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Hormonal, apoptotic, proliferative and inflammatory markers' expression in Desogestrel-treated women with ovarian endometriosis

DANIELA-ROXANA MATASARIU[1,2], LUDMILA LOZNEANU[3,4], IRINA DUMITRAŞCU[1,2], MIHAELA GRIGORE[1,2], ALEXANDRA ELENA CRISTOFOR[1,2], CRISTINA ELENA MANDICI[1], IULIANA-ELENA BUJOR[1,2], ALEXANDRA URSACHE[1], ANCA DANIELA BRĂILA[5,6], ALEXANDRA BAUŞIC[7], ROMEO MICU[8,9]

[1] *Department of Mother and Child, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[2] *Department of Obstetrics and Gynecology, Cuza Vodă Hospital, Iaşi, Romania*

[3] *Department of Morpho-Functional Sciences I – Histology, Pathology, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[4] *Department of Pathology, Sf. Spiridon Emergency County Hospital, Iaşi, Romania*

[5] *Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*

[6] *Department of Obstetrics and Gynecology, Emergency County Hospital, Craiova, Romania*

[7] *Department of Obstetrics and Gynecology, Prof. Dr. Panait Sîrbu Hospital, Bucharest, Romania*

[8] *Department of Obstetrics and Gynecology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[9] *Department of Obstetrics and Gynecology, Regina Maria Maternity, Cluj-Napoca, Romania*

Abstract

Endometriosis is a relatively frequent pathology in gynecological practice. We performed an analysis to demonstrate the molecular changes that occur in endometriosis synthetic progestin-treated patients, hoping to sketch a possible pathophysiological pathway that will help us to better understand and treat this debilitating disease. We conducted a prospective study that included a group of 40 women, evaluated in our hospital between 2020–2021. We evaluated immunohistochemical tissue expression of estrogen receptor (ER), progesterone receptor (PR), B-cell lymphoma 2 (Bcl-2) protein, Ki-67, and serum levels of osteopontin (OPN) and vascular endothelial growth factor (VEGF) in patients with ovarian endometrioma with and without progestin treatment. Our study revealed that Desogestrel treatment increases OPN serum levels, PR and Bcl-2 tissue expression and reduces VEGF serum levels and Ki-67 tissue expression. The results we have obtained are very interesting because the serum levels of OPN seem to be more influenced by progestin treatment, than by endometriosis itself. The study we have conducted gives a molecular complex view of what endometriosis represents and on how Desogestrel treatment works.

Keywords: endometriosis, osteopontin, immunohistopathology, Desogestrel.

Introduction

Endometriosis represents a multifactorial, complex, chronic inflammatory disease, characterized by the presence of endometrial gland and stroma outside the uterus. Most commonly, it affects the ovaries, the peritoneum and in a smaller percentage from 8–12% the bowel [1, 2].

Endometriosis is a relatively frequent pathology in gynecological practice, with an incidence of almost 2% in general population. It affects 7–15% of reproductive-age women, resulting in infertility, persistent pelvic discomfort, dyspareunia, and dysmenorrhea, as well as significant socio-economic consequences [3].

The exact trigger for this pathological process remains yet to be discovered and understood. Despite the vast research in this field, endometriosis is still considered "the disease of theories", because the exact pathophysiological pathway remains unclear [4, 5].

The endometriotic ovarian cysts are usually detected using ultrasound (US) examination but the peritoneal lesions have no US expression, being much more difficult to detect. The histopathological (HP) confirmation is obtained only after the examination of the tissue provided through surgical intervention, more frequently through laparoscopy. Laparoscopy was considered to be until 2021 the "gold standard" in the diagnosis of this pathology [2].

In managing this pathology, we have a variety of options that also includes hormone therapy or surgical intervention to remove the cyst and/or the ectopic endometrial tissue. The hormone therapy can reduce or even eliminate the lesions and the related pain, but its success is highly dependent on the location of endometriosis (superficial or deep implants), having no effect whatsoever on the adhesions of infertility caused by it [6, 7].

Looking deeper into the pathophysiology of endometriosis, studies showed that an abnormal steroidogenesis pathway occurs inside the endometriotic lesions, with large production

of estrogen and progesterone. The estrogen seems to play a significant role in the proliferation and dissemination of the ectopic endometrial cells. The ectopic endometrial tissue proved to be highly receptive to estrogens influence, expressing large amounts of specific receptors for it [8, 9].

B-cell lymphoma 2 (Bcl-2) protein is a founder member of the Bcl family of proteins, which plays an important role in apoptosis regulation. Its function is anti-apoptotic. Elevated Bcl-2 expression and function has been observed in various tumors, including gynecological cancers, such as breast cancer [10, 11], and also ovarian cancer, where it tends to promote survival of the cancerous cells and drug resistance [12].

During cell division, Ki-67 is a marker in the nuclear matrix. Ki-67 appears to play a function in preventing chromosomes from adhering to one another by binding to one end and rejecting other chromosomes [13].

Vascular endothelial growth factor (VEGF) is an angiogenic factor that is both potent and selective. VEGF is found in the epithelium of endometriotic implants in endometriosis patients, notably in hemorrhagic red implants [14]. In a mouse model, progesterone was shown to reduce proliferation of endometrial stromal cells and to suppress VEGF by reducing its transcription [15]. Both normal and pathological angiogenesis are regulated by VEGF. The expression of VEGF is known to be activated by several cytokines, and it is greatly enhanced in the peripheral blood, peritoneal fluid, and endometrium of individuals with endometriosis. The number of endometriotic lesions has been demonstrated to drop significantly when VEGF is inhibited [3, 16].

VEGF and Bcl-2 function in a synergistic way. Studies have proven that high Bcl-2 levels in cancer cells that are under hypoxia, can induce VEGF expression, and promote its secretion and its transcriptional activity, thus having an increased vascularization that is independent of cell survival [17].

Osteopontin (OPN) is a sialoprotein present in many types of tissue, reported to act as a cytokine, proven to have an important role in chronic inflammation. OPN has multiple roles in the organism, acting as a cytokine, cell adhesion protein and cell differentiation antigen. There are discordant conclusions in literature regarding the implications of OPN in the pathophysiology of endometriosis, mainly due to its contribution in cell migration, attachment, and invasion [16, 18, 19].

Desogestrel is a progestin drug, derived from 19-Nortestosterone. Once absorbed in the intestine, it will be transformed in Etonogestrel, which represents its active metabolite [20].

The drug has a light effect over lipid metabolism, by slightly decreasing the levels of high-density lipoprotein (HDL)-cholesterol and also over carbohydrate metabolism [21].

Some studies show that Desogestrel can be effective by itself for the treatment of pelvic pain in women with mild endometriosis [22, 23]. Others [24] stated that Desogestrel was able to control and improve gastrointestinal symptoms and also chronic pelvic pain and dyspareunia in women with rectovaginal endometriosis. Also, it showed a reduction in the volume of the rectovaginal nodules.

Most common adverse effects attributed to Desogestrel are cited to be abnormal bleeding, weight gain and abdominal bloating [23].

Aim

The central idea in our study was to see the differences between the levels/expression of these markers in women with and without treatment and to compare the results, to prove one of these parameters as a possible marker for endometriosis.

Patients, Materials and Methods

We conducted a prospective study that included a group of 40 women (18 to 42 years old) with high suspicion of ovarian endometrioma, admitted in Cuza Vodă Hospital, Iaşi, Romania, between 2020 and 2021. The reduced number of patients was caused by limited elective surgery due to coronavirus disease 2019 (COVID-19) pandemic.

We decided to perform the evaluation of these serum and tissue markers in patients with ovarian endometriosis, with and without Desogestrel treatment, to evaluate the changes due to progestin treatment.

From these 40 patients, only 16 of them accepted Desogestrel treatment prior to surgery due to fearing side effects.

All enrolled patients had a routine US exploration, both transvaginal and abdominal. All of them had a subsequent HP confirmation after the laparoscopic excision and had no other prior surgical intervention or medical treatment for endometriosis in the past.

As exclusion criteria, we imposed: body mass index (BMI) >30, HP diagnosis of tumoral lesions, association of autoimmune or infectious diseases, diabetes, depression, Cushing syndrome, Turner syndrome and pregnancy.

Every patient who participated in the study signed a written informed consent form that was approved by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania (Approval No. 10/2020).

For the evaluation of OPN levels, 6 mL of blood were collected, each in three different moments, from each patient, in Clot Accelerator Tubes: first probe, in the day in which the diagnosis of endometriosis was suggested by the US exploration, without any prior medication; the second probe – six months after the first US –, the moment being also the day of the surgery. At this time, 16 patients were under treatment with 0.075 mg Desogestrel daily, for the past six months, and the rest of 24 patients, without any medication. The third probe was collected six months post-surgery, the 16 patients being under treatment with 0.075 mg Desogestrel continuouslyand the rest of 24 patients, without any medication (Table 1). The samples were centrifuged for 10 minutes at 4000 rotations per minute, and the serum was collected and frozen within an hour of collection and maintained at -20ºC until analysis. All 40 patients were monthly monitored both clinical and by US examination.

OPN levels were determined using human OPN enzyme-linked immunosorbent assay (ELISA) kit (RAB 0436, Sigma-Aldrich, USA). We employed an undiluted serum, a Heidolph Titramax 1000 plate shaker set to 1.5 cycles/s, and a 4°C overnight incubation method. Using a BioRad spectrophotometer, the absorbance was measured at 450 nm. The results were obtained by plotting the mean absorbance

of the samples on a standard curve made with a standard concentration solution, as computed by the software. VEGF-A serum levels were quantified using a commercial ELISA kit (BioVendor, Brno, Czech Republic), following the instructions of the producer. During the incubations, a Heidolph Titramax 100 orbital shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) was used. Absorbances were measured with a BioRad (Austria) spectrophotometer, and the results were calculated using a Magellan software.

Table 1 – ***Serum samples and IHC biopsy in women with endometriosis in three moments of the study***

Day of US diagnosis	Day of surgery				Day of reevaluation (six months after surgery)			
	OPN (serum)		*ER, PR, Bcl-2, Ki-67 (tissue) + VEGF (serum)*		*OPN (serum)*		*VEGF (serum)*	
40 cases without treatment	16 cases (0.075 mg Desogestrel/day)	24 cases (without treatment)	9 cases (0.075 mg Desogestrel/day) for six months	7 cases (without treatment)	16 cases (0.075 mg Desogestrel/day)	24 cases (without treatment)	9 cases (0.075 mg Desogestrel/day) for six months	7 cases (without treatment)

Bcl-2: B-cell lymphoma-2; ER: Estrogen receptor; IHC: Immunohistochemical; OPN: Osteopontin; PR: Progesterone receptor; US: Ultrasound; VEGF: Vascular endothelial growth factor.

After obtaining the tissue biopsies through laparoscopy about 4–7 cm, the samples were originally fixed in 10% neutral buffered formalin solution for 24 hours, dehydrated in xylene, and embedded into paraffin and cutting at 3 μm for immunohistochemical (IHC) staining. During the IHC technique, the histological sections were deparaffinized in xylene, rehydrated in alcohol in the progressive decreasing concentrations and rinsed in distilled water. We used heat-induced epitope retrieval (HIER) method for unmasking the antigenic site with citrate pH 6 antigen retrieval solution (for all antibodies used in the study). 3% Hydrogen peroxide was used for blocking the endogenous peroxidase activity and Labeled Streptavidin Biotin–Horseradish Peroxidase (LSAB–HRP) complex was used for the amplification of the immunoreaction. The IHC positive reaction was considered in the presence of a brown nuclear specificity for anti-ER, anti-PR and anti-Ki-67 antibodies, and cytoplasmic expression for anti-Bcl-2 antibody (Table 2).

Table 2 – ***IHC panel of antibodies used in our study***

Antibody	Clone, manufacturer	Dilution	Expression
Anti-ER	SP1, EDTA, IgG isotype, Biocare	1:1000	Nuclear
Anti-PR	PGR 16, EDTA, IgG1 isotype, Biocare	1:1000	Nuclear
Anti-Bcl-2	100/D5, EDTA, IgG1/kappa, Biocare	1:100	Cytoplasmic
Anti-Ki-67	MM1, EDTA, IgG1 isotype, Biocare	1:250	Nuclear

Bcl-2: B-cell lymphoma-2; EDTA: Ethylenediaminetetraacetic acid; ER: Estrogen receptor; IgG: Immunoglobulin G; IHC: Immunohistochemical; PR: Progesterone receptor.

The immunoexpression of ER, PR, Bcl-2, and Ki-67 was analyzed in both endometriosis lesion's stroma and glandular epithelium. For the semi-quantitative assessment of epithelial component of ER, PR, and Bcl-2, a semi-quantitative score based on literature reports was used [25, 26], while for stromal component, we used a standard 3-point scale scoring system, examined at ×200 magnification, according to the following scoring system: 0 – no cells in the stromal area, 1 – focal cells in the stromal area and 2 – diffuse cells in the stromal area, considered negative score 0 and 1, and positive score 2, regardless of the level of staining intensity.

For ER and PR assessment, we have applied a semi-quantitative score, according to Allred *et al*., based on the percent of positive cells (≤1%: 1; 1–10%: 2; 10–33%: 3; 33–66%: 4; 66–100%: 5) and the intensity of the immunostaining reaction (0: absent; 1: weak +; 2: moderate ++; 3: strong +++). The summing of these two scores represented the final score for the patient (0–2: negative; 3–8: positive) [25].

For Bcl-2 assessment, we used a semiquantitative score, according to Suzuky *et al*., that evaluated the percent of positive cells (0: <1%; 1: 1–25%; 2: 26–50%; 3: 51–75%; 4: >76%). A score between 1 and 4 was considered positive [26].

The immunoexpression of Ki-67 was analyzed in the 10 hot spot area select one high-power field with highest staining rate.

ER, PR and Ki-67 showed nuclear expression in both epithelial and stromal tissue, while Bcl-2 expressed granular cytoplasmic pattern.

Each individual slide was reviewed and independently analyzed by two experienced gynecological pathologists.

For the OPN serum evaluation, the data were processed using Statistical Package for the Social Sciences (SPSS) 18.0 [χ^2 (*chi*-squared) test, Student's *t*-test, analysis of variance (ANOVA) and the Pearson's correlation coefficient]. For VEGF-A serum levels, we used a nonparametric Mann–Whitney *U*-test and Fisher's exact test for comparison of frequencies from SPSS 23.0 software.

Results

Stromal ER concentrations ranged from 20% up to 70% in patients with progesterone treatment and from 0 to 100% in those without treatment, with an average of ER present in stroma slightly lower in those with treatment (44.44% *vs* 45.86%; p=0.933). Epithelial ER was between 0 and 70% in patients that underwent treatment and between 0 and 90% in patients that did not have any treatment at all. Epithelial average ER difference present in epithelium was not statistically significant between treated and untreated patients (23.33% *vs* 25.86%; p=0.885).

Stromal PR concentrations ranged from 70% up to 100% in patients with progesterone treatment and from 5% to 100% in those without treatment, with an average of PR present in stroma slightly higher in those with treatment (90% *vs* 71.43%; p=0.025). Epithelial PR was between 0 to 100% in patients in both study groups: that underwent treatment and in patients that did not have any treatment at all. Epithelial average PR expression was statistically significant lower in patients with treatment (35% *vs* 56.57%; p=0.02).

Stromal Bcl-2 concentrations ranged from 40% to 90%

in patients receiving progesterone treatment and 0 to 90% in those not receiving treatment, with an average of Bcl-2 present in the stroma slightly higher in those receiving treatment (70% *vs* 27.29%; p=0.012). Epithelial Bcl-2 was between 0 to 90% in patients that underwent treatment and between 0 and 90% in patients that did not have any treatment at all. Epithelial average Bcl-2 expression was statistically significant higher in patients with treatment (51.11% *vs* 24.43%; p=0.05).

Stromal Ki-67 concentrations ranged from 0 up to 2% in patients with progesterone treatment and from 0 to 90% in those without treatment, with an average of Ki-67 present in stroma slightly lower in those with treatment (0.67% *vs* 20%; p=0.001). Epithelial Ki-67 was between 0 to 50% in patients that underwent treatment and in 0 to 35% in patients that did not have any treatment at all. Epithelial average Ki-67 expression was statistically significant lower in patients with treatment (1.22% *vs* 8%; p=0.028) (Table 3).

Table 3 – ***The percent and distribution of IHC markers in patients with and without treatment***

Distribution of expression	ER		PR		Bcl-2		Ki-67	
	ER (T)	*ER (non-T)*	*PR (T)*	*PR (non-T)*	*Bcl-2 (T)*	*Bcl-2 (non-T)*	*Ki-67 (T)*	*Ki-67 (non-T)*
Stromal	20–70%	0–100%	70–100%	5–100%	40–90%	0–90%	0–2%	0–90%
Stromal average	Low 44.44%	High 45.86%	High 90%	Low 71.43%	High 70%	Low 27.29%	Low 0.67%	High 20%
p	0.933		0.025		0.012		0.001	
Epithelial	0–70%	0–90%	0–100%	0–100%	0–90%	0–90%	0–50%	0–35%
Epithelial average	Low 23.33%	High 25.86	Low 25%	High 53.57%	High 51.11%	Low 24.43%	Low 1.22%	High 8%
p	0.885		0.02		0.05		0.028	

Bcl-2: B-cell lymphoma-2; ER: Estrogen receptor; IHC: Immunohistochemical; non-T: Cases without treatment; PR: Progesterone receptor; T: Treatment cases.

Figure 1 (A–E) and Figure 2 (A–E) show representative examples of Hematoxylin–Eosin (HE) staining and IHC expression of ER, PR, Bcl-2, and Ki-67 in endometrial ovarian tissue in patients with treatment and without treatment. The two-endometriosis area in both study groups – treatment (Figure 1A) *vs* non-treatment (Figure 2A) –, presented endometriotic cyst lined by simple columnar epithelium, endometrial stroma, abundant hemosiderin-laden macrophages, and fresh hemorrhage in endometrial stroma.

For endometriosis with treatment, we show examples of high percentage of glandular and stromal cells expressing ER (Figure 1B). PR expression was negative in glandular epithelium and positive in surrounding endometrial stroma (Figure 1C). Bcl-2 showed focal, weak, cytoplasmic expression in glandular epithelium and focal positive in stroma, being positive specially in surrounding stromal inflammatory cells (Figure 1D). Ki-67 was predominantly negative in both endometrial glands and stroma (Figure 1E).

For endometriosis without treatment, the expression was found to be to treatment group, except PR that showed stromal positivity but more discontinuous and in the less stromal cells (Figure 2C). Bcl-2 was negative in both comportment: epithelium and stromal cells (Figure 2D). Ki-67 was focal positive in both endometrial glands and stroma (Figure 2E).

The variations of OPN levels in the first moment of our patients' blood evaluation were not statistically significant. But the medium levels of OPN in the day of the surgery were statistically higher in patients with endometriosis that underwent treatment (p=0.05). Also, we have found statistically significant variations between the levels of OPN in the third moment of our serum evaluation, at six months after surgery. The patients treated with progesterone had statistically significant higher levels of OPN (p=0.001).

VEGF serum levels are markedly reduced in progesterone-treated patients with endometriosis compared with untreated patients with endometriosis, but the difference was not statistically significant (Table 4).

Discussions

The continuous attempts in trying to determine a sensitive and specific biomarker to help diagnosing patients with endometriosis, or to help anticipate the response to therapy, or even establish some pathophysiological pathways for this disease, leads to large amounts of research and discordant results that only underlines the need to do more and better in this direction. Our study ads up in this collective effort to beat the maze that endometriosis represent.

OPN is considered to promote the migration of the cells in many types of cancers, determining the supposition that it might be involved also in the dissemination of ectopic endometriotic tissue [27]. The implications of this protein, both in endometriosis and in infertility, attracted the interest of nowadays research. Our study revealed that Desogestrel treatment increases OPN levels. Because OPN is an inflammatory marker, we expected high levels of OPN in untreated patients, with a decrease in its serum amounts, in treated patients. Our study reveals an enhancement of OPN levels under the influence of progestin treatment. There are conflicting results in literature about OPN levels and its implications in women with endometriosis. OPN levels were lower in patients with endometriosis than in women with other kinds of ovarian benign disease, according to Moszynski *et al*. in 2013 [28], but Cho *et al*. in 2009 showed the presence of higher levels of this inflammatory marker in patients with documented endometriosis [29].

ER overexpression was frequently associated with endometriosis. ER was observed in both study groups, and it did not seem to vary significantly with Desogestrel treatment. Our findings underline the limited effect of progesterone treatment over the expression of ER both in stroma and epithelium.

PR expression was negative in glandular epithelium, positive in surrounding endometrial stroma in both groups of patients. It is worth mentioning that PR showed discontinue immunostaining in stroma, compared to patients with treatment.

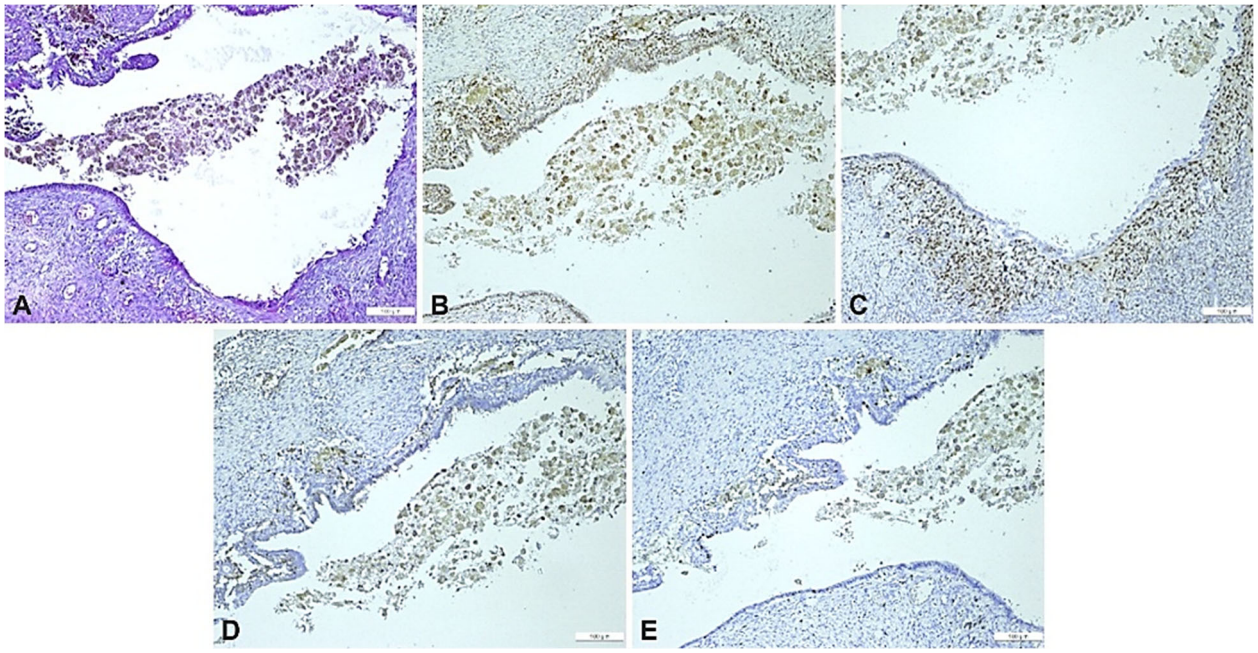

Figure 1 – ***Endometriosis with treatment: (A) Ovarian endometriotic cyst: endometrial glands and stroma, lumen with hemorrhage and hemosiderin-laden macrophage; (B) Epithelial strong, nuclear staining: stromal decidualized cell with moderate and strong positive expression; (C) Epithelial moderate, inconstant, nuclear staining: stromal decidual cell with diffuse, positive expression in next vicinity to the epithelium; (D) Epithelial weak, inconstant cytoplasmic staining: stromal focal, positive expression; (E) Epithelial inconstant nuclear staining: stromal decidual cells with negative expression. HE staining: (A) ×100. Anti-ER antibody immunomarking: (B) ×200. Anti-PR antibody immunomarking: (C) ×200. Anti-Bcl-2 antibody immunomarking: (D) ×200. Anti-Ki-67 antibody immunomarking: (E) ×200. Bcl-2: B-cell lymphoma-2; ER: Estrogen receptor; HE: Hematoxylin–Eosin; PR: Progesterone receptor.***

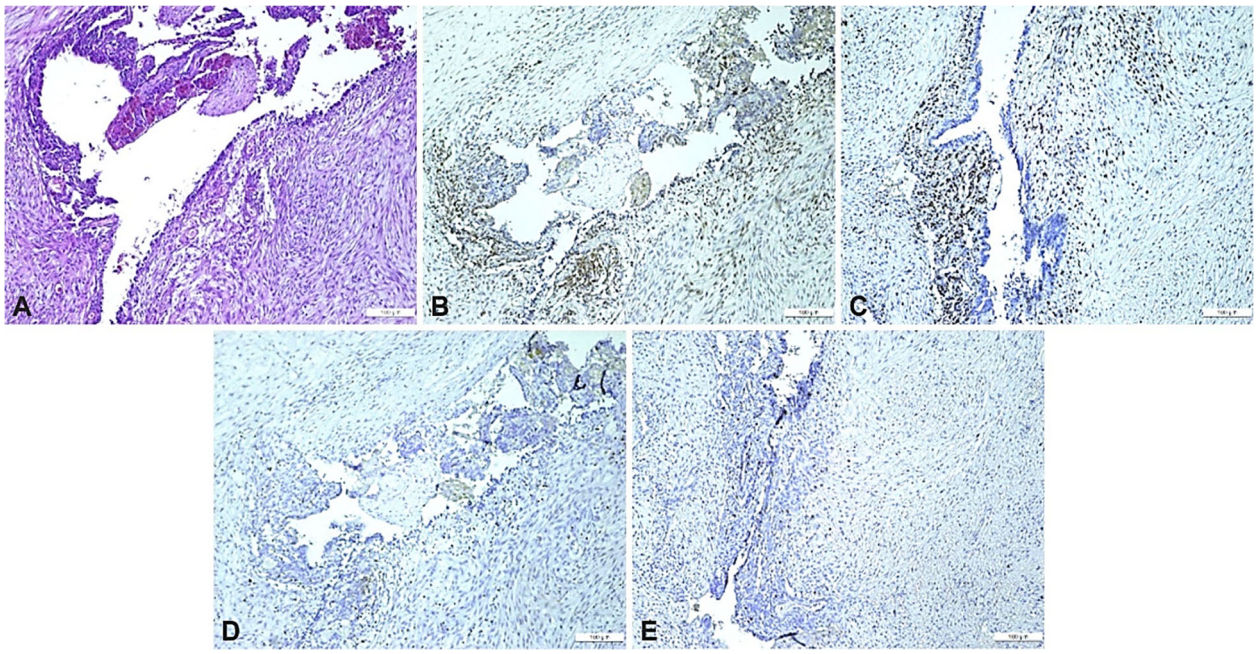

Figure 2 – ***Endometriosis without treatment: (A) Ovarian endometriotic cyst: endometrial glands lined by simple columnar epithelium, hemorrhage in decidualized endometrial stroma; (B) Epithelial and decidualized stroma with strong, diffuse, multifocal, nuclear staining; (C) Epithelial negative and irregular, discontinuous stroma positivity nuclear staining; (D) Epithelial negative: stromal focal, positive expression in inflammatory cells; (E) Epithelial nuclear staining in some epithelial cells: stromal decidual cells with focal positive expression. HE staining: (A) ×100. Anti-ER antibody immunomarking: (B) ×200. Anti-PR antibody immunomarking: (C) ×200. Anti-Bcl-2 antibody immunomarking: (D) ×200. Anti-Ki-67 antibody immunomarking: (E) ×200. Bcl-2: B-cell lymphoma-2; ER: Estrogen receptor; HE: Hematoxylin–Eosin; PR: Progesterone receptor.***

Table 4 – ***Serum samples in women with endometriosis in three moments (M1–M3) of the study***

Group	*n*	OPN (serum pg/mL)			VEGF-A (serum pg/mL)		
		Min.	***Mean***	***Max.***	***Min.***	***Mean***	***Max.***
M1 (day of US diagnosis)							
T	16	<74	220.15	897.24	126.39	955.48	3048.58
non-T	24	<74	337.22	1084.30	150.45	558.41	995.31
M2 (day of surgery)							
T	16	<74	455.13	1740.50	145.24	744.02	2625.08
non-T	24	<74	186.72	569.39	150.45	558.41	995.31
M3 (day of reevaluation – six months after surgery)							
T	16	<74	1178.13	5706.30	147.34	578.47	2750.90
non-T	24	<74	114.25	222.20	419.35	660.82	873.20

n: No. of cases; non-T: Cases without treatment; OPN: Osteopontin; T: Treatment cases; US: Ultrasound; VEGF: Vascular endothelial growth factor.

The study we have conducted showed an increased PR expression in the stroma of Desogestrel-treated women, suggesting increased stromal progesterone sensitivity in this group.

Both Brandenberger *et al*. and Brătilă *et al*. studies revealed an alteration of ER and PR levels in endometriotic lesions, compared to normal endometrium. According to the results reported by Brandenberger *et al*. and Brătilă *et al*., the ER expression level was identically in patients with treatment compared to patients without treatment [30, 31]. The results we have obtained are similar to those obtained by Hayashi *et al*. and Mehedintu *et al*., that reported an increase in PR after progesterone treatment. The aspect underlines the decrease in progesterone resistance in patients with endometriosis, under progesterone treatment [32, 33]. All the patients with high OPN levels also had an increase in the IHC expression of PR and Bcl-2.

Bcl-2 overexpression was frequently associated with endometriosis in a patient with treatment compared to patients without treatment. The Bcl-2 seems to be highly influenced by Desogestrel treatment, both stromal and epithelial expression being increased in the group with treatment.

We observed focal positive Ki-67 immunostaining in the group of patients without treatment. Desogestrel treatment lowers Ki-67 expression in endometriotic tissue. The research of Nguyen *et al.* also showed a decrease of Ki-67 percentage of positive cells in patients under progesterone treatment [34]. The expression of Ki-67, a proliferation immunomarker, was negatively influenced by progesterone treatment.

There are many studies in literature that state that the appearance and development of endometriosis is partially determined by the loss of progesterone signaling in the ectopic endometrial cells and by progesterone resistance [28, 35, 36]. Exogenous administration of progesterone inhibits cell proliferation, resulting in stagnation or even a decrease in the endometriosis cyst's dimensions, an improved cyst's dissection during surgery and a reduction of the bleeding [19].

VEGF suppression determined by progesterone treatment improved patients' outcomes; VEGF being involved in the pathogenesis of this disease. In an animal model meta-analysis from 2016, the anti-VEGF treatment inhibited the size endometriotic lesions. Our study reflects the drop in VEGF levels in treated endometriosis patients, thus sketching a possible future treatment direction [37].

There are studies that show markedly increased values of serum VEGF in patients with endometriosis, compared with controls. Our results indicate that Desogestrel treatment reduces VEGF serum levels in patients with endometriosis but with no statistically significant value [38–40].

In another research done in 2017, we analyzed using 30-Item Endometriosis Health Profile (EHP-30) – health-related quality of life (QoL) –, the impact of progesterone treatment on the QoL in endometriosis affected women, including pain relief evaluation [41]. Becker's *et al*. review from 2017, that evaluated the rate of pain reduction after medical treatment in women with endometriosis, reveals extreme variability between studies [42]. It would be interesting for us to evaluate the degree of pain reduction in women with endometriosis, with and without serum and tissue improved response after treatment.

Conclusions

The study we have conducted gives a complex view over how Desogestrel treatment works. The evaluation of the effects that treatment has on specific tissue receptors, the evaluation of the two markers for cell proliferation and apoptosis and also the examination of OPN and VEGF levels in endometriosis patients' plasma, with and without treatment, are of great interest nowadays. Desogestrel medication has given and continues to give hope to these patients by increasing PR expression and decreasing Ki-67 proliferation marker expression, as well as VEGF levels, on a molecular level. By doing these, exogenous synthetic progestin administration in patients with endometriosis improves their symptoms, decreases the dimensions of the cysts, and possibly enhances intraoperative conditions. OPN does not seem to be a useful marker in endometriosis. This affirmation is supported by the fact that after surgery, the OPN levels did not seem to suffer significant variations. New studies need to be performed to evaluate the mechanism by which the levels of progesterone or the administration of exogenous synthetic progestins affects the serum levels of OPN in endometriosis patients. Our study had a major limitation, that of a low number of patients included. The reduced number of patients was caused by limited elective surgery due to COVID-19 pandemic.

Conflict of interests

The authors declare no conflict of interests.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania (Approval No. 10/2020).

Authors' contribution

First two authors, Daniela-Roxana Matasariu and Ludmila Lozneanu, equally contributed to this article.

References

[1] Giudice LC. Clinical practice. Endometriosis. N Engl J Med, 2010, 362(25):2389–2398. https://doi.org/10.1056/NEJMcp1000274 PMID: 20573927 PMCID: PMC3108065

[2] International Working Group of AAGL, ESGE, ESHRE and WES; Tomassetti C, Johnson NP, Petrozza J, Abrao MS, Einarsson JI, Horne AW, Lee TTM, Missmer S, Vermeulen N, Zondervan KT, Grimbizis G, De Wilde RL. An international terminology for endometriosis, 2021. Facts Views Vis Obgyn, 2021, 13(4):295–304. https://doi.org/10.52054/FVVO.13.4.036 PMID: 34672510 PMCID: PMC9148705

[3] Albu Matasariu DR, Iacob M, Dumitrascu I, Onofriescu M, Vulpoi C. Desogestrel in the preoperative treatment of endometriosis cysts. Rev Med Chir Soc Med Nat Iaşi, 2015, 119(3):710–714. https://www.revmedchir.ro/index.php/revmedchir/article/view/382

[4] Li YZ, Wang LJ, Li X, Li SL, Wang JL, Wu ZH, Gong L, Zhang XD. Vascular endothelial growth factor gene polymorphisms contribute to the risk of endometriosis: an updated systematic review and meta-analysis of 14 case-control studies. Genet Mol Res, 2013, 12(2):1035–1044. https://doi.org/10.4238/2013.April.2.20 PMID: 23613250

[5] Malutan A, Drugan T, Georgescu C, Ciortea R, Bucuri C, Bobric A, Rada MP, Mihu D. Vascular endothelial growth factor serum levels in women with advanced endometriosis. Acta Endocrinol (Bucharest), 2016, 12(1):7–13. https://doi.org/10.4183/aeb.2016.7 PMID: 31258793 PMCID: PMC6586760

[6] Streuli I, de Ziegler D, Santulli P, Marcellin L, Borghese B, Batteux F, Chapron C. An update on the pharmacological management of endometriosis. Expert Opin Pharmacother, 2013, 14(3):291–305. https://doi.org/10.1517/14656566.2013.767334 PMID: 23356536

[7] Aznaurova YB, Zhumataev MB, Roberts TK, Aliper AM, Zhavoronkov AA. Molecular aspects of development and regulation of endometriosis. Reprod Biol Endocrinol, 2014, 12:50. https://doi.org/10.1186/1477-7827-12-50 PMID: 24927773 PMCID: PMC4067518

[8] Brătilă E, Ionescu OM, Badiu DC, Berceanu C, Vlădăreanu S, Pop DM, Mehedinţu C. Umbilical hernia masking primary umbilical endometriosis – a case report. Rom J Morphol Embryol, 2016, 57(2 Suppl):825–829. PMID: 27833977

[9] Czyzyk A, Podfigurna A, Szeliga A, Meczekalski B. Update on endometriosis pathogenesis. Minerva Ginecol, 2017, 69(5):447–461. https://doi.org/10.23736/S0026-4784.17.04048-5 PMID: 28271702

[10] Merino D, Lok SW, Visvader JE, Lindeman GJ. Targeting BCL-2 to enhance vulnerability to therapy in estrogen receptor-positive breast cancer. Oncogene, 2016, 35(15):1877–1887. https://doi.org/10.1038/onc.2015.287 PMID: 26257067

[11] Du C, Zhang X, Yao M, Lv K, Wang J, Chen L, Chen Y, Wang S, Fu P. Bcl-2 promotes metastasis through the epithelial-to-mesenchymal transition in the BCap37 medullary breast cancer cell line. Oncol Lett, 2018, 15(6):8991–8998. https://doi.org/10.3892/ol.2018.8455 PMID: 29844816 PMCID: PMC5958888

[12] Dai Y, Jin S, Li X, Wang D. The involvement of Bcl-2 family proteins in AKT-regulated cell survival in cisplatin resistant epithelial ovarian cancer. Oncotarget, 2017, 8(1):1354–1368. https://doi.org/10.18632/oncotarget.13817 PMID: 27935869 PMCID: PMC5352061

[13] Babaier A, Mal H, Alselwi W, Ghatage P. Low-grade serous carcinoma of the ovary: the current status. Diagnostics (Basel), 2022, 12(2):458. https://doi.org/10.3390/diagnostics12020458 PMID: 35204549 PMCID: PMC8871133

[14] Cristescu C, Velişcu A, Marinescu B, Pătraşcu A, Traşcă ET, Pop OT. Endometriosis – clinical approach based on histological findings. Rom J Morphol Embryol, 2013, 54(1):91–97. PMID: 23529314

[15] Nnoaham KE, Hummelshoj L, Webster P, d'Hooghe T, de Cicco Nardone F, de Cicco Nardone C, Jenkinson C, Kennedy SH, Zondervan KT; World Endometriosis Research Foundation Global Study of Women's Health Consortium. Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries. Fertil Steril, 2011, 96(2):366–373.e8. https://doi.org/10.1016/j.fertnstert.2011.05.090 PMID: 21718982 PMCID: PMC3679489

[16] Streuli I, Santulli P, Chouzenoux S, Chapron C, Batteux F. Serum osteopontin levels are decreased in focal adenomyosis. Reprod Sci, 2017, 24(5):773–782. https://doi.org/10.1177/1933719116669054 PMID: 27678100

[17] Gagliardi M, Ashizawa AT. Making sense of antisense oligonucleotide therapeutics targeting Bcl-2. Pharmaceutics, 2022, 14(1):97. https://doi.org/10.3390/pharmaceutics14010097 PMID: 35056993 PMCID: PMC8778715

[18] Yang M, Jiang C, Chen H, Nian Y, Bai Z, Ha C. The involvement of osteopontin and matrix metalloproteinase-9 in the migration of endometrial epithelial cells in patients with endometriosis. Reprod Biol Endocrinol, 2015, 13:95. https://doi.org/10.1186/s12958-015-0090-4 PMID: 26289107 PMCID: PMC4545920

[19] Albu Matasariu DR, Mihalceanu E, Pangal A, Vulpoi C, Onofriescu M, Nitoi L, Mihaila A, Costachescu G, Constantinescu D, Dumitrascu I. Can osteopontin be considered a biomarker for endometriosis? Rev Chim (Bucharest), 2017, 68(9):2132–2134. https://doi.org/10.37358/RC.17.9.5840 https://revistadechimie.ro/Articles.asp?ID=5840

[20] Buggio L, Somigliana E, Barbara G, Frattaruolo MP, Vercellini P. Oral and depot progestin therapy for endometriosis: towards a personalized medicine. Expert Opin Pharmacother, 2017, 18(15):1569–1581. https://doi.org/10.1080/14656566.2017.1381086 PMID: 28914561

[21] Korver T. A double-blind study comparing the contraceptive efficacy, acceptability and safety of two progestogen-only pills containing Desogestrel 75 µg/day or Levonorgestrel 30 µg/day. Collaborative Study Group on the Desogestrel-containing Progestogen-only Pill. Eur J Contracept Reprod Health Care, 1998, 3(4):169–178. https://doi.org/10.3109/13625189809167250 PMID: 10036599

[22] Razzi S, Luisi S, Ferretti C, Calonaci F, Gabbanini M, Mazzini M, Petraglia F. Use of a progestogen only preparation containing Desogestrel in the treatment of recurrent pelvic pain after conservative surgery for endometriosis. Eur J Obstet Gynecol Reprod Biol, 2007, 135(2):188–190. https://doi.org/10.1016/j.ejogrb.2006.08.002 PMID: 16963174

[23] Remorgida V, Abbamonte LH, Ragni N, Fulcheri E, Ferrero S. Letrozole and Desogestrel-only contraceptive pill for the treatment of stage IV endometriosis. Aust N Z J Obstet Gynaecol, 2007, 47(3):222–225. https://doi.org/10.1111/j.1479-828X.2007.00722.x PMID: 17550490

[24] Leone Roberti Maggiore U, Remorgida V, Scala C, Tafi E, Venturini PL, Ferrero S. Desogestrel-only contraceptive pill *versus* sequential contraceptive vaginal ring in the treatment of rectovaginal endometriosis infiltrating the rectum: a prospective open-label comparative study. Acta Obstet Gynecol Scand, 2014, 93(3):239–247. https://doi.org/10.1111/aogs.12326 PMID: 24372517

[25] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol, 1998, 11(2):155–168. PMID: 9504686

[26] Suzuky Y, Honma T, Hayashi S, Ajioka Y, Asakura H. Bcl-2 expression and frequency of apoptosis correlate with morphogenesis of colorectal neoplasia. J Clin Pathol, 2002, 55(3):212–216. https://doi.org/10.1136/jcp.55.3.212 PMID: 11896074 PMCID: PMC1769608

[27] Ramachandran S, Kwon KY, Shin SJ, Kwon SH, Cha SD, Lee HG, Hong YB, Bae I, Lee GH, Cho CH. Regulatory role of osteopontin in malignant transformation of endometrial cancer. Mol Biol Rep, 2013, 40(5):3623–3629. https://doi.org/10.1007/s11033-012-2436-8 PMID: 23269624

[28] Moszynski R, Szubert S, Szpurek D, Michalak S, Sajdak S. Role of osteopontin in differential diagnosis of ovarian tumors. J Obstet Gynaecol Res, 2013, 39(11):1518–1525. https://doi.org/10.1111/jog.12097 PMID: 23875677

[29] Cho S, Ahn YS, Choi YS, Seo SK, Nam A, Kim HY, Kim JH, Park KH, Cho DJ, Lee BS. Endometrial osteopontin mRNA

expression and plasma osteopontin levels are increased in patients with endometriosis. Am J Reprod Immunol, 2009, 61(4):286–293. https://doi.org/10.1111/j.1600-0897.2009.00692.x PMID: 19260859

[30] Brandenberger AW, Lebovic DI, Tee MK, Ryan IP, Tseng JF, Jaffe RB, Taylor RN. Oestrogen receptor (ER)-alpha and ER-beta isoforms in normal endometrial and endometriosis-derived stromal cells. Mol Hum Reprod, 1999, 5(7):651–655. https://doi.org/10.1093/molehr/5.7.651 PMID: 10381820

[31] Brătilă E, Brătilă CP, Comandaşu DE, Bauşic V, Vlădescu CT, Mehedinţu C, Berceanu C, Cîrstoiu MM, Mitroi G, Stănculescu R. The assessment of immunohistochemical profile of endometriosis implants, a practical method to appreciate the aggressiveness and recurrence risk of endometriosis. Rom J Morphol Embryol, 2015, 56(4):1301–1307. PMID: 26743275

[32] Hayashi A, Tanabe A, Kawabe S, Hayashi M, Yuguchi H, Yamashita Y, Okuda K, Ohmichi M. Dienogest increases the progesterone receptor isoform B/A ratio in patients with ovarian endometriosis. J Ovarian Res, 2012, 5(1):31. https://doi.org/10.1186/1757-2215-5-31 PMID: 23113924 PMCID: PMC3541078

[33] Mehedintu C, Antonovici M, Brinduse L, Bratila E, Stanculescu R, Berceanu C, Bratu O, Pituru S, Onofriescu M, Matasariu DR. The influence of progesterone on immunohistochemical markers in endometriosis. Rev Chim (Bucharest), 2018, 69(3):581–584. https://doi.org/10.37358/RC.18.3.6153 https://revistadechimie.ro/Articles.asp?ID=6153

[34] Nguyen TT, Hachisuga T, Urabe R, Ueda T, Kurita T, Kagami S, Kawagoe T, Hisaoka M. Immunohistochemical analysis of the effect of Dienogest on ovarian endometriotic cysts. J UOEH, 2016, 38(4):271–278. https://doi.org/10.7888/juoeh.38.271 PMID: 27980309

[35] Kapoor R, Stratopoulou CA, Dolmans MM. Pathogenesis of endometriosis: new insights into prospective therapies. Int J Mol Sci, 2021, 22(21):11700. https://doi.org/10.3390/ijms222111700 PMID: 34769130 PMCID: PMC8583778

[36] Brichant G, Laraki I, Henry L, Munaut C, Nisolle M. New therapeutics in endometriosis: a review of hormonal, non-hormonal, and non-coding RNA treatments. Int J Mol Sci, 2021, 22(19):10498. https://doi.org/10.3390/ijms221910498 PMID: 34638843 PMCID: PMC8508913

[37] Liu S, Xin X, Hua T, Shi R, Chi S, Jin Z, Wang H. Efficacy of anti-VEGF/VEGFR agents on animal models of endometriosis: a systematic review and meta-analysis. PLoS One, 2016, 11(11):e0166658. https://doi.org/10.1371/journal.pone.0166658 PMID: 27855197 PMCID: PMC5113963

[38] Kianpour M, Nematbakhsh M, Ahmadi SM, Jafarzadeh M, Hajjarian M, Pezeshki Z, Safari T, Eshraghi-Jazi F. Serum and peritoneal fluid levels of vascular endothelial growth factor in women with endometriosis. Int J Fertil Steril, 2013, 7(2):96–99. PMID: 24520470 PMCID: PMC3850339

[39] Méar L, Herr M, Fauconnier A, Pineau C, Vialard F. Polymorphism and endometriosis: a systematic review and meta-analyses. Hum Reprod Update, 2020, 26(1):73–102. https://doi.org/10.1093/humupd/dmz034 PMID: 31821471

[40] Donnez J, Cacciottola L. Endometriosis: an inflammatory disease that requires new therapeutic options. Int J Mol Sci, 2022, 23(3):1518. https://doi.org/10.3390/ijms23031518 PMID: 35163463 PMCID: PMC8836207

[41] Matasariu RD, Mihaila A, Iacob M, Dumitrascu I, Onofriescu M, Crumpei Tanasa I, Vulpoi C. Psycho-social aspects of quality of life in women with endometriosis. Acta Endocrinol (Bucharest), 2017, 13(3):334–339. https://doi.org/10.4183/aeb.2017.334 PMID: 31149196 PMCID: PMC6516567

[42] Becker CM, Gattrell WT, Gude K, Singh SS. Reevaluating response and failure of medical treatment of endometriosis: a systematic review. Fertil Steril, 2017, 108(1):125–136. https://doi.org/10.1016/j.fertnstert.2017.05.004 PMID: 28668150 PMCID: PMC5494290

Corresponding authors

Alexandra Ursache, Assistant Professor, MD, PhD, Department of Mother and Child, Grigore T. Popa University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iaşi, Romania; Phone +40232–301 600, e-mail: carpalecsandra@yahoo.com

Anca Daniela Brăila, Associate Professor, MD, PhD, Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40351–443 500, e-mail: ancabraila@yahoo.com

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