MD THESIS

VENOUS THROMBOSIS AND WOMEN'S HEALTH: IDENTIFICATION OF RISK FACTORS AND LONG TERM EFFECTS

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MRCPath (1999)

CONTENTS

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1. McColl MD, Ramsay JE, Tait RC, Walker ID, Greer IA, McCall F, Conkie JA, Carty MJ. Risk factors for Pregnancy Associated Venous Thromboembolism. Thrombosis and Haemostasis 1997; 78: 1183-1188.

2. McColl MD, Ramsay JE, Tait RC, Walker ID, McCall F, Conkie JA, Carty MJ, Greer IA. Superficial Vein Thrombosis: Incidence in association with Pregnancy and Prevalence of Thrombophilic abnormalities. Thrombosis and Haemostasis 1998; 79: 741-742.

3. McColl MD, Walker ID, Greer IA. Risk factors for Venous Thromboembolism in Pregnancy. Current Opinion in Pulmonary Medicine 1999 (review) ; 5: 227-232.

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5. McColl MD, Ellison J, Reid F, Tait RC, Walker ID, Greer IA. Prothrombin 20210G→A, MTHFR C677T mutations in women with venous thromboembolism associated with pregnancy. British Journal of Obstetrics and Gynaecology 2000 (in press).

6. McColl MD, Sattar N, Ellison J, Tait RC, Walker ID, Packard CJ, Greer IA. Lipoprotein (a), cholesterol and triglycerides in women with venous thromboembolism. Blood Coagulation and fibrinolysis 2000 (in press).

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CHAPTER 1

INHERITED AND ACQUIRED RISK FACTORS FOR VENOUS THROMBOEMBOLISM

1.1 SUMMARY

1.1.1 Venous thromboembolism (VTE) remains an important cause of morbidity and mortality, with well-recognised inherited and acquired risk factors. Women are particularly at risk of this condition because of pregnancy, the use of combined oral contraceptive pills, and hormone replacement therapy. A number of long-term sequelae may occur following an episode of VTE in women, such as development of the post-thrombotic syndrome, restriction of future contraceptive choice, bleeding events related to anticoagulants, and recurrence of thrombosis. In recent years there has been a marked increase in our understanding of genetic risk factors important for the development of VTE, and of their interaction with acquired risks. In particular, the description of the factor V Leiden and (more recently) prothrombin 20210A mutations have highlighted common genetic variations that are associated with a modest increased risk of thromboembolism. These genetic mutations may also be associated with other conditions that are associated with coagulation activation, such as preeclampsia. Thrombophilia is a rapidly expanding and complex field, and undoubtedly new abnormalities will be described within the next few years.

1.2 INTRODUCTION

1.2.1 Venous Thromboembolism (VTE) is a common condition, particularly in women where pregnancy, the combined oral contraceptive pill (COCP) and hormone replacement therapy (HRT) are recognised risk factors. In particular, recent publicity surrounding the risk of VTE in users of third generation combined oral contraceptives (1-3) and users of HRT (4-7), together with an increased number of deaths from pulmonary embolism during pregnancy (8), has rendered this an important area of research.

1.2.2 It has been reported that between the ages of 15-39, women have a five-fold higher risk of experiencing an episode of VTE compared with arterial thrombosis (9). Women aged 15-24 years have a reported annual incidence of VTE of 0.2 per 1000; between the ages of 25-39 years, this rises to 0.4 per 1000 per year; and between 40-54 years to 0.74 per 1000 per year (9). These figures compare with annual incidences of acute myocardial infarction in women of (for the same age categories) 0.007, 0.19 and 1.76 (9).

1.2.3 VTE is associated not only with mortality from pulmonary embolism, but also significant morbidity, including complications of therapy for VTE, recurrence of thrombosis, development of the post-thrombotic syndrome, and reproductive issues (pregnancy, restriction of future contraceptive choice). Anticoagulants are associated with several potential risks. Bleeding events are increased in patients on heparin and those on warfarin. The incidence of major bleeding events in those prescribed warfarin is estimated at around 2% per year (10,11). The incidence of fatal bleeding events appears less than 1% per year (10,11). Prolonged heparin therapy is also associated

with the development of osteoporosis, which appears to occur commonly, although clinical fractures appear uncommon (<5%) (12-15). Heparin-induced thrombocytopaenia is a serious condition, paradoxically associated with venous and / or arterial thrombosis (16). It is an uncommon complication of heparin therapy that relates to the development of antibodies to the heparin-platelet factor 4 complex (16,17).

1.2.4 Young women who suffer an episode of VTE have many years to develop the post-thrombotic syndrome (PTS). This syndrome consists of continuing or developing symptoms and signs in the previously thrombosed leg, such as pain, swelling, discoloration, varicose veins, eczema, and ulceration. It is likely to relate to venous valvular damage with resultant venous hypertension following the episode of thrombosis, and appears to increase in incidence as time from thrombotic event increases (18). Published data on the prevalence of PTS in unselected groups of patients with previous VTE suggest it is a common clinical problem (19-21). However, there are few data on the prevalence of this condition in young women.

1.2.5 There are no data to assess the possible influence of a previous episode of VTE on future contraceptive choice, the decision to have future pregnancies, or access to hormone replacement therapy. Most family planning physicians would advise against the use of combined oral contraceptives in women with a history of VTE, since these preparations are associated with an increased risk of VTE (1-3,22). Some women may opt for less reliable forms of contraception with the risk of unplanned pregnancies, which in turn are associated with an increased thrombotic risk. Others may decide that they do not wish to risk recurrence of VTE through future pregnancy, and choose sterilisation, or vasectomy in their partner. In the longer term, women with a previous

episode of VTE may face difficulties with access to hormone replacement therapy due to its association with VTE. This aspect of the long-term effects of VTE has not been explored.

1.3 NORMAL HAEMOSTASIS

In order to understand possible mechanisms of thrombosis, a brief outline of the coagulation cascade and natural anticoagulant pathways is given below.

1.3.1 The Coagulation Cascade

1.3.1.1 Virchow's triad states that thrombosis may occur as a result of local abnormalities in the vessel wall, blood stasis, or changes in the composition of blood favouring thrombus formation. There has recently been a tremendous amount of research in elucidating the causes of the third part of this triad. The coagulation cascade consists of a series of enzymatic reactions whose primary goal is the production of fibrin and platelet activation. These enzymatic reactions occur on the surface of platelets, which form a primary haemostatic plug at the site of tissue damage. Platelets adhere to subendothelium via binding of Von Willebrand factor to the platelet glycoprotein Ib-V-IX complex (reviewed in (23)). Platelet-platelet interaction is achieved by fibrinogen binding to the IIb-IIIa platelet glycoprotein. Central to the coagulation cascade is the generation of thrombin. Much research has been directed toward understanding the initiation of the coagulation cascade in-vivo. It is now generally accepted that the extrinsic pathway (involving tissue factor and FVII) is the principal mechanism for initiation of coagulation activation and that the contact system may contribute very little to the initiation of this process (reviewed in (24) and (25)). The principal role of the contact pathway is the amplification of the coagulation process following initiation by the extrinsic pathway. Tissue factor, (TF; also known as

thromboplastin or CD142), the physiologic initiator of blood coagulation, is an integral membrane protein found on the surface of a variety of cell types normally located outside the vasculature (26). TF is expressed particularly by macrophages and smooth muscle cells at sites of atheroma and is likely to be of prime importance in the thrombotic process observed in unstable angina (27) and myocardial infarction (28). It has recently been demonstrated that vascular smooth muscle cells may be stimulated to produce TF which is identified free (although transiently) at the cell surface, in larger pools encrypted at the cell surface, and also intracellularly (29). It is hypothesised that the relatively short-lived surface expression of TF may be critical for limiting the thrombotic potential of intact cells whereas the encrypted surface and intracellular pools may provide a rich source of TF under conditions associated with cellular damage, such as during atherosclerotic plaque rupture (29). In response to vascular injury or thrombotic stimuli, TF binds FVII or FVIIa, enhancing its enzymatic activity considerably (26). This in turn generates FXa and thrombin; thrombin generation cleaves the serine proteases FVIIIa and FVa from their zymogens FVIII and FV respectively. The TF-FVIIa complex also cleaves FIXa from FIX; after generation of thrombin and FVIIIa, the FIXa-FVIIIa complex (tenase complex) may generate further thrombin via liberation of FXa (via the prothrombinase complex). The generation of thrombin appears central to the regulation of haemostasis, since it may both potentiate its own production (by cleavage of FVIIIa and FVa) at sites of tissue damage, and downregulate production of further thrombin at sites distant from tissue damage (via activation of the protein C anticoagulant pathway) (figure 1.1).

1.3.2 Natural Anticoagulants

1.3.2.1 The natural anticoagulants antithrombin, protein C and protein S are of prime importance in limitation of thrombosis to sites of tissue damage in-vivo. Antithrombin is a serine protease inhibitor (SERPIN), synthesised in the liver, which has widespread inhibitory activity on the coagulation proteases, particularly thrombin (30). The action of antithrombin is accelerated greatly by binding to heparin, which alters its structural conformation (30).

1.3.2.2 Protein C is a vitamin K-dependent plasma zymogen of a serine protease, which plays a central role in restraining the coagulation cascade (31). The known components of the protein C anticoagulant pathway are thrombomodulin, which, in complex with thrombin, activates the plasma zymogen protein C, to the anticoagulant activated protein C (APC) (32). APC forms a complex with plasma protein S, which assembles on membrane surfaces to inactivate factors Va and VIIIa (32). Recently, a new protein C receptor has been identified on the surface of endothelial cells (the endothelial cell protein C receptor, EPCR) which appears to augment protein C activation (32). The vascular distribution of the EPCR is such that it appears in high concentration in large arterial vessels and lower concentrations in the microvasculature (32). The precise role of the EPCR, and the reason for its distribution, remain to be demonstrated.

1.3.2.3 Protein S is also a vitamin K-dependent protein that originates predominantly from hepatocytes (33). It is a cofactor for APC. In the circulation 60% of protein S is associated with C4b-binding protein, the resulting complexes being devoid of APC cofactor activity. The remaining free protein S has cofactor activity for APC. FVa inactivation by APC occurs in a biphasic reaction, with rapid cleavage at Arg 506

followed by slower cleavage at Arg 306. The first cleavage only partially affects FVa activity, whereas full inactivation occurs after the second cleavage at Arg 306. It appears that protein S predominantly stimulates APC mediated cleavage at the second site (Arg 306) (33). FVIIIa inactivation by APC is increased by both protein S and FV (but not FVa), the two proteins acting synergistically as cofactors for the reaction (33).

1.3.2.4 Tissue factor pathway inhibitor (TFPI) is the major inhibitor of the tissue factor-FVIIa initiation of coagulation. TFPI is synthesised predominantly in vascular endothelium and has a complex distribution (34). A major pool of TFPI (50-80% of the total intravascular pool) is normally bound to the vessel wall, although it may be released into plasma following injection of heparin (34). The remaining TFPI pool circulates predominantly (~80%) in association with lipoproteins (34).

1.3.3 Markers of Coagulation Activation

1.3.3.1 Coagulation activation peptides are released from their zymogen molecules but, in contrast to the corresponding enzyme, they have a relatively longer half-life and are therefore easier to measure. This makes it possible to study coagulation activation in-vivo. The most extensively studied activation peptides are prothrombin fragment 1+2, fibrinopeptide A and D-Dimers (fibrin and fibrinogen degradation products). Prothrombin fragments 1+2 provide information concerning the amount of thrombin generated whereas fibrinopeptide A indicates the extent to which free thrombin exerts its activity on fibrinogen (35). D-Dimers represent activation of the fibrinolytic system and are particularly useful in the exclusion of venous thromboembolism (36-38).

1.4 RISK FACTORS FOR VTE (TABLE 1.1)

1.4.1 Inherited Risk Factors for VTE

Antithrombin, Protein C and Protein S deficiency

1.4.1.1 Until relatively recently, established thrombophilic abnormalities were found infrequently in those with VTE (table 1.2). For example, collectively deficiencies of the natural anticoagulants antithrombin, protein C or protein S are found in around 5% of patients with a first episode of VTE, although this figure increases in those with recurrent events (39). Mutations affecting the antithrombin gene result in either type I or type II deficiencies (30). Type I antithrombin deficiency is a quantitative deficiency with reduction to approximately 50% of antigen and activity levels. In type II deficiencies, the total antigen level is normal (or near normal) with a reduction in activity level. This may be caused by mutations that produce variants with functional defects primarily affecting the heparin binding site or the reactive site (30). Type I antithrombin deficiency appears rare in the healthy population. In a study of blood donors in the West of Scotland type I antithrombin deficiency was detected in around 1/5000 individuals (40). Type II antithrombin deficiency was detected in 1.45/1000 (40).

1.4.1.2 More than 160 mutations have been identified in the protein C gene (reviewed in (31)). Each mutation leads to absent or defective protein C and therefore type I (quantitative) and type II (functional) defects are observed. Protein C deficiency appears not uncommon in the general population, estimated at approximately 1/500 individuals (41,42). Deficiency of protein S is divided into three subtypes: type I (low total and free protein S), type II (normal free protein S with reduced APC cofactor activity), and type III (low free protein S with normal or near normal total protein S)

(33). Sixty-nine mutations have been associated with type I protein S deficiency (33). Mutations which produce a type III phenotype appear to influence protein S binding to C4b-binding protein (33). Protein S levels are also lower during pregnancy (43) and in users of the combined oral contraceptive pill (44). Currently the prevalence of protein S deficiency in the general population is unknown.

1.4.1.3 The vast majority of individuals who are identified with deficiencies of antithrombin, protein C or protein S are heterozygous for a genetic mutation in the relevant gene. Deficiencies of antithrombin, protein C and protein S are inherited in an autosomal dominant pattern, such that the offspring of an affected parent have a 50% chance of inheritance. Homozygous deficiencies are rare. Homozygous antithrombin deficiency usually results in either fetal death or such severe thrombotic manifestations that death occurs early in childhood (9). Homozygous deficiencies of protein C or protein S are rarely reported and generally associated with a severe thrombotic diathesis which requires lifelong anticoagulation, sometimes with regular protein C or protein S replacement (45-51).

Activated Protein C Resistance and Factor V gene Mutations

1.4.1.4 In 1994, Dahlback found that a significant proportion (around 20%) of patients with a first episode of VTE demonstrated a poor anticoagulant response to activated protein C added in-vitro to their plasma (APC resistance) (52). Bertina and colleagues subsequently demonstrated that such patients had a single point mutation in the gene encoding coagulation factor V (factor V Leiden, FVL) (53). This mutation results in an Arg → Gln substitution at amino acid position 506 of the factor V protein. Factor V is converted to the active cofactor, Va, during assembly of the prothrombinase complex.

Generation of thrombin is limited by cleavage of factor Va by APC at Arg 506, followed by cleavage at Arg 306 and Arg 679 (54). Bertina showed that the mutated factor Va was relatively resistant to degradation by APC (53). Individuals with FVL, even if asymptomatic for VTE, appear to have excessive generation of thrombin as evidenced by elevated markers of coagulation activation (55,56) and consequently a relative thrombotic tendency.

1.4.1.5 The APC sensitivity ratio (the ratio of the APTT performed in the presence and absence of exogenously added APC) can be used as a screening test for the FVL mutation (57). However, the APC sensitivity ratio cannot be used to identify those carrying the mutation if the patient is on oral anticoagulants or heparin, or if the patient has a lupus anticoagulant / elevated anticardiolipin antibodies (57). In these patients, a modified APC test using plasma depleted of coagulation factor V must be used to screen for FVL. The unmodified APC test is also unreliable during pregnancy (58) or in other situations where plasma FVIII is elevated. Acquired APC resistance (an abnormal unmodified APC sensitivity ratio in the absence of the FVL mutation) is seen variably during pregnancy (43,58), in those with lupus anticoagulant activity (59) and in those on the combined oral contraceptive (60). It has recently been reported that users of hormone replacement therapy have lower mean APC-sensitivity ratios compared with post-menopausal non-users (61). It had been noted that there was quite marked variability in APC resistance ratios in those with the FVL mutation, which was considered to be related to variations in levels of FVIIIc. However, recent research has identified a factor V genetic polymorphism (the HR2 haplotype) which appears to contribute to the phenotype of APC resistance (62). Individuals who are doubly heterozygous for both the FVL mutation and the HR2 haplotype appear to have

consistently lower APC sensitivity ratios than individuals with FVL alone (62). This may be clinically significant, since the risk of VTE appears to increase as the APC sensitivity ratio decreases (63). Very recently, a new mutation in the factor V gene has been described which is associated with both APC resistance (Arg 306 → Thr, factor V Cambridge) and an increased risk of VTE (64). However, this mutation appears very rare in thrombotic cohorts (1/602 patients with an episode of VTE in the study by Williamson et al (64)), and as such it is not included in thrombophilia screening in most thrombosis centres.

1.4.1.6 There have been a considerable number of studies that have confirmed that individuals with the FVL mutation are at increased risk of VTE (reviewed in (39)). The risk of VTE appears increased 5-10 fold in those heterozygous for the mutation and approximately 80 fold in homozygotes (65). Those homozygous for FVL may experience thrombosis at a younger age than heterozygotes (65). FVL shows a marked variation in prevalence within different populations, being clearly centred in Europe and, with a few exceptions, relatively rare outside Europe and North America (66). For example, in the UK the prevalence of heterozygous FVL is estimated at 3-5% of the general population. A similar figure is found in Germany. In Greece and Sweden the prevalence is much higher, approaching 12% in some areas. In South East Asia it appears much lower (<1%) and it is almost unknown in Africa. Homozygotes are uncommon, though not rare, occurring in approximately 0.2% of healthy individuals in Germany (66).

1.4.1.7 The FVL mutation has also been studied as a candidate risk factor for arterial thrombosis. However, a relationship to arterial disease appears less well established.

Some researchers have demonstrated an association between FVL and neonatal, childhood and juvenile (<50 years) stroke (67-72). However, not all have found such an association (73,74). FVL has not been established as a risk factor for stroke or myocardial infarction in unselected adult populations (55,75-82). Rather, most reports appear to highlight a risk in selected adult groups, such as young female smokers (83,84).

The Prothrombin 20210 G→A Gene Mutation

1.4.1.8 In November 1996, Poort et al described a common variation in the prothrombin gene that was found in 6% of Dutch patients who presented with a first episode of VTE and in 2% of healthy controls (85). Researchers in other countries have confirmed these findings (86-91). This mutation, a result of a G to A substitution at position 20210 of the 3' untranslated region of the gene, is associated with elevated mean plasma prothrombin levels. The precise mechanism for the elevation of prothrombin levels, or indeed for the increased risk of VTE, is as yet unknown. One study has reported that markers of coagulation activation (prothrombin fragments F1+ F2) in carriers of the prothrombin variant are not higher than in controls, although endogenous thrombin potential was reportedly higher (92). The prothrombin 20210A mutation has been reported in 1.2-2.6% of the healthy population in the UK. (74,87,88). It appears to increase the risk of VTE around 2-4 fold, somewhat less than that posed by FVL. Since both FVL and the prothrombin mutations are not uncommon, individuals with both mutations are occasionally found and appear to have a significantly higher thrombotic risk than those with either mutation alone (93-95). There is emerging evidence that the prothrombin mutation may also be a risk factor for arterial thrombosis in adults, again in selected patient groups (84,86,96). In particular, a

recent large study of selected patients with juvenile stroke (predominantly those aged 15-50 years) has demonstrated a significantly increased risk of stroke in carriers of the prothrombin G→A mutation (97). However, further research is required in this area before widespread screening for this mutation is undertaken in those with arterial disease.

1.4.1.9 There are currently few data on the racial prevalence of the prothrombin mutation, although a recent study has suggested a very low prevalence in non-Caucasians (98). It seems clear that this mutation is not uncommon in those with VTE and many thrombosis centres now test for this as part of first line investigation of possible thrombophilia. Unlike the FVL mutation, which can be detected using the APC sensitivity ratio, there is currently no screening test for the prothrombin gene mutation, and hence detection relies upon polymerase chain reaction techniques.

1.4.2 Mixed Genetic and Acquired Risk Factors for VTE

Hyperhomocysteinaemia

1.4.2.1 Elevation of plasma homocysteine appears to confer an increased risk of both venous and arterial thrombosis (99-107). Two major pathways regulate Homocysteine metabolism: remethylation to methionine, and trans-sulfuration to cysteine (reviewed in (107) (figure 1.2). The conversion of homocysteine to methionine occurs through a vitamin B12-dependent remethylation step involving 5-methyltetrahydrofolate (107). 5-methyltetrahydrofolate, the main form of circulating folate, is formed from 5,10 methylenetetrahydrofolate by the enzyme methylenetetrahydrofolate reductase (MTHFR) (107). In the trans-sulfuration pathway, homocysteine is converted to cystathionine in a reaction which is catalysed by the enzyme cystathionine beta

synthase (CBS) (107). Recently, Frosst described a C→ T mutation at position 677 in the MTHFR gene, which is associated with a reduced enzyme activity and enzymic thermolability (108). Individuals with the homozygous C→T MTHFR (thermolabile) variant have higher homocysteine levels for an equivalent folate level compared to wild type MTHFR subjects (109-111). Subjects homozygous for the C→T MTHFR variant that are folate deficient have significantly elevated fasting levels of homocysteine and may be at increased risk of both VTE and arterial thrombosis (110). Homozygosity for the variant MTHFR gene is common in the general population (11% in the UK) (112). However, the majority of studies which have examined the prevalence of homozygous C→T MTHFR subjects with VTE have failed to demonstrate an increased risk, suggesting that folate deficiency may be an important interacting factor in those who develop VTE (113,114).

1.4.3 Other Coagulation Abnormalities Predisposing to VTE

1.4.3.1 A considerable proportion (10-15%) of individuals that present with VTE are noted to have persistently elevated levels of plasma FVIII (115). Elevation of FVIII (levels > 150 iu/dl) is associated with a 4-5 fold increase in risk of VTE (115). However, FVIII gene studies have failed to demonstrate an associated mutation (116). This elevation in FVIII appears independent of underlying inflammation (117). Abnormalities of the thrombomodulin gene have also been reported in some patients with VTE; however, such defects appear rare in thrombotic cohorts (118). Abnormalities of the fibrinolytic pathway, such as plasminogen deficiency, are considered by some researchers to be a risk for development of VTE (reviewed in (119)). However, plasminogen deficiency has been reported as a not uncommon finding in healthy individuals who are thrombosis-free, and many do not regard this as a

significant thrombotic risk factor (119,120).

1.4.4 Acquired Risk Factors for VTE

Antiphospholipid Syndrome

1.4.4.1 The antiphospholipid syndrome (APS) may be defined as the occurrence of thrombosis (venous and / or arterial), recurrent (3 or more) miscarriage and / or thrombocytopaenia in association with evidence of a lupus anticoagulant or anticardiolipin antibody (reviewed in (121)). The term antiphospholipid antibody refers both to a lupus anticoagulant or the presence of an anticardiolipin antibody. The APS may be primary, or occur in association with connective tissue disorders, lymphoproliferative disorders, infection or complicate drug therapy (121). Despite their in-vivo thrombotic tendency, lupus anticoagulants act as in-vitro coagulation inhibitors, with detection being dependent upon prolongation of a clotting time with demonstration of inhibitory activity on mixing tests, and complete or partial correction of clotting times with addition of phospholipid (122). The most widely used tests for detection of lupus anticoagulants are the activated partial thromboplastin time, dilute Russell's viper venom time (DRVVT), and kaolin clotting time (122). Anticardiolipin antibodies (ACA) are detected using ELISA techniques. A recent epidemiological study demonstrated that individuals with moderate or high (>40 U) titre IgG ACA appear at particularly increased risk of thrombotic events compared to those with lower titres or with other classes of ACA (123). Antiphospholipid antibodies (lupus anticoagulant and / or anticardiolipin antibodies) have complex effects on haemostasis, binding to beta-2-glycoprotein I (β2-Gp1) (124), prothrombin (125,126), protein C (127-129), protein S (125,129), thrombomodulin (130), endothelium (131-133) and platelets (134). Clearly antiphospholipid antibodies are heterogeneous, with no single

mechanism of thrombosis proven. Antiphospholipid antibodies may be transient, and persistently positive tests must be demonstrated in an appropriate clinical setting in order to make a diagnosis of antiphospholipid syndrome. Guidelines on the investigation and management of the antiphospholipid syndrome are in preparation (135).

Pregnancy

1.4.4.2 In women, it has long been recognised that pregnancy is a risk factor for VTE. The recent Report on Confidential Enquiries into Maternal Deaths in the UK (1998) has highlighted death from pulmonary embolism as the major cause of maternal mortality in the United Kingdom (8). VTE associated with pregnancy will be considered in greater detail in Chapters three and four.

Combined oral contraceptives

1.4.4.3 Combined oral contraceptive pills (COCP) were reported as a possible risk factor for VTE in the mid-1960's. Recent research has identified that the newer third-generation preparations (those containing the progestogens desogestrel or gestodene) appear to pose a greater risk of VTE than earlier second-generation preparations (those containing the progestogen levonorgestrel) (1-3). The increased risk of VTE in users of second generation COCP is reported at 3.5 (95% CI 2.6-4.7), compared with 9.1 (4.9-17) for desogestrel-containing preparations and 9.1 (4.9-16.7) for gestodene-containing preparations (1). Women with a body mass index (BMI) greater than 25 kg/m^2 appear to have a higher risk of COC related VTE than those with a lower BMI (2). Third-generation COCP, like second generation preparations, contain 30-35 μg ethinyloestradiol. They have been reported to have significant effects on plasma levels

of natural anticoagulants, procoagulants and the fibrinolytic system (table 1.3) (136). These effects, if considered collectively, appear to promote a relative thrombotic tendency, and may explain the observed increased risk of VTE. Doubt was cast on data showing a higher risk of VTE in users of third-generation COCP compared to second-generation pills, since there appeared to be no plausible physiological explanation for the clinical findings. However, recent research has shown that third-generation COCP appear to produce a significant degree of resistance to activated protein C (comparable to that seen in heterozygous carriers of the factor V Leiden mutation) (60), providing a possible mechanism for these clinical observations.

Hormone replacement therapy

1.4.4.4 Hormone replacement therapy (HRT) has been demonstrated to have significant effects on lipid metabolism, with a reduction in LDL-cholesterol and increase in HDL-cholesterol (137). Users of HRT (both oestrogen-only and oestrogen-progestogen preparations) also experience an overall reduction in plasma fibrinogen concentration (137). These effects may explain, at least in part, the cardioprotective effect that has been demonstrated in users. Users of HRT have also been demonstrated to have significantly lower levels of antithrombin and protein S, and increased levels of coagulation activation markers (137). HRT has recently been reported to be a risk factor for VTE (4-7). The relative risk appears to be around 2-3 fold in current users. Estimates place the extra risk of VTE in women using HRT at around 1 event per 5000 users per year (4,7). The mechanism for an increased risk of VTE is unknown, although the effects on levels of natural anticoagulants may be important. In considering the use of HRT, however, the relatively small risk of VTE should be weighed against the potential benefits, such as a reduction in cardiovascular events and osteoporosis,

particularly as VTE has a low mortality compared with ischaemic heart disease.

Surgery

1.4.4.5 Surgery and immobility are well-recognised periods of increased risk of VTE (138,139). In particular, orthopaedic and neurosurgery are considered high-risk for subsequent development of VTE (138). There are considerable data demonstrating that low molecular weight heparins (LMWH) may significantly reduce the risk of VTE in such high-risk patients with no apparent increased risk of bleeding compared to unfractionated heparins (reviewed in (138)). However, despite thromboprophylaxis with LMWH, some 15-20% of such individuals develop VTE (reviewed in (140)).

Serious medical illness

1.4.4.6 Other hospital inpatients, for example those on medical wards with cardiac failure, nephrotic syndrome or infection are also at increased risk of VTE (141). Most hospitals now have protocols to help prevent this complication, the development of which are aided by publications such as those by the Scottish Intercollegiate Guidelines Network (SIGN) (142).

Malignancy

1.4.4.7 Interest has recently been given to the observation that individuals with cancer are at increased risk of VTE. VTE may be the presenting feature of a variety of malignancies, and not uncommonly develops in those with clinically overt cancer (143). It is also recognised that patients with cancer are at increased risk of postoperative or immobility-related VTE compared to postoperative or immobilised patients without cancer (144). Data suggest that cancer cells may express both tissue

factor and cancer procoagulant, a cysteine protease which may directly activate FX (reviewed in (143)). In addition, fibrinogen levels and platelet count are elevated in 30-40% of patients with cancer (143). Coagulation activation markers, such as prothrombin fragments 1+2, thrombin-antithrombin complexes and D-Dimers are also elevated in a significant proportion of patients with cancer (143). A number of studies have demonstrated that individuals who present with objectively confirmed VTE are significantly more likely to develop cancer within 6-12 months than those who present with possible VTE subsequently excluded by objective testing (reviewed in (145)). It has also been shown that patients who present with idiopathic VTE are significantly more likely to develop cancer (around 10%) than those in whom VTE is associated with a recognised precipitant (145). Controversy surrounds the question of screening for occult malignancy in those who present with idiopathic VTE.

1.4.4.8 There is current interest on the influence of low molecular weight heparins on tumour cell biology (146). LMWH have been demonstrated to prolong survival in subjects with cancer compared to unfractionated heparins (144), stimulating interest on the effect of LMWH on tumour growth and development. Tissue factor expression by tumour cells may be involved in tumour angiogenesis (147), providing a possible mechanism through which anticoagulant therapy may influence tumour growth.

1.5 GENETIC - ENVIRONMENTAL INTERACTIONS IN THE DEVELOPMENT OF VTE

1.5.1 It is apparent that most subjects with inherited thrombophilia develop VTE only when additional prothrombotic triggers are posed. In certain situations the combination of inherited thrombophilia and an additional factor appear to synergistically increase the risk of VTE. For example, it is known that the FVL mutation increases the risk of VTE approximately 5-10 fold (65). The combined oral contraceptive pill (COCP) is associated with an approximate four-fold increased risk of VTE (1-3). Users of COCP who are also carriers of the FVL mutation appear to have an increased risk of VTE of around 35 fold (148). The risk is even higher in those with FVL who are users of third generation oral contraceptive pills (149). A recent study has shown that COCP induces APC resistance in subjects who do not possess FVL, and potentiates the degree of APC resistance in those with FVL, thus providing a biological explanation for these clinical observations (60). It is as yet unknown if there is a similar synergistic effect in users of hormone replacement therapy who are carriers of FVL. It is also recognised that individuals with deficiencies of natural anticoagulants are at increased risk of VTE if taking the COCP (150). At present there are no data regarding a possible interaction between the recently described prothrombin gene variant, the COCP or hormone replacement therapy.

1.6 THROMBOPHILIA AND ADVERSE PREGNANCY OUTCOME

1.6.1 In addition to the well-documented increase in risk of VTE, thrombophilic abnormalities have also been examined as possible risk factors for the development of adverse pregnancy outcomes, such as gestational hypertension, preeclampsia, placental abruption and fetal loss. Several studies have reported that women with the FVL mutation are at increased risk of severe preeclampsia (151-155). The MTHFR TT (thermolabile) genotype may also increase the risk of preeclampsia (152,156), perhaps because pregnancy may be accompanied by folate deficiency, which could lead to the development of elevated plasma homocysteine. There are also data indicating that FVL and the prothrombin mutation are risk factors for the development of gestational hypertension with or without proteinuria (157). A recent study, in addition to demonstrating a risk of severe preeclampsia, showed an increased risk of placental abruption in carriers of FVL and the prothrombin mutation, although the numbers were small (155). There is some evidence that natural anticoagulant deficiencies, and FVL, may be associated with fetal loss (158,159).

1.7 CONCLUSIONS

1.7.1 VTE poses a serious health risk, particularly to women who are at increased risk by virtue of pregnancy and hormonal preparations. Thrombophilic abnormalities are not uncommon in the general population and in some cases may interact synergistically with acquired risk factors to considerably heighten the risk of VTE. Since thrombophilic abnormalities are relatively common, it is increasingly recognised that those at greatest risk of VTE possess either multiple thrombophilias (such as both FVL and natural anticoagulant deficiencies (160-162), or FVL and prothrombin mutations (94,95)) or those with single thrombophilias in risk situations.

Table 1.1: Inherited and Acquired Risk Factors for VTE

Inherited factors	Inherited / Acquired	Acquired factors
Antithrombin deficiency	Hyperhomocysteinaemia †	Combined oral contraceptives
Protein C deficiency	Elevated FVIII ‡	Hormone replacement therapy
Protein S deficiency		Pregnancy
Factor V Leiden		Surgery
Prothrombin 20210A		Immobility
		Cancer
		Medical illness
		Antiphospholipid syndrome

† phenotypic expression appears to be a result of genetic susceptibility and acquired vitamin deficiency

‡ in subjects with VTE, it is uncertain if elevated FVIII is inherited or acquired

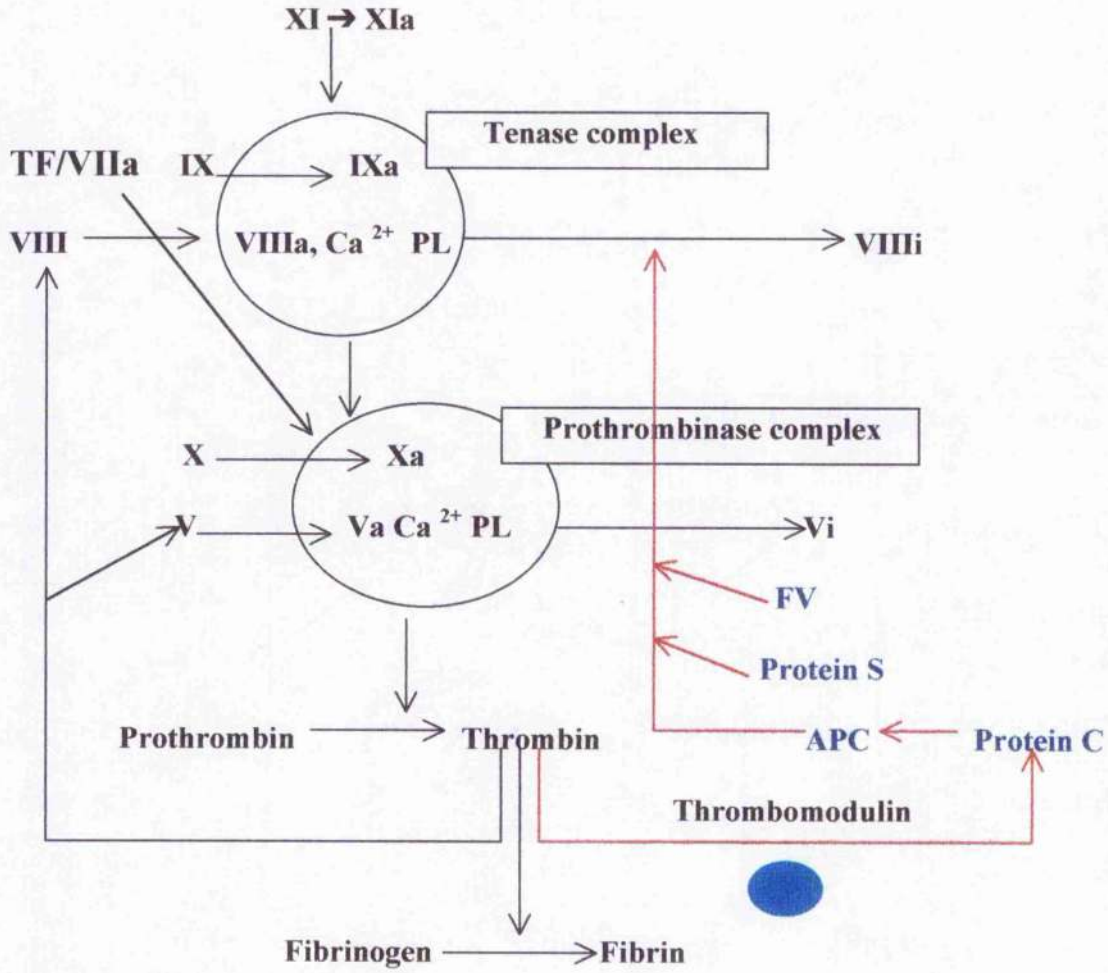
Table 1.2: Prevalence of Inherited Thrombophilia in Consecutive Subjects with a First Episode of VTE and in the General Population [adapted from Lane et al (39)]

Thrombophilia	General population (%)	Consecutive patients with first VTE (%)
Antithrombin deficiency	0.02	1
Protein C deficiency	0.2	3
Protein S deficiency	?	1-2
Factor V Leiden	2.2	20
Prothrombin 20210A	2.2	6-8

Table 1.3: Influence of Third Generation, Low Oestrogen Combined Oral Contraceptives on Various Haemostatic Parameters [from Kluft et al (136)].

Haemostatic Parameter	Increased	Decreased
Natural Anticoagulants	heparin cofactor II	protein S (total & free)
	protein C	APC ratio
		antithrombin
		C4b-BP
Procoagulants	Factors I, II, VII, VIII, IX, X,	
	XI	
	vWf	
Profibrinolytics	plasminogen	tissue plasminogen activator
	Factor XII	
Antifibrinolytics	Factor XIII	PAI-I
		CI-esterase inhibitor

Figure 1.1. The Coagulation Cascade and the Protein C (red / blue) Pathway.



XIa, IXa activated coagulation factors

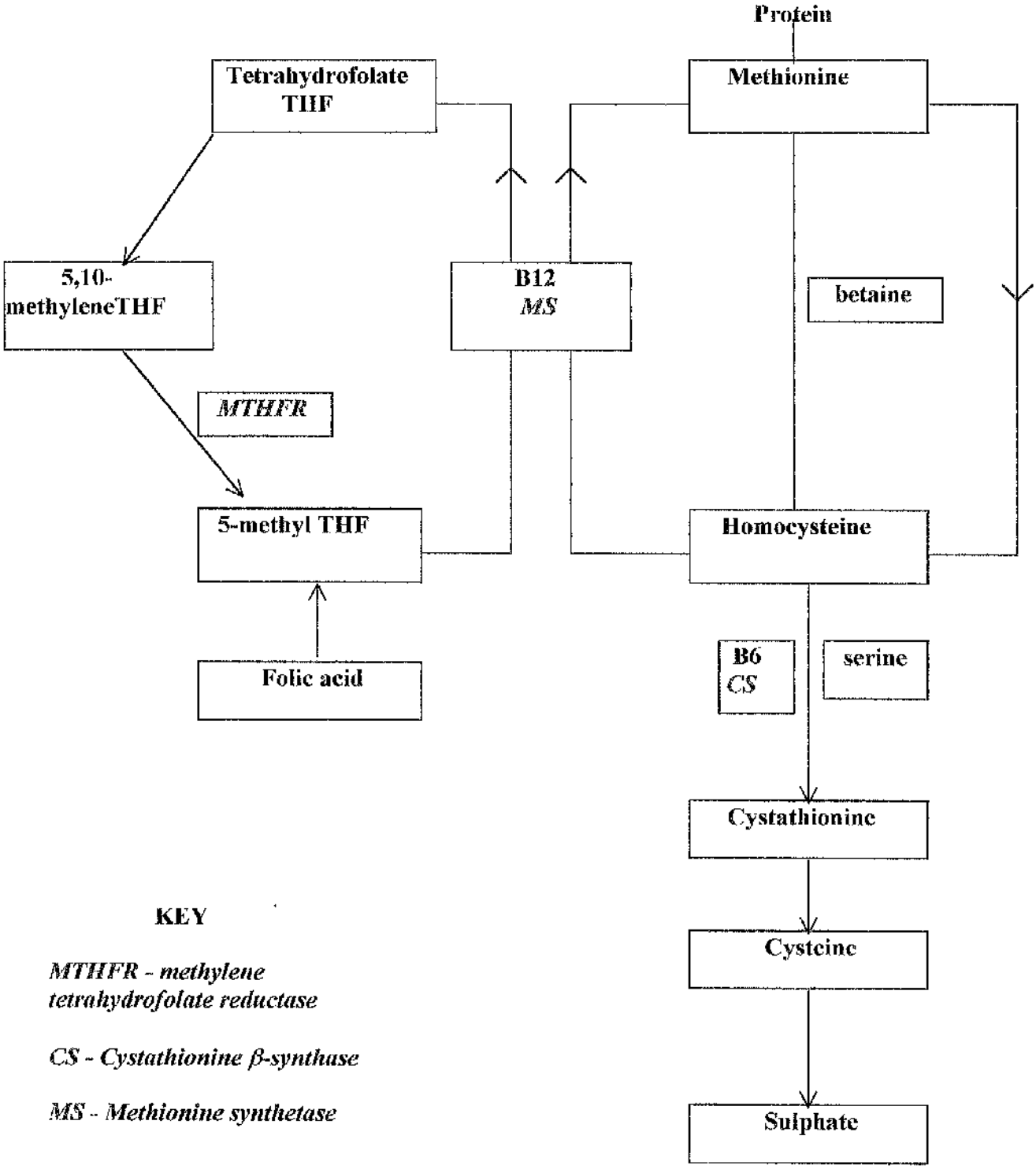
Vi, VIIIi inactivated coagulation factors

APC activated protein C

PL phospholipid

TF tissue factor

Figure 1.2. Methionine / Homocysteine metabolism



KEY

MTHFR - methylene tetrahydrofolate reductase

CS - Cystathionine β-synthase

MS - Methionine synthetase

CHAPTER 2

IDENTIFICATION OF RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN WOMEN IN THE WEST OF SCOTLAND

2.1 SUMMARY

2.1.1 Three hundred and twenty-two consecutive women aged 16-70 years who presented with symptomatic VTE were studied to determine precipitating factors for thrombosis. In 283 women this was a first episode of VTE and in 39 was a recurrent event. 187 presented with DVT, 116 with either definite or probable PE, and 19 with both DVT and PE. The 18 who died from PE (mean age 59.3 years, SD 11.4, n=18) were significantly older than those who survived PE (mean age 50.1 years, SD 14.5, n=117) ($p< 0.005$). Pregnancy was the most common precipitating factor for VTE, associated with 14% of all events and 33.6% of events under 40 years. Intravenous drug abuse was also a common trigger for VTE, associated with 13.7% of all objectively confirmed events and 33.6% of events in women under 40 years. A large number of clinically diagnosed DVT associated with IVDA were documented, suggesting that IVDA may be the most common triggering factor for DVT in our region. None of the IVDA cohort presented with symptomatic PE. Cancer, particularly of breast, colorectal, and lung, was also commonly associated with VTE, detected in 12.8% of the entire cohort and almost 25% of those aged over 50 years.

2.2 INTRODUCTION

2.2.1 Venous thromboembolism (VTE) remains an important cause of morbidity and mortality, particularly in women. This relates predominantly to female exposure to risk factors for VTE, such as pregnancy, the COCP, and HRT, in addition to other risks such as surgery, immobility and cancer. It must be stressed, however, that the absolute risk of VTE in pregnancy, or in users of the COCP or HRT, is low, and that in certain geographical areas other risk factors for VTE may be important. For example, in our area intravenous drug abuse (IVDA) is highly prevalent, with injection into the femoral vein a common mode of administration. It is generally acknowledged that such an injection technique may constitute a risk for development of deep vein thrombosis and / or pulmonary embolism. The contribution of IVDA to the development of VTE has thus far received little attention.

2.3 AIMS

2.3.1 To document risk factors for VTE in a cohort of consecutive females aged 16-70 years diagnosed with either deep vein thrombosis (DVT) and / or pulmonary embolism (PE) and treated at one of two Glasgow hospitals between 1993-1997.

2.4 MATERIALS AND METHODS

2.4.1 Consecutive females aged 16-70 years who had been diagnosed with DVT or PE and treated at one of two Glasgow hospitals (Glasgow Royal Infirmary University NHS trust or the Southern General NHS Trust) between 1993-1997 were the subject of study. Women who developed VTE related to pregnancy were also included, since both hospitals have associated maternity units. Subjects were identified using ICD9 and ICD10 (from 1996) coding data obtained from SMR1 returns to the local health board.

Women with DVT or PE related to pregnancy were identified from SMR2 data. Case-notes from all subjects were recalled and examined. Information extracted from the case-notes included patient demographics, diagnosis, technique used to establish diagnosis, and possible risk factors for VTE (see appendix 1). Only patients in whom the diagnosis was objectively confirmed were included. For the purposes of our analysis, the diagnosis of DVT was accepted if confirmed by compression or duplex Doppler ultrasound scanning or contrast venography (except in the cases associated with IVDA, see below). For PE, we accepted a high probability V/Q scan as definite evidence of PE. A diagnosis of probable PE was made where the V/Q scan was of intermediate probability and patients were treated with at least six months anticoagulation, or cases which were diagnosed on the basis of clinical, ECG, and x-ray findings and received six months anticoagulation. The case-note search identified a large number of women who were intravenous drug abusers (IVDA) who had developed apparent DVT. Many such cases were diagnosed clinically; this data is also presented. The case-notes from 41 women could not be located. These patients were not included in our analysis.

2.4.2 Statistical techniques

2.4.2.1 Normally distributed patient characteristics were assessed by using the Students t-test. p values are two-tailed.

2.5 RESULTS

2.5.1 Three hundred and twenty-two women were admitted with an episode of objectively confirmed VTE during the study period. In 283 women this was a first episode of VTE and in 39 was a recurrent event. 187 presented with DVT, 116 with either definite or probable PE, and 19 with both DVT and PE. The mean age of women with PE (50.0 years) was significantly higher than those with objectively confirmed DVT (41.3 years) ($p < 0.0001$). However, after excluding all cases of VTE related to IVDA, the difference in ages was not statistically significant (mean age of those with DVT 47.6 years, mean age of those with PE 50.0 years, p = NS). Of women with clinically isolated DVT, 103 presented with DVT in the left leg, 77 with right leg DVT and in 7 cases DVT was bilateral. 68 women had definite PE and 48 probable PE. 18 (5.5%) died during hospital admission from PE (table 2.1). 11 women were known to have subsequently died (5 from cancer, 1 from congestive cardiac failure, 1 from respiratory distress syndrome in an IVDA; in the remaining 4 women the cause of death was not available). 23 women had a recurrent thrombosis during the study period. Of this 23, thirteen were IVD abusers; 2 others originally presented with idiopathic VTE.

2.5.2 Risk factors for VTE are shown in table 2.2. Twenty-one percent (68/322) of all episodes had no identified precipitating factor. Pregnancy was the most frequent associated factor, identified in 14% (45/322) of all events. A further 13.7% (44/322) were associated with IVDA. Inclusion of all possible cases of DVT (objective and clinically diagnosed cases) associated with IVDA increased the total number of events related to IVDA to 82, representing 23% (82/360) of all events. Many women with possible DVT related to IVDA self-discharged from hospital before an objective test could be performed, or in some cases the attending clinician felt the diagnosis was

obvious and that further confirmatory tests were unnecessary. Of 82 women with possible VTE secondary to IVDA, all were treated with subcutaneous heparin. Only 2 were discharged on oral anticoagulants.

2.5.3 Malignancy was a common precipitating factor for VTE: 12.8% (41 women) had evidence of malignancy at presentation, or had developed a malignant process within twelve months of VTE (this was the case for one patient). Breast carcinoma was the most common tumour associated with development of VTE (table 2.3), accounting for 46% of all malignancies identified. Colorectal (15%) and bronchial (12%) carcinomas were the next most frequently identified tumours. Of six cases of VTE identified in association with colorectal carcinoma, 4 occurred following surgery for colectomy. The prevalence of cancer-related VTE increased with patient age (figure 2.1): between the ages 15-39 years there were no cancer-related VTE events among 130 VTE cases. This increased between 40-49 years (3/32, 9%), 50-59 years (15/64, 23%) and 60-70 years (23/96, 24%).

2.5.4 Twenty-one (6.5%) women developed VTE in association with synthetic oestrogen containing pills. 5 occurred in users of third (desogestrel or gestodene) generation pills, 3 in users of second generation pills, 5 in other oestrogen-containing preparations (all Dianette®, an oestrogen- containing preparation combined with an antiandrogen rather than a progestogen, used in the treatment of acne and hirsutism), and in 8 cases the preparation was not specified. 24/322 (7.5%) women were users of HRT at the time of VTE. In addition, two women who developed VTE related to surgery were users of HRT, and one woman who developed VTE whilst immobilised due to a fractured ankle was on HRT at time of event. In 25/27 cases, the HRT was an

oral preparation; in 2 cases the preparation was not specified. In most cases the duration of use of COCP or HRT was not specified in the case-notes.

2.5.5 Thirty-three (10.2%) women developed VTE within one month of surgery (this included 5 who also had cancer and 2 who were users of HRT). 10 occurred following orthopaedic surgery. In 22 (6.8%) women VTE was associated with immobility, 9 of which were related to plaster cast immobilisation of the lower limbs. 16 (5%) women had an associated medical illness predisposing to VTE, the most common of which were myocardial infarction / failure (7) and respiratory disease (3). Most women who developed VTE related to surgery, immobility or medical illnesses were over 50 years old (figure 2.2). Whilst only 4% of women had previous VTE as the only risk factor for the present event, 12.1% (39/322) of the entire cohort had previously suffered an episode of VTE.

2.6 DISCUSSION

2.6.1 This analysis of 322 consecutive women aged 16-70 years with objectively confirmed VTE demonstrates that a recognised precipitating factor for thrombosis was present in 79% of all cases. Surprisingly, IVDA was associated with 13.7% of all events (23% of all VTE if clinically diagnosed IVDA events are included). In women under 40 years with objectively confirmed VTE (n=131), IVDA accounted for 33.6% (44/131) of all events. This compares with 33.6% (44/131) related to pregnancy and 15.3% (20/131) related to COCP in this age group. All women with VTE who were IVD abusers developed DVT; none presented with symptomatic PE. Many such women self-discharged from hospital early (and in many cases without follow-up), and therefore we cannot be certain that a proportion did not subsequently develop PE. However, this seems unlikely, since none were readmitted to either hospital with PE.

2.6.2 Fifty-four percent (44/82) of DVT associated with IVDA were objectively confirmed. In the remainder, the diagnosis was made clinically, or the patient self-discharged prior to a radiological examination being performed. There are few data on the prevalence of IVDA as a precipitating factor for VTE. Labropoulos et al studied 47 consecutive patients, who were IVD abusers, presenting with suspected DVT (163). DVT was diagnosed by duplex Doppler ultrasound in 63% of limbs. Ten patients had bilateral DVT. The remainder had a variety of diagnoses, such as groin abscess, haematoma, arteriovenous fistulae, cellulitis, and false aneurysm. In nine patients no diagnosis was reached. It was notable that in this study, 3 patients developed symptomatic pulmonary embolism. This is in contrast to our cohort, in whom none presented with symptomatic PE.

2.6.3 It was of interest that only 2 women with DVT associated with IVDA were treated with oral anticoagulants. In one such patient, warfarin was discontinued after only 4 weeks due to poor and erratic compliance with therapy. Amongst clinicians there is uncertainty regarding the best way to manage these patients. Problems with continued drug abuse, with the risk of vessel puncture whilst on anticoagulants, are clearly of concern and mean that at present few such patients are anticoagulated in the community. Considering these patients represent a significant proportion of women with VTE, studies are needed to determine the correct approach to treatment, since at present there are few data on which to base recommendations.

2.6.4 Cancer was identified in 12.8% of all women with VTE. Cancers of the breast (46%), colon (15%) and lung (12%) were those most frequently identified in association with VTE. The distribution of cancers amongst women with VTE closely followed the prevalence of cancers in women generally: data on almost 120 000 women with cancer in the UK aged 16-75 years demonstrates breast (26.3%), colorectal (13.2%), and lung (11.3%) as the commonest primary tumours (164).

2.6.5 Most patients in the cancer related VTE group had overt cancer at presentation; in one woman cancer was diagnosed within several months of presentation with VTE. However, we do not know how many women developed cancer after presentation since formal follow-up was not undertaken. Monreal recently investigated 832 consecutive patients (male and female) admitted with VTE and identified overt cancer at presentation in 17.7% (165). Other predisposing risks included surgery (26.5%), immobility (20%), previous VTE (13%), COCP / HRT (1%) and pregnancy (1%). In addition, screening tests detected cancer in a further 2% during initial hospital

admission, and in a further 1% during follow-up over several months or years. The subsequent development of malignancy appeared significantly more common in those with idiopathic versus secondary VTE (165). In Monreal's study, cancers of the colon, breast and lung were the most common primary sites associated with VTE, in keeping with our findings. Other studies have supported the idea that those with idiopathic VTE are more likely to subsequently present with cancer compared to patients with secondary VTE (166-168). However, not all studies have confirmed this (169). Screening for occult malignancy in those presenting with VTE has been investigated by Barosi who concluded that screening for cancers of the breast and colon in females might be worthwhile (170). However, screening is expensive and the natural history of the cancers under investigation must be amenable to change. A large Danish study of individuals with VTE recently concluded that there was only a small relative risk of subsequent development of cancer (171). This study demonstrated that those presenting with VTE were more likely to present with cancer in the first 6 months after presentation, and that the relative risk of presentation with cancer rapidly declined to that that seen in the background population after this period. The authors concluded that extensive investigation to detect malignancy was not warranted (171).

2.6.6 In absolute terms, we found that HRT was a slightly more common precipitating factor for VTE than the COCP. However, the median age of those in the HRT group was significantly higher than in the COCP group, which may partly account for this observation. Pregnancy was a common risk factor for development of VTE, accounting for 14% of all cases and 33.6% of those < 40 years. Pregnancy will be considered in detail in Chapter 3.

2.6.7 Eighteen women died from PE after admission to hospital. Four deaths occurred in women who were known to have cancer and two in association with orthopaedic surgery. A further 2 deaths occurred in women with cardiac disease. Seven deaths occurred in women without an obvious precipitant. One woman, who presented with massive PE associated with hypotension and hypoxia, was treated initially with thrombolysis (tissue plasminogen activator) and survived. All others were treated with intravenous heparin followed by oral anticoagulants. The mean age of women who died from PE was compared to those who survived the event. Those who died (mean age 59.3 years, SD 11.4, n=18) were significantly older than those who survived (mean age 50.1 years, SD 14.5, n=117) ($p< 0.005$). A recent study has identified prognostic factors in those presenting with acute PE (172). Older age (>70 years), cancer, heart failure and chronic obstructive pulmonary disease were identified as significant prognostic factors. In addition, certain clinical signs on presentation (systolic arterial hypotension, tachypnoea, and right ventricular hypokinesia on echocardiography) were also of independent prognostic significance.

2.6.8 In summary, this study of precipitating factors for VTE in women aged 16-70 years in the West of Scotland has demonstrated that most events have an underlying triggering factor. Surprisingly, intravenous drug abuse was identified as a highly prevalent risk factor, particularly in women less than 40 years. None of the IVDA cohort presented with symptomatic PE. Pregnancy was associated with 14% of all events and 33.6% of events in those under 40 years. Cancer, particularly those originating from breast, colorectal, or lung, was also commonly associated with VTE, detected in 12.8% of the entire cohort and almost 25% of those aged over 50 years. Further research is needed, particularly in those with VTE associated with IVDA. Data

on this group, to determine the risk of PE, would help in the design of evidence-based management guidelines.

Table 2.1 Details of Eighteen Women who Died from Pulmonary Embolism

Age	Previous VTE	Risk factor(s)	Notes on risk factors	Additional information
68	No	cancer	bronchial carcinoma	V/Q confirmed
65	No	surgery	recent arterial graft immobile	Probable PE Definite DVT
63	No	cancer	breast cancer	Probable PE Definite DVT
68	No	nil		V/Q confirmed
65	No	cancer	pancreatic cancer	V/Q confirmed
64	No	nil		Probable PE. Definite DVT
64	No	surgery	2 days post-op	V/Q confirmed
70	No	medical illness	myocardial infarction	V/Q confirmed
28	No	nil		V/Q confirmed
56	No	nil		V/Q confirmed
38	No	surgery	internal fixation of leg fracture	Post mortem diagnosis
65	No	nil		V/Q confirmed
70	No	nil		V/Q confirmed
56	No	HRT	Premique®	Post mortem diagnosis
68	No	nil		Post mortem diagnosis
55	No	medical illness	CCF	V/Q confirmed
57	No	surgery	post hip replacement	V/Q confirmed
48	No	cancer	bronchial carcinoma	V/Q confirmed

Table 2.2 Risk Factors in 322 Women aged 16-70 years with Objectively Diagnosed VTE between 1993-97.

Risk Factor	Median age (range)	DVT (n)	PE (n)	Total (%)
Nil	58 (17-70)	28	40	68 (21%)
Pregnancy[1]	30 (19-41)	30	15	45 (14%)
IVDA[2]	28 (20-39)	44	0	44 (13.7%)
Cancer[3]	61 (43-70)	22	19	41 (12.8%)
HRT[4]	56 (37-65)	11	13	24 (7.5%)
COCP	30 (19-44)	11	10	21 (6.5%)
Surgery[5]	59.5 (22-69)	12	16	28[†] (8.7%)
Immobility[6]	56 (34-69)	11	11	22 (6.8%)
Medical illness[7]	64.5 (46-70)	6	10	16 (5%)
Previous VTE*	56 (32-69)	12	1	13 (4%)

[†] excludes 5 patients who developed VTE following surgery for cancer

[1] 3 women with VTE related to pregnancy had a previous history of VTE

[2] 9 women with VTE related to IVDA had a previous history of VTE

[3] 4 women with VTE related to cancer had a previous history of VTE

[4] 4 women with VTE related to HRT had a previous history of VTE

[5] 4 women with VTE related to surgery had a previous history of VTE

[6] one woman with VTE related to immobility had a previous history of VTE

[7] one woman with VTE related to medical illness had a previous history of VTE

* Previous VTE as the only risk factor for current event

Table 2.3 Cancers Predisposing to VTE in forty-one Women

Malignancy	Number (%)
Breast	19 (46%)
Colorectal	6 (15%)
Lung	5 (12%)
Ovary	3 (7%)
Unknown Primary	2 (5%)
Cervix	1
Uterus	1
Pancreas	1
Cerebral	1
Haematological	1
Bladder	1

Figure 2.1 Prevalence of Cancer in 322 Consecutive Women aged 16-70 years with VTE

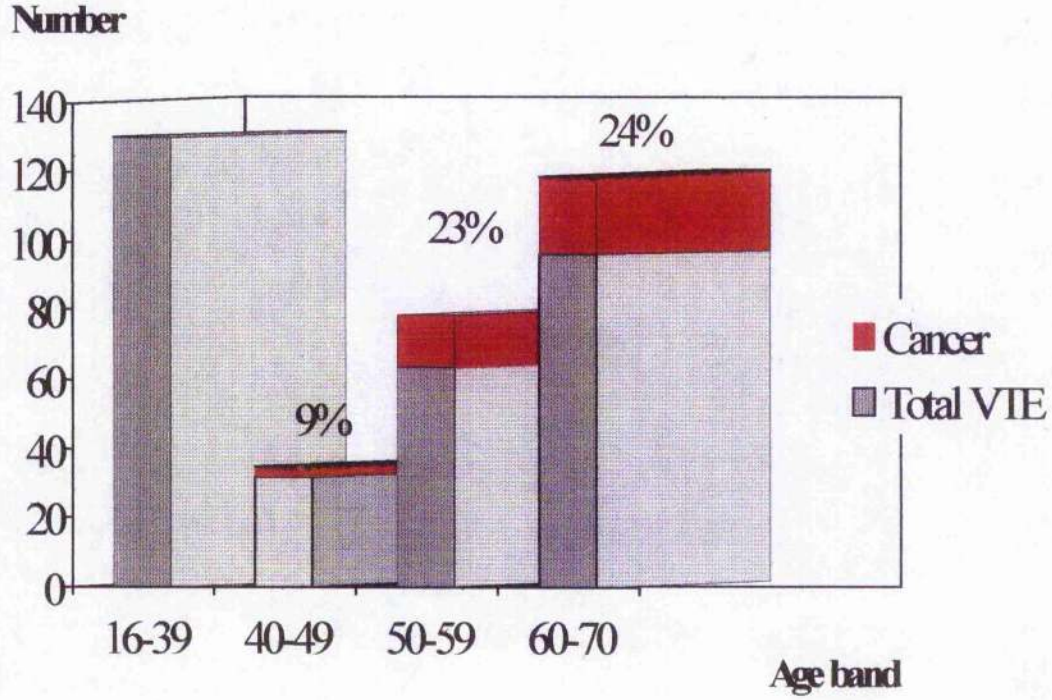
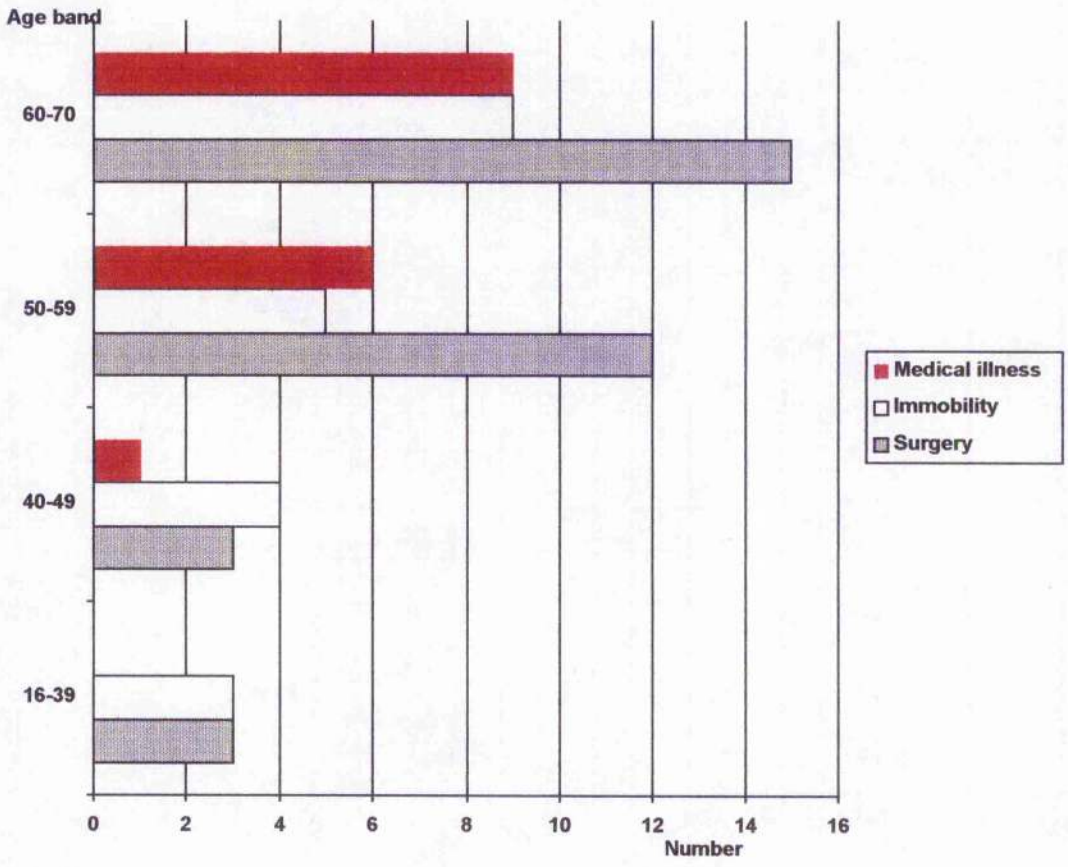


Figure 2.2 Women who Developed VTE Related to Surgery, Immobility or Medical Illness by Age-Band.



CHAPTER 3

CLINICAL AND THROMBOPHILIC RISK FACTORS IN WOMEN WITH VENOUS THROMBOEMBOLISM ASSOCIATED WITH PREGNANCY

3.1 SUMMARY

3.1.1 Venous Thromboembolism remains the commonest cause of maternal mortality in the United Kingdom. We have conducted a large study (encompassing over 93 000 maternity's) to determine the incidence of VTE in association with pregnancy, and to examine the role of inherited thrombophilia and clinical risk factors for this condition. Eighty-seven objectively confirmed VTE events (64 DVT, 23 PE) were recorded at two maternity units in the UK between the years 1985-1998 inclusive. The incidence of DVT was 0.68 per 1000 maternity's (95% CI 0.51-0.85) with 0.47/1000 occurring in the antenatal period (95% CI 0.33-0.61) and 0.21/1000 in the puerperium (95% CI 0.12-0.3). The incidence of PE was 0.25 per 1000 maternity's (95% CI 0.15-0.35), 0.1/1000 antenatal (95% CI 0.04-0.16) and 0.15/1000 in the puerperium (95% CI 0.07-0.22). Of these 87 women, 75 attended for follow-up and thrombophilia screening. The prevalence of thrombophilic abnormalities in cases was compared to that obtained in over 200 random unselected cord blood controls, and to the prevalence of antithrombin deficiency in over 9000 blood donors in our area. Carriers of the factor V Leiden (OR 4.5, 95% CI 2.1-14.5) and prothrombin 20210G→A (OR 4.4, 95% CI 1.2-16) mutations were at increased risk of VTE related to pregnancy. Deficiency of antithrombin was associated with a particularly high risk of VTE (OR for type I deficiency 282, 95% CI 31-2532; for type II deficiency OR 28, 95% CI 5.5-142). The overall prevalence of thrombophilia in the cohort was 26.7%. The homozygous C677T mutation in the MTHFR gene was not associated with an increased risk of VTE (OR

0.45, 95% CI 0.13-1.58). No women were identified with deficiency of protein S. In those with the FVL and prothrombin mutations, additional clinical risk factors for VTE were common. Our study demonstrates that thrombophilic abnormalities not uncommonly underlie maternal VTE. However, since acquired clinical risk factors for VTE are common throughout the group, awareness of such risks remains the key to reducing the incidence of maternal thromboembolism. In addition, selective thrombophilia screening in those with a personal or family history of venous thrombosis may be worthwhile.

3.2 INTRODUCTION

3.2.1 Incidence of Venous Thrombosis associated with Pregnancy

3.2.1.1 Venous Thromboembolism (VTE) remains the leading cause of maternal mortality in the United Kingdom (8). The recent Report on Confidential Enquiries into Maternal Deaths in the UK in the triennium 1994-1996 documented a total of 46 deaths from pulmonary embolism, 15 of which occurred antepartum, 25 postpartum (excluding late postpartum deaths), 3 after abortion / ectopic pregnancy and 3 for which details were unavailable (8). The number of maternal deaths attributable to VTE had increased compared with the previous confidential enquiries report for the preceding triennium. The documented incidence of VTE associated with pregnancy appears to vary considerably. It is only within the past 10-15 years that objective tests have been performed routinely in obstetric patients with suspected VTE. Many of the published figures which document the incidence of VTE in pregnancy include events which were diagnosed clinically, which is now considered unreliable (173,174). There are few data reporting the incidence of objectively confirmed VTE associated with pregnancy. James et al, in a study of 30 000 pregnancies, estimated the incidence of thrombotic complications related to pregnancy at 1 per 1000, although this figure also included superficial thrombophlebitis and pelvic vein thrombosis (175). Kierkegaard et al studied over 14 000 pregnancies and estimated the incidence of objectively proven DVT at 0.74 per 1000 (174). These figures are consistent with a 10-year review of Scottish maternity's which were complicated by VTE (176).

3.2.2 Risk Factors for Pregnancy Associated VTE

3.2.2.1 Pregnancy is accompanied by significant physiological changes in haemostasis (table 3.1). Elevations of coagulation factors Vc, VIIIc, Xc, von Willebrand factor antigen, and decreases in both total and free protein S are reported (reviewed in (177)). A recent prospective cross-sectional study of 239 women throughout pregnancy observed that up to 38% of pregnant subjects develop activated protein C (APC) resistance by the third trimester, which occurs in the absence of the factor V FVL (FVL) gene mutation (43). This acquired APC resistance appears to correlate with increased concentrations of coagulation factors Vc and VIIIc and a decrease in protein S concentration. Coagulation activation markers are also elevated, particularly in the third trimester (43). There are no significant changes in the plasma levels of protein C or antithrombin throughout pregnancy (43,177). Fibrinolytic activity is impaired during pregnancy, relating in part to elevations in placentally-derived plasminogen activator inhibitor type 2 (PAI-2) which is produced in substantial quantities during pregnancy (177). These physiological changes produce a relative thrombotic tendency during pregnancy.

3.2.2.2 The physiological changes in the coagulation and fibrinolytic systems in pregnancy are accompanied by increased venous capacity and distensibility, leading to a reduction in velocity of blood flow within the lower limbs (178). The reduction in blood flow appears more marked in the left leg (178). In addition, it has been noted that women delivered by caesarean section appear to have reduced blood flow in the proximal leg veins compared to those delivered vaginally (179). These effects may be compounded by varying degrees of venous obstruction to both the left iliac vein and the inferior vena cava by the gravid uterus. Other risk factors for VTE may be apparent at

time of booking or develop during pregnancy. These include caesarean section, previous VTE, age over 35 years, obesity (>80 kg), high parity (4 or more), infection, pre-eclampsia, immobility, varicose veins, thrombophilia, or major current illness (8,176,180) (see table 3.2). The paper by Macklon et al (176) describes an approximate two-fold increase in incidence of both antenatal and puerperal DVT in women aged > 35 years, and almost a three-fold increased incidence of PE. This paper also documented a more than two-fold increased incidence of puerperal VTE if delivery was by caesarean section compared with vaginal delivery; the incidence was even higher if delivery was by emergency section.

3.2.3 Inherited Thrombophilia as a risk factor for VTE associated with Pregnancy

Observational Studies in Thrombophilic cohorts

3.2.3.1 Until recently, there were few published data on the contribution of inherited thrombophilic abnormalities to the development of VTE associated with pregnancy. Several papers have documented episodes of VTE during pregnancy or in the postpartum period in cohorts of individuals with known natural anticoagulant deficiencies. Conard et al studied subjects with deficiencies of antithrombin, protein C or protein S from symptomatic families, during pregnancy and the postpartum period for episodes of VTE (181). The incidence of VTE was increased in all three categories. Those with antithrombin deficiency (n=25) had a very high risk of VTE (18% developed VTE antepartum, 33% postpartum). Protein C deficient subjects (n=36) developed events less frequently (7% antepartum, 19% postpartum), and those with protein S deficiency (n=17) also had fewer thrombotic events (0% antepartum, 17% postpartum). Friederich studied 60 previously asymptomatic women with deficiencies of the natural anticoagulants antithrombin (n=13), protein C (n=19) or protein S (n=28),

through 169 pregnancies (182). These women were identified because of a family history of VTE. The incidence of thrombotic events in the anticoagulant factor deficient group was compared to that observed in women from the same families who had normal levels of antithrombin, protein C and protein S (n=69, number of pregnancies in this group =198). 4.1% of women with natural anticoagulant deficiencies developed VTE (all in the third trimester or postpartum) compared with 0.5% in the non-deficient group (odds ratio for VTE in the deficient group = 8). Pabinger studied 230 individuals with natural anticoagulant deficiencies, again from symptomatic families, and noted that 40% of those with antithrombin deficiency developed VTE related to pregnancy (183). Hough retrospectively studied 80 women with thrombophilia during 215 pregnancies and reported VTE in 32% of those with antithrombin deficiency, 22% in those with protein C deficiency, 14% in those with FVL and 12.5% in those with protein S deficiency (184). All of these studies are retrospective and appear to suffer from selection bias, since the patients described are identified from symptomatic families, and in some cases combined thrombophilic abnormalities were present.

Studies documenting prevalence of thrombophilia in obstetric patients with VTE

3.2.3.2 Recent studies have attempted to identify the prevalence of thrombophilic abnormalities in women with pregnancy associated VTE.

3.2.3.3 De Boer et al investigated 30 highly selected women referred to a thrombosis unit with an episode of VTE related to pregnancy for thrombophilia (185). One patient was identified with antithrombin deficiency, 2 with deficiency of protein C and 1 with evidence of a lupus anticoagulant. In addition, 2 patients were reported with fibrinolytic abnormalities, which are not firmly established as risk factors for VTE. Unfortunately,

this paper pre-dated the description of both FVL and the prothrombin 20210A mutations.

3.2.3.4 Hellgren et al investigated 34 women who had suffered an episode of VTE associated with pregnancy and found 20 (59%) with APC resistance (186) (table 3.3). Details of how these women were identified were not given. In addition, not all events were objectively diagnosed, subjects with other thrombophilias were excluded, and PCR was not performed on those with APC resistance to confirm the presence of the FVL mutation. Since it is now appreciated that APC resistance can occur in those without the FVL mutation, it is important to confirm FV genetic status with PCR.

3.2.3.5 Two other small studies have reported on the prevalence of FVL in obstetric patients with VTE. Hallak et al documented results of thrombophilia investigations performed on patients admitted to a high risk pregnancy unit over a two year period (187). This included patients with DVT, PE, transient ischaemic attack and cerebrovascular accidents. 15 patients were described, of whom 7 (4 PE, 2 DVT and 1 sagittal sinus thrombosis) had FVL. This was a small study on a highly select group and therefore only limited conclusions can be drawn. Dizon-Townson et al screened 407 unselected obstetric patients for the FVL mutation (188). 14 (3%) were found to possess the mutation. 4/14 developed DVT, all in either the late second / third trimester or postpartum. The number of pregnancies studied here was very small; in addition, the incidence of VTE related to pregnancy (if taken as representative) appears very high (1 in 100, ten times that reported elsewhere). Of the 4 with FVL who developed DVT, 2 had complicated pregnancies (one was a twin pregnancy with pre-eclampsia, the other was complicated by uterine atony and disseminated intravascular coagulation).

3.2.3.6 Hirsch et al reported that 6 (17%) of 35 women with pregnancy associated VTE possessed the FVL mutation (189). In 5/6 patients with FVL, the episode of VTE occurred in the first trimester. 3 patients in the FVL group developed pulmonary embolism. One patient had coinheritance of both FVL and antithrombin deficiency; prothrombotic abnormalities were identified in a further 4 patients (protein C deficiency in 1, protein S deficiency in 1 and antiphospholipid syndrome in 2). It was noted that additional risks for VTE were present in 40% of the FVL group.

3.2.3.7 Bokarewa investigated 74 women with VTE, placental thrombosis and stroke related to pregnancy and found the FVL mutation in 46% (190). Unfortunately, this study did not describe what, if any, thrombophilic abnormalities were found in the remainder. It was noted that those with FVL had a predilection to develop VTE in the first trimester or postpartum. Women in this study were not investigated for the presence of the prothrombin 20210 G→A mutation, or for the C677T mutation in the MTHFR gene.

3.2.3.8 Thus far there are no data to determine if the prothrombin mutation is associated with a significant number of venous thrombotic events related to pregnancy. This mutation is relatively common in the general population (2.2% in the West of Scotland) (74), although the increased risk of VTE appears somewhat less than that posed by FVL (86-91). One case report has highlighted the prothrombin mutation as a possible risk factor for VTE associated with pregnancy (191), although there are no large studies to support this. Since both the FVL and prothrombin mutations are relatively common, it could be argued that widespread screening for these mutations in early pregnancy

might be worthwhile in order to identify women at increased risk of VTE. However, screening is expensive, and the results may provoke important therapeutic issues. Indeed, unnecessary anticoagulation during pregnancy or the puerperium could itself produce considerable morbidity and mortality (192-194). Also, widespread random screening may identify many asymptomatic females with these mutations, and cause future uncertainty regarding appropriate methods of contraception. Clinicians may not prescribe the combined oral contraceptive in such a situation. Unplanned pregnancies could ensue with, in the presence of thrombophilia, an increased risk of VTE.

3.2.3.9 To examine this important area further, we performed a study to determine

1. The incidence of objectively confirmed VTE associated with pregnancy
2. The prevalence of thrombophilic defects amongst individuals with pregnancy associated VTE
3. The role of acquired risk factors (such as caesarean section, preeclampsia etc.) for VTE associated with pregnancy.

3.3 PATIENTS, MATERIALS AND METHODS

3.3.1 Ethical approval was obtained from the local research ethics committee for this study. All participants gave written informed consent (see appendices 2 and 3). The study was based upon individuals who had attended for antenatal care and delivery at two Glasgow maternity units (Glasgow Royal Maternity Hospital; Southern General Hospital) and who had suffered an objectively confirmed episode of deep vein thrombosis (DVT) and / or pulmonary embolism (PE) during pregnancy or up to six weeks post-partum. The study was commenced in 1995 (retrospectively) and continued prospectively until December 1998. Patient data was obtained from the Information and Statistics Division of the Common Services Agency for the National Health Service in Scotland (SMR2 data). A list of individuals who had been given an ICD code corresponding to either DVT or PE at the two maternity units between January 1985 - December 1998 (14-year period) was obtained. The results from those women identified between January 1985 - December 1995 were published in *Thrombosis and Haemostasis* in 1997 (195). The data presented here are those obtained for the duration of the entire study.

3.3.2 The following diagnostic codes (ICD9) were used to identify retrospective cases: DVT, 6713 and 6714 and PE 6732. Information was also obtained on females up to the age of 49 years who had been admitted to the acute medical receiving units of Glasgow Royal Infirmary or the Southern General Hospital and given heparin therapy, in an attempt to document cases of post-partum VTE who were not admitted to the maternity units. The case records of all individuals were scrutinised to verify that an objectively confirmed diagnosis of either DVT or PE had been obtained. Those with DVT were included for study purposes if compression or Doppler ultrasound, or contrast

venography, were diagnostic. Women with PE were included if ventilation-perfusion scanning was considered of either high or intermediate probability, and they had received at least a six-month course of anticoagulant therapy.

3.3.3 Individuals were invited by letter to attend a clinic for the purposes of interview and blood sampling. Information was obtained on clinical risk factors for thrombosis (outlined by the Royal College of Obstetricians of the UK) during the affected pregnancy (180) (see table 3.2).

Blood Sampling

3.3.4 None of the individuals were pregnant at the time of blood sampling. Blood was obtained by fresh venepuncture, and added to 0.109 mol/L trisodium citrate (9 parts blood:1 part anticoagulant). Samples were mixed gently and centrifuged at 2000g for 10 minutes to obtain platelet poor plasma (PPP). The PPP was then centrifuged for a further 10 minutes at 2000g and the plasma separated into 1ml aliquots within four hours. Plasma samples were then stored in 1ml aliquots at -70^0 C until analysis. Peripheral blood leucocytes were stored and used for DNA analysis.

Sample analysis

3.3.5 Samples were subject to a thrombophilia screen which included prothrombin time, activated partial thromboplastin time, thrombin time, antithrombin (AT) and protein C (PC) activity, free and total protein S antigens, lupus inhibitor screen (APTT performed on a mix of four parts patient platelet poor plasma to one part normal platelet poor plasma, and dilute Russells' viper venom test with platelet neutralisation step), IgG and IgM anticardiolipin antibody assays and activated protein C-sensitivity

ratio (APC-SR). PCR was performed to detect the FVL, prothrombin 20210 G→A and MTHFR C677T mutations.

3.3.6 AT activity was determined by an automated, amidolytic antithrombin based assay (ATIII kit, Boehringer Mannheim; the standard used for this assay was commercial reference plasma Sigma calibrated against WHO International reference preparation for antithrombin III, human code 87/718). Protein C activity was measured by an automated, amidolytic assay using Protac^{TM} C (American Diagnostica Inc) in the activation step and S-2366 (Kabi, Sweden) as the specific chromogenic substrate (the standard used here was the WHO 1st international standard for human plasma protein C, code 86/622). Protein S total and free antigens were measured by an established ELISA method (Dako Ltd, High Wycombe, Bucks). Anticardiolipin antibodies were also measured by an ELISA method (Cambridge Life Sciences kit).

3.3.7 To test for the presence of a lupus anticoagulant, an APTT was performed on patient PPP, on pooled normal plasma, and on a mixture of four parts patient PPP to one part normal PPP. As a more specific test for a lupus anticoagulant, we also performed a Dilute Russell's viper venom time (DRVVT) on double spun plasma. This was performed as follows: 0.1ml dilute phospholipid was incubated with 0.1ml normal control plasma at 37^0 C for 30 seconds. 0.1ml of RVV reagent was added. This was incubated at 37^0 C for 30 seconds 0.1ml $CaCl_2$ added. The time for clot formation was recorded and the test was repeated on patient plasma. If the result with patient plasma was longer than that with normal control plasma, these steps were repeated for normal and patient plasmas, substituting washed freeze-thawed platelets for the dilute phospholipid reagent. The ratio of patient clotting times to normal clotting times was

calculated for both DRVVT and platelet neutralisation procedure (PNP). The normal ratio is 0.9-1.09. Ratios of > 1.1 were retested with the PNP. 10% or greater shortening of the DRVVT with the PNP was considered a positive lupus test.

Test for Resistance to Activated Protein C

3.3.8 APC resistance was assessed by measuring the anticoagulant response in plasma on the addition of APC (Coatest, Chromogenix, Sweden). APC acts as an anticoagulant by degrading factors Va and VIIIa. Thus addition of APC to a plasma sample causes prolongation of the APTT. The APC-sensitivity ratio (APC-SR) represents the ratio of two APTT values, determined in the absence and presence of activated protein C. Normal range for our laboratory at the time of study was 2.6 - 4.5, derived from 76 normal donors of both male and female sex. Resistance to activated protein C was diagnosed when the APC-SR was below 2.6.

Identification of the FVL mutation

3.3.9 DNA was isolated from peripheral blood leucocytes by an established technique (Nucleon, Coatbridge, Lanarkshire). This process involved six steps. Briefly, in the cell preparation stage, reagent A was added to citrated whole blood and centrifuged at 1300g for 5 minutes. Cell lysis was achieved by addition of reagent B to the pellet. Deproteinisation occurs via addition of sodium perchlorate solution. DNA extraction with chloroform and Nucleon® resin was followed by DNA precipitation with cold absolute ethanol, and finally DNA washing. Following the manufacturers instructions, this process took approximately 30-40 minutes.

3.3.10 The G1691A mutation in exon 10 of the factor V gene (nucleotides 1487-1701)

was detected by loss of a cleavage site for *MnlI*. A 223 base-pair fragment was amplified from genomic DNA using the 5' primer (5'-ACCCACAGAAAATGATGCCCAG-3', nucleotides 1566-1587) and the 3' primer (5'TGCCCCATTATTTAGCCAGGAG3', nucleotides -66 to -10 of intron 10). The PCR conditions were as follows: 75-150ng of DNA in 15µg of TRIS-EDTA buffer under 50µl of mineral oil was heated in a thermal cycler to 96^0 C for 60 seconds and 95^0 C for 180 seconds, and then held at 80^0 C for 10 minutes while 35µl of amplification solution was added. The amplification solution contained 5µl of 20mM deoxynucleoside triphosphates, 200ng of each primer, 0.1µl of 1.0 M magnesium chloride and 0.5U of *Taq* polymerase . Thirty cycles of 93^0 C for 60 seconds, 50^0 C for 30 seconds, and 72^0 C for 90 seconds were followed by a final 10 minutes at 72^0 C. Aliquots (10µl) were digested for 12 hours at 37^0 C with 1U *MnlI* (New England Biolabs) after the addition of 2.5µl of digest buffer. The fragments (measuring 37, 82 and 104 bp for the 1691G allele and 82 and 141 bp for the mutant allele) were separated on 2% agarose gel and visualised with ethidium bromide.

Identification of the Prothrombin 20210 G→A mutation

3.3.11 The prothrombin gene was amplified using primers A (5'-TTACAAGCCTGATGAAGGGA-3') and B (5'-CCATGAATAGCACTGGGAGCATTGAAGC-3'). The latter is designed with a C→A substitution at position 20214 to create a restriction site for *Hind III* when the G→A transition is present. The normal allele lacks a *Hind* III restriction site and generates only a 345 bp fragment. A new *Hind* III site introduced into the amplified fragment from the mutant allele yields 2 fragments of 322 bp and 23 bp after restriction enzyme digestion. The PCR conditions were as described for identification of the FVL

mutation, and the fragments were separated on 2% agarose gel and visualised with ethidium bromide.

Analysis of the C677T mutation in the MTHFR gene

3.3.12 A fragment of the MTHFR gene was amplified by PCR using the primers (5'-TGAAGGAGAAGGTGTCTGCGGGA-3') and (5'AGGACGGTGCGGTGAGAGTG-3'). The PCR conditions were as described for detection of the FVL mutation. A fragment of 198 bp was obtained. 10µl of PCR product were digested using the *Hinf* I restriction endonuclease. Electrophoresis was performed on 2% agarose gel. Normal homozygotes were identified by the presence of a 198 bp fragment, C677T homozygotes identified by a 175 bp fragment, and heterozygotes by the presence of both fragments.

Statistical techniques

3.3.13 This study was retrospective, not prospective, with VTE as an endpoint already identified. We calculated odds ratios (OR) with 95% confidence intervals as estimates of the relative risk of thrombosis. Odds ratios were calculated as follows. Data (nominally labelled a,b,c,d) were constructed in standard 2x2 tables (data for those with risk factors in the first row, data for cases in the first column) (196). The OR was calculated as the cross-product ratio (ad/bc) (196). 95% confidence intervals were calculated according to Woolf's method i.e. the standard error of the natural logarithm of the OR (ln OR) was calculated from $\{\sqrt{1/a + 1/b + 1/c + 1/d}\}$ and the 95% confidence intervals derived from ln OR +/- 1.96 {standard error}. An OR was considered to be statistically significant when the lower limit of the 95% CI was ≥ 1.0 (196).

3.4 RESULTS

3.4.1 Maternity's at each unit

3.4.1.1 Maternity unit 1 (GRMH) had a total of 61 383 maternity's for the study period whilst unit 2 (SGH) had a total of 32 110. The study therefore encompassed a total 93 493 maternity's.

3.4.2 Thrombotic events over the 14 year period (tables 3.4, 3.5 and 3.6)

3.4.2.1 During the study period 87 women had an episode of objectively confirmed VTE related to pregnancy. In 78 women this was the first thrombotic event and in 9 this was a recurrent thrombosis (8 previous DVT, 1 previous episode of superficial vein thrombosis). None of the 9 individuals with a recurrent thrombosis had pregnancy as a previous precipitating event. Table 3.4 provides details of diagnosis and gestation at diagnosis in these 87 women. Table 3.5 shows the calculated incidence of thrombosis per 1000 maternity's based upon a total of 93 493 maternity's during the study period. Table 3.6 shows thrombotic events per 10 000 women years at risk. First trimester was defined as up to and including 12 weeks gestation, second trimester 13-28 weeks, third trimester 29-term, puerperium up to 6 weeks following delivery. There were 64 cases of DVT, of which 44 (69%) occurred in the antenatal period and 20 (31%) in the puerperium. Of 44 antenatal cases of DVT, 12 occurred in the first trimester, 8 in the second trimester and 24 in the third trimester. There were 23 cases of pulmonary embolism, 9 (39%) of which occurred in the antenatal period and 14 (61%) in the puerperium. Of 64 patients with DVT, 48 (75%) were left sided and 15 (23.4%) occurred on the right. One case was bilateral. We also identified 6 individuals who had been given a clinical diagnosis of DVT and received anticoagulant therapy but who had not had this confirmed by a radiological investigation. Since this small number were

diagnosed subjectively, we excluded them for the purposes our study.

3.4.3 Patients attending for interview and blood sampling

3.4.3.1 Of the total of 87 individuals with objectively proven venous thrombotic events, 75 attended for interview and blood sampling (follow-up rate of 86%). Of this number, 55 had DVT and 20 had PE. From this cohort we were able to obtain clinical information on risk factors for thrombotic events. All were subject to thrombophilia screening as described above. However, since the prothrombin 20210G→A mutation was described in November 1996 (after the study was commenced), screening for this mutation (and for the MTHFR mutation) was introduced in our centre in 1997. We attempted to recall all women who had already participated in the study to screen for the prothrombin and MTHFR mutations. Not all reattended. In total, 55/75 women had PCR performed to determine the presence or absence of the prothrombin mutation. 52/75 were screened for the C677T mutation in the MTHFR gene. 9 women had a history of VTE prior to the episode of pregnancy associated VTE (8 with previous DVT and 1 with a previous episode of SVT). 14 individuals were on oral anticoagulants at the time of blood sampling, limiting thrombophilia screening.

3.4.4 Clinical risk factors for thrombosis (table 3.7)

3.4.4.1 Table 3.7 demonstrates the prevalence of clinical risk factors for thrombosis detected in women with DVT and PE. It can be seen that clinical risks were common in both the DVT and PE groups. In total, 45/75 (60%) women had additional identifiable clinical risk factors for the development of VTE. 22/55 women with DVT and 8/20 with PE had no evident clinical risk factors (other than pregnancy) for VTE (40% of total cohort). Of these thirty individuals without clinical risk factors, six were

subsequently found to possess thrombophilic defects (two with type I antithrombin deficiency, one with type II antithrombin deficiency, one with protein C deficiency, one with heterozygous FVL, and one with heterozygous prothrombin 20210G→A mutation). Hence 32% (24/75) of episodes of VTE were not associated with either a clinical risk factor for thrombosis (other than pregnancy) or a thrombophilic defect (although 20/75 were not screened for the prothrombin 20210A mutation). 7/20 (35%) cases in the patient group with PE were associated with caesarean section (all were emergency section) prior to the development of thrombosis, compared with 6/55 (11%) (4 emergency section, two elective) cases with DVT associated with caesarean section. The odds ratio for development of PE as compared with DVT associated with caesarean section was 4.4 (95% confidence interval 1.26-15.4).

3.4.5 Identifiable thrombophilic defects (table 3.8)

3.4.5.1 Seventy-five women who attended for follow-up were screened for thrombophilic defects. 7/75 (9.3%) were identified with antithrombin deficiency, 7/75 (9.3%) with the FVL mutation, one with protein C deficiency and one with the antiphospholipid syndrome (consistently elevated IgG anticardiolipin antibody levels and intermittently positive testing in the DRVVT). No women were found to have deficiency of protein S. In addition, of 55 women who were screened for the prothrombin 20210A mutation, 5/55 (9.1%) were found to be heterozygous. One patient was found to possess both the FVL and prothrombin mutations, both in heterozygous form. The total prevalence of established thrombophilia found in the cohort of 75 was 26.7% (20/75). Of 52 women who were screened for the C677T mutation in the MTHFR gene, 3 (6%) were found to be homozygous for the C→T transition, 24 (46%) heterozygous and 25 (48%) homozygous for the normal allele.

Table 3.8 provides detailed information on those with heritable thrombophilia identified in the cohort, including family studies where performed. Of those with AT deficiency, 4 had a type I deficiency phenotype (patients 1-4, table 3.8) and 3 had type II deficiency phenotypes (patients 5-7). Individuals 8-14 were found to have the FVL mutation. In 6 women FVL was identified in the heterozygous state. One woman was homozygous for FVL. Individual 15 had a type I protein C deficiency phenotype. Patients 16-19 were found to possess the prothrombin 20210A mutation (all heterozygous). Of note, 3/7 of those with AT deficiency (all type I antithrombin deficiencies) developed thrombotic events in the first trimester. Four individuals (2 with AT deficiency, one with FVL and one with combined FVL and the prothrombin 20210A mutation) had suffered previous thrombotic events (all DVT). Four of the 19 women who were found to have heritable thrombophilia had a positive family history of VTE. Of 7 women who were found to possess the FVL mutation, six had at least 2 clinical risk factors for VTE. Only one woman with FVL did not have any other clinical risk factors for VTE other than pregnancy. None of the women with FVL had symptoms of PE. Of 5 women who were found to possess the prothrombin 20210A mutation, 3 presented with PE. Four of 5 who were identified with the prothrombin mutation also had two or more additional clinical risk factors for VTE.

3.5 DISCUSSION

3.5.1 This large study, covering over 93 000 maternity's, estimates the overall incidence of objectively proven VTE related to pregnancy at 0.93 per 1000 maternity's (95% confidence interval 0.73-1.13). The incidence of objectively proven DVT was 0.68 per 1000 maternity's (95% confidence interval 0.51-0.85) with 0.47/1000 antenatal and 0.21/1000 puerperal (table 3.5). The incidence of objectively proven PE was 0.25 per 1000 maternity's (95% confidence interval 0.15-0.35), 0.1/1000 antenatal and 0.15/1000 puerperal. These figures are similar to other published data (174-176,197). However, the true incidence of VTE associated with pregnancy may be a little higher, since our figures exclude those treated for DVT who did not have an objective test performed.

3.5.2 It is of interest that the majority (69%) of cases of DVT occurred in the antenatal period, which has been noted in other studies (176,186,198). However, others have found the incidence of puerperal thrombosis to be higher (174,199). It has been suggested that the relative increase in antenatal VTE and comparative decline in incidence of puerperal VTE may reflect changing obstetric practice (200). For example, in the past it was common for physicians to discourage maternal mobilisation in the first week of the puerperium, or to prescribe puerperal oestrogen to suppress lactation. It should be appreciated, however, that the puerperium remains the period of greatest thrombotic risk, since the event rate during this six weeks is significantly greater than the event rate antenatally (table 3.6). Considering the data in terms of women years at risk, the puerperal DVT compared with antenatal DVT event rate appears almost three times greater (18.5 events per 10 000-woman years versus 6.4/10 000 years antenatal). The puerperal PE compared with antenatal PE event rate is increased ten-fold (13.0/10

000 years versus 1.3/10 000 years). The implication from these data is that, whilst antepartum thrombosis may now be more common in absolute terms, potentially a relatively greater number of episodes of VTE may be prevented per week of anticoagulant therapy used in the puerperium than in the antenatal period.

3.5.3 We found that the majority of cases of DVT in relation to pregnancy occurred in the venous system of the left leg (75%), which has been previously noted in one other study (201). This finding may relate to the recently noted reduction in venous flow within the left femoral vein during pregnancy, which may be due to differences in venous anatomy (178). It has also been noted that venous flow within the leg veins appears more sluggish following operative compared with vaginal delivery (179).

3.5.4 A significant number (60%) of women with VTE related to pregnancy had additional clinical risk factors for thrombosis. It was of interest that caesarean section (CS) (particularly emergency CS) was significantly associated with development of PE: 35% of all women who presented with PE had emergency CS as an additional risk for VTE, compared with 11% of those with DVT. Indeed, 50% of all puerperal PE were associated with emergency CS. The odds ratio for development of PE as compared with DVT associated with CS was 4.4 (95% confidence interval 1.26-15.4). The reason for this observation is unclear; we may speculate that CS is a major risk factor for proximal VTE, which is much more likely to lead to PE than more distal events.

3.5.5 Our study is the largest yet reported which has examined the role of thrombophilia as a risk for pregnancy associated VTE. Unlike most other reports, our study considers consecutive women presenting with an episode of VTE related to

pregnancy, and therefore selection bias is less likely. Although we were unable to obtain blood samples from all individuals who developed VTE during the study period, we examined a large cohort and had a high follow-up rate - 75/87 (86%) with VTE were tested for thrombophilic defects. We found a lower prevalence of the FVL mutation in women with VTE related to pregnancy (9.3%) compared with other researchers (186,189,190). In particular, the studies of Bokarewa and Hellgren report that 46-60% of women who suffered pregnancy associated VTE possessed the FVL mutation (186,190). Both of these studies originate from Sweden, where FVL appears significantly more prevalent compared with the UK (202), which may help to explain this observation. Selection bias may also explain the higher prevalence of FVL carriers seen in these studies, since little information is provided in either study of precisely how patients were identified. Neither of these studies documented any associated clinical risk factors for VTE during pregnancy. We found that additional clinical risk factors were common in those with FVL who developed VTE - of seven women with FVL who developed VTE, six had at least two additional clinical risks. It seems likely that in most cases additional clinical risks (or an additional thrombophilia) may be necessary for the development of VTE during pregnancy in carriers of FVL. It is also noteworthy that all seven women with FVL presented with DVT, and none had symptoms of PE. Data indicate a significantly lower prevalence of the FVL mutation in those presenting with isolated PE compared with DVT or DVT and PE (203-206). That is, the relative risk of PE in carriers of FVL appears lower than for DVT (206). The reason for this observation is unclear, although it is hypothesised that higher thrombin levels seen in those with FVL may lead to the production of a more stable thrombus which is less likely to embolise (206). The studies of Bokarewa and Hellgren do not provide details of prevalence of FVL in those with DVT compared with PE (186,190).

The smaller study by Hirsch describes pregnancy associated PE in 6/10 women with FVL (189). However, this paper may also suffer selection bias since patient recruitment criteria are not included.

3.5.6 We found deficiency of antithrombin was as common in our cohort as the FVL mutation. All women who were identified with type I antithrombin deficiency developed VTE in either the first or second trimester, in keeping with the findings of others (183). Three women with type II antithrombin deficiency developed VTE in the puerperium. Only one patient had protein C deficiency and none had deficiency of protein S. One woman was found to have persistently elevated IgG anticardiolipin antibody levels (varying between 30-40 units) and intermittently positive lupus anticoagulant testing, although the presence of this during her pregnancy could not be confirmed.

3.5.7 Of 75 women who were screened for deficiencies of natural anticoagulants, antiphospholipid syndrome and the FVL mutation, 55 were also screened for the recently described prothrombin 20210G→A mutation. Five women were found to be heterozygous for this mutation. One woman was doubly heterozygous for both the FVL and prothrombin mutations. As was the case for those with FVL, additional clinical risk factors were also common in this group. Three of five presented with pulmonary embolism. Currently there are no data on the relative risks of DVT and PE in carriers of the prothrombin mutation.

3.5.8 We compared the prevalence of the FVL and prothrombin mutations in women with VTE to that obtained in over 200 random unselected umbilical cord blood

controls. The cord controls were obtained from a Glasgow maternity unit; this data has previously been published (74). Our control data estimates the prevalence of FVL at 2.2% and the prothrombin mutation at 2.2% (5/224 cord blood controls for both mutations). These figures are similar to those obtained by other researchers in the United Kingdom: Lowe recently documented the prevalence of FVL in Glasgow at 2.5% in a large (almost 1300 subjects) random population study (61), and Brown has estimated the prevalence of the prothrombin mutation at 2.6% (13/508 healthy subjects) (87). The prevalence of the homozygous C677T mutation in cord controls was 12% (19/158 consecutive cord blood samples). This is in agreement with published prevalence figures (112). In addition, we compared the prevalence of types I and II antithrombin deficiency in our cohort to that obtained in over 9000 healthy blood donors in the West of Scotland (published in (40,41)). The risk of VTE related to pregnancy was significantly increased in carriers of both FVL (OR 4.5, 95% confidence interval 2.1-14.5) and prothrombin mutations (OR 4.4, 95% confidence interval 1.2-16) (table 3.9). The calculated risk was particularly high in those with type I (OR 282, 95% confidence interval 31-2532) and type II (OR 28, 95% confidence interval 5.5-142) antithrombin deficiency. These figures represent the first estimates of venous thrombotic risk for women during pregnancy with the FVL and prothrombin mutations. Indeed, this study is the first to comment upon the prevalence of the prothrombin mutation in women with VTE associated with pregnancy. Whilst several other groups have commented upon the prevalence of the FVL mutation, they have not compared them to their general population prevalence. Since FVL shows a marked geographical variation (202), this is an important consideration. Other researchers have estimated the risk of VTE in women with antithrombin deficiency, and also found this to be high during pregnancy (181,183).

3.5.9 Fifty-two women were screened for the C677T mutation in the MTHFR gene. Homozygosity for this mutation is associated with elevated plasma homocysteine levels in the presence of folate deficiency (108,110,207). Girelli recently demonstrated that individuals homozygous for the C677T mutation with adequate folate stores do not have elevated plasma homocysteine levels (110). Since pregnancy is associated with increased folate demands, and supplementation is not generally continued beyond the first trimester, this mutation might theoretically be relevant to pregnancy complications. However, we did not find a higher prevalence of homozygous C→T carriers in cases compared with controls (OR 0.45, 95% confidence interval 0.13-1.58). Kluijtmans recently concluded that this mutation is not a risk factor for VTE (208). However, this mutation may be important in the development of neural tube defects (112), severe preeclampsia and fetal growth retardation (155).

3.5.10 For the purposes of comparison, we should ideally have determined the prevalence of thrombophilia in pregnant women without a history of VTE. However, the study was retrospective (necessary because of the infrequency of maternal VTE), not prospective, and therefore this approach was impractical. In addition, there is no evidence that thrombophilia impairs the ability to conceive, although there is some evidence that women with thrombophilia are at increased risk of fetal loss (158,159). We therefore feel that our comparisons are valid.

3.5.11 In summary, this study of thrombotic events occurring during pregnancy / puerperium has shown that antithrombin deficiency carries a very high risk of maternal VTE. The FVL and more recently described prothrombin mutations are more prevalent,

though less significant, risk factors for the development of pregnancy associated thromboembolism. Most women with the FVL or prothrombin mutations who develop VTE have additional clinical risk factors during pregnancy. Our findings do not lend support to the idea of unselected screening for either mutation during pregnancy, since relatively few thrombotic events would be prevented and the unnecessary use of anticoagulants in such individuals without other risk factors might increase morbidity and mortality. However, selective screening (for example, in those with a personal or strong family history of proven venous thrombosis) may be worthwhile, as suggested by some researchers (209). In order to reduce the incidence of pregnancy associated VTE, clinicians must be aware of clinical risks during pregnancy, and consider the use of anticoagulant therapy in those with multiple risk factors. Assessment for clinical risks should commence at confirmation of pregnancy, and be the subject of continual review throughout pregnancy, delivery and the puerperium. In selected cases, for example women with a previous episode of VTE, preconceptual assessment for clinical risk factors (such as gross obesity, high parity) should be considered.

Table 3.1 Changes in Haemostatic Parameters during Pregnancy

Increased	Decreased
Va	Total PS
VIIIa	Free PS
Xa	
Von Willebrand factor	
PAI-2	
Resistance to APC	
Activation markers	

Table 3.2 Factors that increase the Risk of Venous Thromboembolism in Pregnancy

Risk Factor
Age > 35 years
Obesity (>80 kg)
Para 4 or more
Gross Varicose Veins
Current infection
Pre-eclampsia
Immobility
Major current illness (including nephrotic syndrome and inflammatory bowel disease)
Caesarean section, particularly emergency section
Personal or family history of VTE
Known inherited or acquired thrombophilia
Hyperemesis

Table 3.3 Prevalence of factor V Leiden / APC resistance in Women with VTE related to Pregnancy.

Study	Number of participants	Outcome	Comments
Heilgren **(186)**	34 women with VTE during pregnancy	59% APC resistant	PCR not done ?selection criteria
Hallak **(187)**	15 (not all VTE)	7 FVL	CVA, TIA included Highly selected
Dizon-Townson **(188)**	14 women with FVL	4 developed VTE	
Hirsch **(189)**	35 women with VTE related to pregnancy	17% FVL	?selection criteria
Bokarewa **(190)**	74 (not all VTE)	46% FVL	?selection criteria Placental thrombosis, CVA included

FVL-factor V Leiden

CVA-cerebrovascular accident

TIA-transient ischaemic attack

Table 3.4 Trimester distribution of Thrombotic Events.

	DVT	Pulmonary Embolism
Number	64	23
Total Antenatal Events	44	9
First trimester	12	0
Second trimester	8	1
Third trimester	24	8
Puerperium	20	14

Table 3.5 Incidence of Maternal VTE based upon a Total of 93 493 Maternity's during the Study Period.

Event rate (per 1000 maternity's)	DVT (95% CI)	Pulmonary Embolism (95%CI)
Total	0.68 (0.51-0.85)	0.25 (0.15-0.35)
Antenatal	0.47 (0.33-0.61)	0.1 (0.04-0.16)
Puerperal	0.21 (0.12-0.3)	0.15 (0.07-0.22)

Table 3.6 Event Rates per 10 000 Women-Years at Risk.

Event rate (per 10 000 years at risk)	DVT	Pulmonary Embolism
Antenatal	6.4	1.3
Puerperal	18.5	13.0

Table 3.7 Clinical Risk Factors (CRF) for Thrombosis detected in 75 Women attending for Follow-up. Also Shown are Numbers of Women Subsequently Found with Thrombophilia

Diagnosis	Number	**Number** none	**Of Clinical** one	**Risk factors** two	three or more
DVT	55	22 (40%)	14 (25%)	11 (20%)	8 (15%)
PE	20	8 (40%)	4 (20%)	6 (30%)	2 (10%)
Total	75	30 (40%)	18 (24%)	17 (23%)	10 (13%)
With detectable thrombophilia		6 (20%)	4 (22.2%)	7 (41.2%)	3 (33.3%)

Table 3.8 Details of Women Found to Have Heritable Thrombophilic Defects.

Patient	Diagnosis	Gestation	Thrombophilia	Family Study	Clinical Risk Factors
1	DVT	1	AT 57/55	[1] untested	previous VTE
2	DVT	1	AT 48/45	[1] mother, brother AT deficient	nil
3	DVT	1	AT 57/45	[1] father, sister AT deficient	nil
4	DVT	2	AT 45/49	[1] untested	previous VTE
5	DVT	pp	AT 74/86	[2] mother (symptomatic)	family history
6	PE	pp	AT 73/88	[3] mother CVA aged 32 years	emergency CS twin pregnancy
7	DVT	pp	AT 56/72	[1] untested	nil
8	DVT	1	FVL +/-	[1] untested	previous VTE parity
9	DVT	pp	FVL +/-	[2] sister (symptomatic)	weight family history
10	DVT	3	FVL +/-	[2] sister (symptomatic)	colitis family history
11	DVT	3	FVL +/-	[2] sister (symptomatic)	age, parity family history
12[#]	DVT	3	FVL +/-	[1] untested	previous VTE weight
13	DVT	1	FVL +/-	[1] untested	nil
14	DVT	1	FVL +/+	[1] untested	hyperemesis immobility
15	DVT	3	PC 55/62	[1] untested	nil
16	PE	pp	PT20210A	[1] untested	emergency CS preeclampsia
17	PE	3	PT20210A	[1] untested	nil
18	DVT	1	PT20210A	[1] untested	previous VTE weight
19	PE	pp	PT20210A	[1] untested	emergency CS preeclampsia weight

[#] patient also heterozygous for PT 20210A

Individuals 1-4 type I AT deficiency, 5-7 type II AT deficiency, 8-14 FVL, 15 PC deficiency.

AT, antithrombin: normal range 83-128 IU/dl. Figures given are activity/antigen.

PC, protein C: normal range 74-128 U/dl. Figures given are activity/antigen.

FVL +/- heterozygous; +/+ homozygous

PT20210A heterozygous for prothrombin 20210 G→A mutation

CVA, cerebrovascular accident

[1] families with no history of thrombosis other than in propositus

[2] families with genetically affected relatives, symptomatic for venous thrombosis

[3] genetically affected with arterial thrombotic event

Table 3.9 Prevalence of FVL, Prothrombin 20210A, C677T Mutations and Antithrombin Deficiency in Cases and in the General Population.

Thrombophilia		Cases	General Population*	Odds ratio	95% CI
Prothrombin 20210A	present	5	5	4.4	1.2-16
	absent	50	219		
FVL					
	present	7	5	4.5	2.1-14.5
	absent	68	219		
C677T					
	+/+‡	3	19	0.45	0.13-1.58
	+/- and -/-	49	139		
Antithrombin					
Type 1① deficiency	present	4	1	282	31-2532
	absent	71	4999		
Type 2② deficiency	present	3	3	28	5.5-142
	absent	72	1997		

* Prevalences of FVL and prothrombin 20210A mutations in the general population were obtained by genotyping 224 random unselected cord blood controls in our area [McColl et al (74)]. The prevalence of the three MTHFR genotypes were obtained by analysis of 158 cord controls [McColl et al (74)]. The prevalence of antithrombin deficiency in our area has been published by Tait et al (40).

① quantitative antithrombin deficiency

② qualitative antithrombin deficiency

‡ homozygous for mutant allele

CHAPTER 4

INCIDENCE OF SUPERFICIAL VEIN THROMBOSIS (THROMBOPHLEBITIS) ASSOCIATED WITH PREGNANCY AND PREVALENCE OF THROMBOPHILIC ABNORMALITIES

4.1 SUMMARY

4.4.1 We have determined the incidence of clinically diagnosed SVT associated with pregnancy and the early post-partum period in a retrospective study of 72 000 maternity's. 49 cases occurring in 47 individuals were recorded, with an overall incidence of 0.68/1000 maternity's (95% CI 0.48-0.88). The incidence in the early post-partum period (0.54/1000 maternity's) was markedly higher than in the antenatal period. None had a previous history of deep vein thrombosis or pulmonary embolism. Twenty-four were screened for established thrombophilic abnormalities, with only one abnormality detected (heterozygous FVL). The risk of SVT was increased, though not significantly, in carriers of the FVL mutation (OR 1.83; 95% CI 0.2-16.4). After a mean follow-up period of four years, none of the twenty-four had subsequently developed DVT or PE. Thrombophilia may play a minor role in the aetiology of SVT associated with pregnancy, although a larger study is required to confirm this.

4.2 INTRODUCTION

4.2.1 Pregnancy and the puerperium are recognised as periods of considerable risk for the development of deep venous thrombosis (DVT) and pulmonary embolism (PE) (8). We have documented the incidence of objectively confirmed DVT and PE associated with pregnancy, and examined the role of both inherited and acquired risks for their development (195). There are few data regarding the incidence of superficial venous thrombotic (SVT, thrombophlebitis) events in association with pregnancy. SVT has been reported in association with deficiencies of both protein C and S and also in those with APC resistance (39,54). There are also few data to determine if thrombophilia plays a significant role in the aetiology of SVT. To investigate this further, we have performed a retrospective study of women who developed clinically diagnosed SVT without evidence of DVT or PE associated with pregnancy at two maternity units (Glasgow Royal Maternity Hospital and the Southern General NHS Trust) in Glasgow over the period January 1985 - December 1995. The aims of the study were to determine the incidence of SVT associated with pregnancy, document associated clinical risk factors, and to perform thrombophilia screening to determine the prevalence of prothrombotic abnormalities in this group.

4.3 PATIENTS, MATERIALS AND METHODS

4.3.1 The study was commenced in 1995 and completed in 1996. A list of women with ICD9 codes corresponding to superficial vein thrombosis (superficial thrombophlebitis) (ICD9-671.2) occurring during pregnancy or the early post-partum period (up to the point of discharge after delivery) was obtained from the information and statistics division of the common services agency using SMR2 returns. The study was based upon women attending one of two maternity units in Glasgow between the years 1985-1995 inclusive. From the patient list, case records were examined to confirm that a clinical diagnosis of SVT had been recorded and also to obtain clinical data. For the purposes of our study, SVT was present if the attending doctor felt that there was clinical evidence of superficial thrombus formation in one or both lower limbs with or without limb swelling or inflammation. Women were contacted and invited to attend for interview and blood sampling. All participants gave written informed consent. The study met with the approval of the local ethics committee. Women were screened for deficiency of antithrombin, protein C, protein S, for APC resistance / FVL, and for evidence of lupus anticoagulant / elevated IgG or IgM anticardiolipin antibodies. The study was completed in 1996, and hence women were not screened for the prothrombin 20210G→A mutation, which was described in November 1996.

4.3.2 For the purposes of comparison, the prevalence of thrombophilic abnormalities in cases was compared to that obtained in over 200 random unselected cord controls, as described in Chapter three.

4.3.3 Clinical risk factors (CRF) for development of VTE

4.3.3.1 Data on possible clinical risk factors for VTE (table 1, chapter 3) was obtained

from the patient case-notes, supplemented by information obtained at interview.

4.3.4 Blood Sampling

4.3.4.1 None of the individuals were pregnant at the time of blood sampling and none were on anticoagulants. Women were tested for thrombophilia at least six months after the event. Blood was obtained by fresh venepuncture, and added to 0.109 mol/L trisodium citrate (9 parts blood: 1 part anticoagulant). Samples were mixed gently and centrifuged at 2000g for 10 minutes to obtain platelet poor plasma (PPP). The PPP was then centrifuged for a further 10 minutes at 2000g and the plasma separated into 1ml aliquots within four hours. Plasma samples were then stored in 1ml aliquots at -70^{0} C until analysis. Peripheral blood leucocytes were stored and used for DNA analysis.

4.3.5 Sample analysis

4.3.5.1 Samples were subject to a thrombophilia screen which included prothrombin time, activated partial thromboplastin time, thrombin time, antithrombin and protein C activity, free and total protein S antigens, lupus anticoagulant screen (APTT performed on a mix of four parts patient platelet poor plasma to one part normal platelet poor plasma, and dilute Russells' viper venom test with platelet neutralisation step), IgG and IgM anticardiolipin antibody assays and APC-SR. Testing for the FVL mutation was performed on all individuals in one of the units and in those with a low or borderline APC-SR (APC-SR less than 2.9) in the other unit. The methodology used for the coagulation assays, and PCR for the FVL mutation has been described in Chapter 3.

4.3.6 Statistical techniques

4.3.6.1 Ninety-five percent confidence intervals for the incidence of maternal SVT

were determined by multiplying the standard error by 1.96. Since the study was retrospective with SVT as an endpoint already identified, we calculated odds ratios (OR) with 95% confidence intervals as estimates of the relative risk of thrombosis, as described in Chapter 3.

4.4 RESULTS

4.4.1 During the study period there were 72 200 maternity's, with 49 cases of SVT identified which occurred in 47 women. All cases of puerperal SVT occurred within one week of delivery, with no patients in the cohort readmitted following discharge from hospital with SVT. The median age at time of event was 30 years (range 20-44). None had a previous history of DVT or PE. Table 4.1 shows the calculated incidence of SVT per 1000 maternity's. The overall incidence of SVT was 0.68 per 1000 maternity's (95% confidence interval 0.48-0.88). Puerperal SVT (0.54 per 1000, 95% confidence interval 0.37-0.71) predominated over antenatal SVT (0.14 per 1000, 95% confidence interval 0.06-0.22). 5/47 individuals (3 antenatal, 2 puerperal) were screened to exclude possible DVT using Doppler ultrasound (all were negative), with the remainder not tested objectively for underlying DVT. Clinical risk factors (CRF) identified for the development of thrombosis included pre-existing varicose veins (13 women), age > 35 years (8 women), weight > 80 kg (8 women), parity 4 or greater (6 women) and caesarean section (2 women) (figure 4.1). Most patients were treated symptomatically with non-steroidal anti-inflammatory drugs. Three patients, all with puerperal SVT, received short-term subcutaneous heparin (maximum period 1 week). In two cases heparin was administered following the event and in one case was being given as thromboprophylaxis following emergency caesarean section when SVT occurred.

4.4.2 Twenty-four of forty-seven (51%) attended for follow-up and blood sampling (the others had either moved away from the area or were unwilling to participate for blood sampling). Only one individual of the 24 screened was found to possess a thrombophilic abnormality (heterozygous FVL). No deficiencies of antithrombin,

protein C or protein S, abnormal lupus / anticardiolipin tests were found. Mean interval from time of SVT to follow-up in these 24 women was 48 months (range 12-125), with none having developed either DVT or PE during this period. However, 2 individuals each had a second episode of SVT during subsequent pregnancies. An objective test to exclude DVT was not performed in either subsequent case. Neither of these two individuals was found to possess a thrombophilic abnormality.

4.5 DISCUSSION

4.5.1 The incidence of SVT associated with pregnancy (0.68 per 1000 maternity's, 95% confidence interval 0.48-0.88) appears comparable to that documented for DVT associated with pregnancy (0.68 per 1000, 95% confidence interval 0.51-0.85). The true incidence is likely to be higher since we may have missed some women who developed SVT following discharge from hospital. Such a situation might not result in hospital admission, since SVT is generally regarded as a benign condition compared with DVT or PE. Also, some women with leg pain and / or swelling who underwent radiological examination to exclude DVT may have had SVT, but this may not have been coded as such. Macklon et al documented the incidence of maternal SVT over the period 1983-1992 in Scotland at between 1.36-2.5 per 1000 maternity's in women under 35 years (176). However, this study was based upon national SMR2 discharge data and did not involve an examination of case notes to confirm accuracy. We detected a considerable number of women who had been given a diagnostic code corresponding to SVT but in whom we could find no evidence of this in the case-notes. In many of these instances the patients either had vulval or leg varicosities without clinical evidence of thrombosis.

4.5.2 We noted a marked propensity for SVT to occur in the first week of the post-partum period (39/49, 80%) which has been noted in one smaller study (175). This is in contrast to women who develop DVT related to pregnancy, in which a large proportion present during the antenatal period (69% of our cohort with DVT presented during the antenatal period, chapter 3) (175,176,195). This finding may be explained, at least in part, by selection bias, since women are more likely to be diagnosed with SVT whilst in hospital compared with those who have been discharged.

4.5.3 Over 27% of those affected had pre-existing varicose veins, suggesting that varicosities may be a significant risk factor for the development of SVT. Only 4% of cases were associated with caesarean section. Although no cases of DVT were documented, relatively few women were investigated for underlying DVT (5/47). Without performing objective testing for DVT, we cannot be certain that none had underlying DVT. In addition, we only have limited follow-up data (24/47, 51%) in which none of the women had subsequently developed DVT or PE (mean follow-up period 48 months). The association between SVT and DVT has recently been examined by Bounameaux et al in a study of 551 consecutive patients presenting with SVT (210). This study found coexisting DVT in 5.6% of their cohort, all of whom were screened objectively for DVT. Those found with DVT tended to be older with a history of immobility. Of the remainder, a further 1.7% developed DVT at three months follow-up. Chengelis et al reported that 11% of 263 patients with SVT developed progression of thrombosis from the superficial to the deep veins (211) and Blumenberg reported 8.6% of 232 patients with SVT had coexisting DVT (212). Clearly, SVT may progress to DVT, particularly if SVT involves the proximal superficial saphenous vein (212), and in such a situation imaging to exclude DVT is indicated.

4.5.4 In women whom we were able to follow-up, we found only one individual with the FVL mutation (4.2% of the cohort; 95% CI 0-12.2), and none with deficiencies of PC, PS, antithrombin or antiphospholipid syndrome. The number of women whom we were able to follow-up was rather low, perhaps because the condition is not regarded as a serious health problem to those afflicted, and therefore the motivation to participate in such a study is less. Unfortunately our study was completed before the description of

the prothrombin 20210A mutation, and hence women were not tested for this thrombophilia. The prevalence of the FVL mutation in women with SVT was compared to that obtained in cord blood controls. The odds ratio for development of SVT in carriers of FVL was increased, though the figure is not statistically significant (OR 1.83, 95% confidence interval 0.2-16.4). However, we appreciate that the numbers followed-up are relatively small (51% of the cohort). There are few data on the relationship of thrombophilia to SVT. Since our study, de Moerloose et al have investigated the prevalence of FVL and the prothrombin mutation in 112 consecutive patients with SVT of the lower limbs (213). These patients were not selected for development of SVT in relation to pregnancy. Those with FVL appeared to have an increased risk of SVT compared to controls (OR 2.51, 95% confidence interval 1.04-6.24). However, clearly the increased risk of SVT observed in those with FVL in this study is much lower than the risk of DVT or PE quoted for carriers of the FVL mutation (5-10 fold) (63). The OR was also increased for carriers of the prothrombin mutation (OR 3.28, 95% confidence interval 0.46-36.8), although this was not statistically significant. In this study, BMI > 28 kg/m^2 was also noted to be an independent risk factor for SVT.

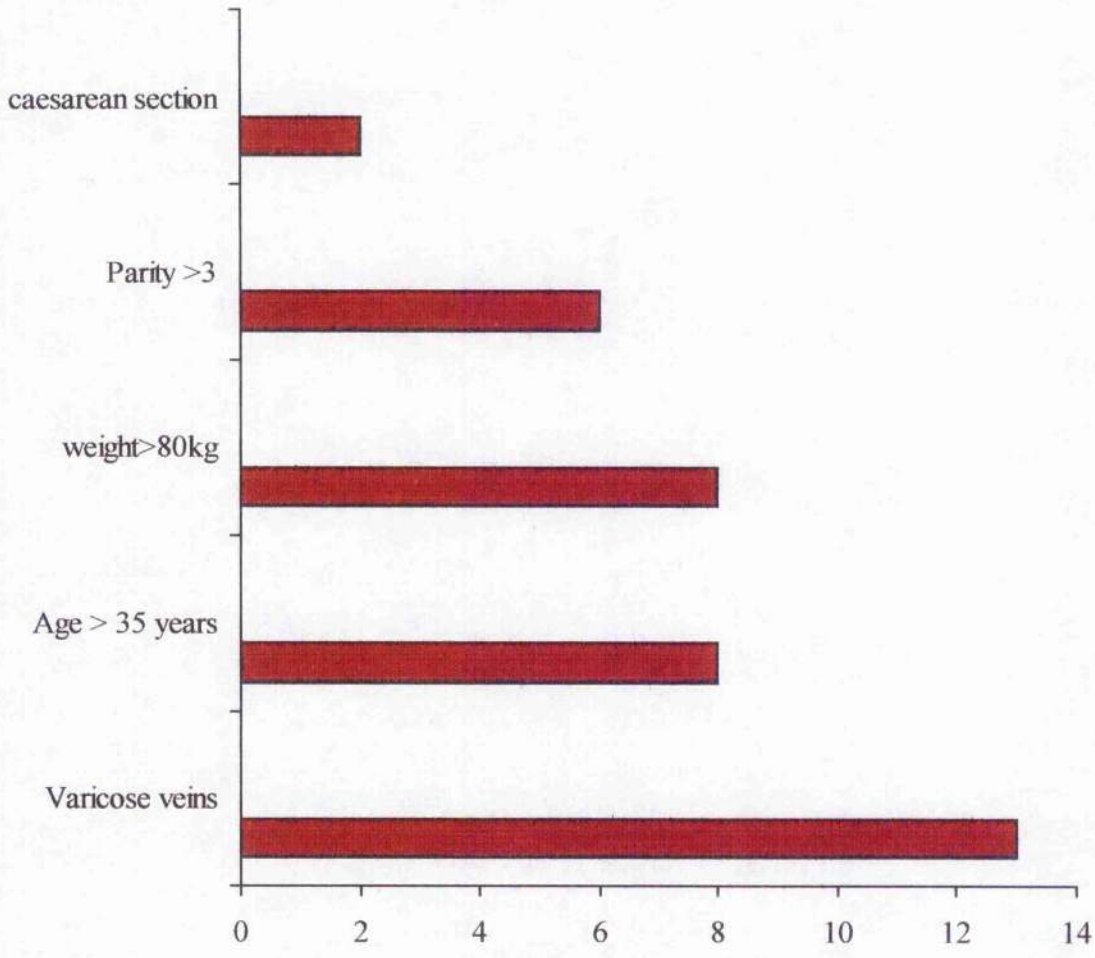
4.5.5 In conclusion, SVT associated with pregnancy appears particularly common in the post-partum period. Additional clinical risk factors for VTE are common in those affected. Thrombophilia may play a relatively minor role in its aetiology, although a larger study is required to fully address this issue. This condition may occur as a result of physical factors associated with pregnancy and delivery (increased venous pressure, venodilation, immobility during delivery) and of the physiological prothrombotic state conferred by pregnancy itself. From our limited data there appears no requirement for

extensive thrombophilia screening in those affected, except perhaps in those with recurrent events or those with a strong family history of venous thrombosis.

Table 4.1 Incidence of Superficial Vein Thrombosis associated with Pregnancy.

	Number	Incidence per 1000 maternity's	95% CI
Antepartum + Post-Partum	49	0.68	0.48-0.88
Antepartum	10	0.14	0.06-0.22
Post-Partum	39	0.54	0.37-0.71

Figure 4.1 Prevalence of Clinical Risk Factors in 47 Women with Superficial Vein Thrombosis Related to Pregnancy.



CHAPTER 5

LIPOPROTEIN (a), CHOLESTEROL AND TRIGLYCERIDES IN WOMEN WITH VENOUS THROMBOEMBOLISM

5.1 SUMMARY

5.1.1 Plasma concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and lipoprotein (a) were measured in 62 women who had suffered an episode of objectively confirmed venous thromboembolism (VTE) at ≤ 50 years of age, and in 98 age-matched female controls. The mean BMI of cases was significantly ($p < 0.001$) higher than that of controls. Plasma triglyceride and LDL-cholesterol correlated directly with BMI, whereas HDL-cholesterol correlated inversely with BMI. Plasma triglyceride was significantly higher, and total cholesterol / HDL-cholesterol significantly lower, in cases compared to controls. After adjustment for BMI, the plasma total cholesterol and LDL-cholesterol remained significantly lower in cases. No significant differences in mean plasma lipoprotein (a) levels were identified between cases and controls. Lipoprotein (a) does not appear to be significantly associated with the development of VTE in young women. The increased risk of VTE in obese subjects may be mediated, at least in part, via hypertriglyceridaemia, through its effects on levels of coagulation factors, natural anticoagulants, and PAI-1. Furthermore, despite increased mean BMI such subjects do not exhibit elevations in LDL-cholesterol concentrations. In fact, LDL-cholesterol concentrations may be reduced in subjects with a history of VTE. Further studies are required to confirm and elucidate mechanisms for these observations.

5.2 INTRODUCTION

5.2.1 In contrast to data linking abnormalities of lipid metabolism to the development of prevalent arterial disease, few studies have examined a possible role for lipids in the aetiology of venous thromboembolism (VTE). This is perhaps surprising considering the advances that have recently been made in elucidating the causes of VTE. Inherited coagulation abnormalities are presently identified in around 25-30% of individuals presenting with a first episode of VTE. The factor V Leiden (FVL) and prothrombin 20210A mutations are the most prevalent genetic defects identified (53,63,85). Elevated plasma homocysteine is also a common finding in those with VTE, identified in approximately 10% (102). Furthermore, approximately 20% of individuals with VTE have persistently elevated levels of coagulation FVIII (> 150 iu/dl), which is associated with a relative risk of VTE of around four-fold (115). Considering FVL, prothrombin 20210A, elevated FVIII, hyperhomocysteinaemia, and natural anticoagulant (antithrombin, protein C and protein S) deficiencies collectively, around 60% of subjects with VTE have an underlying thrombophilia, although not all are readily demonstrable to be inherited, and some are clearly influenced by environmental factors. Such thrombophilic abnormalities exert their effects predominantly via prothrombotic changes in the coagulation pathway.

5.2.2 Lipids have complex effects on the coagulation cascade. For example, triglyceride plays a major role in FVII activity in plasma, and has a direct effect on endothelium to provoke prothrombotic changes, such as an increased expression of plasminogen activator inhibitor type 1 (PAI-1) and von Willebrand factor (214). Triglyceride also has effects on endothelial cell adhesion molecules (214). Despite such complex effects

of lipids, which may be involved in the pathogenesis of VTE, there is a paucity of data in this area. The only published data suggests that individuals with elevated total cholesterol levels and / or triglyceride, may be at increased risk of VTE (215,216). Furthermore, elevated lipoprotein (a), which shares structural similarities to plasminogen, has been reported as a possible risk factor for pulmonary embolism (PE), and for chronic thromboembolic pulmonary hypertension (217,218).

5.2.3 In a study to determine the aetiology of VTE in young women, we investigated plasma lipid parameters (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and lipoprotein (a)) in 62 women with a previous episode of objectively confirmed VTE at age ≤ 50 years, and in 98 healthy age-matched female controls.

5.3 PATIENTS, MATERIALS AND METHODS

5.3.1 The study was approved by the local research ethics committee. Women who had suffered an episode of objectively confirmed VTE at ≤ 50 years of age and treated in our unit were eligible for inclusion in the study. Consecutive females who developed VTE related to pregnancy or up to six weeks post-partum between 1985-1998 were invited to participate. In addition, consecutive women who presented with VTE to our hospital between 1993-1997 at age ≤ 50 years were invited to participate. Women in whom the diagnosis was related to intravenous drug abuse or cancer were excluded from the study. For deep vein thrombosis (DVT), the diagnosis was accepted if duplex Doppler scanning, compression ultrasound, or venography were diagnostic. For pulmonary embolism (PE) the diagnosis was accepted if the ventilation-perfusion scanning result was of either high or intermediate probability, and they had received at least six-months of anticoagulant therapy.

5.3.2 From an eligible cohort of 161 women with a past history of VTE, 2 had died (both from PE), 4 were excluded due to psychiatric disorders, and 84 were either untraceable, had moved from our area, or declined participation. In total, 71 women agreed to participate in the study. Blood for lipid studies was unavailable in 9 women at the time of analysis. Therefore, data is presented on a total of 62 female cases. Triglyceride data is presented on 46 female cases, since 16 women had not performed an adequate 12-hour overnight fast. No women were current users of hormonal preparations or lipid lowering drugs, and none had a personal history of arterial disease. Height (m) and weight (kg) were recorded. Body mass index (BMI) was calculated (kg/m^2).

5.3.3 Ninety-eight healthy aged-matched female controls, free from arterial and venous disease, diabetes mellitus or dyslipidaemia were recruited by local advertisements for volunteers in our area. Although not randomly sampled, the distributions of age, anthropometric and biochemical data are very similar to those found in general population surveys. BMI was recorded in controls, who had blood removed to assay lipid parameters. None of the controls were users of lipid lowering drugs or hormonal preparations.

5.3.4 Blood collection and sample analysis

5.3.4.1 Blood samples were obtained at least 6 months following the thrombotic event. None of the cases or controls were pregnant at the time of blood sampling. Blood was withdrawn after a 12-hour overnight fast. For lipid analysis, 9ml venous blood was collected into tubes containing potassium EDTA as anticoagulant. Plasma was collected following centrifugation, and aliquots were frozen and stored at -70^0 C until analysis. In addition, 20ml venous blood was added to trisodium citrate (9 parts blood: 1 part anticoagulant) for the purpose of thrombophilia investigation.

5.3.4.2 Plasma cholesterol, triglyceride and HDL assays were performed using the standard lipid research clinics protocol (219). LDL-cholesterol was calculated using the Friedwald formula (220). Lipoprotein (a) analysis was carried out on EDTA plasma samples by a latex agglutination assay (Innotest Lp(a), Immunogenetics N.V., Belgium), which has been shown to have an excellent correlation with a manual enzyme immunoassay (R2=97%) in our laboratory (221).

5.3.4.3 Thrombophilia testing was performed in women with previous VTE but not on

healthy controls. This included prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen, plasma FVIIIc, antithrombin (AT) and protein C (PC) activity, free and total protein S antigens, lupus inhibitor screen (APTT performed on a mix of four parts patient platelet poor plasma to one part normal platelet poor plasma, and dilute Russells' viper venom test with platelet neutralisation step), IgG and IgM anticardiolipin antibody assays and unmodified activated protein C-sensitivity ratio (APC-SR). Polymerase chain reaction was used to determine the presence or absence of the factor V Leiden (FVL) and prothrombin 20210A gene mutations in all cases.

5.3.4.4 Plasma FVIIIc was measured in a one-stage assay. Briefly, 0.1ml dilutions of test and standard plasmas were mixed with 0.1mls of FVIII deficient plasma. An APTT was performed for each dilution and the clotting times plotted on log-log graph paper against dilution. The FVIIIc level in the test samples were calculated from the log-log plot. The analytical techniques used in the remaining thrombophilia tests have been described previously.

5.3.5 Statistical analysis

The significance of differences in mean age, BMI, and lipid values in cases and controls were determined by using the Students t-test. Correlation coefficients (Pearson) were calculated to determine the relationship between BMI and lipid parameters. Correlation coefficients and regression plots were calculated using *Minitab* (version 11, PA, USA). Significance was taken at the 95% level. *p* values are two-tailed. Non-parametric data were log-transformed prior to analysis. Multiple linear regression was used to adjust for the influence of BMI on the unadjusted differences in lipid parameters between groups. Linear regression was performed using *Minitab*.

5.4 RESULTS

5.4.1 The characteristics of the 62 cases and 98 controls are shown in table 1. The mean BMI of cases at time of sampling (28.5 ± 6.2) was significantly higher than of controls (24.3 ± 3.8) (p <0.0001). The mean age at presentation with VTE was 31.2 years (range 15-50 years, SD 8.3). Both groups were matched for age at time of blood withdrawal: mean age at time of blood sampling in cases was 35.8 years (range 16-56) and in controls was 37.0 years (range 18-69) (p = NS). Of the 62 women with VTE, 46 presented with DVT and 16 with PE.

5.4.2 Precipitating factors for VTE were as follows: pregnancy (39/62, 63%), combined oral contraceptive pill (10/62, 16%), hormone replacement therapy (2/62, 3%), surgery (1/62), and immobility (1/62). In 9/62 (14.5%) women the event occurred spontaneously. In 8/62 (13%) women the event was recurrent. Twelve women were on oral anticoagulants at the time of blood withdrawal.

5.4.3 We examined the relationship between BMI and total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and lipoprotein (a) concentrations, in cases and controls (table 2). There was a significant positive correlation between BMI and both triglyceride (figure 1) and LDL-cholesterol in cases and controls. Total cholesterol concentration was found to correlate directly with BMI in cases, but not in controls. HDL-cholesterol correlated inversely with BMI only in controls. No significant correlation was observed between lipoprotein (a) and BMI.

5.4.4 In women with a past history of VTE, there were no significant correlations observed between triglyceride concentration and fibrinogen ($r = 0.13$, $p = 0.4$), FVIIIc

(r = 0.01, p = 0.9), or APC-SR (r = -0.16, p = 0.32). Furthermore, no significant correlation was identified between total cholesterol and fibrinogen (r = 0.1, p = 0.4), FVIIIc (r = -0.1, p = 0.4) or APC-SR (r = -0.1, p = 0.5).

5.4.5 Mean concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and lipoprotein (a) in cases and controls are shown in table 1. Mean unadjusted total cholesterol (p = 0.02) and LDL-cholesterol (p = 0.01) were significantly lower, and triglyceride significantly higher (p = 0.023), in cases compared to controls. The unadjusted odds ratio for risk of VTE associated with triglyceride concentration ≥ 1 mmol/l was 2.15 (95% CI 1.06-4.35). After adjustment for BMI, the mean total cholesterol (p < 0.001) and LDL-cholesterol (p < 0.001) remained significantly lower in cases compared to controls, whereas triglyceride levels were not significantly different (p = 0.786). Mean HDL-cholesterol was significantly lower in cases compared to controls (p = 0.042), though this was not significant following adjustment for BMI (p = 0.484). There were no significant differences in mean lipoprotein (a) concentration between cases and controls.

5.4.6 Thrombophilia screening of cases with a past history of VTE identified 4 (6.5%) women with antithrombin deficiency (3 with type I deficiency, 1 with type II deficiency), 1 (1.6%) with protein C deficiency, 1 with antiphospholipid syndrome (persistently elevated IgG anticardiolipin antibody, 15-44 iu/dl), 5 (8.1%) with FVL (4 heterozygous, 1 homozygous) and 4 (6.5%) with the prothrombin 20210A mutation. No combined heritable thrombophilias were detected. Plasma homocysteine was not measured. The overall prevalence of detectable thrombophilia in the cohort of 62 was 24.2% (15/62). 15 women had FVIIIc of > 150 iu/dl. Of these women, 1 had protein C

deficiency, 1 had the prothrombin 20210A mutation, and 2 had FVL. Twelve women were on oral anticoagulants at the time of blood withdrawal. In these women it was not possible to accurately assess protein C and protein S levels, and therefore the prevalence of these deficiencies may have been underestimated.

5.5 DISCUSSION

5.5.1 Lipids have wide-ranging effects on haemostasis that could play a role in the development of VTE. For example, in a large (> 1500 subjects) epidemiological study by Lowe et al, triglyceride and total cholesterol were found to directly correlate with plasma levels of coagulation factors I (women only), VII, VIII (women only), and IX, with elevated levels of natural anticoagulants (antithrombin, protein C, protein S), but not with elevated coagulation activation markers (222). Furthermore, activated protein C sensitivity ratio (APC-SR) is reported to inversely correlate with total cholesterol levels in both sexes, and with triglyceride levels in women (61). Elevated triglyceride concentrations are associated with elevated PAI-1, usually in association with reduced HDL cholesterol, forming part of the insulin resistance syndrome (223). This may be mediated via a direct toxic effect by triglyceride on the endothelium (214). Other potential prothrombotic influences exerted by lipids include effects on tissue factor pathway inhibitor (TFPI), and effects on monocyte expression of tissue factor (TF). It is reported that hypercholesterolaemia may produce a decrease in free TFPI (due to the relative increase in lipoprotein associated TFPI) (34), with recent data demonstrating low levels of heparin-releasable TFPI (a measure of endothelial TFPI) in subjects with previous DVT (224). Monocyte expression of tissue factor (TF) is upregulated following ingestion of oxidised LDL, which occurs at sites of atheroma (147). Animal studies have demonstrated that monocyte-derived TF may trigger thrombosis (225), and it is speculated that monocyte-TF may be important in the development of VTE in situations of blood stasis (226).

5.5.2 The overall influence of changes in coagulation factors, coagulation inhibitors, and expression of tissue factor, which is observed in subjects with

hypertriglyceridaemia and / or hypercholesterolaemia, on the risk of VTE is unknown. There are few data that have examined the role of lipids in the aetiology of VTE. Talbot et al documented serum triglyceride and cholesterol levels in 172 subjects with VTE and compared values with those obtained in over 200 controls (216). Mean levels of cholesterol and triglyceride were higher in cases than in controls, although taken overall the findings did not reach statistical significance. However, subgroup analysis revealed that individuals with recurrent or spontaneous VTE had significantly higher serum cholesterol levels compared with controls. Whilst this study provided an interesting observation, unfortunately the diagnosis of VTE was not objectively confirmed in many individuals, and the BMI of cases and controls was not detailed.

5.5.3 Kawasaki et al have investigated cholesterol and triglyceride levels in 109 consecutive subjects with DVT and compared their findings with those obtained in age, sex and BMI-matched controls (215). The paper did not detail timing of blood samples in relation to the VTE event. Hypercholesterolaemia (with or without hypertriglyceridaemia) was associated with an increased risk of DVT (odds ratio 4.5, 95% CI 2.4-8.3). Hypertriglyceridaemia (with or without hypercholesterolaemia) was also identified as a risk factor for DVT (odds ratio 2.4, 95% CI 1.3-4.6). The finding of hypercholesterolaemia with hypertriglyceridaemia was associated with the greatest risk of DVT (odds ratio 5.1, 95% CI 2-13).

5.5.4 Lipoprotein (a) has recently been the subject of investigation as a potential risk factor for VTE. Lipoprotein (a) shares sequence homology with plasminogen, and may compete for binding to fibrin, thereby inhibiting endogenous fibrinolysis (227). Elevated lipoprotein (a) has been reported in 40 patients with chronic thromboembolic

pulmonary hypertension (217), and also in 25 subjects with previous pulmonary embolism (218). There are currently no data on lipoprotein (a) levels in subjects with DVT.

5.5.5 In the present study total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and lipoprotein (a) concentrations in women aged ≤ 50 years at time of VTE, without a personal history of arterial disease, were compared to those obtained in healthy age-matched female controls. We found that BMI correlated directly with triglyceride and LDL-cholesterol. HDL-cholesterol was inversely correlated with BMI, though this was only significant in controls. We found no significant correlation between triglyceride or total cholesterol and fibrinogen, FVIIIc or APC-SR, unlike the study by Lowe (222). However, it is likely that the number of individuals studied here was too small to detect any significant correlations. Unexpectedly, we found that both total cholesterol and LDL-cholesterol (unadjusted and following adjustment for BMI) were significantly lower in cases compared to controls. Unadjusted triglyceride concentrations were significantly higher in cases, reflecting the direct correlation with BMI; however, the difference was not significant following adjustment for BMI. HDL-cholesterol was significantly lower in cases, though this was not significant following adjustment for BMI. There were no significant differences in lipoprotein (a) concentration in cases compared with controls. The reason for the lower total and LDL-cholesterol levels in cases compared to controls is unknown. We may speculate that since LDL catabolism is effected by the LDL-receptor, women with a history of VTE may have an increased receptor number or affinity. Alternatively, as LDL-receptor regulation is altered by oestrogen status (228), it is conceivable that such women have a heightened endogenous oestrogen status or an increased responsiveness to oestrogens.

This suggestion, however, must remain tentative until further data are available.

5.5.6 We found that the mean BMI of cases was markedly higher than of controls ($p < 0.001$), although we did not record BMI at the time of VTE event. Our control data appears representative of the general population, and it therefore seems likely that the higher BMI observed in cases is a genuine finding. BMI > 25 kg/m^2 is an established risk factor for VTE (2,3,6), although the mechanism is unknown. The observation of a significantly higher BMI in women with VTE, with associated elevated triglyceride and decreased HDL-cholesterol concentrations, is of considerable interest. A pattern of high triglyceride and low HDL is a feature of the insulin resistance phenotype syndrome (223). As yet, there are no studies that have examined insulin and glucose concentrations in subjects with a history of VTE. We hypothesise that the increased risk of VTE associated with increasing BMI may be mediated, in part, via hypertriglyceridaemia, which is accompanied by elevated coagulation factors, elevated PAI-I, increased resistance to the anticoagulant action of APC, and effects on endothelial cells. The question of whether the insulin resistance syndrome may be associated with a higher BMI in a proportion of individuals with VTE requires further research. Although data from Lowe et al (222) demonstrate that the coagulation changes observed with increasing triglyceride and / or cholesterol concentrations may be negated by increased levels of natural anticoagulants, these effects may be of importance in certain circumstances. For example, normal pregnancy is accompanied by a significant rise in triglyceride concentration as gestation progresses, which in turn is accompanied by a marked increase in FVIIc, PAI-1, and D-Dimers (229,230). Furthermore, pregnancies complicated by pre-eclampsia exhibit a more marked rise in plasma triglyceride concentration, suggesting that triglyceride may be involved in the

thrombotic process (231).

5.5.7 We did not observe a significant difference in lipoprotein (a) concentration in cases compared to controls. Lipoprotein (a) may be elevated as part of an acute phase response (232), and recent data suggests that a proportion of individuals with previous VTE demonstrate evidence of inflammation (elevated C-reactive protein) several years following the event (117). This may explain the elevated lipoprotein (a) levels in subjects with VTE reported by other researchers.

5.5.8 We recognise certain limitations of our study. Our subjects were women who had developed VTE at ≤ 50 years of age, and therefore represent a relatively select group. We did\not measure coagulation factor levels (other than FVIII), or investigate for possible insulin resistance, in cases and controls. Our findings raise the possibility that elevated plasma triglyceride concentration, with associated effects on coagulation factors, APC resistance, and PAI-1, may be of some importance in the development of VTE in subjects with high (> 25 kg/m^2) BMI. The higher BMI observed in individuals with VTE is unexplained, though may relate (at least in part) to the insulin resistance syndrome. Data on a larger number of consecutive subjects with VTE, with measurement of lipids, coagulation factors, coagulation inhibitors, and plasma glucose and insulin concentration would be of interest. Clearly with such marked effects on the coagulation cascade, further data are required to determine the precise role of lipids in the aetiology of VTE.

Table 5.1 Mean (±SD) age at time of blood withdrawal, BMI, and total cholesterol (mmol/l), triglyceride (mmol/l), HDL-cholesterol (mmol/l), LDL-cholesterol (mmol/l), and lipoprotein (a) (mg/dl) in 62 women with previous VTE and in 98 controls.

	Cases (n=62)	Controls (n=98)	Unadjusted *p*-value	Adjusted *p*-value †
Mean age (±SD)	35.8 (±9.0)	37.0 (±11.5)	NS	------
Mean BMI (±SD)	28.5 (±6.2)	24.3 (±3.8)	<0.001	------
Total cholesterol	4.74 (±0.97)	5.13 (±1.06)	0.020	<0.001
Triglyceride**	1.29 (±0.55) ‡	1.09 (±0.59)	0.023	0.786
HDL	1.39 (±0.34)	1.44 (±0.35)	0.042	0.484
LDL	2.76 (±0.82)	3.18 (±0.95)	0.011	<0.001
Lp(a)**	23.85 (±25.51)	27.5 (±34.5)*	0.473	------

† adjusted for BMI

‡ triglyceride data available in 46 cases

* Lipoprotein (a) performed on 64 controls

** Non-parametric data (triglyceride, lipoprotein (a) concentrations) were logarithmically transformed prior to analysis

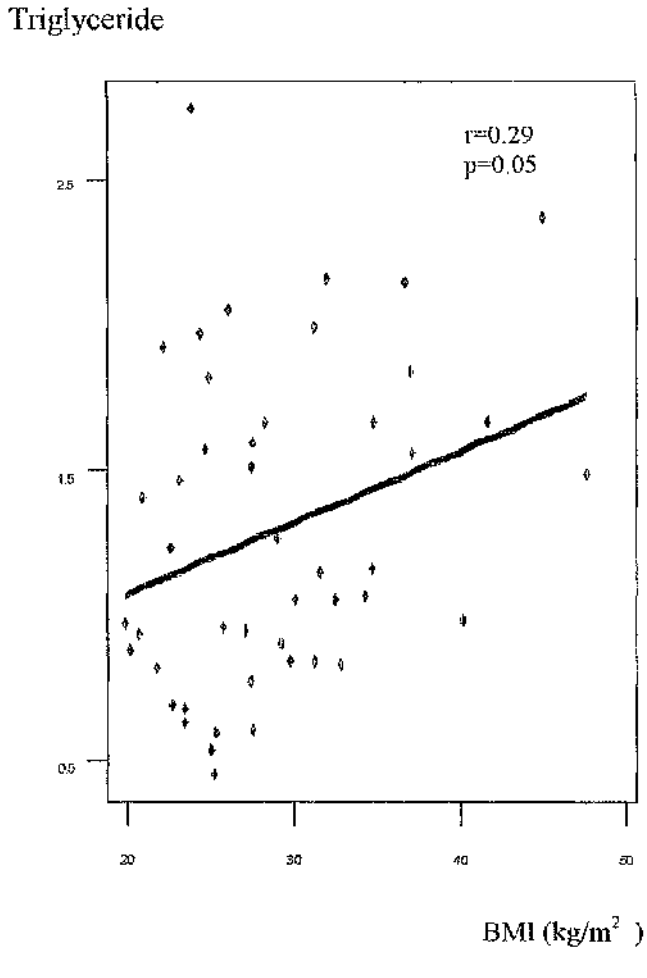
Table 5.2 Correlation coefficients between BMI and total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and lipoprotein (a), in cases and controls.

	Total cholesterol	Triglyceride[1]	LDL	HDL	Lp(a)
Cases (n=62)	0.35‡	0.29**	0.38†	- 0.20	- 0.16
Controls (n=98)	0.19	0.47¶	0.25†	- 0.27‡	0.04

Significance levels: ‡ $p = 0.005$, † $p = 0.01$, ** $p = 0.047$, ¶ $p < 0.001$

[1] Triglyceride measurements in 46 female cases

Figure 5.1 Correlation of triglyceride (mmol/l) with BMI in cases.



CHAPTER 6

PREVALENCE OF THE POST-THROMBOTIC SYNDROME AND CHRONIC VENOUS INSUFFICIENCY IN YOUNG WOMEN WITH VENOUS THROMBOEMBOLISM

6.1 SUMMARY

6.1.1 Seventy-one women who had suffered an episode of objectively proven VTE (52 DVT, 19 PE) at age ≤ 50 years were assessed at a mean of 51 months following the event. Subjects were interviewed and examined to determine the prevalence of the post-thrombotic syndrome (PTS), and had light reflection rheography (LRR) performed on both lower limbs to document the prevalence of chronic venous insufficiency. LRR was also performed on lower limbs of healthy age-matched female controls. Mild PTS was common in women with a previous episode of DVT. Women with a single episode of DVT were more likely to demonstrate evidence of venous insufficiency compared to healthy age-matched female controls (OR 10.9, 95% confidence interval 4.26-28.0). Women with PE were also more likely to have venous insufficiency (OR 3.8, 95% confidence interval 1.2-12.3) compared to controls. There was poor correlation between symptoms of mild PTS and objectively demonstrated venous insufficiency. However, moderate PTS correlated well with LRR findings. Compared with women who had suffered a single previous episode of DVT, women with recurrent DVT were more likely to develop moderate compared with mild PTS (OR 7.7, 95% confidence interval 1.3-44.9), and were more likely to have evidence of venous insufficiency. There was no apparent increase in PTS or venous insufficiency in women with VTE related to pregnancy. 24% of male partners subsequently underwent vasectomy as the preferred mode of contraception following VTE.

6.2 INTRODUCTION

6.2.1 Venous thromboembolism (VTE) appears particularly prevalent in women, being 2-3 times more common than in men during the reproductive years (9). This may largely be attributed to prothrombotic triggers which are experienced by young women, such as the combined oral contraceptive pill and pregnancy. Whilst only a small proportion of individuals who experience an episode of treated VTE die as a result, there are few data on the long-term consequences amongst survivors. There are undoubtedly restrictions on future contraceptive choice, since most clinicians would not prescribe the combined oral contraceptive pill to women with a history of VTE. In addition, it is recognised that a variable proportion develop the post-thrombotic syndrome (PTS) (postphlebitic syndrome), a term used to describe continuing pain, swelling, discoloration, varicosities or ulceration in the previously thrombosed limb. Data suggest that relatively minor symptoms of PTS are common after an episode of VTE (20,21,233-236). However, there are few data on the prevalence of this syndrome in young women with previous VTE. Data on a small number of women with VTE related to pregnancy suggest a higher prevalence of subsequent PTS compared with women who experience VTE in relation to other triggers (237).

6.2.2 It is unclear if symptoms of PTS correlate with objective evidence of chronic venous insufficiency. For example, minor symptoms such as leg pain or leg cramps are common following DVT, and may relate either to venous insufficiency or equally could be psychological in origin. Ambulatory venous pressure monitoring is regarded as the gold standard to demonstrate venous insufficiency (238). However, it is an invasive procedure requiring considerable expertise. Light reflection rheography (LRR) is a simple and reproducible technique which may be used to provide objective evidence of

venous insufficiency (238-240). In previous studies it has demonstrated excellent correlation with ambulatory venous pressure monitoring (238).

6.2.3 The aims of our study were to document the prevalence of symptoms and signs of PTS in women with a previous episode of VTE at age ≤ 50 years, to determine the prevalence of venous insufficiency in this group and compare this to age-matched female controls, and to determine if venous insufficiency / PTS correlated with precipitating factors for VTE (pregnancy versus non-pregnancy), time from event, number of events, or body mass index. We also documented the contraceptive choices made by women who had experienced their episode of VTE during the childbearing years.

6.3 PATIENTS, MATERIALS AND METHODS

6.3.1 A total of 71 women who had suffered an episode of objectively confirmed symptomatic VTE (either deep vein thrombosis or pulmonary embolism) at age ≤ 50 years were the subject of study. In all cases DVT had been diagnosed by compression or Doppler ultrasound, or by venography; in the case of pulmonary embolism, the diagnosis was accepted if V/Q scan was of either high or intermediate probability and the patient received anticoagulation for at least six months. Patient characteristics are presented in table 1. Female cases were recruited from two sources. First, from a cohort of 87 consecutive women with VTE related to pregnancy or the puerperium between 1985-1997 at two nearby maternity units, 45 agreed to participate in a retrospective follow-up study to determine the long-term outcome of VTE. The remainder of this pregnancy related VTE cohort were either untraceable, declined participation or lived an unacceptable distance from our unit. Second, an additional 26 unselected women were recruited from a cohort of 74 consecutive women who had suffered an episode of objectively confirmed VTE aged ≤ 50 years at our hospital between 1993-1997. From this cohort of 74, two died from PE, 4 were excluded due to psychiatric disorders, and 42 were either untraceable, declined participation or lived an unacceptable distance from our unit. Of the 26 who participated, 10 had suffered spontaneous VTE, 10 were associated with COCP, 2 associated with HRT, 2 associated with surgery and 2 were associated with immobility. Women who had cancer or in whom the episode of VTE was related to intravenous drug abuse were excluded from the study.

6.3.2 All of the participants underwent physical examination to evaluate symptoms and signs of PTS. Women were asked about symptoms of PTS (residual pain or cramps), and were examined by a doctor for evidence of venous insufficiency (limb swelling,

skin discoloration, skin hyperpigmentation, varicose veins, ulceration in the previously thrombosed limb). PTS was classified using a simplified version of a validated scoring system (241). We graded PTS as absent (asymptomatic), mild (residual pain or minimal swelling), moderate (skin discoloration, skin hyperpigmentation, major limb swelling, development of varicosities), or severe (healed or acute ulceration). Current use of compression stockings was recorded. Contraceptive choice after the event was recorded in those who suffered VTE related either to pregnancy, the oral contraceptive pill, or those under 40 years. In all participants, height (m) and weight (kg) were recorded to calculate BMI (kg/m^2). Healthy age-matched female controls, without history of VTE or venous disease, were recruited to determine the prevalence of asymptomatic venous insufficiency (using LRR) in a young healthy female population.

6.3.3 Light Reflection Rheography (LRR)

6.3.3.1 The light reflection rheography (LRR) instrument (*AV1000, Haemodynamics, Cheshire, UK)* consists of an electronic sensor head and a combined amplifier-recorder. The sensor head contains three light-emitting diodes that transilluminate the skin to a depth of 0.5-1.5 mm. The emitted light is either absorbed by erythrocytes within the dermal microcirculation, or reflected back to the photodetector on the sensor head. The intensity of reflected light is translated into an electrical signal, which is recorded on a strip chart. The LRR curve, therefore, graphically illustrates dermal blood content. LRR can quantify both parameters of venous function, namely venous emptying and venous refill time (see appendix 4).

6.3.3.2 All LRR traces were recorded with the patient seated in a chair with both feet flat on the floor. Skin temperature was assessed by means of a thermistor probe located

within the sensor head. Patients with extremes of skin temperature (outside the range 28 to 32^0 C) were excluded from examination at that time. The LRR sensor head was affixed 10cm above the medial malleolus by means of a double-sided adhesive ring. After a period of approximately one-minute to allow for calibration, each patient performed 10 dorsiflexions of the foot over a period of 15 seconds (this corresponds to the venous emptying phase on the tracing). The LRR has a metronome, which emits optical and acoustic signals, helping the patient with the exercise regimen. The patient was then instructed to rest the foot, and the instrument continued tracing for a further 40 seconds (venous refill time).

6.3.3.3 LRR was performed on both left and right lower limbs of cases and controls. LRR traces were interpreted by an individual with experience of the technique, who was blind to patient identity and symptomatology (IAG). Venous refill times (VRT) of less than 25 seconds were considered indicative of venous insufficiency, as previously described (239,240). We categorised tracings into those which were either normal (VRT > or equal to 25 seconds) and those which were abnormal (VRT < 25 seconds).

6.3.4 Statistical techniques

6.3.4.1 The significance of differences between mean age at follow-up, follow-up intervals, and BMI were determined using the Students t-test (196). All p values are two-tailed. We calculated odds ratios (OR) with 95% confidence intervals as estimates of the relative risk of development of PTS and venous insufficiency. 95% confidence intervals were calculated according to Woolf's method (Chapter 3). An OR was considered to be statistically significant when the lower limit of the 95% CI was ≥ 1.0. Correlation coefficients (Pearson) were calculated to determine the relationship

between LRR venous refill time and BMI. Correlation coefficients and regression plots were calculated using *Minitab* (version 11, PA, USA).

6.4 RESULTS

6.4.1 Symptoms and clinical signs of PTS

6.4.1.1 Of 71 women evaluated for the PTS, 43 had suffered from a single episode of deep vein thrombosis (DVT), 9 from recurrent DVT, and 19 from clinically isolated pulmonary embolism (PE). The mean age at follow-up of the whole group was 35.5 years (range 16-56). There were no significant differences in age at follow-up between those with DVT or PE. Mean follow-up interval (time from event to interview) was 51.4 months (range 6-156). The mean follow-up interval in women with DVT (56.7 months) was significantly longer than those with PE (38.9 months) ($p < 0.05$, table 6.1). Cases had a significantly higher BMI (28.4) compared with controls (25.6) ($p < 0.005$). 9/71 (12.7%) women, of whom 8 had symptoms of PTS, were using compression stockings at the time of follow-up.

6.4.1.2 None of the cohort had severe PTS by our grading. Of 43 women with a single previous episode of DVT, 11 (25.6%) were asymptomatic for PTS compared with none of the group with recurrent DVT (table 6.2). The majority of women with a single episode of DVT had mild PTS (29/43, 67.4%). Moderate PTS occurred in 3/43 (7%) women with single DVT but in 4/9 (44.4%) women with recurrent DVT. Women with recurrent DVT were more likely to develop moderate compared to mild PTS (OR 7.7, 95% confidence interval 1.3-44.9) in comparison to women with a single episode of DVT. Women presenting with PE, who had no symptoms or signs of DVT at time of diagnosis, were largely asymptomatic (79%) for PTS. Control subjects gave no symptoms to suggest possible PTS.

6.4.2 Light Reflection Rheography

6.4.2.1 LRR was performed on both lower limbs of 68/71 women (49 DVT, 19 PE). LRR was not performed in 3 women, all of whom had previously suffered DVT, due to paraparesis (1), pregnancy (1), and technical difficulty (1). LRR was also performed on both lower limbs of 68 age-matched female controls with no previous history of venous disease (table 1).

6.4.2.2 VRT was < 25 seconds, indicating venous insufficiency, in 33/49 (67.3%) women with previous DVT (55% in the previously thrombosed leg, 33% bilaterally, and 12% in the contralateral limb only). Women with a single episode of DVT who were evaluated by LRR (n = 40) had a prevalence of venous insufficiency of 62.5% (25/40) in either limb. In women with recurrent DVT (n = 9), the prevalence of venous insufficiency in either limb was 88.9% (8/9). 7/19 (36.8%) women with previous PE had evidence of venous insufficiency by LRR (either limb). The overall prevalence of venous insufficiency (as demonstrated by LRR) in the contralateral limbs of women with previous DVT (those with bilateral abnormalities plus those with isolated contralateral limb abnormalities) was 22/49 (45%). Among controls, 9/68 (13%) had a VRT < 25 seconds (7 with an abnormal tracing in one limb, 2 with bilateral abnormalities). Women with a single episode of DVT were significantly more likely to demonstrate evidence of venous insufficiency (in any limb) compared to controls (odds ratio 10.9, 95% confidence interval 4.26-28.0). Women with PE were also more likely to have venous insufficiency (odds ratio 3.8, 95% confidence interval 1.2-12.3) compared to controls. The odds ratio for abnormal LRR in all VTE (DVT plus PE) compared with controls was 9.4 (95% confidence interval 4.0-21.8).

6.4.2.4 Clinical symptoms and signs of PTS were correlated with LRR evidence of venous insufficiency (table 6.3). In women with previous VTE, venous insufficiency was almost as frequent in those asymptomatic for PTS compared to those with symptoms / signs of mild PTS. However, LRR was consistently abnormal in 5/6 women with moderate PTS. One patient with moderate PTS who had normal LRR tracings also had Milroys disease, an inherited lymphoedematous condition resulting in significant limb swelling. In this patient it was difficult to differentiate between symptoms related to previous DVTand those related to lymphoedema. The normal LRR tracings suggest the latter.

6.4.3 PTS / LRR in women with DVT related to pregnancy

6.4.3.1 We performed subgroup analysis to determine if symptoms / signs of PTS, or chronic venous insufficiency, were more common in women in whom DVT was related to pregnancy. From our cohort, 28 women were identified who had suffered a single episode of DVT related to pregnancy. We also identified 15 women who had suffered a single episode of DVT unrelated to pregnancy. There were no significant differences in mean age at follow-up (33.8 ± 8.9 years versus 38.3 ± 9.65 years, $p > 0.1$) or mean follow-up interval (61.5 ± 39.8 months versus 41.9 ± 17.1, $p > 0.05$) in either group. Table 6.4 shows that the prevalence of mild and moderate PTS, and of venous insufficiency (in any limb), was similar in both groups, suggesting that women who suffer a DVT related to pregnancy are not more likely to develop PTS or venous insufficiency compared to women in whom VTE is unrelated to pregnancy

6.4.4 PTS / Venous insufficiency in relation to time from event

6.4.4.1 Symptoms, clinical signs, and objective evidence of chronic venous insufficiency (CVI) were examined in relation to time from event (table 6.5). There was no clear pattern observed either in prevalence of symptoms / clinical signs of PTS, or evidence of CVI, in relation to time from event. However, moderate PTS was only observed when at least 2 years had elapsed since the event.

6.4.5 Effect of BMI on development of PTS and Venous insufficiency

6.4.5.1 We examined the effect of BMI on PTS grading in 40 women with a prior single episode of DVT. Table 6.5 shows that there was no overall clear effect of BMI on grading of PTS. We also assessed the relationship between BMI and LRR refill times in these women. We compared LRR refill times recorded in either the left or right leg (depending on the site of thrombosis) and correlated this with BMI. Overall, the correlation coefficient was not statistically significant ($r = -0.18$, $p = 0.28$), although subgroup analysis demonstrated that BMI correlated inversely with left leg refill time in 26 women with a previous left leg DVT ($r = -0.41$, $p = 0.038$) (figure 6.1). Amongst 68 female controls in whom LRR measurements were performed, we found no correlation between BMI and venous refill time in either the left ($r = -0.08$, $p = 0.55$) or right ($r = -0.01$, $p = 0.97$) leg.

6.4.6 Contraceptive choices following VTE

6.4.6.1 Sixty-three women whose episode of VTE was related to pregnancy (n=45), oral contraceptive pill (n=10), or in whom the event occurred under 40 years (n=8) were asked about contraceptive choices made after VTE. Results are presented in table 6.7. 19% reported that contraception had not been an area of difficulty since they had

not been sexually active since the event. However, in 24% of cases male partners had undergone vasectomy and in 9.5% women had been sterilised. 11% had used or were current users of the progesterone-only contraceptive pill. No women subsequently used the combined oral contraceptive pill.

6.5 DISCUSSION

6.5.1 This retrospective study of 71 women who suffered VTE at age ≤ 50 years demonstrates that mild PTS is common in those with previous DVT. Moderate PTS was particularly likely to occur in women with recurrent DVT. In contrast, women who had suffered a previous episode of PE (unaccompanied by symptoms or signs of DVT) were largely asymptomatic for PTS. However, a degree of caution in interpretation of these findings is required, since women with previous PE were followed-up at a mean shorter interval from diagnosis compared to those with previous DVT. Also, the retrospective nature of our study inevitably introduces a degree of selection bias, since women with continuing symptoms may be more likely to participate than women who are asymptomatic.

6.5.2 The majority of data reporting the long-term outcome of VTE relate predominantly to older subjects. Such data suggests that PTS is common in those with previous DVT (20,21,233-236). However, the prevalence of PTS appears to vary widely, with 29%-79% describing symptoms (21,233-236). This variability may relate to the heterogeneous cohorts that are presented. Data also suggests that symptoms of PTS may increase with time (235,236). There are few data on the prevalence of PTS in young women with previous VTE. Recently, Biguzzi et al followed-up 51 women who had experienced DVT before the age of 40 years at a median of 47 months, and found mild PTS in 55%, moderate PTS in 4% and severe PTS in 4% (242). Bergqvist et al assessed the prevalence of PTS symptomatology by means of a questionnaire in 104 women with VTE related to pregnancy (243). In keeping with our findings, mild PTS was common in this group: only 22% were asymptomatic at median follow-up of 11 years, and few (4%) had symptoms corresponding to severe PTS. The women in this

study were not assessed for evidence of venous insufficiency. Lindhagen et al assessed 23 women following an episode of DVT related to pregnancy and reported PTS in 35% (237). In this study 65% of previously thrombosed limbs had evidence of venous insufficiency. The authors concluded that women with pregnancy-related DVT appeared more likely to develop venous insufficiency compared to women with DVT unrelated to pregnancy, despite such small numbers examined and absence of a comparison group. Our data does not support this idea. We compared women who had suffered a single episode of DVT, either related or unrelated to pregnancy, matched for age and time from event, and found that the prevalence of mild and moderate PTS was similar in both groups. Furthermore, the prevalence of objectively confirmed venous insufficiency was similar in both groups. The numbers involved in our comparison are small, however, and larger numbers of patients are required to confirm this data.

6.5.3 We used the technique of LRR to objectively demonstrate venous insufficiency. This technique is easy to learn, non-invasive, and the traces obtained may be assessed quantitatively. In addition, the equipment is portable. LRR has been extensively used as a technique to exclude acute DVT, with a high negative predictive value (244-249). LRR has also been used in parallel with invasive venous pressure monitoring in the investigation of venous disease, with excellent correlation (238). Using LRR, we demonstrated objective evidence of venous insufficiency in 67% women with previous DVT (in 55% of previously thrombosed limbs and in 45% of contralateral limbs) and in 31.8% of women with previous PE. In women with a single episode of DVT evaluated by LRR the prevalence of venous insufficiency was 62.5% in either limb, and 88.9% in women with recurrent DVT. Amongst 68 age-matched female controls, free from symptomatic venous disease, venous insufficiency was found in 13%. We did not

perform LRR on one patient with previous VTE who was pregnant, since pregnancy is associated with a significant reduction in velocity of venous flow, particularly in the left leg (178). Women with a single episode of DVT were more likely to demonstrate evidence of venous insufficiency (in any limb) compared to controls (odds ratio 10.9, 95% confidence interval 4.26-28.0). Women with PE were also more likely to have venous insufficiency (odds ratio 3.8, 95% confidence interval 1.2-12.3) compared to controls.

6.5.4 We observed poor correlation between symptoms of mild PTS and objective evidence of venous insufficiency (table 6.3). Many women without symptoms or signs of PTS had evidence of venous insufficiency, and a large number who had symptoms of mild PTS did not have objective evidence of insufficiency. This may indicate that a proportion of cases of mild PTS are not related to venous insufficiency, but could relate to other factors (for example, psychological or musculoskeletal). It would appear that LRR is not useful in elucidating the cause of mild post-thrombotic leg symptoms. However, women with moderate PTS generally had objective evidence of venous insufficiency. One woman with "moderate" PTS also had gross lymphoedema; in this patient it was impossible to determine the origin of her symptoms. She had no evidence of venous insufficiency using LRR, suggesting that lymphoedema may be the major cause of her symptoms. The explanation for the high prevalence of venous insufficiency in the contralateral limbs of women with previous VTE is unknown. Asymptomatic contralateral thrombosis is not uncommon in those with DVT, providing a possible explanation (250). Also, venous insufficiency is itself a risk factor for the development of VTE, and we may hypothesise that in a proportion pre-existing venous insufficiency contributed to the development of subsequent VTE.

6.5.5 In the study by Biguzzi et al, 51 women with previous DVT were assessed for evidence of venous insufficiency by an alternative technique (colour Doppler ultrasound) (242). Almost 30% had evidence of superficial vessel reflux; a further 14% had evidence of suboptimal venous recanalisation. No comment was made regarding evidence of deep vessel reflux in this cohort, which may explain the higher prevalence of venous insufficiency detected in our study. This is supported by a study of 386 patients with venous insufficiency, in which deep venous insufficiency was identified more frequently than superficial insufficiency (240). In keeping with our findings, the study of Biguzzi et al reported poor correlation between evidence of venous insufficiency and symptoms of PTS (242). Other researchers employing objective techniques have reported venous insufficiency in 36%-79% of individuals with previous DVT (21,234,251). We examined the relationship between time from event to follow-up to determine if venous insufficiency increased in prevalence with time; we found no evidence of this, in contrast to other studies (236,252). At odds with the study of Biguzzi et al ((242)), we found no overall relationship between BMI and subsequent development of PTS. We demonstrated an inverse correlation between BMI and LRR refill time in women with a previous DVT of the left leg, suggesting that the development of venous insufficiency in this group may relate, at least in part, to BMI. This finding should be interpreted with caution, since the numbers involved are relatively small, and when DVT of the left and right leg were considered collectively, the correlation coefficient was not statistically significant. Further research would be worthwhile in a larger group of patients. No correlation was observed between BMI and refill time in controls.

6.5.6 There are no data on contraceptive choices made by young women following an episode of VTE. We found that male vasectomy was a common mode of contraception following the event (24%). 9.5% of women underwent surgical sterilisation, which is perhaps surprising when one considers that the operation may increase the risk of recurrent VTE. No women were prescribed the combined oral contraceptive. However, 11% had used the progesterone-only pill following their thrombotic event. There are no published reports of an association between this form of oral contraception and development of VTE. Unfortunately, our contraceptive data is uncontrolled, since contraceptive use was not recorded in age-matched female controls. In a future study it would be of interest to record such data in age-matched thrombosis-free women for the purposes of comparison.

6.6.7 In summary, our study provides important evidence that both symptoms and signs of chronic venous insufficiency are common in young women following an episode of symptomatic DVT. Young women with VTE, by virtue of their youth, have a long period to develop venous insufficiency. In this cohort it will be of interest to prospectively monitor for the development of more severe grades of PTS, particularly in those with objective evidence of venous insufficiency.

Table 6.1 Mean age (SD), BMI (SD) (kg/m^2) and follow-up (SD) interval in cases and controls

	Cases			Controls
	All VTE	DVT	PE	
Number	71	52	19	68
Mean age at follow-up	35.7 (8.9)	35.6 (8.8)	35.6 (8.7)	35.5 (9.8)
Mean follow-up interval	51.4 (35.5)	56.7# (37.4)	38.9# (27.2)	-
Mean BMI	28.4‡ (6.4)	27.4 (5.6)	29.9 (7.3)	25.6‡ (4.8)

Significance levels: # $p < 0.05$ for follow-up interval between DVT and PE

‡ $p < 0.005$ for difference between mean BMI of all VTE and controls

$p < 0.02$ for difference between mean BMI of all PE and controls.

Table 6.2 Prevalence of post-thrombotic syndrome in 71 women with previous VTE.

Number / PTS grade	Single DVT	Recurrent DVT	PE
	43	9	19
Absent	11 (25.6%)	0	15 (79%)
Mild	29 (67.4%)	5 (55.6%)	4 (21%)
Moderate	3 (7%)	4† (44.4%)	0
Severe	0	0	0

† odds ratio for moderate Vs. mild PTS in those with recurrent DVT 7.7, 95% CI 1.3-44.9.

Table 6.3 Correlation of clinical PTS with objective evidence of venous insufficiency in 68 women with VTE.

PTS grade	LRR abnormal	LRR normal
Absent (n=26)	14 (54%)	12 (46%)
Mild (n=36)	21 (58%)	15 (42%)
Moderate (n=6[†])	5 (83%)	1 (17%)
Severe (n=0)	-	-

[†] One patient with moderate PTS also had Milroys disease, an inherited lymphoedematous condition resulting in significant limb swelling.

Table 6.4 Comparison of PTS / Venous insufficiency in women with a single episode of DVT related / unrelated to pregnancy.

PTS/LRR	Pregnancy-related DVT (n=28)	DVT unrelated to pregnancy (n=15)
Absent	7 (25%)	4 (26.7%)
Mild	20 (71.4%)	9 (60%)
Moderate	1 (3.6%)	2 (13.3%)
LRR abnormal	18 (64%)	10 (66.7%)

Table 6.5 Correlation of PTS symptoms and LRR findings with follow-up interval in 68 women with VTE.

Follow-up interval	n	Absent	Mild	Moderate	Venous insufficiency by LRR
Up to 1 yr	12	6 (50%)	6 (50%)	0	8 (67%)
1-2 yrs	11	2 (18%)	9 (82%)	0	5 (45%)
2-3 yrs	4	2 (50%)	1 (25%)	1 (25%)	4 (100%)
3-4 yrs	10	4 (40%)	5 (50%)	1 (10%)	8 (80%)
4-5 yrs	9	4 (44.5%)	4 (44.5%)	1 (11%)	4 (44%)
5-6 yrs	12	5 (42%)	6 (50%)	1 (8%)	5 (42%)
> 6 yrs	10	3 (30%)	5 (50%)	2 (20%)	6 (60%)

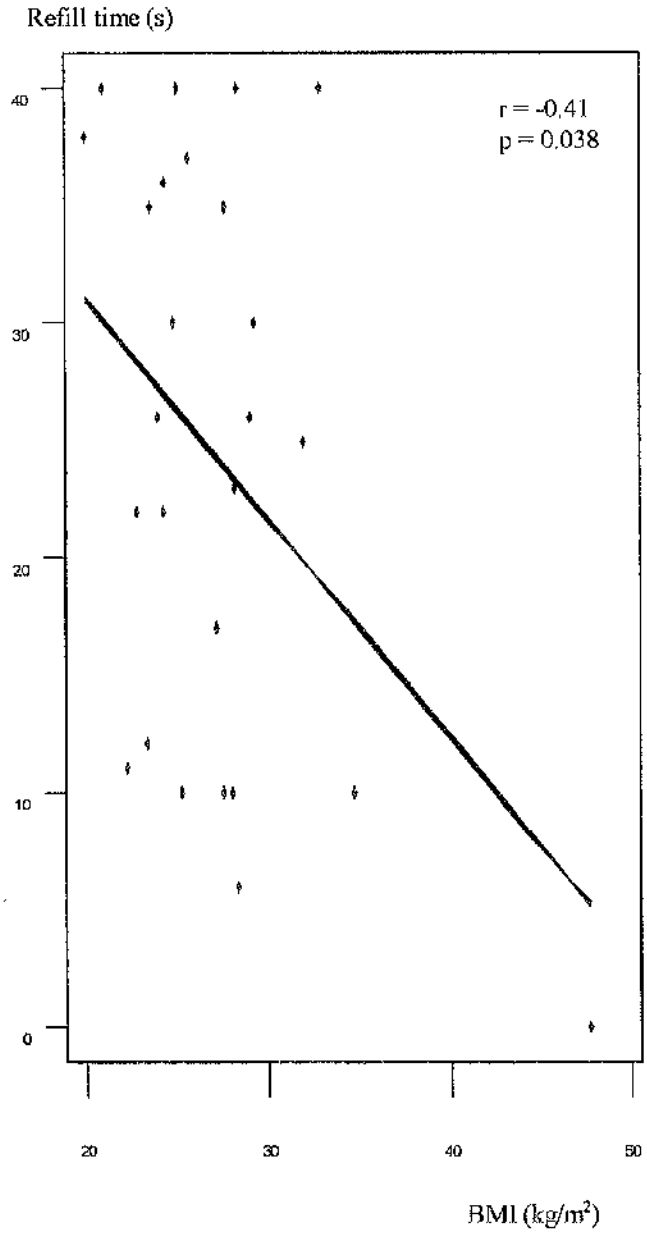
Table 6.6 Influence of BMI on PTS grading in 40 women with a single prior episode of DVT.

BMI	Number	PTS grade		
		Absent	Mild	Moderate
<24	13	2 (15%)	10 (67%)	1(8%)
24-26	9	4 (44.5%)	4 (44.5%)	1(11%)
26-28	6	2 (33%)	3 (50%)	1 (17%)
28-30	6	1 (17%)	5 (83%)	0
>30	6	2 (33%)	4 (67%)	0

Table 6.7 Contraceptive choices made by 63 women following an episode of VTE

Contraceptive choice	Number
Partner had vasectomy	15 (24%)
Patient sterilised	6 (9.5%)
Condoms	16 (25.5%)
Progesterone-only pill	7 (11%)
IUCD	5 (8%)
Diaphragm / cap	2 (3%)
not sexually active	12 (19%)

Figure 6.1 Correlation of left leg venous refill time with BMI in 26 women with a prior episode of left DVT.



CHAPTER 7

7.1 CONCLUSIONS AND FURTHER RESEARCH

7.1.1 Summary of findings and further research from Chapter 2

7.1.1.1 Our data on risks for VTE in women in the West of Scotland (Chapter 2) has highlighted pregnancy and intravenous drug abuse (IVDA) as prevalent triggers for thrombosis. In women < 40 years, 67% of all episodes of VTE were associated with either pregnancy or IVDA. IVDA may be the most common risk factor for VTE in our area in women, since a large number of clinically diagnosed events were also noted. DVT related to IVDA was not associated with clinical evidence of PE in any women. A future study to determine the prevalence of IVDA as a risk factor for VTE in both sexes would be of interest. Such data would help determine the overall contribution of IVDA to VTE in our area, and provide further information on risk of symptomatic PE in this group. Such data may be liable to bias, since a large number of patients who are drug abusers self-discharge from hospital without follow-up, possibly underestimating the risk of PE observed. However, it is likely that symptomatic PE would precipitate re-admission to hospital. This group of subjects would largely be unsuitable for a prospective study involving routine lung scanning to determine the prevalence of asymptomatic PE, because of problems with compliance and venous access. Data on risk of symptomatic PE in DVT associated with IVDA would be of value, since there is uncertainty regarding the optimal duration of anticoagulation in this group.

7.1.1.2 Data from Chapter 2 identified two women who were users of hormone replacement therapy (HRT) at the time of postoperative VTE. There are no data on risk of postoperative VTE in users of HRT compared to non-users. This raises the potential

for a case-control study: all cases of postoperative VTE identified in Chapter 2 (excluding those with active cancer) would be recalled and interviewed. Specific enquiry regarding the use of HRT at the time of surgery would be made. Age-matched female controls, free from venous thrombosis, would be selected and matched for BMI and surgical procedure. The prevalence of HRT use in cases would be compared to their use in controls to determine the risk of postoperative thrombosis in users of HRT.

7.1.2 Summary of findings and further research from Chapter 3

7.1.2.1 In Chapter 3 we derived data on the incidence of objectively confirmed DVT and PE associated with pregnancy. The incidence of DVT was 0.68 per 1000 maternities (95% CI 0.51-0.85) with 0.47/1000 occurring in the antenatal period (95% CI 0.33-0.61) and 0.21/1000 in the puerperium (95% CI 0.12-0.3). The incidence of PE was 0.25 per 1000 maternities (95% CI 0.15-0.35), 0.1/1000 antenatal (95% CI 0.04-0.16) and 0.15/1000 in the puerperium (95% CI 0.07-0.22). However, the puerperal event rate was significantly higher for both DVT (18.5/10 000 versus 6.4/10 000) and PE (13.0 versus 1.3/10 000). This data is based upon > 90 000 maternities and represents the largest study of confirmed VTE events related to pregnancy in the literature. Clinical risk factors during pregnancy were common in women with VTE. Antithrombin deficiency was identified as a particularly high-risk thrombophilia for maternal VTE. The factor V Leiden and prothrombin 20210G→A mutations were associated with a modest increase in risk of VTE. Women were not investigated for hyperhomocysteinaemia as part of our study, since the technique was not established in our centre at the time of our investigations. There are no data regarding homocysteine levels and complications of pregnancy. Ideally, homocysteine should be measured at or near the time of a pregnancy event, since nutritional status (folate, B12) plays an

important role in determining plasma homocysteine levels. In relation to maternal VTE, this approach would be impractical due to the low incidence of the condition. However, other fetomaternal complications, such as pre-eclampsia or intrauterine growth retardation (IUGR), which may be thrombotic in origin, and which are more common than maternal VTE, would be ideal for such a study. For example, it would be of interest to measure fasting plasma homocysteine, serum folate and determine MTHFR genotype in consecutive women with IUGR, and compare the results to those obtained in age- and gestation-matched healthy pregnant controls.

7.1.3 Summary of findings and further research from Chapter 4

7.1.3 In Chapter 4 we determined the incidence of maternal superficial vein thrombosis (SVT) (0.68/1000 maternities, 95% CI 0.48-0.88), comparable to the incidence of maternal DVT. The incidence of SVT in the early post-partum period (0.54/1000 maternities) was higher than in the antenatal period. None of these women had a previous history of deep vein thrombosis or pulmonary embolism, and of those followed-up (n=24/47), none had developed DVT or PE after a mean period of four years. The risk of SVT was increased, though not significantly, in carriers of the FVL mutation (OR 1.83; 95% CI 0.2-16.4). None of the cohort was screened for the prothrombin 20210 mutation. Further investigation into SVT may well be limited by the relatively poor follow-up rate. Women may perceive this condition as relatively minor, and motivation to participate in a research project to investigate this may be low.

7.1.4 Summary of findings and further research from Chapter 5

7.4.1 Our study of lipid parameters in women with VTE (Chapter 5) showed that triglyceride concentrations were significantly higher, and total cholesterol, LDL-

cholesterol, and HDL-cholesterol significantly lower, in cases compared with controls. The elevated triglyceride and lower HDL-cholesterol reflect the higher mean BMI of women with VTE. This phenotype is similar to that seen in the insulin resistance syndrome. The reason for the lower LDL-cholesterol concentrations is unknown. The increased risk of VTE associated with increasing BMI may be partly mediated via hypertriglyceridaemia, which is accompanied by elevated coagulation factors (and inhibitors), increased resistance to the anticoagulant action of APC, and elevated PAI-I. The insulin resistance syndrome may be associated with the higher BMI seen in some individuals with VTE. A large prospective study of consecutive subjects with VTE, incorporating measurement of lipids, coagulation factors, coagulation inhibitors, and plasma glucose and insulin concentration (as measures of insulin resistance) would be of interest to elucidate this area further.

7.1.5 Summary of findings and further research from Chapter 6

7.1.5.1 Our data on the prevalence of the post-thrombotic syndrome (PTS) and chronic venous insufficiency (CVI) has shown that, among 71 women with a previous episode of VTE at age ≤ 50 years, symptoms of PTS were common at a mean 51 months after the event. Most women with a single previous episode of DVT (n = 43) had mild PTS (67.4%); 7% were of moderate severity. In this group the prevalence of venous insufficiency was 62.5%. Women with a single previous episode of DVT were 10.9 times more likely to demonstrate objective evidence of CVI compared with age-matched female controls. There was poor correlation between PTS grading and LRR findings, with the exception of moderate grade PTS. This may indicate that a proportion of cases of mild PTS are not related to venous insufficiency, but could relate

to other factors (for example, psychological or musculoskeletal). Women with recurrent DVT (n = 9) appeared more likely to develop moderate PTS (44%), although the numbers were small. Symptoms suggestive of PTS were uncommon in women with previous PE without clinical evidence of DVT at presentation. We did not observe a higher prevalence of PTS or CVI in women with previous pregnancy-related VTE compared to women in whom VTE was related to other thrombotic triggers. PTS / CVI showed no relationship to BMI, although left leg LRR refill time correlated inversely with BMI in women with a previous left leg DVT. This correlation was observed in 26 women. A larger prospective study of consecutive subjects with a first confirmed episode of DVT would be of interest, with measurement of BMI and LRR to determine if this correlation is confirmed. Whilst taking considerably longer to perform, a prospective study would also be of value to compare the prevalence of the various grades of PTS with our findings, which are retrospective, and therefore subject to a degree of selection bias.

ACKNOWLEDGEMENTS

I am grateful for the advice, support and encouragement of Dr Isobel D Walker, Professor Ian A Greer and Dr RC Tait. I am also grateful to Dr Fiona Reid, who performed the polymerase chain reaction techniques used to identify the genetic mutations mentioned in this thesis, and to Dr Jim Conkie and Frances McCall, who performed the coagulation tests. I am also grateful to colleagues in the department of clinical biochemistry, who performed lipid analysis.

APPENDIX 1

Data extracted from patient case-notes.

Data	Notes
Name	
Date of birth	
Address	
Year of event	
Diagnosis	DVT-left DVT-right DVT-bilateral
	Definite PE Probable PE
Radiological investigation employed	Compression / doppler ultrasound
	V/Q scan Spiral CT Pulmonary angiogram
	Other (specify)
Risk factor(s)	COCP (state preparation)
	HRT (state preparation)
	Pregnancy - $1^{st}/2^{nd}/3^{rd}$ trimester or puerperium
	Surgery (within one month of event)
	Immobility (specify)
	Active Cancer
	IVDA
	Significant medical illness
	Nil
Treatment	Heparin / warfarin (state duration)
Previous VTE	
Outcome	Death (Y or N)

APPENDIX 2

PATIENT INFORMATION LEAFLET

Research Project investigating causes of blood clots

Some time ago you were treated for a blood clot which occurred in either the leg or the lung. The Haematology departments at both Glasgow Royal Infirmary and the Southern General hospitals have been active in researching the causes of such clotting problems. Your clot may have followed, for example, pregnancy, an operation or illness, or may have been related to a hormone preparation. We are now able to test for certain clotting tendencies, and as part of our research we would like to find out how commonly these tendencies occur in women with clots. We are also interested in finding out how often the affected leg continues to give trouble when there has been a previous clot in the leg.

We would be grateful if you are able to help us in our research. We are holding a series of special clinics at Glasgow Royal Infirmary for research into the causes and effects of blood clots. At this visit (which will last about 20 minutes) we will

- Ask you some questions about your general health and in particular about your legs (for example, sore legs, swollen legs, varicose veins),
- Assess the veins of the leg using a special instrument which is placed on your calf (a bit like an ultrasound scan; this is not painful, does not involve any injections and only takes about 10-15 minutes). This test cannot be performed through tights, so the wearing of trousers would be preferable.
- Obtain a blood sample to check your blood count, to look for clotting tendencies, and also to measure cholesterol and blood fat levels.

All information obtained is treated in the strictest confidence, your GP will be informed of your results, and (if you wish) we will also inform you of the test results.

Note: Please do not have any breakfast on the morning of your visit, since some of the blood test results will be affected by intake of food.

APPENDIX 3

PARTICIPANT CONSENT FORM

FULL NAME.....

DATE OF BIRTH ...../...../.....

ADDRESS

.....

.....

....

TELEPHONE Daytime ..... Evening .....

NAME AND ADDRESS OF FAMILY DOCTOR

.....

TO BE COMPLETED BY THE PARTICIPANT

Have you read the Participant Information Sheet? Yes ❒ No ❒
Have you had the opportunity to ask questions and discuss this study? Yes ❒ No ❒
Have you received satisfactory answers to all your questions? Yes ❒ No ❒
Have you received enough information about the study? Yes ❒ No ❒

You are free to withdraw from the study ◆ at any time ◆ without having to give a reason

---

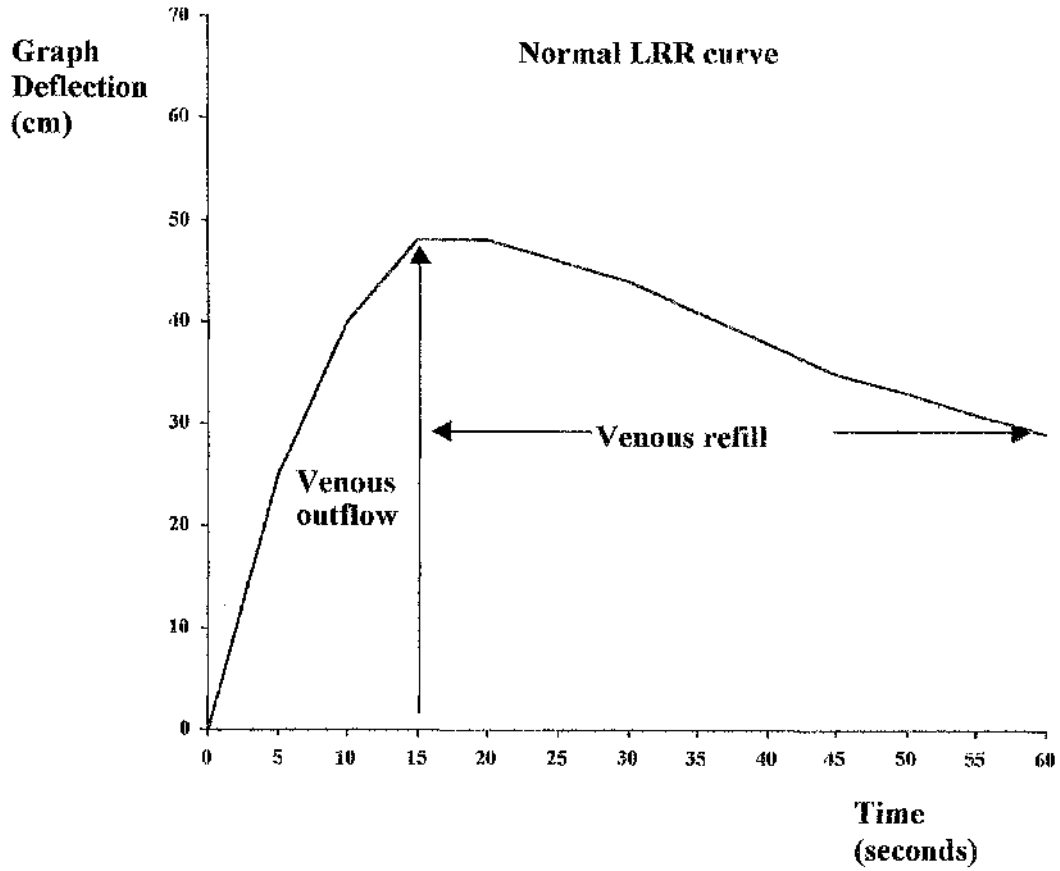
Do you agree to take part in this study? Yes ❒ No ❒
Do you agree to undergo a blood test to investigate your clotting factors? Yes ❒ No ❒
Do you agree to this blood test being used to investigate genes for clotting? Yes ❒ No ❒
Do you agree to the results of tests being sent to your GP? Yes ❒ No ❒

Signed ..... Date .....
Name in block letters

.....

Signature of witness ..... Date .....

APPENDIX 4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Normal LRR curve
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60
50
40
30
20
10
0
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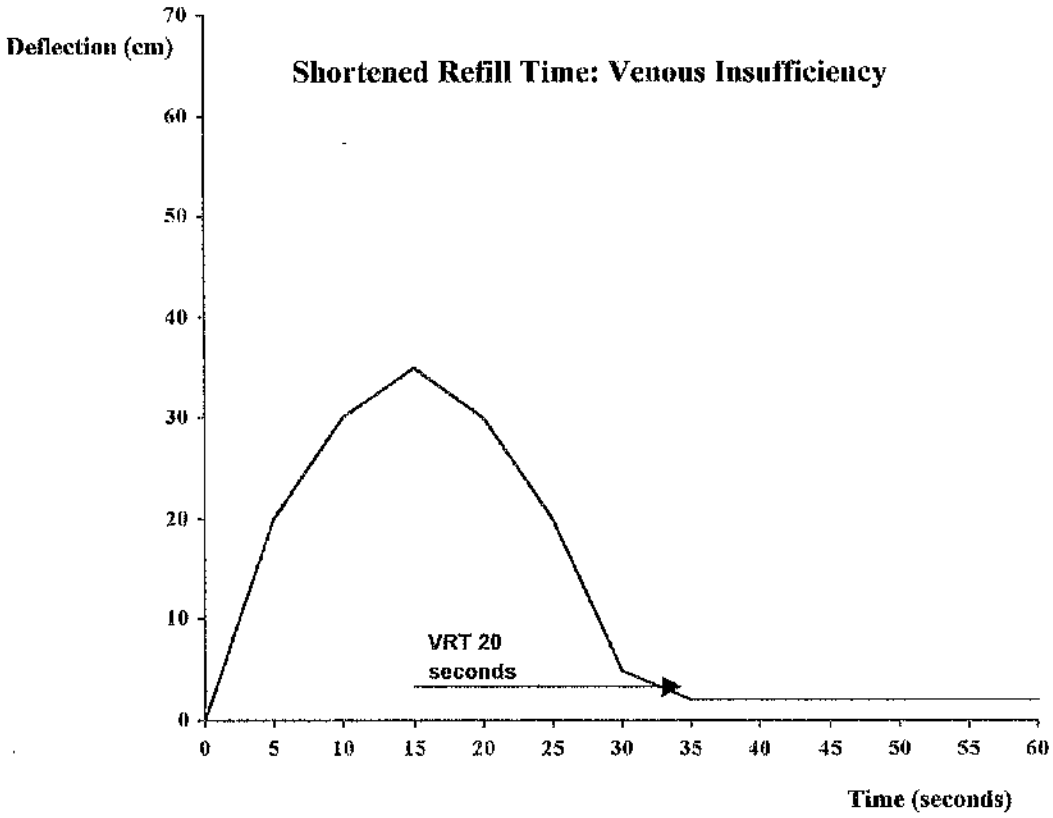
10
15
20
25
30
35
40
45
50
55
60
Venous outflow
Venous refill
Time (seconds)


Shortened Refill Time: Venous Insufficiency
Deflection (cm)
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50
40
30
20
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20
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30
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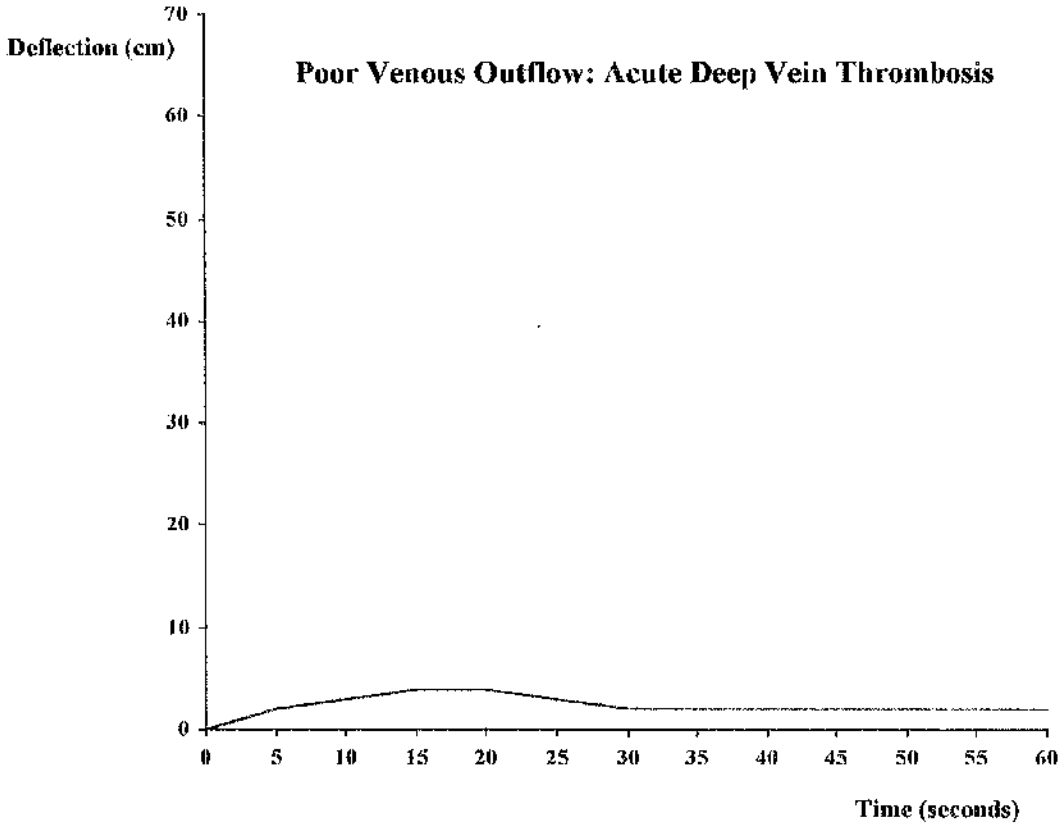
50
55
60
Time (seconds)


Poor Venous Outflow: Acute Deep Vein Thrombosis
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20
10
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (seconds)

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