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Maternal thrombophilia and obstetric complications

Mütterliche Thrombophilie und geburtshilfliche Komplikationen

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Abstract

Women with thrombophilic defects have been shown to be at increased risk, not only of pregnancy associated thromboembolism but also of other vascular complications of pregnancy, including preeclampsia and fetal loss. First trimester fetal loss is associated with factor V Leiden mutation, activated protein C resistance without factor V Leiden mutation and prothrombin G20210A mutation. Late non-recurrent fetal loss is associated with factor V Leiden mutation, prothrombin mutation and protein S deficiency. Concerning acquired thrombophilia, recurrent fetal loss is a well-documented finding in patients with antiphospholipid antibodies. Associations between thrombophilia polymorphisms and an increased risk of intrauterine growth restriction have been discussed in small series of cases but could not be confirmed in large scale studies. Frequencies for anticardiolipin antibodies or lupus anticoagulants and antinuclear antibodies were significantly higher in women with infants small for gestational age compared to controls. Concerning preeclampsia, gestational hypertension and thrombophilia, a number of studies have examined these relationships with conflicting results. For factor V Leiden, MTHFR C677T and prothrombin mutation, no association with preeclampsia was observed, when severe cases were excluded. If studies were restricted to those of severe preeclampsia, an association with the factor V Leiden mutation was apparent and, to a lesser extent, with the MTHFR-mutation.

For antithrombotic therapy, it was shown that in women with antiphospholipid syndrome and recurrent pregnancy loss, unfractionated heparin plus low-dose aspirin results in significantly better gestational outcome than low-dose aspirin alone. Concerning therapy of women with inherited thrombophilia and pregnancy loss, only small, uncontrolled studies are available, demonstrating improved pregnancy outcome when low molecular weight heparin (LMWH) is used for treatment.

In conclusion, heritable thrombophilia and the antiphospholipid-syndrome are major causes of fetal loss after exclusion of other underlying pathologies like chromosomal abnormalities, and screening should be recommended. LMWH with or without aspirin may be used for treatment. There is little value in antenatal screening for prothrombotic polymorphisms to predict the development of small for gestational age infants, preeclampsia or gestational hypertension.

Keywords: thrombophilia; factor V Leiden mutation; prothrombin mutation; MTHFR-mutation; obstetric complications; fetal loss; preeclampsia; gestational hypertension; intrauterine growth restriction; low molecular weight heparin; aspirin.

Zusammenfassung

Frauen mit einer Thrombophilie haben nicht nur ein erhöhtes Risiko für schwangerschaftsassoziierte Thrombosen, sondern können auch andere vaskuläre Komplikationen wie eine Präeklampsie oder eine erhöhte Abortrate aufweisen. Frühaborte im ersten Trimester sind signifikant mit der Faktor V Leiden-Mutation, der aktivierten Protein C-Resistenz ohne Nachweis einer Faktor V Leiden-Mutation und mit einer Prothrombinmutation (G20210A) assoziiert. Bei Frauen mit Spätaborten können eine Faktor V Leiden-Mutation, eine Prothrombinmutation oder ein Protein S-Mangel von klinischer Relevanz sein. Bezüglich der erworbenen thrombophilen Neigungen lassen sich wiederholte Aborte besonders häufig beim Antiphospholipid-Syndrom nachweisen. Assoziationen zwischen thrombophilen Polymorphismen und einem erhöhten Risiko für eine intrauterine Wachstumsretardierung sind wiederholt in Studien mit kleinen Fallzahlen beobachtet worden, konnten jedoch in Studien mit großen Fallzahlen nicht bestätigt werden. Anticardiolipin-Antikörper oder Lupusantikoagulantien und antinukleäre Antikörper waren dagegen signifikant häufiger bei Frauen, die Kinder mit einem zu niedrigen Geburtsgewicht geboren hatten, nachweisbar. Bezüglich einer Präeklampsie sowie des schwangerschaftsinduzierten Hypertonus und einer Thrombophilie zeigten sich in den durchgeführten Studien widersprüchliche Ergebnisse.

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Eine Assoziation zwischen der Faktor V Leiden Mutation, der MTHFR-Mutante und der Prothrombin G20210A-Mutation und einer Präeklampsie konnte nach Ausschluss der schweren Fälle nicht beobachtet werden. Bei Analyse der schweren Fälle zeigte sich jedoch eine Assoziation mit der Faktor V Leiden Mutation, und zu einem geringeren Ausmass, mit der MTHFR-Mutation.

Bezüglich einer antithrombotischen Therapie konnte bei Frauen mit Antiphospholipidsyndrom und wiederholten Aborten gezeigt werden, dass die Gabe von unfraktioniertem Heparin in Kombination mit Aspirin im Vergleich zu alleiniger Gabe von Aspirin signifikant die Anzahl erfolgreicher Schwangerschaften erhöhen konnte. Bezüglich der Behandlung von Frauen mit angeborener Thrombophilie und Aborten existieren derzeit nur unkontrollierte Studien mit kleinen Fallzahlen, die einen Benefit unter Therapie mit niedermolekularem Heparin nachweisen konnten.

Zusammenfassend können sowohl die hereditäre Thrombophilie, als auch das Antiphospholipidsyndrom wesentlich bei der Pathogenese von habituellen Aborten beteiligt sein. In diesen Fällen kann ein Thrombophiliescreening zur weiteren Abklärung empfohlen werden. Niedermolekulares Heparin alleine oder in Kombination mit Aspirin stellt eine mögliche Behandlungsoption bei diesen Frauen dar. Bisher gibt es jedoch keine Rationale für ein generelles Screening bei Patientinnen mit Präeklampsie, schwangerschaftsinduziertem Hypertonus oder intrauteriner Wachstumsretardierung.

Schlüsselwörter: Thrombophilie; Faktor V Leiden Mutation; Prothrombinmutation; MTHFR-Mutation; Abort; Präeklampsie; schwangerschaftsinduzierter Hypertonus; intrauterine Wachstumsretardierung; niedermolekulares Heparin; Aspirin.

Abnormalities in haemostasis that are associated with clinical thrombophilia include heritable defects, such as mutations in the genes encoding the natural anticoagulants antithrombin, protein C and protein S, of clotting factors prothrombin and factor V, and acquired defects, such as antiphospholipids. Women with thrombophilic defects have been shown to be at increased risk, not only of pregnancy-associated thromboembolism but also of other vascular complications of pregnancy, including preeclampsia and fetal loss. More recently, there is a growing interest in the role of thrombophilia in obstetric complications, after several studies have provided evidence for associations.

Pregnancy-associated changes in haemostasis

Normal pregnancy is associated with major changes in all aspects of haemostasis, so that as pregnancy progresses the overall balance is shifted towards hypercoagulability, which additionally may induce exacerbation of the clinical effects of inherited thrombophilias [1, 2]. Pregnancy is associated with an increase in levels of fibrinogen and factors II, VII, VIII, X and XII, whereas concentrations of factors V and IX are unchanged. Endogenous coagulation inhibitors either remain constant (like antithrombin and protein C) or significantly decrease (protein S). Moreover, levels of plasminogen activator inhibitor I (PAI I) increase up to three-fold. As a consequence, these pregnancy-induced changes have to be taken into account when pregnant patients are screened for thrombophilia and are responsible for the increased thromboembolic risk during pregnancy.

Pathophysiology of inherited and acquired thrombophilias with special emphasis on APC-resistance

Inherited resistance to the effect of activated protein C (APC) was first recognized 10 years ago by Dahlbaeck et al. [3]. In the majority of subjects, it results from the Leiden mutation in the gene coding for coagulation factor V [4]. Heterozygosity for the factor V Leiden mutation is inherited in an autosomal dominant fashion [2, 5]. It is present in 5–9% of the white European population [6] and in 20–40% of nonpregnant patients with thromboembolic disease [2]. Homozygosity for the mutation increases the relative risk of venous thromboembolism in nonpregnant patients to 80-fold [7]. Factor V Leiden prothrombotic effects may be increased by pregnancy-induced reductions in protein S. The proportion of pregnant patients with venous thromboembolism attributable to the heterozygote factor V Leiden mutation has been reported to be around 40% [8, 9].

Apart from inherited resistance to activated protein C, the potential significance of resistance occurring in the absence of known mutations of the factor V gene has been recognized and termed “acquired APC-resistance” [10]. In assessment of APC-resistance, a distinction has to be drawn between factor V mutation specific and non-specific assays. For determination of acquired APC-resistance, multiple assay systems have been described [3, 11–13]. Additionally, multiple factors like blood-group, smoking, cholesterol, age, acute phase reactants, blood pressure or body mass index can influence the measurements leading to conflicting results between studies [10]. Apart from the factor V Leiden mutation, polymorphisms of the methylenetetrahydrofolate reductase- (MTHFR) and the prothrombin-gene (G20210A) have been made responsible for thromboembolic complications [14, 15]. Hyperhomocysteinemia and homozygous as well as heterozygous forms of the prothrombin mutation increase the risk of thrombosis, however, the association between homozygosity for the MTHFR-mutation and thrombosis remains controversial [16, 17] since the homocystein-level not only depends on the MTHFR-gene polymorphisms but is also strongly influenced by vitamin B6-, B12- and

Table 1 Factor V Leiden in women with recurrent fetal loss before 13 weeks (modified according to [20]).

	Study design	FVL positive n/N	FVL negative n/N	Odds ratio (95% CI)
Balasch et al. 1997 [21]	retrospective	1/2	54/103	0.91 (0.06–14.9)
Fatini et al. 2000 [22]	retrospective	6/8	53/121	3.85 (0.75–19.85)
Foka et al. 2000 [23]	retrospective	9/13	52/148	4.15 (1.22–14.14)
Grandone et al. 1997 [24]	retrospective	2/7	25/138	1.81 (0.33–9.86)
Rai et al. 2001 [25]	retrospective	59/71	845/983	0.80 (0.42–1.53)
Reznikoff et al. 2000 [26]	retrospective	27/38	233/462	2.41 (1.17–4.98)
Younis et al. 2000 [27]	retrospective	6/14	31/162	3.17 (1.03–9.80)
Total		110/153	1293/2117	2.01 (1.13–3.58)

FVL: factor V Leiden; CI: confidence interval.

folic acid-intake [15]. While the above-mentioned inherited thrombophilic defects are common, inherited deficiencies of protein C, protein S and antithrombin are rarely observed but are strongly associated with clinical thrombosis in nonpregnant and pregnant women [14–16].

The most important acquired thrombophilic defects are the so-called "antiphospholipid-antibodies", which comprise lupus inhibitors and raised anticardiolipin antibody concentrations [14]. This heterogeneous group of antibodies is directed against phospholipid-coagulation protein-complexes and does not only cause venous and arterial thrombosis, but also recurrent fetal loss. Only repeated determination of moderate to high titers of antiphospholipid-antibodies is of clinical relevance.

Inherited thrombophilia and pregnancy loss

Inherited thrombophilia could increase susceptibility to adverse pregnancy outcomes such as fetal loss [18]. Fetal loss is a common and significant problem: about 20% of women have at least one fetal loss and 5% have two or more spontaneous abortions. Furthermore, 30–40% of recurrent fetal losses remain unexplained after standard gynecological, hormonal and karyotype investigations [19].

Results concerning thrombophilia as cause of pregnancy loss differ between studies due to retrospective or prospective design, inclusion criteria, number of included patients and definition, timing and recurrence of fetal loss [20]. A recent meta-analysis, which included 31 studies analyzed the association of factor V Leiden, activated protein C resistance without factor V Leiden, the prothrombin mutation, protein S-, protein C-, antithrombin-deficiency, the methylenetetrahydrofolate mutation and pregnancy loss [20]. Pooled data on recurrent fatal loss associated with the factor V Leiden mutation from 7 studies showed that there was a significant association for recurrent fetal loss before 13 weeks' gestation ([21–27] see Table 1). There was only one study on recurrent pregnancy loss after 22 weeks, which also reported a significant association with factor V Leiden (odds ratio 7.83; 95% confidence interval: 2.83–21.67) [28].

In 12 studies (n = 7932), the association between factor V Leiden and non-recurrent fetal loss was investigated [18, 28–38]. The overall association in the meta-analysis was borderline significant (odds ration 1.73; 95% confidence interval: 1.18–2.54), while significance increased if fetal loss appeared after 19 weeks' gestation (see Table 2, odds ratio 3.26; 95% confidence interval: 1.82–5.83) [18, 28, 29, 32, 36, 37]. No significant relation was observed if fetal loss occurred before 24 weeks (odds ratio 1.40; 95% confidence interval: 0.66–2.97) [29–31, 33, 34].

Concerning activated protein C resistance (APC-R) without factor V Leiden mutation, sensitivity analysis revealed a significant relation between APC-R and recurrent fetal loss before 12 weeks [20, 25, 27, 31], while no significant association between the homozygous MTHFR mutation and recurrent or non-recurrent pregnancy loss was observed in the same meta-analysis [18, 20, 23, 28, 30, 32, 36, 37, 39–44]. Pooled data indicated a significant association between the prothrombin mutation and recurrent fetal loss before 13 weeks and non-recurrent fetal loss after 20 weeks [18, 20, 22, 26, 28, 32, 36, 37, 43, 45]. While no significant association was found in protein C- and antithrombin-deficiency concerning recurrent and non-recurrent pregnancy losses [20, 28, 32, 38, 46–48], protein S-deficiency was significantly associated with recurrent fetal loss [20, 28, 46] and non-recurrent fetal loss occurring after 22 weeks [20, 28, 32, 35].

Concerning acquired thrombophilia, recurrent fetal loss is a well documented finding in patients with antiphospholipid antibodies [49]. The prevalence of persisting antiphospholipid antibodies among women with a history of recurrent fetal loss can be up to 15% with an untreated prospective fetal loss rate of 90% [50, 51]. About two thirds of women with a history of recurrent pregnancy loss and evidence of antiphospholipid-antibodies have first trimester losses only, while the remaining one third suffer both early and late losses or late losses alone [14].

Associations between late pregnancy loss and thrombophilic defects appear to be stronger than in first trimester miscarriages. In the case of first trimester fetal loss, there is a significant confounding factor. A large number of recurrent early pregnancy losses (< 10 weeks of gestation) may be due to chromosomal aberrations in

Table 2 Factor V Leiden in women with non-recurrent fetal loss after 19 weeks (modified according to [20]).

	Study design	FVL positive n/N	FVL negative n/N	Odds ratio (95% CI)
Alfirevic et al. 2001 [32]	retrospective	0/3	18/59	0.32 (0.02–6.52)
Bare et al. 2000 [29]	retrospective	1/128	2/461	1.81 (0.16–20.09)
Gris et al. 1999 [28]	retrospective	15/22	217/674	4.51 (1.81–11.23)
Kupferminc et al. 1999 [18]	retrospective	3/10	9/112	4.90 (1.08–22.30)
Many et al. 2002 [36]	retrospective	3/6	37/114	2.08 (0.4–10.81)
Martinelli et al. 2000 [37]	retrospective	5/11	62/288	3.04 (0.90–10.29)
Total		27/180	345/1708	3.26 (1.82–5.83)

FVL: factor V Leiden; CI: confidence interval.

the fetus, which will invariably cause abortion, irrespective of the presence of thrombophilia. The incidence has been reported to vary between 29 and 60% [52–54]. Only few centers karyotype the abortus, therefore only some studies screened for other underlying pathologies that could explain early fetal loss.

A second confounding factor is the small number of women with the rarer types of thrombophilia. Even after pooling the data in the meta-analysis, only limited data were available which could explain the non-significant associations of protein C- and antithrombin-deficiency with pregnancy loss.

While a higher prevalence of thrombophilic defects can be observed in women with fetal loss, in women with a history of venous thromboembolism (VT) neither stillbirth (intrauterine fetal death $\geq 24^{th}$ week of gestation) nor miscarriage (fetal loss $< 24^{th}$ week) were significantly more often observed compared to control subjects [55]. Therefore, in addition to known thrombophilic defects, yet unknown risk factors may predispose for fetal loss.

Thrombophilia and intrauterine growth restriction

Babies, who are small for their gestational age, have increased early morbidity and mortality [56]. Often a weight threshold below the 10^{th} percentile is used [57]. Associations between thrombophilia polymorphisms and an increased risk of intrauterine growth restriction have been discussed in small series of cases [58, 59]. In a recently published large hospital-based case-control study and a family-based study 493 newborns with intrauterine growth restriction and 472 normal controls were included. The presence or absence in newborns and their parents of the factor V Leiden mutation, the methylenetetrahydrofolate reductase (MTHFR) C677T mutation and the prothrombin G20210A mutation was determined [60]. In the case-control study, the odds ratios, after adjustment for newborn genotype and other risk factors, were 1.55 for the MTHFR mutation (95% confidence interval: 0.83–2.90), 1.18 for the factor V Leiden mutation (95% confidence interval: 0.54–2.55) and 0.92 for the prothrombinmutation (95% confidence interval: 0.36–2.35). Therefore no associations between maternal or newborn thrombophilia polymorphisms and an increased risk of intrauterine growth restriction were observed. A second case-control study included 97 consecutive women, who had pregnancies complicated by unexplained small for gestational age infants and 97 women as controls who delivered infants with a birthweight $\geq 10^{th}$ percentile [61]. Frequencies for anticardiolipin antibodies or lupus anticoagulants and antinuclear antibodies were significantly higher in women with infants small for their gestational age compared to controls ($p = 0.02$ and $p = 0.004$, respectively), while the prevalence of inherited thrombophilia including the homozygous MTHFR-mutation, factor V Leiden mutation, the prothrombin gene mutation and deficiencies of protein C, protein S and antithrombin was comparable in cases and controls. Therefore the aetiology of infants small for their gestational age remains unknown in most cases and may not be associated with inherited thrombophilia.

Association between prothrombotic genotypes, preeclampsia and gestational hypertension

Hypertensive disorders of pregnancy are important causes of maternal and fetal morbidity and mortality, yet their causes remain poorly understood. Preeclampsia effects approximately 5% of primiparous pregnancies, and gestational hypertension a further 20% [62]. Placental ischemia and consequent disturbance of the maternal vascular endothelium are believed to be relevant in the pathogenesis of pregnancy-induced hypertensive disorders [63]. Thus, it is plausible that prothrombotic mutations might predispose to the development of preeclampsia. In a recently published large population-based case-control study, 404 women with preeclampsia, 303 women with gestational hypertension and 164 control women were screened for the factor V Leiden mutation, the prothrombin mutation and the MTHFR C677T mutation. The frequency of genotypes did not differ significantly between cases of preeclampsia or gestational hypertension and controls [62]. A number of studies also have examined these relationships with conflicting results. However, many of these studies have been small or screened only for one or two polymorphisms [18, 30, 58, 64–75], besides, the definition of

preeclampsia and the selection of controls has also varied between studies [62]. A recent literature review summarised data of 26 studies for factor V Leiden, the MTHFR C677T and the prothrombin G20210A mutation, split by severity of disease. For these mutations, no association with preeclampsia was observed when severe cases were excluded. However, if studies were restricted to those of severe preeclampsia, an association was apparent with the factor V Leiden mutation (odds ratio: 2.84; 95% confidence interval: 1.95–4.14) and, to a lesser extent, with the MTHFR-mutation (odds ratio: 1.5; 95% confidence interval: 1.02–2.23) [62].

Clinical implications concerning testing for thrombophilia in women with obstetric complications

In conclusion, heritable thrombophilia and the antiphospholipid-syndrome are major causes of fetal loss after exclusion of other underlying pathologies like chromosomal abnormalities. The association between heritable thrombophilia and fetal loss is strengthened, when late non-recurrent and recurrent fetal loss is focused.

The aetiology of infants small for their gestational age remains unknown in most cases and may not be associated with inherited thrombophilia. Therefore, thrombophilia screening is not recommended in women, who have pregnancies complicated by unexplained small for gestational age infants. There is also little value in antenatal screening for prothrombotic polymorphisms to predict the development of preeclampsia or gestational hypertension.

Treatment and future perspectives

Since heritable thrombophilia and the antiphospholipid-syndrome are major causes of fetal loss, antithrombotic drugs like unfractionated heparin (UFH), low molecular weight heparin (LMWH) and low-dose aspirin may have a potential therapeutic benefit.

In women with antiphospholipid syndrome, who had experienced recurrent pregnancy loss, two prospective randomized studies have shown that UFH plus low-dose aspirin results in significantly better gestational outcome than low-dose aspirin alone [76, 77]. In a study conducted by Kutteh et al. [76] viable infants were delivered in only 11/25 (44%) of women receiving aspirin alone compared to 20/25 (80%) in women receiving aspirin and subcutaneous UFH ($p < 0.05$). These results were confirmed by Rai et al. [77], who observed a rate of live birth in patients treated with low-dose aspirin and heparin of 71% (32/45 pregnancies) compared to only 42% (19/45 pregnancies) in women treated with aspirin alone ($p < 0.01$). Despite these results, the optimal therapeutic regimen has not yet been established in these women, since in Rai's study one fourth of successful pregnancies were delivered prematurely [77].

In contrast, data concerning therapy of women with inherited thrombophilia and pregnancy loss were collected primarily in uncontrolled studies involving only small series of patients using LMWH for treatment. The potential advantages of LMWH over UFH are a longer half-life with only one injection per day, a lower incidence of heparin-induced thrombocytopenia and of heparin-induced osteoporosis [78–80]. A recent collaborative study has demonstrated the safety of using LMWH during 486 gestations [81]. A successful outcome was reported in 89% [83/93] gestations in women with recurrent pregnancy loss. Brenner et al. evaluated efficacy and safety of low molecular weight heparin in 50 women with recurrent pregnancy loss and thrombophilia [82]. Forty-six out of 61 (75%) gestations treated with LMWH resulted in live birth compared to only 38/193 (20%) of the untreated pregnancies in these women prior to diagnosis of thrombophilia ($p < 0.00001$). Whether monitoring of anti-Xa levels is of value is still unknown. However, levels of 0.2–0.6 three hours post-injection are expected in pregnant women who receive prophylactic doses of LMWH [83].

Prospective, randomized, placebo-controlled studies are urgently needed to assess the potential advantage of LMWH in women with thrombophilia and recurrent fetal loss.

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