Factor V Leiden: association with venous thromboembolism in pregnancy and screening issues

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Introduction

Blood coagulation is a biological amplification system.[1] A series of functionally specific plasma proteins (coagulation factors) are involved. These zymogens and accelerators interact in a highly ordered and predetermined sequence to generate thrombin. Thrombin cleaves fibrinogen to generate fibrin monomers, which polymerise spontaneously and are crosslinked by thrombin-activated factor XIIIa to form a stable clot.

This important defence mechanism against bleeding can be activated rapidly when the need arises. The validity of the classical blood coagulation theory, which presents two activation pathways for blood coagulation – the extrinsic and intrinsic pathways – recently has been questioned and revised. It has been replaced by the current concept that takes into account the lack of severe bleeding when there is a deficiency of certain plasma factors (Fig. 1).

The cascade relies on the presence of immunologically and functionally normal plasma proteins. Regulation of coagulation is exerted at each level of the pathway by physiological anticoagulants to overcome the autocatalytic nature of thrombin. Under normal conditions, anticoagulant mechanisms prevail over procoagulant forces. Disturbances of the natural balance between the procoagulant and anticoagulant systems may result in bleeding or thrombotic diseases.[3]

Factor V is an important plasma protein of the coagulation cascade.

Factor V

Factor V is a large, relatively unstable plasma protein (Mr: 330,000). It is synthesised primarily in the liver, although about 20% of the total amount of factor V in blood is synthesised by megakaryocytes and stored in the α-granules of platelets.[4] In conjunction with platelet activation, the

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ABSTRACT

Disturbances of the natural balance between procoagulant and anticoagulant mechanisms can result in bleeding or thrombotic tendencies. Factor V, on activation by thrombin to factor Va, forms an essential component of the prothrombinase complex, in which it demonstrates its cofactor activity for factor Xa. Down-regulation of factor Va by activated protein C (APC) occurs through cleavage of specific peptide bonds in the heavy chain of the molecule. Factor V Leiden (FV Leiden) is a mutation of factor V that renders factor Va resistant to APC, due to loss of one of these cleavage sites. This mutation predisposes the patient to thrombosis. Prevalence of FV Leiden varies; however, heterozygosity for the FV Leiden mutation is recognised as the most common heritable thrombophilic defect in Caucasian populations. The association this inherited thrombophilia has with venous thromboembolism (VTE) is well established. Pregnancy is notably an acquired hypercoagulable state, due in part to physiological changes that occur in the coagulation system. This seems to have potential for interaction with FV Leiden to cause adverse experiences. A role has been suggested for FV Leiden in VTE events during pregnancy. At present, only selected women are screened for FV Leiden. Pregnant women with a history of VTE or with a family history of the mutation are investigated. Whether or not the introduction of a routine screening plan for this mutation is justified remains a matter for debate.

KEY WORDS: Factor V. Pregnancy.
                      Screening. Thrombosis.

stored form of factor V is released and has an important role in normal haemostasis. A concentration of approximately 10 µg/mL factor V is found in plasma.

Factor V is an essential component for the rapid and efficient catalysis of activation of prothrombin to thrombin. It is the non-enzymatic cofactor protein for the enzyme factor (serine protease) X (Xa). However, circulating factor V has little or no procoagulant activity because it is an inactive molecule. Conversion to its active form (Va) is required before cofactor activity is demonstrated.

Factor V structure

Factor V contains triplicated A domains, duplicated C domains and a connecting B region to give the basic domain

structure of A1-A2-B-A3-C1-C2 (Fig. 2). The ceruloplasmin-like A domains are involved in the multiple protein–protein interactions that permit assembly of the prothrombinase complex.[7] Affinity of factor V for the negatively charged phospholipid surface, in the presence of calcium ions (Ca^{2+}), is attributed to the discoidin-like C domains.

The connecting B region is a large, heavily glycosylated domain, the function of which remains a mystery,[7] as B domainless versions of these factors retain full activity. In contrast, Kalafatis et al.[8] refers to its function as an activation peptide.

Factor V activation

Activation of factor V (Va) involves proteolysis by thrombin or factor Xa at Arg 709, Arg 1018 and Arg 1545 (Fig. 2). Cleavage and activation of factor V (Va) transforms the single-chain protein to one with a dimer configuration composed of a heavy and a light chain. The heavy chain (Mr: 105,000) has A1-A2 domains associated through a connecting region (amino acids 304–316), while the light chain (Mr: 74,000) has domains A3-C1-C2. The connecting B domain dissociates when factor V is activated. Instead, the chains are non-covalently associated via the divalent metal ion Ca^{2+}.

Factor Va, together with enzyme factor Xa, Ca^{2+} and phospholipid (from a procoagulant membrane surface) form the prothrombin activating (or prothrombinase) complex. Within this complex, factor Va acts as an accelerator for conversion of phospholipid-bound prothrombin to the active enzyme thrombin.

Regulation by activated protein C

As factor V is a potent procoagulant, its catalytic activity requires down-regulation. Anticoagulant mechanisms ensure careful control of coagulation and it is the protein C anticoagulant system that modulates factor Va activity.

Protein C normally circulates as an inactive enzyme in the blood and requires activation before it can exert its anticoagulant effects. During normal haemostasis, thrombin binds to the thrombomodulin receptor protein on undamaged vascular endothelium and loses its procoagulant activity, becoming instead a major anticoagulant. Thrombin cleaves the protein C heavy chain, which results in APC (Fig. 3).

Activated protein C down-regulates the prothrombinase complex by inactivating factor Va (Vi) proteolytically, with cleavage of three peptide bonds at Arg506, Arg306 and Arg679 in the heavy chain (Fig. 2). The light chain is not cleaved by APC.

The initial and rate-limiting cleavage of the heavy chain is at Arg506. This culminates in a molecule that exhibits 40% cofactor activity in the presence of high concentrations of factor Xa.[10] Subsequent cleavage at Arg306 results in complete loss of activity. Cleavage at Arg 679 occurs slowly and does not appear to play a significant role in Factor Va down-regulation.[10] The A2 domain of factor Va dissociates from the rest of the molecule as a result of cleavage.

Dissociation of the A2 domain impairs the ability of factor Va to interact with other protein components of prothrombinase and results in the arrest of thrombin
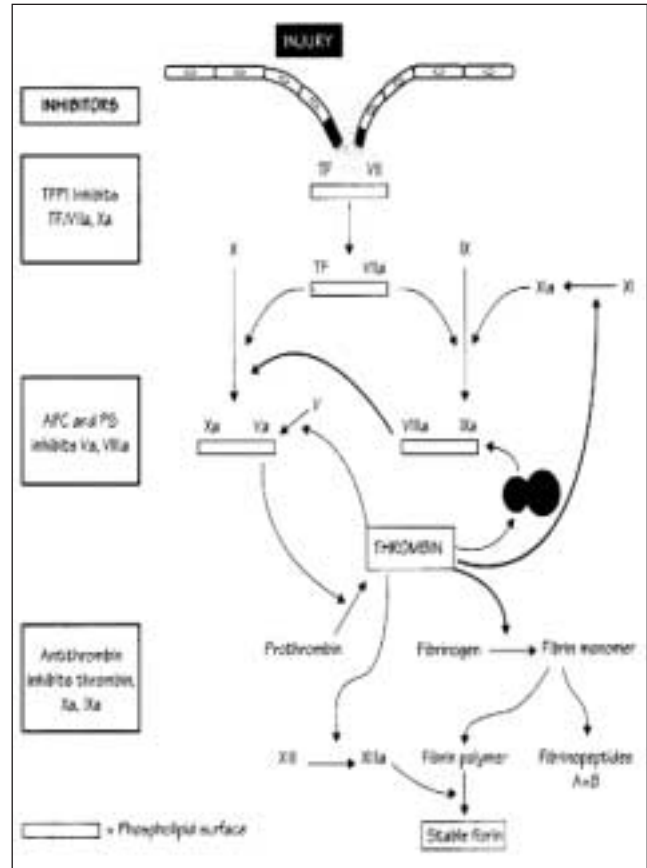

Fig. 1. A simplified schematic of the current concept of coagulation. Adapted from Mehta et al.[2]

formation,[6] thus limiting clot formation. Anticoagulant activity of APC is potentiated and supported by a non-enzymatic plasma protein, protein S.

Factor V, in addition to having the procoagulant function of a precursor for factor Va, also has anticoagulant properties. It functions in synergy with protein S as a cofactor of APC in the inhibition of the factor VIIIa–factor IXa complex.[11]

Factor V mutation and APC resistance

Mutant factor V, commonly referred to as FV Leiden (or V^{R506Q} or V:Q^{506}) is a clinically important coagulation abnormality. In 1993, Dahlbäck et al.[12] observed that the anticoagulant effect of APC was less than normal in plasma samples from a family prone to thrombosis (familial thrombophilia).

The patient's plasma was resistant to prolongation of the activated partial thromboplastin time (APTT) by APC. This phenomenon was referred to as APC resistance (APCR). In 1994, Bertina et al.[13] identified this APCR at the genetic level.

Seminal studies showed that 95% APCR cases had a mutation in the gene coding for factor V synthesis. This single point mutation predicts the replacement of G (codon CGA) with A (codon CAA) at nucleotide 1691 in exon 10 (G1691A). A resultant amino acid substitution occurs in the factor V molecule at position 506, with glutamine (Q) replacing arginine (A). This abnormal molecule was referred
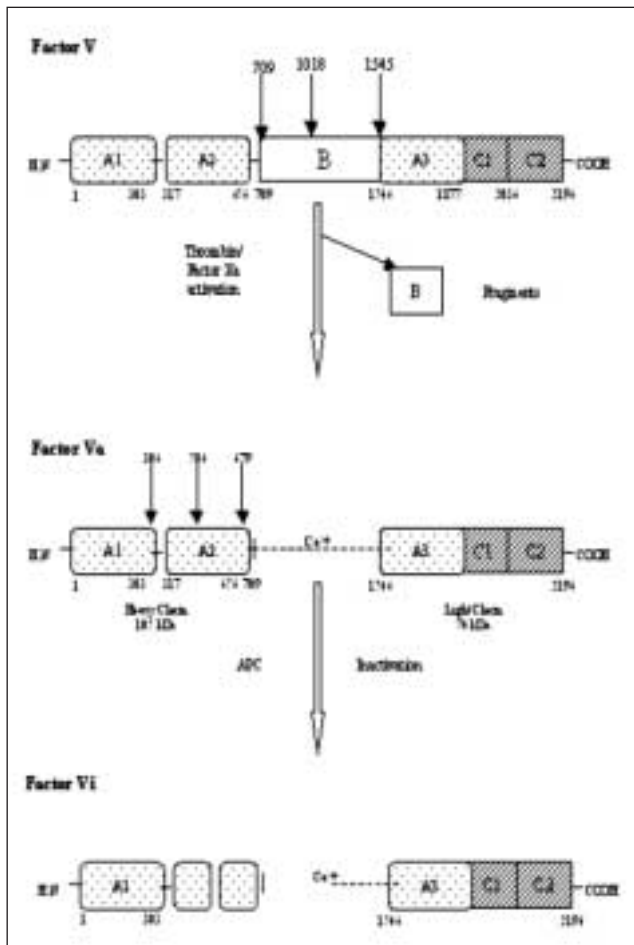
Fig. 2. Schematic diagram of human factor V structure. Activation by thrombin cleavage and inactivation by activated protein C cleavage. Adapted from Cooper *et al*.[5] Amino acid values reproduced from Kalafatis *et al*.[6]

Fig. 3. Activation of protein C, which can then cleave and inactivate either factors VIII or Va. Adapted from Mazza *et al*.[9]

to as FV Leiden (Fig. 4).

Consequently, the APC-cleavage site at Arg506 is absent in FV Leiden and therefore degradation of factor Va by APC is impaired. Thus, FV Leiden renders factor Va resistant to the anticoagulant effect of APC. Inactivation of factor Va Leiden can occur only after cleavage at the Arg306 site, which occurs 10-fold more slowly than in the wild-type protein.[14] However, FV Leiden maintains its full procoagulant activity, being activated in the normal way. The consequence of these mechanisms is that a longer duration of thrombin generation is allowed for and may result in an increased tendency to thrombosis.

Prevalence of FV Leiden

Inheritance can be either heterozygous or the more rare homozygous form. Studies of FV Leiden prevalence show marked variation in different countries. Ridker *et al*.[15] reports it to be present in 5% of the healthy Caucasian population and 1% of the healthy black population.

Rees *et al*.[16] analysed chromosomes from 24 populations when looking for the presence of FV Leiden and found a high prevalence in Europeans (approximately 5%) but FV Leiden was almost non-existent in Asian and African
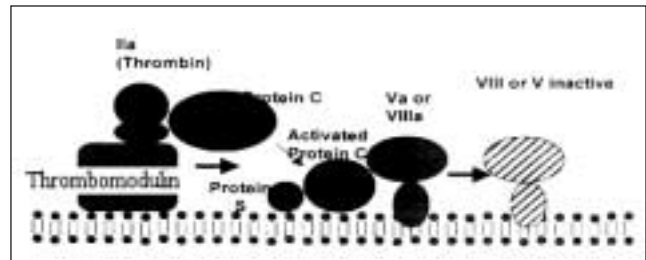
populations. It has been reported to be as high as 12.5% in Sweden and estimates of 2.5% in Italy and 6.8% in Germany are given. The allelic frequency of the mutation in the Dutch population is approximately 2%.

The high prevalence in Caucasian populations could suggest that the FV Leiden mutation arose in an ancestral Caucasian person, approximately 21,000–34,000 years ago,[17] and a subsequent founder effect took place. There is agreement between studies[15,16,18] that heterozygosity for the FV Leiden mutation in Caucasian populations is the most common heritable thrombophilic defect. It may be that FV Leiden is successful as a mutation as heterozygosity may confer a survival advantage (e.g., protecting against post-partum haemorrhage).

FV Leiden is regarded as an independent genetic risk factor for venous thromboembolism (VTE), thus it is an inherited thrombophilia. FV Leiden is currently the most recognised familial thrombophilia.

Thrombophilia

The Haemostasis and Thrombosis Task Force of the British Society for Haematology has agreed that the term 'thrombophilia' can be used to describe the familial or acquired disorders of the haemostatic mechanism that are likely to predispose to thrombosis.[19] VTE is one of many clinical presentations of thrombophilia and refers to deep vein thrombosis (DVT) and pulmonary embolism, which is a complication of DVT. Venous thrombosis and VTE are major causes of concern in the medical profession, and are recognised as threatening complications of pregnancy.

This review aims to look at FV Leiden as a risk factor for VTE in the hypercoagulable state of pregnancy. Should screening for this heritable defect be performed routinely during pregnancy, before a potential thrombotic event has the chance to occur?

Screening tests

In diagnosing any inherited disorder, a complete history should be taken to identify any family history of thrombosis. Other risk factors should be noted. Patients with FV Leiden and no other haemostatic abnormality should have normal clotting results for the simple first-line tests that include prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT).

Those presenting with unexplained thromboembolism should undergo further investigation using a special set of
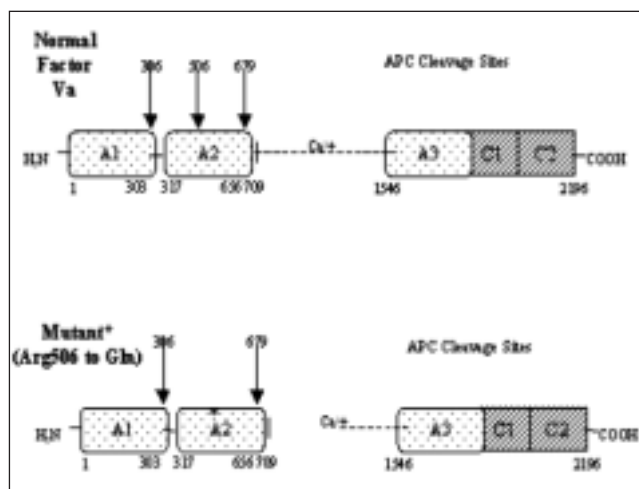
Fig. 4. Schematic diagram to show the structure of normal factor V and the mutant factor V (Leiden) with their APC cleavage sites. Adapted from Cooper *et al*.[5]

tests, collectively referred to as a thrombophilia screen. Laboratory analysis of anticoagulant response to APC is included in this screen. Functional and/or genetic tests can be used for APC resistance (APCR) detection and FV Leiden presence.

Functional assays

APC resistance assays
These provide an indirect measure of factor V inhibition. Based on simple APTT testing methods, addition of purified APC to platelet-poor plasma from normal subjects will prolong clotting time due to degradation of factor V and factor VIII by APC. Plasma containing the mutant factor V shows less prolongation.

Classic APCR method
APC resistance was originally reported as a simple ratio of APTT clotting times, with and without APC. A low sensitivity ratio (APC-SR) is suggestive of APC resistance. To improve precision, reference plasma can be used to normalise the APC-SR. The patient's APC-SR is then simply divided by the APC-SR of the pooled normal plasma to give a normalised ratio. A ratio of <0.84 defines resistance.[13]

Although simple, this assay yields results that are highly sensitive (85–90%) and specific (85–90%) for the single-point factor V gene mutation.[20] This test detected APCR before discovery of the Leiden mutation. When Kapiotis *et al*.[21] correlated the FV Leiden genotype with the APCR assay, a ratio of <1.80 was said to improve the sensitivity of this test to 100%.

Modified APCR method
Robust, simple and rapid, the modified APCR assay has generally replaced the classical method. Patient plasma is prediluted (1 in 4) with factor-V deficient plasma. This dilution allows for normalisation of the concentration of clotting factors involved in the formation and regulation of thrombin, except for factor V (which is derived from patient plasma), rendering the assay more factor-V specific.

This method permits detection of APCR (FV Leiden) in pregnant women and is more sensitive to factor-V dependent APC resistance. There is agreement that this modified test is more highly sensitive and specific (close to 100%) for FV Leiden in both healthy controls and patients with suspected acute VTE.[22]

The classic APCR method has limitations: i) a positive APCR result does not point directly towards a factor-V dependent APC resistance – although 90% APCR cases are associated with FV Leiden, some cases of APCR are not, and other deficiencies (e.g., protein C and protein S) can give a positive APCR, as can pregnancy, which is thought to confer an acquired APCR (in the absence of FV Leiden); ii) the APTT without addition of APC should be within the normal range (25–40 sec) in order to obtain valid APC ratios,[4] so plasma from patients who have a condition that causes deficiency in clotting factors (e.g. pregnancy) will not allow for reliable APCR analysis when using the classic APCR method; and iii) pooled plasma used to normalise the ratio must not contain FV Leiden.

Genetic Assay

The polymerase chain reaction (PCR) is a rapid *in vitro* DNA amplification method, permitting the production of a vast quantity of specific target DNA sequence. Bertina and colleagues[13] employed PCR in their search for the FV Leiden mutation. A loss of the naturally occurring Mnl1 restriction site in the mutant gene was noted. Its use in conjunction with agarose gel electrophoresis allowed normal heterozygous (carriers) and mutant homozygous forms to be detected. Distinction of these forms is extremely important.

Many PCR-based methods for detecting this lesion have since been investigated. PCR is highly sensitive and specific, permitting accurate detection of the mutation. However, contamination can occur in such a sensitive procedure. Enzymes can be expensive, so cost per test is greater than functional APCR assays and the turnaround time is longer.

Molecular methods that differ fundamentally from the original PCR-based techniques are now available for FV Leiden detection. One such method combines extremely rapid PCR with fluorescence resonance energy transfer analysis of the amplified products without the need for DNA extraction.[23] Another approach has employed allele-specific PCR, which is a rapid, simple and cost-effective method.[24]

Pregnancy

Physiological changes within the coagulation system accompany normal pregnancy. During pregnancy (and the puerperium) most clotting factors have been shown to increase in concentration, while there is a decrease in concentration of some of the natural anticoagulants and a reduction in fibrinolytic activity. The resultant hypercoagulable state that is conferred may be a normal physiological response in preparation for childbirth to minimise blood loss during delivery.

Levels of factor V have been shown to rise after 16 weeks gestation, with elevated levels persisting to the third post-natal week.[25] Stirling *et al*.[26] did not report a consistent elevation of factor V, but observed an elevation of factor V

early in pregnancy, followed by a decline until delivery, and then levels rose again.

Levels of protein S appear to decrease throughout pregnancy, while levels of protein C remain normal.[27] Pregnancy seems to cause an acquired APC resistance state.[28] Clark et al.[25] reported that APCR is seen variably during pregnancy and this fall correlated with changes in factor VIII, factor V and protein S. There is, however, very little information at present about the role of acquired APCR during the course of pregnancy.

The haemostatic changes associated with pregnancy that lead to an overall balance shifted towards hypercoagulability has been recognised. It has been suggested that it is advantageous to the pregnant woman to have this hypercoagulable state, but such changes towards hypercoagulability during pregnancy and the post-partum period may confer an increased risk of a thrombotic episode.

Pregnancy is regarded as one of the many acquired risk factors associated with predisposition to thrombosis. The result of such a thrombotic event may lead to adverse pregnancy outcomes and complications, including vascular complications such as VTE.

Pregnancy and VTE: a role for FV Leiden

The fact that FV Leiden is found in 20% of unselected patients and in up to 50% of selected patients with venous thrombosis (e.g., positive family history in first-degree relatives) is widely quoted.[18,29] The population-based Leiden Thrombophilia Study[30] estimated the risk of a VTE event in heterozygous FV Leiden patients to be approximately three- to seven-fold greater compared with persons without the mutation, increasing to an estimated 80-fold in homozygous persons. Homozygosity is found in approximately one case per 5000 in some populations.[29]

The reported risk of developing a thrombosis during pregnancy, compared to the risk for non-pregnant women of the same age, varies from five- to 10-fold.[29,31] Inherited thrombophilias are the leading cause of maternal thromboembolism and are associated with an increased risk of adverse pregnancy outcomes. Venous thromboembolism occurs in one in 1500 pregnancies[32] and is reported to be the leading cause of morbidity and mortality during pregnancy and the post-partum period.

Several studies have looked at obstetric populations and the prevalence of thromboembolic events and a role for FV Leiden mutation has been reported. Women with VTE, during pregnancy or the puerperium, have a higher prevalence of FV Leiden carriage than women in whom no such history has been suggested.

Hallak et al.[33] investigated a group of 15 Israeli patients admitted to a high-risk pregnancy unit with diagnoses of PE, VTE, transient ischaemic attacks and cerebrovascular accidents. Modified APCR and molecular assays were used and seven out of nine thrombotic patients were found to be APC resistant with inheritance of FV Leiden.

Dizon-Townson and colleagues[34] conducted a much larger study of 407 unselected, non-consecutive gravid women (>95% of whom were Caucasian) in Utah who were screened for FV Leiden using genetic analysis. In this cohort, FV Leiden was detected in 14 pregnant women (13 heterozygous [3%] and one homozygous [0.2%] mutant).

Allelic frequency was consistent with the estimated published carrier rate in the general population of 2–4%. Four of 14 carriers of FV Leiden had a DVT (28%), compared to <1% DVT frequency in the obstetric population investigated. High risk of DVT in the presence of FV Leiden was reported.

Hirsh et al.[35] discovered six of the 35 women with pregnancy-associated VTE investigated carried the factor V mutation. Pulmonary embolism was a problem for three of the FV Leiden patients.

Grandone et al.[36] studied 42 Caucasian patients from southern Italy with documented DVT or PE during pregnancy or the post-partum period. Frequency of genetic risk factors, including FV Leiden, was detected and compared with a selected age-matched control group made up of parous women with the same ethnic background and no thrombotic episodes. Ten case patients (23.8%) and four control subjects (1.9%) carried the factor V mutation, providing evidence for a significant risk of a pregnancy-related VTE developing in the presence of prothrombotic risk factors.

In a larger study, Gerhardt et al.[37] investigated 119 German women with a history of pregnancy-related venous thrombosis, together with 233 age-matched normal women, for prevalence of FV Leiden. Functional and genetic assays were employed to detect the mutation, which was found in 44% of case patients compared with 7.7% in matched control subjects, giving a nine-fold increase in thrombotic risk for the case group.

Likewise, in a Swedish study,[38] in a country where prevalence of the mutation is high (10–12%), 32 (46%) of the 70 women investigated, who had a history of thrombosis in pregnancy or during the first three weeks after delivery, had the mutation. Two were homozygous and 30 heterozygous. However, eight of these patients had a normal APC response using the modified APCR assay. Five women in the normal genotype group demonstrated insufficient APC response.

Hough et al.,[39] in a retrospective study of 80 women with familial thrombophilia and a total of 215 pregnancies between them, reported venous thrombosis in 14% of those with FV Leiden. A small Swedish study by Hellgren et al.[40] demonstrated resistance to APC, using the functional assay, in 60% of women with a history of VTE during pregnancy compared to 10% of non-pregnant control women.

In a recent study, Martinelli et al.[41] investigated 119 Caucasian women who had experienced a first episode of VTE during pregnancy or the puerperium. Of this case group, 22 (18.5%) were found to harbour the FV Leiden mutation compared with 2.5% in the control population. These findings led them to report that women with inherited thrombophilia have an increased risk of VTE during pregnancy.

Two large studies have estimated the absolute risk of pregnancy-associated VTE in FV Leiden carriers. Lindqvist et al.[42] screened unselected pregnant patients for FV Leiden, followed them through pregnancy and found that only 1.1% of carriers experienced thrombotic complications. McColl et al.[43] conducted a large retrospective study of 72, 201 deliveries in two Glasgow hospitals over an 11-year period. It was estimated that DVT incidence was 0.71 per 1000 deliveries, while PE was estimated at 0.15 per 1000 deliveries. In this study, approximately 50 patients were identified as having suffered from thrombotic episodes during pregnancy or up

to six weeks post-partum. Further investigations revealed that only 8% of these women had the FV Leiden mutation.

Based on estimates of the prevalence of FV Leiden mutation in the population, the estimated risk of VTE during pregnancy and the puerperium was reported as one in 400–500 FV Leiden-positive pregnancies.

Discussion

The general consensus of opinion is that many pregnant women who carry the mutation do not experience a thrombotic event. However, there is agreement in recent studies for evidence linking FV Leiden to an increased risk of VTE during pregnancy and the post-partum period. Prevalence of FV Leiden in women with pregnancy-associated VTE has been demonstrated to be between 23–60%. The different ethnic populations investigated may account for this variation in prevalence. However, a number of limitations are associated with some of the studies. Hallak et al.[33] investigated a small number of patients and thus only limited conclusions can be drawn. Furthermore, the population was highly preselected, including only women with venous thrombosis related to pregnancy. Selection bias is also seen in the study performed by Grandone et al.,[36] who included only patients from symptomatic families.

When highlighting the estimated risk that FV Leiden carriage poses to development of a VTE during pregnancy, two of the studies do not consider how co-inheritance of other abnormalities affects this risk. In one study,[35] one patient had co-inherited an antithrombin deficiency, one had a protein C deficiency, one presented with a protein S deficiency and two had antiphospholipid syndrome. In another study,[36] six patients presented with combined thrombophilias, some of which are thought to confer an increased risk of VTE.

Pregnancy complications co-existed in two out of four patients with FV Leiden and DVT[36] but were not accounted for. One patient had pre-eclampsia and was expecting twins, another had disseminated intravascular coagulation (DIC) and uterine atony. These conditions may carry a risk themselves for VTE and thus their presence may affect the risk of developing a VTE attributed to FV Leiden carriage. However, these studies do provide convincing evidence that FV Leiden has a role in increasing the risk of VTE during pregnancy.

Guidelines from the BCSH[44] state that women who are homozygous for FV Leiden and have no personal history of venous thrombosis, but have a proven family history of the condition, should be considered at moderate risk of pregnancy-associated thrombosis. The absolute incidence of FV Leiden-related thrombosis during pregnancy was reported to be low,[42] with McColl et al.[43] suggesting it to be 1 in 400–500 pregnancies. Such figures illustrate that despite the high occurrence rate of FV Leiden in pregnant patients with thromboembolic events, there is generally a low prevalence of VTE in pregnancy.

Screening
No protocol exists that indicates which screening test should be used and so individual laboratories must decide, taking into consideration factors such as reliability, efficiency and cost-effectiveness. Current UK guidelines[44] indicate that the modified APC-SR, as opposed to the original APC-SR test, should be used as a phenotypic test for FV Leiden mutation. However, genetic testing for this mutation is highly specific and more selective.

The disagreement observed between the clotting and genetic analyses of 13 patients[38] highlights the advantages of genetic testing. A suppression of the nAPC ratio during pregnancy was seen in the presence and absence of the mutation.

Insensitivity of reagents may account for this disagreement or it may be due to intra-individual variation, with pregnancy causing APCR. DNA-based testing would need to be carried out to confirm whether or not all patients with APCR had the mutation.

Universal routine screening for FV Leiden in pregnancy
Screening for FV Leiden is not performed routinely on all pregnant patients. Only those who have experienced a thrombotic episode or are asymptomatic but have a family history of the mutation are currently screened for FV Leiden. Whether or not it would be reasonable to perform general screening for FV Leiden (APCR) during pregnancy remains a matter for debate.

Owing to the prevalence of this mutation, perhaps consideration should be given to screening all gravid women for this mutation during routine prenatal laboratory evaluation. However, the low figures reported for the absolute incidence of FV Leiden-related thrombosis during pregnancy do not support routine screening.

The benefit of any investigation rests on whether or not any clinical decision (therapy or prevention) would be altered by the information obtained. Asymptomatic pregnant women found to harbour the mutant gene would perhaps benefit from more careful antenatal monitoring, permitting early detection and rapid treatment of a thrombosis. All women with the factor V mutation would benefit from advice about risk factors (e.g., pregnancy, oral contraceptives) thought to have a role in enhancing the risk of VTE. Precautions and measures could then be taken in an attempt to reduce the risk of recurrence.

Investigation of FV Leiden in a routine screening programme may satisfy clinician and patient curiosity; however, identification of this contributory factor to thrombosis may not be deemed worthwhile, especially if there is a risk of generating needless anxiety, as not all carriers suffer a thrombosis during pregnancy.

Generally, routine screening would have a huge impact on the laboratory, medical profession and management, and associated with this are cost implications. Expenditure would increase considerably if the entire population were to be tested. A recent study[45] that analysed cost/benefit showed that universal screening for FV Leiden in pregnancy is not cost-effective.

The study involved 967 unselected pregnant women (of which 99.5% were Caucasian) and was performed on the basis of prevalence of FVL and risk of VTE. It was reported that the additional cost of universal screening would result in prevention of less than one venous thrombotic event. A more moral and philosophical view might suggest that even a small number of maternal complications that have the potential to cause mortality could be averted and cost is but a small matter.

One major problem in advocating routine screening is the different prevalence rates in different countries. Assessment of the benefit-risk ratio for one country will not be the same as that for another geographical region.

Walker[46] agrees that routine screening of all women for thrombophilic defects is not justifiable, further adding that it may cause more harm than good because finding a defect might precipitate unnecessary intervention with antithrombotics. Anticoagulation therapy may be used to treat the patient in an effort to prevent a potential thrombosis. This will impact on the medical profession, as patients will require regular testing and monitoring.

Low molecular weight heparin (LMWH) is used extensively in Europe in the management of VTE in pregnant (and non-pregnant) women. Using this anticoagulant would contribute additional costs to a routine screening programme, as LMWH is more expensive than unfractionated heparin. Also, anticoagulant therapy carries risks to the patient. One author has recommended caution before initiating widespread screening, due to concerns for the safety and efficacy of prophylactic heparin.[47] Among these risks, a bleeding episode at the time of delivery may be more common in the patient on therapy.

Even if heparin is not used to treat the patient for a potential VTE, routine screening would identify those asymptomatic carriers who could benefit from careful monitoring during their pregnancy. All patients, regardless of the treatment strategy used, would require careful and informed counselling to prepare them for the possibility of mutation, living with the condition, and to highlight any risk factors that may enhance a thrombotic event.

Testing positive for hereditary thrombophilia may not just affect a person's health. In the future, some health insurance companies may regard FV Leiden as a health problem. An individual with the mutation may be regarded as a high insurance risk, and thus it may be denied or only offered at a higher premium. Declaration of this inherited thrombophilia may also be required on job applications. If these were enforced, it is unlikely that there would be support for routine screening.

In summary

Factor V Leiden is a common gene mutation. Its prevalence varies in different countries. In Caucasian populations, heterozygosity for FV Leiden is the most common inherited thrombophilia. Carriers of FV Leiden are at increased risk of VTE. There is evidence to suggest that this risk is further increased when this inherited thrombophilia is combined with the prothrombotic influences of pregnancy. Although risk is enhanced, there is evidence to suggest that prevalence of VTE in pregnant women with FV Leiden is generally low.

There is no standard screening test is for the detection of the Leiden mutation. Genetic testing alone or functional assay followed by genetic confirmation would both be reasonable strategies. Only selected patients are screened at present, but should universal routine screening for detecting FV Leiden in pregnant women take place?

The majority of carriers seem to be asymptomatic and although current research into cost-effectiveness is limited, it does not lend support to routine screening. Further research into cost/benefit analysis is recommended before routine

screening can be considered seriously. Even then, screening may only be feasible in those countries where prevalence of the mutation is high. Also, the management of VTE during pregnancy remains controversial. Therefore, widespread routine screening is not advisable at present.   □

References

1   Hoffbrand AV, Petitt JE, Moss PAH. *Essential haematology* (4th edn). London: Blackwell Science, 2001.

2   Mehta A, Hoffbrand V. *Haematology at a glance*. New York: Blackwell Science, 2000.

3   Dahlback B. Blood coagulation. *Lancet* 2000; **355**: 1627–32.

4   Axelsson F, Rosén S. *APC resistance* (Haemostasis Monograph 9). Instrumentation Laboratory, UK. 1997.

5   Cooper DN, Krawczak M. *Venous thrombosis: from genes to clinical medicine*. Oxford: BIOS Scientific, 1997.

6   Kalafatis M, Rand MD, Mann KG. The mechanism of inactivation of human factor V and human factor Va by activated protein C. *J Biol Chem* 1994; **296**(50): 31869–80.

7   Hoffbrand AV, Lewis SM, Tuddenham, EGD. *Postgraduate haematology* (4th edn). Oxford: Butterworth Heinemann, 1999.

8   Kalafatis M, Mann, KG. Factor V: Jeckyll and Mr Hyde. *Adv Exp Med Biol* 2001; **489**: 31–43.

9   Mazza JJ. *Manual of clinical haematology* (3rd edn). London: Lyppincott Williams and Wilkins, 2002.

10 Nicolaes GAF, Tans G, Thomassen CLGD *et al*. Peptide bond cleavages and loss of functional activity during inactivation of factor Va and factor Va^{R506Q} by activated protein C. *J Biol Chem* 1995; **270**: 21158–66.

11 Thorelli E, Kaufman RJ, Dahlbäck B. Cleavage of factor V at Arg 506 by activated protein C and the expression of anticoagulant activity of Factor V. *Blood* 1999; **93**: 2552–8.

12 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognised mechanism characterized by poor anticoagulant response to activated protein C. Prediction of a cofactor to activated protein C. *Proc Natl Acad Sci* 1993; **90**: 1004–8.

13 Bertina RM, Koeleman BPC, Koster T *et al*. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; **369**: 64–7.

14 Heeb MJ, Kojima Y, Greengard JS, Griffin JH. Activated protein C resistance: molecular mechanisms based on studies using purified Gln^{506}- factor V. *Blood* 1995; **85**: 3405–11.

15 Ridker PM, Miletich J. Ethnic distribution of factor V Leiden in 4047 males and females. Implication in venous thromboembolism screening. *JAMA* 1997; **277**(16): 1305–7.

16 Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet* 1995; **346**: 1133–4.

17 Zivelin A, Griffin JH, Xu X, Pabinger I, Samama M, Conard J. A single genetic origin for a common Caucasian risk factor for venous thrombosis. *Blood* 1997; **89**: 397–402.

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

19 Walker ID. Guidelines on the investigation and management of thrombophilia: The British Committee for Standards in Haematology. *J Clin Pathol* 1990; **43**: 703–9.

20 Dahlbäck B. Are we ready for FV Leiden screening? *Lancet* 1996; **347**: 1346–7.

21 Kapiotis S, Quenhenberger P, Jilma B, Handler S, Pabinger-Fasching I, Mannhalter C. Improved characteristic of APC-

resistance assay. *Am J Clin Pathol* 1996; **106**: 588–93.

22 Tripodi A, Negri B, Bertina RM, Mannucci PM. Screening for the FV:Q506 mutation. Evaluation of thirteen plasma-based methods for their diagnostic efficacy in comparison with DNA analysis. *Thromb Haemost* 1997; **77**:436–9.

23 Cooper PC, Cooper SM, Smith JM, Kitchen S, Makris M. Evaluation of the Roche light cycler: a simple and rapid method for the detection of Factor V Leiden and prothrombin G20210A genotypes from blood samples without the need for DNA extraction. *Blood Coag Fibrinolysis* 1999; **14**: 499–503.

24 Mitterer M, Lanthaler AJ, Mair W, Giacomuzzi K, Coser P. Simultaneous detection of FV Q506 and prothrombin 20210A variation by allele-specific PCR. *Haematologica* 1999; **84**: 204-7

25 Clark P, Brennand J, Conkie J, McCall F, Geer I, Walker I. Activated protein C sensitivity, protein C, protein S and coagulation in normal pregnancy. *Thromb Haemost* 1998; **79**: 1166–70.

26 Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. *Thromb Haemost* 1984; **52**: 176–82.

27 Faught W, Garner P, Jones G, Ivey B. Changes in protein C and protein S levels in normal pregnancy. *Am J Obstet Gynecol* 1995; **172**: 147–50.

28 Kjellberg U, Anderson NE, Rosen S. APC resistance and other hemostatic variables during pregnancy and puerperium. *Thromb Haemost* 1999; **81**: 527–31.

29 Bauer KA, Rosendaal FR, Heit JA. Hypercoagulability: too many tests, too much conflicting data. *Hematology* 2002; 353–68.

30 Koster T, Rosendaal FR, De Ronde H. Venous thrombosis due to a poor anticoagulant response to activated protein C: Leiden thrombophilia study. *Lancet* 1993; **342**: 1503–6.

31 Toglia MR, Weg JG. Venous thromboembolism during pregnancy. N *Engl J Med* 1996; **335**(2): 108–14.

32 Lindqvist P. Thrombotic risk during pregnancy: a population study. *Obstet Gynecol* 1999; **94**: 595–9.

33 Hallak M, Senderowicz J, Cassel A *et al*. Activated protein C resistance (factor V Leiden) associated with thrombosis in pregnancy. *Am J Obstet Gynecol* 1997; **176**: 889–93.

34 Dizon-Townson D, Nelson LM, Jang H, Vamer MW, Ward K. The incidence of the factor V Leiden mutation in an obstetric population and its relationship to deep vein thrombosis. *Am J Obstet Gynecol* 1997; **176**(4): 883–6.

35 Hirsch DR, Mikkola KM, Marks PW. Pulmonary embolism and deep vein thrombosis during pregnancy or oral contraceptive use: prevalence of factor V Leiden. *Am Heart J* 1996; **131**: 1145–8.

36 Grandone E, Margaglione M, Colaizzo D *et al*. Genetic susceptibility to pregnancy-related venous thromboembolism: roles of factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase C677T mutations. *Am J Obstet Gynecol* 1998; **179**: 1324–8.

37 Gerhardt A, Scharf RE, Beckman MW *et al*. Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *N Engl J Med* 2000; **342**: 374–80.

38 Bokawera MI, Bremme K, Blomback M. Arg506-Gln mutation in factor V and risk of thrombosis during pregnancy. *Br J Haematol* 1996; **92**: 473–8.

39 Hough RE, Makris M, Preston FE. Pregnancy in women with thrombophilia: incidence of thrombosis and pregnancy outcome. *Br J Haematol* 1996; **93** (Suppl 1).

40 Hellgren M, Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptive. *Am J Obstet Gynecol* 1995; **173**: 210–3.

41 Martinelli I, De Stefano V, Taioli E, Paciaroni K, Rossi E, Mannucci PM. Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium. *Thromb Haemost* 2002; **87**(5): 791–5.

42 Lindqvist PG, Svensson PJ, Marsaal K, Grennert L, Luterkort M, Dahlback B. Activated protein C resistance (FV:Q506) and pregnancy. *Thromb Haemost* 1999; **81**: 532–7.

43 McColl MD, Ramsay JE, Tait RC *et al*. Risk factors for pregnancy-associated venous thromboembolism. *Thromb Haemost* 1997; **78**: 1183–8.

44 Walker ID, Greaves M, Preston FE. Guideline: investigation and management of heritable thrombophilia. British Committee for Standards in Haematology. *Br J Haematol* 2001; **114**: 512–28.

45 Clark P, Twaddle S, Walker ID, Scott L, Greer IA. Cost-effectiveness of screening for factor V Leiden mutation in pregnant women. *Lancet* 2002; **359**: 1919–20.

46 Walker ID. Thrombophilia in pregnancy. *J Clin Pathol* 2000; **53**: 573– 80.

47 Sibai BM. Thrombophilias and adverse outcomes of pregnancy – what should a clinician do? *N Engl J Med* 1999; **340**: 50–1.