THROMBOPHILIA IN WOMEN (AND MEN)

Blue Nude, 1902
Pablo Picasso

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INTERNAL MEDICINE GRAND ROUNDS
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Areas of Interest: Lipid modifications of proteins, lysosomal storage disorders, clinical hematology-oncology, including coagulation disorders.

Introduction

I had a DVT while on oral contraceptives and was told I have Factor V Leiden. What's going to happen when I become pregnant?

I'm her sister. Is it ok if I take birth control pills?

I had a DVT during pregnancy 25 years ago. What about hormone replacement therapy for me now?

I've been prescribed tamoxifen for my early breast cancer, but I had a pulmonary embolism while pregnant. Should I worry?

Recent advances in our understanding of the genetic and acquired causes of venous thromboembolism (VTE) have created great opportunities for intervention but also increasing uncertainty as to how to apply this information to clinical practice. The purpose of this Internal Medicine Grand Rounds is to review new knowledge concerning the genetic basis for VTE with an emphasis on how these genetic factors interact with known effects of female hormones on hemostasis and thrombosis. The implications of this knowledge for clinical decision-making will be discussed.

Venous Thromboembolism and Thrombophilia: Basic Concepts

Venous thromboembolism (VTE) is defined as the formation of a blood clot in the venous circulation with or without migration of clot centrally, usually to the pulmonary circulation, which causes occlusion and infarct. Justification for the "lumping" together of deep venous thrombosis (DVT) and pulmonary embolism (PE) comes from the worrisome observation that nearly half of patients presenting with symptomatic DVT of the thigh but no symptoms suggestive of PE are found to have lung scans that are read as high probability for PE (1, 2). Since DVT and PE so frequently coexist, the current thinking is that these represent two components of a larger syndrome, VTE. The pathophysiologic mechanisms (and hence the risk factors) for venous thromboembolism appear to be quite distinct from arterial vascular occlusion, which occurs under conditions of high shear stress and where local factors on the vascular wall (i. e. plaque rupture) predominate.

The morbidity of VTE includes acute pain and swelling of the affected extremity, chronic changes (the "post-phlebitic syndrome), pulmonary embolus, and paradoxical emboli (through patent foramen ovale). The probability of symptomatic embolization of untreated deep venous thrombosis depends on the site and is estimated to be 30-40%, and the mortality of untreated pulmonary embolism is around 30%, both of which are reduced about 10-fold with treatment (3). The mortality of treated PE in the modern era is between 0.5 and 1% (Figs. 1 and 2). It behooves clinicians to be aware of these risks and

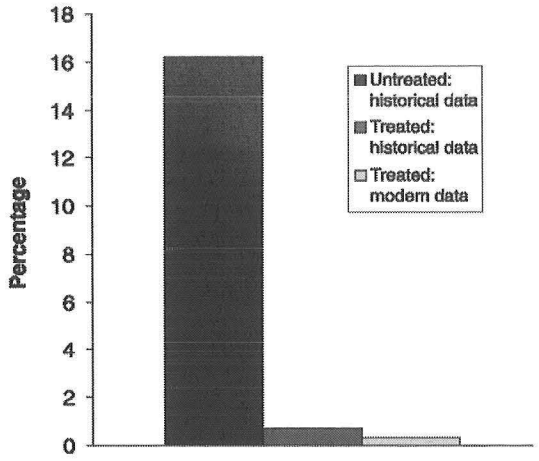


Fig. 1 Risk of fatal pulmonary embolism in untreated and treated proximal deep vein thrombosis. From Ref. (3).

to discuss them with patients so that they understand the seriousness of the disorder and the importance of optimal treatment.

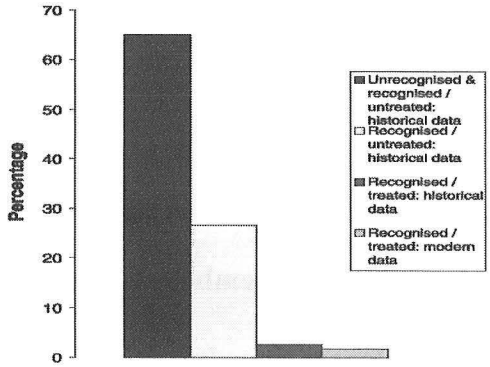


Fig. 2. Risk of fatal or recurrent pulmonary embolism in untreated or treated PE. From Ref. (3).

The revised coagulation pathway: From waterfall to waterwheel

In the 1970s and 1980s Earl Davies and colleagues developed the "cascade model" of coagulation that involves the linking of several proteolytic enzymatic steps that lead to amplification of a signal that initiates and propagates a fibrin clot. This model was been recently revised to emphasize the importance of tissue factor in initiating physiologic coagulation and Factor XI in amplifying the signal. These insights were largely through work done in the Broze laboratory in the 1990's (4).

Blood coagulation consists of a series of highly regulated proteolytic reactions that are self-amplifying and that lead to the activation of thrombin and formation of a fibrin clot (Fig. 3). The reaction is initiated when Factor VIIa (the only factor that circulates to any degree in active form) binds to tissue factor on endothelial cells and monocytes at the site of vascular injury. The tissue factor-Factor VIIa complex proteolytically activates Factor X to Xa and Factor IX to IXa. Factor Xa, in the presence of its cofactor Va, cleaves prothrombin to thrombin. Thrombin clips the soluble protein fibrinogen to form insoluble fibrin, which forms the hemostatic plug. Other prothrombotic actions of thrombin include activation of Factors XI, V and VIII, activation of platelets through a platelet cell surface receptor, activation of Factor XIII,

which covalently crosslinks fibrin to stabilize the clot, and activation of a thrombin-activatable fibrinolytic inhibitor (TAFI).

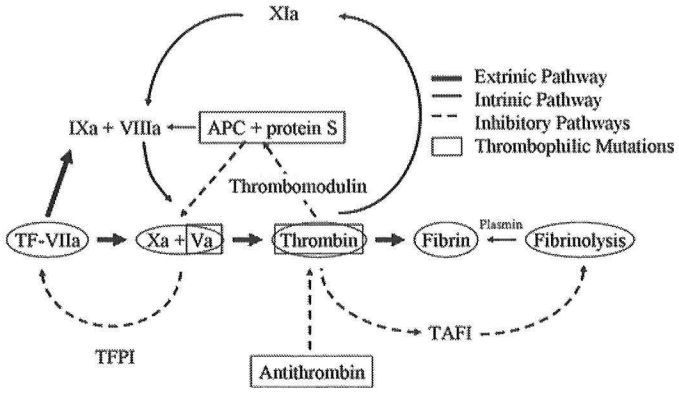


Fig. 3. Revised "Classical" Coagulation Pathway. Factors in which thrombophilic mutations have been described are boxed. Adapted from Ref. (5).

Anticoagulant mechanisms: Four inhibitory pathways (and counting)

Through a feedback mechanism that limits the extent of the fibrin clot, thrombin also initiates an important anticoagulant mechanism, by binding to thrombomodulin and forming a complex that activates protein C. Other regulatory proteins limit the generation of thrombin. The tissue factor-Factor VIIa complex is rapidly inactivated in plasma by a specific tissue factor pathway inhibitor (TFPI). Antithrombin forms covalent complexes with Factor Xa, and to a lesser extent with thrombin, Factor IXa and Factor XIa. Heparin accelerates the inactivation of these factors by about 1000-fold, and antithrombin deficiency is a cause of heparin resistance.

Acquired factors (Table 1) and hereditable mutations in a number of these anticoagulant pathways predispose to thrombosis (Tables 2 and 3). Mutations leading to functional deficiencies of antithrombin, Protein C and Protein S have all been associated with a high (>10-fold) risk of venous thromboembolism. These deficiencies are rare (<1% each in the general population). Hundreds of different mutations in the genes encoding each of these anticoagulant proteins have been described, precluding a simple genetic test using currently available technology. Mutations in procoagulant proteins that predispose to thrombosis are more common than mutations in inhibitory pathways. A single mutation in Factor V (termed Factor V Leiden) is present in about 6% of the Caucasian population. The mutant Factor V is resistant to inactivating cleavage by activated protein C (so-called "APC resistance") and circulates in an active form much longer than is normal--on the order of hours instead of minutes. An abnormality in the gene encoding prothrombin at position 20210 was described in 1996 that leads to elevated levels of prothrombin and a higher incidence of thrombosis (6). The mutation causes a gain of function with a completely novel mechanism (7). It occurs at the 3' most nucleotide of the gene just

Table 1

Acquired Causes of Hypercoagulability

Recent surgery, immobilization or trauma
Active malignancy
SLE
Inflammatory bowel disease
Myeloproliferative disorder
Antiphospholipid antibodies
Heparin-induced thrombocytopenia

Prevalence of Biologic Defects in Patients with Venous Thrombosis

Activated Protein C Resistance [Factor V Leiden]	12 – 40%*
Prothrombin G20210A Mutation	6 – 18%*
Deficiencies of Antithrombin III, Protein C, Protein S	5 – 15%
Hyperhomocysteinemia	10 – 20%
Antiphospholipid Antibody Syndrome	5 – 10%

* Prevalence restricted to Caucasian populations

prior to the poly-adenylation signal and greatly enhances the processing of the mRNA, leading to increased cleavage site recognition, increased 3' end processing and increased mRNA accumulation and protein synthesis. It is present in about 4% of the Caucasian population and is found in about 15% of patients under investigation for thrombophilia.

Like Factor V Leiden, it is only rarely found in Africans and Asian populations (8). It is a relatively weak risk factor for thrombosis ; most patients with this mutation will not have had a thrombosis by the age of 50 (9). Importantly, mutations in one of the above factors can be identified in 60% of patients with a first episode of idiopathic VTE.

Similar small increases in risk of thrombosis of 2-3 fold are seen in patients with elevated levels of coagulation factors VIII, IX, and XI (10-13). The genetic basis for the increase in protein levels is currently unknown and how testing for these additional risk factors will add to the overall predictive ability of these tests is also unknown.

Table 3

Relative Risk of Thrombosis in Individuals with Thrombophilic Defects

Activated Protein C Resistance [Factor V Leiden]	3-8X
Prothrombin G20210A Mutation	2-3X
Antithrombin Deficiency	>10X
Protein C or Protein S Deficiency	~10X
Hyperhomocysteinemia	>>10X
Antiphospholipid Antibody Syndrome	2X

Role of thrombophilic mutations in arterial thromboembolic disease

The role that thrombophilic mutations plays in arterial thromboembolic disease is uncertain, but appears to be small relative to the well documented risk factors that lead to vascular disease; i.e., smoking, hyperlipidemia, hypertension, and diabetes. In patients who have these risk factors, the probability of finding a thrombophilic mutation is quite small—not different from the unaffected population. These observations suggest that factors that affect the arterial wall, rather than the soluble factors, play an overriding role in causing arterial disease.

Blood levels of homocysteine may be genetically determined and a number of conflicting reports on genetic polymorphisms in genes that determine these levels have appeared. It is considered reasonable to consider measuring homocysteine levels in patients with arterial VTE disease as hyperhomocystenemia can be easily controlled by

folate supplementation. There does not appear to be a role for genetic testing for hyperhomocysteinemia outside of research laboratories. Acquisition of anti-phospholipid antibodies in patients with arterial or venous thromboembolism is increasingly recognized as a major risk factor and is considered part of the routine work-up of VTE and should also be considered in selected patients with arterial disease (stroke or TIA in the young).

Approach to the patient with idiopathic VTE – where are we going with testing?

Currently, nearly half of patients with idiopathic VTE will have a positive test for a hereditary or acquired thrombophilic defect. Patients most likely to be identified as harboring genetic thrombophilic defects are young, have recurrent events, have unusual locations for VTE, such as splenic, mesenteric veins, cerebral veins (not retinal or upper extremity), or who have a family history of VTE (Table 4). Women with a history of recurrent spontaneous abortion have a high incidence of acquired anti-phospholipid antibodies, but there is some evidence that thrombophilic mutations may contribute to late fetal loss as well (see below).

When to do a hypercoagulable work-up?
Young (<50)
Positive family history
Recurrent
Unusual site
Recurrent spontaneous abortion

Ideally, testing for thrombophilic mutations could be used to prevent future events through selection of appropriate patients for long-term anticoagulation. A suggested work up is shown in Table 5. However, the risks of carrying thrombophilic mutations are overestimated due to ascertainment bias inherent in case-control studies. This has led to a closer scrutiny of the utility of such testing for making clinical decisions, as discussed in recent literature (14, 15).

In favor of testing, it is likely to be useful in first-time idiopathic VTE patients with a high risk of recurrent events (antiphospholipid syndrome, antithrombin deficiency, homozygous carriers of Factor V Leiden, and patients with more than one thrombophilic defect). In addition, identification of at-risk family members allows for primary prophylaxis in asymptomatic carriers during high-risk situations. It has also been argued that testing could guide counseling regarding the use of oral contraceptives and hormone replacement therapy in at-risk family members.

On the negative side, it is argued that testing is expensive, that individuals meeting the criteria for genetic deficiencies listed above are rare (less than 2% of those tested) and that because not all risk factors have been identified, false reassurance to family members is likely to result.

How is the dilemma being addressed? Late last year an important prospective cohort study was reported that examined the value of predictive testing after a first episode of VTE (16). This study, from Cambridge, followed 570 patients for two years following discontinuation of warfarin. (Of note, other studies have shown the highest risk of recurrence occurs in this two year period). Patients with active malignancy, antiphospholipid antibodies, and mesenteric and cerebral vein thrombosis were excluded,

Table 5

Laboratory Evaluation for Hypercoagulability

- Screen for resistance to activated protein C [APC] with a clotting assay that dilutes patient plasma in factor-V deficient plasma [confirm positive APC resistance assay genetically] or genetic test for Factor V- Arg506Gin [Factor V Leiden]
- Genetic test for prothrombin G20210A mutation
- Functional assay of antithrombin III [heparin-cofactor assay]
- Functional assay of Protein C
- Functional assay of Protein S along with immunological assays of total and free protein S
- Clotting assay for anticoagulant/ELISA for cardiolipin antibodies [IgG and IgM]
- Measurement of fasting total plasma homocysteine levels

because patients with these disorders have been shown to have a high risk of recurrence and are normally anticoagulated indefinitely. Four subgroups of patients were analyzed separately; patients with antecedent surgery within 6 weeks of the VTE (Group A), patients who were pregnant or postpartum (Group B), patients with idiopathic VTE (Group C) and patients with a variety of antecedent non-surgical risk factors, including use of estrogens, prolonged travel, fracture with casting of a limb, and immobilization (Group D). Consecutive patients were enrolled between 1997 and January 2002. Thrombophilic mutations tested for were Factor V Leiden, prothrombin G20210A, antithrombin, protein C and protein S deficiencies. The cumulative risk of recurrence was 11%. None of the recurrences were in Groups A and B, and the rate of recurrence in Groups C and D were 20% and 8% respectively (Fig. 4). Most importantly, the presence or absence of a thrombophilic defect was poorly predictive of recurrence (Fig. 5).

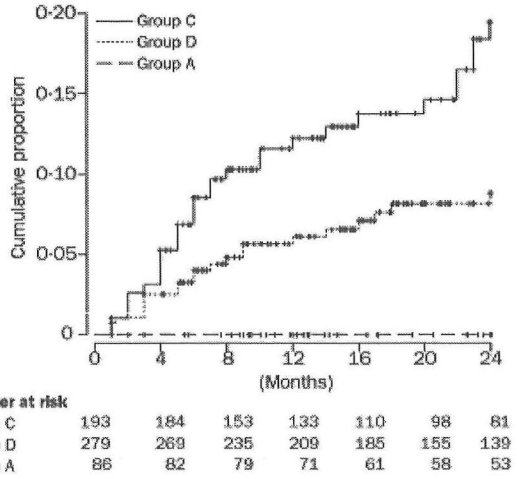


Fig. 4. Cumulative proportions of recurrent thrombosis after cessation of anticoagulant therapy. Data for Group B are not included because it was a small group with no recurrences. From ref. (16)

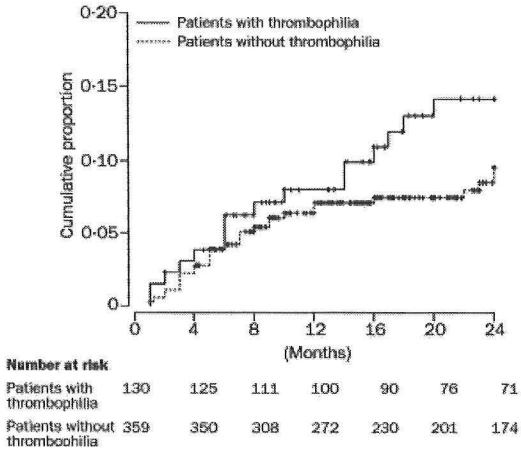


Fig. 5. Cumulative proportions of recurrent thrombosis in patients with and without laboratory evidence of a heritable thrombophilic defect. Note the poor discrimination provided by testing. . From ref. (16).

The low risk of recurrence in patients who have a postoperative VTE has been confirmed in many previous studies. The high risk of recurrence (20%) in patients with idiopathic VTE was also consistent with previous studies (17). This paper provides fairly strong justification for not screening for thrombophilic defects in unselected patients with a first episode of VTE—given the current available tests. It will be interesting to see what new recommendations, if any, will result from this important prospective study as current recommendations for indefinite coagulation include results of screening tests (18) (Table 6). Also of note, children with idiopathic venous thrombosis have a higher risk of recurrence and a higher incidence of thrombophilic mutations (19). Therefore, screening in this population may prove to be warranted, and a definitive study is warranted in this population.

Table 6

Recommendations for indefinite anticoagulation (INR 2-3)

1. Two or more spontaneous thromboses
2. One spontaneous thrombosis in AT deficiency or anti-phospholipid antibody syndrome
3. One spontaneous life-threatening (near fatal PE), cerebral, mesenteric, orportal vein thrombosis)
4. One spontaneous thrombosis at an unusual site (cerebral, mesenteric, or portal vein thrombosis)
5. One spontaneous thrombosis in the presence of more than a single genetic defect

Hormonal effects on hemostatic pathways

Use of female hormones first began in the 1960s with the widespread availability of oral contraceptives. It is estimated that 100 million women use an oral contraceptive worldwide (20). Hormone replacement therapy for menopausal symptoms gained popularity from the 1970s onward, and recent estimates show that 30 – 40% of postmenopausal women in the US use hormone substitution (21). A relationship between hormone use and an increased incidence of VTE was apparent within a few years of their introduction. Compared with non-users, users of oral contraceptives have about a 3-4 fold increased risk for VTE (22).

What do we know about the mechanism whereby female hormones increase the risk of VTE? An increase in procoagulant effects, a decrease in anticoagulant effects, and variable and canceling effects on fibrinolysis produce an overall effect that favors the formation of VTE (5). Increases in the levels of prothrombin, Factor VII, Factor VIII, Factor X, fibrinogen, and prothrombin fragment 1 + 2 and decreases in the level of Factor V were recently documented in a randomized, crossover study of second and third generation oral contraceptive use (23). The increases were greater for third generation as compared to second generation hormones, which is consistent with the increased incidence of VTE in users of third generation drugs. The magnitude of the increases (though in general less than 1.5-fold) were consistent with increased levels of prothrombin and Factor VIII that are associated with an increased risk of VTE.

Much has been published recently on an acquired resistance to activated protein C that develops in women who use oral contraceptives. Resistance to activated protein C is an independent risk factor for thrombosis, even in the absence of Factor V Leiden (24, 25).While the molecular basis of the resistance is unknown, decreased levels of plasma protein S, the cofactor of activated protein C, may play a role (26).

Interactions between hormonal and genetic thrombophilic states

Oral contraceptives

An increase in the risk of VTE was apparent shortly after the introduction of oral contraceptives in the 1960's. First generation pills contained 50 mg or more of ethinyl estradiol or mestranol and a progestin, usually norethindrone. In response to the increased thrombotic risks, second generation oral contraceptives, containing a lower dose of estradiol and a different progestational agent (levonorgestrel) were introduced, and a lower thrombotic risk, still about 4-fold elevated, was confirmed. Unfortunately, third generation pills, formulated to have less estrogen but with newer and perhaps more potent progestins, have about a two-fold increased risk relative to second generation pills. It is now recommended that third generation pills be avoided for new users due to this increased risk. A list of second and third generation brands are shown in Table 7 (27). Progesterone-only contraceptives probably have

Table 7

Progestins used in oral contraceptives (OCP), with selected OCP brands.

Second Generation

Ethynodiol (*Demulin®, Zovia®*)
Levonorgestrel (*Alesse®, Tri/Levlen®, Nordette®, Triphasil®*)
Norethindrone (*Brevicon®, Micronor®*, Ortho-Novum®, Modicon®*)
Norethindrone acetate (*Estrostep®, Loestrin®*)
Norgestrel (*Lo/Ovral®, Ovrette®**)

Third Generation

Desogestrel (*Desogen®, Mircette®*)
Gestodene (*Not used in U.S.*)
Norgestimate (*Ortho Tri-Cyclen®*)

*Progestin only pills

a slightly less increased risk as compared to those containing estrogen and progestin (2-fold as compared to 3-4 fold) (28). No data is available concerning progesterone-releasing IUDs, though no changes in surrogate coagulation markers seen to occur with use. Short term treatment (so-called emergency contraception or the "morning-after" pill) is not associated with a significant risk of thrombosis. In a study of over 100,000 prescriptions for post-coital contraceptives between 1989 and 1996 in the UK, no episodes of VTE were reported within a 45 day period following use (29).

Identification of common hereditary risk factors for VTE has allowed investigations into how these interact in the setting of oral contraceptive use. Women who are heterozygous for the Factor V Leiden mutation and who use oral contraceptives have between a 20- and 35-fold risk of VTE as compared to women without either of these risk factors (30, 31) (Fig. 6). The risk is even higher (about 50-fold) in users of third generation contraceptives (32). Deficiencies of antithrombin, protein C and protein S also appear to increase the risk of VTE in concert with oral contraceptive use, although the analytical methods used in these studies were insufficient to determine the magnitude of the effect (33, 34). An increased risk of cerebral vein thrombosis in carriers of the prothrombin 20210 mutation and users of oral contraceptives has also been reported (35).

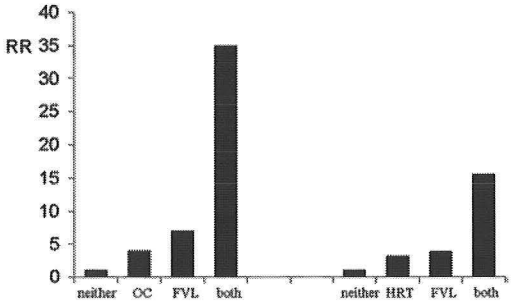


Fig. 6 Interaction of factor V Leiden and oral contraceptive use (left panel), and factor V Leiden and hormonal replacement therapy (right panel). The bars show the risk of those with only factor V Leiden, only oral contraceptives/hormonal replacement therapy, and those with both oral contraceptives/hormone replacement therapy and factor V Leiden, all relative to those with neither. From ref. (30, 36).

Although not currently included in the standard evaluation of thrombophilia, elevated levels of Factor VIII correlate with an increased risk of VTE, and a probably multiplicative interaction with oral contraceptives has also been described, with about a 10-fold increased risk (10, 37).

Although it is important to consider these relative risks, it is the absolute risks that mandate clinical decision making, and the absolute risks are relatively low. The baseline incidence of VTE is 1 per 10,000 women per years, so a 35-fold increased risk is still quite small (Table 8). Furthermore, VTE is a treatable condition; with modern treatment fatality or irreversible damage to health should occur in less than 1% of episodes.

Should women be screened for thrombophilic mutations prior to oral contraceptive use? One analysis has estimated that 500,000 women would have to be screened in order to prevent one death from PE (38). When taken with all attendant morbidity and mortality, screening all women for Factor V Leiden may make economic sense, but only if the testing could be done for less than $10 per person (39). More importantly, the absence of a recognized defect does not rule out the possibility of

thrombosis, as only 60% of individuals with recurrent thromboses have such a defect. Even in the absence of a defect, an individual with a strong family history cannot be assured that the identified weak risk factor contributes significantly to the absolute risk.

Table 8

Estimated risks of venous thromboembolism (VTE) in patients exposed to hormonal therapy or pregnancy.* From Ref. (27)

	Baseline relative risk	20 year old with thrombophilia; est. events per 10,000 women-yrs**	60 year old with thrombophilia; est. events per 10,000 women-yrs**	***Risk in Factor V Leiden heterozygote	***Risk in Prothrombin 20210 heterozygote
2nd generation OCP	4	4		*20 – 35*	
3rd generation OCP	6 – 8	5 – 6		*50*	*16***
Pregnancy	5 – 6				
HRT	2 – 4		20 – 40	*13*	
Tamoxifen/Raloxifene	3 – 7		30 – 70		
Tamoxifen w/ adjuvant chemotherapy	*5 – 15*		*50 – 150*		

* Estimated from available literature. Numbers shown in italics are based on limited data
** Calculated based on incidence of VTE in 20 year old of 1/10,000 and in 60 year old of 10/10,000
*** Risk compared to women without the mutation and not receiving hormonal therapy or pregnant
**** Estimated based on combined 2nd and 3rd generation OCP data, but 73% of women enrolled in study were on 3rd generation OCP

Hormone Replacement Therapy (HRT)

Recent studies have questioned the role of hormone replacement therapy in the prevention of cardiovascular disease, and have prompted a reassessment of risks and benefits of HRT. A number of case-control and prospective studies have come to the conclusion that HRT elevates the risk of VTE between 2 and 3 fold (40-46). Two very large randomized prospective trials have provided absolute risk data for combined estrogen/progesterone vs. placebo regarding a number of cardiovascular and non-cardiovascular outcomes. The Women's Health Initiative (47) randomized about 16,000 healthy women between the ages of 50 and 79 to estrogen/progestin or placebo and followed them an average of 5.2 years. (Interestingly, equal numbers of drug and placebo groups (40%) stopped taking the study medication, which is probably similar to what occurs in practice. Furthermore, 10% of the placebo group "dropped in" and began taking exogenous estrogens whereas 6.2% of the treated group did the same). The cumulative incidence of VTE at 7 years was about 1% for the estrogen/progestin group, which was twice that of the placebo group (34 vs. 16 events per 10,000 woman-years) (Fig. 7). Similar trends for cardiovascular heart disease (37 vs. 30) and stroke (29 vs. 21) were observed. Significantly, overall survival was unaffected (Fig. 8).

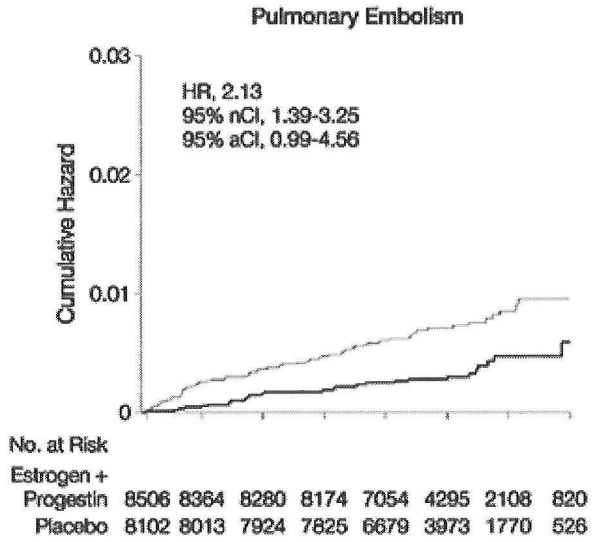


Fig. 7. Kaplan-Meier Estimates of Cumulative Hazards for Pulmonary Embolism. From Ref. (47).

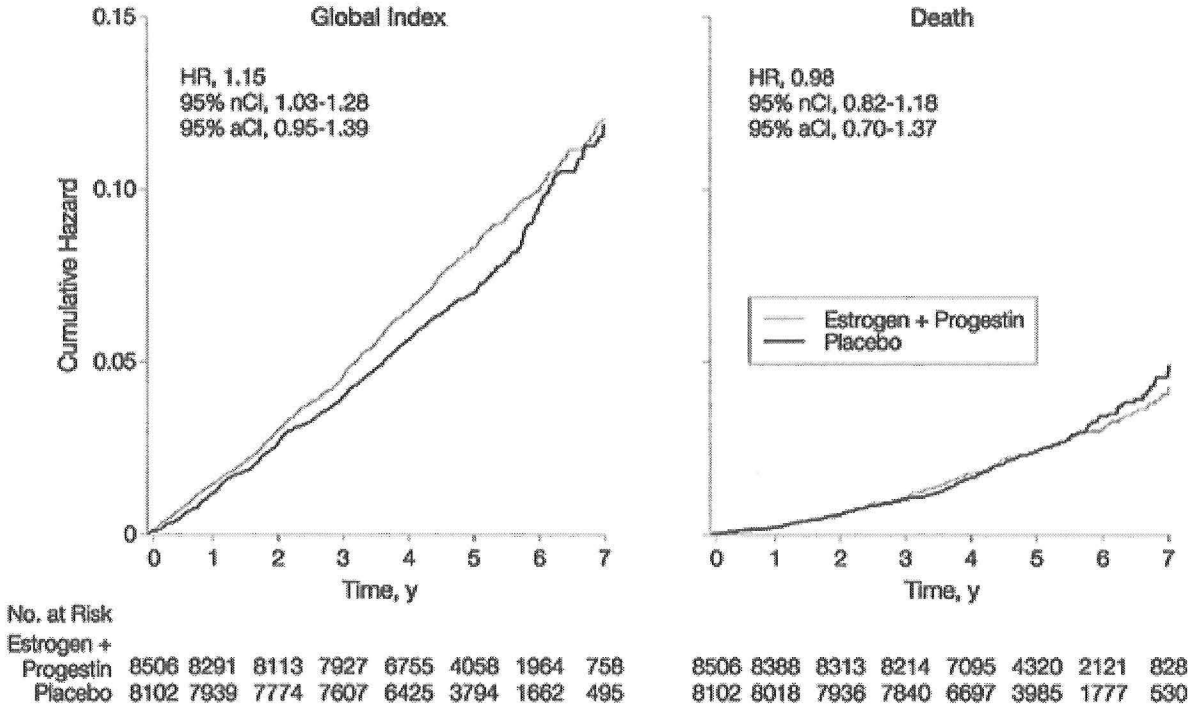
The HERS trial (a secondary prevention trial) involved women with pre-existing cardiovascular disease (46, 48). It enrolled 2763 women with coronary disease and a mean age of 66.7 years. The cumulative incidence of VTE was 2.5% in the treated group and 0.9% in the control group. There was no significant difference in survival. Follow up data published at 7 years indicated 59 events per 10000 woman-years vs. 28 per 10000 woman-years in the control group. The relative risk was highest during the first two years and approached 1 at years 5-7.

The EVTET trial from Norway (49) was a smaller but very informative study that looked at the effect of HRT on VTE incidence in high-risk women. These were 140 women with previous DVT randomized to HRT or placebo. (Of note, study was begun before there was a clear consensus on the elevated risk associated with HRT and was terminated early). Such a randomized study could not be performed today for ethical reasons, but it is especially informative, because it provides data as to whether knowing thrombophilic risk factors could have guided choice of HRT. Women with antithrombin deficiency or malignancy were excluded. Mean duration of follow up was 484 days. The incident rates were 10.7% in the treated group and 2.3% in the placebo group. Of the nine patients with VTE, only 3 had a genetic thrombophilia (one homozygous and two heterozygous for Factor V Leiden), two had anticardiolipin antibodies (which today would warrant treatment) and 4 had no abnormalities detected. Therefore, testing for genetic thrombophilia would have only identified one-third of women at risk and provided false reassurance to the rest. In this case it is clear that avoidance of HRT by women with previous VTE would have been more effective than testing, and it is consistent with the prospective trial discussed above (16).

The absolute risk of thrombosis is highly dependent on age, so the impact of HRT (and attendant thrombophilic mutations) will be greater as compared to oral contraceptives. Two case-control studies have looked at the associations. The relative risks, as with OCPs, are multiplicative (36, 50). In a study similar to the HERS trial (secondary coronary prevention) (50) the increased risk for HRT alone was 3-4 and for

the common thrombophilic mutations (Factor V Leiden and prothrombin G20210A) it was about 5, with an increased risk of both at 15. This corresponds to an absolute incidence of 15 per 1000 per year vs. 2 for women without thrombophilia on placebo. The estimated number needed to screen for Factor V Leiden to avoid an HRT-asociated VTE durng 5 years of treatment was about 400 in women with coronary disease. This translates to a cost of $50,000 per VTE hospitalization, which is probably prohibitive. Thus, there is no current recommendation for screening in this population.

Fig. 8 Kaplan-Meier Estimates of Cumulative Hazards for Global Index and Death. From Ref. (47).



A recent case-control study (51) of *transdermal* vs. oral estrogen on risk of VTE was reported from France, where the use of transdermal estrogen is especially high. Interestingly, the odds ratio for VTE in current users of oral and transdermal estrogen compared with non-users was 3.5 (95% CI 1.8-6.8) and 0.9 (95% CI 0.5-1.6), suggesting that transdermal estrogen may be safer than oral. This is a plausible idea, because transdermal estrogen has been reported to have little effect on hemostatic parameters (52). It would not be surprising to see a shift in popularity to transdermal estrogen, especially given the recent data from the Women's Health Initiative.

Breast cancer therapy

The nonsteroidal estrogen receptor modulators (tamoxifen and raloxifene) have combined estrogenic and anti-estrogenic effects. This is significant because the compounds posssess antitumor activity without some of the antiestrogenic side effects (such as decreased bone mineralization and increased cardiovascular risk). These agents

increase the risk of VTE in a magnitude very similar to OCPs and HRT. Findings from several studies suggest that chemotherapy, often given in conjunction with tamoxifen for the treatment of breast cancer, contributes to the risk of thrombosis (53-58). The absolute risk in this setting is extraordinarily high, approaching 8% in post-menopausal women. Metastatic disease further increases the already high risk. The issue is currently being studied prospectively, but some advocate prophylaxis for patients undergoing combined chemo/hormonal therapy, particularly in women who have a previous personal history of thrombosis. No information is available concerning genetic thrombophilic risk factors in this group.

Pregnancy

Pregnancy and the post-partum period is associated with a 5-6 fold increase in the incidence of VTE (59) and pulmonary embolism remains as a leading cause of maternal mortality in the modern era. Untreated DVT results in PE 16% of the time in this setting, of which 13% are fatal, for an overall mortality of 2%, which can be prevented 90% by appropriate anticoagulant treatment (60). Interestingly, most DVTs in pregnancy (90%) involve the left leg, suggesting that local anatomic factors as well as plasma factors play a role. A greater proportion of these are iliofemoral, which may be more dangerous, with a higher propensity to embolize. The baseline risk is about 1 per 1000 deliveries, and the incidence of younger mothers (<35) is half that of older mothers. The post-partum period, extending to at least 6 weeks after delivery, may be the period of highest risk, particularly for pulmonary embolism. For this reason, women with a previous episode of VTE are routinely anticoagulated with coumadin for 6 weeks post-partum. Coumadin is not excreted into breast milk and is considered safe in lactating women.

There is an ongoing debate as to whether women with a previous episode of VTE should receive prophylactic therapeutic heparin during pregnancy. Estimates of the risk of antepartum VTE have ranged from 0-13% in mostly small studies (reviewed in(61)) . A recent prospective study of the incidence of VTE in 125 pregnant women with a prior episode of VTE has been highly cited (62). It found an incidence of recurrent VTE of 3/125 during the antepartum period and 3/125 in the post-partum period (who for various reasons did not receive recommended post-partum coumadin). The conclusion of the study was that it is safe to withhold heparin in patients who have had a previous VTE due to the low incidence. However, women with known thrombophilic defects were excluded. Furthermore, because all three patients with VTE later had thrombophilic risk factors identified, the study recommended testing for Factor V Leiden and prothrombin G20210A for this subgroup, probably inappropriately. The study has largely been ignored by the obstetrics community, as the incidence of events could be regarded as unacceptable. Furthermore, in light of the prospective studies of thrombophilic testing above, the numbers in this study were too small to justify the recommendation for testing. Current obstetrical practice is to anticoagulate with prophylactic doses of low molecular weight heparin in women with previous personal history of idiopathic VTE. Women

with documented antithrombin deficiency or the antiphospholipid syndrome or recurrent VTE warrant more intensive anticoagulation with adjusted dose heparin (61, 63).

The influence of thrombophilic mutations on the incidence of VTE during pregnancy and the postpartum period has been examined in numerous case-control studies (reviewed in (60)). As with the other hormonal influences, the incidence of VTE is multiplicative with the risk of individual thrombophilic risk factors.

Thrombophilia and adverse pregnancy outcomes

Antiphospholipid antibodies, an acquired thrombophilic defect, have recently been established as an important and treatable cause of recurrent miscarriage. The potential role of other thrombophilic defects is now an area of active investigation. Women with Factor V Leiden or the prothrombin 20210 mutations have a 2- to 3-fold increased risk of late fetal loss (64-67). It would be of interest to know whether women with pregnancy loss and thrombophilic defects other than anti-phospholipid syndrome would benefit from anticoagulation with heparin. No randomized trials are yet available. However, an unrandomized study was done in Israel comparing 37 patients with hereditary thrombophilia and 3 or more consecutive pregnancy losses treated with enoxaparin 40 mg per day with 48 similar untreated historical controls. Routine evaluation for recurrent pregnancy loss (including anti-phospholipid antibodies, chromosome analysis, and anatomical evalution) had excluded these other causes. The live birth rate was 70.2% in the treated group and 44% in the untreated group. The relatively high birth rate in the treated group is encouraging and is consistent with similar smaller studies (68-70). A randomized trial placebo-controlled trial would be needed to address the issue, but because of ethical concerns, a larger trial comparing two doses of enoxaparin is in progress as is a trial of lovenox in women without prospectively identified thrombophilic mutations.

Maternal thrombophilias are not associated with pregnancy wastage prior to 10 weeks of gestation (71).

Implications for application of medicine in complex genetic disorders: lessons learned

Thrombophilic defects can now be identified in over half of patients with idiopathic VTE. However, most of these mutations impart only a modest increase in relative risk compared to the unaffected population (about 3-fold). Therefore, the predictive value of any one test is limited and clearly, more such defects remain to be determined.

The value of genetic predictive tests will depend largely on the risks and benefits of available treatment or preventive measures. In the case of thrombophilia, the treatment (anticoagulation) carries significant risks. Therefore, the "bar" must be set fairly high with respect to making decisions based on testing. The positive predictive value of the tests (combined) must be in line with those currently used for clinical

decision-making. This has not yet been achieved, and unfortunately, the current testing algorithm (utilizing a combination of traditional and DNA-based methods) taxes all but the most well-equipped of laboratories. Ultimately, if a high proportion of relevant defects (say, greater than 95%) can be identified, genetic testing may yet prove to be useful, but the number of individual mutations involved will be high (probably on the order of thousands)--much higher than previously suspected.

Case wrap-up

I had a DVT while on oral contraceptives and was told I have Factor V Leiden. What's going to happen when I become pregnant?

Answer: Recurrence rates for women with previous DVT (regardless of thrombophilic risk factors) may be as high as 13%. Anticoagulation for 6 weeks during the post-partum period is indicated regardless of testing. If the Factor V Leiden were positive, antepartum anticoagulation with heparin is indicated. If the Factor V Leiden were negative, whether or not to withhold anticoagulation is controversial. Many obstetricians would recommend heparin during this pregnancy regardless of the Factor V status.

I'm her sister. Is it ok if I take birth control pills?

Answer: The risk of DVT with oral contraceptives is increased. Factor V testing has not been shown to predict risk of thrombosis in a prospective setting. Alternative methods of birth control should be explored.

I had a DVT during pregnancy 25 years ago. What about hormone replacement therapy for me now

Answer: The risk of recurrent DVT is about 10% in the first year. Alternatives to HRT should be strongly considered.

I've been prescribed tamoxifen for my early breast cancer, but I had a pulmonary embolism while pregnant. Should I worry?

Answer: There is good reason to worry. Anticoagulation should be strongly considered, especially if you have metastatic breast cancer and/or are receiving chemo-therapy. Enrollment in a clinical trial is encouraged.

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References

1. Nielsen, H.K., Husted, S.E., Krusell, L.R., Fasting, H., Charles, P. and Hansen, H.H. (1994) Silent pulmonary embolism in patients with deep venous thrombosis. Incidence and fate in a randomized, controlled trial of anticoagulation versus no anticoagulation. *J Intern Med,* 235, 457-61.
2. Monreal, M., Rey-Joly Barroso, C., Ruiz Manzano, J., Salvador Tarrason, R., Lafoz Naval, E. and Viver Manresa, E. (1989) Asymptomatic pulmonary embolism in patients with deep vein thrombosis. Is it useful to take a lung scan to rule out this condition? *J Cardiovasc Surg (Torino),* 30, 104-7.
3. Kelly, J. and Hunt, B.J. (2003) Do anticoagulants improve survival in patients presenting with venous thromboembolism? *J Intern Med,* 254, 527-39.
4. Luchtman-Jones, L. and Broze, G.J., Jr. (1995) The current status of coagulation. *Ann Med,* 27, 47-52.
5. Vandenbroucke, J.P., Rosing, J., Bloemenkamp, K.W., Middeldorp, S., Helmerhorst, F.M., Bouma, B.N. and Rosendaal, F.R. (2001) Oral contraceptives and the risk of venous thrombosis. *N Engl J Med,* 344, 1527-35.
6. Poort, S.R., Rosendaal, F.R., Reitsma, P.H. and Bertina, R.M. (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood,* 88, 3698-703.
7. Gehring, N.H., Frede, U., Neu-Yilik, G., Hundsdoerfer, P., Vetter, B., Hentze, M.W. and Kulozik, A.E. (2001) Increased efficiency of mRNA 3' end formation: a new genetic mechanism contributing to hereditary thrombophilia. *Nat Genet,* 28, 389-92.
8. Rosendaal, F.R., Doggen, C.J., Zivelin, A., Arruda, V.R., Aiach, M., Siscovick, D.S., Hillarp, A., Watzke, H.H., Bernardi, F., Cumming, A.M. *et al.* (1998) Geographic distribution of the 20210 G to A prothrombin variant. *Thromb Haemost,* 79, 706-8.
9. Salomon, O., Steinberg, D.M., Zivelin, A., Gitel, S., Dardik, R., Rosenberg, N., Berliner, S., Inbal, A., Many, A., Lubetsky, A. *et al.* (1999) Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism: prevalence and risk assessment. *Arterioscler Thromb Vasc Biol,* 19, 511-8.
10. Kraaijenhagen, R.A., in't Anker, P.S., Koopman, M.M., Reitsma, P.H., Prins, M.H., van den Ende, A. and Buller, H.R. (2000) High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. *Thromb Haemost,* 83, 5-9.
11. Schambeck, C.M., Hinney, K., Haubitz, I., Mansouri Taleghani, B., Wahler, D. and Keller, F. (2001) Familial clustering of high factor VIII levels in patients with venous thromboembolism. *Arterioscler Thromb Vasc Biol,* 21, 289-92.

12. Meijers, J.C., Tekelenburg, W.L., Bouma, B.N., Bertina, R.M. and Rosendaal, F.R. (2000) High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med,* 342, 696-701.
13. van Hylckama Vlieg, A., van der Linden, I.K., Bertina, R.M. and Rosendaal, F.R. (2000) High levels of factor IX increase the risk of venous thrombosis. *Blood,* 95, 3678-82.
14. Martinelli, I. (2003) Pros and cons of thrombophilia testing: pros. *J Thromb Haemost,* 1, 410-1.
15. Machin, S.J. (2003) Pros and cons of thrombophilia testing: cons. *J Thromb Haemost,* 1, 412-3.
16. Baglin, T., Luddington, R., Brown, K. and Baglin, C. (2003) Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. *Lancet,* 362, 523-6.
17. Schulman, S., Rhedin, A.S., Lindmarker, P., Carlsson, A., Larfars, G., Nicol, P., Loogna, E., Svensson, E., Ljungberg, B. and Walter, H. (1995) A comparison of six weeks with six months of oral anticoagulant therapy after a first episode of venous thromboembolism. Duration of Anticoagulation Trial Study Group. *N Engl J Med,* 332, 1661-5.
18. Bauer, K.A. (2003) Management of thrombophilia. *J Thromb Haemost,* 1, 1429-34.
19. Nowak-Gottl, U., Junker, R., Kreuz, W., von Eckardstein, A., Kosch, A., Nohe, N., Schobess, R. and Ehrenforth, S. (2001) Risk of recurrent venous thrombosis in children with combined prothrombotic risk factors. *Blood,* 97, 858-62.
20. Organization, W.H. (1998) Cardiovascular Disease and Soeroid Hormone Contraception. Report of a WHO Scientific Group. WHO Technical Report Series 877. World Health Organization, Geneva.
21. Lemaitre, R.N., Heckbert, S.R., Psaty, B.M., Smith, N.L., Kaplan, R.C. and Longstreth, W.T., Jr. (2002) Hormone replacement therapy and associated risk of stroke in postmenopausal women. *Arch Intern Med,* 162, 1954-60.
22. (1995) Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Lancet,* 346, 1575-82.
23. Middeldorp, S., Meijers, J.C., van den Ende, A.E., van Enk, A., Bouma, B.N., Tans, G., Rosing, J., Prins, M.H. and Buller, H.R. (2000) Effects on coagulation of levonorgestrel- and desogestrel-containing low dose oral contraceptives: a cross-over study. *Thromb Haemost,* 84, 4-8.
24. de Visser, M.C., Rosendaal, F.R. and Bertina, R.M. (1999) A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. *Blood,* 93, 1271-6.
25. Rodeghiero, F. and Tosetto, A. (1999) Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. *Ann Intern Med,* 130, 643-50.
26. Tans, G., Curvers, J., Middeldorp, S., Thomassen, M.C., Meijers, J.C., Prins, M.H., Bouma, B.N., Buller, H.R. and Rosing, J. (2000) A randomized cross-over

study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. *Thromb Haemost,* 84, 15-21.
27. Macik, B.G., Rand, J.H. and Konkle, B.A. (2001) Thrombophilia: what's a practitioner to do? *Hematology (Am Soc Hematol Educ Program)*, 322-38.
28. Vasilakis, C., Jick, H. and del Mar Melero-Montes, M. (1999) Risk of idiopathic venous thromboembolism in users of progestagens alone. *Lancet,* 354, 1610-1.
29. Vasilakis, C., Jick, S.S. and Jick, H. (1999) The risk of venous thromboembolism in users of postcoital contraceptive pills. *Contraception,* 59, 79-83.
30. Vandenbroucke, J.P., Koster, T., Briet, E., Reitsma, P.H., Bertina, R.M. and Rosendaal, F.R. (1994) Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation [see comments]. *Lancet,* 344, 1453-7.
31. Martinelli, I., Taioli, E., Bucciarelli, P., Akhavan, S. and Mannucci, P.M. (1999) Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. *Arterioscler Thromb Vasc Biol,* 19, 700-3.
32. Bloemenkamp, K.W., Rosendaal, F.R., Buller, H.R., Helmerhorst, F.M., Colly, L.P. and Vandenbroucke, J.P. (1999) Risk of venous thrombosis with use of current low-dose oral contraceptives is not explained by diagnostic suspicion and referral bias. *Arch Intern Med,* 159, 65-70.
33. Pabinger, I. and Schneider, B. (1994) Thrombotic risk of women with hereditary antithrombin III-, protein C- and protein S-deficiency taking oral contraceptive medication. The GTH Study Group on Natural Inhibitors. *Thromb Haemost,* 71, 548-52.
34. Simioni, P., Sanson, B.J., Prandoni, P., Tormene, D., Friederich, P.W., Girolami, B., Gavasso, S., Huisman, M.V., Buller, H.R., Wouter ten Cate, J. *et al.* (1999) Incidence of venous thromboembolism in families with inherited thrombophilia. *Thromb Haemost,* 81, 198-202.
35. Martinelli, I., Sacchi, E., Landi, G., Taioli, E., Duca, F. and Mannucci, P.M. (1998) High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med,* 338, 1793-7.
36. Rosendaal, F.R., Vessey, M., Rumley, A., Daly, E., Woodward, M., Helmerhorst, F.M. and Lowe, G.D. (2002) Hormonal replacement therapy, prothrombotic mutations and the risk of venous thrombosis. *Br J Haematol,* 116, 851-4.
37. Bloemenkamp, K.W., Helmerhorst, F.M., Rosendaal, F.R. and Vandenbroucke, J.P. (1999) Venous thrombosis, oral contraceptives and high factor VIII levels. *Thromb Haemost,* 82, 1024-7.
38. Vandenbroucke, J.P., van der Meer, F.J., Helmerhorst, F.M. and Rosendaal, F.R. (1996) Factor V Leiden: should we screen oral contraceptive users and pregnant women? *Bmj,* 313, 1127-30.
39. Szucs, T., Osterkorn, D. and Schramm, W. (1996) [Public health economic evaluation of screening for APC resistance (Leiden mutation) in new oral contraceptive users]. *Med Klin (Munich),* 91, 317-9.
40. Daly, E., Vessey, M.P., Hawkins, M.M., Carson, J.L., Gough, P. and Marsh, S. (1996) Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet,* 348, 977-80.

41. Jick, H., Derby, L.E., Myers, M.W., Vasilakis, C. and Newton, K.M. (1996) Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet,* 348, 981-3.
42. Perez Gutthann, S., Garcia Rodriguez, L.A., Castellsague, J. and Duque Oliart, A. (1997) Hormone replacement therapy and risk of venous thromboembolism: population based case-control study. *Bmj,* 314, 796-800.
43. Grodstein, F., Stampfer, M.J., Goldhaber, S.Z., Manson, J.E., Colditz, G.A., Speizer, F.E., Willett, W.C. and Hennekens, C.H. (1996) Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet,* 348, 983-7.
44. Varas-Lorenzo, C., Garcia-Rodriguez, L.A., Cattaruzzi, C., Troncon, M.G., Agostinis, L. and Perez-Gutthann, S. (1998) Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a population-based study in southern Europe. *Am J Epidemiol,* 147, 387-90.
45. Hoibraaten, E., Abdelnoor, M. and Sandset, P.M. (1999) Hormone replacement therapy with estradiol and risk of venous thromboembolism--a population-based case-control study. *Thromb Haemost,* 82, 1218-21.
46. Hulley, S., Grady, D., Bush, T., Furberg, C., Herrington, D., Riggs, B. and Vittinghoff, E. (1998) Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *Jama,* 280, 605-13.
47. Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C. *et al.* (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama,* 288, 321-33.
48. Furberg, C.D., Vittinghoff, E., Davidson, M., Herrington, D.M., Simon, J.A., Wenger, N.K. and Hulley, S. (2002) Subgroup interactions in the Heart and Estrogen/Progestin Replacement Study: lessons learned. *Circulation,* 105, 917-22.
49. Hoibraaten, E., Qvigstad, E., Arnesen, H., Larsen, S., Wickstrom, E. and Sandset, P.M. (2000) Increased risk of recurrent venous thromboembolism during hormone replacement therapy--results of the randomized, double-blind, placebo-controlled estrogen in venous thromboembolism trial (EVTET). *Thromb Haemost,* 84, 961-7.
50. Herrington, D.M., Vittinghoff, E., Howard, T.D., Major, D.A., Owen, J., Reboussin, D.M., Bowden, D., Bittner, V., Simon, J.A., Grady, D. *et al.* (2002) Factor V Leiden, hormone replacement therapy, and risk of venous thromboembolic events in women with coronary disease. *Arterioscler Thromb Vasc Biol,* 22, 1012-7.
51. Scarabin, P.Y., Oger, E. and Plu-Bureau, G. (2003) Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet,* 362, 428-32.
52. Scarabin, P.Y., Alhenc-Gelas, M., Plu-Bureau, G., Taisne, P., Agher, R. and Aiach, M. (1997) Effects of oral and transdermal estrogen/progesterone regimens on blood coagulation and fibrinolysis in postmenopausal women. A randomized controlled trial. *Arterioscler Thromb Vasc Biol,* 17, 3071-8.

53. Levine, M.N., Gent, M., Hirsh, J., Arnold, A., Goodyear, M.D., Hryniuk, W. and De Pauw, S. (1988) The thrombogenic effect of anticancer drug therapy in women with stage II breast cancer. *N Engl J Med,* 318, 404-7.
54. Saphner, T., Tormey, D.C. and Gray, R. (1991) Venous and arterial thrombosis in patients who received adjuvant therapy for breast cancer. *J Clin Oncol,* 9, 286-94.
55. Pritchard, K.I., Paterson, A.H., Paul, N.A., Zee, B., Fine, S. and Pater, J. (1996) Increased thromboembolic complications with concurrent tamoxifen and chemotherapy in a randomized trial of adjuvant therapy for women with breast cancer. National Cancer Institute of Canada Clinical Trials Group Breast Cancer Site Group. *J Clin Oncol,* 14, 2731-7.
56. Fisher, B., Costantino, J.P., Wickerham, D.L., Redmond, C.K., Kavanah, M., Cronin, W.M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J. *et al.* (1998) Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst,* 90, 1371-88.
57. Fisher, B., Dignam, J., Wolmark, N., Wickerham, D.L., Fisher, E.R., Mamounas, E., Smith, R., Begovic, M., Dimitrov, N.V., Margolese, R.G. *et al.* (1999) Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. *Lancet,* 353, 1993-2000.
58. Ettinger, B., Black, D.M., Mitlak, B.H., Knickerbocker, R.K., Nickelsen, T., Genant, H.K., Christiansen, C., Delmas, P.D., Zanchetta, J.R., Stakkestad, J. *et al.* (1999) Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. *Jama,* 282, 637-45.
59. Gerhardt, A., Scharf, R.E., Beckmann, M.W., Struve, S., Bender, H.G., Pillny, M., Sandmann, W. and Zotz, R.B. (2000) Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *N Engl J Med,* 342, 374-80.
60. Zotz, R.B., Gerhardt, A. and Scharf, R.E. (2003) Inherited thrombophilia and gestational venous thromboembolism. *Best Pract Res Clin Haematol,* 16, 243-59.
61. Bates, S.M. and Ginsberg, J.S. (2002) How we manage venous thromboembolism during pregnancy. *Blood,* 100, 3470-8.
62. Brill-Edwards, P., Ginsberg, J.S., Gent, M., Hirsh, J., Burrows, R., Kearon, C., Geerts, W., Kovacs, M., Weitz, J.I., Robinson, K.S. *et al.* (2000) Safety of withholding heparin in pregnant women with a history of venous thromboembolism. Recurrence of Clot in This Pregnancy Study Group. *N Engl J Med,* 343, 1439-44.
63. Bowles, L. and Cohen, H. (2003) Inherited thrombophilias and anticoagulation in pregnancy. *Best Pract Res Clin Obstet Gynaecol,* 17, 471-89.
64. Kupferminc, M.J., Eldor, A., Steinman, N., Many, A., Bar-Am, A., Jaffa, A., Fait, G. and Lessing, J.B. (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med,* 340, 9-13.
65. Meinardi, J.R., Middeldorp, S., de Kam, P.J., Koopman, M.M., van Pampus, E.C., Hamulyak, K., Prins, M.H., Buller, H.R. and van der Meer, J. (1999) Increased

risk for fetal loss in carriers of the factor V Leiden mutation. *Ann Intern Med,* 130, 736-9.

66. Martinelli, I., Taioli, E., Cetin, I., Marinoni, A., Gerosa, S., Villa, M.V., Bozzo, M. and Mannucci, P.M. (2000) Mutations in coagulation factors in women with unexplained late fetal loss. *N Engl J Med,* 343, 1015-8.
67. Sarig, G., Younis, J.S., Hoffman, R., Lanir, N., Blumenfeld, Z. and Brenner, B. (2002) Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. *Fertil Steril,* 77, 342-7.
68. Gris, J.C., Neveu, S., Tailland, M.L., Courtieu, C., Mares, P. and Schved, J.F. (1995) Use of a low-molecular weight heparin (enoxaparin) or of a phenformin-like substance (moroxydine chloride) in primary early recurrent aborters with an impaired fibrinolytic capacity. *Thromb Haemost,* 73, 362-7.
69. Brenner, B., Hoffman, R., Blumenfeld, Z., Weiner, Z. and Younis, J.S. (2000) Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin. *Thromb Haemost,* 83, 693-7.
70. Brenner B, f.t.L.E.I. (2002) Efficacy and safety of two doses of Enoxaparin in pregnant women with thrombophilia and recurrent pregnancy loss. *Blood,* 100, 2765, P702a (Abstract).
71. Roque, H., Paidas, M.J., Funai, E.F., Kuczynski, E. and Lockwood, C.J. (2004) Maternal thrombophilias are not associated with early pregnancy loss. *Thromb Haemost,* 91, 290-5.