The prevalence of non organ specific and thyroid autoimmunity in patients with polycystic ovary syndrome

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Background. Polycystic ovary syndrome (PCOS) is commonly associated with endocrine, metabolic, cardiovascular and other morbidities. However its association with autoimmune diseases is still controversial.

Aim. The aim of this study was to assess the prevalence of non organ-specific and antithyroid, antibodies in PCOS women compared to healthy controls.

Methods. The study included 152 women with PCOS and 76 healthy controls for the evaluation of non organ-specific autoimmunity and 64 PCOS and 68 controls for the study of organ-specific autoimmunity. All sera were tested for autoantibodies using the ELISA method.

Results. There were no significant differences in the prevalence of ANA, SSA, SSB, anti-dsDNA, anti-RNP, ANCA/MPO or ANCA/PR3 between PCOS and controls. The prevalence of ACLA IgG was higher in controls than PCOS (5.4% v.s. 0%, $P=0.011$). Patients had a higher prevalence of anti-TPO antibodies (18.75% v.s. 7.35%, $P=0.045$) and slightly but not significantly higher prevalence of autoimmune thyroiditis (18.75% v.s. 10.29%) than controls.

Conclusion. The prevalence of non organ-specific autoantibodies in PCOS women is low and similar to controls. On the other hand, we found a slightly higher prevalence of thyroid autoimmunity in PCOS women.

Key words: autoimmunity, autoimmune thyroid disease, polycystic ovary syndrome, systemic autoantibodies, hyperandrogenism

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of child-bearing age. It is typically identified during the early reproductive years or adolescence and its prevalence is as high as 15% when the broader Rotterdam criteria are applied and about 10% according to the criteria of the Androgen Excess Society (AES) (ref.1). PCOS is associated with significant endocrine, metabolic, cardiovascular, reproductive as well as psychiatric morbidities2,3.

The etiology of PCOS remains obscure but evidence exists for a multifactorial origin with a genetic predisposition. An autoimmune mechanism has been suggested in some cases of PCOS especially those with other autoimmune disorders4,5. Unfortunately the heterogeneity of the syndrome, variety of ovarian antigens as well as antibody tests have led to conflicting results.

It is well accepted that sex hormones (estrogens and androgens) may play a significant role in the mosaic of autoimmunity. High levels of androgens are presented in patients who suffer from PCOS seeming to have a protective role against the development of autoimmune diseases; however several mechanisms related to estrogen effects on the immune system oppose this activity. Estrogens increase the secretion of IL-4 in Th2 lymphocytes, IL-1 in monocytes, IL-6 in T-lymphocytes and interferon-γ in Th1 cells and their stimulatory effect on the immune system was inhibited by progesterone (ref.8-10). Patients with PCOS present low levels of progesterone due to oligo/anovulation leading to over-stimulation of the immune system and to autoantibody production. Whether the estrogen or androgen component more strongly influence the immune system, is still unknown. Therefore, we suggest, this unique hormonal equilibrium in women with PCOS could be a suitable model for studying the hormonal influence on autoimmune diseases.

In the first part of this study we examined the prevalence of non-organ specific autoimmunity in PCOS women and compared it with non PCOS controls. Secondly we studied the occurrence of thyroid autoimmunity and autoimmune thyroid disease.
SUBJECTS AND METHODS

The study included 152 women with PCOS that were primarily identified in an outpatient department of gynecology, endocrinology and diabetology and subsequently examined in the 1st internal medicine department, University Hospital of Louis Pasteur, Kosice, Slovak Republic. The average age was 30.23 ± 6.7 (range 14-50 years). All patients fulfilled the Rotterdam diagnostic criteria for PCOS\(^1\). The exclusion criteria were history of known autoimmune disease and concurrent thyroid dysfunction. Other pathologic conditions which might influence menstrual cycle including pituitary diseases (hyperprolactinemia, hypopituitarism etc.), adrenal disorders and severe comorbidities were ruled out by careful examination. Among all PCOS women 49\% of them had clinical hyperandrogenism manifested as hirsutism, 51\% of patients had no hirsutism. 26\% had normal regular menstrual cycle and 74\% presented with menstrual irregularities such as oligomenorrhea or amenorrhea. According to body weight 42\% of patients had normal BMI, 19\% were overweight, and 39\% were obese.

The control group consisted of 76 healthy regularly menstruating randomly selected age matched women with no history of autoimmune disease or apparent hyperandrogenism. The average age was 29 ± 4 (range 22-45 years). None of them was taking hormonal contraception. All subjects signed an informed consent. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

The study was divided into 2 parts: 1. organ non-specific autoimmunity in PCOS patients and 2. Organ specific, i.e. anti-thyroidal autoimmunity in PCOS patients.

Laboratory assessments

Blood samples were taken in an early follicular phase of a menstrual cycle. In case of amenorrhea, blood was taken after excluding pregnancy. Hormone levels of sex hormone binding globulin (SHBG), luteinizing hormone (LH), and follicule-stimulating hormone (FSH) were determined using chemiluminiscent immunoassay (analyzer Architect, module C). Free testosterone (fTST) was detected by radio-immunoassay using commercially available kits of DiaSource.

Study of non organ-specific autoimmunity

In this part of the study, blood samples were collected to assess a wide spectrum of non organ specific autoantibodies, such as: antinuclear (ANA), extractable nuclear (ENA), double stranded DNA (dsDNA) antibodies, antibodies against ribonucleoprotein (RNP) and vasculitis associated antibodies, such as antineutrophil cytoplasmic (ANCA) and antcardioliopin antibodies (ACLA).

Serum was obtained by centrifugation on centrifuge Capricorn, in 3g during 7 min and consequently frozen in minus 40 °C. In parallel, a large profile belonging to 2 groups of autoantibodies was tested in all sera from the first study.

ANA/Hep2 IFT screening: screening for autoantibodies implemented in all sera using an indirect immunofluorescence test (IFT) (diagnostic kit Hep-2 cell IFA Kit, serum dilution 1:80). Only sera positive in ANA/Hep2 IFT were further evaluated by ELISA on 8S ELISA ANA screening (ANA ELISA diagnostic kit 8S, dilution of sera 1:101, a positive reaction > cut off).

dsDNA screen performed by ELISA method (diagnostic kit AIDA dsDNA-G, Germany, dilution of sera 1:101, positive titer > 18 U/mL)

ANCA screen: performed by ELISA (diagnostic tests AIDA VASCULITIS SCREEN, sera dilution 1:101, positive titer > 18 U/mL)

ANCA specification: sera positive in screening were further tested for antibodies against MPO and PR3 antigens using ELISA diagnostic set AIDA MPO and AIDA PR3 Sensitive. Sera dilution was 1:101 and titers > 18 U/mL were considered positive in both cases

ACLA screen: antcardioliopin antibodies were tested using ELISA CardiolipinCheck tests, sera dilution 1:101, cut-off >24.0 U/mL

ACLA specification: sera positive in screening were tested for isotypes ACLA IgG and ACLA IgM with ELISA Cardiolipin GM tests, sera dilution 1:101, cut-off >18.0 U/mL

Study of thyroid autoimmunity

This part of the study, included 64 PCOS patients, age 31 ± 4.5 (range 23-42) years and 68 controls, average age 29 ± 3.9 (range 22-45) years. Blood samples were taken for the assessment of serum fT3, fT4, TSH and thyroid autoimmunity (anti-TG, anti-TPO). We also studied the prevalence of autoimmune thyroid disease (AIT). In both groups, the Diagnosis of AIT was based on elevated serum antibodies against thyroglobulin (anti-TG) and/or antibodies against thyroperoxidase (anti-TPO) with simultaneous finding of hypoechoic thyroid gland on ultrasound. FT3 and FT4 were measured by radioimmunoassay using the automatic analyser Statrec (normal ranges FT3 2.5-5.8 pmol/L, FT4 11.0-25.0 pmol/L), ultrasensitive TSH was evaluated using the kits Immunotech (France), normal range 0.17-0.45 mIU/L. Chemiluminiscent immunoanalysis was used to evaluate antithyroid autoantibodies.

Thyroid ultrasonography was performed using 2-dimensional ultrasonography ESAOTE TECHNOX MPx with linear 12 MHz probe.

Statistical analysis

Values are given as means ± SD. Groups were compared using t-tests or Mann Whitney U test depending on the distribution. Two-tailed Fisher’s exact tests or Chi-square tests were used as appropriate for comparison of categorical variables between groups. \( P \) values of < 0.05 were considered statistically significant. For statistical analysis, we used SPSS statistical software system (SPSS 17.0; SPSS Inc., Chicago, IL). Confidence intervals were estimated using the Wilson method with continuity corrections for low expected counts.
RESULTS

As expected, PCOS women had significantly higher BMI compared to controls (28.08 ± 6.91 vs 21.31 ± 3.05, *P*<0.001), significantly higher fasting glucose level (4.81 ± 0.98 vs. 4.58 ± 0.41 mmol/L, *P*=0.023), higher free testosterone level (3.82 ± 1.31 vs. 3.18 ± 0.88 pg/mL, *P*<0.002) and higher LH (8.42 ± 6.41 vs. 5.47 ± 2.61 IU/L, *P*<0.001). We also confirmed lower levels of SHBG in PCOS women in comparison to the healthy population (43.44 ± 31.84 vs. 69.29 ± 28 nmol/L, *P*<0.001).

**PCOS and non organ specific autoimmunity**

The prevalence of non organ specific autoantibodies in PCOS women and controls is shown in Table 1. There were no significant differences in the prevalence of ANA screening, SSA/Ro, SSB/La, anti RNP, anti ds-DNA between PCOS and control women, which was very low in both groups. The prevalence of ANCA screen positivity was significantly higher in controls than PCOS patients (*P*=0.042), however when specified, the frequencies of ANCA/MPO and ANCA/PR3 were similar in both evaluated groups.

There were significant differences in the positivity of ACLA IgG between examined groups, i.e. the control group had significantly higher prevalence of ACLA IgG than the PCOS women (*P*=0.01). There were no significant differences in occurrence of the ACLA IgM positivity.

**PCOS and antithyroid autoimmunity**

Mean values of serum TSH, FT4, anti-TG and anti-TPO are demonstrated in Table 2.

There were no significant differences in serum TSH and FT4 levels between PCOS and controls. In addition serum levels of anti-TG and anti-TPO were similar in both evaluated groups.

The prevalence of anti-TG, anti-TPO positivity and the prevalence of AIT, hypo- and hyperthyroidism are shown in Table 3.

The prevalence of autoimmune thyroid disease (based on the above criteria) was slightly but not significantly

### Table 1. Prevalence of non organ specific autoantibodies in PCOS women and controls.

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>PCOS (n=152)</th>
<th>Controls (n=74)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>Percentage prevalence (95% CI)</td>
<td>Number of cases</td>
</tr>
<tr>
<td>ANA screen</td>
<td>1</td>
<td>0.66% (0.03-0.417)</td>
<td>2</td>
</tr>
<tr>
<td>Anti-SSA</td>
<td>1</td>
<td>0.66% (0.03-0.417)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-SSB</td>
<td>0</td>
<td>0% (0-3.07)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>0</td>
<td>0% (0-3.07)</td>
<td>0</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>3</td>
<td>1.97% (0.51-6.11)</td>
<td>0</td>
</tr>
<tr>
<td>ANCA screen</td>
<td>6</td>
<td>3.95% (1.62-8.78)</td>
<td>9</td>
</tr>
<tr>
<td>ANCA/MPO</td>
<td>0</td>
<td>0% (0-3.07)</td>
<td>0</td>
</tr>
<tr>
<td>ANCA/PR3</td>
<td>0</td>
<td>0% (0-3.07)</td>
<td>1</td>
</tr>
<tr>
<td>ACLA IgG</td>
<td>0</td>
<td>0% (0-3.07)</td>
<td>4</td>
</tr>
<tr>
<td>ACLA IgM</td>
<td>1</td>
<td>0.66% (0.03-0.417)</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2. Mean serum levels of thyroid parameters in PCOS women and controls.

<table>
<thead>
<tr>
<th>Mean serum levels</th>
<th>PCOS (n=64)</th>
<th>Controls (n=68)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (mIU/L)</td>
<td>2.37 ± 1.46</td>
<td>2.37 ± 1.46</td>
<td><em>P</em>=0.937</td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td>16.36 ± 5.34</td>
<td>16.49 ± 2.32</td>
<td><em>P</em>=0.852</td>
</tr>
<tr>
<td>aTG (IU/mL)</td>
<td>53.09 ± 157.07</td>
<td>29.8 ± 100.77</td>
<td><em>P</em>=0.386</td>
</tr>
<tr>
<td>aTPO (IU/mL)</td>
<td>59.74 ± 149.03</td>
<td>45 ± 204.77</td>
<td><em>P</em>=0.805</td>
</tr>
</tbody>
</table>

### Table 3. Prevalence of antithyroid antibodies, AIT and thyroid dysfunction in PCOS women and controls.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PCOS (n=64)</th>
<th>Controls (n=68)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ aTG</td>
<td>5</td>
<td>7.81%</td>
<td>3</td>
</tr>
<tr>
<td>+ aTPO</td>
<td>12</td>
<td>18.75%</td>
<td>5</td>
</tr>
<tr>
<td>AIT</td>
<td>12</td>
<td>18.75%</td>
<td>7</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>7</td>
<td>10.94%</td>
<td>9</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>1</td>
<td>1.56%</td>
<td>0</td>
</tr>
</tbody>
</table>
higher in PCOS patients. Moreover, there were no significant differences in the occurrence of thyroid dysfunction ($P=0.082$ for hypothyroidism, and $P=0.301$ for hyperthyroidism) between groups. The frequency of anti-TG positivity was similar in PCOS and controls however the prevalence of anti-TPO positivity was significantly higher in PCOS women. The relative risk for positivity of aTG and aTPO in patients with PCOS in comparison with healthy women was better estimated by calculating the odds ratios with 95% CI using the Mantel-Haenszel test. As shown in Table 4, women with PCOS had a more than 2-fold higher risk for AIT and almost a double risk for positivity of both types of anti-thyroid antibodies.

**DISCUSSION**

According to the best our knowledge this is the biggest study on autoimmunity in PCOS patients and the widest spectrum of non organ specific autoantibodies examined to date. The results did not confirm any higher frequency of non organ specific autoantibodies in PCOS women than healthy women. Moreover, there was significantly higher prevalence of ACLA IgG antibodies in controls, suggesting that the hyperandrogenic state in PCOS may be a protective factor against the autoimmune response.

Data on the prevalence of non organ-specific autoimmune disorders in PCOS are poor and controversial. There is evidence that estrogen excess has been linked to different autoimmune diseases\(^{14,15}\). Because of chronic anovulation and luteal insufficiency, PCOS women have relative hyperestrogenism which could promote the autoimmune response. On the other hand, high levels of androgens presenting in these patients seem to have a protective role in the development of autoimmune diseases, which is in agreement with our results.

There are few papers demonstrating the presence of various non organ-specific antibodies in PCOS women. Former studies on relatively small groups of patients demonstrated a higher prevalence of ANA positivity in PCOS women\(^{14,15}\). Recently, Heffter-Frischmuth et al., evaluated sera from PCOS patients for the presence of non organ-specific autoantibodies (ANA, anti-histone, anti-nucleosome and anti-dsDNA). Statistical significance was found only in the levels of anti-histone and anti dsDNA antibodies\(^{16}\). In another study, a significantly higher prevalence of ANA and SMA was found in PCOS patients compared to controls (19.4% v.s. 5.1%). The prevalence of other antibodies was very low and similar to that in the control group\(^{16}\). This is particularly in contrast to our study, demonstrating very low occurrence of the ANA positivity in the PCOS group. The prevalence of anti SSA, SSB, anti dsDNA, anti RNP antibodies was also very low and comparable with the results of above mentioned study.

Although ANCA screen in our study demonstrated higher frequency of positivity in controls, further evaluating of ANCA (ANCA/MPO) did not detect any differences between PCOS and controls. In contrast we found a higher prevalence of ACLA IgG in control group as compared to PCOS women. Recently one study showed lower levels of IgM anti-phosphorylcholine antibodies in PCOS patients as compared to healthy women which is in agreement with our results\(^{16}\). All these findings might indicate that PCOS women could be protected against autoimmune diseases, which is probably related to hyperandrogenemia.

In the second part of this study we demonstrated similar plasma levels of fT4, TSH and also significantly higher prevalence of anti-TPO positivity in patients compared to controls. The overall prevalence of AIT was slightly but not significantly higher in PCOS women. Previous data on the association between PCOS and AIT are controversial. Some reports documented higher prevalence of AIT and higher levels of TSH in PCOS women. Although we found 13% prevalence of hypothyroidism in apparently healthy women, some data suggest that up to 18% of the general population might suffer from hypothyroidism\(^ {17}\). In a Chinese population of PCOS women higher levels of TSH were documented only in women with a LH/FSH ratio over 3 (ref.\(^ {18}\)). Janssen et al. presented significantly higher prevalence of anti-TPO and/or anti-TG antibodies in PCOS patients, higher prevalence of AIT and also higher levels of TSH in PCOS women\(^ {19}\). Comparably, significantly higher titer of anti-TPO antibodies and a higher prevalence of goiter with AIT characteristics were shown in 78 PCOS Iranian women compared to a healthy population\(^ {20}\). In contrast, a higher prevalence of PCOS in euthyroid girls with AIT has been demonstrated in a recent study\(^ {21}\). These data suggest a possible role of autoimmunity in the etiopathogenesis of PCOS but this hypothesis is unproven as a recent study clearly documented that PCOS alone was not associated with thyroid disease and the metabolic syndrome or its components appear to be related to thyroid volume, function and antithyroid antibody levels\(^ {22}\). Because the BMI in our patients was significantly higher than in controls, we assume that slight differences in the occurrence of anti-TPO prevalence may be related to obesity and insulin resistance.

Finally an autoimmune mechanism of PCOS has been suggested by some authors. According to Gleicher et al. this disease might be an opposite condition to premature ovarian failure\(^ {23}\). Suh et al. reported histological findings in a case of PCOS consistent with autoimmune oophoritis\(^ {24}\) and Lee et al. described a case of a patient with combined PCOS and autoimmune polyglandular syndrome type 2 (ref.\(^ {25}\)). Recently Chapman described a fundamental role of thymus and T cells in estrogen

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>aTG</td>
<td>1.836</td>
<td>0.428-8.019</td>
</tr>
<tr>
<td>aTPO</td>
<td>2.988</td>
<td>0.986-9.056</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>0.922</td>
<td>0.314-2.710</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>AIT</td>
<td>2.244</td>
<td>0.829-6.071</td>
</tr>
</tbody>
</table>

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induced follicle cyst formation. However studies of auto-antibiotic antibodies performed so far have yielded conflicting results. The group of Lonsdale demonstrated ovarian antibodies, and lymphocytic infiltration of ovaries in patients with PCOS (ref.27). Many other targeting antigens of ovarian immune pathology have been described in PCOS women. In another study, positive auto-antibiotic antibodies for at least one isotype were present in 44% of the PCOS women. However these results could not be confirmed by others. Nevertheless this aspect of PCOS pathogenesis is still open for further research and requires further studies of other antibodies against various ovarian structures.

We conclude that the prevalence of non organ-specific autoantibodies in PCOS women is very low and similar or even lower than in healthy controls. On the other hand, we found a slightly higher prevalence of thyroid autoimmunity in PCOS women but this finding may be related to the metabolic syndrome rather than to PCOS alone.

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Authorship contributions: JP, JF: manuscript drafting; JV: supplied sera from PCOS patients; ID: provided PCOS patients; DK, ZV, JR: laboratory investigations; IL: manuscript revising.

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