STUDY OF BIOLOGICAL MARKERS IN ENDOMETRIOSIS

EDUARD CRAUCIUC[1]*, SINDILAR ALIA[1], OVIDIU TOMA[2], DRAGOS CRAUCIUC[3]

Abstract. Endometriosis is an estrogen-dependent inflammatory disorder. Researches proved the fact that there are changes in serum marker concentrations that precede the occurrence of infertility symptoms, with endometriosis as the background. The objectives of the present study were to find out if the changes CA-125 and IL-6 correlate with the clinical and biochemical state of the patient with infertility and if they would be good predictors for infertility, in the case of the patients with endometriosis. We compared the values of the markers in the patients with infertility and ovarian endometriosis (n=94) with those of a control group with ovarian endometriosis (n=106) for which the diagnosis of infertility was not confirmed, in order to establish the prognostic factors of infertility. The values CA-125 and IL-6 were significantly higher for the group with infertility.

INTRODUCTION

Endometriosis is a disease that affects a woman in ten worldwide (Johnson, N.P., Hummelshoj, L., 2013).

Endometriosis – the growth of the stroma and endometrial glands outside the uterus, represents the third cause of gynaecologic consult for chronic pelvic pain, dysmenorrhea, dyspareunia and infertility (Giudice, L.C., 2010; Holicov-Lutuc, M., 2014; May, K.E., et al., 2010; Verkauf, B.S., 1987).

Patients with ovarian endometriosis face a lot of inconveniences daily, which causes deterioration in the quality of life, an increased stress due to infertility, depression and anxiety (Bulun, S.E., 2009; Burney, R.O. Giudice, L.C., 2012).

The data based on our cases is in accordance with the data in the specialized literature, which describes the fact that there is a prevalence of 30-50% for the women with endometriosis, who also suffer of infertility (Bulun, S.E., 2009; Streuli, I., et al., 2013).

Protective factors (Giudice, L.C., 2010; Holicov-Lutuc, M., 2014):
- multiparity
- COC (combined oral contraceptive)
- Regular physical exercise

Increased incidence (Giudice, L.C., 2010; Holicov-Lutuc, M., 2014):
- Pregnancy over 35 years old
- High socioeconomic level
- menstrual cycles ‹ 27 days
- abnormally long menstruations › 8 days
- malformations connected to Muller duct.

The results of our study come to confirm the importance of determining these markers for the diagnosis and for monitoring the patients with endometriosis and infertility.

PURPOSE AND OBJECTIVES

The main purpose is, on the one hand, a better understanding of the problems our patients have to face, by offering them more social and psychological support to overcome these obstacles (Barbieri, R.L., et al., 1986; Bedaiwy, M.A., et al., 2002, Bedaiwy, M.A. Falcone, T., 2004; Giudice, L.C., 2010; Holicov-Lutuc, M., 2014; May, K.E., et al., 2010; Pittaway, D.E., et al., 1989), and on the other hand, improved therapeutic options (Peltecu, Gh., 2014).

MATERIAL AND METHOD

In this study we assessed a number of 200 patients aged between 18 and 45 (the mean age being about 32) with endometriotic ovarian cysts, who were investigated at "Elena Doamna" Clinical Hospital of Obstetrics and Gynaecology in Iaşi, in the period of time 2012–2016, 94 of which infertility associated with endometriosis.
We performed:

- a Doppler endovaginal ultrasound examination (Giudice, L.C., 2010; Peltecu, Gh., 2014);
- the anatomo-pathologic exam (the patients who were performed ablation or tissue excision during surgery had an extemporaneous examination, and also a histologic examination for paraffin, in order to confirm or infirm endometriosis) (Giudice, L.C., 2010; Holicov-Lutuc, M., 2014; May, K.E., et al., 2010;).

The assessment of serum markers
We observed the changes of the serum markers in endometriosis, studying CA-125 and cytokines IL-6 and TNF-α, as indicators for diagnosing endometriosis (Barbieri, R.L., et al., 1986; Bedaiwy, M.A., et al., 2002, Bedaiwy, M.A. Falcone, T., 2004; Keenan, J.A., et al., 1995; Koyama, N., et al., 1993).

Statistical analysis
Data was loaded and processed with the help of statistical functions in SPSS 18. We applied: ANOVA significance tests – using the descriptive indicators of the monitored parameters, with 95% interval of trust; t-Student test – quantitative test applied to study the significant difference between three environments; χ^2 test – a qualitative test by which two or more frequencies of the same population were compared; Pearson correlation to determine the relation between the parameters considered in the study. By drawing the ROC curve, predictability of serum markers was determined in infertility determinism.

RESULTS AND DISCUSSIONS

The social status from the perspective of its association with the degree of addressability to the doctor for the patients showed the fact that, generally, they were part of families with an income above the average (94%).
The age of the patients varied from 18 to 45 years old, with a mean of 31.77±5.23 years old and a median of 35, which suggests the homogeneity of the series of values.
Marital status of the patients with endometriosis: 72.5% married; 15.34% single and 12.1% in a relationship.
Living environment was mainly urban 86%.
The location of endometriosis was unique or mixt:
- ovary 100%;
- anterior cul de sac or in the bladder, broad ligament, uterus, parietal peritoneum (44%);
- Douglas pouch and utero-saccharal ligaments (3%);
- Fallopian tube (2%):
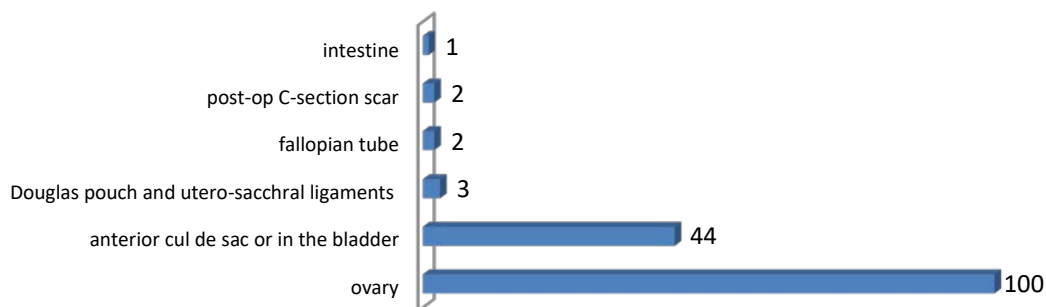- Post-op C-section scar (2%);
- intestine (1%).

Fig. 1. Share of cases depending on the location of endometriosis

Signs and symptoms

Among the main symptoms identified in the cases studied, we noticed: dysmenorrhea (90%), pelvic pain (87.5%), irregular menstrual cycles (67.5%). Gastrointestinal tract manifestations were predominantly constipation / diarrhea (28%), and from the urinary tract manifestations haematuria was identified more frequently (42.5%) (tab. I)

Table I. Signs and symptoms for the patients with ovarian endometriosis

Signs and symptoms	n	%
In the reproductive organs		
Dysmenorrhea	180	90.0
Pelvic pain	175	87.5
Lumbosacral pain	84	41.0
Irregular menstrual cycles	135	67.5
Torsion / rupture of the endometrium	44	22.0
Infertility	94	47.0
Gastro-intestinal tract		
Cyclic rectorragia	27	13.5
Rectal tenesmus	40	20.0
Alternant constipation/diarrhea	56	28.0
Urinary tract		
Haematuria	85	42.5
Dysuria	28	14.0
Ureteral obstruction	8	4.0

Based on the cases studied. infertility was associated in 47% of the cases with ovarian endometriosis, which allowed us to split the study group in two sub-groups:
- 94 patients with ovarian endometriosis and infertility;
- 106 patients with ovarian endometriosis without infertility.

Based on the demographic characteristics, we noted an increased frequency of infertility cases for the overweight patients (31.9%), with a mean age of about 35 years old, smoking (18.1%), and a sedentary life style (95.7%) (tab. II).

Table II. Characteristics of the group with endometriosis depending on the presence of infertility

Characteristics	Lot with infertility (n=94)	Lot without infertility (n=106)	p
Demographic data			
Mean age (years)	34.91±9.34	28.63±12.15	**0.001**
Obesity	30 (31.9%)	28 (26.4%)	0.484
Smoking	17 (18.1%)	3 (2.8%)	**0.001**
Alcohol consumption	7 (7.5%)	9 (8.5%)	0.991
Sedentary life style	90 (95.7%)	98 (92.5%)	0.496

Characteristics	Lot with infertility (n=94)	Lot without infertility (n=106)	p
Personal pathologic history			
Cardiovascular damage	92 (97.9%)	98 (92.5%)	0.153
Chronic kidney disease	54 (57.4%)	27 (25.5%)	**0.001**
Respiratory damage	13 (13.8%)	11 (10.4%)	0.595
Psychiatric damage	12 (12.8%)	22 (20.8%)	0.189
Neoplasia	7 (7.4%)	6 (5.7%)	0.823
Gastro-intestinal damage	4 (5.3%)	3 (2.8%)	0.593
Autoimmune diseases	1 (1.1%)	5 (4.7%)	0.273
Diagnostic			
Laparoscopy	63 (67.0%)	75 (70.9%)	0.680
Laparotomy	27 (28.7%)	13 (12.3%)	**0.006**
Transvaginal ultrasound exam	33 (35.1%)	22 (20.8%)	**0.035**
CT scan	19 (20.2%)	6 (5.7%)	**0.004**
MRI	46 (48.9%)	36 (34.0%)	**0.045**
Lab parameters			
Haemoglobin, g/dL	13.14±1.48	13.26±1.33	0.561
Total cholesterol, mg/dL	181.45±43.23	187.95±45.84	0.305
Serum triglycerides, mg/dL	116.86±46.66	115.13±55.63	0.853
Glycemia, mg/dL	115.33±49.89	100.99±19.92	**0.007**
Creatinine Clearance	67.96±32.07	92.01±29.30	**0.001**
Creatinine, mg/dL	1.44±0.52	0.88±0.34	**0.001**
Proteinuria, mg/dL	195.63±126.12	98.68±23.00	**0.050**
Urea, mg/dL	29.18±18.41	18.09±4.42	**0.005**
C-Reactive Protein, mg/dL	12.00±4.58	6.72±1.99	**0.002**
CA-125, U/mL	33.21±15.02	27.10±10.10	**0.021**
IL-6, pg/mL	1.99±0.15	0.95±0.10	**0.049**
TNF-α	16.15±9.90	14.13±8.71	0.625

Considering the *personal history*, we noted by frequency: cardiovascular damage (97.9%) and chronic renal disease (57.4%) which apparently induce a twice higher relative risk of atherosclerosis (RR=2.26; IC95%: 1.56-3.26).

The lab parameters showed significantly increased values of glycaemia for the patients with infertility (115.33 *vs* 100.99 mg/dl; p=0.007), also of creatinine (1.44 *vs* 0.88 mg/dl; p=0.001), proteinuria (195.63 *vs* 98.68 mg/dl; p=0.05), urea (29.18 *vs* 18.09 mg/dl; p=0.005) and **CRP** (12 *vs* 6.72 mg/dl; p=0.002) and the mean value of creatinine clearance was significantly reduced (67.96 *vs* 92.01; p=0.001).

CA-125 marker (33.21 vs 27.10 U/ml; p=0.021) and **IL-6** (1.99 *vs* 0.95 pg/ml; p=0.049) recorded mean values that were significantly higher for the patients with infertility. CA-125 is associated with ovarian cancer most of the times and there is a wrong impression that an increased level of CA-125 in the blood means cancer ovarian, or that a normal level means the absence of cancer (Barbieri, R.L., et al., 1986; Lucidi, R.S., et al., 2005).

By drawing ROC curve we noticed that CA-125 marker was a good predictor in identifying infertility (AUC=0.747; IC95%: 0.555–0.939). The analysis proved the fact that in the analysed group there is no significant correlation between the values of CA-125 and the patients' age (r=0.1006. p=0.618).

We assessed the size of the ovarian endometrium (cm) by performing an endovaginal or abdominal ultrasound exam, using a GE Voluson 750 ultrasound machine. The investigation proved the fact that, for the analysed group, there is a significant correlation between the values of CA-125 and the size of endometrium (r=0.41. p=0.038). In the presence of adhesions, significantly higher values were found for CA-125 (F=3.71. p=0.041). The analysis showed that in case of tubal damage (the uni- or bilateral hydrosalpinx) the values of CA-125 were significantly higheri (F=11.059. p=0.031). It was also proved that. in case of peritoneal damage through endometriotic implants. the values of CA-125 were significantly higher (F=7.118077; p=0.037).

The serum dosage of IL- 6 de 1.99 pg/mL have a sensitivity of 90% and a specificity of 67% in diagnosis and **TNF-α** dosed from the peritoneal fluid, with a value of over 16 pg/mL has 100% sensitivity and 89% specificity when establishing the diagnostic. These values are similar with those from the specialized literature (Keenan, J.A., et al., 1995; Pittaway, D.E., Douglas, J.W., 1989).
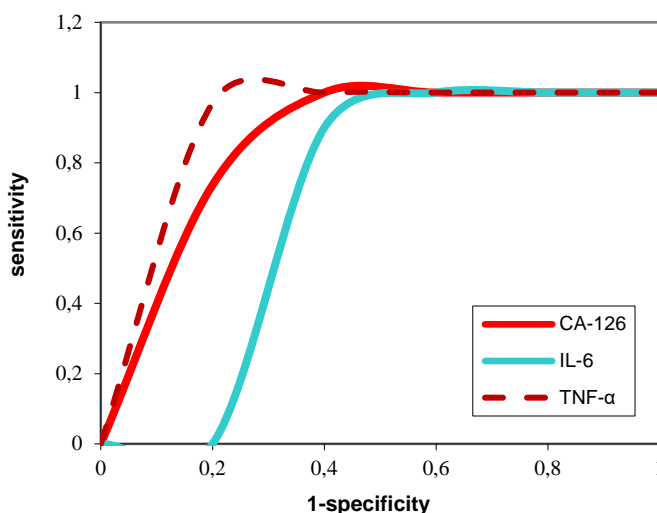

Fig. 2. Predictability of serum markers for detecting infertility

The mean values of haemoglobin, total cholesterol and serum triglycerides did not show significant differences depending on the absence or presence of infertility (p>0.05) (tab. II).

CONCLUSIONS

Endometriosis is considered a tabu disease, with serious consequences on the physical and mental health.
Endometriosis is the first cause of feminine infertility.

The disease is translated into insufferable pain, long term treatment and their side effects, many surgeries and infertility.

For the patients with ovarian endometriosis, CA-125 markers with a mean level of 33 U/ml and IL- 6 with a mean level of 2 pg/ml, proved to be good predictors of infertility.

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[1] "Gr.T.Popa" University of Medicine and Pharmacy, Iași, România, „Elena Doamna" Iași Clinical Hospital

[2] "Alexandru Ioan Cuza" University, Iasi, Romania

[3] Forensic Institute, Iași, Romania

* crauciuc@yahoo.com