Heterozygous PT mutation was found only in 7 (6%) of patients with recurrent VTE. Two (2%) had both heterozygous PT mutation and FVL, fifty-seven (49%) patients were without the mutations and data on this type of thrombophilia were missing in 14 patients.

Recurrent VTE tended to be more frequent among men than among women (77[14%] versus 54 [10%]; p=0.052). Time to recurrence in men and women were 20±19 months and 25±22 months respectively (p=0.16). In a subgroup of 964 patients we were able to evaluate recurrence among patients with unprovoked thrombosis versus those with provoked thrombosis (i.e. with one or more genetic and/or acquired risk factors) and found no significant difference.

A Kaplan-Meier analysis was made to estimate recurrence risk during follow-up in the 260 patients heterozygous for FVL and the 640 patients with normal genotype. The groups separated already during the first year of follow up, and after 8 years nearly 25% in the FVL group had suffered a new VTE episode compared to 10% in the group with normal genotype (p<0.01). After adjustment for age, this difference was still significant (p<0.01). The yearly incidence for VTE recurrence was 3.3% (CI 2.5– 4.4%) in the FVL group compared to 1.5% (CI 1.2 – 2.0 %) in the group with normal genotype (OR 2.4 (CI 1.6–3.6)) (**figure 7**).

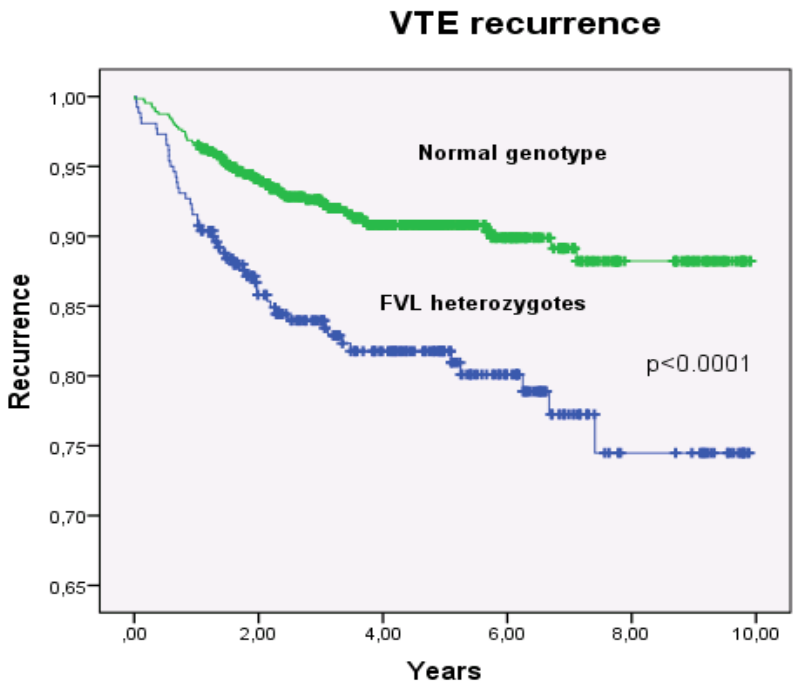

Figure 7.
Kaplan-Meier analysis showing the risk for VTE recurrence during up to 10 years of follow-up after first VTE episode. A significant difference is shown between patients with heterozygous Factor V Leiden (FVL) mutation and patients with normal genotype.

PAPER II

Of the 1,465 patients in the MATS cohort, 36 (2.5%) were found to be homozygous for FVL (21 women and 15 men). No patient was homozygous for PTM, 12 patients (0.8%, 6 women and 6 men) had a double mutation of heterozygous FVL and PTM. Mean age of the 48 subjects with homozygous FVL or compound FVL and PTM was 57 ±17 at inclusion VTE and women seemed to be younger than men (53 ±20 vs 62 ±13, ns). Ninety-two percent had DVT at inclusion, 15% PE and 6% had both DVT/PE. Of the 44 patients with DVT, 27% was in v. iliaca, 44% in v. femuropoplitea and 14% localized to the calf. Positive family history of VTE in first degree relatives was reported in 20 subjects (42%). In 26 (54%) of the subjects had at least one additional acquired risk factor. Mean age of first VTE was 52 ±18 where women were significantly younger than men (46 ±20 vs 59 ±12, $p < 0.02$).

The FVL homozygous group had a mean age of 56 ±18 at inclusion VTE, which was significantly younger, compared to controls (63 ±17), p < 0.02). Women were younger than men (50 ±17 vs 65 ±14, p < 0.02) and homozygous women were younger than female controls at inclusion VTE (50 ±19 vs 63 ±17, p< 0.001). This difference was not seen among men. **Table 2** describes the distribution of DVT/PE. Positive family history of VTE in first degree relatives was reported in 14 subjects (39%) and at least one additional acquired risk factor was seen in 20 subjects (56%). Of these, three women were using oral contraceptives and three women were in postpartum.

At study end 13 patients (36%) had been diagnosed with more than one VTE event during their lifetime thus far. Mean age at first VTE was 53 ±18 and women were significantly younger than men (47 ±19 vs 64 ±14, p <0.01) (**table2**). Odds ratio for VTE in individuals homozygous for FVL compared to controls was 13.9 (95% CI 9.9 – 19.7) assuming a prevalence of 0.18% homozygosity for FVL and 8.1% in heterozygous form in the south of Sweden (288).

Table 2.
Location of thrombus and age at thrombosis

Homozygous FVL	All n = 36	Male n = 15	Female n = 21	Controls n = 1429
Clinical features				
Age at first VTE	53 ±18	64 ±14	47 ±19	62 ±17
Age at inclusion VTE	56 ±18	65 ±14	50 ±19	63 ±17
DVT	32 (89)	13 (87)	19 (90)	1078 (75)
PE	6 (17)	4 (27)	2 (10)	435 (30)
DVT + PE	2 (6)	2 (13)	0 (0)	86 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity[a]	23 (72)	9 (69)	14 (74)	662 (61)
Distal thrombosis of the lower extremity[a]	5 (16)	3 (23)	2 (11)	300 (28)
Upper extremity	3 (9)	0 (0)	3 (16)	300 (28)
Other location or clinical diagnosis	1 (3)	1 (8)	0 (0)	50 (5)

Mean ± SD and *n* (%)
[a]The distribution of thrombi in the left lower extremity compared to the right was equal among men whereas 74 % female subjects had thrombosis of the left leg.

Patients with double heterozygosity of FVL and PTM had a mean age of 59 ±16 at inclusion VTE and there was no difference in the age at inclusion compared with controls (59 ±16 vs 63 ±17, ns). The mean age of women in this group was slightly higher than that of men, although not significantly. Positive family history of VTE in first degree relatives was reported in six subjects (50%) and at least one additional acquired risk factor was seen in six subjects (50%). At the study end, eight patients (67%) had been diagnosed with more than one VTE during their lifetime thus far. All males had recurrent VTE (100%) and women had a recurrence rate of 33%. Men were significantly younger than women at first VTE (53 ±9 vs 60 ±21, $p<0.03$).

Table 3.
Location of thrombus and age at thrombosis

Double heterozygote FVL/PTM	All n = 12	Male n = 6	Female n = 6	Controls n = 1453
Clinical features				
Age at first VTE	56 ±16	53 ±9	60 ±21	62 ±17
Age at inclusion VTE	59 ±16	57 ±9	62 ±21	63 ±17
DVT	11 (92)	5 (83)	16 (100)	1099 (76)
PE	1 (8)	1 (17)	0 (0)	440 (30)
DVT + PE	0 (0)	0 (0)	0 (0)	88 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity[a]	10 (90)	5 (100)	5 (83)	662 (61)
Distal thrombosis of the lower extremity[a]	1 (9)	0 (0)	1 (17)	300 (28)
Upper extremity	0 (0)	0 (0)	0 (0)	66 (6)
Other location or clinical diagnosis	0 (0)	0 (0)	0 (0)	50 (5)

Mean ± SD and *n* (%)

PAPER III

Out of the whole cohort (n=6,068), 398 (6.6%) had a venous thromboembolism during the follow-up time, which was the mean of 26.2 years. Of the VTE cases, 116 had cancer before or up to 180 days after VTE. The mean age and BMI was 46.8 years and 25.0 kg/m^2 respectively. Those who had no elevated ISPs were 2,448

men, those with one raised were 1,559, 906 had two elevated proteins and 1,155 had three or more raised ISPs. All the basic characteristics, i.e. age, BMI, smoking, diabetes mellitus, cholesterol, triglycerides were significantly associated with number of raised ISPs (**table 4**).

Age and BMI were significant risk factors for developing a VTE when adjusted for other risk factors (HR=1.05, p<0.01 (95% CI 1.01 - 1.08) and HR=1.10, p<0.001 (95% CI 1.06 - 1.14 respectively)). Diabetes mellitus was also a significant risk factor for VTE with a HR=1.78 and p value of <0.05 (95% CI 1.13 to 2.81), see **table 4.**

When we excluded the VTE cases with cancer during follow-up or within 180 days after VTE (n=109) we had similar results, i.e. age, BMI and diabetes mellitus were significant risk factors (HR=1.05, p<0.01 (95% CI 1.01 - 1.08), HR=1.10, p<0.01 (95% CI 1.06 - 1.14) and HR=1.78, p<0.05 (95% CI 1.13 - 2.81), respectively) whereas the other factors were not.

Table 4.
Various risk factors in relation to VTE risk

	VTE, no	VTE yes	HR, crude (95%CI)	HR age, adjusted(95%CI)	HR, risk factors adjusted (95%CI)
N	5,670	398	---	---	---
Age, years (±SD)	46.8 (3.7)	47.3 (4.4)	1.06 (1.03-1.09)	---	1.05 (1.01-1.08)
Cholesterol mmol/l (±SD)	5.7(1.1)	5.7(0.9)	0.99 (0.90-1.09)	0.97 (0.88-1.07)	0.96 (0.80-1.08)
BMI, kg/m2 (±SD)	24.9 (3.3)	25.9 (3.8)	1.09 (1.06-1.12)	1.09 (1.05-1.12)	1.10 (1.06-1.14)
SBP, mmHg (±SD)	129.0 (15.5)	130.0 (16.6)	1.01 (1.00-1.01)	1.00 (0.00-1.01)	1.00 (1.00-1.01)
Triglycerides, mmol/l (±SD)	1.6 (1.1)	1.5 (1.8)	0.98 (0.88-1.09)	0.98 (0.88-1.09)	0.88 (0.76-1.03)
Current smoking yes (%)	2735 (48.2)	184 (46.0)	1.08 (0.90-1.31)	1.09 (0.90-1.33)	1.20 (0.94-1.52)
Diabetes Mellitus yes (%)	260 (4.6)	27 (7.2)	1.93 (1.12-3.00)	1.95 (1.25-3.05)	1.78 (1.13-2.81)

When we looked at inflammation in relation to VTE development, the risk was adjusted for the same possible confounding factors as seen in table 4. The HR for venous thromboembolism was not significantly related to the number of raised inflammatory proteins (p for trend =0.37) adjusted for age, BMI, diabetes mellitus, smoking and cholesterol (**table 5**). The hazard ratio (HR) was 1.00 for those without elevated ISPs (reference), and the HR was 1.11 (95% CI 0.87 to 1.42), 1.18 (95% CI 0.87 to 1.59) and 1.09 (95% CI 0.81 to 1.47), respectively in men with 1, 2 or ≥3 raised ISPs and non-significance remained after we excluded the cancer VTE cases.

None of the five individual inflammatory proteins, i.e. fibrinogen, haptoglobin, ceruloplasmin, A1-antitrypsin and orosomucoid, were significant as potential risk factors for VTE. The plasma levels of the proteins were measured and divided into quartiles, see **table 5**. The HRs for the fourth quartiles (vs first quartiles) were 0.81 (95% CI 0.61 to 1.08), 1.12 (95% CI 0.83 to 1.51), 1.18 (95% CI 0.87 to 1.58), 1.07 (95% CI 0.81 to 1.41) and 1.21 (95% CI 0.90 to 1.64), respectively, for the above mentioned proteins. As expected, the non-significance remained when VTE cases within the cancer subjects were excluded.

Table 5.

Incidence of thrombosis in relation to number of elevated acute phase proteins (ie in the 4th quartile. Based on number of acute phase proteins in the fourth quartile (of Fibrinogen, Haptoglobin, Ceruloplasmin, orosomucoid, a1-antitrypsin).

	None	One	Two	≥ three	P, trend
N	2448	1559	906	1155	
VTE † n, (% of N)	156 (6.4%)	109 (7.3%)	63 (7,0%)	70 (6.0%)	
HR, age-adjusted	1.00	1.11 (0.83-1.48)	1.35 (0.97-1.89)	1.17 (0.84-1.63)	0.15
+risk factors*	1.00	1.11 (0.87-1.42)	1.18 (0.87-1.59)	1.09 (0.81-1.47)	0.42
VTE † (without history of cancer) n, (% of N)	113 (4.6%)	75 (4.8%)	50 (5.5%)	51 (4.4%)	0.16
HR (95% CI) age+risk factor adjusted*	1.00	1.06 (0.79-1.42)	1.29 (0.91-1.81)	1.09 (0.78-1.54)	0.37

†VTE: DVT, PE or both
*Risk factors: BMI, diabetes, smoking and cholesterol

PAPER IV

In the whole material, 2,894 patients had target INR 2.0-3.0 where the adjusted mean iTTR was 74.5 %. During total of 2,875 treatment years, 75 major bleeding and 51 thromboembolic events were registered indicating a 2.6% (95% CI 2.0 – 3.2) bleeding risk/patient year, (**table 6**). Of the total bleedings, 18 were among patients with a VTE indication for OAT where of 17 patients had an INR target of 2.0-3.0.

Bleeding events

In patients with major bleedings the mean INR (SD) was 3.5 (1.7) and the patients presented an increased time with INR>3.0 (21.2% ± 19.5) compared to those with no bleeding (13.9% ± 17.2) ($p< 0.001$. As expected, increasing age was a risk factor for major bleedings ($p< 0.001$).

Thromboembolic events

A total of 51 thromboembolic events were registered in the study, of which 41 (80%) were arterial, and 10 (20%) were venous thrombosis. Mean INR at the time of the thrombotic event was 2.3 (1.0) and a trend was seen in percentage of time INR <2.0 in patients with thromboembolic episodes ($p=0.058$).

Renal function and complications

There was significantly lower mean eGFR among those patients with a major bleeding episode compared to those with no major bleeding ($p=0.003$). The difference was consistent using both Lund-Malmö and the MDRD equations. Additionally, patients with major bleeding episodes had a significantly ($p<0.001$ in both cases) higher proportion with eGFR<30 ml/min/1.73m^2 and eGFR<45 ml/min/1.73m^2 compared to those without major bleeding. No differences were seen within patients with thromboembolic complications.

Table 6. Patient characteristics, complication frequencies and estimated risk per patient year. Patients can have more than one indication of anticoagulation treatment with warfarin.

	Total (n)	Mean age (years)	Treatment Years	Bleeding n (%)	Thrombosis n (%)	Bleeding risk/ patient year (%)	Thrombosis risk/patient year (%)
Whole population	3536	72	2875	75 (2.6)	51 (1.4)	2.6 (2.1-3.2)	1.8 (1.3-2.3)
-men	1925	70	1561	37 (1.9)	27 (1.4)	2.4 (1.7-3.2)	1.7 (1.2-2.5)
-women	1611	74	1314	38 (2.4)	24 (2.4)	2.9 (2.1-3.8)	1.8 (1.2-2.7)
Venous thromboembolism	963	67	696	18 (1.9)	12 (1.3)	2.6 (1.6-4.0)	1.7 (0.9-2.9)
-INR 2.0-3.0	931	67	662	17 (1.8)	11 (1.2)	2.5 (1.5-4.0)	1.6 (0.9-2.8)
-INR Other	32	67	34	1 (3.0)	1 (3.0)	3.5 (0.2-15.3)	3.5 (0.2-15.3)
Other	2634	73	2179	59 (2.9)	39 (1.5)	2.6 (2.0-3.4)	1.8 (1.3-2.4)
-INR 2.0-3.0	2023	75	1693	45 (2.2)	28 (1.4)	2.6 (2.0-3.4)	1.7(1.2-2.3)
-INR Other	611	68	486	14 (2.3)	11 (1.8)	1.7 (0.1-7.4)	5.0 (1.2-13.0)

Patients with VTE. Patients with VTE as an indication for warfarin treatment were significantly younger than the group with other indications, with a mean age (SD) of 67 (16) compared to 73 (11) ($p< 0.001$).

As seen in **table 7**, major bleeding events were relatively more frequent with decreasing levels of eGFR (although no patient in the VTE group had an eGFR <30 and a major bleeding event) leading to a tendency towards a higher risk for bleeding per patient year. However, here was no significant increase in the relative risk for bleeding event for every 10 ml/min difference in eGFR within the VTE group (RR=1.006 per 10 ml/min, p=0.96, CI (0.80-1.27)) whereas the risk was significantly increased for the group with other indications, RR= 1.385 per 10 ml/min decrease in eGFR ($p<0.0001$, CI (1.20-1.61)).

Mean eGFR(LM) (SD) in the patients with VTE and major bleeding event was 63.3 (23.1) and 46.8 (22.8) in the group with other indications for warfarin treatment (p=0.009). However, mean age and total percentage of time INR >3.0 (SD) were the same for different levels of eGFR in both the VTE and other indications group.

In the VTE group there were 12 (1.2%) thromboembolic events and 39 (1.5%) in the group of other indications, respectively. When we looked at the risk for thromboembolic events in relation to renal function, there was no significant correlation in either of the two different indication groups, RR= 0.876 per 10 units eGFR, p= 0.359 (CI 0.66 – 1.17) and RR= 1.04, p=0.71 (CI 0.86 – 1.24) respectively.

Table 7.

Risks for major bleeding or thromboembolic events with respect to renal function in ml/min/1.73m^2. Risks in percent per patient year with 95% CI in brackets. eGFR is calculated using the LM-equation in 3536 patients.

	(n)	Mean age	Patient Years	Bleeding events (%)	Risk/ patient year (%)	% INR >3.0	Thrombosis (%)	Risk/ Patient Year (%)	% INR <2.0
Venous thromboembolism	963	67	696	18 (1.9)	2.6	14.2	12 (1.2)	1.7 (0.9-2.9)	14.6
eGFR>60	512	67	351	8 (1.6)	2.2 (1.0-4.9)	15.1	9 (1.7)	2.4 (1.2-4.4)	16.2
eGFR 45-60	206	77	157	6 (2.9)	4.2 (1.7-8.4)	13.6	1 (0.5)	0.7 (0.0-3.3)	14.2
eGFR 30-45	114	80	84	4 (3.5)	5.0 (1.6-11.7)	12.4	0 (0.0)	---	13.7
eGFR<30	39	79	27	0	---	15.1	2 (5.1)	7.4 (1.2-22.8)	11.6
Other	2573	73	2179	57 (1.3)	2.6 (2.0-3.4)	14.1	39 (1.5)	1.8 (1.3-22.8)	13.3
eGFR>60	1087	67	895	14 (1.4)	1.5 (0.8-2.4)	15.0	15 (1.3)	1.6 (0.9-2.5)	13.4
eGFR 45-60	807	77	689	16 1.9)	2.4 (1.4-3.8)	12.3	3 (1.7)	2.0 (1.1-3.2)	13.0
eGFR 30-45	399	80	354	12(3.0)	3.7 (2.0-6.3)	13.8	9 (2.5)	2.8 (1.3-5.0)	12.0
eGFR<30	186	79	154	14 (7.0)	9.1 (5.1-14.7)	15.5	1 (0.5)	0.7(0.0-2.9)	15.9

RR, relative risk

RR =1.006 per 10 units lower eGFR in the ”VTE” group, p=0.96, CI (0.80-1.27)

RR=1.385 per 10 units lower eGFR in the ”other” group, p<0.001, CI (1.20-1.61

GENERAL DISCUSSION

Although much is known about VTE and its risk factors, this major worldwide health problem world widely still causes great mortality and morbidity and its incidence varies greatly between studies. Until the 1990s, VTE was considered to be primarily a complication of major surgery or a late stage of terminal illness. Since then many trials have described the relationship between VTE and various acquired risk factors as well as some inherited. These still need further documentation for better understanding (289) and to be able to identify the risk for first as well as recurrent episodes of VTE. In this thesis, we have looked further into the most common known inherited risk factor, FVL, both in heterozygous and homozygous form. Up to now, there has been no general consensus on the recurrence risk among patients with first unprovoked VTE and this common mutation has given conflicting results in previously published studies (204, 210, 211, 213, 216, 290, 291). Those, that describe the incidence of VTE and its risk factors, have mostly been performed in Northern or Western Europe or in the United States. These studies show that the incidence varies widely, ranging from 71 to 192 peer 100, 000 individuals yearly (45, 61-63, 65). These previous studies have been designed differently; either using inpatient records only, both in- and outpatient records, or patients from different geographic areas, and they have not taken into account concomitant diseases such as malignancy. Some of the studies did not separate first-time from recurrent VTE episodes although others have.

In **Papers I and II** in this thesis, we describe a cohort from a well-defined geographic area with both in-and an outpatients diagnosed with VTE at UMAS (later called SUS). Since that is the only hospital in the region treating these patients, our results most likely represents a true population based VTE epidemiology. The cohort therefore reflects subjects from the general population and not only subgroups. The incidences of VTE, DVT and PE in our population were lower compared to other epidemiological studies (45, 62, 65, 292), which could in part be explained by more strict diagnostic criteria nowadays and better thromboprophylactic therapy, and also by the fact that we did not include patients < 18 years and VTE cases verified by autopsy.

Although several studies have established inherited thrombophilia as a common risk factor for VTE, some of these conditions are still not completely understood. Previous studies regarding epidemiology of VTE have mostly been of retrospective design and performed within subgroups of the general population and only a few

prospective studies have been performed to estimate the community based incidence and risk factors among unselected VTE patients (203, 216, 232). To our knowledge, the studies presented in **Paper I and II** are based on one of the largest cohort of consecutive patients with homozygous FVL to date, as well as evaluating the recurrence risk of VTE among FVL carriers. Studies describing clinical features associated with homozygous FVL have mostly been derived from selected materials (156, 160). Of the total MATS material in which we had information on mutation status, 27 % had FVL in heterozygous form alone and 3% had the mutation in homozygous form. This prevalence of the most common inherited thrombophilia among VTE patients is in accordance with previous literature (149).

Of the total MATS material, women suffering their first VTE episodes were, not surprisingly, younger than men **(Paper I)** . That can most likely can be explained by acquired VTE risk factors such as use of contraceptive pills and pregnancies in younger women. In **Paper II**, the mean age at inclusion thrombosis was 56 years which was significantly younger compared to controls which had a mean age of 63 years ($p<0,02$) and evident that it was because of the younger age of women with homozygous FVL and VTE compared to men. However, the age difference was also significant even when compared to female controls (50 ± 19 vs 61 ± 20, $p <0.02$) where this was not observed within the male gender. Women seem to have two peaks of incidence with the first around 30 years of age and the second around 60 years of age. These findings support previous studies that women with known homozygous FVL should avoid oral contraceptives because of greatly increased thrombosis risk (166, 293).

It is of interest to look at the distribution of VTE. In **Paper I** when we look at the whole material, PE was significantly more frequent among women than men (27% vs 21%, $p=0.0013$) where the opposite was true for DVT (68% vs 72%, $p=0.0013$). Furthermore, in **Paper II**, it seemed that homozygous FVL subjects had less frequent PE (17%) compared to controls (30%) with a p value close to significance ($p=0.051$). This is in accordance with earlier studies that have indicated that heterozygous FVL patients suffer from pulmonary embolisms to a lesser degree than controls (163, 164, 294). However, according to our results, this only applies to female FVL subjects since 10 % of the homozygous FVL women had PE versus 27 % in the male group. The reason for this lower frequency of PE in FVL patients is still unknown, but previous data have shown that thrombi in FVL patients are more distally located than in controls (162) although in our study, the majority of the homozygous subjects had proximal thrombus (84 %) compared to 71 % in controls. Van Stralen et al showed similar results (294) and our findings support the theory that thrombus in FVL patients has a different structure, such as a stronger adhesive properties, leading to a decreased risk for embolism. However, no concrete conclusion can be drawn from our findings since only patients with symptoms of PE underwent computer tomography.

In our unselected population of homozygous FVL patients (**Paper II**), the mean age at first thrombosis was higher than in previous studies or 64 ±14 among men and 47 ±19 among women, with a significant age difference between the genders ($p<0.01$). As mentioned earlier, oral contraceptives and pregnancy increase the risk of thrombosis and, according to other observations, there seems to be some synergistic effect with both FVL mutation and hormonal changes present (295). Rosendaal et al (159) found a mean age of 31 in 7 homozygous FVL patients and the Procare group (160), that analysed 85 such patients, showed a mean age of 40 among men and only 28 among women. It has to be kept in mind that these previous studies are conducted on selected material, probably leading to this younger age where our population is likely to reflect better the actual age at which first thrombosis occurs within homozygous FVL patients. In our study (**Paper II**) it seems that the risk for VTE among homozygous subjects is lower (OR= 13.9, 95 % CI 9.9-19.7) than previously thought in many studies, where, for example, in the LETS study an 80-fold risk increase was reported (159).

When we looked at double heterozygous FVL and PTM mutation (**Paper II**) there was no significant difference in age between female and male subjects at inclusion thrombosis as well as first thrombotic event. In the group, there were no reports of oral contraception or pregnancies in the compound group which might explain these results. These subjects seemed to be older at first thrombosis than previously reported, however on a selected material, with mean age of 52 ±17 compared to 34.7 (161), respectively.

The aim of **Paper I** was mainly to estimate the recurrence risk for VTE among patients with FVL in heterozygous form. A Kaplan-Meier analysis, including 900 VTE patients, showed that patients heterozygous for the FVL mutation ran a significantly increased risk for a new VTE episode during 4.8 ±2.3 years of follow up. The yearly incidence for recurrence was 3.3% for the FVL group compared to 1.5% in the group with normal genotype, with OR= 2.4. We could not demonstrate any differences between the thrombophilia and normal genotype groups when we compared common acquired VTE risk factors that could explain this difference in recurrence risk. Positive family history was, as expected, more common in the thrombophilia group since mutations tend to run in families. Interestingly, recurrence was more frequent in men compared to women.

Previously there has been no general consensus on the recurrence risk among patients with first unprovoked VTE and this common mutation and studies have given conflicting results. Most of the previous data has been derived from retrospective (210, 211, 291) or small prospective studies (203, 204, 215, 216, 290, 296).The relative risk in these studies ranged from 1.4-4.0 and in a systematic review by Ho et al. (205), of both prospective and retrospective studies, heterozygous FVL carriers had an odds ratio of 1.4 (95 % CI 1.2 – 1.7) for VTE

recurrence after a first episode. However, there are also a few studies that do not support our findings, De Stefano et al. (210), found that subjects with the mutation in heterozygous form ran the same risk for recurrent VTE as those without the mutation in a retrospective cohort of 624 patients in 1999. Lindmarker et al (215), Rintelen et al (291) and Eichinger et al (297) all presented similar results as well, although the observation periods were short in some of these studies.

When we evaluated the risk for recurrence (**Paper I**), we excluded patients with previous VTE episodes as well as patients that received anticoagulation during the follow up time. These facts, as well as some missing data, led to a relatively large loss of subjects for follow up, equivalent to about 20 % drop-out rate. However, since we could demonstrate the differences between the heterozygous FVL group and normal genotype group, and our results being in accordance to many of the previous literature, we presume the data is valid and reflect both true VTE epidemiology in the general populations as well as the recurrence risk among heterozygote carriers of the FVL mutation in the community, thus presenting real world data.

Although the recurrence risk in patients with heterozygous FVL was more than twofold compared to normal genotype subjects, the annual risk for recurrence, however, was substantially low in both the groups. It is therefore difficult to recommend lifelong anticoagulation therapy after a VTE episode within this group of patients. However, this information on the recurrence risk could be valuable when determining duration of therapy, especially with other risk factors present. Moreover, it could be helpful when deciding whether to do a mutation test or not.

As previously mentioned in this thesis, there are many known acquired factors that increase the risk for VTE. Inflammation has been of interest in the last decades, as its role in arterial thrombosis has been reported. However, it is still controversial whether inflammation is a cause or only a consequence of the thromboembolic process. Presumably, some inflammatory mediators contribute to the VTE process which in turn induces an inflammatory reaction.

In our study (**Paper III**), we could not find a significant relationship between inflammation and the risk for venous thromboembolism. The number of raised inflammation-sensitive proteins in the fourth quartile was not related to the VTE risk. Furthermore, no significant relationship was found when we looked at the different proteins individually, i.e. fibrinogen, haptoglobin, ceruloplsamin, orosomucoid and alfa1-antitrypsin. Many studies have shown similar results, i.e. that inflammation is not a significant risk factor for VTE. However, most of the studies have not analysed the same inflammation-sensitive proteins that we did, apart from fibrinogen.

Tsai et al studied various inflammation markers as well as coagulation factors as a potential VTE risk and published the results in the LITE study in 2002. They found, not surprisingly, that levels of the clotting factors VIII and VII as well as von Willebrand factor were positively and independently associated with the incidence of venous thromboembolism in their large population-based cohort study (261). However, the inflammation markers fibrinogen and C-reactive protein were not significantly related to the risk for having a VTE (261) which is in accordance to our observations, i.e. fibrinogen was not associated with increased VTE risk. A case-control study from 2000 made by Austin et al gave the same results, i.e. fibrinogen is not a risk factor for VTE (298). Controversially, a few case-control studies (299, 300) have indicated a relationship between elevated fibrinogen and VTE. Recently, a study by Saghazadeh et al in 2016 (301) reviewed evidence supporting the role of inflammation as a cause of VTE and present evidence suggesting the role of inflammation as a cause of VTE and found that current literature indicates that people suffering from certain inflammatory or inflammation- related medical conditions are more likely to develop VTE.

Furthermore, some genetic association studies have indicated a link between inflammation-related genetic variants, including pro- and anti-inflammatory cytokines, pathways, chemokines and other inflammation-related genes, and VTE, both first occurrence and recurrence risk (302-304).

Additional biomarkers that have been studied are many cytokines such as IL-6, sP-selectin TNF-alfa, IL-8 and MCP-1 (305-308). Inhibition of these factors has been found to reduce VTE incidence and recurrence (306, 307), thereby, and by lowering serum cholesterol and/or triglycerides, it has been hypothesized and indicated that statins could reduce VTE (247, 250). However, other studies could not verify this relationship (71, 309, 310) and in our study (**Paper III**), cholesterol or triglycerides were no significant risk factors for developing VTE.

In the study, we also found that age and BMI, as expected according to the literature (309), gave significantly higher risk for VTE. Diabetes mellitus was similarly associated with incidence of VTE, which also has been shown by the large LITE study (71), although the Copenhagen City Heart Study could not verify this relationship (309).

During an inflammatory process more than one factor is involved and therefore it is difficult to find a good biomarker to predict VTE and guide the anticoagulation therapy. Additionally, those studies that show a relationship between inflammation and VTE could have been biased, as inflammation markers were elevated after the thrombotic event. One of the strengths of our study was the large prospective cohort. We wanted to see if inflammation in general could induce VTE by looking at the number of ISPs, but no significant relationship was observed. Another factor increasing the study´s strength was the very clear end-point, i.e. an objectively

verified VTE according to the hospital´s register. We assumed the end-point registration was reliable and comprehensive. The Swedish national inpatient register has been valued with an overall positive predictive value for all diagnoses between 85-95% (311), a Danish validation (312) and another from the USA (313) has shown that data on VTE obtained from administrative registries are a valuable source of information although they should be used with caution. Furthermore, the assessment of the ISPs, cholesterol and triglycerides with electroimmunoassay is an established and reliable method (281).

After a VTE has been diagnosed, treatment with one of the anticoagulant agents is initiated. The main challenge for the physician is to determine the duration of therapy but also estimate the risk for major bleedings during the therapy. As mentioned earlier, chronic renal failure increases the risk for bleeding, why we wanted to estimate the risk for major bleeding within patients with VTE on warfarin, the most frequent anticoagulation therapy in relation to renal function.

In the study in **Paper IV**, the patients with venous thromboembolism as an indication for warfarin treatment were, as expected, younger than the patients in the other indication group, which mainly consisted of atrial fibrillation patients. The bleeding risk per/patient year was the same for both the groups (2.6% in both cases). Our data show that the risk for bleeding in the group with other indications for OAT was significantly higher within patients with impaired renal function (table 3), and it has been suggested that these patients stay longer out of range (>3.0) in INR (271, 272). However, in our study, the percentage with INR >3.0 between different eGFR groups did not significantly vary. Furthermore, in our group with VTE as an indication, the same was seen as a trend, i.e. inverse relationship between bleeding risk and eGFR but without an increased time of INR >3.0 (table 3). However, that particular group suffered from a lack of power, which probably explains the trend and not significance. The VTE group is younger and this could reflect a population with less frail patients with lower percentage of impaired renal function, and hence fewer bleeding events, whereas a previous study has demonstrated no difference in the incidence of major bleeding between different age groups at the same levels of eGFR (274). Limdi et al (271) describe in their study that impaired renal function influences warfarin dosage, anticoagulation control and hence increases the risk for hemorrhage. The TTR in their study, however, was low and ranged from 40.1 % to 49.7 % with percentages of INR > 3.0 around 20%. Since we demonstrate high TTR (74.5%) in our study, and do not show significant over-anticoagulation, data indicates that the bleeding could be due to other factors of renal impairment and its consequences, i.e. rheology, anaemia and platelet dysfunction and not because of poorly controlled anticoagulation. Also, in a recently submitted publication (314) of 321 bleedings within patients with various OAT indications in Malmö, Sweden, where subjects in the present study were included, the authors found that a very low

proportion of the patients who suffered major bleedings on warfarin were treated with platelet inhibitors concomitantly.

The RE-COVER study, where the direct oral anticoagulant dabigatran and warfarin were compared as treatments in VTE (277), showed no significant interaction between given anticoagulant and age or renal function. However, the study had a limited number of patients in the subgroups of >80 years and with CrCl<5, leading to a lack of power, as in our study (**Paper IV**). Furthermore, subjects with eGFR <30 ml/min/1.73m^2 were excluded from the RE-COVER study. In our VTE group, around 15% of patients have eGFR <45 ml/min/1.73m^2 and some of these are subjects where caution is advised using, for example, some of the direct oral anticoagulants which are eliminated via renal route.

Due to too few events there was a lack of power in the present study to distinguish a clear relationship between impaired renal function and complications to OAT with warfarin. Parikh et al concluded in their cohort of VTE patients that severe decreases in eGFR not only increase the risk for bleeding but also for long-term recurrent VTE (315). This is in accordance to other studies (263, 316), but due to few events we could not demonstrate a significantly higher proportion of patients with INR <2.0 in the subjects with eGFR <30 and thrombotic complication.

LIMITATIONS

The studies in this thesis suffered from some limitations. In **Papers I and II**, although in total we had a large consecutive community based cohort, there was a substantial drop-out, approximately 30% when recruiting patients to the MATS database due to previously mentioned reasons. This figure, 30 %, is an estimation based on a review of hospital records of all excluded VTE patients in 1 year during the study period by a study nurse. Additionally, when doing many of the different analyses, there was a further drop-out rate, in part due to missing data but also because we excluded patients with previous VTE episodes as well as patients that received anticoagulation during the follow-up time. When looking into some smaller subgroups, such as in **Paper II**, the sample size was too small to enable statistically stable analyses.

In **Paper I**, we did not take into account that some patients could also have had deficiencies of the natural anticoagulants (i.e. protein S, C and antithrombin) leading to an even higher risk for recurrence. However, since these are extremely rare, they are, in our opinion, are highly unlikely to affect our results. Furthermore, information about the presence of antiphospholipid syndrome as well as hereditary elevated FVIII level, which is now is considered as a risk factor for VTE, were not taken into account, although we expect these individuals to be very few. Another possible error in our study (**Paper I**) was not including subjects <18 years of age in our analyses but not excluding them from the Malmö population in our VTE incidence calculation. Rask et al (68), on the other hand, who did a paediatric retrospective study from our hospital with the same catchment area showed an annual incidence of only 5 per 100,000 children. We therefore conclude that previously mentioned factors should not affect our results. Additionally, not including upper extremity DVT (UEDVT) cases or autopsy diagnoses might have contributed to our lower incidence. However, in the same MATS material, Isma et al evaluated UEDVT cases separately, finding the yearly incidence of UEDVT only 3.6 per 100,000 inhabitants (317).

In the study of **Paper III**, the subjects were only male,s which might decrease the valuation of the results. Furthermore, the biomarkers we tested were based on a single blood test at study inclusion with an obvious possibility of intraindividual variation. The level of the inflammatory markers is therefore not presented at the time of VTE event. Analysing these inflammatory biomarkers at the time of VTE episode could, however, give conflicting results as these could be increased as a

consequence of VTE. Ideally, the biomarker measurement within all the subjects would be just before a VTE event which obviously would be difficult to accomplish. A change of exposure could also cause a bias in the study. Smokers according to questionnaire at inclusion could have stopped smoking during the follow up time, blood pressure changed etc. However, several previous studies have shown that these proteins predict incidence of cardiovascular diseases and stroke even over very long follow-up periods (262, 282, 318).

The limitations of the study in **Paper IV** can also be easily acknowledged since it is an observational study and only epidemiological conclusions regarding association between low eGFR and major bleeding events can be made from our analyses. The data was only from one year, i.e. 2008, and from complications studied from only one centre which led to lower patient numbers and treatment years. Only hospital records from the Skåne University Hospital were used in the review of events. However, it cannot be ruled out that some patients had an event and were treated at another hospital, leading to an underestimation of events. However, this is real world data, not excluding any patients on OAT at the present time. Moreover, when evaluating renal function, we could not use an absolute eGFR value since we lacked information about height and weight of the patients. Instead, we used relative eGFR, which does not give accurate information about renal clearance, which ideally should be used to assess the appropriate dosing of drugs. In future studies one would need different eGFR equations. The general population in AuriculA is heterogeneous and there is a difference in cTTR between hospitals, implying that there are differences in patient selection, co-morbidities and adherence to guidelines. The organization of anticoagulation treatment in Sweden is founded on anticoagulation centres that includes both primary care and in hospital-based settings. Consequently, the patients enrolled in AuriculA represent the whole patient community that are treated with anticoagulants in Sweden. However, although there can be differences between centres there is no selection bias of patients in AuriculA. Out of the 3,536 patients, 187 did not have a p-Cr measured during the study period and two patients with complications did not have a p-Cr value at the time of event, which perhaps can have led to underestimation in our results. Moreover, we did not have more extensive patient profiles regarding co-morbidities, risk factors for bleeding and thromboembolism, smoking status, medications or alcohol consumptions that could have influenced our results.

As already discussed, the VTE group was underpowered both with regards to the presence of renal impairment, but also regarding the endpoints, which is probably why we could not reach significance in our analyses as we did in the group of other indications for OAT. We would need larger cohort studies taking these factors into account.

CONCLUSIONS

In **Paper I**, of this relatively large population based study, we conclude that approximately 1/3 of unselected VTE patients have inherited thrombophilia, with FVL in heterozygous form dominating. There is a more than two-fold higher risk for VTE recurrence after anticoagulation therapy in subjects with heterozygous FVL compared to individuals with normal genotype, although the recurrence is generally low.

In **Paper II**, looking into the same unselected, community based population of VTE patients, we found that homozygous FVL female patients have lower PE frequency than DVT, indicating a different structure of the thrombus. The odds ratio for thrombosis among homozygous FVL subjects seems to be lower than previously described compared to non FVL carriers.

In **Paper III**, we conclude that in our cohort, that inflammation is not associated to the risk for developing VTE. Age, BMI and diabetes are significant risk factors for VTE event whereas cholesterol- and triglyceride levels are not.

In **Paper IV**, we conclude that among VTE patients on oral anticoagulation with warfarin, there is not significantly increased bleeding with impaired renal function, although a trend could be seen.

FUTURE CONSIDERATIONS

Venous thromboembolism is a relatively common major public health problem associated with reduced survival, morbidities and substantial health-care cost. Although this disease has been widely studied and many independent VTE risk factors and predictors of recurrence identified, leading to effective primary and secondary prophylaxis, the problem remains extensive. It is still necessary to identify additional risk factors and gaining an even better understanding of the pathogenesis, to continue to get a better handle of the problem.

The studies in **Papers I and II** describe the prevalence and incidence of VTE within the most common inherited thrombophilias. The results indicate that FVL mutation in heterozygous form is only a small risk factor regarding recurrence and even lower than in many other studies. We can use this information in the future to estimate the recurrence risk, when other risk factors are present when determining the anticoagulation therapy. Moreover, in Paper II it seems that the same mutation in homozygous form also might also be a weaker risk factor than previously thought, although still a very significant one. In the light of this, and the fact that family history alone increases the odds for VTE approximately 2.5 fold, screening for these types of thrombophilias should still be conservative unless further genetic risk factors can be found. In fact, since VTE can run in families without the known mutations being present, the main goal in the future research on inherited thrombophilia should be identifying new mutations. Our lower incidence of VTE compared to other studies might, in part, be due to better prophylactic therapy but there is still much to learn and improve in that field.

Although our study (**Paper III)** could not show a relationship between inflammation and VTE, there is yet an area to investigate further. There are many other inflammatory biomarkers to explore and with better understanding on the pathogenesis, the role of inflammation and the immune system in VTE susceptibility might become clearer in the future.

As previously mentioned, the physician's biggest challenge in the treatment of VTE is deciding the length of anticoagulation and estimating the risk of bleeding versus a new thrombotic event. Therefore, it is of value to estimate the bleeding risk in relation to renal function. Although there was not significantly increased bleeding with decreased eGFR within VTE patients on warfarin (**Paper IV**), there was a trend and the relationship has been indicated within patients with atrial fibrillation (274).

As the VTE group is younger and healthier, this problem might be of a lesser degree. However, keeping the results of **Paper IV** kept in mind, renal function must be considered within this group of patients, especially with the increasing use of the new oral anticoagulants, many of which are mainly eliminated by the renal route. At present, the monitoring of renal function is recommended at the start of therapy, especially in conjunction to hospitalizations and initiation of other medications that may affect renal function. Therefore, it is important in the future to continue with registers such as AuriculA to monitor eGFR, not only in patients on warfarin but also on the new oral anticoagulants. Such registers play central role in collecting data on complication frequencies for comparison between different oral anticoagulation treatments in a clinical setting. Time will then tell how these will turn out in the real world with on-going studies.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Hemostas eller blodstillning är kroppens naturliga förmåga att levra blodet för att förebygga blödningar. Detta är ett dynamiskt system och samtidigt undviker det att blodproppar bildas. Rubbningar i hemostasen, av olika skäl, kan leda till antingen blödning eller blodpropp. När blodproppar förekommer i det venösa kärlsystemet kallas det venös tromboembolism (VTE) och innebär att blodproppar bildas i kroppens djupa ådror (djup ventrombos, DVT), vanligast i benen, eller propp i lungorna (lungemboli, LE). VTE är en relativt vanlig sjukdom som kan leda till svår sjuklighet och även dödlighet. Insjuknandefrekvensen i VTE är ungefär 1-3 / 1000 individer årligen varav 2/3 är DVT.

Många orsaker till VTE är kända, både ärftliga och förvärvade, men oftast är det ett samspel mellan dessa riskfaktorer som bidrar till sjukdomen. Vanligaste förvärvade riskfaktorer är hög ålder, kirurgi, nedsatt rörlighet (immobilisering), cancer, hormonbehandling och graviditet samt svåra kroppsskador. Utav de ärftliga riskfaktorerna är den s.k. faktor V Leiden (FVL) mutationen den vanligaste och förekommer hos ca 7-10 % i den svenska befolkningen.

Det övergripande syftet med denna avhandling har varit att kartlägga de två vanligaste ärftliga riskfaktorerna för VTE, dvs. faktor V Leiden (FVL) och prothrombingen (PT) mutationerna och hur dessa påverkar risken för VTE och att få återfall efter avslutad behandling. Ytterligare syften var att undersöka inflammation som en potentiell riskfaktor för VTE samt att utvärdera blödningsrisken hos warfarin (Waran) behandlade VTE patienter i förhållande till njurfunktion.

I **delarbete 1-II** beskrivs 1,465 oselekterade VTE patienter som rekryterades till Malmö Thrombophilia Study (MATS) under åren 1998-2008 på Universitetssjukhuset i Malmö (UMAS), numera Skånes Universitetssjukhus (SUS). Utav dessa 1,465 VTE patienter hade 1021 (70%) DVT, 352 (24%) LE och 91 (6%) hade både DVT och LE. Lika många män (49%) som kvinnor (51%) hade VTE, med medelåldern 62±16 och 61±20 år respektiv. Utav alla VTE patienterna där information om FVL och PT mutation fanns (n=1,267), hade 27% FVL mutation i heterozygot form, 3% i homozygot form, PT mutation förekom i heterozygot form hos 4% medan ingen var homozygot. Enbart 1% hade både FVL mutation och PT mutationen. Patienterna följdes upp under 4.8 ± 2.3 år och analys med

överlevnadskurvor visade att VTE patienter som är heterozygota för FVL mutationen (n=260) hade dubbelt så stor risk som patienter med normal genotyp (n=640) för återfall i VTE efter avslutad behandling med en årlig incidens för återinsjuknande på 3.3% respektive 1.5%. Detta är något lägre risk än som tidigare beskrivits.

I hela materialet hade 36 VTE patienter FVL i homozygot form. Kvinnor inom denna grupp var signifikant yngre än män (50 vs 65 år) som sannolikt kan förklaras av användande av p-piller samt graviditet. Dessutom verkade det som att FVL mutation bidrog oftare till DVT än LE som kan tyda på att blodproppen hos denna VTE grupp kan ha en annan struktur och egenskap. I delarbete II beräknades risken för VTE hos FVL homozygota patienter till ca 14 gånger ökat jämfört med kontroller, vilket är lägre än tidigare rapporterat.

I **delarbete III** studerades inflammations markörers (fibrinogen, haptoglobin, ceruloplasmin och α-1 antitrypsin) roll vid VTE. Studien utgår från ” The Malmö Preventive Study” där patienter inkluderades mellan 1974-1982 och följdes upp till 2008. Resultaten var att ålder och BMI var signifikant associerade med VTE. Däremot kunde man inte påvisa att inflammation ökade risken för VTE, varken generellt eller för enskilda inflammationsmarkörer.

I **delarbete IV** var huvudsyftet att mäta relationen mellan nedsatt njurfunktion och komplikationer hos VTE patienter under blodförtunnande behandling med warfarin. Studien är av samtliga 3536 patienter under warfarin behandling, på olika indikationer, i Malmö under året 2008 och ingår i den svenska nationella databasen och kvalitetsregistret, AuriculA. Utav alla 3536 patienterna hade 963 patienter indikation VTE för behandlingen med warfarin. I studien kunde vi inte visa på signifikant ökad risk för blödning med nedsatt njurfunktion även om en trend sågs. Detta kan förklaras av att VTE patienter är yngre och generellt friskare än patienter med warfarin på andra indikationer, så som förmaksflimmer och hjärtklaffsjukdomar. Ökat risk för arteriell trombos eller VTE återinsjuknande kunde inte påvisas tydande på en god behandlingseffekt.

Sammanfattningsvis är den röda tråden i avhandlingen att beskriva kända genetiska VTE riskfaktorer hos olika kohorter av VTE patienter samt att bedöma nya potentiella riskfaktorer. Huvudfynden och nyhetsvärden är att återfallsrisken vid VTE och FVL mutation i heterozygot form är lägre än tidigare beskrivits och att FVL mutationen i homozygot form i tidigare studier sannolikt övervärderat VTE risken. Inflammationens betydelse tycks inte vara stark kopplat till VTE utveckling enligt den aktuella undersökningen. Hos warfarin behandlade VTE patienter är behandlingseffekten god men nedsatt njurfunktion har en benägenhet att öka risken för blödningar även om en signifikant koppling inte kunde påvisas.

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REFERENCES

1. ANNING ST. The historical aspects of venous thrombosis. Med Hist. 1957;1(1):28-37.
2. Dexter L. The chair and venous thrombosis. Trans Am Clin Climatol Assoc. 1973;84:1-15.
3. Isma NaBL. *Sublavia-ventrombos. I: Klinisk handläggning av venös tromboembolism. Gottsäter A, Svensson PJ (eds)* Lund: Studentlitteratur.; 2010.
4. Virchow R. *Phlogose und Thrombose im Gefässystem. Gesammelte abhandlungen zur wissenschaftlichen medizin.* Frankfurt, Germany: Staatdruckerei.
5. Schmidt A. *Untersuchungen über die Faserstoffgerinnung* European Journal of Physiology. 1872;6(1):413-538.
6. Morawitz P. *Die Chemie der Blutgerinnung.* Ergebn Physiol. **1905**:307-422.
7. Brewer DB. Max Schultze (1865), G. Bizzozero (1882) and the discovery of the platelet. Br J Haematol. 2006;133(3):251-8.
8. Quick AJ. *The prothrombin time in haemophilia and in obstructive jaundice.* . J Biol Chem. 1935(**109**):73-4.
9. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med. 2008;359(9):938-49.
10. George JN. Platelets. Lancet. 2000;355(9214):1531-9.
11. Sadler JE. A new name in thrombosis, ADAMTS13. Proc Natl Acad Sci U S A. 2002;99(18):11552-4.
12. Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. Arterioscler Thromb Vasc Biol. 2002;22(9):1381-9.
13. MACFARLANE RG. AN ENZYME CASCADE IN THE BLOOD CLOTTING MECHANISM, AND ITS FUNCTION AS A BIOCHEMICAL AMPLIFIER. Nature. 1964;202:498-9.
14. DAVIE EW, RATNOFF OD. WATERFALL SEQUENCE FOR INTRINSIC BLOOD CLOTTING. Science. 1964;145(3638):1310-2.
15. Davie EW. Biochemical and molecular aspects of the coagulation cascade. Thromb Haemost. 1995;74(1):1-6.
16. Monroe DM, Hoffman M. What does it take to make the perfect clot? Arterioscler Thromb Vasc Biol. 2006;26(1):41-8.
17. Hoffman M, Monroe DM. Coagulation 2006: a modern view of hemostasis. Hematol Oncol Clin North Am. 2007;21(1):1-11.
18. Heemskerk JW, Bevers EM, Lindhout T. Platelet activation and blood coagulation. Thromb Haemost. 2002;88(2):186-93.

19. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol. 2004;24(6):1015-22.
20. Hoffman M. A cell-based model of coagulation and the role of factor VIIa. Blood Rev. 2003;17 Suppl 1:S1-5.
21. Hoffman M. Remodeling the blood coagulation cascade. J Thromb Thrombolysis. 2003;16(1-2):17-20.
22. Mann KG, Brummel K, Butenas S. What is all that thrombin for? J Thromb Haemost. 2003;1(7):1504-14.
23. Mann KG. Thrombin formation. Chest. 2003;124(3 Suppl):4S-10S.
24. Krishnaswamy S, Nesheim ME, Pryzdial EL, Mann KG. Assembly of prothrombinase complex. Methods Enzymol. 1993;222:260-80.
25. Ichinose A. Physiopathology and regulation of factor XIII. Thromb Haemost. 2001;86(1):57-65.
26. Bouma BN, Mosnier LO. Thrombin activatable fibrinolysis inhibitor (TAFI) at the interface between coagulation and fibrinolysis. Pathophysiol Haemost Thromb. 2003;33(5-6):375-81.
27. Dahlbäck B. Advances in understanding pathogenic mechanisms of thrombophilic disorders. Blood. 2008;112(1):19-27.
28. Dahlbäck B. Physiological anticoagulation. Resistance to activated protein C and venous thromboembolism. J Clin Invest. 1994;94(3):923-7.
29. Broze GJ. Tissue factor pathway inhibitor. Thromb Haemost. 1995;74(1):90-3.
30. Weitz JI. Heparan sulfate: antithrombotic or not? J Clin Invest. 2003;111(7):952-4.
31. Van de Wouwer M, Collen D, Conway EM. Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation. Arterioscler Thromb Vasc Biol. 2004;24(8):1374-83.
32. Dahlbäck B, Villoutreix BO. Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. Arterioscler Thromb Vasc Biol. 2005;25(7):1311-20.
33. Dahlbäck B, Villoutreix BO. The anticoagulant protein C pathway. FEBS Lett. 2005;579(15):3310-6.
34. Dahlbäck B. Progress in the understanding of the protein C anticoagulant pathway. Int J Hematol. 2004;79(2):109-16.
35. Mann KG, Kalafatis M. Factor V: a combination of Dr Jekyll and Mr Hyde. Blood. 2003;101(1):20-30.
36. Dahlbäck B. The tale of protein S and C4b-binding protein, a story of affection. Thromb Haemost. 2007;98(1):90-6.
37. Dahlbäck B. Pro- and anticoagulant properties of factor V in pathogenesis of thrombosis and bleeding disorders. Int J Lab Hematol. 2016.
38. Fay PJ. Activation of factor VIII and mechanisms of cofactor action. Blood Rev. 2004;18(1):1-15.

39. Rijken DC, Wijngaards G, Welbergen J. Immunological characterization of plasminogen activator activities in human tissues and body fluids. J Lab Clin Med. 1981;97(4):477-86.
40. Marder VJ, Matchett MO, Sherry S. Detection of serum fibrinogen and fibrin degradation products. Comparison of six technics using purified products and application in clinical studies. Am J Med. 1971;51(1):71-82.
41. Robbie LA, Bennett B, Croll AM, Brown PA, Booth NA. Proteins of the fibrinolytic system in human thrombi. Thromb Haemost. 1996;75(1):127-33.
42. Mosnier LO, Bouma BN. Regulation of fibrinolysis by thrombin activatable fibrinolysis inhibitor, an unstable carboxypeptidase B that unites the pathways of coagulation and fibrinolysis. Arterioscler Thromb Vasc Biol. 2006;26(11):2445-53.
43. Booth NA. Fibrinolysis and thrombosis. Baillieres Best Pract Res Clin Haematol. 1999;12(3):423-33.
44. Anderson FA, Wheeler HB, Goldberg RJ, Hosmer DW, Forcier A. The prevalence of risk factors for venous thromboembolism among hospital patients. Arch Intern Med. 1992;152(8):1660-4.
45. Nordström M, Lindblad B, Bergqvist D, Kjellström T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med. 1992;232(2):155-60.
46. Chen J, López JA. Interactions of platelets with subendothelium and endothelium. Microcirculation. 2005;12(3):235-46.
47. Sevitt S. The structure and growth of valve-pocket thrombi in femoral veins. J Clin Pathol. 1974;27(7):517-28.
48. Friedman MH, Brinkman AM, Qin JJ, Seed WA. Relation between coronary artery geometry and the distribution of early sudanophilic lesions. Atherosclerosis. 1993;98(2):193-9.
49. PATERSON JC, McLACHLIN J. Precipitating factors in venous thrombosis. Surg Gynecol Obstet. 1954;98(1):96-102.
50. Wakefield TW, Myers DD, Henke PK. Mechanisms of venous thrombosis and resolution. Arterioscler Thromb Vasc Biol. 2008;28(3):387-91.
51. Becker BF, Heindl B, Kupatt C, Zahler S. Endothelial function and hemostasis. Z Kardiol. 2000;89(3):160-7.
52. GIBBS NM. Venous thrombosis of the lower limbs with particular reference to bed-rest. Br J Surg. 1957;45(191):209-36.
53. Pottier P, Hardouin JB, Lejeune S, Jolliet P, Gillet B, Planchon B. Immobilization and the risk of venous thromboembolism. A meta-analysis on epidemiological studies. Thromb Res. 2009;124(4):468-76.
54. Warlow C, Ogston D, Douglas AS. Deep venous thrombosis of the legs after strokes. Part I--incidence and predisposing factors. Br Med J. 1976;1(6019):1178-81.
55. Hamer JD, Malone PC, Silver IA. The PO2 in venous valve pockets: its possible bearing on thrombogenesis. Br J Surg. 1981;68(3):166-70.

56. Closse C, Seigneur M, Renard M, Pruvost A, Dumain P, Belloc F, et al. Influence of hypoxia and hypoxia-reoxygenation on endothelial P-selectin expression. Thromb Res. 1997;85(2):159-64.

57. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. J Thromb Haemost. 2005;3(8):1590-6.

58. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. Thromb Haemost. 2009;101(3):439-51.

59. Lawson CA, Yan SD, Yan SF, Liao H, Zhou YS, Sobel J, et al. Monocytes and tissue factor promote thrombosis in a murine model of oxygen deprivation. J Clin Invest. 1997;99(7):1729-38.

60. Rao LV. Tissue factor as a tumor procoagulant. Cancer Metastasis Rev. 1992;11(3-4):249-66.

61. Anderson FA, Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern Med. 1991;151(5):933-8.

62. Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med. 2004;117(1):19-25.

63. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998;158(6):585-93.

64. Hansson PO, Welin L, Tibblin G, Eriksson H. Deep vein thrombosis and pulmonary embolism i n the general population. 'The Study of Men Born in 1913'. Arch Intern Med. 1997;157(15):1665-70.

65. Oger E. Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. Thromb Haemost. 2000;83(5):657-60.

66. 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(6):663-6.

67. Rosendaal FR. Thrombosis in the young: epidemiology and risk factors. A focus on venous thrombosis. Thromb Haemost. 1997;78(1):1-6.

68. Rask O, Berntorp E, Ljung R. Risk factors for venous thrombosis in Swedish children and adolescents. Acta Paediatr. 2005;94(6):717-22.

69. Heit JA. The epidemiology of venous thromboembolism in the community: implications for prevention and management. J Thromb Thrombolysis. 2006;21(1):23-9.

70. Baglin T, Luddington R, Brown K, Baglin C. High risk of recurrent venous thromboembolism in men. J Thromb Haemost. 2004;2(12):2152-5.

71. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Arch Intern Med. 2002;162(10):1182-9.

72. White RH. The epidemiology of venous thromboembolism. Circulation. 2003;107(23 Suppl 1):I4-8.
73. Heit JA, Melton LJ, Lohse CM, Petterson TM, Silverstein MD, Mohr DN, et al. Incidence of venous thromboembolism in hospitalized patients vs community residents. Mayo Clin Proc. 2001;76(11):1102-10.
74. Spencer FA, Emery C, Lessard D, Anderson F, Emani S, Aragam J, et al. The Worcester Venous Thromboembolism study: a population-based study of the clinical epidemiology of venous thromboembolism. J Gen Intern Med. 2006;21(7):722-7.
75. Keenan CR, White RH. The effects of race/ethnicity and sex on the risk of venous thromboembolism. Curr Opin Pulm Med. 2007;13(5):377-83.
76. White RH, Zhou H, Murin S, Harvey D. Effect of ethnicity and gender on the incidence of venous thromboembolism in a diverse population in California in 1996. Thromb Haemost. 2005;93(2):298-305.
77. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. JAMA. 1997;277(16):1305-7.
78. Fujimura H, Kambayash J, Monden M, Kato H, Miyata T. Coagulation factor V Leiden mutation may have a racial background. Thromb Haemost. 1995;74(5):1381-2.
79. Uthman I, Khamashta M. Ethnic and geographical variation in antiphospholipid (Hughes) syndrome. Ann Rheum Dis. 2005;64(12):1671-6.
80. Patel RK, Ford E, Thumpston J, Arya R. Risk factors for venous thrombosis in the black population. Thromb Haemost. 2003;90(5):835-8.
81. Anderson FA, Spencer FA. Risk factors for venous thromboembolism. Circulation. 2003;107(23 Suppl 1):I9-16.
82. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost. 2005;3(8):1611-7.
83. Rosendaal FR. Risk factors for venous thrombotic disease. Thromb Haemost. 1999;82(2):610-9.
84. Simioni P, Tormene D, Spiezia L, Tognin G, Rossetto V, Radu C, et al. Inherited thrombophilia and venous thromboembolism. Semin Thromb Hemost. 2006;32(7):700-8.
85. Young G, Albisetti M, Bonduel M, Brandao L, Chan A, Friedrichs F, et al. Impact of inherited thrombophilia on venous thromboembolism in children: a systematic review and meta-analysis of observational studies. Circulation. 2008;118(13):1373-82.
86. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369(6475):64-7.
87. Hillarp A, Zöller B, Svensson PJ, Dahlbäck B. The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis. Thromb Haemost. 1997;78(3):990-2.
88. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost. 1998;79(4):706-8.

89. Wheeler HB, Anderson FA, Cardullo PA, Patwardhan NA, Jian-Ming L, Cutler BS. Suspected deep vein thrombosis. Management by impedance plethysmography. Arch Surg. 1982;117(9):1206-9.

90. Prandoni P, Lensing AW, Cogo A, Cuppini S, Villalta S, Carta M, et al. The long-term clinical course of acute deep venous thrombosis. Ann Intern Med. 1996;125(1):1-7.

91. White RH, Zhou H, Romano PS. Incidence of symptomatic venous thromboembolism after different elective or urgent surgical procedures. Thromb Haemost. 2003;90(3):446-55.

92. White RH, Romano PS, Zhou H, Rodrigo J, Bargar W. Incidence and time course of thromboembolic outcomes following total hip or knee arthroplasty. Arch Intern Med. 1998;158(14):1525-31.

93. Geerts WH, Heit JA, Clagett GP, Pineo GF, Colwell CW, Anderson FA, et al. Prevention of venous thromboembolism. Chest. 2001;119(1 Suppl):132S-75S.

94. Raabe A, Gerlach R, Zimmermann M, Seifert V. [Practice of perioperative thromboembolic prophylaxis in neurosurgery: results of a German survey]. Zentralbl Neurochir. 2000;61(2):103-10.

95. Collins R, Scrimgeour A, Yusuf S, Peto R. Reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin. Overview of results of randomized trials in general, orthopedic, and urologic surgery. N Engl J Med. 1988;318(18):1162-73.

96. Prandoni P, Sabbion P, Tanduo C, Errigo G, Zanon E, Bernardi E. Prevention of venous thromboembolism in high-risk surgical and medical patients. Semin Vasc Med. 2001;1(1):61-70.

97. Azu MC, McCormack JE, Huang EC, Lee TK, Shapiro MJ. Venous thromboembolic events in hospitalized trauma patients. Am Surg. 2007;73(12):1228-31.

98. Paffrath T, Wafaisade A, Lefering R, Simanski C, Bouillon B, Spanholtz T, et al. Venous t hromboembolism after severe trauma: incidence, risk factors and outcome. Injury. 2010;41(1):97-101.

99. Warlow C, Ogston D, Douglas AS. Venous thrombosis following strokes. Lancet. 1972;1(7764):1305-6.

100. Ageno W, Agnelli G, Checchia G, Cimminiello C, Paciaroni M, Palareti G, et al. Prevention of venous thromboembolism in immobilized neurological patients: Guidelines of the Italian Society for Haemostasis and Thrombosis (SISET). Thromb Res. 2009;124(5):e26-31.

101. Testroote M, Stigter W, de Visser DC, Janzing H. Low molecular weight heparin for prevention of venous thromboembolism in patients with lower-leg immobilization. Cochrane Database Syst Rev. 2008(4):CD006681.

102. Scurr JH, Machin SJ, Bailey-King S, Mackie IJ, McDonald S, Smith PD. Frequency and prevention of symptomless deep-vein thrombosis in long-haul flights: a randomised trial. Lancet. 2001;357(9267):1485-9.

103. Lapostolle F, Surget V, Borron SW, Desmaizières M, Sordelet D, Lapandry C, et al. Severe pulmonary embolism associated with air travel. N Engl J Med. 2001;345(11):779-83.

104. Kakkar VV, Howe CT, Nicolaides AN, Renney JT, Clarke MB. Deep vein thrombosis of the leg. Is there a "high risk" group? Am J Surg. 1970;120(4):527-30.
105. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. Arch Intern Med. 2000;160(6):809-15.
106. Printen KJ, Miller EV, Mason EE, Barnes RW. Venous thromboembolism in the morbidly obese. Surg Gynecol Obstet. 1978;147(1):63-4.
107. Semrad TJ, O'Donnell R, Wun T, Chew H, Harvey D, Zhou H, et al. Epidemiology of venous thromboembolism in 9489 patients with malignant glioma. J Neurosurg. 2007;106(4):601-8.
108. Blom JW, Vanderschoot JP, Oostindiër MJ, Osanto S, van der Meer FJ, Rosendaal FR. Incidence of venous thrombosis in a large cohort of 66,329 cancer patients: results of a record linkage study. J Thromb Haemost. 2006;4(3):529-35.
109. Chew HK, Wun T, Harvey D, Zhou H, White RH. Incidence of venous thromboembolism and its effect on survival among patients with common cancers. Arch Intern Med. 2006;166(4):458-64.
110. Alcalay A, Wun T, Khatri V, Chew HK, Harvey D, Zhou H, et al. Venous thromboembolism in patients with colorectal cancer: incidence and effect on survival. J Clin Oncol. 2006;24(7):1112-8.
111. Chew HK, Wun T, Harvey DJ, Zhou H, White RH. Incidence of venous thromboembolism and the impact on survival in breast cancer patients. J Clin Oncol. 2007;25(1):70-6.
112. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005;293(6):715-22.
113. Rahr HB, Sørensen JV. Venous thromboembolism and cancer. Blood Coagul Fibrinolysis. 1992;3(4):451-60.
114. LIEBERMAN JS, BORRERO J, URDANETA E, WRIGHT IS. Thrombophlebitis and cancer. JAMA. 1961;177:542-5.
115. Baron JA, Gridley G, Weiderpass E, Nyrén O, Linet M. Venous thromboembolism and cancer. Lancet. 1998;351(9109):1077-80.
116. Zangari M, Anaissie E, Barlogie B, Badros A, Desikan R, Gopal AV, et al. Increased risk of deep-vein thrombosis in patients with multiple myeloma receiving thalidomide and chemotherapy. Blood. 2001;98(5):1614-5.
117. Sørensen HT, Mellemkjaer L, Olsen JH, Baron JA. Prognosis of cancers associated with venous thromboembolism. N Engl J Med. 2000;343(25):1846-50.
118. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med. 2005;143(10):697-706.
119. Hellgren M, Blombäck M. Studies on blood coagulation and fibrinolysis in pregnancy, during delivery and in the puerperium. I. Normal condition. Gynecol Obstet Invest. 1981;12(3):141-54.

120. Chang J, Elam-Evans LD, Berg CJ, Herndon J, Flowers L, Seed KA, et al. Pregnancy-related mortality surveillance--United States, 1991--1999. MMWR Surveill Summ. 2003;52(2):1-8.
121. James AH, Bushnell CD, Jamison MG, Myers ER. Incidence and risk factors for stroke in pregnancy and the puerperium. Obstet Gynecol. 2005;106(3):509-16.
122. Lindqvist P, Dahlbäck B, Marŝál K. Thrombotic risk during pregnancy: a population study. Obstet Gynecol. 1999;94(4):595-9.
123. Vessey MP, Doll R. Investigation of relation between use of oral contraceptives and thromboembolic disease. A further report. Br Med J. 1969;2(5658):651-7.
124. Olié V, Canonico M, Scarabin PY. Postmenopausal hormone therapy and venous thromboembolism. Thromb Res. 2011;127 Suppl 3:S26-9.
125. Vessey M, Mant D, Smith A, Yeates D. Oral contraceptives and venous thromboembolism: findings in a large prospective study. Br Med J (Clin Res Ed). 1986;292(6519):526.
126. van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJ, Rosendaal FR. The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. BMJ. 2009;339:b2921.
127. Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. BMJ. 2001;323(7305):131-4.
128. Lidegaard Ø, Edström B, Kreiner S. Oral contraceptives and venous thromboembolism: a five-year national case-control study. Contraception. 2002;65(3):187-96.
129. Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ. 2009;339:b2890.
130. Grady D, Applegate W, Bush T, Furberg C, Riggs B, Hulley SB. Heart and Estrogen/progestin Replacement Study (HERS): design, methods, and baseline characteristics. Control Clin Trials. 1998;19(4):314-35.
131. Grodstein F, Stampfer MJ, Goldhaber SZ, Manson JE, Colditz GA, Speizer FE, et al. Prospective study of exogenous hormones and risk of pulmonary embolism in women. Lancet. 1996;348(9033):983-7.
132. Varas-Lorenzo C, García-Rodríguez LA, Cattaruzzi C, Troncon MG, Agostinis L, Perez-Gutthann S. Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a population-based study in southern Europe. Am J Epidemiol. 1998;147(4):387-90.
133. Lowe G, Woodward M, Vessey M, Rumley A, Gough P, Daly E. Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45-64 years. Relationships to hormone replacement therapy. Thromb Haemost. 2000;83(4):530-5.
134. Olié V, Canonico M, Scarabin PY. Risk of venous thrombosis with oral versus transdermal estrogen therapy among postmenopausal women. Curr Opin Hematol. 2010;17(5):457-63.
135. EGEBERG O. INHERITED ANTITHROMBIN DEFICIENCY CAUSING THROMBOPHILIA. Thromb Diath Haemorrh. 1965;13:516-30.

136. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. J Clin Invest. 1981;68(5):1370-3.
137. Comp PC, Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med. 1984;311(24):1525-8.
138. Tait RC, Walker ID, Perry DJ, Islam SI, Daly ME, McCall F, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol. 1994;87(1):106-12.
139. Tait RC, Walker ID, Reitsma PH, Islam SI, McCall F, Poort SR, et al. Prevalence of protein C deficiency in the healthy population. Thromb Haemost. 1995;73(1):87-93.
140. Franco RF, Reitsma PH. Genetic risk factors of venous thrombosis. Hum Genet. 2001;109(4):369-84.
141. Zöller B, Berntsdotter A, García de Frutos P, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood. 1995;85(12):3518-23.
142. Gladson CL, Scharrer I, Hach V, Beck KH, Griffin JH. The frequency of type I heterozygous protein S and protein C deficiency in 141 unrelated young patients with venous thrombosis. Thromb Haemost. 1988;59(1):18-22.
143. Malm J, Laurell M, Nilsson IM, Dahlbäck B. Thromboembolic disease--critical evaluation of l aboratory investigation. Thromb Haemost. 1992;68(1):7-13.
144. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci U S A. 1993;90(3):1004-8.
145. Dahlbäck B. Thrombophilia: the discovery of activated protein C resistance. Adv Genet. 1995;33:135-75.
146. Dahlbäck B. Procoagulant and anticoagulant properties of coagulation factor V: factor V Leiden (APC resistance) causes hypercoagulability by dual mechanisms. J Lab Clin Med. 1999;133(5):415-22.
147. Griffin JH, Evatt B, Wideman C, Fernández JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood. 1993;82(7):1989-93.
148. Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet. 1993;342(8886-8887):1503-6.
149. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med. 1994;330(8):517-22.
150. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet. 1995;346(8983):1133-4.
151. Svensson PJ, Zöller B, Mattiasson I, Dahlbäck B. The factor VR506Q mutation causing APC resistance is highly prevalent amongst unselected outpatients with clinically suspected deep venous thrombosis. J Intern Med. 1997;241(5):379-85.
152. Lindqvist PG, Dahlbäck B. Carriership of Factor V Leiden and evolutionary selection advantage. Curr Med Chem. 2008;15(15):1541-4.

153. Irani-Hakime N, Tamim H, Elias G, Finan RR, Daccache JL, Almawi WY. High prevalence of factor V mutation (Leiden) in the Eastern Mediterranean. Clin Chem. 2000;46(1):134-6.

154. Zivelin A, Griffin JH, Xu X, Pabinger I, Samama M, Conard J, et al. A single genetic origin for a common Caucasian risk factor for venous thrombosis. Blood. 1997;89(2):397-402.

155. Dahlbäck B. Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism. Blood. 1995;85(3):607-14.

156. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T, et al. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism--pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. Thromb Haemost. 2001;86(3):809-16.

157. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353(9159):1167-73.

158. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL, et al. A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. Blood. 2002;99(8):2720-5.

159. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood. 1995;85(6):1504-8.

160. Group P. Comparison of thrombotic risk between 85 homozygotes and 481 heterozygotes carriers of the factor V Leiden mutation: retrospective analysis from the Procare Study. Blood Coagul Fibrinolysis. 2000;11(6):511-8.

161. Emmerich J, Alhenc-Gelas M, Aillaud MF, Juhan-Vague I, Jude B, Garcin JM, et al. Clinical features in 36 patients homozygous for the ARG 506-->GLN factor V mutation. Thromb Haemost. 1997;77(4):620-3.

162. Björgell O, Nilsson PE, Nilsson JA, Svensson PJ. Location and extent of deep vein thrombosis in patients with and without FV:R 506Q mutation. Thromb Haemost. 2000;83(5):648-51.

163. Martinelli I, Cattaneo M, Panzeri D, Mannucci PM. Low prevalence of factor V:Q506 in 41 patients with isolated pulmonary embolism. Thromb Haemost. 1997;77(3):440-3.

164. Manten B, Westendorp RG, Koster T, Reitsma PH, Rosendaal FR. Risk factor profiles in patients with different clinical manifestations of venous thromboembolism: a focus on the factor V Leiden mutation. Thromb Haemost. 1996;76(4):510-3.

165. Ridker PM, Glynn RJ, Miletich JP, Goldhaber SZ, Stampfer MJ, Hennekens CH. Age-specific incidence rates of venous thromboembolism among heterozygous carriers of factor V Leiden mutation. Ann Intern Med. 1997;126(7):528-31.

166. Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet. 1994;344(8935):1453-7.

167. Lindqvist PG, Svensson PJ, Marsaál K, Grennert L, Luterkort M, Dahlbäck B. Activated protein C resistance (FV:Q506) and pregnancy. Thromb Haemost. 1999;81(4):532-7.

168. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996;88(10):3698-703.

169. Souto JC, Coll I, Llobet D, del Río E, Oliver A, Mateo J, et al. The prothrombin 20210A allele is the most prevalent genetic risk factor for venous thromboembolism in the Spanish population. Thromb Haemost. 1998;80(3):366-9.

170. Rees DC, Chapman NH, Webster MT, Guerreiro JF, Rochette J, Clegg JB. Born to clot: the European burden. Br J Haematol. 1999;105(2):564-6.

171. Franco RF, Santos SE, Elion J, Tavella MH, Zago MA. Prevalence of the G20210A polymorphism in the 3'-untranslated region of the prothrombin gene in different human populations. Acta Haematol. 1998;100(1):9-12.

172. Rahimi Z, Vaisi-Raygani A, Mozafari H, Kharrazi H, Rezaei M, Nagel RL. Prevalence of factor V Leiden (G1691A) and prothrombin (G20210A) among Kurdish population from Western Iran. J Thromb Thrombolysis. 2008;25(3):280-3.

173. Kyrle PA, Mannhalter C, Béguin S, Stümpflen A, Hirschl M, Weltermann A, et al. Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene. Arterioscler Thromb Vasc Biol. 1998;18(8):1287-91.

174. Morange PE, Barthet MC, Henry M, Fontanet H, Aillaud MF, Alessi MC, et al. A three-generation family presenting five cases of homozygosity for the 20210 G to A prothrombin variant. Thromb Haemost. 1998;80(5):859-60.

175. Zivelin A, Rosenberg N, Faier S, Kornbrot N, Peretz H, Mannhalter C, et al. A single genetic origin for the common prothrombotic G20210A polymorphism in the prothrombin gene. Blood. 1998;92(4):1119-24.

176. Samama MM, Trossaërt M, Horellou MH, Elalamy I, Conard J, Deschamps A. Risk of t hrombosis in patients homozygous for factor V Leiden. Blood. 1995;86(12):4700-2.

177. Souto JC, Mateo J, Soria JM, Llobet D, Coll I, Borrell M, et al. Homozygotes for prothrombin gene 20210 A allele in a thrombophilic family without clinical manifestations of venous thromboembolism. Haematologica. 1999;84(7):627-32.

178. Lim W. Antiphospholipid antibody syndrome. Hematology Am Soc Hematol Educ Program. 2009:233-9.

179. Petri M. Epidemiology of the antiphospholipid antibody syndrome. J Autoimmun. 2000;15(2):145-51.

180. Schved JF, Dupuy-Fons C, Biron C, Quére I, Janbon C. A prospective epidemiological study on the occurrence of antiphospholipid antibody: the Montpellier Antiphospholipid (MAP) Study. Haemostasis. 1994;24(3):175-82.

181. Cervera R, Boffa MC, Khamashta MA, Hughes GR. The Euro-Phospholipid project: epidemiology of the antiphospholipid syndrome in Europe. Lupus. 2009;18(10):889-93.

182. Kearon C, Gent M, Hirsh J, Weitz J, Kovacs MJ, Anderson DR, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med. 1999;340(12):901-7.
183. Ginsburg KS, Liang MH, Newcomer L, Goldhaber SZ, Schur PH, Hennekens CH, et al. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. Ann Intern Med. 1992;117(12):997-1002.
184. den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. Thromb Haemost. 1998;80(6):874-7.
185. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med. 1996;334(12):759-62.
186. Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost. 2005;3(2):292-9.
187. Torbicki A, Perrier A, Konstantinides S, Agnelli G, Galiè N, Pruszczyk P, et al. Guidelines on the diagnosis and management of acute pulmonary embolism: the Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur Heart J. 2008;29(18):2276-315.
188. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest. 2016;149(2):315-52.
189. Wells PS, Lensing AW, Davidson BL, Prins MH, Hirsh J. Accuracy of ultrasound for the diagnosis of deep venous thrombosis in asymptomatic patients after orthopedic surgery. A meta-analysis. Ann Intern Med. 1995;122(1):47-53.
190. Kearon C, Julian JA, Newman TE, Ginsberg JS. Noninvasive diagnosis of deep venous thrombosis. McMaster Diagnostic Imaging Practice Guidelines Initiative. Ann Intern Med. 1998;128(8):663-77.
191. Becker DM, Philbrick JT, Abbitt PL. Real-time ultrasonography for the diagnosis of lower extremity deep venous thrombosis. The wave of the future? Arch Intern Med. 1989;149(8):1731-4.
192. Lensing AW, Büller HR, Prandoni P, Batchelor D, Molenaar AH, Cogo A, et al. Contrast venography, the gold standard for the diagnosis of deep-vein thrombosis: improvement in observer agreement. Thromb Haemost. 1992;67(1):8-12.
193. Segal JB, Eng J, Tamariz LJ, Bass EB. Review of the evidence on diagnosis of deep venous thrombosis and pulmonary embolism. Ann Fam Med. 2007;5(1):63-73.
194. Rathbun SW, Raskob GE, Whitsett TL. Sensitivity and specificity of helical computed tomography in the diagnosis of pulmonary embolism: a systematic review. Ann Intern Med. 2000;132(3):227-32.
195. Roy PM, Colombet I, Durieux P, Chatellier G, Sors H, Meyer G. Systematic review and meta-analysis of strategies for the diagnosis of suspected pulmonary embolism. BMJ. 2005;331(7511):259.
196. Wardrop D, Keeling D. The story of the discovery of heparin and warfarin. Br J Haematol. 2008;141(6):757-63.

197. Hirsh J. Heparin. N Engl J Med. 1991;324(22):1565-74.
198. O'REILLY RA, AGGELER PM, LEONG LS. STUDIES ON THE COUMARIN ANTICOAGULANT DRUGS: THE PHARMACODYNAMICS OF WARFARIN IN MAN. J Clin Invest. 1963;42:1542-51.
199. BARRITT DW, JORDAN SC. Anticoagulant drugs in the treatment of pulmonary embolism. A controlled trial. Lancet. 1960;1(7138):1309-12.
200. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E. A method to determine the optimal i ntensity of oral anticoagulant therapy. Thromb Haemost. 1993;69(3):236-9.
201. Sandén P, Renlund H, Svensson PJ, Själander A. Warfarin treatment complications do not correlate to cTTR when above 70. Thromb Res. 2015;136(6):1185-9.
202. Wallentin L, Yusuf S, Ezekowitz MD, Alings M, Flather M, Franzosi MG, et al. Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. Lancet. 2010;376(9745):975-83.
203. Simioni P, Prandoni P, Lensing AW, Manfrin D, Tormene D, Gavasso S, et al. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood. 2000;96(10):3329-33.
204. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation. 1995;92(10):2800-2.
205. Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. Arch Intern Med. 2006;166(7):729-36.
206. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet. 2003;362(9383):523-6.
207. Vossen CY, Walker ID, Svensson P, Souto JC, Scharrer I, Preston FE, et al. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol. 2005;25(9):1992-7.
208. Eichinger S, Minar E, Hirschl M, Bialonczyk C, Stain M, Mannhalter C, et al. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost. 1999;81(1):14-7.
209. Rintelen C, Pabinger I, Knobl P, Lechner K, Mannhalter C. Probability of recurrence of thrombosis in patients with and without factor V Leiden. Thromb Haemost. 1996;75(2):229-32.
210. De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med. 1999;341(11):801-6.
211. Kovac M, Mikovic D, Antonijevic N, Rakicevic L, Djordjevic V, Radojkovic D, et al. FV Leiden mutation and risk of recurrent venous thromboembolism in Serbian population. J Thromb Thrombolysis. 2008;25(3):284-7.

212. Marchiori A, Mosena L, Prins MH, Prandoni P. The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. A systematic review of prospective studies. Haematologica. 2007;92(8):1107-14.
213. Simioni P, Prandoni P, Lensing AW, Scudeller A, Sardella C, Prins MH, et al. The risk of recurrent venous thromboembolism in patients with an Arg506-->Gln mutation in the gene for factor V (factor V Leiden). N Engl J Med. 1997;336(6):399-403.
214. Margaglione M, D'Andrea G, Colaizzo D, Cappucci G, del Popolo A, Brancaccio V, et al. Coexistence of factor V Leiden and Factor II A20210 mutations and recurrent venous thromboembolism. Thromb Haemost. 1999;82(6):1583-7.
215. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost. 1999;81(5):684-9.
216. Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica. 2007;92(2):199-205.
217. Santamaria MG, Agnelli G, Taliani MR, Prandoni P, Moia M, Bazzan M, et al. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res. 2005;116(4):301-6.
218. De Stefano V, Simioni P, Rossi E, Tormene D, Za T, Pagnan A, et al. The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. Haematologica. 2006;91(5):695-8.
219. van den Belt AG, Sanson BJ, Simioni P, Prandoni P, Buller HR, Girolami A, et al. Recurrence of venous thromboembolism in patients with familial thrombophilia. Arch Intern Med. 1997;157(19):2227-32.
220. JORDAN FL, NANDORFF A. The familial tendency in thrombo-embolic disease. Acta Med Scand. 1956;156(4):267-75.
221. JB. B. Recurring phlebitis of obscure origins. John Hopkins Hosp Bull. 1905;16:228-33.
222. Couturaud F, Leroyer C, Julian JA, Kahn SR, Ginsberg JS, Wells PS, et al. Factors that predict risk of thrombosis in relatives of patients with unprovoked venous thromboembolism. Chest. 2009;136(6):1537-45.
223. Couturaud F, Leroyer C, Tromeur C, Julian JA, Kahn SR, Ginsberg JS, et al. Factors that predict thrombosis in relatives of patients with venous thromboembolism. Blood. 2014;124(13):2124-30.
224. Zöller B, Li X, Sundquist J, Sundquist K. Age- and gender-specific familial risks for venous thromboembolism: a nationwide epidemiological study based on hospitalizations in Sweden. Circulation. 2011;124(9):1012-20.

225. Zöller B, Ohlsson H, Sundquist J, Sundquist K. Familial risk of venous thromboembolism in first-, second- and third-degree relatives: a nationwide family study in Sweden. Thromb Haemost. 2013;109(3):458-63.

226. Zöller B, Li X, Sundquist J, Sundquist K. Determination of age-specific and sex-specific f amilial risks for the different manifestations of venous thromboembolism: a nationwide family study in Sweden. Thromb Haemost. 2011;106(1):102-12.

227. Sørensen HT, Riis AH, Diaz LJ, Andersen EW, Baron JA, Andersen PK. Familial risk of venous thromboembolism: a nationwide cohort study. J Thromb Haemost. 2011;9(2):320-4.

228. Hron G, Eichinger S, Weltermann A, Minar E, Bialonczyk C, Hirschl M, et al. Family history for venous thromboembolism and the risk for recurrence. Am J Med. 2006;119(1):50-3.

229. Gauthier K, Kovacs MJ, Wells PS, Le Gal G, Rodger M, investigators R. Family history of venous thromboembolism (VTE) as a predictor for recurrent VTE in unprovoked VTE patients. J Thromb Haemost. 2013;11(1):200-3.

230. Zöller B, Ohlsson H, Sundquist J, Sundquist K. Family history of venous thromboembolism (VTE) and risk of recurrent hospitalization for VTE: a nationwide family study in Sweden. J Thromb Haemost. 2014;12(3):306-12.

231. Sundquist K, Sundquist J, Svensson PJ, Zöller B, Memon AA. Role of family history of venous thromboembolism and thrombophilia as predictors of recurrence: a prospective follow-up study. J Thromb Haemost. 2015;13(12):2180-6.

232. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA. 2005;293(19):2352-61.

233. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? Br J Haematol. 2008;143(3):321-35.

234. Middeldorp S. Evidence-based approach to thrombophilia testing. J Thromb Thrombolysis. 2011;31(3):275-81.

235. Coppens M, Reijnders JH, Middeldorp S, Doggen CJ, Rosendaal FR. Testing for inherited thrombophilia does not reduce the recurrence of venous thrombosis. J Thromb Haemost. 2008;6(9):1474-7.

236. Bank I, Libourel EJ, Middeldorp S, Van Der Meer J, Büller HR. High rate of skin complications due to low-molecular-weight heparins in pregnant women. J Thromb Haemost. 2003;1(4):859-61.

237. Bates SM, Greer IA, Pabinger I, Sofaer S, Hirsh J, Physicians ACoC. Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133(6 Suppl):844S-86S.

238. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. The New England journal of medicine. 1997;336(14):973-9.

239. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Seminars in thrombosis and hemostasis. 2011;37(8):885-96.

240. Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. Circulation. 1996;94(8):2013-20.
241. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, et al. An association between atherosclerosis and venous thrombosis. The New England journal of medicine. 2003;348(15):1435-41.
242. Jezovnik MK, Poredos P. Idiopathic venous thrombosis is related to systemic inflammatory response and to increased levels of circulating markers of endothelial dysfunction. International angiology : a journal of the International Union of Angiology. 2010;29(3):226-31.
243. Hong C, Zhu F, Du D, Pilgram TK, Sicard GA, Bae KT. Coronary artery calcification and risk factors for atherosclerosis in patients with venous thromboembolism. Atherosclerosis. 2005;183(1):169-74.
244. Jezovnik MK, Poredos P, Stalc M. Impairment of the vasodilatation capability of the brachial artery in patients with idiopathic venous thrombosis. Journal of atherosclerosis and thrombosis. 2010;17(11):1190-8.
245. Cogo A, Bernardi E, Prandoni P, Girolami B, Noventa F, Simioni P, et al. Acquired risk factors for deep-vein thrombosis in symptomatic outpatients. Archives of internal medicine. 1994;154(2):164-8.
246. Libertiny G, Hands L. Deep venous thrombosis in peripheral vascular disease. The British journal of surgery. 1999;86(7):907-10.
247. Goldhaber SZ, Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, et al. A prospective study of risk factors for pulmonary embolism in women. JAMA : the journal of the American Medical Association. 1997;277(8):642-5.
248. Vaya A, Mira Y, Ferrando F, Contreras M, Estelles A, Espana F, et al. Hyperlipidaemia and venous thromboembolism in patients lacking thrombophilic risk factors. British journal of haematology. 2002;118(1):255-9.
249. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation. 2008;117(1):93-102.
250. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. The New England journal of medicine. 2009;360(18):1851-61.
251. Conde I, Lopez JA. Classification of venous thromboembolism (VTE). Role of acute inflammatory stress in venous thromboembolism. Journal of thrombosis and haemostasis : JTH. 2005;3(11):2573-5.
252. Collen D, Hoylaerts MF. Relationship between inflammation and venous thromboembolism as studied by microparticle assessment in plasma. Journal of the American College of Cardiology. 2005;45(9):1472-3.
253. Wakefield TW, Strieter RM, Prince MR, Downing LJ, Greenfield LJ. Pathogenesis of venous thrombosis: a new insight. Cardiovasc Surg. 1997;5(1):6-15.
254. Libby P. Multiple mechanisms of thrombosis complicating atherosclerotic plaques. Clinical cardiology. 2000;23 Suppl 6:VI-3-7.

255. Holvoet P, Collen D. Thrombosis and atherosclerosis. Current opinion in lipidology. 1997;8(5):320-8.
256. Cushman M, Folsom AR, Wang L, Aleksic N, Rosamond WD, Tracy RP, et al. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003;101(4):1243-8.
257. Verhovsek M, Douketis JD, Yi Q, Shrivastava S, Tait RC, Baglin T, et al. Systematic review: D-dimer to predict recurrent disease after stopping anticoagulant therapy for unprovoked venous thromboembolism. Annals of internal medicine. 2008;149(7):481-90, W94.
258. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet. 1995;345(8943):152-5.
259. Legnani C, Cini M, Cosmi B, Poggi M, Boggian O, Palareti G. Risk of deep vein thrombosis: interaction between oral contraceptives and high factor VIII levels. Haematologica. 2004;89(11):1347-51.
260. Lowe G, Rumley A, Woodward M, Vessey M. C-reactive protein, idiopathic venous thromboembolism and hormone replacement therapy. Thrombosis and haemostasis. 2000;84(4):730-1.
261. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Tracy RP, Aleksic N, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). The American journal of medicine. 2002;113(8):636-42.
262. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F. Effects of cholesterol and inflammation-sensitive plasma proteins on incidence of myocardial infarction and stroke in men. Circulation. 2002;105(22):2632-7.
263. Wattanakit K, Cushman M, Stehman-Breen C, Heckbert SR, Folsom AR. Chronic kidney disease increases risk for venous thromboembolism. J Am Soc Nephrol. 2008;19(1):135-40.
264. Pavord S, Myers B. Bleeding and thrombotic complications of kidney disease. Blood Rev. 2011;25(6):271-8.
265. Ocak G, Verduijn M, Vossen CY, Lijfering WM, Dekker FW, Rosendaal FR, et al. Chronic kidney disease stages 1-3 increase the risk of venous thrombosis. J Thromb Haemost. 2010;8(11):2428-35.
266. Barbano B, Gigante A, Amoroso A, Cianci R. Thrombosis in nephrotic syndrome. Semin Thromb Hemost. 2013;39(5):469-76.
267. Wattanakit K, Cushman M. Chronic kidney disease and venous thromboembolism: epidemiology and mechanisms. Curr Opin Pulm Med. 2009;15(5):408-12.
268. Jalal DI, Chonchol M, Targher G. Disorders of hemostasis associated with chronic kidney disease. Semin Thromb Hemost. 2010;36(1):34-40.
269. Kaw D, Malhotra D. Platelet dysfunction and end-stage renal disease. Semin Dial. 2006;19(4):317-22.

270. Bos MJ, Koudstaal PJ, Hofman A, Breteler MM. Decreased glomerular filtration rate is a risk factor for hemorrhagic but not for ischemic stroke: the Rotterdam Study. Stroke. 2007;38(12):3127-32.

271. Limdi NA, Beasley TM, Baird MF, Goldstein JA, McGwin G, Arnett DK, et al. Kidney function influences warfarin responsiveness and hemorrhagic complications. J Am Soc Nephrol. 2009;20(4):912-21.

272. Limdi NA, Limdi MA, Cavallari L, Anderson AM, Crowley MR, Baird MF, et al. Warfarin dosing in patients with impaired kidney function. Am J Kidney Dis. 2010;56(5):823-31.

273. Poli D, Antonucci E, Testa S, Tosetto A, Ageno W, Palareti G, et al. Bleeding risk in very old patients on vitamin K antagonist treatment: results of a prospective collaborative study on elderly patients followed by Italian Centres for Anticoagulation. Circulation. 2011;124(7):824-9.

274. Wieloch M, Jönsson KM, Själander A, Lip GY, Eriksson N, Svensson PJ. Estimated glomerular filtration rate is associated with major bleeding complications but not thromboembolic events, in anticoagulated patients taking warfarin. Thromb Res. 2013;131(6):481-6.

275. Wieloch M, Själander A, Frykman V, Rosenqvist M, Eriksson N, Svensson PJ. Anticoagulation control in Sweden: reports of time in therapeutic range, major bleeding, and thrombo-embolic complications from the national quality registry AuriculA. Eur Heart J. 2011;32(18):2282-9.

276. Connolly SJ, Pogue J, Eikelboom J, Flaker G, Commerford P, Franzosi MG, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. Circulation. 2008;118(20):2029-37.

277. Schulman S, Kearon C, Kakkar AK, Mismetti P, Schellong S, Eriksson H, et al. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. N Engl J Med. 2009;361(24):2342-52.

278. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med. 2009;361(12):1139-51.

279. Bajc Mea. För regionala medicinska rådet i koagulationssjukdomar. Venös tromboembolism: Vårdprogram för södra sjukvårdsregionen 2009, Malmö.2009.

280. Jönsson KM, Wieloch M, Sterner G, Nyman U, Elmståhl S, Engström G, et al. Glomerular filtration rate in patients with atrial fibrillation on warfarin treatment: a subgroup analysis from the AURICULA registry in Sweden. Thromb Res. 2011;128(4):341-5.

281. Laurell CB. Electroimmuno assay. Scandinavian journal of clinical and laboratory investigation Supplementum. 1972;124:21-37.

282. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F. Long-term effects of inflammation-sensitive plasma proteins and systolic blood pressure on incidence of stroke. Stroke; a journal of cerebral circulation. 2002;33(12):2744-9.

283. Schulman S, Angerås U, Bergqvist D, Eriksson B, Lassen MR, Fisher W, et al. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in surgical patients. J Thromb Haemost. 2010;8(1):202-4.

284. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. N Engl J Med. 2006;354(23):2473-83.

285. Björk J, Bäck SE, Sterner G, Carlson J, Lindstrom V, Bakoush O, et al. Prediction of relative glomerular filtration rate in adults: new improved equations based on Swedish Caucasians and standardized plasma-creatinine assays. Scand J Clin Lab Invest. 2007;67(7):678-95.

286. Foundation NK. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1-266.

287. Archibald G, Bartlett W, Brown A, Christie B, Elliott A, Griffith K, et al. UK Consensus Conference on Early Chronic Kidney Disease--6 and 7 February 2007. Nephrol Dial Transplant. 2007;22(9):2455-7.

288. Kjellberg U, van Rooijen M, Bremme K, Hellgren M. Factor V Leiden mutation and pregnancy-related complications. Am J Obstet Gynecol. 2010;203(5):469.e1-8.

289. Rosendaal FR. Venous thrombosis: the role of genes, environment, and behavior. Hematology Am Soc Hematol Educ Program. 2005:1-12.

290. Eichinger S, Minar E, Hirschl M, Bialonczyk C, Stain M, Mannhalter C, et al. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost. 1999;81(1):14-7.

291. Rintelen C, Pabinger I, Knöbl P, Lechner K, Mannhalter C. Probability of recurrence of thrombosis in patients with and without factor V Leiden. Thromb Haemost. 1996;75(2):229-32.

292. Palareti G, Legnani C, Cosmi B, Valdré L, Lunghi B, Bernardi F, et al. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation. 2003;108(3):313-8.

293. Pabinger I, Nemes L, Rintelen C, Koder S, Lechler E, Loreth RM, et al. Pregnancy-associated risk for venous thromboembolism and pregnancy outcome in women homozygous for factor V Leiden. Hematol J. 2000;1(1):37-41.

294. van Stralen KJ, Doggen CJ, Bezemer ID, Pomp ER, Lisman T, Rosendaal FR. Mechanisms of the factor V Leiden paradox. Arterioscler Thromb Vasc Biol. 2008;28(10):1872-7.

295. Wu O, Robertson L, Langhorne P, Twaddle S, Lowe GD, Clark P, et al. Oral contraceptives, hormone replacement therapy, thrombophilias and risk of venous thromboembolism: a systematic review. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Thromb Haemost. 2005;94(1):17-25.

296. Santamaria MG, Agnelli G, Taliani MR, Prandoni P, Moia M, Bazzan M, et al. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res. 2005;116(4):301-6.

297. Eichinger S, Pabinger I, Stümpflen A, Hirschl M, Bialonczyk C, Schneider B, et al. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. Thromb Haemost. 1997;77(4):624-8.

298. Austin H, Hooper WC, Lally C, Dilley A, Ellingsen D, Wideman C, et al. Venous thrombosis in relation to fibrinogen and factor VII genes among African-Americans. Journal of clinical epidemiology. 2000;53(10):997-1001.

299. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms--the Leiden Thrombophilia Study (LETS). Thrombosis and haemostasis. 1994;71(6):719-22.

300. Jude B, Amrouni N, Deguine I, Marey A, Asseman P, Watel A, et al. Coupled D dimer and fibrinogen levels during thrombolytic therapy of venous thromboembolism. Thromb Res. 1992;65(3):457-62.

301. Saghazadeh A, Rezaei N. Inflammation as a cause of venous thromboembolism. Crit Rev Oncol Hematol. 2016;99:272-85.

302. Tang B, Chen YK, Luo WJ, Fu J, Sun JM. Association between interleukin-10 -1082A/G, -819C/T and -592C/A polymorphisms with deep venous thrombosis. Hum Immunol. 2014;75(3):203-7.

303. Beckers MM, Ruven HJ, Haas FJ, Doevendans PA, ten Cate H, Prins MH, et al. Single nucleotide polymorphisms in inflammation-related genes are associated with venous thromboembolism. Eur J Intern Med. 2010;21(4):289-92.

304. Mahemuti A, Abudureheman K, Aihemaiti X, Hu XM, Xia YN, Tang BP, et al. Association of interleukin-6 and C-reactive protein genetic polymorphisms levels with venous thromboembolism. Chin Med J (Engl). 2012;125(22):3997-4002.

305. Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D, et al. Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. Arteriosclerosis, thrombosis, and vascular biology. 2005;25(6):1231-6.

306. Meier TR, Myers DD, Jr., Wrobleski SK, Zajkowski PJ, Hawley AE, Bedard PW, et al. Prophylactic P-selectin inhibition with PSI-421 promotes resolution of venous thrombosis without anticoagulation. Thrombosis and haemostasis. 2008;99(2):343-51.

307. Wakefield TW, Strieter RM, Downing LJ, Kadell AM, Wilke CA, Burdick MD, et al. P-selectin and TNF inhibition reduce venous thrombosis inflammation. The Journal of surgical research. 1996;64(1):26-31.

308. Jougasaki M, Ichiki T, Takenoshita Y, Setoguchi M. Statins suppress interleukin-6-induced monocyte chemo-attractant protein-1 by inhibiting Janus kinase/signal transducers and activators of transcription pathways in human vascular endothelial cells. Br J Pharmacol. 2010;159(6):1294-303.

309. Holst AG, Jensen G, Prescott E. Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. Circulation. 2010;121(17):1896-903.

310. Chamberlain AM, Folsom AR, Heckbert SR, Rosamond WD, Cushman M. High-density lipoprotein cholesterol and venous thromboembolism in the Longitudinal Investigation of Thromboembolism Etiology (LITE). Blood. 2008;112(7):2675-80.

311. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC public health. 2011;11:450.

312. Severinsen MT, Kristensen SR, Overvad K, Dethlefsen C, Tjonneland A, Johnsen SP. Venous thromboembolism discharge diagnoses in the Danish National Patient Registry should be used with caution. Journal of clinical epidemiology. 2010;63(2):223-8.

313. Tamariz L, Harkins T, Nair V. A systematic review of validated methods for identifying venous thromboembolism using administrative and claims data. Pharmacoepidemiology and drug safety. 2012;21 Suppl 1:154-62.

314. Janson D WM. Emergency treatment of major bleeding in warfarin treated patients in a "real world" setting. A retrospective evaluation of the Auricula Malmö cohort., submtted. 2016.

315. Parikh AM, Spencer FA, Lessard D, Emery C, Baylin A, Linkletter C, et al. Venous thromboembolism in patients with reduced estimated GFR: a population-based perspective. Am J Kidney Dis. 2011;58(5):746-55.

316. Ocak G, Lijfering WM, Verduijn M, Dekker FW, Rosendaal FR, Cannegieter SC, et al. Risk of venous thrombosis in patients with chronic kidney disease: identification of high-risk groups. J Thromb Haemost. 2013;11(4):627-33.

317. 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(6):e335-8.

318. Lind P, Hedblad B, Stavenow L, Janzon L, Eriksson KF, Lindgarde F. Influence of plasma fibrinogen levels on the incidence of myocardial infarction and death is modified by other inflammation-sensitive proteins: a long-term cohort study. Arteriosclerosis, thrombosis, and vascular biology. 2001;21(3):452-8.

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Evaluation of recurrent venous thromboembolism in patients with Factor V Leiden mutation in heterozygous form

Signy V. Sveinsdottir [a,b,*], Ymir Saemundsson [a,b], Nazim Isma [a,b], Anders Gottsäter [a,c], Peter J. Svensson [a,b]

[a] University of Lund, Skåne University Hospital, Malmö, Sweden
[b] Centre for Thrombosis and Haemostasis, Skåne University Hospital, Malmö, Sweden
[c] Vascular Centre, Skåne University Hospital, Malmö, Sweden

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ABSTRACT

Introduction: To evaluate the risk for recurrence after first venous thromboembolism (VTE) among patients with or without Factor V Leiden (FVL) mutation.
Materials and Methods: A prospective population based study of 1465 consecutive unselected VTE patients was performed at Skåne University Hospital 1998–2008. The VTE was objectively verified and the patients answered questionnaire and left blood samples for evaluation.
Results: Out of 1465 patients (721[49%] men and 744[51%] women) thrombophilia data were available for 1267, and FVL mutation was found in heterozygous form in 339 (27). The homozygous form and prothrombin mutation (PTM) were much less common. Patients were followed during 4.8 ± 2.3 years (total 6133 patient years) and recurrence after first VTE (evaluated in 1108 patients) occurred in 131 (12%, 95%CI 10–14%), where of 49(37%) had heterozygous FVL mutation and 57(44%) were without thrombophilia. The remaining 25(19%) patients had either PTM, FVL in homozygous form, compound PTM/FVL or unknown thrombophilia status. Having FVL mutation in heterozygous form significantly increased the risk for VTE recurrence (odds ratio 2.4 (95 %CI 1.6–3.6; $p<0.01$). In a Kaplan-Meier analysis the FVL group also differed significantly ($p<0.01$) from the other patients concerning time to recurrence (almost 25% vs. 10% after 8 years).
Conclusions: FVL mutation in heterozygous form is common among VTE patients and significantly increases the risk for VTE recurrence.

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Introduction

Venous thromboembolism (VTE) is a relatively common cause of morbidity and mortality with an annual incidence of around 0.1-0.3% [1,2]. Multiple risk factors that can either be transient or persistent are known. The most important acquired risk factors for VTE include high age, malignancy, trauma, major surgery, immobilisation, hormone therapy, obesity, pregnancy and the postpartum period [3–5]. Many patients have more than one interacting risk factor. Several genetic risk factors for VTE have also previously been described [6,7]. The most common inherited risk factor is resistance to activated protein C (APC resistance), occurring in 20–60% of VTE patients, and almost always caused by the Factor V Leiden mutation (FVL) [6,7]. The mutation occurs in heterozygous form in about 5–8% of the Caucasian population leading to a 4–7 times increased risk for VTE [8–10]. The homozygous form of the mutation is found in only 0.18% [11] but is associated with an up to 80 times increased risk for VTE [12]. A rarer form of genetic thrombophilia is the prothrombin mutation (PTM), found in its heterozygous form among 1–4% of Caucasians [8] conferring about 3–4 times increased risk for VTE [9,10]. Genetic deficiencies of the natural anticoagulants, i.e. protein C, S and antithrombin are considered to increase the risk for VTE even more, but are even less frequently occurring in the background population.

Studies have shown that patients are at increased risk for recurrence after first episode of VTE, especially after an unprovoked thrombosis. The cumulative incidence of recurrent VTE after first deep vein thrombosis has been shown to be about 17% at 2 years of follow up and 30% at 8 years of follow [13]. This knowledge has influenced recommendations on the duration of anticoagulation therapy for VTE patients. Since long-term anticoagulation can both be inconvenient and cause major bleedings [14,15], it is desirable to give prolonged treatment only to patients at the highest risk and to limit treatment duration in patients with lower risk of recurrence.

Although much is known about the risk for recurrence after a first episode of VTE, it has been controversial whether the most common thrombophilic mutation, heterozygous Factor V Leiden, confers increased risk of VTE recurrence or not [16–23]. Previous studies have been of both prospective [14–16,24–26] and retrospective [17–19] design as well as a few meta-analyses [20,21]. Strong

* Corresponding author at: Centre for Thrombosis and Haemostasis, Skåne University Hospital (SUS), 20502, Malmö, Sweden. Tel.: +46 40332392; fax: +46 40336255.
E-mail address: signy.sveinsdottir@med.lu.se (S.V. Sveinsdottir).

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prospective studies are preferable, but are dependent on adequate cohorts of consecutive patients to generate reliable data.

A higher incidence of recurrent VTE events has been shown among patients with the rare thrombophilias, i.e. protein C, S and antithrombin deficiencies, homozygous mutations of FVL and PTM as well as multiple defects. However, the data mainly stem from small and/or retrospective studies [27–29]. Results concerning acquired risk factors and distribution of VTE have been published from the Malmö Thrombophilia Study (MATS) [30]. We now evaluated MATS regarding the distribution of the two most common thrombophilias, i.e. FVL and PTM, in a total material of VTE patients, but mainly we studied FVL as a risk factor for recurrence of VTE.

Methods

MATS is a prospective population-based study conducted at Skåne University Hospital (SUS) in Malmö, a city of 300.000 inhabitants in southern Sweden. SUS is the only hospital treating VTE patients in the area. The study ran from March 1998 to December 2008 during which 1465 consecutive unselected VTE patients were collected, representing 70% of all patients diagnosed at the Emergency Department with VTE (DVT and/or PE) [30]. The remaining 30% were excluded due to unwillingness to participate, language problems, dementia or other illness. The patients had to have objectively verified DVT and/or PE with phlebography, duplex ultrasound, computed tomography (CT), lung scintigraphy or magnetic resonance imaging (MRI). Other inclusion criteria in MATS were age >18 years and possibility to communicate in the Swedish language. All participants provided written informed consent and the study was approved by the Lund University Ethical Committee.

Included patients were required to leave blood samples, answer a questionnaire and were evaluated concerning risk factors for VTE. The DNA mutations for factor V and II were analysed using Taqman allele discrimination with gene specific assays for the two factors (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA). Body mass index (BMI) in kg/m^2 was recorded as well as tobacco use before VTE diagnosis (defined as smoking ≥ 5 cigarettes/day or ≥ 25 g pipe tobacco/week,). We did not take into account if the patients stopped smoking under the follow-up period. We also recorded surgical intervention, immobilisation or cast therapy within the last month, travel more than three hours by bus, car, train or air within the last month. Other risk factors such as malignancies diagnosed prior to or at the diagnosis of VTE, heredity (defined as a history of VTE in first-degree relatives), and use of contraceptive pills, hormonal therapy, pregnancy and postpartum period (defined as first 6 weeks after delivery) among women were assessed. The location of VTE at inclusion, VTE events prior to study inclusion, and all VTE recurrences during follow-up was recorded.

All patients were treated with low molecular weight (LMH) or unfractionated (UFH) heparin during initiation and then warfarin as oral anticoagulation (OAC). The hospital treatment protocol suggests therapy for 3–6 months for first-time VTE with consideration of extended treatment in case of recurrent VTE. Thrombolysis was considered in specific cases according to protocol. The treating physician had no knowledge of the patient´s thrombophilia status when determining the duration of anticoagulation treatment.

Statistical analyses including descriptive analyses, Chi-2 test, Cox-regression and Kaplan-Meier tables were made in SPSS 19.0 (IBM, Markham, Canada). Results are expressed as mean ± SD, n (%), odds ratio (95%CI). P-values <0.05 were considered as significant

Results

Patient Characteristics

A total of 1465 consecutive patients were included in the study, of which 721 (49%) were men with mean age of 64 ± 15 years and 744 (51%) women with mean age 63 ± 19 years (Table 1). Body mass index (BMI, in kg/m^2) could be evaluated in 1376 patients and was equal in men (26.8 ± 4.1) and women (26.7 ± 5.4). DVT was diagnosed in 1021 (70%) patients, PE in 352 (24%), whereas 91 (6%) had both DVT and PE. Data was missing in one patient. Out of those patients with DVT, 300 (27%) were distal thrombosis, 682 (61%) were proximal. Other locations of venous thrombosis was found in 130 (12%) patients, i.e. arm (69), portal vein (6), mesenteric vein (6) and other (8) or unknown sites (41). PE was significantly more frequent among women than men (27% versus 21%; p = 0.013) whereas the opposite was true for DVT (68% versus 72%; p = 0.013).

Acquired Risk Factors

The 1267 VTE patients from whom data regarding FVL and PTM status were available were divided into a group with either or both the mutations (n = 432) and a group without these mutations (n = 835). Common acquired risk factors for VTE were compared between the two groups (Table 2). Positive heredity (a first degree relative with VTE) existed in 145 (34%) patients with FLV and/or PTM and 172 (21%) patients without (p<0.01). Previous surgical intervention, cast therapy and malignancy, on the other hand, were all more prevalent in the group without the mutations (Table 2). There were no significant differences between the two groups regarding other acquired risk factors.

Thrombophilia

Among the 1267 patients with VTE, 835 (66%) had normal genotypes, whereas 339 (27%) patients were heterozygous and 36 (3%) homozygous for FVL. Fourty-five (4%) were heterozygous and none homozygous for the PTM, whereas 12 (1%) were compound (FVL + PTM) heterozygous. No significant differences existed between genders (Table 3).

VTE and Recurrence

All the 1465 patients in the study were followed up for 4.8 ± 2.3 years and the total follow up time was 6133 patient years. When doing the statistical analyses for VTE recurrence after inclusion we excluded patients (n = 25) with thrombotic events at any time before study inclusion or recurrernce during anticoagulation therapy (n = 281). Complete information on recurrences was missing in 51 subjects. Among the remaining 1108 patients, 131 (12%, CI 10–14%) suffered a VTE recurrence after study inclusion. In this group, 49 (42%) were heterozygous and 2 (2%) homozygous for the FVL mutation. Heterozygous PTM was found only in 7 (6%) of patients with recurrent VTE. Two (2%) had both heterozygous PTM and FVL, Fifty-seven (44%) patients were without the mutations and data on this type of thrombophilia were missing in 14 patients.

Age at first VTE was 62.4 ± 15.1 years in men and 61.4 ± 19 years in women (p = 0.241). Recurrent VTE tended to be more frequent among men than among women (77[14%] versus 54 [10%]; p = 0.052). Time to recurrence in men and women were 20 ±

Table 1
Characteristics of 1465 consecutive patients with venous thromboembolism at study inclusion. Mean ± SD or n(%).

	Men (n = 721)	Women (n = 744)	All patients (n = 1465)
Age (years)	64 ± 15	63 ± 19	63 ± 17
Body mass index (kg/m^2)	27 ± 4	27 ± 5	27 ± 5
DVT	517 (72)	504 (68)	1021 (70)
PE	151 (21)	201 (27)	352 (24)
DVT + PE	52 (7)	39 (5)	91 (6)

SD = Standard deviation, DVT = Deep vein thrombosis, PE = Pulmonary embolism

Table 2
Acquired risk factors among patients with venous thromboembolism (VTE) with or without thrombophilia in the form of FVL and/or PTM. N (%).

Risk factor	Thrombophilia (FVL and/or PTM) (n = 432)	Normal genotype (without FVL and/or PTM) (n = 835)	All patients (n = 1465)[‡]	*P*
Present smoking[γ]	68 (17)	125 (16)	216 (16)	0.75
Surgical intervention	40 (9)	138 (16)	197 (13)	<0.01[†]
Immobilisation	47 (11)	106 (13)	174 (12)	0.35
Cast therapy	12 (3)	43 (5)	57 (4)	0.05[†]
Travel > 3 hours	37 (9)	53 (6)	97 (7)	0.15
Positive heredity for VTE	145 (34)	172 (21)	357 (24)	<0.01
Malignancy	29 (7)	90 (11)	167 (11)	0.018[†]
Ongoing VTE prophylaxis	10 (2)	18 (2)	29 (2)	0.86
Hormone therapy[*]	57 (27)	93 (21)		0.12
Pregnancy[*]	6 (3)	10 (2)		0.68
Postpartum[*]	3 (1)	8 (1)		0.70

[*] Female patients only, n = total patients;
[‡] n = 1358 for present smoking, data missing for n = 107;
[†] Significantly lower in the thrombophilia group.
[γ] Present smoking defined as smoking ≥ 5 cigarettes/day or using ≥ 25 gr pipe tobacco/week.

19 months and 25 ± 22 months respectively (p = 0.16). In a subgroup of 964 patients we were able to evaluate recurrence among patients with unprovoked thrombosis versus those with provoked thrombosis (i.e. with one or more genetic and/or acquired risk factors) and found no significant difference. A Kaplan-Meier analysis was made to estimate recurrence risk during follow-up in the 260 patients heterozygous for FVL and the 640 patients with normal genotype. The groups separated already during the first year of follow up, and after 8 years nearly 25% in the FVL group had suffered a new VTE episode compared to 10% in the group with normal genotype (p < 0.01). After adjustment for age in a Cox-regression analysis this difference was still significant (p < 0.01). The yearly incidence for VTE recurrence was 3.3% (CI 2.5– 4.4%) in the FVL group compared to 1.5% (CI 1.2 – 2.0%) in the group with normal genotype (OR 2.4 [CI 1.6–3.6]) (Fig. 1).

Discussion

In our present study a Kaplan-Meier analysis including 900 VTE patients, showed that patients heterozygous for the FVL mutation ran a significantly increased risk for new VTE recurrence during 4.8 ± 2.3 years of follow up. Although the risk for a new VTE episode in the FVL group was increased by nearly 150%, the annual risk for recurrence was comparably low in both groups, however, 1.5% among patients without the FVL mutation and 3.3% among carriers. Up to now there has been no general consensus on the recurrence risk among patients with first unprovoked VTE and this common mutation, and previously published studies have given conflicting results [15–20,22–24,26]. However, other types of inherited thrombophilias such as deficiencies of protein S, C and antithrombin, homozygous FVL and PTM or double heterozygosity in VTE patients have been associated with an increased risk of recurrent thrombotic events [27,29,31].

Table 3
Thrombophilia (FVL and/or PTM) among 1267 consecutive patients with venous thromboembolism. N (%).

Type of thrombophilia	All patients (n = 1267)	Men (n = 620)	Women (n = 647)
Without thrombophilia	835 (66)	400 (64)	435 (67)
Heterozygous FVL	339 (27)	178 (29)	161 (25)
Homozygous FVL	36 (3)	15 (2)	21 (3)
Heterozygous PTM	45 (4)	21 (3)	24 (4)
Homozygous PTM	0 (0)	0 (0)	0 (0)
Compound PTM and FVL	12 (1)	6 (1)	6 (1)

FVL = Factor V Leiden, PTM = Prothrombin mutation

Most of previous data in this field has been derived from retrospective [17–19] or small prospective [14–16,24–26] studies. Our analysis is one of the largest prospective studies that have evaluated recurrence risk among VTE patients with the FVL mutation. Our findings are consistent with those in many previous studies in which relative risks for FVL patients compared to subjects without the mutation have ranged from 1.4-4.0 [15,21,22,26]. Ho *et al.* [20] in a systematic review of both prospective and retrospective studies showed that heterozygous FVL carriers had an odds ratio of 1.4 (CI 1.1 – 1.8) for VTE recurrence after a first episode Marchiori *et al.* [21] presented comparable results in a meta-analysis published 2007; a relative risk of 1.4 (CI 1.2 – 1.7) [21]. The small prospective studies of Simioni *et al.* [14] and Ridker *et al.* [15] have also showed significant differences although they suffered from limitations related to the small number of patients.. A small retrospective study from 2007 of 56 patients from the Serbian population also showed a high risk of recurrent VTE among patients with the FVL mutation (relative risk 1.7 (CI 1.0-2.8) [19]. However, there are also a few studies that do not support our findings. De Stefano *et al.* [18] in a retrospective cohort of 624 patients in 1999 found that heterozygous FVL carriers ran the same risk for recurrent VTE as those without the mutation. Such results have also been presented in the prospective studies of Rintelen *et al.* [17] Lindmarker *et*

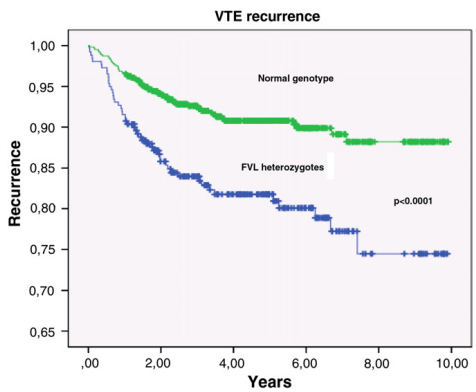

Fig. 1. Kaplan-Meier analysis showing the risk for VTE recurrence during up to 10 years of follow-up after first VTE episode. A significant difference is shown between patients with heterozygous Factor V Leiden (FVL) mutation and patients with normal genotype.

al. [24] and Eichinger *et al.* [32] but it has to be taken into account that some of these studies had short observation periods.

Since heterozygous FVL mutation this is the most common inherited thrombophilia, it is of special importance to estimate the VTE recurrence risk within these patients. Our analysis showed that about one fourth (27%) of VTE patients had FVL heterozygosity alone, a proportion in accordance with previous literature [33]. Previous studies regarding epidemiology of VTE have most been of retrospective design and performed within subgroups of the general population, however, and only a few prospective studies have been performed to estimate the community based incidence and risk factors among unselected VTE patients [14,25,34]. Our study includes both in- and out-patients diagnosed with VTE at the Skåne University Hospital, and, since that is the only hospital in the region treating these patients, our results most likely reflect true population based VTE epidemiology described more in detail by Isma *et al.* [30] in a part of the same material as in this study.

Women suffering their first VTE episodes were, not surprisingly, younger then men. This can probably be explained by acquired VTE risk factors such as use of contraceptive pills and pregnancies in younger women. However, we observed that men seemed to have a higher risk than women for VTE recurrence. This has also previously been showed in a large meta-analysis [35]. As demonstrated in the study of Isma *et al.* [30], the incidences of VTE, DVT and PE in our population were lower compared to previous epidemiological studies [2,36]. This might be explained by the fact that we nowadays use more strict diagnostic criteria and have implanted early mobilisation and improved thrombophrophylactic therapy in inpatients at risk for VTE. As PE is a diagnosis that is often established post mortem, the lower PE incidence in modern epidemiological materials could also reflect a lower frequency of autopsies.

When we compared common acquired VTE risk factors between the group of patients with the two most common thrombophilias (FVL and/or PTM) and the group with normal genotype we could not demonstrate any differences explaining why the patients with this type of thrombophilia were more or less likely to have recurrent VTE episodes. That positive family history of VTE was more common within the thrombophilia (FVL and/or PTM) group was of course expected as mutations run in families.

As previously mentioned, this is to the best of our knowledge one of the largest prospective studies evaluating VTE recurrence risk in FVL carriers. As it is community based it reflects subjects from the general population, not only patient subgroups. When we evaluated the risk for recurrence we excluded patients with previous VTE episodes as well as patients that received anticoagulation during the follow up time. These facts, as well as missing data, led to a relatively large loss of subjects for follow-up, equivalent to an about 20% drop-out rate. As we found evident differences between patients with and without the FVL mutation in heterozygous form, the above-mentioned limitations did not affect the strength of the study or significances of the findings. Furthermore, as our results are in accordance with previous literature we presume the data is valid and reflect both true VTE epidemiology in the general population and the VTE recurrence risk among heterozygote carriers of the FVL mutation in the community. We did not take into account, however, that some patients could also have had deficiencies of the natural anticoagulants (protein S, C and antithrombin) leading to an even more pronounced increase of the risk for recurrence. As these deficiencies are extremely rare, in our opinion they are highly unlikely to have affected the results of the study. Other limitations to be mentioned, are infomation about the presence of antiphospholipid syndrome as well as hereditary elevated FVIII level, which is now considered as a risk factor for venous thrombosis and may modify thrombosis risk in patients having other risk factors such as FVL.

In conclusion, this study shows that subjects carrying the Factor V Leiden mutation in its heterozygous form after a first episode of VTE run a 2–2.5 times increased risk for VTE recurrence. Since the risk for new VTE episodes among patients with normal genotype is low, however, recurrence risk among patients with the FVL mutation is reasonably low as well. It is therefore difficult to recommend lifelong anticoagulation therapy after VTE in this group, when all the risks and inconveniences associated with such treatment is taken into account. It is still useful though to know that this group of patients has a significantly higher recurrence risk when determining duration of therapy, when evaluating other risk factors, and when deciding which patients that should undergo testing for the mutation.

Conflict of Interest Statement

None.

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References

[1] Anderson Jr FA, Wheeler HB, Goldberg RJ, Hosmer DW, Forcier A. The prevalence of risk factors for venous thromboembolism among hospital patients. Arch Intern Med 1992;152:1660–4.
[2] Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med 1992;232:155–60.
[3] Anderson Jr FA, Spencer FA. Risk factors for venous thromboembolism. Circulation 2003;107:I9–I16.
[4] Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost 2005;3:1611–7.
[5] Rosendaal FR. Risk factors for venous thrombotic disease. Thromb Haemost 1999;82:610–9.
[6] Dahlback B. Physiological anticoagulation. Resistance to activated protein C and venous thromboembolism. J Clin Invest 1994;94:923–7.
[7] Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–7.
[8] Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79:706–8.
[9] Hillarp A, Zoller B, Svensson PJ, Dahlback B. The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis. Thromb Haemost 1997;78:990–2.
[10] Leroyer C, Mercier B, Oger E, Chenu E, Abgrall JF, Ferec C, et al. 20210 A allele of the prothrombin gene in venous thromboembolism patients. Thromb Haemost 1998;80:49–51.
[11] Kjellberg U, van Rooijen M, Bremme K, Hellgren M. Factor V Leiden mutation and pregnancy-related complications. Am J Obstet Gynecol 2010;203(469):e1–8.
[12] Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995;85:1504–8.
[13] Prandoni P, Lensing AW, Cogo A, Cuppini S, Villalta S, Carta M, et al. The long-term clinical course of acute deep venous thrombosis. Ann Intern Med 1996;125:1–7.
[14] Simioni P, Prandoni P, Lensing AW, Manfrin D, Tormene D, Gavasso S, et al. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000;96:3329–33.
[15] Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation 1995;92:2800–2.
[16] Eichinger S, Minar E, Hirschl M, Bialonczyk C, Stain M, Mannhalter C, et al. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost 1999;81:14–7.
[17] Rintelen C, Pabinger I, Knobl P, Lechner K, Mannhalter C. Probability of recurrence of thrombosis in patients with and without factor V Leiden. Thromb Haemost 1996;75:229–32.
[18] De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999;341:801–6.
[19] Kovac M, Mikovic D, Antonijevic N, Rakicevic L, Djordjevic V, Radojkovic D, et al. FV Leiden mutation and risk of recurrent venous thromboembolism in Serbian population. J Thromb Thrombolysis 2008;25:284–7.
[20] Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. Arch Intern Med 2006;166:729–36.

[21] Marchiori A, Mosena L, Prins MH, Prandoni P. The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. A systematic review of prospective studies. Haematologica 2007;92:1107–14.
[22] Simioni P, Prandoni P, Lensing AW, Scudeller A, Sardella C, Prins MH, et al. The risk of recurrent venous thromboembolism in patients with an Arg506–>Gln mutation in the gene for factor V (factor V Leiden). N Engl J Med 1997;336:399–403.
[23] Margaglione M, D'Andrea G, Colaizzo D, Capicci G, del Popolo A, Brancaccio V, et al. Coexistence of factor V Leiden and Factor II A20210 mutations and recurrent venous thromboembolism. Thromb Haemost 1999;82:1583–7.
[24] Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999;81:684–9.
[25] Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007;92:199–205.
[26] Santamaria MG, Agnelli G, Taliani MR, Prandoni P, Moia M, Bazzan M, et al. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res 2005;116:301–6.
[27] De Stefano V, Simioni P, Rossi E, Tormene D, Za T, Pagnan A, et al. The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. Haematologica 2006;91:695–8.
[28] Heit JA. The epidemiology of venous thromboembolism in the community: implications for prevention and management. J Thromb Thrombolysis 2006;21: 23–9.
[29] van den Belt AG, Sanson BJ, Simioni P, Prandoni P, Buller HR, Girolami A, et al. Recurrence of venous thromboembolism in patients with familial thrombophilia. Arch Intern Med 1997;157:2227–32.
[30] Isma N, Svensson PJ, Gottsater A, Lindblad B. Prospective analysis of risk factors and distribution of venous thromboembolism in the population-based Malmo Thrombophilia Study (MATS). Thromb Res 2009;124:663–6.
[31] Emmerich J, Alhenc-Gelas M, Aillaud MF, Juhan-Vague I, Jude B, Garcin JM, et al. Clinical features in 36 patients homozygous for the ARG 506–>GLN factor V mutation. Thromb Haemost 1997;77:620–3.
[32] Eichinger S, Pabinger I, Stumpflen A, Hirschl M, Bialonczyk C, Schneider B, et al. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. Thromb Haemost 1997;77:624–8.
[33] Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994;330:517–22.
[34] Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005;293:2352–61.
[35] Douketis J, Tosetto A, Marcucci M, Baglin T, Cosmi B, Cushman M, et al. Risk of recurrence after venous thromboembolism in men and women: patient level meta-analysis. BMJ 2011;342:d813.
[36] Palareti G, Legnani C, Cosmi B, Valdre L, Lunghi B, Bernardi F, et al. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003;108:313–8.

Paper II

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Homozygous factor V Leiden and double heterozygosity for factor V Leiden and prothrombin mutation

Ymir Saemundsson · Signý Vala Sveinsdottir · Henrik Svantesson · Peter J. Svensson

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Abstract The most common forms of familial thrombophilia are factor V Leiden (FVL) and prothrombin mutation (PTM). Homozygous FVL and PTM have long been feared conditions thought to cause high rates of morbidity and mortality. To analyse clinical features in patients with homozygous FVL and PTM, as well as patients with double heterozygosity for FVL and PTM. All patients with homozygous FVL, PTM or double heterozygosity in the MATS database of 1465 consecutive unselected patients were analysed regarding age at inclusion venous thromboembolism (VTE), age at first thrombosis, recurrence, clinical course and acquired risk factors. We found 36 patients homozygous for FVL. Patients homozygous for FVL were younger than controls at group level (56 ± 18 vs. 63 ± 17, $p < 0.02$). Homozygous women were younger than female controls (50 ± 19 vs. 63 ± 18, $p < 0.002$). No difference was observed when comparing male subjects. Women were younger than men at inclusion thrombosis (50 ± 19 vs. 65 ± 14, $p < 0.02$) and at first thrombosis (47 ± 19 vs. 64 ± 14, $p < 0.01$). Deep venous thrombosis (DVT) was seen in 33 patients (92 %), 6 (17 %) had pulmonary embolism (PE) and 3 (8 %) had combined DVT and PE. PE was less frequent in homozygous FVL women compared to female controls ($p < 0.03$). VTE recurred in 3 subjects during the duration of the study. Odds ratio for VTE in homozygous FVL patients compared to controls was 13.9 (95 % CI 9.9–19.7). We found no subjects with homozygous PTM. Double heterozygosity for FVL and PTM was seen in 12 subjects. There was no difference in age at inclusion VTE between double heterozygotes and controls (59 ± 16 vs. 63 ± 17, ns.). DVT was seen in 92 % at inclusion, 8 % had PE. Mean age at first VTE was 52 ± 17 (27–82). Consecutive homozygous FVL patients had a higher age at first thrombosis than previously described. Homozygous females are affected at an earlier age than homozygous men and female controls. It seems that thrombi in homozygous FVL have a different pattern compared to controls i.e. more prone for thrombosis in the lower extremity. The odds ratio for thrombosis among homozygous FVL seems to be lower than previously described.

Keywords Homozygous factor V Leiden · APC-resistance · Thrombosis · Venous thromboembolism · Thrombophilia · Heterozygous prothrombin mutation

Y. Saemundsson · S. V. Sveinsdottir · P. J. Svensson (✉)
Department for Coagulation Disorders, Lund University, Centre for Thrombosis and Haemostasis, Skåne University Hospital, S-205 02 Malmö, Sweden
e-mail: peter.svensson@med.lu.se

H. Svantesson
Kungälv Hospital, Kungälv, Sweden

Introduction

Venous thromboembolism (VTE) is a common disorder and an ever present differential diagnosis for the practicing clinician. It affects one in 1,000 individuals annually [1]. Today there are several effective methods for treating VTE and preventing VTE in patients at risk [1]. To minimize patient suffering and potential death caused by VTE it is essential that we are aware of the risk factors that predispose to the disorder, thereby identifying patients that may benefit from prophylactic treatment. Virchow [2] postulated in 1856 that the triad of changes to the vessel wall, blood composition and blood flow were involved in the formation of a thrombus. Virchow's conclusions are still valid, however, since then several underlying specific risk factors have been identified. Many of these are thoroughly investigated, thereby enabling effective

prophylactic treatment to patients at risk of VTE. There are still a few conditions predisposing for VTE that need further documentation to be better understood [3]. Roughly a hundred years after Virchows discoveries were published Nandorff and Jordan [4] introduced the term familial thrombophilia after publishing 43 cases of inherited thrombophilia. Since then a number of different hereditary conditions have been described. The most common inherited risk factor known is the so called factor V Leiden mutation (FVL) with a prevalence in Caucasians of around 5 % [5]. This mutation leads to resistance to activated protein C, a condition first described by Dahlbäck in 1993 [6]. Subjects heterozygous for this mutation have been shown to have approximately a 5-fold risk increase of VTE compared to controls [7]. The second most common form of inherited thrombophilia is the Prothrombin G20210A mutation (PTM). The prevalence of this mutation is 1–4 % in Europeans [8], and studies have indicated a 4-fold increased risk of VTE compared to controls [7]. Less is known, however, about those homozygous for these conditions. There are only a few studies describing patients with homozygous FVL [7, 9–11]. They indicate that these patients suffer from their first thrombosis at a far younger age and have a 10- to 80-fold increased risk of VTE, compared to controls. Furthermore, individuals with homozygous FVL have been shown to have a higher rate of recurrence of VTE than controls [12]. Earlier studies have debated whether the clinical course of VTE-events in FVL patients differs from that of normal controls. For example some studies have indicated a lower frequency of pulmonary embolism (PE) in this group of patients where DVT is common. It has been hypothesized that a different structure or location of thrombi in FVL patients leads to a decreased risk of embolic events [13–15].

Another group of patients that are generally considered to have a similar risk profile of VTE as those homozygous for FVL are patients with double heterozygosity for both FVL and PTM. A meta-analysis by Emmerich et al. [7] describing 51 cases of such patients indicates that they have approximately a 20-fold risk increase, and like homozygous individuals, have their first VTE at a very young age. The purpose of this study is 2-fold. Firstly we wish to further examine the clinical features associated with occurrence of VTE in patients with homozygous FVL and PTM. Secondly, as double heterozygosity for FVL and PTM is considered to have a similar risk profile for VTE as homozygous individuals, and are generally treated in the same way, we choose to include them in the analysis.

Materials and methods

The Malmö thrombofilia study (MATS) is a prospective population based study conducted at Malmö University Hospital. This hospital is the only hospital treating VTE patients in a catchment area of approximately 280,000 people. All subjects were recruited between March of 1998 and December 2008. MATS recruited patients >18 years of age that had an objectively verified diagnosis of VTE through phlebografy, venous duplex or computed tomography. Patients were required to leave blood samples, answer a questionnaire and participate in a complete analysis of risk factors for VTE. Seventy percent of all patients treated for VTE at Malmö University Hospital were included in the study. The remaining 30 % were excluded due to unwillingness to participate, language problems, dementia or other severe illness that prevented the patient from participating. The control group constituted of all patients in the MATS database excluding the 36 patients with homozygous FVL when analysing homozygous FVL and excluding the 12 patients with double heterozygosity when analysing double heterozygosity. The control group was comparable to the control group in age and sex distribution [16]. The study was approved by the Lund University Ethical Committee and all patients provided written consent.

DNA analysis was performed using Taqman allele discrimination with gene specific assays for factor V and factor II (Applied Biosystems). Subjects homozygous for FVL, PTM and double heterozygotes were analyzed using the MATS database regarding patient age at inclusion thrombosis and patient age at first thrombosis. We also included data about level of thrombosis at inclusion VTE, total number of VTE-events in each patient, complete analysis of risk factors and heredity.

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All results are expressed as mean ± SD. Odds ratios were estimated by using data on prevalence published by Kjellberg et al. [17]. p values were calculated using Fischer's exact test and student's t test when appropriate. Results were considered significant if $p < 0.05$.

Results

In the MATS database, constituting 1,465 VTE patients, 36 (2.5 %) were found to be homozygous for FVL (21 women and 15 men). We found no patients homozygous for PTM, 12 patients (0.8 %) (6 women and 6 men) had a double mutation of heterozygous FVL and PTM. Baseline characteristics are presented in Table 1. There were no patients with protein C or S deficiency among these homozygous and compound subjects. The mean age of the 48 subjects with homozygous FVL or compound FVL and PTM was 57 ± 17 (22–91) at inclusion VTE. Women were younger than men (53 ± 20 vs. 62 ± 13, ns.). Ninety-two percent

Table 1 Baseline characteristics

	Homozygous FVL	Double heterozygosity FVL and PTM
Patient characteristics	$n = 36$	$n = 12$
Male/female sex	15/21	6/6
Ongoing VTE prophylaxis	1 (3)	0 (0)
Ongoing use of compression socks	1 (3)	1 (8)
Immobilisation	2 (6)	3 (25)
Cancer	5 (14)	0 (0)
Oral contraceptives[a]	3 (14)	0 (0)
Pregnancy[a]	3 (14)	0 (0)
Surgical intervention	2 (6)	1 (8)
Cast therapy	0 (0)	1 (8)
BMI > 30	9 (25)	3 (25)
Present tobacco use	9 (25)	2 (17)
Heredity for VTE in 1st degree relative	14 (39)	6 (50)
No known acquired risk factor	16 (44)	6 (50)

n (%)

[a] Percentage of female patients only

Table 2 Location of thrombus and age at thrombosis

Homozygous FVL	All $n = 36$	Male $n = 15$	Female $n = 21$	Controls $n = 1{,}429$
Clinical features				
Age at first VTE	53 ± 18	64 ± 14	47 ± 19	62 ± 17
Age at inclusion VTE	56 ± 18	65 ± 14	50 ± 19	63 ± 17
DVT	32 (89)	13 (87)	19 (90)	1,078 (75)
PE	6 (17)	4 (27)	2 (10)	435 (30)
DVT + PE	2 (6)	2 (13)	0 (0)	86 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity[a]	23 (72)	9 (69)	14 (74)	662 (61)
Distal thrombosis of the lower extremity[a]	5 (16)	3 (23)	2 (11)	300 (28)
Upper extremity	3 (9)	0 (0)	3 (16)	66 (6)
Other location or clinical diagnosis	1 (3)	1 (8)	0 (0)	50 (5)

Mean ± SD and *n* (%)

[a] The distribution of thrombi in the left lower extremity compared to the right was equal among men whereas 74 % female subjects had thrombosis of the left leg

of the patients had deep vein thrombosis (DVT) at inclusion, 15 % had PE and 6 % had combined DVT and PE. The distribution of thrombus location in the DVT group of 44 subjects was 27 % v.iliaca, 44 % v.femuropoplitea and 14 % localized to the calf. Three patients (6 %) had thrombosis of the arm (Tables 2, 3). Positive family history of VTE in first degree relatives was reported in 20 subjects (42 %). At least one additional acquired risk factor was seen in 26 subjects (54 %). At study end 21 patients (44 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. Three patients (6 %) had a total of 3 VTE-events and 3 patients (6 %) had a total of 4. Mean age at first VTE was 52 ± 18 (11–91). Women were significantly younger than men (46 ± 20 vs. 59 ± 12, $p < 0.02$).

Homozygous FVL

Patients homozygous for FVL had a mean age of 56 ± 18 (22–91) at inclusion VTE (Table 2). This was significantly younger when comparing to controls (63 ± 17, $p < 0.02$). Women were younger than men (50 ± 19 vs. 65 ± 14, $p < 0.02$). Homozygous women were younger than female controls at inclusion VTE (50 ± 19 vs. 63 ± 18, $p < 0.01$). No difference was observed when comparing male subjects (65 ± 14 vs. 63 ± 15, ns.). DVT was seen in 92 % of the patients at inclusion, 17 % had PE and 8 % had combined DVT and PE. In the female group 10 % were suffering from pulmonary embolism compared to 27 % in the male group. The distribution of thrombus location in the DVT group of 33 subjects was 33 % v.iliaca, 39 % v.femuropoplitea and 15 % localized to the calf. Three patients (9 %) had thrombosis of the arm. One subject with combined DVT and PE did not have a radiologically verified DVT, the exact localization of which thus remains unknown. The DVT diagnosis, in this patient, was set clinically after a positive PE diagnosis was verified by computed tomography. Positive family history of VTE in first degree relatives was reported in 14 subjects (39 %) (Table 1). At least one additional acquired risk factor was seen in 20 subjects (56 %). Three women were using oral contraceptives and three women were in post partum. Two patients had been immobilized >10 h. One patient had undergone major surgery prior to VTE-event. BMI > 30 was seen in nine patients. Malignant diseases had been diagnosed in five patients. One patient had cancer of the lung and died during the study period. One woman had breast cancer and one man cancer of the prostate. Two patients suffered from haematological malignancies, one of which had his inclusion VTE during warfarin treatment. At study end 13 patients (36 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far (Table 2). Two patients (6 %) had a total of 3 VTE-events and 2 patients (6 %) had a total of 4. Mean age at first VTE was 53 ± 18 (17–91). Women were significantly younger than men (47 ± 19 vs. 64 ± 14, $p < 0.01$). Odds ratio for VTE in individuals homozygous for FVL compared to controls was 13.9 (95 % CI 9.9–19.7) assuming a prevalence of 0.18 % homozygosity for FVL and 8.1 % heterozygosity for FVL in the south of Sweden as described by Kjellberg et al. [17].

Table 3 Location of thrombus and age at thrombosis

Double heterozygote FVL/PTM	All $n = 12$	Male $n = 6$	Female $n = 6$	Controls $n = 1{,}429$
Clinical features				
Age at first VTE	52 ± 17	53 ± 9	53 ± 24	62 ± 17
Age at inclusion VTE	59 ± 16	57 ± 9	62 ± 21	63 ± 17
DVT	11 (92)	5 (83)	6 (100)	1,099 (77)
PE	1 (8)	1 (17)	0 (0)	440 (30)
DVT + PE	0 (0)	0 (0)	0 (0)	88 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity	10 (90)	5 (100)	5 (83)	662 (61)
Distal thrombosis of the lower extremity	1 (9)	0 (0)	1 (17)	300 (28)
Upper extremity	0 (0)	0 (0)	0 (0)	66 (6)
Other location or clinical diagnosis	0 (0)	0 (0)	0 (0)	50 (5)

Mean ± SD and *n* (%)

Double heterozygosity

Patients with double heterozygosity of FVL and PTM had a mean age of 59 ± 16 (30–82) at inclusion VTE (Table 3). There was no difference in age at inclusion VTE when comparing with controls (59 ± 16 vs. 63 ± 17, ns.). Mean age of women in this group was slightly higher than that of men (62 ± 21 vs. 57 ± 9, ns). DVT was seen in 92 % of the patients at inclusion, 8 % had PE while no patient had combined DVT and PE. The distribution of thrombus location in the DVT group of 11 subjects was 18 % v.iliaca, 73 % v.femuropoplitea and 9 % localized to the calf. Positive family history of VTE in first degree relatives was reported in six subjects (50 %) (Table 1). At least one additional acquired risk factor was seen in six subjects (50 %). Two patients had been immobilized >10 h. One patient had undergone major surgery prior to VTE-event. Three patients had a BMI > 30. At study end eight patients (67 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. One patient (8 %) had a total of 3 VTE-events and one patient (8 %) had a total of 4. All males had recurrent VTE (100 %), females had a recurrence rate of 33 %. Mean age at first VTE was 56 ± 16 (27–82). Women had a mean age of 60 ± 21 and men were significantly younger with a mean age of 53 ± 9 ($p < 0.03$).

Discussion

Homozygous FVL

Since first described in the mid fifties several studies have established inherited thrombophilia as a common risk factor for VTE. Still some of these conditions remain to be fully understood. Earlier studies of homozygous FVL have shed some light on the clinical features associated with this condition, however the largest studies were derived from selected materials [7, 11]. We present to our knowledge the largest cohort of consecutive patients with homozygous FVL. The mean age at inclusion thrombosis was 56 years. This was significantly younger when comparing to controls that had a mean age of 63 ($p < 0.02$). It was evident that the female subjects, with a mean age of 50, were the reason for the young age. Male subjects had a mean age of 65. When performing a gender specific comparison a significant age difference between homozygous women and female controls was revealed (50 ± 19 vs. 61 ± 20, $p < 0.02$). No such difference was observed when comparing male subjects (65 ± 14 vs. 62 ± 16, ns.). These findings indicate that women homozygous for FVL would be affected by VTE-events in a different way than males and female controls. It is well documented that oral contraceptive use and pregnancy (particularly post partum) predispose for VTE in FVL females [18–21]. We noticed a similar pattern in our material where women had two peaks of incidence (Figs. 1, 2). The first peak came at around 30 years of age and the second peak was around 60 years of age, the latter much like the male peak of incidence. Out of the nine women with thrombosis around the age of 30, six had known acquired risk factors. Three were using oral contraceptives, two were in post partum and one had ongoing pregnancy. This supports previous studies, arguing that women with known homozygous FVL should avoid oral contraceptives because of a greatly increased risk of thrombosis [19, 21]. An interesting observation is that the only women with homozygous FVL in the study that did have PE were under 30 years of age. One was pregnant with a gestational age of 23 weeks and the other was using oral contraceptives. Earlier studies have indicated that heterozygous FVL patients suffer from pulmonary embolisms to a lesser degree than controls [14, 15, 22], and it has been shown that there is no difference in frequency of PE between homozygotes and heterozygotes [11]. In our study 17 % of the subjects with homozygous FVL were suffering from PE compared to 30 % in the control group. There was no significant difference, although there was a tendency ($p < 0.051$). However, when comparing female subjects with male subjects there was a significant difference. In the group of female homozygous FVL patients 10 % were suffering from PE compared to 27 % in the male group. Our findings are consequently in accordance with previous observations that FVL patients suffer from PE to a lesser extent compared to controls but only regarding female subjects. The reason for the lower frequency of PE in FVL patients still remains undiscovered. Proximal DVT:s concerning the iliofemoral vein have been shown to cause PE

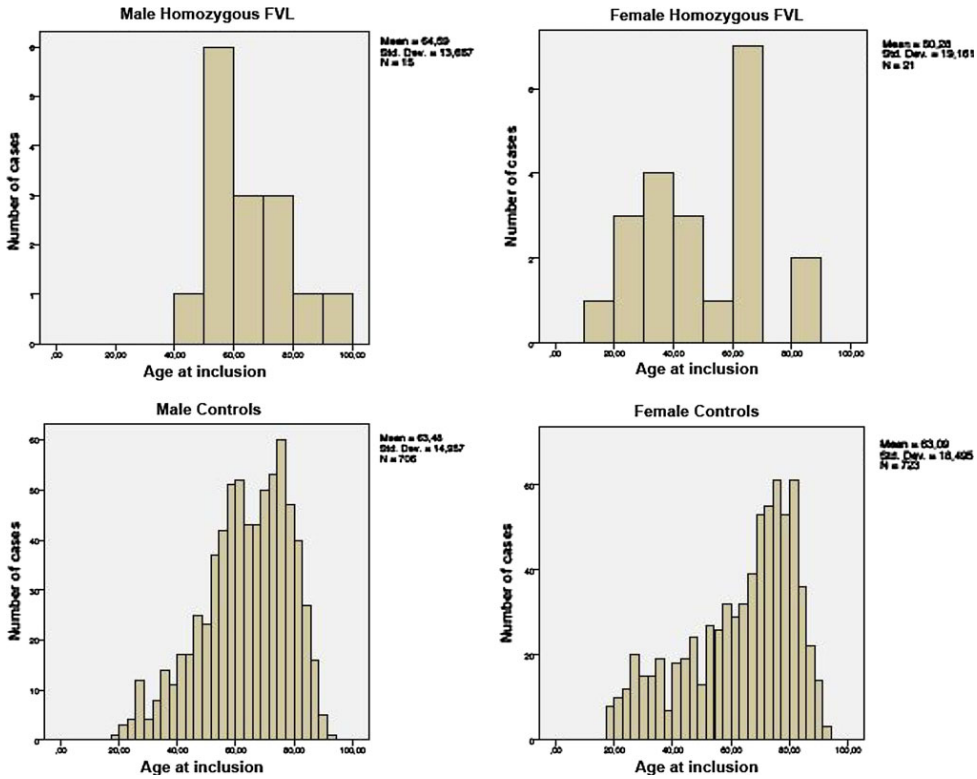

Fig. 1 Distribution of age at inclusion thrombosis

more frequently than distal DVT:s [23]. It has also been observed that thrombi in FVL patients are more distally located than thrombi in controls [13]. Combining these observations makes an appealing explanation to this phenomenon. However our results seem to contradict this theory. In our study 84 % of homozygous FVL patients had a proximal DVT (i.e. all thrombosis from the popliteal vein and above excluding PE), compared to 71 % in controls. These results are supported by similar observations made by van Stralen et al. [22]. Our findings would therefore support the theory that the thrombus in a FVL patient has a different structure, perhaps with stronger adhesive properties, thereby decreasing the risk of an embolic event. No extensive conclusions can however be drawn from our findings since only patients with symptoms of PE underwent computer tomography. At study end 13 patients (36 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. Unfortunately it was impossible to draw any conclusions from this data since a majority of patients diagnosed with homozygous FVL were given lifelong warfarin treatment. Nevertheless, the risk of recurrence after a thrombotic event is today a well described phenomena illustrated by several studies, for instance Prandoni et al. [24] where 1,626 consecutive VTE patients had a recurrence rate of 22.9 % at median 50 months' follow up without oral anticoagulation. A very recent study conducted in Malmö using the MATS database shows that patients with heterozygote FVL have an increased risk of recurrence compared to controls with an odds ratio of 2.4 [25]. However this is contradictory to the findings in an article by Willem et al. [26] showing no increased risk of recurrence compared to non thrombophilia patients. This study was not designed to look at recurrence for VTE in homozygous or double heterozygotes since several patients received lifelong treatment with anticoagulants.

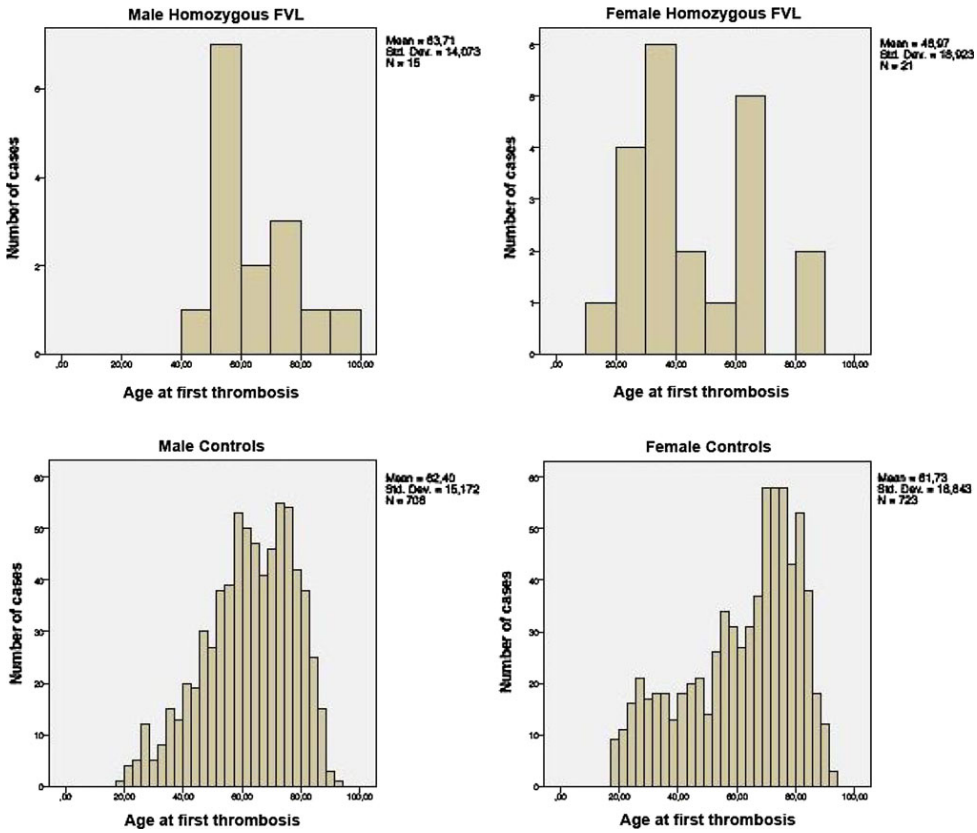

Fig. 2 Distribution of age at first thrombosis

Previous studies have indicated that patients homozygous for FVL suffer their first thrombotic event at an early age. For instance Rosendaal et al. [10] found a mean age of 31 at first thrombosis in 7 homozygous FVL patients. A large study of 85 patients with homozygous FVL conducted by the Procare group showed a mean age of 40 among men and 28 among women [11]. In our unselected population of homozygous FVL patients the mean age at first thrombosis was 64 ± 14 among men and 47 ± 19 among women (Fig. 2). In our opinion, the discrepancy in age between our study and the two aforementioned studies can be explained mainly by the fact that their materials were selected. All studies conducted on selected materials will render a young study population. We believe that our results better reflect at which age first thrombosis occurs in homozygous FVL patients. One remarkable finding is the high age at which male homozygous FVL carriers suffered their first thrombosis. In fact the homozygous FVL men were even slightly older when comparing to age at first thrombosis in male controls (64 ± 14 vs. 62 ± 15). This suggests that although males homozygous for FVL have a higher incidence of thrombosis, they resemble the average male VTE patient in terms of other clinical features. Women had a mean age of 47 ± 19 at first thrombotic event. Thus women were significantly younger than men ($p < 0.01$). As previously mentioned FVL and oral contraceptives greatly increase risk of thrombosis. Observations point to a synergistic effect when both are present [27]. More than half of the female subjects had their first VTE during fertile age, while only one male was younger than 50. Hormonal factors likely play an important role in the pathogenesis of VTE. In our study we found an odds ratio for VTE among homozygous FVL patients to be 13.9 (95 % CI 9.9–19.7) compared to non FVL carriers.

Emmerich et al. [7] showed an odds ratio of 9.85 in 30 homozygous FVL patients. These numbers stand in strong contrast to earlier observations in the LETS study where an 80-fold risk increase was reported [10]. In our material we also observed that patient age at first VTE was widely distributed (11–91). This confirms earlier observations made by Emmerich et al. [12] and suggests that factors other than genetic predisposition alone are important.

Homozygous PTM

Among the 1,480 patients in the MATS database we found no patients with homozygous PTM. This points to the rarity of this form of mutation and is confirmed by a study conducted by Rosendaal et al. [8] where 5,527 individuals, mainly Caucasians, were tested and no homozygous prothrombin mutation was found.

Double heterozygosity

The 12 subjects with double heterozygous FVL and PTM had a mean age of 52 ± 17 at first thrombosis. A meta-analysis by Emmerich et al. [7] on 51 patients with double heterozygosity reported a mean age of 34.7 at first thrombotic event. Although larger in sample size this meta-analysis consists mainly of selected materials. In contrast to the homozygous group there was no significant difference in age between female and male subjects at inclusion thrombosis and first thrombosis. There was even a tendency towards that females were older than males. One factor that might explain this is that no reports of oral contraception or pregnancies occurred in the compound group. Eight out of 12 (67 %) patients had more than one thrombotic event. There was no significant difference in age at inclusion thrombosis compared to controls (59 ± 16 vs. 63 ± 17). Recurrence rates were high, especially in males, however no definite conclusions can be drawn from this mainly because of the small sample size.

Study limitations

One of the limitations of our study is that patients <18 years of age were not included. This could potentially have meant that the mean age of inclusion thrombosis would have been somewhat lower. Another limitation is the fact that approximately 30 % of the patients that had a VTE during the study period did not participate due to reasons described in materials and methods. The figure 30 % is an estimation based on a review of hospital records of all excluded VTE patients in 1 year during the study period by a study nurse. This group matched the enrolled patients in terms of all important parameters. The sample size in this study is still too small to enable statistically stable analyses. Larger prospective studies or collaborations between centres are needed.

Conclusions

Homozygous consecutive patients had a higher age at first thrombosis than previously described. Somewhat higher risk of recurrence compared to heterozygotes and patients without thrombophilia. Homozygous females are affected at an earlier age than homozygous men and female controls. It seems that thrombi in homozygous FVL have a different pattern compared to non-thrombofilia patients, i.e. more prone for thrombosis in the lower extremity. The odds ratio for thrombosis among homozygous FVL seems to be lower than previously described. Further investigations of thrombosis in homozygous FVL are necessary.

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Conflict of interest The authors of this study report no conflict of interests.

References

1. Dahlbäck B (2008) Advances in understanding pathogenic mechanisms of thrombophilic disorders. Blood 112:19–27
2. Virchow R (1856) Phlogose und Thrombose im Gefäßsystem. Gesammalte Abhandlungen zur Wissenschaftlichen Medtzin. Medinger Sohn, Frankfurt
3. Rosendaal FR Venous thrombosis: the role of genes, environment, and behavior. Hematology Am Soc Hematol Educ Program 2005:1–12
4. Jordan FL, Nandorff A (1956) The familial tendency in thromboembolic disease. Acta Med Scand 156(4):267–275
5. Rees DC, Cox M, Clegg JB (1995) World distribution of factor V Leiden. Lancet 346:1133–1134
6. Dahlbäck B, Carlsson M, Svensson PJ (1993) Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 90:1004–1008
7. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T et al (2001) Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism. Thromb Haemost 86:809–816
8. Rosendaal FR, Doggen CJM, Zivelin A, Arruda VR, Aiach M, Siscovick DS et al (1998) Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 79:706–708
9. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL et al (2002) A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. Blood 99:2720–2725
10. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH (1995) High risk of thrombosis in patients homozygous for factor V Leiden. Blood 85:1504–1508

11. The Procare Group (2000) Comparison of thrombotic risk between 85 homozygotes and 481 heterozygotes carriers of the factor V Leiden mutation: retrospective analysis from the procare study. Blood Coagul Fibrinolysis 11(6):511–518
12. Emmerich J, Alhenc-Gelas M, Aillaud MF, Juhan-Vague I, Jude B, Garcin JM et al (1997) Clinical features in 36 patients homozygous for the ARG 506–>GLN factor V mutation. Thromb Haemost 77(4):620–623
13. Björgell O, Nilsson PE, Nilsson JA, Svensson PJ (2000) Location and extent of deep vein thrombosis in patients with and without FV:R 506Q mutation. Thromb Haemost 83(5):648–651
14. Martinelli I, Cattaneo M, Panzeri D, Mannucci PM (1997) Low prevalence of factor V:Q506 in 41 patients with isolated pulmonary embolism. Thromb Haemost 77(3):440–443
15. Manten B, Westendorp RG, Koster T, Reitsma PH, Rosendaal FR (1996) Risk factor profiles in patients with different clinical manifestations of venous thromboembolism: a focus on the factor V Leiden mutation. Thromb Haemost 76(4):510–513
16. Isma N, Svensson PJ, Gottsäter A, Lindblad B (2009) Prospective analysis of risk factors and distribution of venous thromboembolism in the population-based Malmö thrombophilia study (MATS). Thromb Res 124(6):663–666
17. Kjellberg U, van Roojen M, Bremme K, Hellgren M (2010) Factor V Leiden mutation and pregnancy-related complications. Am J Obstet Gynecol 203(5):469; e1–8
18. Rintelen C, Mannhalter C, Ireland H, Lane DA, Knöbl P, Lechner K, Pabinger I (1996) Oral contraceptives enhance the risk of clinical manifestation of venous thrombosis at a young age in females homozygous for factor V Leiden. Br J Haematol 93(2):487–490
19. Pabinger I, Nemes L, Rintelen C, Koder S, Lechler E, Loreth RM et al (2000) Pregnancy-associated risk for venous thromboembolism and pregnancy outcome in women homozygous for factor V Leiden. Hematol J 1(1):37–41
20. Middeldorp S, Libourel EJ, Hamulyák K, Van Der Meer J, Büller HR (2001) The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. Br J Haematol 113:553–555
21. Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR (1994) Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet 344(8935):1453–1479
22. van Stralen KJ, Doggen CJ, Bezemer ID, Pomp ER, Lisman T, Rosendaal FR (2008) Mechanisms of the factor V Leiden paradox. Arterioscler Thromb Vasc Biol 28(10):1872–1877
23. Hull RD, Carter CJ, Jay RM, Ockelford PA, Hirsch J, Turpie AG et al (1983) The diagnosis of acute, recurrent, deep-vein thrombosis: a diagnostic challenge. Circulation 67(4):901–906
24. Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R et al (2007) The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 92(2):199–205
25. Sveinsdottir S, Saemundsson Y, Isma N, Svensson PJ (2012) Evaluation of recurrent venous thromboembolism in patients with factor V Leiden mutation in heterozygous form. Thromb Res 130(3):467–471
26. Lijfering Willem M, Middledorp Saskia, Veeger Nic JGM, Hamulyák Karly, Prins Martin H, Bűller Harry R, van den Meer Jan (2010) Risk of recurrent venous thrombosis in homozygous carriers an double heterozygous carriers of factor V Leiden an prothrombin G20210A. Circulation 121:1706–1712
27. Wu O, Robertson L, Langhorne P, Twaddle S, Lowe GD, Clark P et al (2005) Oral contraceptives, hormone replacement therapy, thrombophilias and risk of venous thromboembolism: a systematic review. The thrombosis: risk and economic assessment of thrombophilia screening (TREATS) study. Thromb Haemost 94(1):17–25

Paper III

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Inflammatory plasma markers and risk for venous thromboembolism

Signy V. Sveinsdottir · Peter J. Svensson · Gunnar Engström

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Abstract Venous thromboembolism (VTE) and arterial thrombosis have been thought to result from two different mechanisms. Recent data indicate that the two diseases may share some common risk factors, such as the activity of inflammation on haemostasis. In this population-based study we explored whether raised levels of inflammation-sensitive plasma markers (ISPs) increase the risk for venous thromboembolism. Measurements of five ISPs (fibrinogen, haptoglobin, ceruloplasmin, α1-antitrypsin and orosomucoid) were performed in 6,068 subjects from "the Malmö Preventive Study". These apparently healthy men from the city of Malmö in Sweden, were included in the study between 1974 and 1982 and followed up until 2008. We calculated the hazard ratio (HR) for VTE in relation to the number of raised ISPs as well as individual ISPs in the fourth quartile. Mean follow-up time was 26.2 years. Out of the cohort (n = 6,068), 398 (6.6 %) had a venous thromboembolism during the follow-up. The number of raised ISPs was significantly associated with age, BMI and smoking. Age, BMI and diabetes mellitus type 2 were also significant risk factors for developing a VTE (HR = 1.05 with $p < 0.01$ and 95 % CI 1.01–1.08, HR = 1.10 with $p < 0.001$ and 95 % CI 1.06–1.14 and HR = 1.78 with $p < 0.05$ and 95 % CI 1.13–2.81, respectively). Incidence of venous thromboembolism was not significantly related to number of raised inflammatory proteins (p for trend = 0.37) or any of the individual ISPs. Age and BMI is significantly associated with the risk for developing VTE. Incidence of VTE was not associated with any of the inflammatory proteins.

Keywords Venous thromboembolism · Risk factors · Inflammation · Inflammation-sensitive proteins

S. V. Sveinsdottir · P. J. Svensson
University of Lund, Skåne University Hospital, Malmö, Sweden

S. V. Sveinsdottir (✉) · P. J. Svensson
Centre for Thrombosis and Haemostasis, Skåne University Hospital (SUS), 20502 Malmö, Sweden
e-mail: signyvs@gmail.com; signy.sveinsdottir@med.lu.se

G. Engström
Department of Clinical Sciences, University of Lund, Malmö, Lund, Sweden

Background

Venous thromboembolism (VTE) and arterial thrombosis have been thought to result from two different mechanisms [1]. During the last two decades, studies have shown the important role of inflammation in the pathophysiology of arterial atherothrombosis [1] and its cross linkage to the coagulation system [2]. Although much is known about the mechanism and risk factors for the venous thromboembolism, many questions still remain. Data has now shown that atherosclerosis is more prevalent in patients with VTE [3] indicating that the two diseases may share some common mechanisms [1, 3, 4] and risk factors. In both the conditions endothelial dysfunction and vascular inflammation are part of the pathogenesis. Furthermore, arterial and venous thrombosis are thought to share risk factors such as hypertension, hyperlipidemia, chronic arterial disease of the legs, diabetes mellitus and obesity even though the evidence is controversial [5–9].

Damage to the vein wall has been thought to be necessary for the initiation of a thrombotic event. However, data now indicate that the process is more complex and the hemostatic balance can be disturbed by inflammation with

increased production of procoagulant factors activating the blood coagulation as well as inhibiting the fibrinolytic pathway [10]. The thrombus in turn increases the inflammatory process, thereby activating many factors such as platelets, leucocytes, various interleukins and cell-derived microparticles [3, 4, 11]. A large population based cohort study, the Longitudinal Investigation of Thromboembolism Etiology (LITE) [12] has shown that elevated levels of the coagulation factor VIII as well as von Willebrand factor were positively and independently associated with the risk of VTE. However, the same study did not show a significant relationship between inflammatory markers (fibrinogen and C-reactive protein) and VTE.

Previous studies from the Malmö Preventive study have shown that raised concentrations of five inflammatory proteins (fibrinogen, haptoglobin, ceruloplasmin, orosomucoid, and α1-antitrypsin) are associated with myocardial infarction, stroke and other cardiovascular outcomes. Whether these plasma proteins are associated with VTE is unclear [13]. Considering that venous thromboembolism is a relatively common cause of morbidity and mortality with an age-adjusted incidence of 1–2 per 1,000 of the general population and high frequency of recurrence, it would be helpful to be able to identify those at risk. As much is known about the VTE risk factors, biomarkers would add further information in preventing and treating patients with VTE. The aim of this study was to investigate inflammatory plasma markers in a large cohort of men followed for more than 20 years for the association to VTE.

Methods

During the period between 1974 and 1984, a screening program was conducted to detect individuals with a high risk for cardiovascular diseases [14]. Complete birth cohorts from the city of Malmö were used to invite men to take part in the program and the participation rate was 71 %, total of 22,444 men. Out of those, 6,193 men were randomly selected from the birth cohorts examined between 1974 and 1982. Men with history of myocardial infarction, stroke or cancer (according to questionnaire) and former VTE (according to hospital registers) were then excluded leaving 6,068 men in the study. Within those men five plasma proteins were determined, i.e. haptoglobin, fibrinogen, ceruloplasmin, orosomucoid and α1-antitrypsin, at the time of inclusion.

Since cancer is a potential confounding factor, which could increase inflammation as well as increase the risk of VTE, we performed the analysis in two steps. First, we analysed the cohort without considering whether they had history of cancer. Then we did the same analyses without including VTE cases with cancer before or up to 180 days after VTE episode.

Baseline examinations

The age of the subjects was considered when they were included into the study.

Smoking was assessed in a questionnaire and subjects were categorised as current smokers and non-smokers. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). When looking at blood pressure in our analyses we used systolic blood pressure that was measured twice in the right arm after a 10-minute rest. The average value of these two measurements was used.

Blood samples were taken after an overnight fast. Diabetes mellitus was recorded when venous blood glucose was ≥6.1 mmol/l (according to The American Diabetes Association Guidelines, ADA, 2007) measured in whole blood and in those using anti-diabetic medication. Serum cholesterol and triglyceride concentrations were analysed with standard methods at the laboratory of the hospital and expressed as mmol/l.

Inflammation-sensitive plasma proteins (ISPs)

The plasma levels of acute phase proteins were measured using electroimmunoassay [15] consecutively at the time of study entry. The coefficient of variation of this method is considered to be <5 % [16]. Using the same material, it has been previously shown that these proteins are highly correlated and that the cardiovascular risk increases with the number of ISP in the top quartile [13, 17]. For fibrinogen, haptoglobin and orosumucoid we used detection limits 350, 50 mg/l α1-antitrypsin and 20 mg/l for ceruloplasmin. Median (interquartile range) levels for the ISPs were 3.46 (3.0–4.0) g/l for fibrinogen, 0.80 (0.67–0.93) g/l for orosomucoid, 1.28 (1.09–1.42) g/l for α1-antitrypsin, 1.30 (0.89–1.75) g/l for haptoglobin and 0.30 (0.26–0.35) g/l for ceruloplasmin.

Previously, all the five ISPs have been associated with different cardiovascular diseases (i.e. ischemic stroke, cardiac events and cardiovascular death) [13]. In accordance with several previous studies from this cohort, we constructed a composite score (i.e. the number of ISPs in the fourth quartile) from these five proteins [13, 18].

Follow-up

To retrieve the cases during the whole follow-up period, the Swedish hospital discharge register was used. This register covers all hospitalizations in south of Sweden during the entire follow-up period and the register became nation-wide in 1987. VTE was defined as ICD-8 codes 450–451, ICD-9 codes 415B or 451, and ICD-10 codes I26 and I80. All the men were followed from the baseline examination until VTE, death, emigration from Sweden or until the end of the follow-up time, 31 December 2008.

Table 1 Baseline characteristics in relation to number of raised inflammation-sensitive protein (ISP) levels

	Number of raised IPSs				
	0 (n = 2,448)	1 (n = 1,559)	2 (n = 906)	≥3 (n = 1,155)	*p*
Age (years)	46.6 (3.6)	46.8 (3.7)	47.0 (3.5)	47.3 (4.0)	<0.001
BMI (kg/m^2)	24.8 (3.1)	25.1 (3.3)	25.1 (3.4)	25.1 (3.8)	<0.01
Current smokers (%)	30	49	65	74	<0.001
Diabetes mellitus (%)	3.8	4.9	5.1	6.2	<0.01
Cholesterol (mmol/l)	5.6 (1.0)	5.7 (1.1)	5.8 (1.1)	5.9 (1.1)	<0.001
Triglycerides (mmol/l)	1.4 (0.8)	1.6 (1.4)	1.7 (1.1)	1.7 (1.1)	<0.001
SBP (mmHg)	127.9 (14.6)	129.5 (15.6)	129.6 (16.8)	130.4 (16.6)	<0.001

Data presented as mean (SD) or proportions (%). BMI body mass index

Statistical analyses

SPSS software (version 20.0) was used in all statistical calculations. When [14] we looked at baseline characteristics in relation to number of raised ISPs we used one-way ANOVA for age and BMI and Pearson Chi square for smoking. As triglycerides showed a skewed distribution, we used Mann–Whitney test for the analysis. When the VTE risk was assessed in relation to number of elevated ISPs Cox proportional hazards model was performed. Age, BMI and cholesterol were modeled as continuous variables whereas current smoking, diabetes mellitus were used as dichotomous variables. The number of raised ISPs was used as an ordinal variable to get the p value for trend. The same statistical tests were used regarding the individual ISPs.

Results

Out of the whole cohort (n = 6,068), 398 (6.6 %) had a venous thromboembolism during the follow-up time, which was the mean of 26.2 years. Those men who did not have any VTE episode were 5,670 (93.4 %).

Of the VTE cases, 116 had cancer before or up to 180 days after VTE.

Baseline characteristics

The baseline characteristics of the subjects (n = 6,068) in relation to the raised ISP are presented in Table 1. Those who had no elevated ISPs were 2,448 men, those with one raised were 1,559, 906 had two elevated proteins and 1,155 with three or more raised ISPs. The mean age and BMI was 46.8 years and 25.0 kg/m^2 respectively. Smoking ($p < 0.001$), high BMI ($p < 0.001$), cholesterol ($p < 0.001$) and high age ($p < 0.01$) were significantly associated with number of raised ISPs.

Potential risk factors for venous thromboembolism

Within the whole cohort, the risk for venous thromboembolism in relation to age, BMI, cholesterol, triglycerides, diabetes mellitus, blood pressure and smoking can be viewed in Table 2. As expected, age and BMI were significant risk factors for developing a VTE when adjusted for other risk factors (HR = 1.05, $p < 0.01$ with 95 % CI 1.01–1.08 and HR = 1.10, $p < 0.001$ with 95 % CI 1.06–1.14 respectively). Diabetes mellitus was also a significant risk factor for VTE with a HR = 1.78 and *p* value of <0.05 (95 % CI 1.13–2.81). The other risk factors did not reach significance.

When we excluded the VTE cases with cancer during follow-up or within 180 days after VTE (n = 109) we had similar results, i.e. age, BMI and diabetes mellitus were significant risk factors (HR = 1.05, $p < 0.01$ with 95 % CI 1.01–1.08, HR = 1.10, $p < 0.01$ with 95 % CI 1.06–1.14) and HR = 1.78, $p < 0.05$ with 95 % CI 1.13–2.81 respectively) whereas the other factors were not.

Inflammation and venous thromboembolism

A total of 6,056 men were available for the multivariate Cox regression analysis (i.e. cholesterol level was missing in 12 subjects). The risk for VTE in relation to raised ISPs was adjusted for possible confounding factors as seen in Table 3.

The HR for venous thromboembolism was not significantly related to number of raised inflammatory proteins (*p* for trend = 0.37) adjusted for age, BMI, diabetes mellitus, smoking and cholesterol. The hazard ratio (HR) was 1.00 for those without elevated ISPs (reference), and the HR was 1.11 (95 % CI 0.87–1.42), 1.18 (95 % CI 0.87–1.59) and 1.09 (95 % CI 0.81–1.47), respectively in men with 1, 2 or ≥3 raised ISPs.

The results were still non-significant when the cancer VTE cases were excluded from the analysis. HR for the

Table 2 Various risk factors in relation to VTE risk

	VTE, no	VTE yes	HR, crude (95 % CI)	HR age, adjusted (95 % CI)	HR, risk factors adjusted (95 % CI)
N	5,670	398	–	–	–
Age, years (±SD)	46.8 (3.7)	47.3 (4.4)	1.06 (1.03–1.09)	–	1.05 (1.01–1.08)
Cholesterol (mmol/l) (±SD)	5.7 (1.1)	5.7 (0.9)	0.99 (0.90–1.09)	0.97 (0.88–1.07)	0.96 (0.80–1.08)
BMI (kg/m^2) (±SD)	24.9 (3.3)	25.9 (3.8)	1.09 (1.06–1.12)	1.09 (1.05–1.12)	1.10 (1.06–1.14)
SBP (mmHg) (±SD)	129.0 (15.5)	130.0 (16.6)	1.01 (1.00–1.01)	1.00 (0.00–1.01)	1.00 (1.00–1.01)
Triglycerides (mmol/l) (±SD)	1.6 (1.1)	1.5 (1.8)	0.98 (0.88–1.09)	0.98 (0.88–1.09)	0.88 (0.76–1.03)
Current smoking yes (%)	2,735 (48.2)	184 (46.0)	1.08 (0.90–1.31)	1.09 (0.90–1.33)	1.20 (0.94–1.52)
Diabetes mellitus yes (%)	260 (4.6)	27 (7.2)	1.93 (1.12–3.00)	1.95 (1.25–3.05)	1.78 (1.13–2.81)

Table 3 Incidence of thrombosis in relation to number of elevated acute phase proteins (i.e. in the 4th quartile)

	None	One	Two	≥Three	*p*, trend
N	2,448	1,559	906	1,155	
VTE[a], n (% of N)	156 (6.4 %)	109 (7.3 %)	63 (7.0 %)	70 (6.0 %)	
HR, age-adjusted	1.00	1.11 (0.83–1.48)	1.35 (0.97–1.89)	1.17 (0.84–1.63)	0.15
+risk factors[b]	1.00	1.11 (0.87–1.42)	1.18 (0.87–1.59)	1.09 (0.81–1.47)	0.42
VTE[a] (without history of cancer), n (% of N)	113 (4.6 %)	75 (4.8 %)	50 (5.5 %)	51 (4.4 %)	0.16
HR (95 % CI) age + risk factor adjusted[b]	1.00	1.06 (0.79–1.42)	1.29 (0.91–1.81)	1.09 (0.78–1.54)	0.37

Based on number of acute phase proteins in the fourth quartile (of fibrinogen, haptoglobin, ceruloplasmin, orosomucoid, α1-antitrypsin)

[a] VTE: DVT, PE or both

[b] Risk factors: BMI, diabetes, smoking and cholesterol

Table 4 Incidence of thrombosis in relation to quartiles of individual acute phase proteins

	Q1	Q2	Q3	Q4	*p**
Fibrinogen	1.00	0.79 (0.60–1.04)	0.93 (0.71–1.23)	0.81 (0.61–1.08)	0.56
Haptoglobin	1.00	1.09 (0.82–1.45)	1.19 (0.90–1.58)	1.12 (0.83–1.51)	0.23
Ceruloplasmin	1.00	1.05 (0.80–1.37)	0.97 (0.73–1.29)	1.18 (0.87–1.58)	0.77
Orosomucoid	1.00	1.30 (0.99–1.73)	1.24 (0.92–1.67)	1.21 (0.90–1.64)	0.58
α1-Antitrypsin	1.00	0.97 (0.74–1.21)	0.96 (0.72–1.28)	1.07 (0.81–1.41)	0.86

* *p* for trend, adjusted for age, BMI and current smoking and cholesterol

men with 0, 1, 2, ≥3 raised ISPs had HR 1.00 (reference), 1.06 (95 % CI 0.79–1.42), 1.29 (95 % CI 0.91–1.81), 1.09 (95 % CI 0.77–1.54), respectively.

Individual ISPs and risk for venous thromboembolism

We looked at the five individual inflammatory proteins separately, i.e. fibrinogen, haptoglobin, ceruloplasmin, A1-antitrypsin and orosomucoid, as potential risk factors for VTE. The plasma levels of the proteins were measured and divided into quartiles. None of the proteins gave significantly increased risk for VTE, see Table 4. The HRs for the fourth quartiles (vs first quartiles) were 0.81 (95 % CI 0.61–1.08), 1.12 (95 % CI 0.83–1.51), 1.18 (95 % CI 0.87–1.58), 1.07 (95 % CI 0.81–1.41) and 1.21 (95 % CI 0.90–1.64), respectively, for the above mentioned proteins. As expected, the non-significance remained when VTE cases within the cancer subjects were excluded.

Discussion

In our present study, we could not show a significant relationship between inflammation and the risk for venous thromboembolism. The number of raised inflammation-sensitive proteins in the fourth quartile was not related to the VTE risk. Furthermore, we found no significant relationship when we looked at the proteins individually, i.e.

fibrinogen, haptoglobin, ceruloplasmin, orosomucoid and α1-antitrypsin. Age and BMI, as expected according to the literature [19], gave significantly higher risk for VTE. Diabetes mellitus was similarly associated with incidence of VTE, which also has been shown by the large LITE study [8] although the Copenhagen City Heart Study could not verify this relationship [19].

Many previous studies have been performed to evaluate different biomarkers with regard to their role in predicting VTE. Most studies that explored inflammation as potential risk factor for VTE have not analyzed those inflammation-sensitive proteins that we did apart from fibrinogen. Our main results are well in accordance to many studies, i.e. that inflammation is not a significant risk factor for VTE.

One of the biomarkers that have been most analyzed is d-dimer, the well known risk factor for first event and recurrence of VTE [20]. Another biomarker that has been much at focus as a potential risk factor is the clotting factor VIII. Various studies, both case–control [21], prospective [12] as well as prospective observational studies [22] have indicated factor VIII as a risk factor although its relevance for guidance on anticoagulation duration is not clear. C-reactive protein, the inflammation marker, has been investigated in a few case–control studies [23, 24] and has not been accepted as a risk factor for developing VTE.

Tsai et al. studied various inflammation markers as well as coagulation factors as a potential VTE risk and published the results in the LITE study in 2002. They found that levels of factor VIII, von Willebrand factor and factor VII were positively and independently associated with the incidence of venous thromboembolism in their large population-based cohort study [12]. However, the inflammation markers fibrinogen and C-reactive protein were not significantly related to the risk for having a VTE [12]. This is in accordance to our observations, i.e. fibrinogen was not associated with increased VTE risk. A case–control study from 2000 made by Austin et al. got the same results, i.e. fibrinogen is not a risk factor for VTE [25]. Controversially, a few case–control studies [26, 27] have been able to show a relationship between elevated fibrinogen and VTE.

Other inflammatory biomarkers that have been studied are many cytokines such as IL-6, sP-selectin TNF-α, IL-8 and MCP-1 [28–30] where inhibition of these factors have been found to reduce VTE incidence and recurrence [29]. Through this inhibition and by lowering serum cholesterol and/or triglycerides, it has been hypothesized and indicated that statins could reduce VTE [9]. However, other studies could not verify this relationship [8, 19, 31]. In our study, cholesterol or triglycerides were no significant risk factors for developing VTE.

There are many various inflammatory markers that can be analyzed individually as potential VTE risk factors. However, during an inflammatory process more than one factor is involved and therefore it is difficult to find a good biomarker to guide the anticoagulation therapy. Additionally, those studies that show a relationship between inflammation and VTE could have been biased, as inflammation markers were elevated after the thrombotic event. We wanted to see if inflammation in general could induce VTE by looking at the number of ISPs, but no significant relationship was observed. Furthermore, none of the analyzed individual biomarkers were found to increase the VTE risk.

The strengths of this large prospective cohort study were the large number of subjects and its long follow-up time. The end-point was very clear, an objectively verified VTE according to the hospital's register. We assume the end-point registration is reliable and comprehensive. The Swedish national inpatient register has been valued with an overall positive predictive value for all diagnoses between 85 and 95 % [32]. Furthermore, a validation from the USA [33] has shown that data on VTE obtained from administrative registries are a valuable source of information although they should be used with caution. The assessment of the ISPs, cholesterol and triglycerides with electroimmunoassay is an established and reliable method [15]. They were based on a single blood test at inclusion and an intraindividual variation is possible. The level of the inflammatory markers is therefore not presented at the time of VTE event. A change of exposure could also cause a bias in the study. Smokers according to questionnaire at inclusion could have stopped smoking during the follow up time, blood pressure changed etc. However, several previous studies have shown that these proteins predict incidence of cardiovascular diseases and stroke even over very long follow-up periods [13, 16, 17].

We conclude in our cohort that inflammation is not associated to the risk for developing VTE, age, BMI and diabetes are significant risk factors for thrombotic event. Cholesterol- and triglyceride levels are not related to VTE risk.

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References

1. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC (2011) Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Semin Thromb Hemost 37(8):885–896. Epub 2011/12/27

2. Davies MJ (1996) Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. Circulation 94(8):2013–2020. Epub 1996/10/15
3. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW et al (2003) An association between atherosclerosis and venous thrombosis. N Engl J Med 348(15):1435–1441. Epub 2003/04/11
4. Hong C, Zhu F, Du D, Pilgram TK, Sicard GA, Bae KT (2005) Coronary artery calcification and risk factors for atherosclerosis in patients with venous thromboembolism. Atherosclerosis 183(1):169–174. Epub 2005/06/09
5. Libertiny G, Hands L (1999) Deep venous thrombosis in peripheral vascular disease. Br J Surg 86(7):907–910. Epub 1999/07/27
6. Vaya A, Mira Y, Ferrando F, Contreras M, Estelles A, Espana F et al (2002) Hyperlipidaemia and venous thromboembolism in patients lacking thrombophilic risk factors. Br J Haematol 118(1):255–259. Epub 2002/07/09
7. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW (2008) Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation 117(1):93–102. Epub 2007/12/19
8. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR (2002) Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Arch Intern Med 162(10):1182–1189. Epub 2002/05/22
9. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ et al (2009) A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med 360(18):1851–1861. Epub 2009/03/31
10. Conde I, Lopez JA (2005) Classification of venous thromboembolism (VTE). Role of acute inflammatory stress in venous thromboembolism. J Thromb Haemost 3(11):2573–2575. Epub 2005/10/26
11. Jezovnik MK, Poredos P (2010) Idiopathic venous thrombosis is related to systemic inflammatory response and to increased levels of circulating markers of endothelial dysfunction. Int Angiol 29(3):226–231. Epub 2010/05/27
12. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Tracy RP, Aleksic N et al (2002) Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). Am J Med 113(8):636–642. Epub 2002/12/31
13. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F (2002) Effects of cholesterol and inflammation-sensitive plasma proteins on incidence of myocardial infarction and stroke in men. Circulation 105(22):2632–2637. Epub 2002/06/05
14. Berglund G, Eriksson KF, Israelsson B, Kjellstrom T, Lindgarde F, Mattiasson I et al (1996) Cardiovascular risk groups and mortality in an urban Swedish male population: the Malmo Preventive Project. J Intern Med 239(6):489–497 .Epub 1996/06/01
15. Laurell CB (1972) Electroimmuno assay. Scand J Clin Lab Investig Suppl 124:21–37. Epub 1972/01/01
16. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F (2002) Long-term effects of inflammation-sensitive plasma proteins and systolic blood pressure on incidence of stroke. Stroke 33(12):2744–2749. Epub 2002/12/07
17. Lind P, Hedblad B, Stavenow L, Janzon L, Eriksson KF, Lindgarde F (2001) Influence of plasma fibrinogen levels on the incidence of myocardial infarction and death is modified by other inflammation-sensitive proteins: a long-term cohort study. Arterioscler Thromb Vasc Biol 21(3):452–458. Epub 2001/03/07
18. Engstrom G, Lind P, Hedblad B, Wollmer P, Stavenow L, Janzon L et al (2002) Lung function and cardiovascular risk: relationship with inflammation-sensitive plasma proteins. Circulation 106(20):2555–2560. Epub 2002/11/13
19. Holst AG, Jensen G, Prescott E (2010) Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. Circulation 121(17):1896–1903. Epub 2010/04/21
20. Verhovsek M, Douketis JD, Yi Q, Shrivastava S, Tait RC, Baglin T et al (2008) Systematic review: D-dimer to predict recurrent disease after stopping anticoagulant therapy for unprovoked venous thromboembolism. Ann Intern Med 149(7):481–490 W94. Epub 2008/10/08
21. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR (1995) Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 345(8943):152–155. Epub 1995/01/21
22. Legnani C, Cini M, Cosmi B, Poggi M, Boggian O, Palareti G (2004) Risk of deep vein thrombosis: interaction between oral contraceptives and high factor VIII levels. Haematologica 89(11):1347–1351. Epub 2004/11/09
23. O'Donnell J, Mumford AD, Manning RA, Laffan M (2000) Elevation of FVIII: C in venous thromboembolism is persistent and independent of the acute phase response. Thromb Haemost 83(1):10–13. Epub 2000/02/11
24. Lowe G, Rumley A, Woodward M, Vessey M (2000) C-reactive protein, idiopathic venous thromboembolism and hormone replacement therapy. Thromb Haemost 84(4):730–731. Epub 2000/11/01
25. Austin H, Hooper WC, Lally C, Dilley A, Ellingsen D, Wideman C et al (2000) Venous thrombosis in relation to fibrinogen and factor VII genes among African-Americans. J Clin Epidemiol 53(10):997–1001. Epub 2000/10/12
26. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP (1994) Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case–control study of plasma levels and DNA polymorphisms—the Leiden Thrombophilia Study (LETS). Thromb Haemost 71(6):719–722. Epub 1994/06/01
27. Jude B, Amrouni N, Deguine I, Marey A, Asseman P, Watel A et al (1992) Coupled D dimer and fibrinogen levels during thrombolytic therapy of venous thromboembolism. Thromb Res 65(3):457–462. Epub 1992/02/01
28. Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D et al (2005) Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. Arterioscler Thromb Vasc Biol 25(6):1231–1236. Epub 2005/03/26
29. Wakefield TW, Strieter RM, Downing LJ, Kadell AM, Wilke CA, Burdick MD et al (1996) P-selectin and TNF inhibition reduce venous thrombosis inflammation. J Surg Res 64(1):26–31. Epub 1996/07/15
30. Jougasaki M, Ichiki T, Takenoshita Y, Setoguchi M (2010) Statins suppress interleukin-6-induced monocyte chemo-attractant protein-1 by inhibiting Janus kinase/signal transducers and activators of transcription pathways in human vascular endothelial cells. Br J Pharmacol 159(6):1294–1303. Epub 2010/02/09
31. Chamberlain AM, Folsom AR, Heckbert SR, Rosamond WD, Cushman M (2008) High-density lipoprotein cholesterol and venous thromboembolism in the Longitudinal Investigation of Thromboembolism Etiology (LITE). Blood 112(7):2675–2680. Epub 2008/07/11
32. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C et al (2011) External review and validation of the Swedish national inpatient register. BMC Public Health 11:450. Epub 2011/06/11
33. Tamariz L, Harkins T, Nair V (2012) A systematic review of validated methods for identifying venous thromboembolism using administrative and claims data. Pharmacoepidemiol Drug Saf 21(Suppl 1):154–162. Epub 2012/01/25

Paper IV