

PhDTHESIS

**Examination of Activated Protein C related Morbidity and its Prevention in an
Obstetrics and Gynecology Cohort**

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1. Introduction

Thromboembolism is one of the leading causes of morbidity and mortality. Although the incidence of thromboembolism related complications has decreased in developed countries in the past 30 years, it is still a major cause of in-patient mortality. Unfortunately, on comparison to the Western European average, thromboembolia related mortality in Hungary is 2.5 folds. Furthermore, the incidence of thromboembolic complications is higher in women than in men.

Thrombophilic predisposition has an important role in obstetrics and gynecology, it influences the mode of contraception, outcome of pregnancy and delivery, and menopausal hormone therapy. The risk of thrombosis is significantly increased in women with acquired or inherited thrombophilia, especially while on oral contraceptives or menopausal hormone therapy, and during pregnancy/maternity.

Thromboembolism at unusual sites (cerebral, mesenterial, extremities, etc.), at a young age (usually below the age of 40 years) or recurrent thrombosis are usually the presenting signs of thrombophilia. For quite some time it was believed that inherited thrombophilia resulted from deficiency of natural anticoagulant factors (antithrombin-III, protein C or protein S), but now it is known that only 1-3% of the cases can be explained by these defects. Subsequent research identified a number of inherited factors (plasminogen deficiency, prothrombin polymorphism, factor XII deficiency, etc.) which can be responsible for unexplained thromboembolic events, but these too could explain only a few percent of the cases. In 1994 Dahlback et al presented a tremendous breakthrough, where they described the resistance to activated protein C (APC). It is believed that almost 40% of hereditary thrombophilia can be attributed to APC resistance.

Bertina et al elucidated a point mutation (1691 G to A) that underlies a single amino acid substitution in coagulation factor V (Arg506 to Gln), that is responsible for APC resistance. The mutation (Leiden) in the gene coding for coagulation factor V renders factor V resistant to the proteolytic effect of APC, as such preventing the feedback inhibition of APC on further coagulation, essentially allowing coagulation to continue inappropriately.

One of the most important achievements in 20th century gynecology is the safe and effective contraception. It can be assumed that the contraceptive pill changed the life of millions of women. Initially the pills had a higher hormonal content, with

marked side effects which in some cases were even fatal. Later on pills with lower hormonal content were developed that showed excellent efficacy and safety profile. Soon after the introduction of oral contraceptives (OC), it was realized that their use increases the risk of thromboembolic complications. The incidence of myocardial infarct, ischemic stroke and thrombosis were noted to be increased among OC users. Although the risk for thromboembolic complications has decreased with the decrease in the estrogen content of OC, the risk has not been eliminated all together.

One of the most frequent causes of hereditary thrombophilia is the Leiden mutation of coagulation factor V (LFV), the phenotypic manifestation of which is activated protein C (APC) resistance. Its prevalence in the Caucasian population is 4-7%, and it can be shown in 33% of patients with thromboembolism. The relative risk for venous thromboembolic events in factor V Leiden heterozygous patients is estimated to be seven folds compared to patients without a known hereditary predisposition. Furthermore, the reported rate of thromboembolic complications during pill use has been estimated to be about 25-35 folds higher among Leiden carriers than in normal individuals. The Leiden homozygous patients have 80-folds relative risk for thrombosis without OC use and there is no data about the same risk with OC use, but could be estimated to be about a few hundred folds. The carrier frequency of LFV among ethnic Hungarians is about 9.3%. With such a high frequency of individuals carrying LFV, one would expect thromboembolism to occur more commonly. A 7-fold increase in the risk of thromboembolism among carriers should at least theoretically result in a much higher morbidity. It can be stipulated that thromboembolic complications of OC use among LFV carriers require other risk factors to be present. Additional risk factors may include inherited forms of thrombophilia, such as prothrombin variant 20210A, protein S deficiency, antithrombin- III deficiency and inborn errors of lipid metabolism. Equally important additional risk factors could be the presence of acquired forms of thrombophilia such as antiphospholipid antibodies, lupus anticoagulant, hyperhomocysteinemia, hyperlipidemia, platelet disorders, obesity, immobilization and malignant disease.

Antiphospholipid antibodies are associated with arterial and venous thrombosis and recurrent pregnancy failure. Antiphospholipid antibodies may be detected in 3% of healthy pregnant women, and may not be associated with increased risk of thrombosis or miscarriage. The frequency of antiphospholipid antibodies in asymptomatic young women with OC use is higher than in those not using the pill.

The majority of cases with elevated antiphospholipid antibody titer have an elevated level of immunoglobulin G (IgG) type anti- β 2- glycoprotein I antibody.

Despite similarities between clinical effects of oral contraceptives and antiphospholipid antibodies, no one has analysed their links. Drugs that are known to be associated with antiphospholipid antibodies include phenothiazines, quinidine, hydralazine, procainamide and phenytoin. So far, no clinical data has been published regarding the possible effect of steroid hormones in oral contraceptives on antiphospholipid antibodies in asymptomatic women.

Thromboembolism has been a major cause of maternal mortality and morbidity for decades. Pregnancy often triggers the first thromboembolic event for women with inherited thrombophilia. Thromboprophylaxis should be considered for patients at moderate or high risk based on age, weight, parity, personal or familiar history of deep vein thrombosis, the presence of antiphospholipid antibodies, varicose veins, current infection, preeclampsia, immobility, major illness and emergency cesarean delivery in labor.

The crucial dilemma of thromboprophylaxis in pregnancy for women with asymptomatic thrombophilia is that the large majority of these women would not have obstetric complications even without prophylaxis. The important questions are safety and efficacy. Both questions receive controversial answers in different studies. Instead of a categorical yes or no, the selective prophylaxis would be a reasonable approach.

As per the guidelines of the Hungarian Obstetrics and Gynecology College, prior to OC prescription a routine physical and gynecological examination, and if due, a cervical cancer screening is compulsory. Blood testing is not compulsory. With regards to thrombosis, the only recommendation is that OC is not recommended for those who have a known personal and/or family history of thromboembolism. Furthermore OC is not recommended for women above 35, particularly those who smoke, due to an increased risk for thrombosis.

During the follow up for pregnancy there is no recommendation with regards to examination for risk of thrombosis. This is true even if there is a history of unexplained obstetrical events (habitual abortion, fetal death in utero, intrauterine retardation, etc.). Thrombophilia examination is recommended only if there is a history of thromboembolic complication.

There can be multiple genetic reasons for thrombophilia. Among these, in Hungary, Leiden mutation of coagulation factor V is the most common. The high carrier

frequency in the Hungarian population and inexactness of family anamnesis makes it crucial for the Professional College to renew its guidelines.

2. Aims of the thesis

2.1. To characterize the reproductive health status in those with APC resistance

2.2. To examine the effect of OC on APC sensitivity

2.3. To examine antiphospholipid antibody titers in OC users with LFV carriers and FV wildtype genotype.

2.4. To identify factors that significantly influence the development of thromboembolism during pregnancy and in the postpartum period among factor V Leiden carriers.

2.5. To examine the efficacy of LMWH prophylaxis in pregnancy and postpartum period in factor V Leiden carriers

3. Materials and patients

3.1. Study population

Analysis of APC-resistance

We examined 3140 women for resistance to activated protein C using a commercially available kit. We compared the relative risk of thrombosis with and without OC use in the positive and negative result group. We calculated the average and standard deviation (SD) of APC rate from a large population. APC resistance was defined as those have an APC rate below 1 SD of the population average and those with values above this were considered APC sensitive. We analyzed the relative thrombosis risk in the whole study population, in those who used OC in the past, and current OC users. We compared the relative thrombosis risk in participants with positive family history of thrombosis to those who did not. Fertility is one of the most important indicators of reproductive health status. Reproductive problems were estimated as frequency of miscarriages and infertility disorders. We compared the relative risk of miscarriage and infertility in the APC resistant and APC sensitive groups. Among the study population we had detailed obstetric history in 1049 cases, and anamnesis regarding infertility in 1045 cases. Sterility was defined as the inability to conceive even after one year of copulation without contraception, irrespective of the subsequent

outcomes. History of deep vein thrombosis was available in 972 cases, and in 971 cases family history of thromboembolism was also available. During the time of APC sensitivity examination, 570 were using OC, 338 were past OC users and 133 never used OC. Relative risk for thrombosis was determined in all three groups.

In a cross-sectional study, 223 consecutive cases were screened for the presence of antiphospholipid antibodies. All cases were apparently healthy non-pregnant young women and were seeking contraceptive advice. The mean age was 22.5 years (range 11-51 years). Ninety-six of them were on the pill at the time of the study. Comparability of the groups was assessed by generating statistical differences between pill users and non-users in distribution of age, mean total length of past gestations, number of miscarriages, frequency of infertility, family and personal history of deep vein thrombosis and the day of menstrual cycle on which the sample was drawn for antiphospholipid antibody testing. There were no statistically significant differences between pill-users and non-users in mean age (21.8 SD=5.6 years vs. 23.3 SD=8.4 years, $p=0.1379$), mean total length of past gestations (10.1 months vs. 12.7 months, $p=0.5166$), frequency of miscarriage (5/96 vs. 1/127, OR=6.92 95%CI 0.79-60.3), frequency of infertility (3/96 vs. 1/127, OR=4.06 95%CI 0.42-39.7), family history of deep vein thrombosis (13/96 vs. 15/127, OR=1.17 95%CI 0.53-2.59), personal history of deep vein thrombosis (3/127 vs. 0/96, NA) and in the mean day of menstrual cycle on which blood was drawn for antiphospholipid antibody screening (21 vs. 17, $p=0.1709$).

Peripheral blood sample of 10 ml volume was drawn by cubital venipuncture between 8 and 9 o'clock in the morning after 12 hours fasting. Each woman gave consent to participation in the study. The Institutional Ethics Board approved the study protocol.

A case control study enrolled 313 asymptomatic, unrelated, non-pregnant women, with a mean age of 22.9 years, who were seeking contraceptive advice. We compared the anti- β 2-glycoprotein IgG titers with and without oral contraceptive use in Leiden carriers and non-carriers. Sixty-six women were Leiden carriers with heterozygous genotype, and 137 were on pills at the time of the study. The relative frequency of LFV carriers is 9.3% in Hungary. The frequency of LFV women in this study population was much higher (21%) because only a randomly preselected cohort of wild-type women were entered into the study.

A controlled retrospective study was performed on 301 pregnancies of a cohort of 145 LFV-carrier women. Data of individual pregnancies, deliveries, complications and prophylaxis were obtained by structured interview and by review of case-notes. Cases with the presence of additional procoagulant factors such as homozygous LFV, protein S deficiency, prothrombin 20210A variant, antiphospholipid antibodies, lupus anticoagulant, elevated factor VIII level or elevated lipoprotein (a) level were considered as cases with combined thrombophilia. Body mass index (BMI) was calculated by dividing the non-pregnant weight in kilograms by the square of height in meters. The length of gestation was ascertained by first trimester ultrasound measurements. Early pregnancy loss was defined as spontaneous abortion before 13 completed weeks. Cases of late fetal loss included spontaneous abortions between 13 and 24 completed weeks and intrauterine fetal death beyond 24 weeks. Preterm birth was defined as delivery of the fetus before 37 completed weeks of pregnancy. All cases requiring oxytocin augmentation during labor were considered as cases of inertia. We defined placental insufficiency as a case, which is complicated with positive functional tests before labor or with signs of fetal distress during labor. Preeclampsia was defined as persistent hypertension exceeding 140/90 mmHg with >0.3 g/L proteinuria in the absence of pyuria. Fetal growth retardation was defined as fetal weight below the 10th centile for the gestational age. Postpartum hemorrhage was defined as an estimated blood loss exceeding 500mL following delivery of the fetus. Febrile morbidity included cases with a temperature exceeding 38°C on two consecutive days more than 24 hours after delivery. The diagnosis of DVT was based on the results of compression Doppler ultrasound studies. Prophylaxis was started before the end of the first trimester and continued until at least four weeks after the end of the pregnancy. Various regimens were used for prophylaxis with either unfractionated heparin or low molecular weight heparin (LMWH) including enoxaparin, dalteparin and nadroparin. The dosage was based on the manufacturers' recommendation taking into consideration the length of gestation and the patient's weight.

3.2. Resistance to activated protein C (APC)

Resistance to activated protein C (APC) were investigated further for the presence of the Leiden mutation. The sensitivity to APC was determined as the ratio of activated

partial thromboplastin time values measured in the presence and in the absence of APC. APC resistance was defined as a sensitivity ratio below 2.0. The tests were carried out with commercially available kits (Diagnostica Stago, Asnieres, France).

3.3. FV Leiden and FII 20210 A polymorphism detection by PCR

Cases with the Leiden mutation and prothrombin polymorphism were investigated for by polymerase chain reaction according to the protocol. Polymerase chain reaction was used to amplify the gene segment around the site of the mutation. The DNA segments were then digested with endonuclease HindIII and Mn-II. These are then distinguished from wild-type fragments by electrophoresis.

3.4. Measurement of anti-phospholipid antibodies

Anti- β 2-glycoprotein I IgG, IgA and IgM antibodies

Anti- β 2-glycoprotein I antibody levels were determined by ELISA method. Purified human β 2-glycoprotein I was used as antigen (Chrystal Chem.Inc, USA) in 10mg/ml concentration on irradiated 96-well polystyrene plates (Cellstar No 655 180, Greiner Labortechnik, Germany). Antigen-bound antibodies were detected by horseradish peroxidase-labelled anti-human IgG, IgA and IgM antibodies (DAKO AS, Glostrup, Denmark) using O-phenylendiamin- H_2O_2 substrate. According to calibration curves, the level of IgG type antibodies and IgA or IgM were expressed in standardised IgG units (SGU) per ml and in U/ml, respectively. One SGU is equivalent to 1 μ g of affinity purified IgG serum. The upper limit of normal levels of IgG type and IgA or IgM type antibodies were 14.6 SGU/ml and 43.0 U/ml or 34.0 U/ml, respectively. Our Immunology Laboratory where the measurements were carried out is under continuous national and international quality control. The upper limit of normal antiphospholipid antibody levels were set at two SD above the mean of many hundreds of samples from healthy adults.

Anticardiolipin IgG, IgA and IgM antibodies

Anticardiolipin antibodies were measured by ELISA method. Bovine cardiolipin was used as antigen (Sigma Immunochemicals Inc., Mo, USA). The blocking diluents was phosphate buffered saline with 10 % calf serum. The samples were analysed in 1:100

dilution. Antigen-bound antibodies were detected by horseradish peroxidase-labelled anti-human IgG, IgA and IgM antibodies (DAKO AS, Glostrup, Denmark) using O-phenyldiamin-H₂O₂ substrate. According to calibration curves, the level of IgG type antibodies and IgA or IgM were expressed in SGU/ml and in U/ml, respectively. The upper limit of normal levels of IgG type and IgA or IgM type antibodies were 22.0 SGU/ml and 8.0 U/ml or 16.0 U/ml, respectively.

3.5. Statistical analysis

Means of continuous variables were compared with t-test ($p < 0.05$ was significant). Comparison of the two groups in respect to the various categorical and continuous variables was performed with χ^2 - and t -tests, respectively. Differences in the frequency of abnormal results were expressed by odds ratios and 95% confidence intervals (CI) were generated for the assessment of statistical significance.

Logistic regression analysis was performed to identify factors that significantly influence the occurrence of deep vein thrombosis or thromboembolism during pregnancy and within six weeks after the end of the pregnancy. Non-significant factors were removed from the model one by one in the order of decreasing p-values until only significant predictors remained in the model.. All statistical analyses were carried out with the StatView 5.0.1. Software-package (SAS Inc., Cary, NC, USA).

4. Results

4.1 Activated protein C sensitivity as a measure of reproductive health status.

4.2. Antiphospholipid antibodies among LFV carriers and non-carriers with and without OC use.

In the study population the mean age, history, the length of previous pregnancy, number of miscarriage, occurrence of infertility and family history of thrombosis did not differ significantly between users and non-users of oral contraceptives.

The frequency of elevated antiphospholipid antibody levels among pill-users was higher than in non-users. The odds of serum levels above the normal limit of any type of antiphospholipid antibodies were significantly higher among pill-users than in non-users. Pill-users also had significantly higher odds of having an anti- β 2-glycoprotein I IgG level above the normal limit. ANOVA of anti- β 2-glycoprotein I levels between OC users and non-users resulted in an F value of 1.876 corresponding to $p = 0.1723$.

Among pill-users and non-users with high anti- β 2-glycoprotein I antibodies the mean IgG type antibody levels were 19.2 SGU/ml and 20.6 SGU/ml ($p=0.68$). Among pill-users and non-users with high anticardiolipin antibody levels the mean IgG type antibody levels were 37.2 SGU/ml and 32.1 SGU/ml ($p=0.59$), respectively. 19.8% of pill-users and 9.4% of non-users had elevated titer of at least one type of antiphospholipid antibody.

Twenty-three women used a second-generation combined oral contraceptive preparation and seventy-three were taking a third generation pill. The mean duration of use among pill users with and without elevated anti- β 2-glycoprotein I antibody level were 14 months and 25 months ($p=0.3801$). Women with and without elevated level of any type of antiphospholipid antibody had used the pill on average for 14.9 months and 21.3 months ($p=0.4111$), respectively.

Only one case with lupus anticoagulant was found. The woman also had elevated level of IgG type anticardiolipin antibody and was not on the pill. Plasma samples of pill users had a significantly lower sensitivity to activated protein C than those of non-users (2.364 vs. 2.606, $p=0.0102$). APC sensitivity ratio of women with elevated level of any type of the measured antiphospholipid antibodies did not differ from that of women without elevated antibodies (2.442 vs. 2.524, $p=0.4716$). There was no statistically significant difference in APC sensitivity ratio between women with and without elevated anti- β 2-glycoprotein I antibody level (2.396 vs. 2.511, $p=0.4587$). The difference that is apparent, however, was due to the significant effect of pill use (ANOVA $p=0.0139$).

4.3. Pill-use and anti- β 2-glycoprotein I IgG antibody titer among LFV women

Characteristics of pill users and nonusers among LFV carriers and non-carriers

The mean age of pill users (22.3 years, SD \pm 6.6 years) and nonusers (23.4 years, SD \pm 8.7 years) did not differ significantly ($p = 0.1861$). The mean duration of OC use among women with elevated (7.8 months, SD \pm 16 months) and normal (8 months, SD \pm 21.1 months) anti- β 2-glycoprotein I IgG antibody titer was similar ($p = 0.9662$). The mean age of heterozygous women with (23.8 years, SD \pm 8.5 years) and without pill use (28.4 years, SD \pm 12.2 years) did not differ significantly ($p = 0.0778$). There was no significant difference ($p = 0.5844$) in mean age between wild-type genotype

women with (21.8 years, SD \pm 5.8 years) and without pill use (22.3 years, SD \pm 7.3 years).

Among LFV heterozygous women we found elevated anti- β_2 -glycoprotein I IgG antibody titer in six cases during the pill use. Among heterozygous contraceptive users with elevated antibody titer, five were taking a combined pill with second generation progestogen (levonorgestrel, cipropterone or norgestimate) and only one was taking a third-generation formulation (gestodene). Fourteen out of the 26 heterozygous women with normal anti- β_2 -glycoprotein I IgG level were using a pill with second-generation progestogen component. Twelve women were using a third-generation pill (with desogestrel or gestodene). *Among LFV carriers, contraceptive pill users had a higher mean anti- β_2 -glycoprotein I IgG titer than nonusers (9.2 SGU/mL vs. 4.7 SGU/mL, $p = 0.0485$).*

Among women with FV wild-type genotype and elevated anti- β_2 -glycoprotein I IgG antibody titer five, two and two were using a contraceptive formulation with 20 μ g, triphasic 30-40-30 μ g and 30 μ g ethinyl-estradiol, respectively. Out of their 95 wild-type counterparts with normal anti- β_2 -glycoprotein I IgG antibody titer 33, 26, 14, 19 and 3 were taking a pill with 20 μ g, 30 μ g, triphasic 30-40-30 μ g, and 35 μ g and 50 μ g ethinylestradiol content, respectively. The frequency of elevated anti- β_2 -glycoprotein I IgG titer among women with wild-type FV genotype is shown in Table 3. Seven out of the nine wild-type genotype women with elevated anti- β_2 -glycoprotein I IgG titer were using third-generation pill, the other two used a second-generation formulation. Among 95 FV wild-type genotype women with normal anti- β_2 -glycoprotein I IgG titer, 60 and 35 were using third-generation and second-generation pill, respectively. *Among women with FV wild-type genotype there was no significant difference in anti- β_2 -glycoprotein I IgG titers between users and nonusers of OCs (6.4 SGU/mL and 6.0 SGU/mL, respectively; $p = 0.7010$).* The mean titer of anti- β_2 -glycoprotein I IgG among those nine FV wildtype women who had elevated antibody levels during pill use was 23.6 SGU/mL (SD \pm 8.6 SGU/mL). Wild-type nonusers with elevated antibody levels (n = 7) had a mean anti- β_2 -glycoprotein I IgG titer of 27.6 SGU/mL (SD \pm 14.4 SGU/mL). *The odds of an elevated anti- β_2 -glycoprotein I IgG titer during OC use in LFV heterozygous women was 2.41 (95% CI: 0.79–7.39) relative to users with wild-type genotype.*

4.4. Factors affecting thrombosis risk during pregnancy and in the postpartum period among factor V Leiden carriers: indication for selective prophylaxis

Two-hundred out of the 301 pregnancies ended with childbirth and there were 40 spontaneous abortions. Preterm birth, preeclampsia, placental insufficiency, fetal growth retardation, placental abruption, premature rupture of the membranes, dystocia, Caesarean section, febrile complication and major haemorrhage occurred in 29, 13, 27, 9, 4, 14, 12, 33, 10 and 7 cases, respectively. Twenty-five pregnancies were complicated by deep vein thrombosis (DVT) or thromboembolism. Thirty-six pregnancies were conceived with past history of deep vein thrombosis and 43 pregnancies were carried in the presence of combined thrombophilia. Prophylaxis alone did not affect the occurrence of pregnancy associated DVT among factor V Leiden carriers ($p=0.2571$). Prophylaxis did not affect the occurrence of DVT among women with a history of DVT ($p=0.7199$) or among those without a history of DVT ($p=0.1077$), however, in the latter group, DVT occurred only when no prophylaxis was given. Pregnancy associated DVT was strongly associated with combined thrombophilia among factor V Leiden carriers ($p=0.0001$). Among pregnancies that ended with a Caesarean section (18 without prophylaxis, 15 with prophylaxis) DVT occurred only when no prophylaxis was given (5 cases, $p=0.0267$). *In a logistic regression model, factors that significantly influenced the development of deep vein thrombosis included the year of the pregnancy ($OR=1.066$, $p=0.033$), gestational age ($OR=1.058$, $p=0.0089$), the use of thrombosis prophylaxis ($OR=0.118$, $p=0.0227$) and the presence of combined thrombophilia ($OR=5.835$, $p=0.0005$). The frequency of premature rupture of the membranes and Caesarean section was significantly higher in those pregnancies in which thrombosis prophylaxis was employed ($OR=7.102$, $p=0.027$; $OR=4.129$, $p=0.0378$, respectively). Even with adjustment for the presence of combined thrombophilia, the use of prophylaxis significantly reduced the risk of early pregnancy loss ($OR=0.220$, $p=0.0434$).*

5. Discussion

5.1. APC resistance and reproductive health status.

It has been a long standing medical desire to make OC use as safe as possible. The discovery of APC resistance in the 90s and its high prevalence raises the need for

screening. It is a known fact that those with Leiden mutation are at increased thrombosis risk, but population genetic screening is not economical. Furthermore, in those countries where the prevalence of Leiden mutation is below 7% the rationale for APC resistance examination is also questionable. Our study shows that the heterozygous Leiden mutation in the studied population (9.3%) is higher than the Western European average.

Although the American and British guidelines do not suggest population screening, they do emphasise the need for prophylaxis among carriers. The APC sensitivity test seems to be a plausible approach on a population level to screen for carriers.

We carried out the APC resistance functional examination in a total of 3140 women. These results provide appropriate interpretation of the Leiden mutation status. Present OC users were had a significantly lower APC rate as compared to non users. As yet it has not been possible to identify the increased thrombosis risk on the basis of APTT, the relation between APTT and APC rate has not been established.

In our study population the prevalence of APC resistance was 10.91% (n = 317). Our findings prove that a positive family history does not identify those at increased risk for thrombosis. In one of our another study, we showed that the predictive value for thrombosis of a positive family history was only 0.24 as compared to 0.65 for a positive genetic examination result. It can be concluded that the genetic examination are more competent in selecting those who are at increased risk for thrombosis. Although a positive family history has a high sensitivity as a screening method, its predictive value at the individual level is quite poor.

The value of APC resistance screening is highlighted by other reproductive health status indicators as well. Those with APC resistance have a 2.72 fold higher incidence of spontaneous abortion, a 4.04 fold higher incidence of sterility. The experience gained till now indicates that preconceptional thrombosis prophylaxis can, at least in a certain number of cases, result in successful conception. In those who were past OC users the relative risk for thrombosis was 2.51 fold and in current users it was 25.8 fold.

Keeping in mind that APC resistance seriously endangers the reproductive health of Hungarian women, we recommend APC resistance examination for OC users. There is no need to exclude anyone from OC use in case of a positive finding, the ultimate decision lies with the patient, and appropriate knowledge is the key to appropriate decision.

5.2. Antiphospholipid antibodies and use of oral contraception

Both antiphospholipid antibodies and oral contraceptives have been associated with increased risk of thrombosis, ischaemic stroke and myocardial infarction. Regardless of the different magnitudes of their impact on cardiovascular and cerebrovascular morbidity, their combined presence may result in an exacerbated effect. Overlapping characteristics of morbidity of antiphospholipid antibodies and oral contraceptives suggest that there may be common features in their pathomechanisms.

In vitro studies confirmed that anti-b₂-glycoprotein I antibodies may hamper inactivation of factor Va by endogenous activated protein C. Acquired resistance to activated protein C of this mechanism was shown to increase the risk of thrombosis in affected women. Without elaboration on the possible mechanisms involved, the development of acquired resistance to protein C was also observed among oral contraceptive users and in pregnant women.

Results of our study show that elevated antiphospholipid antibody levels occur significantly more frequently among oral contraceptive pill users than among non-users. Combining all types of antiphospholipid antibodies, pill-users had an elevated antibody titre more than twice as frequently as non-users. The higher frequency of elevated antibody titre was related most commonly to IgG type anti-b₂-glycoprotein I antibodies. Among pill-users, the frequency of elevated IgG type anti-b₂-glycoprotein I antibody level was 8.3%. According to data in the literature, 3 % of healthy parous women can be expected to have elevated level titre of this antibody. In our series, 9.4% of healthy women who did not use oral contraceptive pills had elevated levels of antiphospholipid antibodies. It remains unclear whether positive antiphospholipid antibody titres contribute to the morbidity of oral contraceptives causally or they are only concomitant features. Antiphospholipid antibodies are known to play an etiological role in early pregnancy failure and a number of obstetric complications. Screening for antiphospholipid antibodies among asymptomatic oral contraceptive users may not be feasible. However, women that are incidentally found to have elevated antiphospholipid antibodies may do so for the simple reason of being on the pill. Further studies are needed to assess longitudinal changes in antiphospholipid antibody levels during oral contraception, and to explore their relation to cardiovascular and cerebrovascular morbidity.

The higher frequency of elevated anti- α_2 -glycoprotein I IgG titer among OC users is largely due to that among LFV carriers. In other words, LFV carriers seem to respond to OCs with elevated anti- α_2 -glycoprotein I IgG titer more frequently than women with FV wild-type genotype. There is an increase of mean anti- α_2 -glycoprotein I IgG titer among pill users who are carriers of LFV. LFV carriers with elevated anti- α_2 -glycoprotein I IgG titer during OC use might be at an increased risk of thromboembolism. Users and nonusers have similar mean titers among women with wild-type genotype. LFV carriers on the pill have significantly higher mean titer of IgG type anti- α_2 -glycoprotein I antibody than those not using OCs. The clinical relevance of these findings need to tested in future studies.

5.3. Thrombosis profilaxis among pregnant faktor V Leiden carriers

Thromboembolism is a leading cause of maternal mortality. Identifying women at high risk of venous thrombosis and providing them with prophylactic therapy may decrease the incidence of thromboembolism. According to the results of our study, thrombosis prophylaxis decreases the risk of pregnancy associated DVT among factor V Leiden carriers. The effect increases with the length of gestation and is most significant when combined thrombophilia is present or delivery is affected by Caesarean section. Indications for selective thrombosis prophylaxis among asymptomatic pregnant factor V Leiden carriers might be the presence of combined thrombophilia and Caesarean section.

Contradictory data exist in the literature regarding the relation between LFV carriership and fetal loss. Prophylactic therapy with low-molecular-weight-heparin was shown to be effective in preventing maternal floor infarction in a case report of a factor V Leiden carrier with previous fetal demises. Data presented that the use of thromboprophylaxis reduces the risk of early pregnancy loss even with adjustment for the presence of combined thrombophilia.

Carriership of factor V Leiden has been shown to confer evolutionary survival advantage by reducing the risk massive intrapartum haemorrhage. The frequency of massive haemorrhage among carriers (n=56) and non-carriers (n=426) was 2% and 14% respectively. Since the mean blood loss of carriers and non-carriers was 322 ml and 379 ml, respectively. Though significant in statistical terms, the difference is clinically not remarkable. The association between non-carriership and massive

intrapartum blood loss is not uniform. In our series, the relative frequency of massive postpartum haemorrhage was higher than 2% in women who received prophylaxis, however, it was statistically not different from that among women without prophylaxis. The use of low molecular weight heparin for obstetric thromboprophylaxis was shown to be safe in high-risk women. Thrombophilia screening continues to be debated on grounds of predictive value and cost-effectiveness. Despite the growing scientific interest in thrombophilia related issues, there seems to be a resistance to suggestions of screening. Seeking laboratory evidence of heritable thrombophilia in most cases of venous thrombosis is unlikely to lead to information of value in the clinical management of the individual case. The effectiveness and risks of testing for thrombophilia in relation to preventing the first venous thromboembolic event have not been formally assessed.

The rate of venous thromboembolism in asymptomatic family members carrying the factor V Leiden mutation was shown to be too low to warrant continuous anticoagulant prophylaxis. However, the 3.6-times higher incidence of risk period-related venous thromboembolism in carriers compared to noncarriers suggests that asymptomatic carriers might benefit from thromboprophylaxis during risk periods.

Retrospective analysis of the risk of recurrent thrombosis among women who had at least one pregnancy after an episode of VTE showed that the recurrence is 3.5-times more likely to occur during pregnancy than outside pregnancy. Our conclusion that women with previous thrombosis should receive prophylaxis during pregnancy and the postpartum period. The risk of pregnancy-associated recurrence of VTE was shown to be so low that it highly questions the use of routine prophylactic anticoagulation. Universal screening for factor V Leiden together with full-length prophylaxis for carriers in pregnancy was reported to be not cost effective. Closer inspection of the published data allow for a contradictory conclusion. Calculations based on the published model together with its assumptions show that the number needed to treat in order to prevent one event is 324 with universal screening and 1818 with selective screening. These results clearly favor universal screening to selective screening.

Asymptomatic pregnant women who are heterozygous carriers of factor V Leiden are at only a slightly increased risk of venous thromboembolism, therefore pharmacological prophylaxis is not mandatory. However, the beneficial effect of universal prophylaxis on the frequency of early pregnancy loss may support non-

selective use at least in the first trimester. The risk of thromboembolism is moderately increased among women with combination defects. Thromboprophylaxis should be considered for pregnant women in this risk category. Our data suggest that the indication for selective prophylaxis among pregnant factor V Leiden carriers should be based on the presence of additional determinants of thrombophilia.

List of publication related to the thesis

Full length

1. **Vad S**, Lakos G, Kiss E, Sipka S, Csorba R, Póka R: Antiphospholipid antibodies in young women with and without oral contraceptive use. *Blood Coag Fibrinolys* 2003;**14**: 57-60., IF: 1,938
2. Póka R, **Vad S**, Lakos G, Bereczki Z, Kiss E, Sipka S: Increased titer of anti-beta2-glycoprotein I IgG antibody among factor V Leiden carriers during oral contraceptive use. *Contraception* 2004;**69**: 27-30., IF:1,443.
2. Póka R, **Vad S** , Balogh I, Ajzner E: Variable effect of prothrombotic factors on fetomaternal circulation. *Am J of Obst Gyn.* 2005;**193(6)**:2180-2181. IF:3,083
3. **Vad S**, Hermann T, Balogh I et al: Factors affecting thrombosis risk during pregnancy and in the postpartum period among factor V Leiden carriers: indication for selective prophylaxis. *Blood Coag Fibrinolys* 2007 (in press)
4. **Vad S**, Lakos G, Kiss E, Pető E, Póka R: The presence of antiphospholipid antibodies in young women during use of oral contraceptives. *Magyar Nőorv L.*2003;**66**: 3-7.
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6. Póka R, Ajzner É, **Vad Sz**, Pető E.: Activated protein C sensitivity as a measure of reproductive health. *Magyar Nőorv L.*2002;**65**: 267-271.

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1. R Póka, **S Vad**, E Ajzner, I Balogh, G Pfliegler, Z Boda: retrospective analysis of factors affecting pregnancy associated thrombosis risk among factor V Leiden carriers. *Thromb Research* 2005;**1155**, 109-149.
2. **Vad S**, Lakos G, Kiss E, Sipka S, Csorba R, Póka R: Antiphospholipid antibodies in young women with and without oral contraceptive use. 17th European Congress of Obstetrics and Gynecology, Prague, 2002, May 22-25.
3. **Vad S**, Póka R, Ajzner É: Sensitivity to activated protein C as a measure of women's reproductive health. 17th European Congress of Obstetrics and Gynecology, Prague, 2002, május 22-25.

4. Póka R, **Vad S**. Pattern of pill switch in oral contraception. 17th European Congress of Obstetrics and Gynecology, Prague, 2002, May 22-25.
5. **Vad S**, Póka R.: The presence of antiphospholipid antibodies in young women during use of oral contraceptives. Magyar Nőorvos Társaság Észak- Kelet Magyarországi szakcsoport ülése, Mátészalka, 2002. szeptember 21.
6. Póka R, Juhász R, **Vad S**: Factors affecting pregnancy- associated thrombosis risk in factor V Leiden carriers. XVIIth World Congress of Obstetrics and Gynecology, Santiago, Chile, 2003. november 7.
7. **Vad S**, Bereczky Z: Increased titer of anti-beta2-glycoprotein I IgG antibody among factor V Leiden carriers during oral contraceptive use. Ph.D. and Student Scientific Circle Meeting, Debrecen, 2003. February 28.
8. **Vad S**, Póka R, Balla G: Preterm delivery in a lupus anticoagulant positive patient presenting with HELLP syndrome. Magyar Immunológiai Társaság XXXV. Vándorgyűlése, Sopron, 2005. October 19-21.