

THE REASSESSMENT OF ANTIOVARIAN ANTIBODIES TEST PERFORMANCE IN THE DIAGNOSIS OF PREMATURE OVARIAN FAILURE IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

MIHAELA CIOCAN¹, I. GH. TOTOIANU², L. PUȘCAȘIU³

¹Doctorand. Universitatea „Lucian Blaga“ din Sibiu, ² Universitatea „Lucian Blaga“ din Sibiu, ³ Universitatea de Medicină și Farmacie Tîrgu-Mureș

Keywords: premature ovarian failure, antiovarian antibodies, autoimmune thyroid disease

Abstract: Objective: To reassess the antiovarian antibodies test performance in the diagnosis of premature ovarian failure of the patients with autoimmune thyroid disease. Material and method: The study involved 70 patients with autoimmune thyroid disease, from which ten cases (10/70, 14.4%) were associated with premature ovarian failure and were found to have serum antiovarian antibodies, using as reagent a mixture of ovarian proteins; the values greater than 10 IU/mL were considered positive. Results: Antiovarian antibodies were positive in one case (1.4%, 1/70) and was not a case of premature ovarian failure; in the same case antinuclear antibodies were positive. Conclusions: Determination of the circulating antiovarian antibodies as a possible predictive factor for premature ovarian failure is not really sustained, especially because it doesn't exist a recognized specific marker of the ovarian autoimmunity, and the study results are statistically insignificant.

Cuvinte cheie: insuficiență ovariană prematură, anticorpi antiovariari, boală tiroidiană autoimună

Rezumat: Obiective: Reevaluarea performanțelor determinării anticorpilor antiovariari circulanți în diagnosticul insuficienței ovariene premature la pacientele cu boală tiroidiană autoimună. Material și metodă: Studiul include 70 de paciente cu boală tiroidiană autoimună, din care 10 (10/70, 14.4%) au avut insuficiență ovariană prematură, la care s-au determinat anticorpii antiovariari din ser, utilizând ca reagent o mixtură de proteine ovariene; valorile mai mari de 10 UI/mL au fost considerate pozitive. Rezultate: Anticorpii antiovariari au fost pozitivi într-un singur caz (1.4%, 1/70), care nu a fost dintre cazurile cu insuficiență ovariană; în același caz anticorpii antinucleari au fost pozitivi. Concluzii: Determinarea anticorpilor antiovariari circulanți ca posibil factor predictiv pentru insuficiența ovariană prematură nu este argumentată, în condițiile în care nu există un marker specific recunoscut al autoimunității ovariene și rezultatele studiului sunt nesemnificative statistic.

INTRODUCTION

Highlighting specific autoantibodies is widely accepted as a marker of autoimmune diseases (1, 2). Methods for identifying ovarian autoimmunity, such as detecting antiovarian antibodies (AOA) in circulation or the presence of lymphocytic infiltration on sections of ovary biopsy, are controversial. The onset of the ovarian failure following the autoimmune oophoritis is difficult to prove through the detection of circulating AOA, as it is actually the clinical expression of a final stage of the autoimmune disease, characterised by the disappearance of ovarian follicles and, therefore, of the antigen target (3).

Premature ovarian failure (POF) is rare, affecting ~ 1% of women. It is conventionally defined as secondary amenorrhea of at least 4-6 months before the age of 40 with at least two postmenopausal levels of follicle stimulating hormone (FSH) > 40IU/L at intervals of 1-3 months (4, 5, 6). It represents the nonphysiologic cessation of the ovarian activity before the The aetiology remains unknown in most cases, even up to 90%, idiopathic POF. The remaining cases can have a wide range of causes, some clearly identifiable: genetic, autoimmune, iatrogenic, viral, toxic, others less (2, 4-6).

The autoimmune cause has been assigned even 20 to 30% of the POF. Three severe autoimmune association have been described called autoimmune polyglandular syndromes

(APS), which can reach between 39-72% prevalence of POF in APS I and 10% in APS II. Its onset precedes the onset of adrenal insufficiency with a few years. Among the autoimmune endocrine diseases the most common association has been reported with autoimmune thyroid disease (ATD), especially chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), between 12-33%, the other associations actually being very rare (2-3,6-7).

ATD is the most common autoimmune disease in the population, with an incidence of 10-12%, with female predominance (8). The association found between the two clinical entities could be the consequence of this higher incidence of thyroid disease, but could be favoured by the existence of an autoimmune field, demonstrated by the presence of the thyroid autoimmunity. Highlighting the AOA in the serum of these patients could select a group of ovarian autoimmunity and risk of developing POF and infertility.

OBJECTIVE OF STUDY

To reassess the antiovarian antibodies test performance in the diagnosis of premature ovarian failure of the patients with autoimmune thyroid disease.

MATERIAL AND METHOD

The study was conducted during January 15, 2009 and

¹Autor Corespondent: Mihaela Ciocan, Spitalul Clinic Județean Mureș, Str Sportivilor, nr. 1, Tîrgu Mureș, Romania; e-mail: mciocan2001@yahoo.com; tel +40-0265 262288

Articol intrat în redacție în 18.04.2011 și acceptat spre publicare în 08.08.2011
ACTA MEDICA TRANSILVANICA Septembrie 2011; 2(3)414-417

CLINICAL ASPECTS

January 15, 2011 and includes a total of 70 patients, aged 16-49 years, diagnosed with autoimmune thyroid disease, patients in whom the serum level of antiovarian antibodies was determined.

The purpose of this study is to reassess the antiovarian antibodies test performance in the diagnosis of POF of the patients with ATD.

The inclusion criteria were: a) women with ATD - Hashimoto's thyroiditis, antithyroperoxidase antibodies (ATPO) > 50 IU / mL and Graves-Basedow disease, anti-receptor antibodies for thyroid stimulating hormone (TSH) > 1.5 IU/L and with menstrual cycle, age up to 40 and premenopausal levels of FSH, and b) women with menopause before the age of 45, amenorrhea for at least six months, with postmenopausal FSH levels of > 40 IU/mL, with at least two determinations every 1 month.

The exclusion criteria were: a) women with ATD in the context of APS, women suffering from POF of a precise cause: genetic (agenesis or ovarian dysgenesis, galactosemia) or iatrogenic (pelvic surgery, radiotherapy, chemotherapy).

In all the cases we had the consent of the patients'.

In all patients the serum level of antiovarian antibodies was determined. Blood sampling was performed by venous puncture, and after 30 minutes it was followed of serum separation by centrifugation at room temperature. The sealed serum tubes were frozen to -20 °C. Determination of serum AOA was done quantitatively after thawing at room temperature using the "Enzyme Linked Immunosorbent Assay" (ELISA) technique, called "double sandwich" with solid phase, using EIA-2937 kit, with a mixture of ovarian proteins as a reagent. Values higher than 10 IU/ mL were considered positive.

At the time of sampling all patients had euthyroidism, with or without thyroid hormone replacement therapy or with synthetic antithyroids. None of the patients under went immunosuppressive treatment before or during the study, none of the patients with menopause was under estrogen hormone therapy at the time of sampling

RESULTS

The study included 70 patients suffering from autoimmune thyroid disease, 63/70 of which (90%) with Hashimoto's thyroiditis and 7/70 (10%) with Graves-Basedow disease.

At the moment of enrolment in the study, 12.9% (9/70) of the cases had euthyroidism, without substitution therapy, 72.8% (51/70) had hypothyroidism under substitution therapy with thyroid hormones, and 14.3% (10/70) had hyperthyroidism under treatment with synthesis antithyroids.

Table no. 1. The distribution of cases according to the type of thyroid autoimmune disease and to thyroid function

Autoimmune thyroid disease	Hashimoto's thyroiditis	Graves-Basedow disease	
Thyroid function	Number patients (%)	Number patients (%)	Total
Euthyroidism	9		9 (12,9%)
Hypothyroidism	48	3	51 (72,8%)
Hiperthyroidism	6	4	10 (14,3%)
Total	63 (90%)	7 (10%)	70

The age of patients included in the study was between 16-49, with an average age of 36.20±7.43 years. The age at which the autoimmune thyroid disease was diagnosed was between 11 and 42 with an average age of 31.20±6.61 years.

From the study group, at 14 patients (20%) ovarian

failure started before the age of 45, of which 10 had premature ovarian failure under the age of 40 (14.4%). The average age of onset of menopause was 38.28±4.14 years and in the subgroup with POF was 36.3±2.9 years.

Table no. 2. Comparison table for average age

	Age, years, mean±DS
Age of cohort	36.2±7.43
The onset of ATD	31.2±6.61
The onset of menopause	38.28±4.14
The onset of POF	36.3±2.9

The ovarian failure was diagnosed in 8 cases (57.14%) after the diagnosis of the autoimmune thyroid disease; in 3 cases (21.43%) the diagnosis was concurrent with the investigation of secondary amenorrhea, and in other 3 cases (21,43%) after some years following the appearance of menopause.

Regarding the aspect of the menstrual cycle: 35 patients had regular menstrual cycles (62.5%), 19 patients presented bradimenorrhea (33.9%), 2 patients presented tahimenorrhea (3.6%).

Table no. 3. The distribution of cases according to menstrual cycles

Menstrual cycles	Number of patients	(%)
Regular menstrual cycles	35	50 %
Bradimenorrhea	19	27,1%
POF < 40 years	10	14,3%
Menopause 40-45 years	4	5,7%
Tahimenorrhea	2	2,9%

Antiovarian antibodies over 10 IU/ l were detected in only one case of the 70 (1.4%) Three other cases had border-line values 9-10 IU/ l (4.2%).

Table no. 4. The distribution of patients according to levels of AOA

Values of AAO	Number of patients	(%)
>10 IU/ml (positive)	1	1,4%
9-10 IU/ml (border line)	3	4.3%
<10 (negative)	69	98.6%

That particular case of the 34-year-old patient had been diagnosed two years before with chronic lymphocytic thyroiditis, without a high titre of antithyroid antibodies (60 IU/ ml), the patient was under replacement therapy, she had given birth and had no menstrual abnormalities, antinuclear antibodies (ANA) were positive - titre 1/ 320, with fine speckled appearance. In 8 patients with high titres, over 500 IU/ ml, non-organ-specific antibodies - antinuclear antibodies were identified. These were negative.

One of the patients with thyroiditis and early menopause had elevated serum transaminase levels, interpreted as autoimmune hepatitis, which normalized after 3 months of thyroid hormone replacement treatment. In this case also AOA and ANA were negative. Other associated autoimmune pathologies were: one case of vitiligo and one case of insulin independent diabetes.

Of the entire study group 6 patients (8.5%) were recorded as infertile, 5 with primary sterility (7.1%) and 1 with secondary infertility (1.4%); from these, 4 (5.6%) were included in the group of premature ovarian failure, three of them with primary infertility.

DISCUSSIONS

The onset of unpredictable POF faces the clinician and the patient with the case of permanent infertility (4).

Two mechanisms that can lead to primary ovarian

CLINICAL ASPECTS

failure can be described: follicular depletion, as in some genetic mutations such as Turner syndrome and ovarian follicle dysfunction, as in FSH receptor mutations or of the LH (luteinising hormone) receptor mutations and as in autoimmune oophoritis (2,3,5,9-10).

The literature describes the possibility of temporary and unpredictable resuming of the ovarian function as a feature of POF. Pregnancy and childbirth have been reported after the diagnosis of POF reaching a percentage of 1-10% of POF (fluctuating ovarian function) (6). Ovarian ultrasound, and biopsy in some cases show follicles in various stages of development (9, 11). These facts might suggest partial or reversible action on ovarian tissue by mechanism of follicular dysfunction of a factor that is of no interest to the entire follicular population or to all the stages of development and which may be autoimmune, unlike genetic factors which act through the mechanism of follicle depletion (5, 12).

Ovarian reserve assessment is difficult, using a combination of markers: FSH, LH, E₂, inhibin B, anti-Müllerian hormone (AMH), antral follicle diameter and ovarian volume or surface, or with a better predictive value: laparoscopic ovarian biopsy and histology (4, 11).

Studies on groups of patients with POF, conducted on with other endocrine pathology, especially thyroid or conducted on groups of infertile patients subjected to "in vitro" fertilisation procedures (IVF), patients who underwent repeated ovarian punctures, revealed the presence of circulating antiovarian antibodies, evidence of the presence of an ovarian autoimmune diseases (2-3,6). Laboratory methods used are of the antigen-antibody reaction types: immunofluorescence, radioimmunoassay (RIA), ELISA, chemiluminescence, immuno-blotting (gel electrophoresis separation, solid phase transfer spots, identification of protein fractions with RIA, ELISA or chemiluminescence) (11-18).

Multiple antigenic structures have been reported in the ovary in the germinal components: the oocyte - human protein Maternal Antigen That Embryos Require (HuMATER), heat shock protein 90- β (HSP90 β), and the zonapellucida, but also in the somatic components (corpus luteum, granulosa and theca cells) antigens in steroid-producing cells (SCA), as in POF associated with Addison's disease, such as enzymes 3 β -hydroxysteroid dehydrogenase, 17-hydroxylase and the enzyme of the cleavage site of the cytochrome P450 chain (SCC) located predominantly in the internal theca, the 67-kDa protein in the corpus luteum of women with systemic lupus erythematosus, 51-kDa protein in granulosa and gonadotropins and their receptors (antiFSH and antiLH antibodies, antibodies antiLHreceptor and antiFSHreceptor) (2-3,7,16,19). Another antigen adds to these, still insufficiently characterised, α -enolase (50-kDa protein) (18).

Research results maintain the lack of consensus and certainty to a particular antiovarian specific autoantibody as a marker of ovarian autoimmunity, suggesting the autoimmunity nature of the multiple antigenic targets (7, 16).

The correlation between the presence of AOA and histology is a matter unsystematically dealt with in studies because of the invasiveness of ovarian biopsy (20). Until now the correlation between the presence of antiadrenal antibodies in autoimmune POF and autoimmune oophoritis, between anti-3 β -hydroxysteroid dehydrogenase of POF associated with autoimmune adrenal disease has been demonstrated. (2,11,18).

AOA incidence varies widely in different studies. In studies that used the ELISA method, AOA were detected in 59% (27/46) of idiopathic POF patients (1997, Fenichel et al.), 53% (44/ 83) of idiopathic POF (1997, Luborski et al.) and 46.5% (20/43) of idiopathic POF compared with 41% (16/39) in the

control group (2008, Mahnaz et. al). The incidence of ATPO antibodies was 30% (1997, Luborski et al.) and 32.6% in the POF group and 10.3% in the control group (2008, Mahnaz et. al) and 72.7% in the POF and 35.3% respectively in the control group (Soytac et al, 2010) (12-13,15,17).

Pires et al. (2006, 2007) used immunoblotting with chemiluminescence to detect AOA instead of a mixture of ovarian antigens with a molecular weight between 30-120 k-Da ("novel blocking protocol") and obtained AOA positive results in 30% (15/ 50) of the cases in the POF group and 26% (13/50) of cases in the group with IVF. They noticed the 90-kDa fraction dominance found in 46% (7/15) and 46% (6/ 13) of cases, consequently suggesting it as a possible marker of ovarian autoimmunity. This test based on the principle of antigenic targets and multiple histological levels has a better specificity (7.16).

Sunblad et al. report the positive results of 19.1% (21/110) of POF cases for α -enolase for a 50 k-Da antigen by immuno-blotting with ELISA (18).

POF-GIS Study Group, which investigated 357 cases with POF, of which 10.1% with a history of autoimmune disease, in 2009 reported positive results to AOA by indirect immunofluorescence and RIA in 2.3% of cases (7/298), antiadrenal antibody anti21- α -hydroxylase through RIA in 1.2% (3/249) of cases and antithyroglobulinantithyroid antibodies and ATPO through hemagglutination and RIA in 14.8% (44/297). The incidence of autoimmune thyroid disease and antithyroid antibodies was not significantly different than in the general population. The detection of AOA seems to have a low specificity in the prediction of ovarian autoimmunity in patients with POF, despite the use of tests with better sensitivity and specificity. The correlation with POF in adrenal autoimmune disease is much better in the case of anti21- α -hydroxylase antibodies (11).

The great differences are due to small batches studied, variable criteria for inclusion in the study or for comparison, different methods used in the preparation of antigens, the use of different antigens or laboratory methods used (1-2,12,14).

The ELISA method is more widely used in practice and research because of its affordability and in our study it was the method of determining the AOA. The result of 1,4 % (1/70) positive AOA reported to the small number of cases is statistically insignificant. The studies mentioned that used the same method, with a comparable or higher number of cases, had POF criterion for inclusion in the study, not the autoimmune thyroid disease, a fact which could lead to a concentration of cases with ovarian autoimmunity.

The average age group of cohort with autoimmune thyroid disease was 36.20 \pm 7.43 years, similar with the average age of onset of menopause in the subgroup with POF, which was 36.3 \pm 2.9 years.

AOA positivity was not correlated with a high titre of ATPO, which would require a more intense autoimmune reaction, but it was associated with the positivity of non-specific antinuclear antibodies, ANA, which would require the presence of an autoimmune reaction background.

CONCLUSIONS

The significance of circulating AOA remains a concern today. Their detection in the serum of patients suggests an autoimmune process that can be primary or secondary to ovarian aggression. The sequence of events that occur in the ovary before the manifestation of ovarian failure is difficult to predict.

Although finding POF association with autoimmune thyroid disease, as well as the simultaneous detection of ATPO

CLINICAL ASPECTS

and AOA suggests the autoimmune mechanism of POF, the usage of AOA as a possible predictor for POF is not grounded, given that there is no specific marker of ovarian autoimmunity and the results are very discordant.

It is difficult to imagine a single and clear pathophysiological model as in other autoimmune diseases due to the multitude of antigenic targets in the ovary that were highlighted. The rational conclusion is that ovarian autoimmunity tests should be explored and studied through tests using a set of antigens and not one single antigen.

REFERENCES

1. Luborsky Judith, Ovarian Autoimmune Disease and Ovarian Autoantibodies. *Journal of Women's Health & Gender-Based Medicine* 2002; 11 (7): 579-584.
2. Forges T., Monnier-Barbarino Patricia, Faure G.C. et al. Autoimmunity and antigenic targets in ovarian pathology. *Human Reproduction Update* 2004; 10 (2):163-175.
3. Gleicher N. Autoantibodies in infertility: current opinion. *Human Reproduction Update* 1998; 4(2): 169-176.
4. Rees Margaret, Purdie D.W. Management of the Menopause. Ediția a 4-a. Royal Society of Medicine Press Ltd and British Menopause Society Publications Ltd, London, 2006: 141-149.
5. Nelson L.M. Primary Ovarian Insufficiency, *N Engl J Med* 2009; 360:606-14.
6. Goswami D., Conway G. Premature ovarian failure. *Horm Res* 2007; 68: 196-202.
7. Pires E.S., Meherji P.K., Vaidya R.R. et al. Specific and Sensitive Immunoassays Detect Multiple Anti-ovarian Antibodies in Women With Infertility. *Journal of Histochemistry&Cytochemistry* 2007; 55 (12): 1181-1190.
8. Greenspan F. S., Gardner David G., Basic & Clinical Endocrinology. Ediția a 6-a, The McGraw-Hill Companies, New York 2001: 240-241, 248-250, 258.
9. Welt K. Corine, Falorni A., Taylor E. Ann et al. Selective Theca Cell Dysfunction in Autoimmune Oophoritis Results in Multifollicular Development, Decreased Estradiol, and Elevated Inhibin B Levels. *The Journal of Clinical Endocrinology & Metabolism* 2005; 90(5): 3069-3076.
10. Bakalov V.K., Anasti J.N., Calis A.K. et al. Autoimmune oophoritis as a mechanism of follicular dysfunction in women with 46,XX spontaneous premature ovarian failure. *Fertility and Sterility* 2005; 84(4): 958 -965.
11. Bachelot Anne, RouxelAgnès, Massin Nathalie et al. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. *European Journal of Endocrinology* 2009;161(1):179-187.
12. Fènichel P., Sosset Catherine, Barbarino-Mounier Patricia et al. Prevalence, specificity and significance of ovarian antibodies during spontaneous premature ovarian failure. *Human Reproduction* 1997;12(12): 2663-2628.
13. Luborsky Judith, Llanes B., Davies S. et al. Ovarian Autoimmunity: Greater Frequency of Autoantibodies in Premature Menopause and Unexplained Infertility Than in the General Population. *Clinical Immunology*1999; 90(3): 368-374.
14. WheatcroftN.J., Toogood A.A., Li T.C. et al. Detection af antibodies to ovarian antigens in women with premature ovarian failure. *Clin Exp Immunol* 1994; 96(1):122-128.
15. Mahnaz A., Fallahian M., Eshrati B. et al. The Presence of Anti Thyroid and Anti Ovarian Auto-Antibodies in Familial Premature Ovarian Failure. *International Journal of Fertility and Sterility* 2008; 1(4): 171-174.
16. Pires E.P. Multiplicity of molecular and cellular targets in human ovarian autoimmunity: an update. *J Assist Reprod Genet* 2010; DOI 10.1007/s10815-0-9440-5
17. Serap S.I., Mutlucan K., Baser H. et al. An association between autoimmune thyroiditis and premature ovarian failure: a cross-sectional study. *European Congress of Endocrinology, Endocrine Abstract* 2010; 22 : 477.
18. Sundblad Victoria, Bussmann L., Chiauzzi A. Violeta et al.. α -enolase: a novel autoantigen in patients with premature ovarian failure. *Clinical Endocrinology* (2006) 65, 745-751.
19. La Marca A, Marzotti S et al. – Primary ovarian insufficiency due to steroidogenic cell autoimmunity is associated with a preserved pool of functioning follicles. *J ClinEndocrinolMetab.* 2006; 94(10): 3816-23.
20. Bats A.Sophie, Barbarino- Monnier Patricia, Benne Marie-Christine et al. – Local lymphocytic and epithelial activation in a case of autoimmune oophoritis. *Fertility and Sterility* 2008; 90(3): 849.5-849.8.