The frequency of antiphospholipid syndrome and inherited thrombophilia in women with pregnancy loss. A multicentre study in Poland

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Abstract
Antiphospholipid syndrome (APS) and inherited thrombophilia are still viewed as an important cause of pregnancy loss. The aim of our study was to determine the prevalence of antiphospholipid syndrome and inherited thrombophilia in women with pregnancy loss in Poland. We analyzed 155 couples in three groups. The first group included women with at least 3 early miscarriages. The second group consisted of women who had 2 late pregnancy failures. In the third group there were patients with pregnancy loss in the 22nd week of gestation. There were 59 healthy women in the control group. APS was found in 5 (3.2%) of the 155 patients. The frequencies of APS in women with early miscarriages, late miscarriages and stillbirth were 3.9, 3.2 and 2.4% respectively. Inherited thrombophilia was diagnosed in 24 (15.5%) patients. This group involved 5 women (9.8%) with REM, 9 women (14.3%) with LFL and 10 (24.4%) with stillbirth. Antiphospholipid antibodies screening should be offered to women with both recurrent early and late pregnancy loss. In patient experiencing stillbirth screening for the factor V Leiden mutation should be considered despite negative history of thrombosis.

Key words: antiphospholipid syndrome, inherited thrombophilia, pregnancy loss, recurrent miscarriages, venous thromboembolism, placental insufficiency

Introduction
Antiphospholipid syndrome and inherited thrombophilia are still viewed as an important cause of pregnancy loss. Extensive investigations have improved our knowledge of the disease but have also brought new questions warranting of answers. The controversy concerns the frequency of these complications in women with obstetric failure, the mechanisms of pregnancy loss as well as the necessity and method of treatments [1].

Antiphospholipid syndrome (APS) is an autoimmune condition characterized by the production of antiphospholipid antibodies (aPL) combined with certain clinical features. The presence of aPL including antycardiolipins antibodies, lupus anticoagulant and antibodies against β2 glicoprotein I determines high risk of pregnancy loss as well as severe preeclampsia and placental insufficiency [2]. There is no obvious reason for increase for aPL: infection and genetic factors are taken under consideration [3].

Amongst all the mechanisms by which aPL impair fetal growth and development, three are the most important: placenta thrombosis, inhibition of normal placentation and local destruction caused by inflammation [4].

In large meta analyses, the frequency of APS among women with recurrent pregnancy loss is 15-20%, compared with about 5% in non-pregnant women without a history of obstetric complication [5, 6].

How is this issue reflected in Poland? Is the prevalence of APS in women with fetal loss comparable to the incidence of inherited thrombophilia in this group of women?
The incidence of inherited thrombophilia, which is genetic predisposition to venous thromboembolism – VTE is observed in 3-42% of women with pregnancy complications and 15-28% of healthy women without a history of complicated pregnancies [7, 8]. These numbers suggest that the impact of inherited thrombophilia on pregnancy development is less important than it has been assumed. Some authors [9, 10] even conclude that there is no relationship between congenital thrombophilia and recurrent pregnancy losses.

However, the results from the studies of experimentally induced thrombophilia in animals show that both maternal and fetal thrombophilia may lead to abnormal pregnancy development, since maternal and embryonic components regulate local haemostasis in the feto-maternal space and may together determine the risk of disturbances [11].

The aim of our study was to determine the prevalence of antiphospholipid syndrome and inherited thrombophilia in women with pregnancy loss in Poland.

Material and methods

We analyzed 374 couples with pregnancy loss and finally included 155 for further investigations. Patients were recruited from 5 university centers in Poland and were divided into 3 groups according to their pattern of pregnancy failure.

The first group consisted of 51 women with a mean age of 31.6 years who had experienced at least 3 early miscarriages before 10 weeks of gestation (recurrent early miscarriage – REM). The total number of miscarriages in this group was 176. One patient (1.96%) had deep vein thrombosis in the lower left extremity.

In the second group, we enrolled 63 women with a mean age of 30.4 years who had 2 pregnancy failures between 10-21+6 week of gestation (late fetal loss – LFL). In this group, 2 women (3.17%) experienced DVT events.

The third group included 41 patients with a mean age of 30.6 years who had lost their pregnancy in the 22nd week of gestation or later (stillbirth) due to unknown causes. Forty-six pregnancies were lost in this group. Two (4.8%) women had a history of DVT and thrombosis of the pelvis.

The control group consisted of 59 healthy women with a mean age of 32.0 who had each delivered at least one baby at term. None of the women in the is group had a history of miscarriage, stillbirth, thrombosis or autoimmune disease (thyroid gland disorder, rheumatic disease), and none had used hormonal treatment nor smoked, abused alcohol or narcotics during the previous 3 months.

The blood was drawn at least 3 months after pregnancy failure. All 155 patients and 59 controls were tested for the presence of antiphospholipid antibodies (lupus anticoagulant LA, antiphospholipid antibodies-ACA and anti-ß2-glycoprotein-I antibodies – ß2GPI) and congenital thrombophilia (factor V Leiden mutation, prothrombin G20210A mutation, protein C deficiency, protein S deficiency).

When antiphospholipid antibodies were present, tests were repeated after 12 weeks (according to Sydney’s criteria, 2005). Patients with positive antibodies for a second time were classified as patients with antiphospholipid syndrome (APS).

Anticardiolipin antibodies were tested in human plasma using enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin/beta-2-glycoprotein I.

Anti-ß2-glycoprotein-I antibodies were tested using enzyme immunoassay (ELISA) too, for the quantitative measurement of IgG class autoantibodies against beta-2-glycoprotein I in human plasma.

A lupus anticoagulant test was performed using activated partial thromboplastin time (APTT)-based assay and dilute Russell’s viper venom time (dRVVT). Both tests were composed of three steps: screening, mixing and confirmation.

Blood for genetic tests was drawn regardless of the time interval since the pregnancy’s end. Purification of total DNA was performed using QIAamp DNA Blood Mini Kit (Qiagen Inc. Germany). Factor V G1691A mutation and prothrombin G20210A variant was diagnosed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Free protein S was tested using The Monoclonal Free Protein S Antigen assay (ELISA method). Diluted patient plasma was incubated in the wells coated with antibodies specific for human protein S. Bound free protein S was quantified using a horseradish peroxidase. A chromogenic substrate of tetramethylbenzidine and hydrogen peroxide were added to develop a colored reaction. The intensity of the color was measured spectrophotometrically at 450 nm in optical density units.

Protein C was measured using chromogenic assay method. Protein C was activated by a specific fraction from the Agkistrodon conorix snake venom. The amount of activated protein C was determined by monitoring the rate of hydrolysis of a specific chromogenic substrate. The release of pNA (p-nitroaniline) was measured at 405 nm in optical density units.
All tests were done in the Hemostasis Laboratory of J. Strusia Hospital in Poznań. This laboratory has a TUV Rheinland ISO9001.

For statistical analysis StatSoft Statistica v. 10 software was used. Statistical analysis was performed with chi-square contingency tables. \( p < 0.05 \) was considered statistically significant.

**Results**

Antiphospholipid syndrome (APS) was found in 5 (3.2%) of the 155 patients enrolled in the study. The frequencies of APS in women with early recurrent miscarriages (REM), \((n = 2)\) late miscarriages (LFL) \((n = 2)\) and stillbirth \((n = 1)\) were 3.9, 3.2 and 2.4% respectively.

Anticardiolipin antibodies (ACA) were present in both patients with REM. All three types of antibodies (ACA, LA, aβ₂GPI) were found in the serum of one of the two women with LFL; two types of antibodies (LA and ACA) were detected in the serum of the second woman. In the case of a patient with a stillbirth in the 37th week of gestation, anticardiolipin antibodies (ACA) were present.

Antiphospholipid antibodies were found in none of the 59 women in the control group.

Inherited thrombophilia was diagnosed in 24 (15.5%) of the patients included in the study. This group involved 5 women (9.8%) with REM, 9 women (14.3%) with LFL and 10 (24.4%) with stillbirth.

The most common mutation was factor V Leiden, found in 15 of the tested women (9.7%); in 2 (3.9%) of patients with REM, in 5 (7.9%) of the patients with LFL and in 8 (19.5%) with stillbirth.

Prothrombin G20210A gene polymorphism was detected in total in 3 (1.9%) women (2 with REM and 1 with stillbirth).

Protein S deficiency was found in 4 (2.6%) patients, and protein C deficiency was found in 3 (1.9%) patients. In one patient with REM, protein S and C deficiencies were found simultaneously. Also, one patient with REM had both antiphospholipid antibodies and the prothrombin G20210A gene polymorphism.

In the control group, there were 3 cases (5.5%) of inherited thrombophilia. Two women were carriers of the factor V Leiden mutation, and 1 exhibited protein C deficiency (tab. 1).

**Discussion**

Laboratory criteria of APS were fulfilled only by 3.2% women with pregnancy loss. The highest 3.9% prevalence was found in women with early recurrent miscarriage and the lowest – 2.4% in patients with stillbirth.

In comparison with other authors it is low APS frequency. According to Petri et al. the positive LA, IgG or IgM ACA test is present in more than 20% of patients with recurrent pregnancy loss or recurrent miscarriages [12]. It should be pointed out that above mentioned authors took into consideration only two types of antibodies: LA and ACA. Oshiro et al. in his study involving 366 women with two or more consecutive pregnancy losses, found presence of LA and ACA in titer of > 20 GPL in 21.5% women [13].

Current APS criteria, modified in Sydney in 2005, include the third type of antibody – anti B2GPI and prolong testing intervals to 12 weeks [14]. Despite these new criteria, the frequency of APS as the most common reversible cause of recurrent pregnancy loss, according to RCOG guidelines published in April 2011, has been estimated still on 15% [15].

Robertson found much higher rate of aPL positive in recurrent pregnancy loss women – 40% [16].

**Tab. 1. Results of APS and hereditary thrombophilia tests in women with pregnancy loss**

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>REM ( n = 51 )</th>
<th>LFL ( n = 63 )</th>
<th>Stillbirth ( n = 41 )</th>
<th>Controls ( n = 59 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>APS</td>
<td>2 (3.9%)</td>
<td>2 (3.2%)</td>
<td>1 (2.4%) ACA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2-ACA</td>
<td>1-LA, ACA, anti-B₂-GPI</td>
<td>1-LA, ACA</td>
<td></td>
</tr>
<tr>
<td>Inherited</td>
<td>5 (9.8%)</td>
<td>9 (14.3%)</td>
<td>10 (24.4%) *</td>
<td>3 (5.5%) *</td>
</tr>
<tr>
<td>thrombophilia</td>
<td>2-FV Leiden</td>
<td>5-FV Leiden</td>
<td>8-FV Leiden</td>
<td>2-FV Leiden</td>
</tr>
<tr>
<td></td>
<td>1-PC + PS def.</td>
<td>2-PC def.</td>
<td>1-PS def.</td>
<td></td>
</tr>
</tbody>
</table>

REM – recurrent early miscarriage; LFL – late fetal loss; APS – antiphospholipid syndrome;
ACA – anticardiolipin antibodies; LA – lupus anticoagulant; anti-B₂-GPI – anti-B₂-Glycoprotein I antibodies;
PS def. – deficiency of protein S; PC def. – deficiency of protein C; * – significant difference with controls \( (p < 0.001) \)
Substantial discrepancy in frequency of APS (2.4-40%) in women with pregnancy loss is a result of the use of non-standardized serums, single aPL test or varied criteria of early and late pregnancy loss [17].

In our material the frequency of APS in women with early recurrent pregnancy loss (< 10 week gestation) was 3.9%. Simultaneously, in Branch et al. study, including 288 patients with recurrent miscarriage, only 2.4% met APS criteria [18].

It must be underlined that presence of aPL in low titters (< 99 percentyl or < 40 GPL or MPL) does not justify the diagnosis of APS. On the other hand strict adherence to the APS criteria would lower the frequency of the disease in women with repeated pregnancy loss (RPL). It is because one of the criteria for APS testing is the presence of morphologically healthy fetus. However scanning of fetal anatomy at or before 12 week gestation is often very challenging or sometimes impossible. Only cytogenetic screening at 7 or 10 weeks gestation can confirm normal fetal development. RCOG guidelines recommend genetic testing in diagnosis of recurrent miscarriage only after third consecutive miscarriage [15]. According to Stephenson genetic screening of all miscarried embryos would help in over 40% of patients to reduce overdiagnosis of APS in women with RPL [19].

In our country cytogenetic examination of conception products is limited and expensive. The most common molecular probes specific for chromosomes X, Y, 13, 16, 18 and 21, can only detect a total of 85% chromosomal aberrations found in all miscarriages [20]. Microarray technology is much more effective method [21]. We expect that lowering costs of genetic testing will allow it to become a standard management. We must bear in mind that chromosomal aberration in the embryo do not exclude APS in the mother. Finding a chromosomal aberration in the material from third miscarriage should not exclude the presence of maternal aPL, no matter if the father is the same or different. Evidently, aPL carrier status in the father has a much worse prognosis.

Question arises, whether we should wait until third miscarriage to start aPL screening? Although aPL screening after just one miscarriage does not have any medical neither economical indication, it is believed it is worth investigating the present of LA and ACA to offer already after one miscarriage in women over 35 years old.

There are many women with pregnancy losses typical for APS seeking medical advice, but none of them have any of aPL (ACA, LA or aB2GPI) detected. For these women McCarthy coined a definition of seronegative APS [22]. However is this syndrome an indication for pharmacological prophylaxis in consecutive pregnancy? From multicentre studies published in 2012 it is believed that LA but not aCL is the strongest predictor of recurrent pregnancy loss [23].

We did not confirm these observation in our study of Polish population. All women diagnosed with APS had anticardiolipin antibodies.

In 3 patients with late pregnancy losses we found all three antibody types. The presence of all three antibody types according to Ruffatti et al. has a negative impact on future pregnancy [24]. On their report involving 97 pregnancies in 79 patients with and without APS triple positivity conferred a risk of late fetal loss of 52.6% compared with a loss rate of 2.2% when only two tests were positive. In our material in one of patients diagnosed with three types of antibodies, in both subsequent pregnancies intrauterine fetal demise at 24 week gestation occurred despite the fact that proper treatment was employed. In both pregnancies rising levels of ACA IgM was observed. Nielsen and Christiansen found that IgM ACA is of better predictive value on pregnancy outcome than any other aPL type [25].

From our observations it seems that APS is either over diagnosed or under estimated. It is a result of only single antibody testing or diagnosing APS relying on low titers of aPL.

In our mind the establishment of central laboratories is the proper direction, in order to avoid false positive results and thus unnecessary treatment. Perhaps APS laboratory criteria should be validated? The need to wait 12 weeks for the second test from the first aPL screening in women with pregnancy loss causes controversies and many women resign from the diagnostic process and become pregnant before the potential cause of pregnancy failure is clarified.

Inherited thrombophilia was much more common in our patients (15.5%). Unlike APS, the highest frequency (24.4%) was observed in the stillbirth group and the lowest (9.8%) in patients with recurrent early miscarriages.

The common presence of inherited thrombophilia in healthy Caucasian population questions its influence on pregnancy course. Bellver et al. [7] estimated the frequency of inherited thrombophilia in healthy population at 28.1% and Kuperminc et al. [26] at 15-17%. That is why above mentioned authors do not recommend screening for inherited thrombophilia in Caucasian population. In our control group consisting of 59 healthy women, with previous successful pregnancy, the frequency
of inherited thrombophilia was 5.5%. Two women were carriers of factor V Leiden mutation, in one protein C deficiency was found and in the study group the frequency of inherited thrombophilia was estimated at 15.5%.

Results of our study revealed substantial correlation between factor V Leiden mutation and stillbirth but not with early recurrent miscarriages neither late miscarriage. From 41 women with stillbirth, 17.8% were factor V Leiden mutation carriers. Very similar conclusion had Abu-Asab et al. [27] in the study of Palestinian women employing identical classification of recurrent pregnancy losses and stillbirth. Also in EPCOT [28] report the risk of stillbirth in thrombophilic women was higher than the miscarriage risk (3.6 vs 1.3).

In the light of these results it is hard to accept in clinical practice American College of Chest Physicians (ACCP) guidelines [29] which in women with inherited thrombophilia and pregnancy failures in the history, do not recommend screening nor antithrombotic prophylaxis.

In the literature there is no consensus upon the impact of G20210A prothrombin gene mutation on intrauterine demise. Only Norwegian authors found correlation with intrauterine fetal death with OR of 4.0 (95% CI 1.1-1.4). There were no such correlation neither for factor V Leiden mutation or antithrombin deficiency [30].

In our study from 41 women with stillbirth only one was a G20120A prothrombin gene mutation carrier.

Even more controversial is relationship between inherited thrombophilia and early recurrent miscarriages. Reznikoff-Etievant et al. [31] examined 260 women with two or more miscarriages before 10 week gestation and found that 10.3% of these women were factor V Leiden mutation carriers in comparison to 4.6% women in the control group. Authors concluded that factor V Leiden mutation is related to early recurrent pregnancy losses. Adopting the same time criteria for early miscarriages (<10 weeks gestation) we had different results. Among 51 women with early recurrent pregnancy loss only 3.9% were factor V Leiden carriers with almost the same frequency of this mutation in the control group (3.4%).

There were 79 studies included in Robertson et al. [16] metaanalysis. Twenty five papers (7167 women) analysed an association between thrombophilia and early pregnancy loss and 15 (4038 women) between thrombophilia and late losses. The OR for early pregnancy loss for heterozygotes of factor V Leiden mutation was 1.68 (95% CI 1.09-2.58) and for late pregnancy loss was 2.06 (95% CI 1.10-3.86). Authors concluded that indeed thrombophilic women are at risk of thrombotic events and increased pregnancy complications, however the absolute risk still remains low.

Screening of 101 German women did not confirm correlation between factor V Leiden mutation, factor II and early (<12) or late pregnancy losses. The occurrence of factor V Leiden mutation was 8.7 and 3.1% respectively and 7.4% in the control group [10].

Recent analysis revealed that the factor V Leiden mutation and the presence of aPL may play a role in pathogenesis of stroke in perinatal period not only in mothers but also in the foetuses [32].

The variable association between inherited thrombophilia including APS and pregnancy loss cannot be currently viewed as a causal one [17, 33]. That is why the decision for inherited thrombophilia screening should be based on individual risk and benefits from testing. Nevertheless, finding coagulation disorder, does not always require therapeutic intervention.

Conclusions

Antiphospholipid antibodies screening should be offered to women with both recurrent early and late miscarriages

In patient experiencing stillbirth, testing for the factor V Leiden mutation should be considered despite negative thrombotic history.

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References

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