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Review



# Thrombophilia and pregnancy

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## Abstract

Pregnancy is hypercoagulable state. The field of thrombophilia; the tendency to thrombosis, has been developed rapidly and has been linked to many aspects of pregnancy. It is recently that severe pregnancy complications such as severe preeclampsia intrauterine growth retardation abruptio placentae and stillbirth has been shown to be associated with thrombophilia. Recurrent miscarriage and has also been associated with thrombophilia. Finally, thromboembolism in pregnancy as in the non-pregnant state is linked to thrombophilia. In this review all aspects of thrombophilia in pregnancy are discussed, and also all prophylactic and therapeutic implications.

Thrombophilias are inherited or acquired conditions which predispose an individual to thromboembolism. Deficiencies of protein S C and antithrombin are rare and each of them is found in about 3% of patients with thrombosis. Recently, three important inherited thrombophilias were discovered which are responsible of the majority of thromboembolic events in patients with otherwise no apparent risk for thrombosis. Resistance to activated protein C caused by an adenine 506 guanine (A506G) mutation in factor V (factor V Leiden) has been linked with an increased risk for venous thromboembolism [1-3]. Heterozygosity for the factor V (FV) Leiden mutation is found in about 5% of the population and the mutation is responsible of 20–30% of venous thromboembolism events. A recently described guanine 20210 adenine mutation in prothrombin is associated with higher plasma prothrombin concentrations and increased risk for venous thromboembolism [4] and cerebral-vein thrombosis [5]. Homozygosity for the cytosine 677 thymine (C677T) mutation in methylenetetrahydrofolate reductase (MTHFR) results in decreased synthesis of 5-methyltetrahydrofolate, the primary methyl donor in the conversion of homocysteine to methionine and the resulting increase in plasma homocysteine concentrations is a risk factor for thrombosis [6,7]. The mutation is responsible for reduced MTHFR activity and is the most frequent cause of mild hyperhomocysteinemia and can be found in 5–15% of the population.

Homocysteine is an independent risk factor for atherosclerosis, stroke, peripheral vascular disease and cardiovascular diseases [8,9]. Homocysteine concentrations are affected by nutrition. A deficiency in folate, B-6, and/or B-I2 causes elevation of homocysteine. Homocysteine concentrations are also affected by genetics such as cystathionine beta-synthase deficiency (10) and C677T MTHFR gene mutation [7]. Hyperhomocysteinemia promotes vascular damage by several mechanisms. Many of the endothelial vascular changes associated with hyperhomocysteinemia can be found in preeclampsia [11-15].

The risk of venous thromboembolism (VTE) associated with acquired and inherited thrombophilias is amplified by other risk factors, for example, the post-surgical state and immobilization. Recent evidence suggests that the risk of maternal VTE in cases with underlying

thrombophilia is substantially increased. The risk of VTE in pregnant women may be further amplified by the type of underlying genetic predisposition, like, homozygosity for a mutation, the presence of multiple mutations (multigenic defects) or thrombophilic anomalies [16-19].

#### *Thrombophilia and adverse pregnancy outcome*

Preeclampsia, abruptio placenta, intrauterine growth restriction (IUGR) and intrauterine fetal death (IUFD) greatly contribute to maternal and fetal morbidity and mortality. Their causes are unknown, but all of them may be associated with abnormal placental vasculature and disturbances of hemostasis leading to inadequate maternal-fetal circulation [11-15,20,21].

The etiology of preeclampsia is unknown. It is associated with abnormal placental development and disturbances of hemostasis leading to inadequate fetomaternal circulation. Recent data suggest that endothelial dysfunction, vasoconstriction, placental ischemia and enhanced coagulation are associated with abnormal placental development which may lead to inadequate fetomaternal circulation and decreased placental perfusion [22]. In normal pregnancy the trophoblast invades the spiral arteries which lose their muscular wall and become flaccid allowing maximum blood flow to the placenta. The abnormal interaction between mother and fetal allograft in abnormal pregnancies leads to abnormal trophoblastic invasion of the spiral arteries, resulting in small narrowed vessels. The subsequent vasculopathy and secondary thrombosis from hypercoagulability may result in inadequate perfusion of the intervillous space, preeclampsia, placental infarcts, IUGR, placental abruption and IUFD. Placental pathologists use the term placental vasculopathy to describe pathological placental changes characterized by superficial endovascular cytotrophoblast invasion in the spiral arteries, acute atherosis and thrombotic processes in the spiral arteries and/or the intervillous space. Clinically, placental vasculopathy is associated with preeclampsia, IUGR, placental abruption and some cases of fetal loss and preterm labor [22].

The known thrombotic nature of the placental vascular lesions and the increased thrombotic risk associated with the existence of thrombophilias strongly suggest a cause-and-effect relationship between inherited and acquired thrombophilias and the listed severe obstetric complications.

## Placental findings associated with thrombophilias and pregnancy complications

The group from Tel-Aviv, [23] described placental findings in women who had severe complications during pregnancy and were carriers of thrombophilias, and compared them to women with severe complications during pregnancy but who had no thrombophilias [23]. Sixty eight women with singleton pregnancies who had severe preeclampsia, IUGR, abruptio placentae or IUFD comprised the study population. They were evaluated after delivery for the occurrence of mutations of FV Leiden, MTHFR, and prothrombin gene, and deficiencies of protein S, protein C and antithrombin. All were negative for antiphospholipid antibodies. Thirty two women carried a thrombophilia and 36 women did not. All placentas were evaluated by a single pathologist who was blinded for the results of thrombophilia assessment. There was no difference in the maternal age, parity, type of pregnancy complication and fetoplacental weight ratio between the groups. The number of women with villous infarcts was significantly higher in women with thrombophilias (72% vs. 39%, $p < 0.01$) as was the number of women with multiple infarcts, $p < 0.05$. The incidence of placentas with fibrinoid necrosis of decidual vessels was also significantly higher in women with thrombophilias ($p < 0.05$). However, in a recent study with a very similar design and which also examined the relationship between placental histology and thrombophilia in women with severe complications, no specific histological pattern could be identified when thrombophilia positive and thrombophilia negative groups were compared [24]. Nevertheless, a high rate of placental infracts (50%) and thrombosis was confirmed in both women with and without thrombophilias [24]. Likewise, placental pathology in early onset preeclampsia and FGR was similar in women with and without thrombophilia although a high rate of placental abnormalities was found [25]. Arias et al. [26], evaluated 13 women with thrombotic lesions of the placenta. All women had obstetric complications such as preeclampsia, preterm labor, IUGR or stillbirth. In 10 of 13 women (77%) inherited thrombophilias were found; 7 were heterozygous for the FV Leiden mutation, and 3 had protein S deficiency. The most prominent placental lesions were fetal stem vessel thrombosis, infarcts, hypoplasia, spiral artery thrombosis and perivillous fibrin deposition.

The high rate of placental lesions found in both thrombophilic women and non thrombophilic women with severe complications in these studies may explain the difficulty to find a difference between thrombophilic and non thrombophilic women, or may reflect an as yet unknown state of thrombophilia. It should be noted however, that all these reports evaluated mixed clinical conditions and not just severe preeclampsia.

## Aquired thrombophilia

#### *The antiphospholipid syndrome and pregnancy complications*

The antiphospholipid syndrome (APS), an acquired autoimmune condition, is characterized by the presence of certain features and circulating antibodies. It is defined

as the presence of lupus anticoagulant (LAC) and/or anticardiolipin antibodies (aCL) with recurrent miscarriage (RM), thrombosis, preeclampsia, IUGR and placental abruption. The most specific clinical features are thrombosis (both venous and arterial thrombosis), RM and fetal loss in the second and third trimester and autoimmune thrombocytopenia [27-29].

The APS is associated with placental vascular thrombosis, decidual vasculopathy, intervillous fibrin deposition, and placental infarction [21,30]. These pathological changes in the placenta may result in miscarriage, IUGR, stillbirth, and early severe preeclampsia. In relation to fetal loss, positive test for aCL or presence of LAC may be found in up to 20% of women with recurrent pregnancy loss [31-36]. It may present with either recurrent embryonic loss [37], or fetal demise beyond 10 weeks of gestation [38] and is found in 10–15% of women with fetal death beyond 20 weeks of gestation [39,40]. The relation between APS and preeclampsia has been shown in several studies [40-46]. In a series of more than 300 patients with severe preeclampsia, reported by the Amsterdam group [44], an overall incidence of 21% was found related to detectable ACA (> 10 GPL and/or MPL), with a 27.4% incidence in the group with delivery at less than 28 weeks' gestation, and a 19.3% incidence in the group with delivery more than 28 weeks' gestation. However, after considering the nearly 20% low positive IgG and/or IgM titers (< 15 GPL and/or MPL) in their control population of healthy female volunteers, the authors concluded [43] that 16% is a realistic estimate of the incidence of aCL positive patients in patients with a history of severe preeclampsia, which is concordant with other studies [42,45,47]. It should be noted that several investigators found no correlation between APS and preeclampsia [48-50]. Since in early-onset severe preeclampsia most studies found an association with positive tests for aCL, testing in these patients may have therapeutic implications for future pregnancies. Women with APS are also at a substantial risk for IUGR [40,51,53] which is around 30%. In one study, 24% of mothers delivered of IUGR infants had medium or high positive tests for aCL [54].

### Inherited thrombophilias and pregnancy complications severe preeclampsia and inherited thrombophilia

Dekker et al. [43], tested women with severe preeclampsia at least 10 weeks post partum for the presence of inherited and acquired thrombophilias. A high rate of protein S deficiency, APCR, hyperhomocysteinemia and aCL IgG or IgM was found. Dizon-Townson et al. [55] and Nagy et al. [56] described a higher prevalence of FV Leiden mutation in women with severe preeclampsia compared to controls. In the study of Dizon-Townson et al [55], 158 nulliparous women with severe preeclampsia at a mean gestational age of 33 weeks were compared to 403 normotensive women. Nagy et al. [56] in Hungary described a high prevalence of the FV Leiden mutation in 69 women with severe preeclampsia compared to 71 healthy controls. Rigo et al. [57] investigated 120 women with severe preeclampsia, (72% nulliparous) and 101 healthy matched for age and parity. 18.3% of preeclamptic women were carriers of the FV Leiden mutation compared to 3% in controls ($p < 0.001$). However, there was no difference in homozygosity for MTHFR. Among FV Leiden positives there was a statistically higher prevalence of HELLP syndrome compared to FV Leiden negatives. Perinatal outcomes were comparable in FV Leiden positive and negative women. Kupferminc et al. [47] conducted a study in order to determine whether obstetric complications are associated with thrombophilias. 110 healthy women who had during pregnancy severe preeclampsia, IUGR (<5th percentile), severe abruptio placentae and stillbirth were enrolled in the study. The control group comprised 110 healthy matched women with normal pregnancies. All 220 patients were tested for all known thrombophilias at least 2 months after delivery. 34 out of 110 patients had severe preeclampsia. All were nulliparous. The FV and MTHFR mutations were significantly higher in women with preeclampsia (26% vs. 6.4% and 20.6% vs. 8.2%, respectively). The prothrombin mutation was not more prevalent in women with preeclampsia. Overall, 52.9% of patients with severe preeclampsia had a genetic thrombophilic mutation compared to 17.3% in the control group. In additional 14.7% of the study group other types of thrombophilia were found. Thus, the total prevalence of thrombophilias in the women with preeclampsia was 64.7% compared to 18% in controls. In women with IUGR, abruptio placentae and stillbirth the thrombophilia rate was 61.4%, 70% and 50%, respectively. The total prevalence of all thrombophilias detected in the 110 women with complications was 65% compared to 18% in controls. Of the 18 multiparous women in this group, 15 had had obstetric complications in a previous pregnancy. In 10 of these 15 multiparous women (67%) thrombophilia was found. This indicates a high rate of recurrence in multiparous women with thrombophilias. van Pampus et al. [44], in Netherlands described 345 women with a history of severe preeclampsia diagnosed before 34 weeks, who were investigated postpartum for the presence of thrombophilias. The control group consisted of 67 healthy women with a history of uncomplicated pregnancies.

The women with preeclampsia were further divided into those who had delivered at less than or more than 28 weeks' gestation. In both sub-groups and in all women study patients, a higher prevalence of APCR was found compared to controls, but the prevalence of the FV Leiden mutation was similar to controls. Hyperhomocysteinemia was more prevalent in women with severe disease who

had delivered at less than 28 weeks. Kupferminc et al. [58] in another study tested 63 healthy women with severe preeclampsia (54 nulliparous) and 126 matched controls for all thrombophilias. Again, FV Leiden mutation and MTHFR mutations were significantly more prevalent in women with severe preeclampsia but not the prothrombin mutation. Overall 56% of women with severe preeclampsia had a genetic thrombophilic mutation compared to 19% in the control group and the incidence of all thrombophilias was 67% in severe preeclampsia. Women with severe preeclampsia and thrombophilia delivered earlier and the neonates were of lower birth weight compared to preeclampsia and no thrombophilia (31 weeks vs. 33 weeks). The incidence of combined thrombophilias was also more prevalent in severe preeclampsia. Thrombophilia was found in four of the seven multiparous women with preeclampsia (57%) who had had obstetric complications in a previous pregnancy which indicates high rate of recurrence in these women. Von Tempelhof et al. [59] in Germany examined the FV Leiden mutation, PS, PC, AT III, aCL and LAC in 61 women with severe preeclampsia (44 nulliparous) of whom 32 had HELLP syndrome. The FV Leiden mutation prevalence was higher in both severe preeclampsia and HELLP as were also aCL or LAC. Livingstone et al. [60], in USA tested the genetic thrombophilic mutations in 110 women with severe preeclampsia and 97 controls. Most women were nulliparous and 60% of them were African Americans. No difference was found in the prevalence of thrombophilias between the women with severe preeclampsia and control women groups, or in fetal genetic thrombophilias. Laivuori et al in Finland [61] tested 113 nulliparous women with preeclampsia: 100 with severe disease, 13 with mild disease and 103 controls for the C677T polymorphism of the MTHFR gene. No difference in homozygosity for MTHFR was found between the 2 groups (preeclampsia 3% vs controls 6%). Higgins et al. [62], tested the prothrombin mutation in 13 eclamptic and 74 preeclamptic women with severe disease coming from 34 families of mostly Anglo-Saxon origin. They were compared to 119 controls. The prothrombin mutation was found in only one family of the 34 tested and was similar in cases and controls. Kupferminc et al. [63] tested for the prothrombin mutation 222 patients with: severe preeclampsia (n = 55), mild preeclampsia (n = 25), and other complications and also 156 healthy women. Twenty eight (13%) were heterozygotes of the prothrombin mutation compared to 5 (3.2%) of the controls (p = 0.001). In women with severe preeclampsia the prevalence of the mutation was 9% compared to 3.2% in controls [p = 0.07, OR 2.8 (0.9–9.4)]. Abruptio placentae and IUGR were significantly associated with the mutation. Within the study group, of the 9 multiparous carriers of the mutation, six (66%) had had complications in previous pregnancies compared to 27 out of 112 (24%) multiparous women in the study group without the mutation p= 0.01. Krauss et al., [64] in Germany, detected a higher incidence of APC resistance in 21 women who had HELLP syndrome,6 months to 9 years after completion of pregnancy, compared to normal values obtained from 70 healthy non pregnant females.

### Mild or unknown type of preeclampsia and inherited thrombophilia

Grandone et al. [65] in Italy, compared women with Gestational hypertension (GH) to 129 healthy controls with similar ethnic background. Forty five had preeclampsia and 51 had non proteinuric hypertension. Seventy six percent of women were nulliparous. The prevalence of FV Leiden mutation and C677T MTHFR mutations were significantly higher in women with preeclampsia. In a later study, Grandone et al. [66] investigated the prevalence of prothrombin mutation and FV Leiden mutation in 140 women (62% nulliparous) with gestational hypertension with (n = 70) or without proteinuria (n = 70) and 216 controls. The FV and prothrombin mutations prevalence were higher in women with hypertension compared to controls while MTHFR mutation prevalence was similar in preeclamptic and controls. In the 70 women with preeclampsia the prothrombin and MTHFR mutations were more prevalent compared to the controls but not the FV Leiden mutation. Among non-proteinuric patients a significant association with FV Leiden mutation was found. O'Shaughnessy et al., [67] compared in a prospective study 283 women (230 primigravidas) with preeclampsia to 100 age-matched normal women and to another 100 normotensive women. There was no difference in the frequency of FV Leiden mutation or MTHFR mutations compared to the control group. A significant increase in homozygosity for the C677T MTHFR mutation in preeclampsia was reported by Shoda et al. in Japan [68] who tested 67 women and 98 pregnant controls Lindoff et al. [69], in Sweden compared 50 women with previous preeclampsia to 50 controls and found that the APC ratios were significantly lower in women with preeclampsia but the difference in FV was not significant. Mello et al. [70], in Italy, investigated the prevalence of thrombophilias in 46 nulliparous women with preeclampsia and in women with a history of fetal loss in the second and third trimester. The frequency of APCR and FV Leiden mutation was significantly higher in women with preeclampsia and in the fetal loss group compared to the control group. Lindqvist et al. [71], in a retrospective study investigated the role of APCR and FV Leiden mutation in 2,480 women enrolled in early pregnancy. The overall prevalence of APC resistance was 11% (270/2480). The APC-resistant subgroup (n = 270) did not differ significantly from the non-APC-resistant subgroup (n = 2210) in terms of preeclampsia or IUGR, but was characterized by an 8-fold risk of venous thromboembolism. De Groot et al. [72] in

Netherlands in a retrospective study examined 163 women who had preeclampsia in their first pregnancy and 163 controls matched for age gravidity and date of delivery. The prevalence of FV Leiden mutation and prothrombin mutations was similar in the groups. Morison et al. [73] in Scotland in a retrospective study examined nulliparous women with preeclampsia or gestational hypertension and healthy controls. They tested for genetic thrombophilias and plasminogen activator inhibitor (PAI-1) polymorphism. No difference was found in the prevalence of thrombophilias between women with preeclampsia, gestational hypertension and controls. Murphy et al. [74] screened 584 primigravid subjects for MTHFR and FV Leiden mutation mutations. 2.7% were heterozygous for FV Leiden and 10.6% homozygote for MTHFR mutation. The frequency of these polymorphisms was not higher in those who subsequently developed preeclampsia (n = 12) or IUGR ($10^{th}$ percentile) (n = 9). Kim et al. [75] in a retrospective study in the USA, tested white women with preeclampsia and controls for C677T MTHFR and FV Leiden mutations and mutation of cystathionine beta synthase. There was no difference in the prevalence of these mutations either independently or in combination in women with mild or severe preeclampsia or HELLP syndrome. Among multiparous women with preeclampsia the prevalence of MTHFR was 15.7% compared to 10.9% among nulliparous with preeclampsia. This may indicate that women with this mutation are at high risk for developing recurrent preeclampsia [75]. Powers et al. [76], compared the prevalence of MTHFR mutation between 99 nulliparous women with preeclampsia, 24 women with transient hypertension, 14 controls and their infants. No differences were noted in the mutation prevalence between women with preeclampsia and controls or between infants of preeclamptic mothers and controls. Lachmeijer et al. [77] in Netherlands studied the MTHFR C677T and MTHFR A1298C mutations is association with preeclampsia. One group consisted of 47 consecutive unrelated women with preeclampsia. Another group included 127 unrelated women with preeclampsia from affected sib-pair families and a control group of 120 healthy women. Another 85 women with preeclampsia and known homocysteine status were tested for the relation between the C677T mutation and hyperhomocysteinemia. Both MTHFR mutations were not more frequent in women with preeclampsia. Women with preeclampsia and hyperhomocysteinemia had a higher prevalence of the C677T mutation compared to women with preeclampsia but without hyperhomocysteinemia, but not the A1298C mutation.

Zusterzeel et al. [78] from the Netherlands and Kaiser et al. [79], in Australia also could not find a difference between women with preeclampsia or HELLP syndrome and controls in the prevalence of both these MTHFR mutations, while Hauge et al. [80] found higher prevalence of homozygosity for the A1298C mutation in 64 women with severe preeclampsia and hyperhomocysteinemia.

Several studies described higher levels of homocysteine in preeclampsia. Rajkovic et al. [81], reported that homocysteine levels were doubled in 20 women with preeclampsia compared to 20 healthy controls. Vollset, [82] In the Hodarland homocysteine study which is the largest performed to date, plasma homocysteine levels were evaluated in 5883 women with 14,492 pregnancies. It was shown that when comparing the upper with the lower quartile of plasma homocysteine, the adjusted risk for preeclampsia was 1.32 (95% CI 0.98–1.77), for very low birth weight 2.01 (95% CI 1.23–3.27), and for stillbirth 2.03 (95% CI 0.98–4.21). Cotter et al. [83] found that in 56 women who developed severe preeclampsia homocysteine levels measured at 15.3 weeks were higher compared to those obtained from 112 healthy controls with normal pregnancy.

Inherited hypofibrinolytic mutations are also associated with pregnancy complications. [84] Glueck et al. [85] investigated the hypofibrinolytic 4G/4G mutation of PAI-1 gene as a possible factor contributing to obstetric complications. They compared women who had obstetric complications including 31 with severe preeclampsia to matched control women with normal pregnancies. Women with obstetric complications were more likely than controls to be 4G/4G homozygotes.

#### *Recurrence rate of adverse pregnancy outcome*

Several studies demonstrate a high recurrence rate in women with severe preeclampsia/ HELLP syndrome. [86-88] Several studies albeit small, showed that in multiparous women with thrombophilias and severe pregnancy complications there is high (66–83%) recurrence rate in subsequent pregnancies, while the type of complication may change from one pregnancy to the other; e.g. severe preeclampsia to IUGR [47,58,59,63].

#### *Summary: Preeclampsia and thrombophilia*

The differences between reports may be related to different populations studied, study design and different definitions of preeclampsia. Some studies deal with mild preeclampsia and other with severe disease. Several studies include only primigravidas and other both primigravidas and multiparous. Some include also women with recurrent preeclampsia. The fetus may also play a role: When the fetus has inherited thrombophilia from the mother there may be an accelerated rate of thrombosis in the placenta with ensuing complications compared to a situation with a unaffected fetus. It is also possible that other, as yet undefined genes need to be activated in order

**Table 1: Association of pregnancy complications and thrombophilias**

| | Mild preeclampsia | Severe preeclampsia | IUGR | Placental abruption |
|---|---|---|---|---|
| Antithrombin deficiency | | ++ | ++ | + |
| Protein S deficiency | | ++ | ++ | ++ |
| Protein C deficiency | | ++ | ++ | |
| APC resistance | | ++ | ++ | ++ |
| Factor V Leiden | | ++ | | ++ |
| MTHFR C677T | | | | + |
| Hyperhomocysteinemia | | ++ | ++ | ++ |
| Factor II G 20210A | | + | ++ | ++ |
| Antiphospholipid syndrome | | ++ | ++ | ++ |
| Combined defects | | ++ | ++ | ++ |

Degree of association : + possible association; established association ++

to induce thrombophilic states with clinical significance in preeclampsia.

Since the rate of venous and arterial thrombosis and of placental thrombosis in pregnancy complications is not essentially different between ethnic groups and races, it may be that other thrombophilias as yet unknown play a role. For example, the FV Leiden mutation is highly prevalent among the Caucasian population, the prevalence ranging from 10–15% in Sweden, 4–8% in central Europe, and 2% in the south, and 5% in USA. The mutation is almost non-existent in Asia, Japan, Africa and South America. Preeclampsia is a multigenetic disease, and there are important difference in prognosis and management between late mild preeclampsia and early onset severe disease. Most studies and a recent meta analysis [73] suggest that there is an association between thrombophilias and the development of severe preeclampsia but not in mild preeclampsia [73]. The low-pressure intervillous blood flow in the presence of a maternal hypercoagulable state may trigger fibrin deposition in the placenta and cause placental infarcts which may incite development of early severe disease.

The evidence in the literature summarized also in a recent meta-analysis [73] suggests that severe preeclampsia but not mild preeclampsia is associated with trombophilias. Mainly severe preeclampsia is associated with FV Leiden mutation hyperhomocyteinemia, and deficiencies of protein S C and AT III (Table 1). It is not clear as yet, whether severe preeclampsia is associated with the prothrombin and MTHFR mutations.

## Inherited thrombophilias and IUGR

Only few studies reported on an association between IUGR and thrombophilias. de Vries et al. [89], studied thrombophilias in 62 women with obstetric complications and compared the results to historical data. Thirty one women had abruptio placentae, 18 had IUFD and 13 IUGR. Most prominent in women with IUGR was the high incidence of hyperhomocysteinemia (38%), protein S deficiency (23%) and FV Leiden mutation (12.5%). Kupferminc et al. [47] investigated 110 women with severe preeclampsia, IUGR below <5th percentile, severe abruptio placentae and IUFD > 23 weeks. Forty-four women had IUGR. The mean birth weight was 1387 ± 616 grams and 64% of patients delivered at less than 36 weeks, with a mean gestational age of 33.2 ± 3.9. In 50% of the women with severe IUGR a genetic thrombophilic mutation was found. In additional 11.4% of women with IUGR, other thrombophilias were found. Thus, the total prevalence of thrombophilias in women with IUGR was 61.4%. The total prevalence of all thrombophilias in the 110 women with severe obstetric complications was 65% compared to 18% in control women. The women with obstetric complications had also significantly higher incidence of combined thrombophilias. Kupferminc et al. [63], in another study, tested the prothrombin mutation in patients with severe preeclampsia (n = 55), mild preeclampsia (n = 25), IUGR defined as birtweight below the $10^{th}$ percentile (n = 72), severe abruptio placentae (n = 27), unexplained stillbirth > 23 weeks' gestation (n = 16), second trimester fetal loss, three or more consecutive first trimester losses and 156 healthy control women who had at least one normal pregnancy. Twenty-eight women of the study group (13%) were found to be heterozygous carriers of the 20210 variant of the prothrombin gene compared to five (3.2%) of the control group (p = 0.001, OR 2.9; 95% CI 1.3–6.5). Compared to controls, the prothrombin gene mutation was significantly more prevalent in women with IUGR, abruptio placentae, and second trimester loss but not in women with mild or severe preeclampsia, stillbirth and habitual abortion. The 28 women in the study group, carriers of the prothrombin mutation had 62 pregnancies of which only 7 (11.3%) were normal. Within the study group, of the 9 multiparous

patients carriers of the mutation, six (66%) had had complications in previous pregnancies compared to 27 out of 112 (24%) multiparous women in the study group without the mutation (p= 0.01). This again indicates a high rate of recurrence in women with complications of pregnancy associated with thrombophilias.

Martinelli et al. [90], tested 63 women with history of IUGR defined as birth-weight under the tenth percentile and 93 parous women with uneventful pregnancies for the mutations of FV Leiden and prothrombin gene. In 42 women IUGR was associated with gestational hypertension. Thirteen women were under the fifth percentile and five under the third percentile. The women with IUGR delivered at mean gestational age of 34.6 ± 3.4 weeks and the mean birth weight was 1584 ± 586 grams. 95% were delivered by cesarean section indicating high risk deliveries. Among the women with IUGR 13% had the FV Leiden mutation compared to 2.2% in the controls (OR 6.9, 95%CI 1.4–33.5), and 12% had the prothrombin mutation compared to 2.2% in controls (OR 5.9, 95%CI 1.2–29.4). Six women had combined thrombophilias. In regression analysis model these thrombophilias were again independently associated with IUGR. In a later report from the same group [91], these mutations were tested in neonates < 2500 grams. Three hundred ninety six women with history of recurrent pregnancy losses or gestational hypertension and 359 women with one or more uneventful pregnancies were tested for these mutations. Neonates delivered by mothers with thrombophilic mutations comprised 30% of babies weighting < 1000 g, 18.7% of those ranging from 1001–2499 g and 9.5% among babies weighting ≥ 2500 g. Overall, 27.6% of neonates delivered by of mothers with the mutations weighted less than 2500 g compared to 13.9% neonates from mothers without these mutations (OR 2.4, 95% CI 1.5–3.7). A logistic regression analysis taking into account different diagnoses, parity, twin birth showed again that carrier status – of prothrombotic mutations was significantly associated with a birth-weight < 2500 g (OR 2, 95% CI 1.1–3.6). Overall 22.8% of neonates under the 10$^{th}$ percentile were from mothers with thrombophilic mutations compared to 14.5% of neonates under the 10$^{th}$ percentile from mothers without thrombophilic mutations (OR 1.7, 95% CI 1.1–2.7). Recently, Infante-Rivard C et al. [92] did not find an association between thrombophilic mutations and IUGR < 10th percentile. In this study 493 newborns with IUGR and 472 controls and their parents were tested for thrombophilic mutations. The risk of IUGR was not increased among mothers with thrombophilias. In addition these polymorphisms in the newborns were not associated with an increased risk of IUGR. Several important differences between this study and the previous studies are apparent. In this study, the mean birth weight was 2393 ± 606.2 grams, and 83% of the patients delivered at 36–40 weeks' gestation. In the study of Kupferminc et al. [47] the mean birth weight was 1387 ± 616 grams and the mean gestational week was 33.2 ± 3.9. Similarly, Martinelli et al. [90], reported a mean gestational week at delivery of 34.6 ± 3.4 and a mean birth weight of 1584 ± 586 grams. In the later study this group reported that neonates from thrombophilic mothers formed 30% of those weighting < 1000 grams [91]. It is known that IUGR is associated with increased morbidity and mortality, both antepartum and postpartum [90]. However, the use of the 10th percentile may include many constitutionally small fetuses which carry none of the clinical risks of growth restricted fetuses [90]. Conversely, the combination of prematurity and IUGR carries a high risk of long-term serious sequelae [90]. It is evident from these differences in birth weights and gestational age that these studies are dealing with different fetal and neonatal populations with different clinical relevance. In the Hodarland homocysteine study [82], plasma homocysteine levels were evaluated in 5883 women with 14,492 pregnancies. It was shown that when comparing the upper with the lower quartile of plasma homocysteine, the adjusted risk for preeclampsia was 1. 32 (95% CI 0.98–1.77), for prematurity 1.38 (95% CI 1.09–1.75), for very low birth weight 2.01 (95% CI 1.23–3.27), and for stillbirth 2.03 (95% CI 0.98–4.21). Placental abruption had no correlation with the homocysteine quartile, but the adjusted OR when homocysteine concentrations >15 μmol/L were compared with lower values was 3.13 (95% CI 1.63–6.03).

Kupferminc et al. [94] tested a very selective group with severe midtrimester IUGR (22–26 weeks' gestation) defined as birth weight <3$^{rd}$ percentile and associated with oligohydramions (n = 26). The frequency of thrombophilias was 69% in the study group compared to 14% in the control group (n = 56) [odds ratio (OR) 4.5; 95% confidence interval (CI) 2.3–9, P < 0.001]. The frequencies of FV Leiden mutation, prothrombin gene mutation and protein S deficiency were significantly increased in the study group compared to the control group. The frequency of multiple thrombophilias was 33% in the study group versus none among the controls. Of the 26 pregnancies with severe mid-trimester IUGR, 13 ended in IUFD before 25 weeks' gestation: 10 of these women had thrombophilia. Table 1 summarizes the association between individual thrombophilias and IUGR.

## Inherited thrombophilias and abruptio placentae

Van der Molen et al. investigated coagulation inhibitors and abnormalities of the homocysteine metabolism as risk factors for placental vasculopathy [95]. They compared non-pregnant women with a history of placental vasculopathy defined as abruption or placental infarction with non-pregnant women matched for age and

occupation. Placental infarction was defined as villous necrosis associated with a stillborn baby or IUGR at less than the 10th percentile. Twenty-two of 101 had also hypertension during pregnancy. Protein C activity was significantly lower. Homozygotes for the MTHFR mutation and carriers of the FV Leiden mutation were significantly more frequent in the study group. The median homocysteine levels, APCR ratio, protein S and AT-III were not different between the groups. However, a significant OR for homocysteine was found above 14.4 micromole which was the 80th percentile of the controls. Also, combination of risk factors such as homocysteine levels above 14.4 micromol and protein S deficiency resulted in a significant increased OR. So that the risk factors for placental vasculopathy which emerge in this study are decreased levels of APCR and protein C, elevated homocysteine and the MTHFR mutation or combinations of these.

Wiener-Megnagi et al. [96], studied 27 women who had abruptio placentae and 29 control subjects matched for age, parity, and ethnic origin. Sixty three % of case patients had an activated protein C ratio ≤ 2.5, compared with 17% of control subjects with an OR of 8.16 (p =.00125). Only participants with APCR ≤ 2.5 underwent DNA analysis. Eight of 15 case patients tested were found to have the FV Leiden mutation (5 heterozygous and 3 homozygous, 29.6%), compared with 1 heterozygote among the control subjects who were tested (3.4%). Goddjin-Wessel et al. [97] found hyperhomocysteinemia in 31% of women with abruption or infarction compared to 9% in controls ($p < 0.05$). Kupferminc et al. [47] found a 70% incidence of thrombophilias in women with abruptio placentae. Sixty percent of these had thrombophilic mutations and 10% had AT-III deficiency or APS. Twenty women had abruptio placentae, among whom three also had mild preeclampsia, seven had antepartum or postpartum hypertension, and eleven of the neonates were below the 10th percentile for gestational age. In this study, which was the first to examine the prothrombin mutation in women with pregnancy complications, the OR for this mutation was 8.9; 95% CI 1.8–43.6, whereas the OR for FV Leiden mutation were 4.9; CI 1.-17.4, and for MTHFR mutation the OR was 2; CI 0.5–8.1. In another study [63], the rate of the prothrombin mutation in 27 women with abruptio placentae was 18.5% compared to 3.2% in controls (OR 5.8; 95% CI 1.8–18.6), P = 0.01. In the study of de Vries et al. [89] abruptio placentae was associated with 26% hyperhomocysteinemia and 29% protein S deficiency. Table 1 summarizes the association between individual thrombophilias and abruptio placentae.

## Inherited thrombophilias and fetal loss

About 15% of all pregnancies will terminate in miscarriage. Recurrent miscarriage (RM) is a condition defined as three consecutive miscarriages and affects 1%-2% of women of reproductive age. Up to 5% have ≥ 2 recurrent losses. These sporadic miscarriages are the commonest complication of pregnancy and are mainly due to chromosomal abnormalities in the fetus.

It is now widely accepted that RM is a heterogeneous condition, with several etiological factors such as prothrombolic states, structural uterine anomalies, chromosomal anomalies, and endocrinological defects. In up to 80%, however, the underlying cause is not apparent and the condition is therefore considered unexplained.

Pregnancy is a hypercoagulable state, and women with thrombophilia are at increased risk for thrombosis during pregnancy and adverse maternal and fetal sequelae [98]. The hemostatic system plays an important role in the success of pregnancy and the process of implantation, and placentation. Implantation of the fertilized egg into the uterine decidua establishes a contact between the fetus, the placenta and the maternal circulation. This contact between placenta and maternal circulation is crucial for the success of pregnancy. Pro-thrombotic changes and thrombosis may interfere with these processes leading to miscarriage. This may explain many cases of previously unexplained RM. In support of the role of thrombophilia in RM are direct and indirect evidences.

**Indirect evidence:** Many women with a history of RM are at greater risk of preeclampsia, IUGR and intra-uterine fetal death, which suggests that these adverse pregnancy outcomes represent a spectrum of disorders which share a common origin [98]. Women with a history of RM are in a prothrombotic state while not pregnant [98-102]. Thrombin antithrombin (TAT) complex levels are significantly higher in women with both first and second trimester fetal losses compared to controls. It has also been reported that women with RM are in a chronic state of endothelial stimulation associated with activation of the coagulation system [98]. Women with a history of RM have an excess of thromboxane production at weeks 4–7 of gestation and between gestational weeks' 8 and 11 they are relatively prostacyclin deficient compared with women with no previous history of pregnancy loss [99]. These changes were greatest among those whose pregnancy ended in miscarriage. The shift in the thromboxane/prostacyclin ratio in favor of the prothrombotic agent thromboxane may lead to vasospasm and platelet aggregation in the trophoblast, causing the development of microthrombi and placental necrosis [98].

**Direct evidence** comes from studies finding higher prevalence of thrombophilias in women with RM. The results vary between studies reflecting the fact that most studies have included small numbers of women, have been prone

**Table 2: Association of fetal loss and thrombophilias**

| | Early recurrent miscarriage (< 12–13 weeks) | Late fetal loss |
|---|---|---|
| Antithrombin deficiency | | |
| Protein S deficiency | ++ | ++ |
| Protein C deficiency | | |
| APC resistance | ++ | |
| Factor V Leiden | ++ | ++ |
| MTHFR C677T | | |
| Hyperhomocysteinemia | ++ | ++ |
| Factor II G 20210A | ++ | ++ |
| Antiphospholipid syndrome | ++ | ++ |
| Combined thrombophilias | ++ | ++ |

Degree of association : established association ++

to selection bias and frequently failed to differentiate between women with recurrent first trimester miscarriage and those with later pregnancy complications.

Prospective studies [98] have demonstrated an increased prevalence of antiphospholipid antibodies (aPL) among women with first trimester RM. Some 15% of women with RM have persistently positive tests for aPL. Several studies have reported that the early fetal loss rate in women with aPL is in the range of 50–70% [100-104]. The majority of miscarriages amongst aPL-positive women occurred in the first trimester of pregnancy after the establishment of fetal heart activity. The outcome of pregnancy in untreated women with a history of RM in association with APS is poor with a live birth rate of about 20% whereas treatment with antithrombotic therapy, such as aspirin and heparin or low molecular weight heparin started early in the first trimester of pregnancy has been shown to significantly improve the live birth rate [98,103,104].

## Prevalence of inherited thrombophilias in first and second trimester miscarriage

Studies reporting the prevalence of FV Leiden mutation among women with RM cite figures ranging from 3 to 42%. Of all inherited thrombophilias many reports find an association between high prevalence of the FV Leiden mutation and RM. Brenner et al [105]. tested women with 3 or more first trimester losses, 2 or more second trimester losses or one or more third trimester loss. The FV Leiden mutation was more frequent in the fetal loss group compared to controls. Thrombophilias were more common in second and third trimester losses but first trimester recurrent abortion was not associated with thrombophilia. The MTHFR or prothrombin mutations were not more common in women with fetal loss. Overall, 49% of women with pregnancy loss had a thrombophilia compared to 22% of controls. Brenner et al. [106], analyzed 39 consecutive women with recurrent fetal loss of unknown cause for activated protein C resistance, FV Leiden mutation, or APCR without FV Leiden mutation. Evaluation of 128 pregnancies in 19 patients with FV Leiden mutation and 56 gestations in nine women with acquired APCr, revealed over 50% first-trimester abortions and 17% late abortions. Ridker et al [107], compared the frequency of FV Leiden mutation in 113 women with recurrent fetal loss to 437 controls. The mutation was found in 8% in women with RM and in 3.7% of controls OR 2.3, 95% CI,1.0–5.2. In a subgroup of patients with 3 or more pregnancy losses and no successful pregnancies, the prevalence of the mutation was 9% OR 2.6, 95% CI, 1–6.7. Younis et al [108]. evaluated APCR and the FV Leiden mutation in women with first trimester recurrent embryonic loss, in women with second trimester loss and controls. The prevalence of APCR and FV Leiden mutation was significantly higher in women with first and second trimester recurrent pregnancy loss compared to the control group. Sarig et al. [109], evaluated 145 patients with RM and 145 matched controls. At least one thrombophilic defect was found in 66% of study group patients compared with 28% in controls. Combined thrombophilic defects were documented in 21% of women with pregnancy loss compared with 5.5% of control patients. Late pregnancy wastage occurred more frequently in women with thrombophilia compared with women without thrombophilia. The FV Leiden mutation was more common in women with pregnancy loss but not factor II G20210A and homozygosity for MTHFR. Tal et al. [110] studied prospectively 125 patients with one or more first or second trimester pregnancy losses and matched controls for the prevalence of APCR. Pregnancy losses were divided into preclinical, first trimester clinical and second trimester abortion. The FV Leiden mutation was found in 14.4% of patients compared with 5. 6% in controls ($P < 0.05$). The rate of preclinical pregnancy losses in Leiden mutation carriers was significantly higher compared to no APCR patients. Finan et al. [111], tested 110 women with

first trimester unexplained losses (≥ 2) and 67 controls. Forty one % were carriers of the FV Leiden mutation compared to 16.4% in controls, (p = 0.002). 13.6% were carriers of the prothrombin mutation compared to 3% in controls (p = 0.001). Foka et al. [112] tested 80 women with recurrent (≥ 2) losses and 100 controls. Sixty one out of the 70 had first trimester losses. The prevalence of the FV Leiden mutation was significantly higher in the women with RM (19% vs. 4%, p = 0.003). The prevalence of the prothrombin mutation was also significantly higher in the study group (9% vs. 2%, p = 0,038). The MTHFR mutation prevalence was similar between the groups. Reznikoff-Etievan et al. [113] investigated 260 women with 2 or more losses before 10 weeks of pregnancy and 240 controls. The FV Leiden mutation and G20210A mutations were found to be associated with early recurrent spontaneous miscarriage before 10 weeks of pregnancy, the OR being 2.4 (95% CI 1–5) and 2.7 (95% CI 1–7), respectively. Similar results were found whether or not women had had a previous live birth. Alonso et al. [114] recently investigated inherited and acquired thrombophilia in women with unexplained abortions ≥ 1 first trimester abortion after exclusion of abnormal karyotype and other causes of abortions, and intrauterine fetal death. Late fetal loss was associated with thrombophilia but not early abortions. Other studies did not find an association between the FV Leiden mutation and RM. Rai et al. [115], investigated 1111 consecutive Caucasian women with a history of either recurrent early miscarriage (three or more consecutive pregnancy losses at <12 weeks gestation; n = 904) or a history of at least one late miscarriage. A control group of 150 parous Caucasian women with no previous history of adverse pregnancy outcome was also studied. Acquired APCR was significantly more common among both women with recurrent early miscarriage (8.8% vs. 3.3%; P = 0.02) and those with late miscarriage (8.7% vs. 3.3%; P = 0.04) compared with controls (3.3%: 5/150). In contrast, the frequency of the FV Leiden mutation allele was similar among women with recurrent early miscarriage (3.3%) those with late miscarriage (3.9%) and the control group (4.0%). Grandone et al. [116] investigated the FV Leiden mutation in 43 women with 2 or more unexplained fetal losses and 118 controls. The mutation was more frequent in women with fetal loss. The FV Leiden mutation was more frequent in women with second trimester loss, but the prevalence of the mutation in women with first trimester loss and controls was similar. Kutteh et al. [117] tested 50 women with recurrent pregnancy loss based on well-documented history. 189 pregnancies were before completion of week 12. The prevalence of the genetic thrombophilias was similar in the study and control group. Dizon-Townson et al. also did not find an association between RM and the FV Leiden mutation [118]. Studies regarding the association of MTHFR and recurrent pregnancy loss are contradicting with some who negate an association between MTHFR and recurrent abortions [105,119], and others who find such association [120,121].

Nelen et al. [122] performed a meta analysis to evaluate the relation between recurrent early pregnancy loss and hyperhomocyteinemia. In the papers included was their own data. Evaluated were fasting or afterload homocysteine concentrations and the MTHFR C677T mutation. Overall, the pooled OR for elevated homocysteine were 2.7 (1.5–5.2), for afterload homocysteine 4.2 (2.0–8.8) and for MTHFR 1.4 (1.0–2.0). These data support hyperhomocysteinemia as a risk factor for recurrent early pregnancy loss. Homozygosity for the MTHFR mutation represents a small increase in women's risk for recurrent pregnancy loss. Some studies do not find an association between the prothrombin mutation and RM [123-125], while other studies do [111,112]. In addition, Pihusch et al. [126] studied 102 patients with two or more consecutive abortions and 128 women without miscarriage. No differences in the prevalence of FV Leiden mutation, MTHFR mutation or glycoprotein IIIa, and beta-fibrinogen polymorphism was found. The prothrombin mutation occurred more often in patients with RM. This effect was significant in 75 women with abortions exclusively in the first trimester (6.7%, vs. 0.8%, P = 0.027, OR 8.5). Sanson et al. [127], investigated women with deficiencies of antithrombin, protein S and protein C. In the 60 deficient subjects 22.3% of the 188 pregnancies resulted in miscarriage or stillbirth as compared to 11.4% of the 202 pregnancies in the 69 non-deficient subjects. The relative risk of abortion and stillbirth per pregnancy for deficient women as compared to non-deficient women was 2.0 (95% C.I. 1.2–3.3). Women with dysfibrinogemia are also candidates for miscarriage and of 64 pregnancies in women with dysfibrinogemia 39% ended by miscarriage [128].

## Late fetal loss and thrombophilia

Preston et al. [129], reported on the relation between heritable thrombophilic defects and fetal loss in a cohort of women with FV Leiden mutation or deficiency of antithrombin, protein C, or protein S. The authors studied 1384 women enrolled in the European Prospective Cohort on Thrombophilia (EPCOT). Of 843 women with thrombophilia 571 had 1524 pregnancies; of 541 control women 395 had 1019 pregnancies. The controls were partners of male members of the EPCOT cohort or acquaintances of cases. They analyzed the frequencies of miscarriage (fetal loss at or before 28 weeks of gestation) and stillbirth (fetal loss after 28 weeks of gestation) jointly and separately. The risk of fetal loss was increased in women with thrombophilia (OR 1.35 [95% CI 1.01–1.82]). The OR was higher for stillbirth than for miscarriage (3.6 [1.4–9.4] vs. 1.27 [0.94–1.71]). The highest OR

for stillbirth was in women with combined defects (14.3 [2.4–86.0]) compared with 5.2 (1.5–18.1), in antithrombin deficiency, 2.3 (0.6–8.3) in protein-C deficiency, 3.3 (1.0–11.3) in protein-S deficiency, and 2.0 (0.5–7.7) with FV Leiden mutation.

The authors concluded that women with familial thrombophilia, especially those with combined defects or antithrombin deficiency, have an increased risk of fetal loss. Gris et al. [130], performed a case-control study in 232 women with a history of one or more second or third trimester losses but no thrombosis who were matched with 464 controls and tested for thrombophilia and APS. They found at least one thrombophilia in 21.1% of the patients and in 3.9% of the controls ($p < 10(-4)$). In women, the crude OR for stillbirth associated with any positive thrombophilia was 5.5, 95% CI [3.4–9.0]. Using conditional logistic regression analysis, 4 adjusted risk factors for stillbirth remained: protein S deficiency, positive anti-beta2 glycoprotein IgG antibodies, positive aCL IgG antibodies and the FV Leiden mutation. The C677T mutation in the MTHFR gene was not an individual risk factor but an homozygous genotype was strongly associated with the former 4 risk factors (16.8% of patients vs. 0.9% of controls). In women with such associations, stillbirths always occurred in absence of folic acid supplementation during pregnancy. Available conclusions of pathological analysis of placentas were found to have a very high proportion of "maternal vascular disease of the placenta" in patients with at least one positive risk marker for thromboembolism, specially in case of association with the C677T MTHFR homozygous genotype, compared to patients with negative markers ($p < 10(-4)$). The conclusion was that late fetal loss, through placenta thrombosis, might sometimes be the consequence of a maternal multifactorial prothrombotic state.

Kupferminc et al. [47] found a 50% prevalence of Thrombophilias in women with IUFD more than 23 weeks. Martinelli et al. [131], in a recent study studied 67 women with fetal loss after the 20 weeks of pregnancy and 232 controls. 16% of the 67 women with fetal loss and 6% of the controls had either the FV Leiden mutation or the prothrombin mutations. The relative risks of late fetal loss in carriers of the FV and prothrombin mutations were 3.2 (95% CI, 1.0–10.9), and 3.3 (95% CI, 1.1–10.3), respectively. Placental pathology found histological evidence of thrombosis in 76% of examined placentas examined. Another study performed by the Tel-Aviv group, investigated women with IUFD at 27 weeks' gestation or more [132]. In 40 women with unexplained IUFD, the prevalence of inherited thrombophilias was 42.5% in the study group compared with 15% in controls (OR 2.8, 95% confidence interval 1.5, 5.3, P =.001).

***Summary: fetal loss and thrombophilias***

A recent meta-analysis [133] included 31 studies regarding thrombophilic disorders and fetal loss. Factor V Leiden was associated with early and late recurrent fetal loss, and late non-recurrent fetal loss. Activated protein C resistance was associated with early recurrent fetal loss, and prothrombin G20210A mutation with early recurrent and late non-recurrent fetal loss. Protein S deficiency was associated with recurrent fetal loss and late non-recurrent fetal loss. Methylenetetrahydrofolate mutation, protein C, and antithrombin deficiencies were not significantly associated with fetal loss [133]. Table 2 summarize the association between fetal loss and thrombophilias.

## Management of fetal loss associated with thrombophilia

Recently, two prospective randomized studies have shown that treatment with heparin plus low dose aspirin results in significantly better gestational outcome that low dose aspirin alone in women with APS syndrome who experienced recurrent pregnancy loss (RPL) [100-104]. In the study by Kutteh et al. [100], viable infants were delivered in only 44% of women receiving aspirin compared to 80% in women receiving also heparin ($p < 0.05$). The study of Rai et al. [104], was a randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with RM associated with antiphospholipid antibodies. The rate of live birth in patients treated with aspirin and heparin was 71% compared to 42% with aspirin alone ($p < 0.01$). Still, 25% of successful pregnancies were delivered prematurely. Treatment consisted of prophylactic heparin (5,000 IU b.i.d.), or LMW heparin, and low-dose aspirin (0.1 g per day). The data for inherited thrombophilias is even more limited and no controlled trials exists. Brenner et al. [134], evaluated 149 women with recurrent first trimester abortion, two or more second trimester abortions or IUFD. There were 254 pregnancies with 67% pregnancy loss. They were evaluated for thrombophilia which was found in 50. Twenty three had a single defect with FV Leiden mutation most common and 27 had combined thrombophilia. These 50 women had only 20% live births. The women with thrombophilia were treated during pregnancy with Enoxaparin; 40 mg/day in women with solitary thrombophilia and 80 mg/day in women with combined thrombophilias in addition to aspirin in women with APS. 45 of 61 (75%) gestations treated by Enoxaparin resulted in live birth compared to only 38/193 (20%) of the untreated pregnancies in these 50 women prior to diagnosis of thrombophilia ($p < 0.00001$). In 23 women without a single living child following 82 untreated gestations, antithrombotic therapy resulted in 26/31 (84%) successful deliveries ($p < 0.0001$). Younis et al. [135], measured activated protein C (APC) resistance and FV Leiden mutation in 56 non-pregnant women, with a history of two or more unexplained

recurrent pregnancy losses. During the same study period, seven women carrying the FV Leiden mutation conceived and were subsequently followed throughout their pregnancy.

Subcutaneous LMW heparin, (Enoxaparin, 40 mg/day) and oral low dose aspirin (100 mg/day) were administered throughout the pregnancies, starting at early first trimester. Five of the seven pregnancies occurring progressed uneventfully to term with normal fetal growth, normal doppler flow studies and uneventful neonatal outcome. Two of the seven women had early missed abortions.

### Management of adverese pregnancy outcome associated with thrombophilia

Although at the moment our knowledge as for the optimal treatment during pregnancy is limited, the data suggest that certain risk groups such as pregnant women with a personal or family history of thromboembolism should be screened for thrombophilia. Testing should be also performed to women with a history of recurrent first trimester loss, second trimester loss, IUFD, severe preeclampsia, IUGR or abruptio placentae. Are women with pregnancy complications and/or placental thrombosis and thrombophilias candidates for anti-thrombotic therapy as certainly are those with venous and arterial thrombosis? There are no controlled trials as to guide us how to manage women with thrombophilia and previous placental thrombosis and/or severe pregnancy complications. However, some data suggest a high recurrence rate of complications in future pregnancies in women who had previously severe pregnancy complications and women with severe complications who are carriers of thrombophilias.

Recently, the Cochrane Collaboration [136] reported a 15% reduction in the risk of preeclampsia (32 trials with 29,331 women; relative risk (RR) 0.85; 95% CI 0.78–0.92) and a 14% reduction in fetal and/or neonatal death (30 trials with 30,093 women; RR 0.86 95% CI 0.75–0.99). This reduction in death was the greatest amongst high-risk women (4134 women; RR 0.73 95% CI 0.56–0.96). The combination of aspirin and heparin or low molecular weight (LMW) heparin is effective in recurrent fetal loss in APS syndrome and could be considered for women with inherited thrombophilias and history of severe preeclampsia, IUGR, abruptio placentae or fetal loss, although no controlled studies on the subject are currently available.

An interest in the potential therapeutic value of heparin to prevent and treat pregnancy complications has existed for many years. One of the larger retrospective cohort studies was performed by North et al. [137] who reported on women with renal disease in pregnancy divided into a control group, patients treated by low-dose aspirin, and women reciving prophylactic heparin combined with aspirin and/or dipyridamole. Preeclampsia was less common in the heparin group compared with the no-treatment group and the aspirin group. Kupferminc et al. [138], reported on the use of LMW heparin in prevention of recurrent adverse pregnancy outcome. Women with a history of severe preeclampsia, abruptio placentae, IUGR or stillbirth and a known thrombophilia (n = 33) were treated with Enoxaparin 40 mg/day and 100 mg aspirin beginning from 8–12 weeks' gestation. The mean gestational age at delivery in the index pregnancies was 32.1 ± 5.0 weeks as compared to 37.6 ± 2.3 weeks' gestation in the ensuing pregnancies treated with LMW heparin ($P < .0001$). The mean birth-weight of the infants in the index pregnancies was 1175 ± 590 g compared to 2719 ± 526 g in the treated pregnancies ($P < .0001$). Pregnancy complications occurred in only three (9.1%) of the women and severe preeclampsia did not occur in the treated pregnancies. There were no perinatal deaths in the treated pregnancies. Ryazi et al. [139] evaluated treatment with LMW heparin combined with aspirin in pregnant women with thrombophilia and a history of early-onset preeclampsia and/or IUGR. Twenty-six patients with thrombophilias had a subsequent pregnancy and were treated with LMW heparin plus aspirin. Their pregnancy outcome was compared with all patients having a subsequent pregnancy without thrombophilias receiving only aspirin (n = 19). There was no difference in the overall birth-weight between the groups. However, when considering the 18 patients with single coagulation abnormalities (i.e. excluding 8 patients with multiple thrombophilias), birth weights were significantly higher ($p = 0.019$) compared to the 19 with no abnormality. In addition, two perinatal deaths occurred in the aspirin group versus no perinatal death in the aspirin plus LMW heparin group. These preliminary studies suggest that LMW heparin may have an additional favorable effect on pregnancy outcome of women with a history of severe preeclampsia and/or IUGR and documented thrombophilia. Large randomized controlled are urgently needed.

### Venous thromboembolism (VTE) in pregnancy

The risk of VTE in pregnancy is approximately six times greater than in non-pregnant women and is a major cause of death among women during pregnancy and the puerperium. Pulmonary embolism occurs in approximately 16 percent of patients with untreated deep vein thrombosis (DVT), and is the most common cause of maternal death [140-146]. The overall risk of DVT in pregnancy (0.05–1.8 percent) [144] is higher in women with a previous history of VTE, with a recurrence rate of about 1 in 71 women [147]. Maternal DVT is more common in the left leg (accounting for about 85 percent of leg thrombosis), occurs more commonly in iliofemoral veins than in calf veins (72 percent compared with 9 percent, respectively),

and is more often associated with pulmonary embolism (PE) [148].

Underlying factors increasing the risk of VTE in pregnant women include obstruction of venous return by the enlarging uterus, venous atonia [149] and the acquired prothrombotic changes that occur in hemostatic factors. The physiologic changes in the hemostatic system include elevation of fibrinogen and FVIII, acquired functional resistance to activated protein C, a decrease in protein S and increases in plasminogen activator inhibitors 1 and 2 that decrease fibrinolysis and platelet activation [140,145,150,151]. Advanced maternal age, obesity, immobilization and the presence of maternal thrombophilias are additional factors. All these contribute to the hypercoagulable state which occurs in a normal pregnancy. The incidence of clinical DVT is estimated at 0.08–1.2 percent following vaginal delivery, rising to 2.2–3.0 percent following cesarean section [152]. Emergency cesarean section is associated with the highest risk, and maternal age and weight are also important predictive factors [153]. A high proportion of both post-partum DVT and pulmonary embolism manifests after discharge from hospital [154], highlighting the need for careful surveillance in the puerperium [155].

If venous thrombosis is suspected during pregnancy, an objective diagnosis must be obtained. With proper precautions, the radiation dose from the necessary investigations is small, and the risk to the fetus is negligible [154]. If VTE is suspected but unconfirmed by test results, treatment should be started and tests repeated within 7 days; therapy is discontinued if findings remain negative [146].

Pulmonary embolism usually occurs during the third trimester or the post-partum period. Diagnosis during pregnancy is difficult as many of the signs and symptoms also occur in healthy pregnant women, and pulmonary emboli often arise from the pelvic veins while the leg veins remain normal [152]. The elevation of D-dimers during pregnancy may be unrelated to VTE [151]. In many cases anticoagulant therapy should be initiated on the basis of clinical awareness and suspicion only, but in view of the potential hazards, an objective diagnosis is mandatory.

### Hereditary thrombophilias and venous thrombosis during pregnancy

The risk of venous thrombosis in women with an inherited or acquired thrombophilia is increased in pregnancy. However, not all women with thrombophilia will develop VTE during pregnancy, suggesting the existence of additional, yet unidentified, environmental factors. The risk of VTE depends on the type of thrombophilia and the existence of additional risk factors.

AT III deficiency is the most thrombogenic of the hereditary thrombophilias, with a 50 percent life chance of thrombosis [155]. The frequency of ATIII deficiency in the general population is 0.02–0.17 percent and is higher in patients with VTE (1.1 percent). The risk of thromboembolism in AT III deficient pregnant women not receiving anticoagulant therapy is about 50 percent [156].

Abnormalities of the protein C and protein S system are present in 0.14-0.5 percent of the general population and 3.2 percent of patients with thrombosis. The risk of thrombosis in pregnancy is 3–10 percent for protein C deficiency and 0–6 percent for protein S deficiency, substantially lower than for antithrombin-deficient women. In postpartum women, the thrombosis risk is 7–19 percent for protein C deficiency and 7–22 percent for protein S deficiency [140,157,158].(While antigenic and functional assays of protein C levels during pregnancy remain unchanged, there is a marked decrease in protein S levels (free protein S). Protein S levels are frequently abnormal in pregnancy, in 25 percent of healthy women in the first trimester, 60 percent in the second and 83–100 percent in the third trimester [150]. Hence, when protein S deficiency may be suspected during pregnancy, reliable assay results are obtainable only in the first months of pregnancy. An alternative approach is to test the parents of the patient.

Activated protein C resistance (APCR) is present in 3–7 percent of healthy Caucasians and in 20–30 percent of patients with thrombosis. APCR has been found in up to 78 percent of women investigated for venous thrombosis in pregnancy [160], whereas the FV Leiden mutation was found in up to 46 percent of cases [143].

Recently, Gerhardt et al. described the prevalence of congenital thrombophilia in 352 women, 119 of whom had a VTE during, or immediately after pregnancy [142]. In women with VTE, the prevalence of FV Leiden mutation was 43.7 percent, compared with 7.7 percent among the age-matched normal women (RR 9.3; 95% CI, 5.1 to 16.9). The prevalence of the prothrombin-gene mutation was 16.9 percent in women with VTE as compared with 1.3 percent in the control group (RR 15.2; 95% CI, 4.2 to 52.6). The prevalence of the combined defects of both FV Leiden and prothrombin-gene mutation was 9.3 percent, as compared with zero in the control group. The presence of both mutations substantially increased the risk, with an OR estimated at 107 for the combination of mutations. Additional risk factors, such as AT III deficiency or protein C and protein S deficiencies were present in 25 percent of women with a history of VTE, as compared with 11 percent of women with no history. In this study it was demonstrated that the prothrombin-gene mutation and FV Leiden mutation are individually associated with an

increased risk of VTE during pregnancy and the puerperium, and that the risk among women with both mutations is disproportionately higher than that among women with only one mutation [142]. A calculation of the positive predictive value for each genetic defect, assuming an underlying rate of VTE of 0.67 per 1000 pregnancies in Western populations gave values of 1:500 for FV Leiden mutation, 1:200 for the prothrombin-gene mutation, and 4.6:100 for the combination of the two defects.

Further insight into the risk of thrombosis in previously symptom-free women with FV Leiden mutation has been provided in a study of 43 women from symptomatic families. Overall, the incidence of pregnancy-associated thrombosis was 14 percent [157], but it appears that the risk may be higher postpartum. McColl and colleagues estimated the risk of VTE in pregnancy to be 1 in 437 for FV Leiden mutation, 1 in 113 for protein C deficiency, 1 in 2·8 for Type I (quantitative) antithrombin deficiency, and 1 in 42 for Type II (qualitative) antithrombin deficiency [160].

APCR can be caused by disorders other than FV Leiden mutation, including antiphospholipid antibody syndrome and other genetic defects in the FV molecule. The resistance can also be acquired during the second and third trimester of a normal pregnancy as a result of increases in FV and factor VIII and decreased protein S levels [1,155,148,157]. The precise mechanism for this "physiological" APCR is still not known, nor its contribution to the higher risk of VTE during pregnancy [1,154,148,158]. The prothrombin mutation is associated with elevated plasma prothrombin levels (factor II activity >130 percent) and is present in about 2–5 percent of healthy individuals. This mutation has been associated with a three-fold increased risk of VTE. A higher risk of thrombosis was found in women using oral contraceptives and in women with obstetric complications.(.(. [4,6,160,162].

Hyperhomocysteinaemia is frequently associated with homozygosity for the thermolabile variant of MTHFR (C677T) [156] and is present in about 8–10 percent of healthy individuals [6]. Pregnancy is associated with decreased concentrations of homocysteine and folic acid supplements will also lower homocysteine concentrations. However, the contribution of homocysteine to VTE in pregnancy remains unclear. Gerhardt et al revealed that homozygosity for the C677T MTHFR mutation was not associated with an increase in risk for VTE during pregnancy [142].

This may be explained by the fact that plasma homocysteine concentrations decrease during pregnancy and most pregnant women take folic acid supplements, which ameliorate hyperhomocysteinemia [143].

Interestingly, the presence of an inherited thrombophilia in a pregnant woman may have positive effects. FV Leiden mutation has been associated with a reduced risk of intrapartum bleeding complications, conferring a possible survival advantage for carriers [161].

## The management of thrombophilia during pregnancy

The management of thrombophilia during pregnancy encompasses primary thromboprophylaxis in asymptomatic women, secondary prophylaxis of recurrences in women who have previously developed thrombosis, and the treatment of acute thrombotic episodes. It is rather difficult to establish guidelines for antithrombotic therapy due to the paucity of relevant and well-controlled trials. Thus, the recommendations regarding prophylactic and therapeutic strategies in pregnancy are largely based on clinical trials in non-pregnant populations [163]. An additional problem in assessing the response to antithrombotic therapy during pregnancy is the danger of imaging procedures. An objective diagnosis of VTE during pregnancy is crucial [163]. The management of women with VTE during pregnancy is summarized in Table 3.

**Table 3: Management of Women with VTE**

| Category | Patients | Recommendation |
|---|---|---|
| Very high risk for VTE | Previous VTE on anticoagulants; VTE in current pregnancy; Antithrombin deficiency | LMW heparin (Enoxaparin) mg/kg Twice day OR heparin adjusted dose with confirmation of pregnancy |
| High risk for VTE | Previuos VTE; Protein C, S deficiency plus family history of VTE;homozygote FV or prothrombin mutation; combined thrombophilia | LMW heparin (Enoxaparin) 40 mg/day until 6–12 weeks postpartum Or fixed dose heparin |
| Moderate risk for VTE | Heterozygote FV or prothrombin mutation, PS deficiency, and family history VTE | Postpartum anticoagulation LMW heparin (Enoxaparin) 40 mg/day |
| Relatively low risk for VTE | Heterozygote FV or prothrombin mutation; no personal or family history VTE | Monitor for additional risks for VTE |

The diagnosis has serious implications not only for the immediate management of the pregnancy, but also for the management of future pregnancies.

Heparin is currently the drug of choice for the prevention and treatment of VTE during pregnancy, though it is gradually being replaced by low-molecular-weight heparins (LMWH). LMWHs exhibit a number of advantages over unfractionated heparin (UFH), including improved bioavailability, a longer half-life, ease of administration, no monitoring requirement and fewer side-effects [163-169]. Animal and human studies have shown that LMWHs are not teratogenic or fetotoxic and do not cross the placenta [170].

Oral anticoagulants (OACs) are rarely employed during pregnancy because of substantial side-effects [140,145,163]. Coumarin derivatives cross the placenta and are associated with embryopathy in 4–5 percent of exposed fetuses, especially during the first trimester [163]. Central nervous system anomalies can occur in any trimester. OACs are reserved for conditions in which the effectiveness of heparin and LMWH may be limited. These include the management of women with artificial heart valves and in cases where heparin therapy is contraindicated, including cases of heparin-induced thrombocytopenia (HIT) or skin allergy. Heparin, LMWH and coumarin derivatives are not secreted in the breast milk and can be given safely to nursing mothers [163].

#### *Primary prophylaxis of thrombosis in asymptomatic women*

In asymptomatic women with known protein C deficiency, protein S deficiency, FV Leiden or prothrombin mutation, who have never experienced VTE, we recommend either clinical surveillance or prophylactic therapy during the last weeks of pregnancy and 2–6 weeks in the puerperium. Subcutaneous heparin, 5000 IU twice daily or a LMWH (enoxaparin, 40 mg once daily, or dalteparin, 5000 IU daily) is used. This thromboprophylactic treatment is firmly indicated in women undergoing cesarean section. In women with AT III deficiency, the risk may be substantially greater and prophylactic therapy is probably indicated throughout the pregnancy. Recently, Martinelli et al. [170], investigated the management of women during pregnancy who are homozygote for the FV Leiden mutation or who had both mutations of the FV Leiden and the prothrombin gene mutations. Most cases of thrombosis occurred in the post-partum. Compared to controls the OR for risk for thrombosis were 41.3 and 9.2, respectively. Therefore, they recommended that in these women prophylaxis should be given in the postpartum while in homozygote women for the FV Leiden mutation prophylaxis throughout pregnancy and post-partum should be considered [169]. Clinical surveillance is usually reserved for women who are allergic to heparin, refuse to use heparin or LMWH, or who have experienced a previous VTE in association with a transient risk factor [163]. The effectiveness of a surveillance approach is dependent on the early detection and treatment of VTE disease, especially before the development of pulmonary embolism. Compression stockings, which are effective in women with recurrent DVT, may be useful in pregnancy and should be recommended to women with previous DVT or varicose veins [171].

#### *Secondary prophylaxis in women with previous thrombosis*

All patients with a personal or family history of VTE should be considered for antenatal prophylaxis and be screened for a thrombophilia. The two general approaches recommended for pregnant women with previous VTE are active prophylactic therapy with heparin or LMWH and clinical surveillance [143,163]. Thromboprophylaxis is firmly indicated for women who exhibit additional risk factors such as hyperemesis, obesity, immobilization or surgery, and particularly if they have pre-eclampsia or concurrent medical conditions associated with thrombosis, such as nephrotic syndrome, inflammatory bowel disease, or infection.

Women with thrombophilia and a history of previous VTE should receive thromboprophylaxis during pregnancy and the puerperium [172]. Symptom-free carriers of thrombophilia require special consideration. The risk of thrombosis varies with the type of thrombophilia and currently no controlled guidelines are available. Some investigators find it useful to risk stratify patients as low risk or high risk to help guide clinical evaluation. Asymptomatic patients at high risk of VTE may be defined as those with ATIII deficiency, more than one thrombophilic anomaly or a homozygote mutation, and those with first degree relatives who have experienced severe VTE. All other patients are considered low risk.

#### *The treatment of an acute thrombotic episode*

Acute DVT during pregnancy in women with or without thrombophilia is usually treated with full dose intravenous heparin for 5–10 days, followed by maintenance subcutaneous heparin given twice daily, adjusted to prolong the activated partial thromboplastin time (aPTT) into the therapeutic range [163].

Changes in the metabolism and clearance of heparin during pregnancy complicate dosing and equivalent doses of subcutaneous heparin produce lower plasma concentrations in pregnant women than in non-pregnant women [173]. It is not clear whether the dose of heparin should be adjusted with increasing weight [171]. Although some centers advocate weight-adjusted doses, others recommend frequent aPTT testing or measurements of anti-fac-

**Table 4: Treatment of VTE during Pregnancy – Recommended LMWH Doses from Controlled Studies in Medical Patients [175]**

| LMWH | Commercial name | Recommended Dose |
|---|---|---|
| Dalteparin | Fragmin | 200 anti-factor Xa units/kg once daily |
| Enoxaparin | Clexane, Lovenox | 1.0 mg/kg twice daily or 1.5 mg/kg once daily |
| Nadroparin | Fraxiparin | 200 anti-factor Xa units/kg once daily |
| Tinzaparin | Innohep | 175 anti-factor Xa units/kg once daily |

tor Xa taken four hours after injection as a guide to reaching the desired level of 0.5–1.2 U/ml [163]. Heparin is given until term, discontinued shortly before delivery, and restarted in conjunction with warfarin post-partum. Heparin is subsequently discontinued when the international normalized ratio (INR) is 2–3. The optimal duration of post-partum warfarin therapy in women with thrombophilia who have developed DVT during pregnancy is currently undefined, and no guidelines are available based on clinical trials. The duration of therapy depends on the magnitude of risk of a recurrent VTE. Individual risk assessment should be performed, taking into consideration the type of thrombophilia, the presence of multigenic defects, the extent and site of thrombosis, the time-frame of occurrence, the history of VTE and the family history. Low risk mothers are given prophylactic therapy for a further 4–6 weeks, and patients with high risk should receive extended thromboprophylaxis. Patients with APS or AT III deficiency who have experienced a VTE require indefinite treatment because of the high risk of recurrence. LMWHs continue to replace UFH in the management of acute VTE. The recommended doses for the treatment of VTE are shown in Table 4[175]. LMWH should be injected subcutaneously into the abdominal wall, and in later months of pregnancy, the anterior aspect of the thigh. These simple, once daily, treatment regimens offer the potential for home or outpatient treatment of DVT, with obvious resource implications, and are feasible in patients with proximal deep vein thrombosis who do not require hospitalization. After therapeutic administration of LMWH for 10–14 days, a prophylactic dose of LMWH should be continued throughout the pregnancy and then replaced with heparin for 6–8 weeks post-partum as described for UFH [140,164,168]. Published studies indicate that the effective maintenance dose of enoxaparin is 40 mg once daily and of dalteparin, 5000 IU once daily [163-167,175,176]. However, in certain thrombophilic conditions these doses should be increased depending on the risk of recurrent VTE. It has been suggested that levels of enoxaparin, given at 40 mg once daily, are not affected by gestational age [165], but others report that because of increased renal clearance during pregnancy once daily dose may not be enough [178]. Monitoring of LMWH levels is not required, but some investigators advocate measurements of peak anti-factor Xa concentrations 3–6 hours after the last injection, with the goal of achieving target plasma levels of 0.4–0.6 IU per ml [179]. The dose of LMWH should be reduced during delivery, and the timing of administration adjusted to allow epidural or spinal anesthesia. Epidural anesthesia may be managed by omitting the last dose of LMWH or by delaying placement of the epidural catheter for 6–12 hours [157]. LMWH can be restarted 2 hours after catheter removal [140]. Warfarin should be used post-partum, particularly to avoid the risk of osteoporosis associated with prolonged LMWH administration [179].

A recent systematic review of all published clinical reports employing LMWH in at-risk pregnancies, and data from an international interest group, reported the incidence of recurrent VTE as 3 in 486 pregnancies [164]. No congenital malformations in the newborns were observed. Adverse effects were generally uncommon, and the most frequently described were minor hemorrhagic complications [164].

The risk of osteoporosis is lower in pregnant women treated with LMWH than with UFH [166]. Insertion of a vena caval filter may be indicated in pregnant women. Patients with pulmonary embolism despite adequate antithrombotic therapy, free-floating iliofemoral DVT, iliofemoral DVT in the weeks prior to delivery, or severe femoropopliteal DVT may benefit from filter insertion. The clinical impression is that filters prevent pulmonary embolism, but some patients develop significant leg swelling despite adequate maintenance with low-dose subcutaneous heparin. There does not appear to be any fetal morbidity or mortality associated with filter insertion [180].

## The anti-phospholipid syndrome

The clinical manifestations of APS include DVT and PE, coronary or peripheral artery thrombosis, cerebrovascular or retinal vessel thrombosis and pregnancy morbidity [181,182]. Women with no history of thrombosis or prior fetal losses who are found to have APS during a first pregnancy do not need prophylactic therapy.

Women with APS who have had prior thromboses, whether or not associated with pregnancy, must receive thromboprophylactic therapy throughout pregnancy and the post-partum period. The recommended therapy is either heparin (5,000 IU b.i.d.) or LMWH and low-dose aspirin (0.1 g per day) [183]. The dose of LMWH depends on the risk of thrombosis. In "high-risk" patients we usually recommend LMWH at therapeutic doses, such as those used for the treatment of DVT (enoxaparin, 1 mg per kg b.i.d). Postpartum, LMWH is replaced by warfarin.

The treatment of an acute venous thrombosis in an APS carrier is similar to that indicated for women with other types of thrombophilia (described above). However, in our opinion there are differences in the maintenance doses and duration of prophylactic therapy required. In women with APS, we tend to administer higher doses of LMWH depending on the severity of the thrombotic event, the history of previous thromboembolic events and the antibody titer. LMWH or UFH is given until term and discontinued shortly before delivery. After delivery, the heparin therapy is reinstated with concomitant OAC, which are then administered for an extended period of time. In women with APS it is usual to recommend low dose aspirin (0.1 g per day) in addition for the prevention of arterial thrombosis (cerebral events). For women with APS who present with recurrent arterial or venous thrombotic events during pregnancy, high dose aspirin (0.3–0.5 g per day) and full therapeutic doses of UFH or LMWH are recommended.

## Arterial thrombosis during pregnancy

Stroke carries a high mortality and morbidity, and is a severe complication during pregnancy and the puerperium. Among 50 million deliveries in the USA, 17.7 cases of stroke and 11.4 cases of intracranial venous thrombosis occurred per 100,000 deliveries [184]. Stroke was strongly associated with pregnancy-related hypertension and eclampsia [185] and its incidence was increased after delivery (relative risk, 8.7) but not during pregnancy itself [186]. Intracranial venous thrombosis has been associated with maternal age [184]. The association of thrombotic cerebral events and inherited thrombophilia is still controversial. Several studies have reported that patients with prothrombin or FV Leiden mutations are at higher risk of developing cerebral vein thrombosis [151,154,187]. However, other studies have failed to show a significant link between thrombophilia and the occurrence of cerebral venous or arterial thrombosis [1,145,146,152,154-156,188] No studies have described the association between thrombophilia and cerebral ischemic events during pregnancy.

We have recently investigated twelve previously healthy pregnant women who had transient cerebral ischemic event during pregnancy [189]. The incidence of inherited thrombophilia was significantly higher in the 12 women with neurological symptoms (83 percent), compared with healthy pregnant women matched for age, ethnicity and smoking habits (17 percent). Hence it is possible that pregnancy and the puerperium may precipitate arterial thrombotic events in patients with inherited thrombophilia, similar to the effects of smoking and the use of oral contraceptives. These agents were found to substantially increase the relative risk of myocardial infarction and cerebral vein thrombosis in patients with the factor II and FV mutations [190]. We suggest that women with transient neurological events appearing during pregnancy should be investigated for inherited thrombophilia.

Women who develop stroke or transient focal neurologic deficits during pregnancy should be treated for a prolonged period with aspirin, 0.3–0.5 g per day. Women with APS should be treated with a combination of aspirin and heparin or LMWH [183]. No guidelines are available for the women with other types of thrombophilia and the decision whether to add heparin or LMWH should be made individually on the basis of the type of thrombophilia, additional known risk factors, recurrence of thrombotic events and their severity.

## References


1. Dahlback B: **Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene.** *Haemostasis* 1994, **24:**139-151.
2. Bertina RM, Koeleman RPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH: **Mutation in blood coagulation factor V associated with resistance to activated protein C.** *Nature* 1994, **369:**64-67.
3. Svensson PJ, Dahlback B: **Resistance to activated protein C as a basis for venous thrombosis.** *N Engl J Med* 1994, **330:**517-552.
4. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM: **A genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis.** *Blood* 1996, **88:**3698-3703.
5. Maertinelli I, Sagghi E, Landi G, Taioli E, Duca F, Mannucci PM: **High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in the user of oral contraceptives.** *N Engl J Med* 1988, **338:**1793-1797.
6. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP *et al.*: **A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase.** *Nat Genet* 1995, **10:**111-113.
7. Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van OostBA, den Heijer M, Trijbels FJ, Rozen R, Blom HJ: **Molecular genetic analysis in mild hyperhomocystinemia: a common mutation in the methylene-tetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease.** *Am J Hum Genet* 1996, **58:**35-41.
8. McCully KS: **Homocysteine and vascular disease.** *Nat Med* 1996, **2:**386-389.
9. Mayer EL, Jacobsen DW, Robinson K: **Homocysteine and coronary atherosclerosis.** *J Am Coll Cardiol* 1996, **27:**517-527.
10. Sebastio G, Sperandeo MP, Panico M, de Franchis R, Kraus JP, Andria G: **The molecular basis of homocysteinuria due to cystathionie beta-synthase deficiency in Italian families, and report for four novel mutations.** *Am J Hum Genet* 1995, **56:**1324-1333.
11. Roberts JM, Taylor RN, Musici TJ, Rodgers GM, Hubel CA, McLaughlin MK: **Preeclampsia an endothelial cell disorder.** *Am J Obstet Gynecol* 1989, **161:**1200-1204.

12. Salafia CM, Pezzulo JC, Lopez-Zeno JA, Minior VK, Vintzileos AM: **Placental pathologic features of preterm preeclampsia.** *Am J Obstet Gynecol* 1995, **173:**1079-1105.
13. Shanklin DR, Sibai BM: **Ultrastructural aspects of preeclampsia.** *Am J Obstet Gynecol* 1989, **161:**735-741.
14. Khong TY, Pearce JM, Robertson WB: **Acute atherosis in preeclampsia: maternal determination and fetal outcome in the presence of the lesion.** *Am J Obstet Gynecol* 1987, **157:**360-363.
15. Salafia CM, Minior VK, Pezzulo JC, Popek EJ, Rosenkrantz TS, Vintzileos AM: **Intrauterine growth restriction in infants of less than thirty-two weeks' gestation: associated placental pathologic features.** *Am J Obstet Gynecol* 1995, **173:**1049-1057.
16. Greer IA: **Thrombosis in Pregnancy: Maternal and Fetal Issues.** *Lancet* 1999, **353:**1258-1265.
17. Seligsohn U, Zivelin A: **Thrombophilia As a Multigenic Disorder.** *Thromb Haemost* 1997, **78:**297-301.
18. Gerhardt A, Scharf RE, Beckmann MW, Struve S, Bender HG, Pillny M, Sandmann W, Zotz RB: **Prothrombin and Factor V mutations in women with a history of thrombosis during pregnancy and the puerperium.** *N Engl J Med* 2000, **342:**374-380.
19. Greer IA: **The Challenge of Thrombophilia in Maternal-Fetal Medicine.** *N Engl J Med* 2000, **342:**424-425.
20. Green JR: **Placenta previa and abruptio placentae.** In: *Maternal Fetal Medicine: Principles and Practice* Edited by: *Creasy RK, Resnik R. Philadelphia: W.B. Saunders*; 1994:609-610.
21. Infante-Rievard C, David M, Gauthier R, Ribard GE: **Lupus anticoagulants, anticardiolipin antibodies and fetal loss.** *N Engl J Med* 1991, **325:**1063-1066.
22. Dekker GA, Sibai BM: **Etiology and pathophysiology of preeclampsia Current concepts. AJOG Review.** *Am J Obstet Gynecol* 1998, **179:**1359-1375.
23. Many A, Schreiber L, Rosner S, Lessing JB, Eldor A, Kupferminc MJ: **Pathologic features of the placenta in women with severe pregnancy complications and thrombophilia.** *Obstet Gynecol* 2001, **98:**1041-1044.
24. Mousa HA, Alfirevic Z: **Do placental lesions reflect thrombophilia state in women with adverse pregnancy outcome?** *Hum Reprod* 2000, **15:**1830-1833.
25. Sikkema JM, Franx A, Bruinse HW, van der Wijk NG, de Valk HW, Nikkels PG: **Placental pathology in early onset pre-eclampsia and intra-uterinegrowth restriction in women with and without thrombophilia.** *Placenta* 2002, **23:**337-342.
26. Arias F, Romero R, Joist H, Kraus FT: **Thrombophilia: a mechanism of disease in women with adverse pregnancy outcome and thrombotic lesions in the placenta.** *J Matern Fetal Med* 1998, **7:**277-286.
27. Alarcon-Segovia D, Delete M, Oria CV, Sanchez-Guerrero J, Gomet-Pacheco L, Cabiedes I, Fernandez L, Ponce de Leon S: **Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus: a prospective analysis of 500 consecutive patients.** *Medicine* 1989, **68:**353-365.
28. Asherson RA, Khamashta MA, Ordi-Ros J, Derksen RHWM, Machin SJ, Barquinero J, Outt HH, Harris EN, Vilardell-Torres M, Hughes GR: **The "primary" anti-phospholipid syndrome: major clinical and serological features.** *Medicine* 1989, **68:**366-374.
29. Harris EN: **Syndrome of the black swan.** *Br J Rheumatol* 1987, **26:**324-326.
30. Rand JH, Wu XX, Andree HA, Lockwood CJ, Guller S, Scher J, Harpel PC: **Pregnancy loss in the antiphospholid-antibody syndrome – a possible thrombogenic mechanism.** *N Engl J Med* 1997, **337:**154-160.
31. Branch DW, Silver RM, Pierangeli S, van Leeuwen I, Harris EN: **Antiphospholipid antibodies other than lupus anticoagulant and anticardiolipin antibodies in women with recurrent pregnancy loss, fertile controls, and anliphospholipid syndrome.** *Obstet Gynecol* 1997, **89:**549-555.
32. MacLean MA, Cumming GP, McCall F, Walker ID, Wallter JJ: **The prevalence of lupus anticoagulant and anticardiolipin antibodies in women with a history of first trimester miscarriages.** *BJOG* 1994, **101:**103-110.
33. Out HJ, Bruinse HW, Christiaens GCML, van Vliet M, Meilof IF, de Groot PG, Smeenk RJ, Derksen RH: **Prevalence of antiphospholipid antibodies in patients with fetal loss.** *Ann Rheum Dis* 1991, **50:**553-557.
34. Parazzini F, Acaia B, Faden D, Lovotti M, Marelli G, Cortelazzo S: **Antiphospholipid antibodies and recurrent abortion.** *Obstet Gynecol* 1991, **77:**854-858.
35. Petri M, Golbus M, Anderson R, Whiting-O'Keefe Q, Corash L, Hellmann D: **Antinuclear antibody, lupus antiroagulant, and anticardiolipin antibody in women with idiopathic habitual abortion. A controlled prospective study of forty-four women.** *Arthritis Rheum* 1987, **30:**601-606.
36. Yetman DL, Kutteh WH: **Antiphospholipid antibody panels and recurrent pregnancy loss: prevalence of anticardiolipin antibodies compared with other antiphospholipid antibodies.** *Fertil Steril* 1996, **66:**540-546.
37. Rai RS, Clifford K, Cohen H, Regan L: **High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies.** *Hum Reprod* 1995, **10:**3301-3304.
38. Oshiro BT, Silver RM, Scott JR, Yu H, Branch DW: **Antiphospholipid antibodies and fetal death.** *Obstet Gynecol* 1996, **87:**489-493.
39. Bocciolone L, Meroni P, Parazrini F, Tincani A, Radici E, Tarantini M, Rossi E, Bianchi C, Mezzanotte C, D'Angelo A: **Antiphospholipid antibodies and risk of intrauterine late fetal death.** *Acta Obstet Gynecol Scand* 1994, **73:**389-392.
40. Branch DW, Silver RM, Blackwell L, Reading JC, Scott JR: **Outcome of treated pregnancies in women with antiphospholipid syndrome: an update of the Utah experience.** *Obstet Gynecol* 1992, **80:**614-620.
41. Scott RAH: **Anti-cardiolipin antibodies and pre-eclampsia.** *BJOG* 1987, **94:**604-605.
42. Branch DW, Andres R, Digre KB, Rote NS, Scott JR: **The association of antiphospholipid antibodies with severe preeclampsia.** *Obstet Gynecol* 1989, **73:**541-545.
43. Dekker GA, de Vries JIP, Doelitzsch PM, Huijgens PC, Blomberg von BME, Jakobs C, Geijn van HP: **Underlying disorders associated with severe early-onset preeclampsia.** *Am J Obstet Gynecol* 1995, **173:**1042-1048.
44. Pampus van MG, van MP, Dekker GA, Wolf HW, Huijgens PC, Koopman MMW, Blomberg BME, von Büller HR: **High prevalence of hemostatic abnormalities in women with a history of severe preeclampsia.** *Am J Obstet Gynecol* 1999, **180:**1146-1150.
45. Pattison NS, Chamley LW, McKay W, Liggins GC, Butler WS: **Antiphospholipid antibodies in pregnancy: prevalence and clinical associations.** *BJOG* 1993, **100:**909-913.
46. Yasuda M, Takakuwa K, Tokunaga A, Tanaka K: **Prospective studies of the association between anticardiolipin antibody and outcome of pregnancy.** *Obstet Gynecol* 1995, **86:**555-559.
47. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB: **Increased frequency of the genetic thrombophilia in women with complications of pregnancy.** *N Eng J Med* 1999, **340:**9-13.
48. Rajah SB, Moodley J, Pudifin D, Duursma J: **Anticardiolipin antibodies in hypertensive emergencies. Clin Exp Hypertens B.** *Hypertension in Pregnancy* 1990, **B9:**267-271.
49. Out HJ, Bruinse HW, Christiaens GCML, Van Vliet M, De Groot PG, Nieuwenhuis HK, Derksen RHWM: **A prospective, controlled multicenter study on the obstetric risks of pregnant women with antiphospholipid antibodies.** *Am J Obstet Gynecol* 1992, **167:**20-26.
50. Lynch A, Marlar R, Murphy J, Davila G, Santos M, Rutledge J, Emlen Wl: **Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study.** *Ann Intern Med* 1994, **120:**470-475.
51. Branch DW, Scott JR, Kochenour NK, Hershgold E: **Obstetric complications associated with lupus anticoagulant.** *N Engl J Med* 1985, **313:**1322-1326.
52. Caruso A, De Carolis S, Ferrazzani S, Valesini G, Caforio L, Manusco S: **Pregnancy outcome in relation to uterine artery now velocity waveforms and clinical characteristics in women with antiphospholipid syndrome.** *Obstet Gynecol* 1993, **82:**970-976.
53. Lima F, Khamashta MA, Buchanan NMM, Kerslake S, Hunt BI, Hughes GRV: **A study of sixty pregnancies in patients with the antiphospholipid syndrome.** *Clin Exp Rheumatol* 1996, **14:**131-136.
54. Polzin WJ, Kopelman JN, Robinson RD, Read JA, Brady K: **The association of antiphospholipid antibodies with pregnancy com-**

**plicated by fetal growth restriction.** *Obstet Gynecol* 1991, **78:**1108-1111.
55. Dizon-Townson DS, Nelson LM, Easton K, Ward K: **The factor V Leiden mutation may predispose women to severe preeclampsia.** *Am J Obstet Gynecol* 1996, **175:**902-5.
56. Nagy B, Tóth T, Rigó JJR, Karádi I, Romics L, Papp Z: **Detection of factor v Leiden in severe pre-eclamptic Hungarian women.** *Clin genet* 1998, **53:**478-481.
57. Rigo J Jr, Nagy B, Fintor L, Tanyi J, Beke A, Karadi I, Papp Z: **Maternal and neonatal outcome of preeclamptic pregnancies: the potential roles of factor V Leiden mutation and 5,10 methylenetetrahydrofolate reductase.** *Hypertens Pregnancy* 2000, **19:**163-172.
58. Kupferminc MJ, Fait G, Many A, Gordon D, Eldor A, Lessing JB: **Severe preeclampsia and high frequency of genetic thrombophilic mutations.** *Obstet Gynecol* 2000, **96:**45-49.
59. von Tempelhoff GF, Heilmann L, Spanuth E, Kunzmann E, Hommel G: **Incidence of the factor V Leiden mutation, coagulation inhibitor deficiency, and elevated antiphospholipid antibodies in patients with pre-eclampsia or HELLP-syndrome.** *Thromb Res* 2000, **100:**363-365.
60. Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM: **Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia.** *Am J Obstet Gynecol* 2001, **185:**153-157.
61. Laivuori H, Kaaja R, Ylikorkala O, Hiltunen T, Kontula K: **677$\tilde{C}$T polymorphism of the MTHFR gene and pre-eclampsia.** *Obstet Gynecol* 2000, **96:**277-280.
62. Higgins JR, Kaiser T, Moses EK, North R, Brennecke SP: **Prothrombin G20210A mutation: is it associated with pre-eclampsia?** *Gynecol Obstet Invest* 2000, **50:**254-256.
63. Kupferminc MJ, Peri H, Zwang E, Yaron Y, Wolman I, Eldor A: **High prevalence of the prothrombin gene mutation in women with intrauterine growth retardation, abruptio placentae and 2nd trimester loss.** *Acta Obstet Gynecol Scand* 2000, **79:**963-967.
64. Krauss T, Augustin HG, Osmers R, Meden H, Unterhalt M, Kuhn W: **Activated protein C resistance and factor V Leiden in patients with hemolysis, elevated liver enzymes, low platelets syndrome.** *Obstet Gynecol* 1998, **92:**457-460.
65. Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, Martinelli P, Montanaro S, Pavone G, Di Minno G: **Factor V Leiden, C >T MTHFR polymorphism and genetic susceptibility to preeclampsia.** *Thromb Haemost* 1997, **77:**1052-1054.
66. Grandone E, Margaglione M, Colaizzo D, Cappucci G, Scianname N, Montanaro S, Paladini D, Martinelli P, Di Minno G: **Prothrombotic genetic risk factors and the occurrence of gestational hypertension with or without proteinuria.** *Thromb Haemost* 1999, **81:**349-352.
67. Shaughnessy KM, Fu B, Ferraro F, Lewis I, Downing S, Morris NH: **Factor V Leiden and thermolable methylenetetrahydrofolate reductase gene variants in an east Anglian preeclampsia cohort.** *Hypertension* 1999, **33:**1338-1341.
68. Sohda S, Arinami T, Hamada HM, Yamada N, Hamaguchi H, Kubo T: **Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia.** *J Med Genet* 1997, **34:**525-526.
69. Lindoff C, Ingemarsson I, Martinsson G, Segelmark M, Thysell H, Astedt B: **Preeclampsia is associated with reduced response to activated protein C.** *Am J Obstet Gynecol* 1997, **176:**457-460.
70. Mello G, Parretti E, Martini E, Mecacci F, La Torre P, Cioni R, Lucchetti R, Fedi S, Gori AM, Pepe G, Prisco D, Abbate R: **Usefulness of screening for congenital or acquired hemostatic abnormalities in women with previous complicated pregnancies.** *Haemostasis* 1999, **29:**197-203.
71. Lindqvist PG, Svensson PJ, Marsaal K, Grennert L, Luterkort M, Dahlback B: **Activated protein C resistance (FV:Q506) and pregnancy.** *Thromb Haemost* 1999, **81:**532-537.
72. De Groot CJM, Bloemenkamp KWM, Duvekot EJ, Helmerhorst FM, Bertina RM, Van Der Meer F, De Ronde H, Oei SG, Kanhai HHH, Rosendaal FR: **Preeclampsia and genetic risk factors for thrombosis: a case control study.** *Am J Obstet Gynecol* 1999, **181:**975-980.
73. Morrison ER, Miedzybrodzka ZH, Campbell DM, Haites NE, Wilson BJ, Watson MS, Greaves M, Vickers MA: **Prothrombotic genotypes are not associated with pre-eclampsia and gestational hypertension: results from a large population-based study and systematic review.** *Thromb Haemost* 2002, **87:**779-785. Review
74. Murphy RP, Donoghue C, Nallen RJ, D'Mello M, Regan C, Whitehead AS, Fitzgerald DJ: **Prospective evaluation of the risk conferred by factor V Leiden and the thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy.** *Arterioscler Thromb Vasc Biol* 2000, **20:**266-270.
75. Kim YJ, Williamson RA, Murray JC, Andrews J, Pietscher JJ, Peraud PJ, Merrill DC: **Genetic susceptibility to pre-eclampsia: roles of cytosineto-thymine substitution at nucleotide 677 of the gene for methylenetetrahydrofolate reductase, 68-basepair insertion at nucleotide 844 of the gene for cystathione-synthase, and factor V Leiden mutation.** *Am J Obstet Gynecol* 2001, **184:**1211-1217.
76. Powers RW, Minich LA, Lykins DL, Ness RB, Crombleholme WR, Roberts JM: **Methylenetetrahydrofolate reductase polymorphism, folate, and susceptibility to preeclampsia.** *J Soc Gynecol Invest* 1999, **6:**74-79.
77. Lachmeijer AMA, Arngrimsson R, Bastiaans EJ, Pals G, ten Kate LP, de Vries JIP, Kostense PJ, Aarnoudse JG, Dekker GA: **Mutations in the gene for MTHFR, homocysteine levels, and vitamin status in women with a history of PE.** *Am J Obstet Gynecol* 2001, **184:**394-402.
78. Zusterzeel PLM, Visser W, Blom HJ, Peters WHM, Heil SG, Steegers EAP: **Methylenetetrahydrofolate reductase polymorphisms in pre-eclampsia and the HELLP syndrome.** *Hypertens Preg* 2000, **19:**299-307.
79. Kaiser T, Brennecke SP, Moses EK: **Methylenetetrahydrofolate reductase polymorphisms are not a risk factor for pre-eclampsia/eclampsia in Australian women.** *Gynecol Obstet Invest* 2000, **50:**100-102.
80. Hauge B, Wiltshire E, Nelson P, Dekker G, Robinson J, Thomas D: **Polymorphisms of Methylenetetrahydrofolate reductase in women with pre-eclampsia 12th International society for the study of hypertension in pregnancy.** *Hypertens Pregnancy* 2000, **19:**23. abstract # O46
81. Rajkovic A, Catlano PM, Malinow MR: **Elevated homocysteine levels with preeclampsia.** *Obstet Gynecol* 1997, **90:**168-171.
82. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, Monsen AL, Ueland PM: **Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study.** *Am J Clin Nutr* 2000, **71:**962-968.
83. Cotter AM, Molloy AM, Scott JM, Daly SF: **Elevated plasma homocysteine in early pregnancy: a risk factor for the development of severe preeclampsia.** *Am J Obstet Gynecol* 2001, **185:**781-785.
84. Glueck CJ, Phillips H, Cameron D, Wang P, Fontaine RN, Moore SK, Sieve-Smith L, Tracy T: **The 4G/4G polymorphism of the hypofibrinolytic PAI-1 gene: an independent risk factor for serious pregnancy complications.** *Metabolism* 2000, **49:**845-852.
85. Glueck CJ, Kupferminc MJ, Fontaine RN, Wang P, Eldor A: **Increased Frequency of the Hypofibrinolytic 4G/4G Polymorphism of the Plasminogen Activator Inhibitor-1 (PAI-1) Gene in Women with Obstetric Complications.** *Obstet Gynecol* 2001, **97:**44-48.
86. Sibai BM, Ramadan MK, Chari RS, Friedman SA: **Pregnancies complicated by HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets): subsequent pregnancy outcome and long-term prognosis.** *Am J Obstet Gynecol* 1995, **172(1 Pt 1):**125-129.
87. Hnat MD, Sibai BM, Caritis S, Hauth J, Lindheimer MD, MacPherson C, VanDorsten JP, Landon M, Miodovnik M, Paul R, Meis P, Thurnau G, Dombrowski M: **Perinatal outcome in women with recurrent preeclampsia compared with women who develop preeclampsia as nulliparas.** *Am J Obstet Gynecol* 2002, **186:**422-426.
88. Sibai BM, Mercer B, Sarinoglu C: **Severe preeclampsia in the second trimester: recurrence risk and long-term prognosis.** *Am J Obstet Gynecol* 1991, **165:**1408-1412.
89. de Vries JI, Dekker GA, Huijgens PC, Jakobs C, Blomberg BM, van Geijn HP: **Hyperhomocystinemia and protein S deficiency in complicated pregnancies.** *BJOG* 1997, **104:**1248-1254.
90. Martinelli P, Grandone E, Colaizzo D, Paladini D, Scianname N, Margaglione M, Di Minno G: **Familial thrombophilia and the occur-**

**rence of fetal growth restriction.** *Haematologica* 2001, **86:**428-431.
91. Grandone E, Margaglione M, Colaizzo D, Pavone G, Paladini D, Martinelli P, Di Minni G: **low birth-weight in neonates of mothers carrying factor V G1691A and factor A 20210 mutations.** *Haematologica* 2002, **87:**177-181.
92. Infante-Rivard C, Rivard GE, Yotov WV, Génin E, Guiguet M, Weinberg C, Gauthier R, Feoli-Fonseca JC: **Absence of Association of Thrombophilia Polymorphisms with Intrauterine Growth Restriction.** *N Engl J Med* 2002, **347:**19-25.
93. Reznik R: **Intrauterine growth restriction.** *Obstet Gynecol* 2002, **99:**490-496.
94. Kupferminc MJ, Many A, Bar-Am A, Lessing JB, Landsberg JA: **Mid-Trimester Severe intrauterine growth restriction is associated with a high prevalence of thrombophilia.** *BJOG* 2002, **109:**1373-1376.
95. van der Molen EF, Verbruggen B, Novakova I, Eskes TK, Monnens LA, Blom HJ: **Hyperhomocysteinemia and other thrombotic risk factors in women with placental vasculopathy.** *BJOG* 2000, **107:**785-791.
96. Wiener-Megnagi Z, Ben-Shlomo I, Goldberg Y, Shalev E: **Resistance to activated protein C and the Leiden mutation: high prevalence in patients with abruptio placentae.** *Am J Obstet Gynecol* 1998, **179:**1565-1567.
97. Goddijn-Wessel TA, Wouters MG, van de Molen EF, Spuijbroek MD, Steegers-Theunissen RP, Blom HJ, Boers GH, Eskes TK: **Hyperhomocystinemia: a risk factor for placental abruption or infarction.** *Eur J Obstet Gynecol Reprod Biol* 1996, **66:**23-29.
98. Regan L, Rai R: **Thrombophilia and pregnancy loss.** *J Reprod Immunol* 2002, **55:**163-180. Review
99. Tulppala M, Viinikka L, Ylikorkala O: **Thromboxane dominance and prostacyclin deficiency in habitual abortion.** *Lancet* 1991, **337:**879-881.
100. Vincent T, Rai R, Regan L, Cohen H: **Increased thrombin generation in women with recurrent miscarriage.** *Lancet* 1998, **352:**116.
101. Woodhams J, Candotti G, Shaw R, Kernoff PBA: **Changes in coagulation and fibrinolysis during pregnancy: evidence of activation of coagulation preceding spontaneous abortion.** *Thromb Res* 1989, **55:**99-107.
102. Rai R, Tuddenham E, Backos M, Regan L: **Pre-pregnancy thrombophilic abnormalities are associated with subsequent spontaneous abortion.** *Hum Reprod* 2000, **15:**168.
103. Kutteh WH: **Antiphospholipid antibody-associated recurrent pregnancy loss: Treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone.** *Am J Obstet Gynecol* 1996, **174:**1584-1589.
104. Rai R, Cohen H, Dave M, Regan L: **Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies).** *BMJ* 1997, **314:**253-257.
105. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N: **Thrombophilic polymorphisms are common in women with fetal loss without apparent cause.** *Thromb Haemost* 1999, **82:**6-9.
106. Brenner B, Mandel H, Lanir N, Younis J, Rothbart H, Ohel G, Blumenfeld Z: **Activated protein C resistance can be associated with recurrent fetal loss.** *Br J Haematol* 1997, **97:**551-554.
107. Ridker PM, Miletich JP, Buring JE, Ariyo AA, Price DT, Manson JE, Hill JA: **Factor V Leiden mutation as a risk factor for recurrent pregnancy loss.** *Ann Intern Med* 1998, **128:**1000-1003.
108. Younis JS, Brenner B, Ohel G, Tal J, Lanir N, Ben-Ami M: **Activated protein C resistance and factor V Leiden mutation can be associated with first-as well as second-trimester recurrent pregnancy loss.** *Am J Reprod Immunol* 2000, **43:**31-35.
109. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B: **Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage.** *Fertil Steril* 2002, **77:**342-347.
110. Tal J, Schliamser LM, Leibovitz Z, Ohel G, Attias D: **A possible role for activated protein C resistance in patients with first and second trimester pregnancy failure.** *Hum Reprod* 1999, **14:**1624-1627.
111. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY: **Prevalence of factor V G1691A (factor V-Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population.** *Am J Hematol* 2002, **71:**300-305.
112. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, Zournatzi V, Makris PE, Bontis J, Kotsis A: **Factor V leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages.** *Hum Reprod* 2000, **15:**458-462.
113. Reznikoff-Etievan MF, Cayol V, Carbonne B, Robert A, Coulet F, Milliez J: **Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage.** *BJOG* 2001, **108:**1251-1254.
114. Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ, Pinto CR: **Acquired and inherited thrombophilia in women with unexplained fetal losses.** *Am J Obstet Gynecol* 2002, **187:**1337-1342.
115. Rai R, Shlebak A, Cohen H, Backos M, Holmes Z, Marriott K, Regan L: **Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage.** *Hum Reprod* 2001, **16:**961-965.
116. Grandone E, Margaglione M, Colaizzo D, d'Addedda M, Cappucci G, Vecchione G, Scianname N, Pavone G, Di Minno G: **Factor V Leiden is associated with repeated and recurrent unexplained fetal losses.** *Thromb Haemost* 1997, **77:**822-824.
117. Kutteh WH, Park VM, Deitcher SR: **Hypercoagulable state mutation analysis in white patients with early first-trimester recurrent pregnancy loss.** *Fertil Steril* 1999, **71:**1048-1053.
118. Dizon-Townson DS, Kinney S, Branch DW, Ward K: **The factor V Leiden mutation is not a common cause of recurrent miscarriage.** *J Reprod Immunol* 1997, **34:**217-223.
119. Deitcher SR, Park VM, Kutteh WH: **Methylene tetrahydrofolate reductase 677$\tilde{C}$T mutation analysis in Caucasian women with early first trimester recurrent pregnancy loss.** *Blood* 1998, **92(Suppl 1):**117b.
120. Kornberg A, Raziel A, Rahimini-Levene N, Yona R, Sela BA, Ron-El R: **Hypercoagulability and recurrent abortions.** *Blood* 1998, **92(Suppl 1):**121b.
121. Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers CH, Eskes TK: **Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages.** *J Nutr* 1998, **128:**1336-1341.
122. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Eskes TK: **Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis.** *Fertil Steril* 2000, **74:**1196-1199.
123. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF: **Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion.** *Hum Reprod* 1999, **14:**2448-2450.
124. Pickering W, Marriott K, Regan L: **G20210A prothrombin gene mutation: prevalence in a recurrent miscarriage population.** *Clin Appl Thromb Hemost* 2001, **7:**25-28.
125. Deitcher SR, Park VM, Kutteh WH: **Prothrombin 20210 G A mutation analysis in Caucasian women with early first trimester recurrent pregnancy loss.** *Blood* 1998, **92(Suppl 1):**118b.
126. Pihusch R, Buchholz T, Lohse P, Rubsamen H, Rogenhofer N, Hasbargen U, Hiller E, Thaler CJ: **Thrombophilic gene mutations and recurrent spontaneous abortion: prothrombin mutation increases the risk in the first trimester.** *Am J Reprod Immunol* 2001, **46:**124-131.
127. Sanson BJ, Friederich PW, Simioni P, Zanardi S, Hilsman MV, Girolami A, ten Cate JW, Prins MH: **The risk of abortion and stillbirth in antithrombin-, protein C-, and protein S-deficient women.** *Thromb Haemost* 1996, **75:**387-388.
128. Brenner B: **Inherited thrombophilia and fetal loss: Curr Opin.** *Hematol* 2000, **7:**290-295. Review
129. Preston FE, Rosendaal FR, Walker ID, Briet E, Berntorp E, Conard J, Fontcuberta J, Makris M, Mariani G, Noteboom W *et al.*: **Increased fetal loss in women with heritable thrombophilia.** *Lancet* 1996, **348:**913-916.
130. Gris JC, Quere I, Monpeyroux F, Mercier E, Ripart-Neveu S, Tailland ML, Hoffet M, Berlan J, Daures JP, Mares P: **Case-control study of the frequency of thrombophilic disorders in couples with late foetal loss and no thrombotic antecedent – the Nimes Obstetricians and Haematologists Study5 (NOHA5).** *Thromb Haemost* 1999, **81:**891-899.

131. Martinelli I, Taioli E, Cetin I, Marinoni A, Gerosa S, Villa MV, Bozzo M, Mannucci PM: **Mutations in coagulation factors in women with unexplained late fetal loss.** *N Engl J Med* 2000, **343:**1015-1018.
132. Many A, Elad R, Yaron Y, Eldor A, Lessing JB, Kupferminc MJ: **Third-trimester unexplained intrauterine fetal death is associated with inherited thrombophilia.** *Obstet Gynecol* 2002, **99:**684-687.
133. Rey E, Kahn SR, David M, Shrier I: **Thrombophilic disorders and fetal loss: a meta-analysis.** *Lancet* 2003, **361:**901-9088.
134. Brenner B, Hoffman R, Blumenfeld Z, Weiner Z, Younis JS: **Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin.** *Thromb Haemost* 2000, **83:**693-697.
135. Younis JS, Ohel G, Brenner B, Haddad S, Lanir N, Ben-Ami M: **The effect of thrombophylaxis on pregnancy outcome in patients with recurrent pregnancy loss associated with factor V Leiden mutation.** *BJOG* 2000, **107:**415-419.
136. Duley L, Henderson-Smart D, Knight M, King J: **Antiplatelet drugs for prevention of pre-eclampsia and its consequences: Systematic review.** *BMJ* 2001, **322:**329-333.
137. North RA, Ferrier C, Gamble G, Fairley KF, Kincaid-Smith P: **Prevention of preeclampsia with heparin and antiplatelet drugs in women with renal disease.** *Aust N Z J Obstet Gynaecol* 1995, **35:**357-362.
138. Kupferminc MJ, Fait G, Many A, Lessing JB, Yair D, Bar-Am A, Eldor A: **A Low molecular weight heparin for the prevention of obstetric complications in women with thrombophilia.** *Hypertens Pregnancy* 2001, **20:**35-44.
139. Riyazi N, Leeda M, de Vries JI, Huijgens PC, van Geijn HP, Dekker GA: **Low-molecular-weight heparin combined with aspirin in pregnant women with thrombophilia and a history of preeclampsia or fetal growth restriction: a preliminary study.** *Eur J Obstet Gynecol Reprod Biol* 1998, **80:**49-54.
140. Greer IA: **Thrombosis in Pregnancy: Maternal and Fetal Issues.** *Lancet* 1999, **353:**4-10.
141. Seligsohn U, Zivelin A: **Thrombophilia As a Multigenic Disorder.** *Thromb Haemost* 1997, **78:**297-301.
142. Gerhardt A, Scharf RE, Beckmann MW, Struve S, Bender HG, Pillny M, Sandmann W, Zotz RB: **Prothrombin and Factor V Mutations in Women With a History of Thrombosis During Pregnancy and the Puerperium.** *N Engl Med* 2000, **342:**374-380.
143. Greer IA: **The Challenge of Thrombophilia in Maternal-Fetal Medicine.** *N Engl Med* 2000, **342:**424-425.
144. Turnball A, Tindall VR, Beard RW: **Report on confidential enquiries into maternal death in England and Wales 1982–1984.** *London: HMSO* 1989. Report No:28
145. Greer IA: **Epidemiology, risk factors and prophylaxis of venous thrombo-embolism in obstetrics and gynaecology.** *Baillieres Clin. Obstet Gynaecol* 1997, **11:**403-430.
146. Ferrari E, Jambou D, Fischer F, Appert-Flory A: **Maladie Thromboembolique Veineuse Chez La Femme Enceinte: Sang Thrombose Vaisseaux.** 1999, **11:**16-19.
147. Badracco MA, Vessey M: **Recurrence of Venous Thromboembolism and the Use of Oral Contraceptives.** *BMJ* 1974, **2:**215-217.
148. Ginsberg JS, Brill-Edwards P, Burrows RF, Bona R, Prandoni P, Buller HR, Lensing A: **Venous Thrombosis During Pregnancy: Leg and Trimester of Presentation.** *Thromb Haemost* 1992, **67:**519-520.
149. Macklon NS, Greer IA, Bowman AW: **An Ultrasound Study of Gestational and Postural Changes in the Deep Venous System of the Leg in Pregnancy.** *Br J Obstet Gynaecol* 1997, **104:**191-197.
150. Clark P, Brennand J, Conkie JA, McCall F, Greer IA, Walker ID: **Activated Protein C Sensitivity, Protein C, Protein S and Coagulation in Normal Pregnancy.** *Thromb Haemost* 1998, **79:**1166-1170.
151. Greer IA: **Haemostasis and thrombosis in pregnancy.** Edited by: *Bloom AL, Forbes CD, Thomas DP, and Tuddenham EGD. Edinburgh: Churchill Livingston*; 1994:987-999.
152. Friend JR, Kakkar VV: **The diagnosis of deep venous thrombosis in the puerperium.** *J Obstet Gynaecol Br Commonw* 1970, **77:**820-823.
153. Macklon NS, Greer IA: **Venous thromboembolic disease in obstetrics and gynaecology: the Scottish Experience.** *Scott Med* 1996, **41:**83-86.
154. Ginsberg JS, Hirsh J, Rainbow AJ, Coates G: **Risks to the fetus of radiologic procedures used in the diagnosis of maternal venous thromboembolic disease.** *Thromb Haemost* 1989, **61:**189-196.
155. Finazzi G, Barbui T: **Different incidence of venous thrombosis in patients with inherited deficiencies of antithrombin III, protein C and protein S.** *Thromb Haemost* 1994, **71:**15-18.
156. Conard J, Horellou MH, Van Dreden P, Lecompte T, Samama M: **Thrombosis and pregnancy in congenital deficiencies in AT III, protein C or protein S: study of 78 women.** *Thromb Haemost* 1990, **63:**319-320.
157. Hunt BJ, Doughty HA, Majumdar G, Copplestone A, Kerslake S, Buchanan N, Hughes G, Khamashta M: **Thromboprophylaxis with low molecular weight heparin (Fragmin) in high risk pregnancies.** *Thromb Haemost* 1997, **77:**39-43.
158. Friederich PW, Sanson BJ, Simioni P, Zanardi S, Huisman MV, Kindt I, Prandoni P, Buller HR, Girolami A, Prins MH: **Frequency of pregnancy-related venous thromboembolism in anticoagulant factor-deficient women: implications for prophylaxis.** *Ann Intern Med* 1996, **125:**955-960.
159. Hallak M, Senderowicz J, Cassel A, Shapira C, Aghai E, Auslender R, Abramovici H: **Activated protein C resistance (factor V Leiden) associated with thrombosis in pregnancy.** *Am J Obstet Gynecol* 1997, **176:**889-893.
160. McColl MD, Walker ID, Greer IA: **A Mutation in the prothrombin gene contributing to venous thrombosis during pregnancy.** *Br J Obstet Gynaecol* 1998, **105:**923-925.
161. Lindqvist PG, Svensson PJ, Dahlback B, Marsal K: **Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss-a possible evolutionary selection mechanism.** *Thromb Haemost* 1998, **79:**69-73.
162. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL: **A Common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women.** *Blood* 1997, **90:**1747-1750.
163. Ginsberg JS, Hirsh J: **Use of Antithrombotic agents during pregnancy.** *Chest* 1998, **114:**524S-530S.
164. Sanson BJ, Lensing AW, Prins MH, Ginsberg JS, Barkagan ZS, Lavenne-Pardonge E, Brenner B, Dulitzky M, Nielsen JD, Boda Z *et al.*: **Low molecular-weight heparin in pregnancy: a systematic review.** *Thromb Haemost* 1999, **81:**668-672.
165. Nelson-Piercy C, Letsky EA, De Swiet M: **Low-molecular-weight heparin for obstetric thromboprophylaxis: experience of sixty-nine pregnancies in sixty-one women at high risk.** *Am J Obstet Gynecol* 1997, **176:**1062-1068.
166. Melissari E, Parker CJ, Wilson NV, Monte G, Kanthou C, Pemberton KD, Nicolaides KH, Barrett JJ, Kakkar VV: **Use of low molecular weight heparin in pregnancy.** *Thromb Haemost* 1992, **68:**652-656.
167. Gillis S, Shushan A, Eldor A: **Use of low molecular weight heparin for prophylaxis and treatment of thromboembolism in pregnancy.** *Int Gynaecol Obstet* 1992, **39:**297-301.
168. Fejgin MD, Lourwood DL: **Low molecular weight heparins and their use in obstetrics and gynecology.** *Obstet Gynecol Surv* 1994, **49:**424-431.
169. Harenberg J, Schneider D, Heilmann L, Wolf H: **Lack of anti-factor Xa activity in umbilical cord vein samples after subcutaneous administration of heparin or low molecular mass heparin in pregnant women.** *Haemostasis* 1993, **23:**314-320.
170. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM: **Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia.** *Thromb Haemost* 2001, **86:**800-803.
171. Macklon NS, Greer IA: **Technical note: Compression stockings and posture: a comparative study of their effects on the proximal deep veins of the leg at rest.** *Br J Radiol* 1995, **68:**515-518.
172. Brill-Edwards P, Ginsberg JS, Gent M, Hirsh J, Burrows R, Kearon C, Geerts W, Kovacs M, Weitz JI, Robinson KS, Whittom R, Couture G: **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* 2000, **343:**1439-1444.
173. Brancazio LR, Roperti KA, Stierer R, Laifer SA: **Pharmacokinetics and pharmacodynamics of subcutaneous heparin during the early third trimester of pregnancy.** *Am J Obstet Gynecol* 1995, **173:**1240-1245.

174. Barbour LA, Smith JM, Marlar RA: **Heparin levels to guide thromboembolism prophylaxis during pregnancy.** *Am J Obstet Gynecol* 1995, **173:**1869-1873.
175. Hirsh J: **Low-molecular-weight heparin for the treatment of venous thromboembolism.** *Am Heart J* 1998, **135:**S336-S342.
176. Rasmussen C, Wadt J, Jacobsen B: **Thromboembolic prophylaxis with low molecular weight heparin during pregnancy.** *Int Gynaecol Obstet* 1994, **47:**121-125.
177. Dulitzki M, Pauzner R, Langevitz P, Pras M, Many A, Schiff E: **Low-molecular-weight heparin during pregnancy and delivery: preliminary experience with 41 pregnancies.** *Obstet Gynecol* 1996, **87:**380-383.
178. Casele HL, Laifer SA, Woelkers DA, Venkataramanan R: **Changes in the pharmacokinetics of the low-molecular-weight heparin enoxaparin sodium during pregnancy.** *Am J Obstet Gynecol* 1999, **181:**1113-1117.
179. Thomson AJ, Walker ID, Greer IA: **Low-molecular-weight heparin for immediate management of thromboembolic disease in pregnancy.** *Lancet* 1998, **352:**1904.
180. Aburahma AF, Boland JP: **Management of Deep Vein Thrombosis of the lower extremity in pregnancy: a challenging dilemma.** *Am Surg* 1999, **65:**164-167.
181. Greaves M: **Antiphospholipid antibodies and thrombosis [Letter, Comment].** *Lancet* 1999, **354:**1031.
182. Lockshin MD: **Pregnancy loss in the antiphospholipid syndrome.** *Thromb Haemost* 1999, **82:**641-648.
183. Kutteh WH, Ermel LD: **A clinical trial for the treatment of antiphospholipid antibody-associated recurrent pregnancy loss with lower dose heparin and aspirin.** *Am J Reprod Immunol* 1996, **35:**402-407.
184. Lanska DJ, Kryscio RJ: **Stroke and Intracranial Venous Thrombosis During Pregnancy and Puerperium.** *Neurology* 1998, **51:**1622-1628.
185. Mas JL, Lamy C: **Stroke in pregnancy and the puerperium.** *J Neurol* 1998, **245:**305-313.
186. Kittner SJ, Stern BJ, Feeser BR, Hebel R, Nagey DA, Buchholz DW, Earley CJ, Johnson CJ, Macko RF, Sloan MA, Wityk RJ, Wozniak MA: **Pregnancy and the risk of stroke.** *N Engl Med* 1996, **335:**768-774.
187. Rizk B, Meagher S, Fisher AM: **Severe ovarian hyperstimulation syndrome and cerebrovascular accidents.** *Hum Reprod* 1990, **5:**697-698.
188. Kaaja R, Siegberg R, Tiitinen A, Koskimies A: **Severe ovarian hyperstimulation syndrome and deep venous thrombosis [Letter].** *Lancet* 1989, **2:**1043.
189. Kupferminc MJ, Yair D, Bornstein NM, Lessing JB: **Transient focal neurological deficits during pregnancy in carriers of inherited thrombophilia.** *Stroke* 2000, **31:**892-895.
190. Bertina RM, Rosendaal FR: **Venous thrombosis-the Interaction of genes and environment.** *N Engl J Med* 1998, **338:**1840-1841.