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Clinical, morphological and immunohistochemical survey in different types of endometriosis

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Abstract

Endometriosis is a benign pathology, commonly found in women at reproductive age. It is represented by the ectopic presence of the endometrial glandular epithelium in several tissues and organs. This ectopically located tissue can display premalignant or even malignant changes under the influence of certain factors that affect cell structure, function and proliferation. Our study includes a total of 28 patients, with endometriosis of different localizations: ovarian or pelvic endometriosis, adenomyosis or endometriosis of the abdominal wall. We performed a clinical and statistical analysis upon the collected clinical and laboratory data, together with the results obtained by using classical histological and immunohistochemical (IHC) profiling. The classical staining revealed the existence of the ectopic glandular epithelium, while the IHC reactions obtained with the anti-cytokeratin (CK) 7/anti-CK20, anti-estrogen receptor alpha (ERα)/anti-progesterone receptor (PR) antibodies, ascertained that these tissues were of endometrial origin. The environmental, hormonal or inflammatory factors influence these areas, so that the ER/PR scores may be modified, the cellular proliferation might be increased (Ki67+ marker), the anti-apoptotic B-cell lymphoma 2 (BCL2) protein expression and phosphatase and tensin homolog (PTEN) may also be modified. Moreover, tumor protein 53 (p53) was positive in cases with atypia, density of inflammatory cells clearly increased compared to the adjacent normal endometrium, respectively with cluster of differentiation (CD) 3+, CD20+, CD68+, CD79α+, and tryptase+ cells, all of which may influence the cellular structure, histological architecture of the surrounding microenvironment and cause premalignant or even malignant changes in endometriosis outbreaks.

Keywords: endometriosis, clinical statistics, inflammatory cells, microscopic analysis.

Introduction

Endometriosis is a benign pathology, represented by the ectopic location of endometrial glands, outside the uterus. It is commonly found in the pelvis, peritoneum, rectovaginal septum [1], ovaries, abdominal wall [2], Caesarean section (CS) or hysterectomy scar [3], umbilicus, and less commonly in other organs (kidney, urinary tract, colon, lung or brain). It can also be found in the myometrium, as adenomyosis, or primary umbilical endometriosis (PUE), which is also rare [4, 5]. It affects especially women at reproductive age.

The most common symptoms in this pathology are pain, low fever, bleeding, but also dysmenorrhea, dysuria, dyspareunia. The severity and complexity of pain may involve major problems in the management of therapeutic resources [6, 7].

Dysmenorrhea affects approximately 62% of women with endometriosis [8], 20–30% of women experience infertility, and 40–60% chronic pelvic pain [9]. Most commonly, endometriosis is discovered by laparoscopy or laparotomy, being localized in the ovary or in the Douglas' pouch [10, 11]; extraperitoneal lesions are found less frequently [12, 13].

There are many hypotheses regarding the etiopathogenicity of the disease: the most accepted theory being that of retrograde menstruation (Sampson's theory). Other hypotheses postulated in time were coelomic metaplasia; lymphatic or vascular dissemination, an origin in embryonic stem cells, or the so-called induction theory [14–16]. Although endometriosis is a benign condition, it may have malignancy characteristics: local or distance dissemination, damage of adjacent tissues or cell invasion [9, 11]. Risk factors for endometriosis are early menarche, short interval of menstrual periods, late menopause, or null parity. It can also be associated with CS, tubal ligation, hysterectomy, contraceptive use or pregnancy [17].

The most commonly known system of staging in endometriosis was developed by the *American Society for Reproductive Medicine* (ASRM), which divides this pathology into four stages dependent on the intraoperative evaluation of localization and spread of the lesions, as follows: I – minimum, II – mild, III – moderate, IV – severe. In advanced stages, profound implantation of endometriosis is described and defined by deep penetration (>5 mm) of subperitoneal tissues [18, 19].

In the absence of specific symptoms, the positive diagnosis may encounter several traps. Clinical gynecological examination may be normal or may indicate painful nodules in the uterosacral ligaments or in the Douglas' pouch [20]. Among the imaging investigations, transvaginal sonography (TVS) is widely used, being considered the first imaging line in diagnosing this pathology [21]. This can identify ovarian endometriosis or urinary tract endometriosis, but in order to detect endometriosis of the posterior pelvic compartment, vaginal, uterosacral ligaments' or rectovaginal septum, it has a low sensitivity. Therefore, sonovaginography (SVG) with saline solution has been used, representing a combination of TVS and introduction of a saline solution in the vagina, creating an acoustic window between the transvaginal probe and the structures surrounding the vagina. This technique can diagnose rectovaginal endometriosis with much higher accuracy than TVS (sensitivity of 90.6% and a specificity of 85.7%) [19, 22].

It should also be kept in mind that, in addition to adverse symptoms, there may also be associated risk factors that alter the structure of endometrial glands, leading to premalignant or even malignant transformation of endometriosis outbreaks. Studies have shown that genes, the immune system, environmental factors and hormonal influences can lead to transformation into endometrial hyperplasia, dysplasia and even malignant transformation in outbreaks of the ectopic endometrium [23–26]. The most common malignant tumors developed from endometriosis are ovarian epithelial carcinomas (clear cell adenocarcinoma, endometrioid adenocarcinoma), and more rarely carcinosarcomas, Müllerian adenosarcomas and other tumor types [27].

Purpose

The purpose of this study was to evaluate the clinical characteristics of patients with endometriosis, to compare certain histological and immunohistochemical (IHC) aspects of the ectopic endometrial outbreaks, according to localization, and to demonstrate the involvement of some risk factors in the evolution of this pathology.

Patients, Materials and Methods

Our study was conducted on 28 cases of endometriosis: seven cases of ovarian endometriosis, seven cases of pelvic endometriosis, seven cases of adenomyosis (myometrial endometriosis) and seven cases of abdominal wall endometriosis. The patients were hospitalized and investigated in the Department of Obstetrics and Gynecology, Emergency County Hospital of Craiova, Romania, between 2010 and 2018.

A clinical and statistical study was performed, using the Microsoft Excel 2010 program, based on: year of hospitalization, location of endometriosis (ovarian, pelvic, adenomyosis, abdominal wall endometriosis), patient age, environment of origin, height, weight, body mass index (BMI), behaviors (*e.g.*, coffee, alcohol, smoking), living and working conditions (housewife/employee/student), symptoms (bleeding, pain), personal history (menarche, regular or irregular menstruation, spontaneous or on request abortions, number of natural or CS deliveries, infertility, surgical procedures), laboratory data [leukocyte formula, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, erythrocyte sedimentation rate (ESR), serum value of cancer antigen 125 (CA125)] and the medication used to alleviate the symptomatology. All patient data were collected and compared.

The surgical management of the studied cases is described in Table 1.

Table 1 – *Surgical techniques depending on the localization of endometriosis*

Endometriosis – localization	Surgical management
Ovarian	▪ Cystectomy (three cases); ▪ Partial ovariectomy (four cases).
Pelvic	▪ Excision of endometriosis outbreaks (seven cases).
Adenomyosis	▪ Excision of adenomyosis outbreaks (seven cases).
Abdominal wall	▪ Excision of endometriotic nodules (seven cases).

After surgery, the specimens were sent for histopathological study at the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova. The tissues were fixed in 10% neutral buffered formalin, at room temperature.

After fixation, the specimens were washed in tap water for one hour, passed through successive alcohols 70% (overnight), 90%, 96%, 100% (one hour in each concentration), three incubations in xylene (3×1 hour), paraffin impregnation (56°C, overnight) and paraffin embedding as blocks the next day. They were cut to a thickness of 4 μm, using the HMB350 microtome (ThermoScientific), equipped with a water section transfer system, the sections obtained were next applied to poly-L-lysine treated slides for better adhesion, and left to dry in the thermostat (37°C) for 24 hours.

Subsequently, we continued with the protocol for the classical histological and special immunohistochemistry stainings.

For the classical Hematoxylin–Eosin (HE) staining, slides were deparaffinized, re-hydrated in increasing alcohol concentrations until distillate water (dH_2O), the nuclei stained with Hematoxylin (1 minute), and the cytoplasms with Eosin (2 minutes).

For IHC techniques, we dewaxed the samples in three xylene baths (3×10 minutes), dried the samples, encircled the tissue with a hydrophobic marker, and re-hydrated the tissue in alcoholic baths with decreasing concentration, 100%, 96%, 90%, 70% (5 minutes each), until dH_2O. Next, the slides were processed for antigen retrieval by microwaving (650 W, seven cycles × 3 minutes) in citrate, pH 6 or ethylenediaminetetraacetic acid (EDTA), pH 9, then left to cool down at room temperature. After thorough washing in dH_2O (3×5 minutes), the endogenous peroxidase was blocked in 3% hydrogen peroxide (H_2O_2) solution for 30 minutes, then the sections were washed in dH_2O (2×5 minutes), 1% phosphate-buffered saline (PBS) (1×5 minutes), and then the non-specific antigenic sites were blocked using 3% skimmed milk (30 minutes). The sections were then incubated with the primary antibody (Table 2) and kept at 4°C for 18 hours.

Next day, the samples were brought at ambient temperature (30 minutes), washed in PBS (3×5 minutes), incubated for one hour with the secondary antibody [mouse/rabbit immunoglobulin G (IgG) antibody, VC002-025, R&D Systems, VisUCyte Horseradish peroxidase (HRP) Polymer], and color was developed with 3,3'-Diaminobenzidine (DAB) (Dako). The nuclei were next labeled with Hematoxylin, and the samples were dehydrated in alcohols with increasing concentrations 70%, 90%, 96%, 100% (5 minutes each), clarified in xylene for 30–45 minutes and coversliped with a xylene-based mounting medium.

In order to demonstrate the existence of endometrial ectopic glands, we used tissue specific markers [cytokeratin (CK) and hormone receptors], and in order to view the cellular division we applied markers of cell proliferation and markers that highlighted the antiapoptotic transformations (Table 2). We counted the inflammatory cells around the endometriosis foci and those near the normal endometrium, and compared the obtained cell densities.

The slides were imaged with a Nikon DS-5Mc 5 megapixels cooled charged-coupled device (CCD) camera mounted on a Nikon Eclipse 44i microscope, and controlled by the Image ProPlus AMS 7 (Media Cybernetics, USA) image capture and analysis package. For all images captured for semi-quantitative analysis, we utilized the same exposure and illumination settings, and based on the red–green–blue (RGB) profile of the DAB signal, the regions of interest (ROIs) were segmented and automatically counted as individual elements (cells). All data was collected in Microsoft Excel spreadsheets, and were subjected to statistical analysis, showing the involvement of risk factors in the evolution, dissemination, proliferation and transformation of endometriosis foci.

Table 2 – *Immunohistochemical panel of antibodies used by us*

Antibody	Manufacturer	Clone	Antigen retrieval	Target	Dilution	Labeling
Anti-CK7	Dako	OV-TL 12/30	Citrate, pH 6	Monoclonal mouse anti-human CK7	1:50	Glandular epithelia
Anti-CK20	Dako	Ks20.8	Citrate, pH 6	Monoclonal mouse anti-human CK20	1:25	Cellular protein of mature enterocytes and goblet cells
Anti-ER	Dako	1D5	EDTA, pH 9	Monoclonal mouse anti-human ER*α*	1:50	Estrogen receptor *α*
Anti-PR	Dako	PgR 636	EDTA, pH 9	Monoclonal mouse anti-human PR	1:50	Progesterone receptor
Anti-Ki67	Dako	MIB-1	EDTA, pH 9	Monoclonal mouse anti-human Ki67	1:50	Cells in division in the G1, S, G2 and M phase
Anti-p53	Dako	DO-7	EDTA, pH 9	Monoclonal mouse anti-human p53 protein	1:50	Nuclear marker
Anti-BCL2	Dako	124	EDTA, pH 9	Monoclonal mouse anti-human BCL2 oncoprotein	1:50	B-cell lymphoma 2
Anti-PTEN	Abcam	ab31392	Citrate, pH 6	Polyclonal rabbit	1:50	Tumor suppressor gene
Anti-CD3	Dako	–	Citrate, pH 6	Polyclonal rabbit anti-human CD3	1:50	T-lymphocytes
Anti-CD20	Dako	L26	Citrate, pH 6	Monoclonal mouse anti-human CD20cy	1:50	B-lymphocytes
Anti-CD68	Dako	KP1	Citrate, pH 6	Monoclonal mouse anti-human CD68	1:100	Macrophages
Anti-CD79α	Dako	JCB117	EDTA, pH 9	Monoclonal mouse anti-human CD79*α*	1:50	B-lymphocytes
Anti-tryptase	Dako	AA1	Citrate, pH 6	Monoclonal mouse anti-human mast cell tryptase	1:500	Mast cells

CK: Cytokeratin; ER: Estrogen receptor; PR: Progesterone receptor; p53: Tumor protein 53; BCL2: B-cell lymphoma 2; PTEN: Phosphatase and tensin homolog; CD: Cluster of differentiation; EDTA: Ethylenediaminetetraacetic acid.

We next calculated the means, standard deviations, then compared the results and applied the appropriate statistical tests [analysis of variance (ANOVA): single factor and χ^2 (*chi*-square) test].

Results

Each of the 28 cases was investigated clinically, imagistically, and subsequently surgically treated using various techniques depending on the location of endometriosis. Upon the admission of each patient, common specific biological samples were collected and comparative data were obtained. The medical records, accompanied by the written consent of each patient for the use of personal data, or photographing of surgical specimens during and after surgery, were obtained in order to include the patients in the study.

The age at diagnosis of endometriosis varied according to the localization: ovarian endometriosis (22–36 years old), pelvic (30–47 years old), adenomyosis (30–44 years old), endometriosis of the abdominal wall (27–31 years old). We observed that the lowest average age of occurrence was for the endometriosis of the abdominal wall, the cause being the most common CS, and the highest average age of occurrence appeared for pelvic endometriosis. The mean age, for localization of ovarian endometriosis (3.71±4.6 years old) and abdominal wall endometriosis (28.42±2.22 years old) is significantly lower than the mean age of pelvic endometriosis (37.14±5.45 years old) and adenomyosis (34.57±4.6 years old) [$F(3.27)=5.39$, $p<0.005$] (Figure 1).

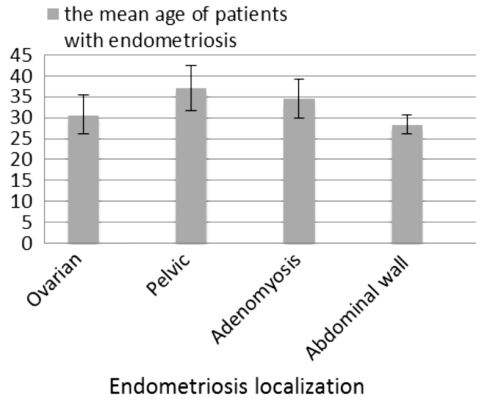

Figure 1 – ***The mean age of patients with endometriosis.***

Depending on the urban/rural environment, we showed that the most common cases of ovarian endometriosis were diagnosed in urban environment women, and the most common in the rural area were cases of endometriosis of the abdominal wall, but there was no predilection between the home environment and the location of endometriosis ($p=0.86$).

Depending on the year of diagnosis of endometriosis, it was observed that most cases of ovarian endometriosis were recorded in 2018, pelvic in 2017, adenomyosis in 2012 and 2017, and endometriosis of the abdominal wall in the years 2017 and 2018.

Using the height and weight of each patient with endometriosis, we calculated the body mass index (weight in kg/height in m), using the following intervals (underweight: <18.5; normoponderal: 18.5–25; overweight: 25.1–30; grade I obesity: 30.1–35, grade II obesity: 35.1–40, morbid obesity: >40) and obtaining results, depending on the location of endometriosis. Most patients were normoponderal.

The most common symptoms reported as reasons for admission were vaginal bleeding and pelviabdominal pain. Pain was present in all cases of ovarian or pelvic endometriosis and adenomyosis, and in the case of endometriosis of the abdominal wall, only one patient did not experience pelviabdominal pain. Only one case of endometriosis of the abdominal wall showed no symptoms.

Also, depending on the location, we compared the personal physiological history (the presence of regular/irregular menstrual periods, spontaneous/on demand abortions, the number of normal births/CS) and personal pathological antecedents (infertility, surgical interventions to remove endometriosis outbreaks: cystectomy, partial hysterectomy, hysterectomy, excision of endometriotic nodules from CS scarring). The average age of menarche was between 11 and 14 years old. Regarding the mean age of the menarche, it increased from abdominal wall endometriosis (11±0.77 years old) to adenomyosis (13±1.15 years old), ovarian endometriosis (13±0.81 years old) and pelvic endometriosis (13.14±0.89 years old) (Figure 6). Most patients with endometriosis of the abdominal wall had regular menstruation and most patients with pelvic or ovarian endometriosis had irregular menstruation; spontaneous abortions occurred only in pelvic endometriosis; most cases of abdominal wall endometriosis were associated with CS; primary infertility appeared most frequently in ovarian endometriosis, and most of the surgical cases were those diagnosed with ovarian endometriosis (Figure 2).

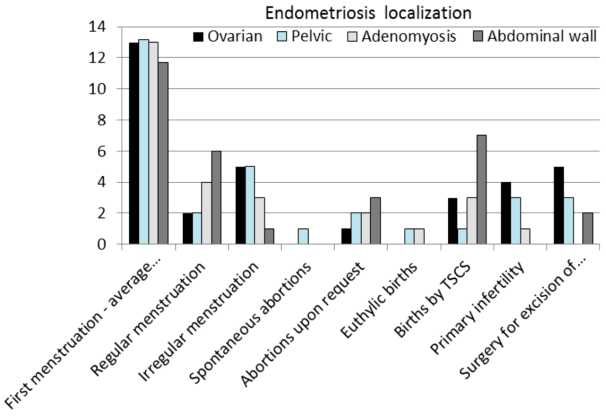

Figure 2 – ***Personal physiological and medical history. TSCS: Transverse segmental Caesarean section.***

In terms of laboratory tests, we compared the leukocyte formula, complete blood count (CBC), ESR (one hour) and patient serum CA125 marker using the same reference values (Table 3).

We have noticed that in patients with pelvic endometriosis, the number of leukocytes increased due to the number of neutrophils (four cases with leukocytosis from seven, three of which are attributable to neutrophils and one case to monocytes). Also, in ovarian endometriosis, there were cases with leukocytosis (three cases of leukocytosis from seven, three with increased neutrophils, one case with increased basophils), and for the cases of adenomyosis and endometriosis of the abdominal wall,

only one case of leukocytosis was present due to numerical growth of neutrophils (Figure 3).

Table 3 – ***Normal ranges of laboratory tests***

Parameter	Normal values
Leukocytes	4–10%
Neutrophils	2–8%
Lymphocytes	1–4%
Monocytes	0.3–1%
Eosinophils	0–0.7%
Basophils	0–0.2%
Erythrocytes	$3.8–5.1\times10^{6}$
Hemoglobin	11.7–15.5 g/dL
Hematocrit	35–45%
MCV	81–100 fL
MCH	27–34 $pg/10^{-12}$ g
MCHC	32–36 g/dL
Platelets	150 000–450 000
ESR (one hour)	1–12
CA125 serum	0–35 IU/mL

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; ESR: Erythrocyte sedimentation rate; CA125: Cancer antigen 125.

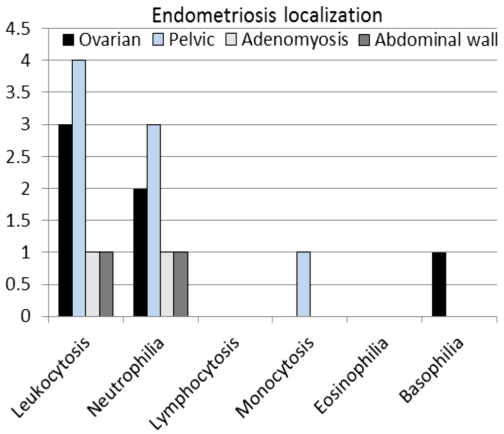

Figure 3 – ***Inflammatory changes depending on the location of endometriosis.***

By comparing the CBC, we found seven patients with microcytic anemia [mean corpuscular volume (MCV) <80 fL) (one patient with ovarian endometriosis, two patients with adenomyosis, three patients with endometriosis of the abdominal wall)] (Figure 4). The number of platelets was normal in all 28 patients. The increase of ESR above normal, as a marker of inflammation, was present in five patients (two with ovarian endometriosis, two with pelvic endometriosis, one with adenomyosis), and in the abdominal wall no cases with ESR increase have been reported. The increase in the CA125 serum marker was observed in one case of ovarian endometriosis and two cases of pelvic endometriosis (Figure 5) (*chi*-square test = 0.25).

The behaviors of patients varied, some being coffee-consumers, other smokers, or their associations, and only one patient claimed to be alcohol-consumer, diagnosed with adenomyosis. Most coffee-consumers were those with adenomyosis, while most smokers were identified for the group of abdominal wall endometriosis. These vices associated with other risk factors can accelerate periendometrial inflammatory processes. Most patients diagnosed with endometriosis are employed, but there was no association between work conditions and the localization of endometriosis. Before surgery, only three patients received hormonal treatment, 16 patients received antialgic treatment and nine patients did not receive any treatment (Figure 6).

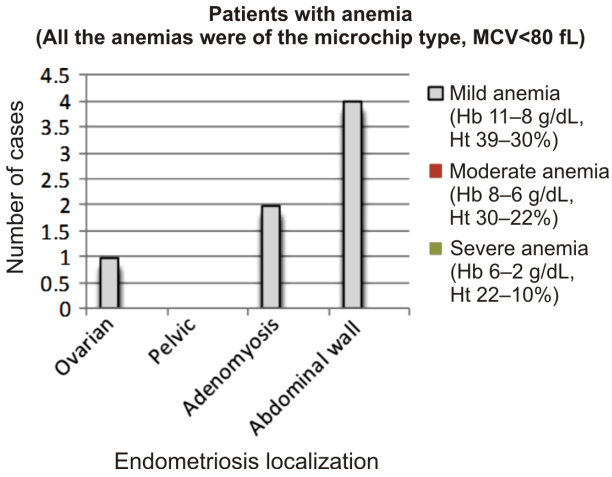

Figure 4 – ***Cases of anemia correlated with disease localization. MCV: Mean corpuscular volume; Hb: Hemoglobin; Ht: Hematocrit.***

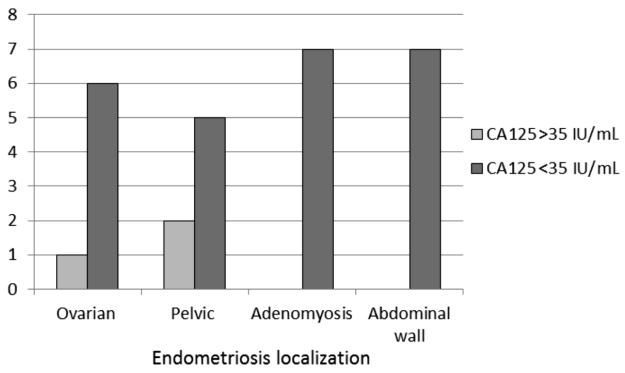

Figure 5 – ***Serum values of CA125. CA125: Cancer antigen 125.***

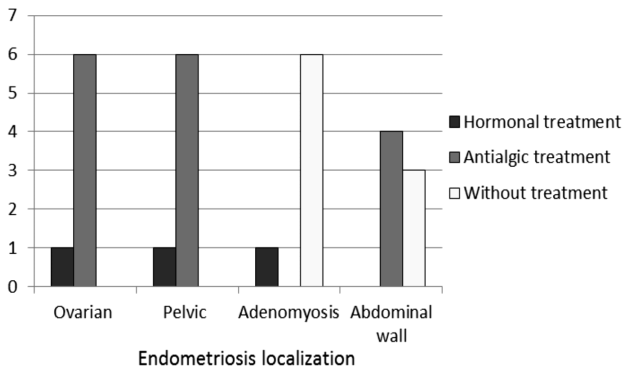

Figure 6 – ***Drug therapy.***

The surgical treatment for ovarian endometriosis consisted in cystectomy, unilateral or bilateral partial ovariectomy (Figure 7), depending on endometriotic cysts, or in the elderly, with associated pathologies, hysterectomy with bilateral anexectomy (Figure 8). Neglected endometriotic cysts in young women, can significantly affect fertility through multiple mechanisms, but also through pressure on ovarian tissue, significantly destroying and reducing the ovarian reserve.

Pelvic endometriosis was most commonly diagnosed during laparoscopy or opening the pelviabdominal cavity, performed for a certain genital pathology. Surgical conduct meant excision of endometriosis outbreaks from the pelvis, peritoneum, and rigorous cleaning of neighboring structures.

In case of adenomyosis, depending on age, the excision of myometrial endometriosis foci was performed with the reconstruction of the uterine cavity, in young women who wanted to maintain menstrual function and reproductive capacity, or total hysterectomy with bilateral oophorectomy in certain clinical circumstances.

Given that CS delivery is more and more common, endometriosis in the abdominal wall is more frequent. Endometriotic foci can be painful, intensify menstruation and create discomfort to the patient; that is why excision of endometriosis foci is practiced. All excised structures are sent for histopathological examination, which assures the diagnosis of certainty in endometriosis with different localizations.

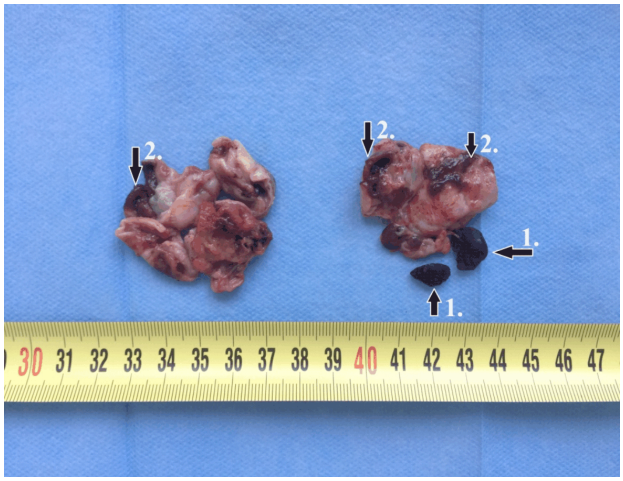

Figure 7 – ***Ovarian endometriotic cysts: bilateral ovarian cystectomy. Note the dark areas with tar-like appearance (1) and dark-violet areas, representing the endometrial glands in the cystic wall structure (2).***

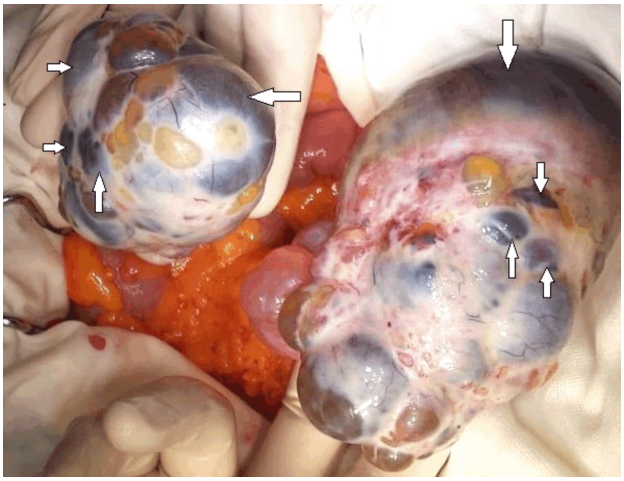

Figure 8 – ***Bilateral ovarian endometriomas indicated by arrows: surgical pathology.***

In the classical HE staining, we noticed the existence of ectopically located endometrium, in the structure of various tissues (ovarian, myometrial, peritoneal, *rectus abdominis* muscle). This ectopic endometrial tissue might have an organized or diffuse structure.

In ovarian endometriosis, the presence of ciliated cylindrical unstratified epithelium can be observed, as well as cysts with variable sizes, having a dark matter-content, chocolate appearance (tar), or multiple small, cystic structures. Also, in classical stainings, adenomyosis is visualized in the structure of the myometrium, at more than 2 mm from the junction with the endometrium. This ectopic endometrial tissue can be disposed as islands, which communicates with the uterine cavity. This might be a functional tissue that accumulates a chocolate-like content following the menstrual cycle, but it is often non-functional. External, pelvic or abdominal wall endometriosis is characterized by the presence of glandular simple stratified epithelia, stroma and frequent striated muscle fibers (Figure 9), performing similar structures and imitating uterine function, with cyclic hemorrhages, inflammatory reactions, forming adhesions or scars and deforming adjacent tissues.

Based on immunohistochemistry, we could show that the ectopic tissue was of endometrial type. With CK7, we showed that the areas of interest which exhibit glandular epithelium, are similar to the normal endometrium (Figure 10). In order to make a differential diagnosis with a digestive tract metastasis, the CK20 immunolabeling was performed, which gave a negative reaction, and showed that the tissue did not originate in the digestive tract. This negative reaction was observed in both endometriosis and normal endometrial areas (Figure 11). Estrogen receptor (ER) and progesterone receptor (PR) are present in the endometrial cells, and the positive immunolabeling reaction showed once again the endometrial origin (Figures 12 and 13).

Eutopic and ectopic endometrial cells, under hormonal influence, are in a continuous process of multiplication and development; Ki67 marks the cells in phases G1, S, G2 and mitosis of the cell cycle, and is missing in G0. The proliferation was more intense in foci of endometriosis (Figure 14), and the tumor protein 53 (p53) immunolabeling shows cells that undergo functional changes and have acquired anti-apoptotic ability (Figure 15).

B-cell lymphoma 2 (BCL2)-positive immunoassay showed that some endometrial ectopically localized cells suffer alterations that inhibit cellular apoptosis and may

lead to preneoplastic changes. In the normal endometrium, the reactivity had a much lower intensity (Figure 16).

The positive reaction with the anti-phosphatase and tensin homolog (PTEN) antibody showed that certain endometrial cells undergo structural changes, and their growth and division occurs more rapidly than normal. In the normal endometrium, there was a negative immunoreaction (Figure 17).

Considering that the inflammatory process may be involved in structural and functional cellular changes, we used several antibodies to determine the number of inflammatory cells around endometriosis foci and those adjacent to the normal endometrium, so we utilized primary antibodies raised against cluster of differentiation (CD) 3 for T-lymphocytes (Figure 18), CD20 for B-lymphocyte labeling (Figure 19), CD68 for macrophages (Figure 20), tryptase for mast cells (Figure 21), CD79*α* for plasma cells (Figure 22).

We compared these results and we noticed that most CD3+, CD20+ T-lymphocytes, tryptase+ mast cells, CD79*α*+ B-lymphocytes were present around the endometriosis of the abdominal wall, and most CD68+ macrophages were identified around adenomyosis (Figures 23–26). In ovarian endometriosis cases, there were no numerical differences between CD68+ macrophages and CD79*α*+ B-lymphocytes (Figure 23).

In pelvic endometriosis, there were significant differences between the number of CD3+, CD68+, CD79*α*+ cells and respectively the number of CD20+ and tryptase+ cells (Figure 24).

In cases of adenomyosis, there were no numerical differences between trypase+ mast cells and CD79*α*+ lymphocytes, but there were large differences between CD3+ or CD68+ cells, and CD20+, tryptase+, CD79*α*+ cell groups (Figure 25).

In the cases of endometriosis of the abdominal wall, the CD3+ lymphocyte count was significantly higher than the other cell groups.

Around the normal endometrium, there were present more CD3+ and CD20+ cells, compared to ovarian endometriosis, and considerably less CD68+, tryptase+, CD79*α*+ cells, compared to all other endometriosis outbreaks, and in the case of normal endometrium, the CD3+, CD68+, CD79*α*+ cell groups were significantly larger than CD20+, tryptase+ cells groups (Figure 27).

We compared the averages obtained for each category and obtained a global result. There was a statistically significant difference between the values of CD3, CD68, CD79*α*, CD20 and tryptase for ovarian endometriosis $F(4.34)=86.203$, $p<0.001$; for pelvic endometriosis, $F(3.34)=115.405$, $p<0.001$; for adenomyosis, $F(4.34)=64.555$, $p<0.001$; for endometriosis of the abdominal wall, $F(4.34)=55.795$, $p<0.001$, and for the normal endometrium, $F(4.34)=58.261$, $p<0.001$.

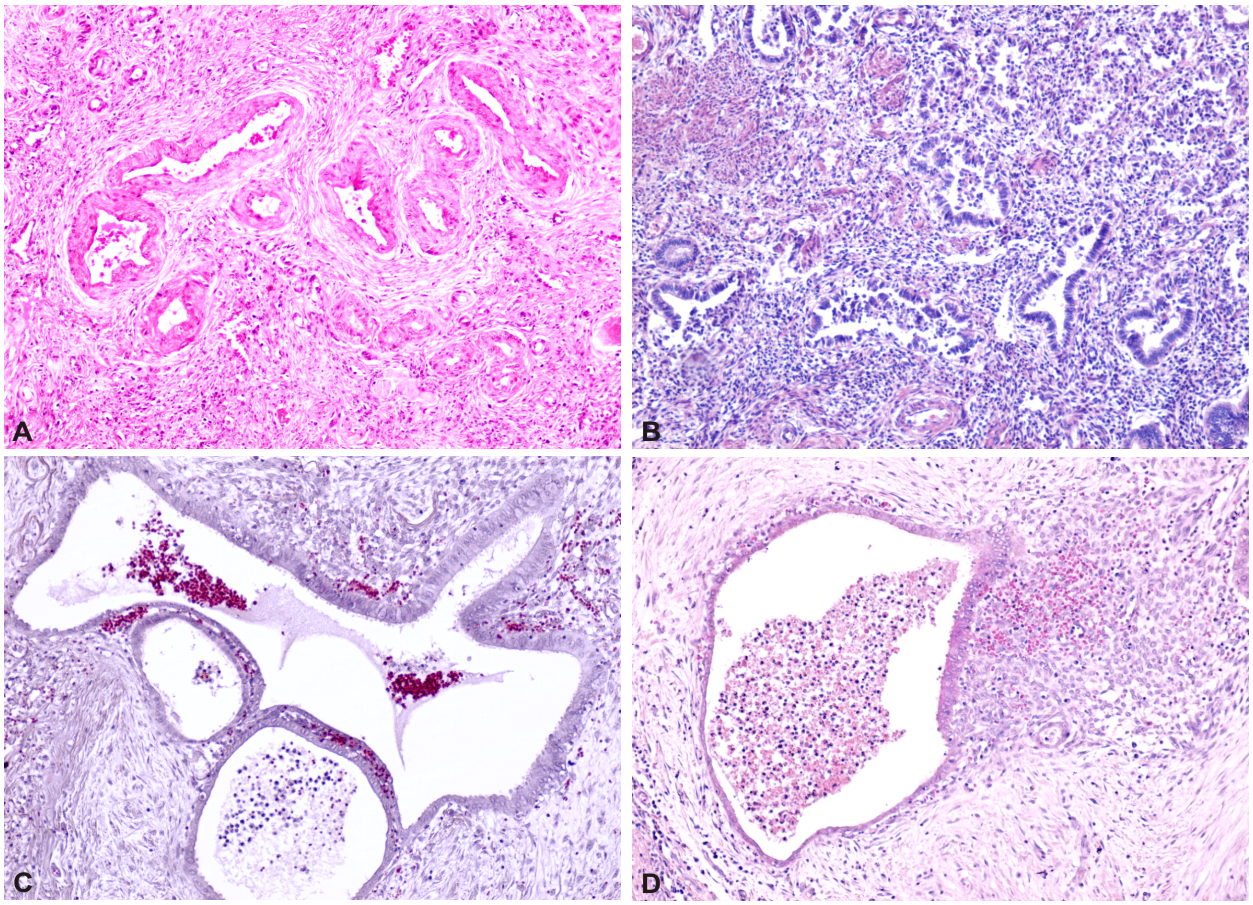

Figure 9 – ***(A) Ovarian endometriosis – simple stratified epithelia of endometrial glandular tissue; (B) Myometrial endometriosis (adenomyosis) – the endometrial glands are seen in the structure of the myometrium; (C) Peritoneal pelvic endometriosis – simple stratified glandular tissue and stroma; (D) Endometriosis of the abdominal wall – simple stratified glandular tissue, stroma and striated muscle fibers. HE staining: (A–D) ×100.***

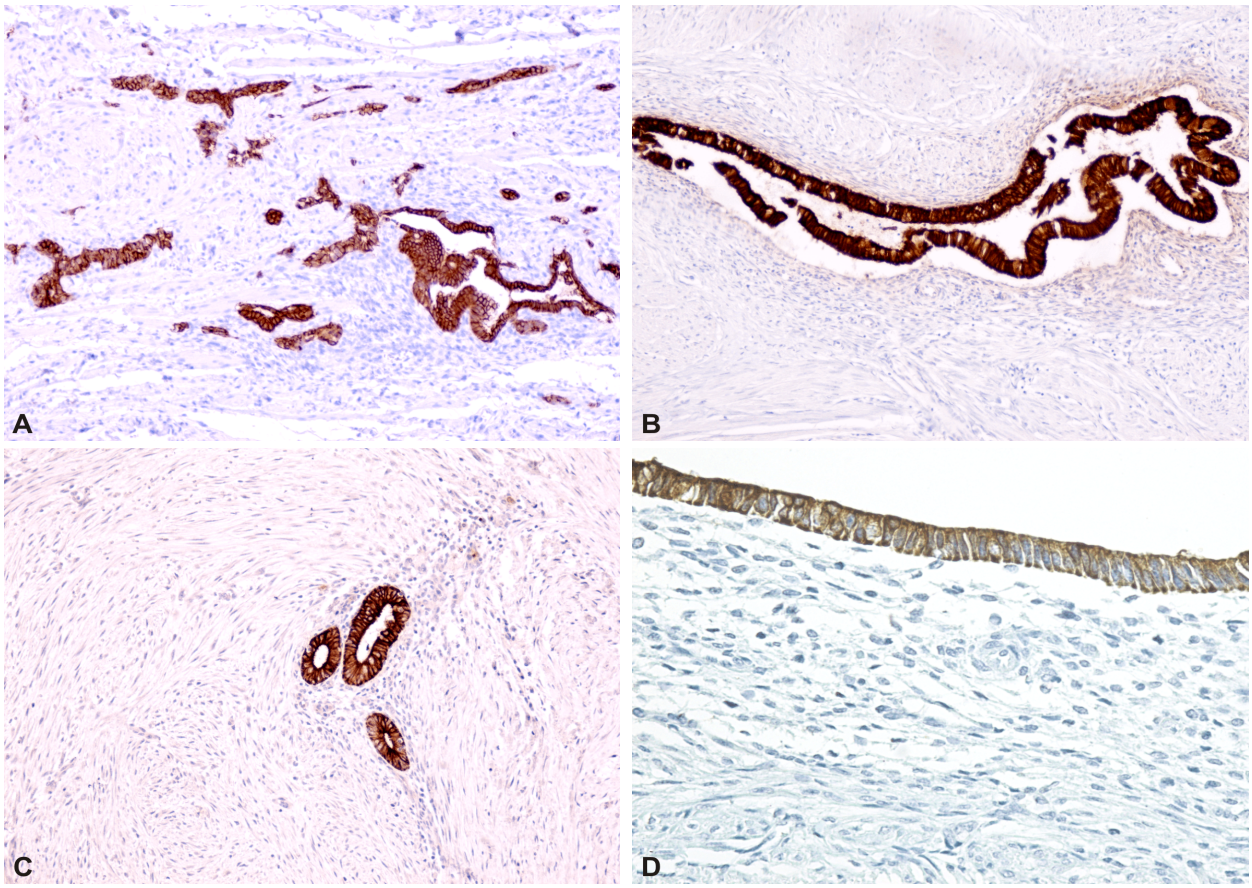

Figure 10 – ***(A) Ovarian endometriosis; (B) Myometrial endometriosis (adenomyosis); (C) Endometriosis of the abdominal wall – note the presence of ectopic endometrial tissue in the structure of the ovary, myometrium and rectus abdominis muscle; (D) Normal endometrium with positive reaction. Anti-CK7 antibody immunolabeling: (A–C) ×100; (D) ×200. CK7: Cytokeratin 7.***

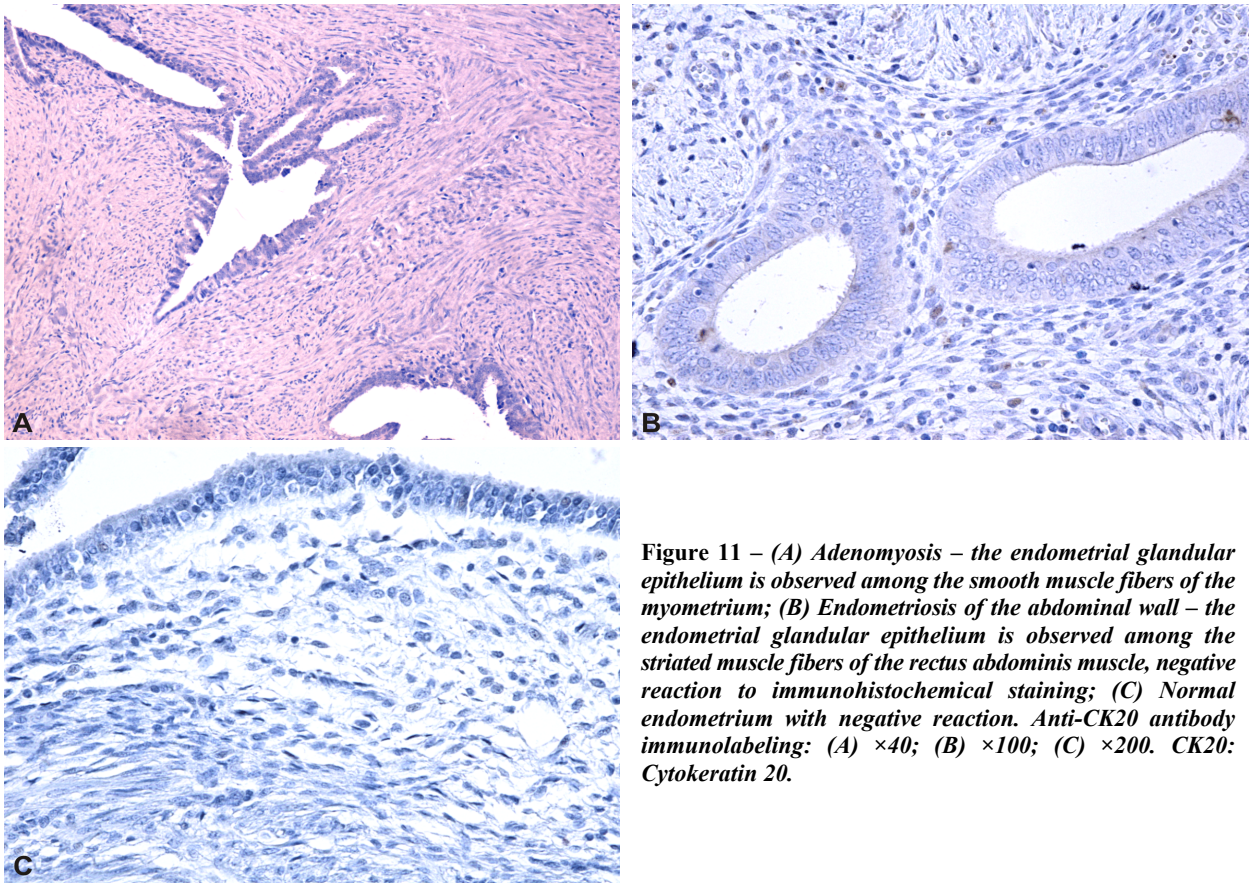

Figure 11 – ***(A) Adenomyosis – the endometrial glandular epithelium is observed among the smooth muscle fibers of the myometrium; (B) Endometriosis of the abdominal wall – the endometrial glandular epithelium is observed among the striated muscle fibers of the rectus abdominis muscle, negative reaction to immunohistochemical staining; (C) Normal endometrium with negative reaction. Anti-CK20 antibody immunolabeling: (A) ×40; (B) ×100; (C) ×200. CK20: Cytokeratin 20.***

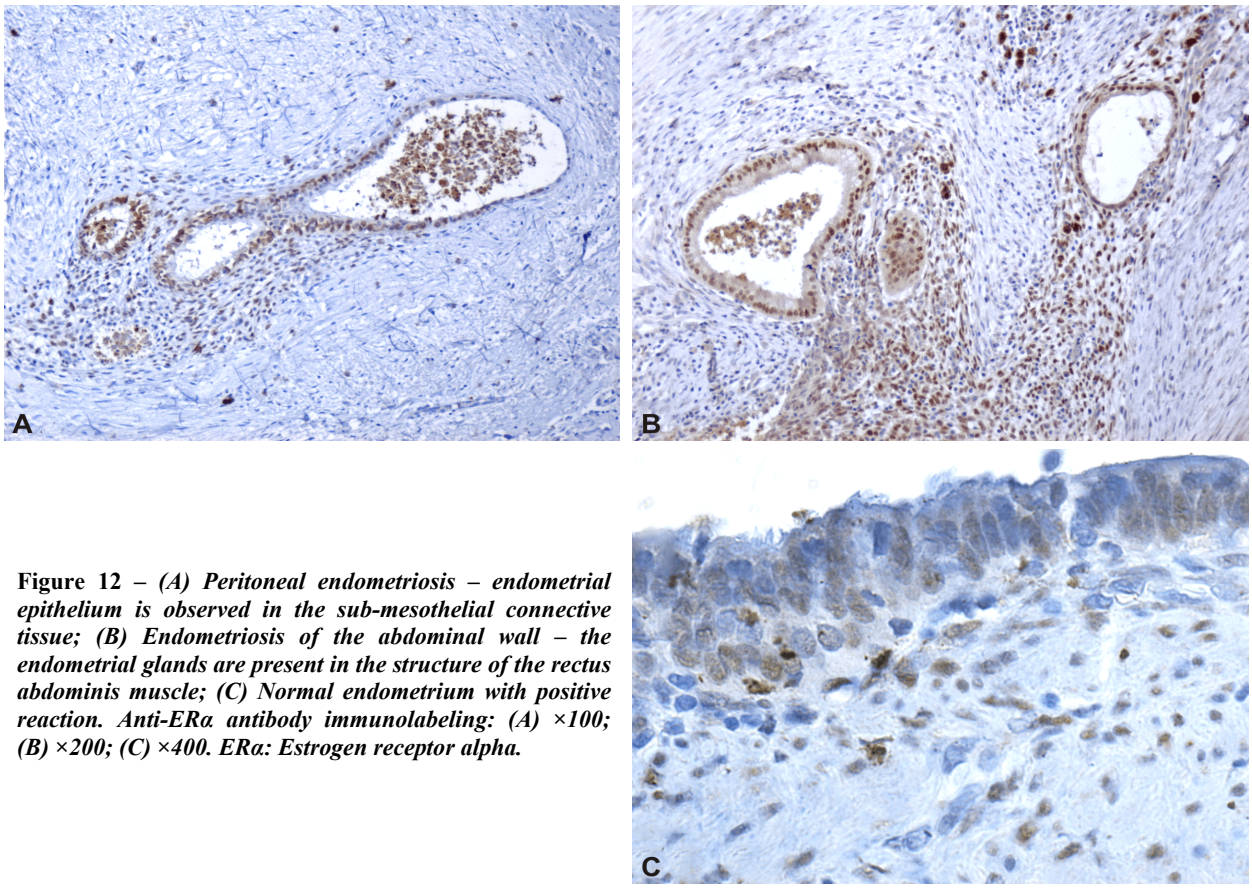

Figure 12 – *(A) Peritoneal endometriosis – endometrial epithelium is observed in the sub-mesothelial connective tissue; (B) Endometriosis of the abdominal wall – the endometrial glands are present in the structure of the rectus abdominis muscle; (C) Normal endometrium with positive reaction. Anti-ERα antibody immunolabeling: (A) ×100; (B) ×200; (C) ×400. ERα: Estrogen receptor alpha.*

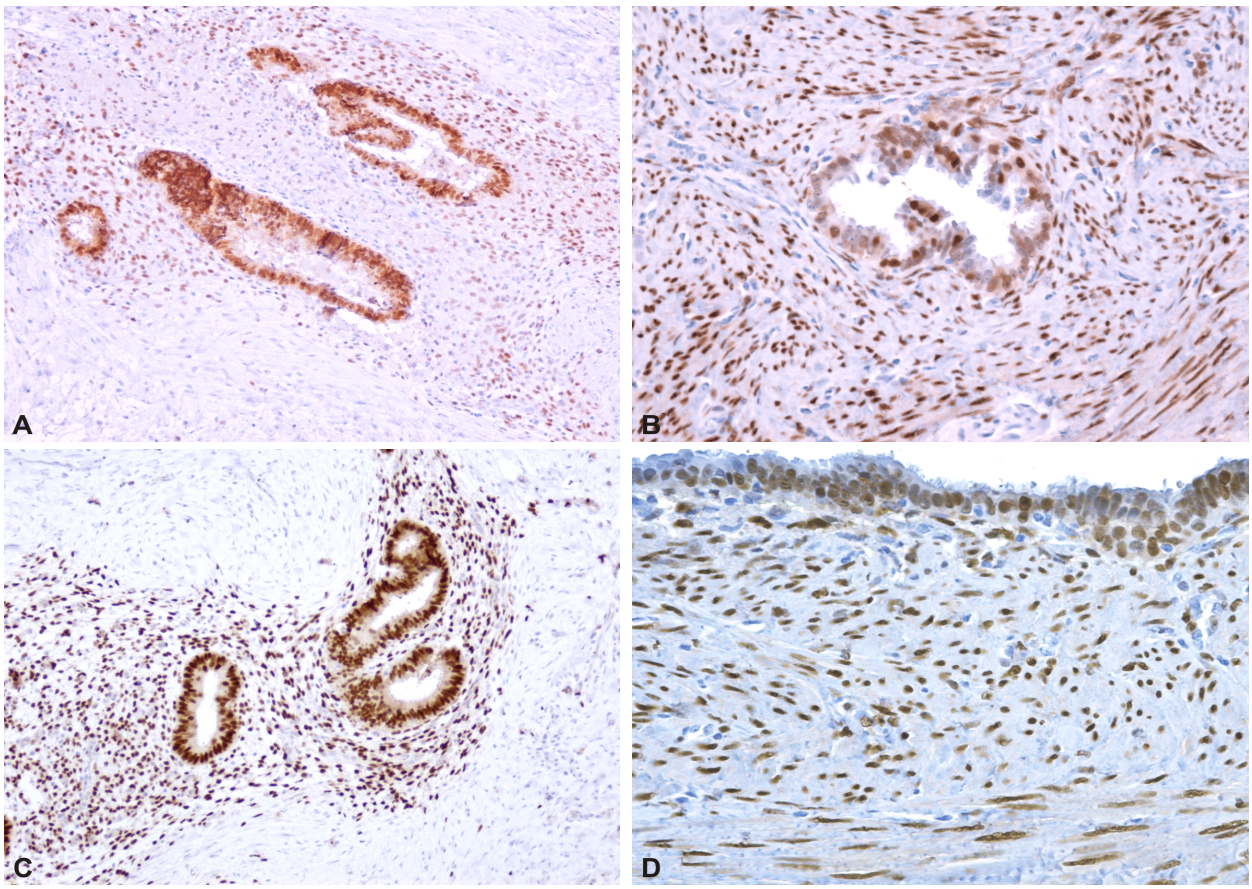

Figure 13 – *(A) Peritoneal endometriosis – note the endometrial epithelium in the peritoneal structure; (B) Adenomyosis – endometrial epithelium is observed among the myometrial muscle fibers; (C) Endometriosis of the abdominal wall – endometrial glands in the structure of the rectus abdominis muscle; (D) Normal endometrium with positive reaction. Anti-PR antibody immunolabeling: (A and C) ×100; (B and D) ×200. PR: Progesterone receptor.*

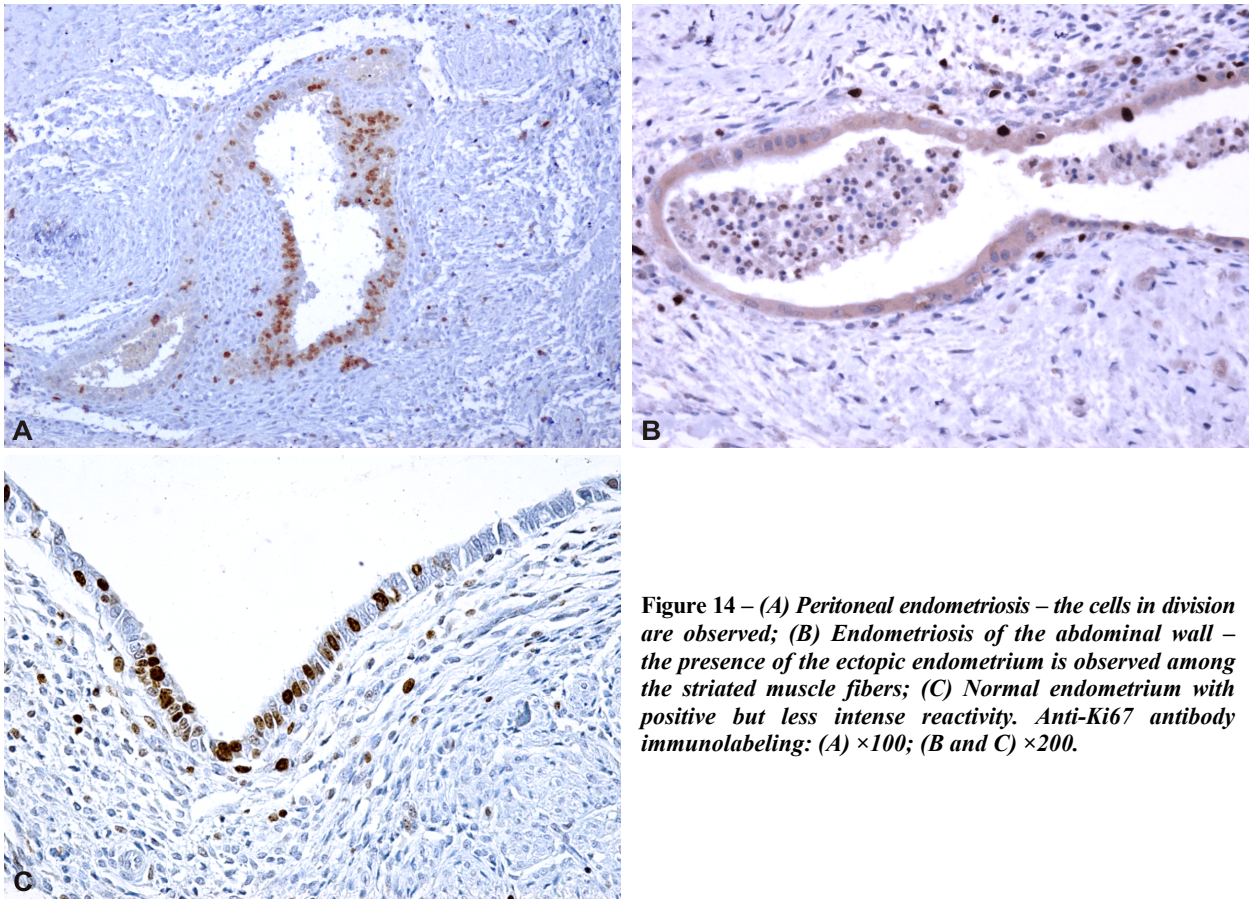

Figure 14 – *(A) Peritoneal endometriosis – the cells in division are observed; (B) Endometriosis of the abdominal wall – the presence of the ectopic endometrium is observed among the striated muscle fibers; (C) Normal endometrium with positive but less intense reactivity. Anti-Ki67 antibody immunolabeling: (A) ×100; (B and C) ×200.*

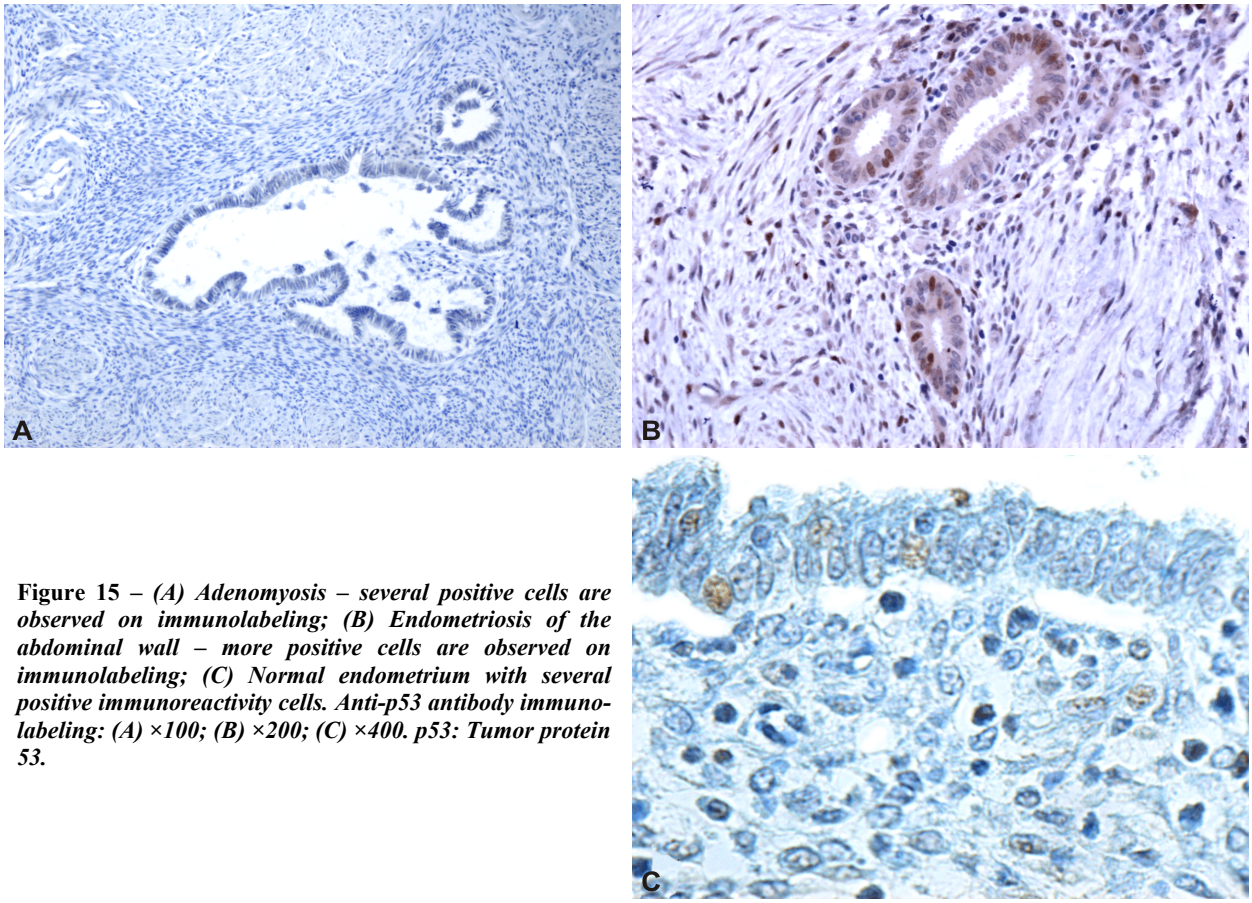

Figure 15 – *(A) Adenomyosis – several positive cells are observed on immunolabeling; (B) Endometriosis of the abdominal wall – more positive cells are observed on immunolabeling; (C) Normal endometrium with several positive immunoreactivity cells. Anti-p53 antibody immunolabeling: (A) ×100; (B) ×200; (C) ×400. p53: Tumor protein 53.*

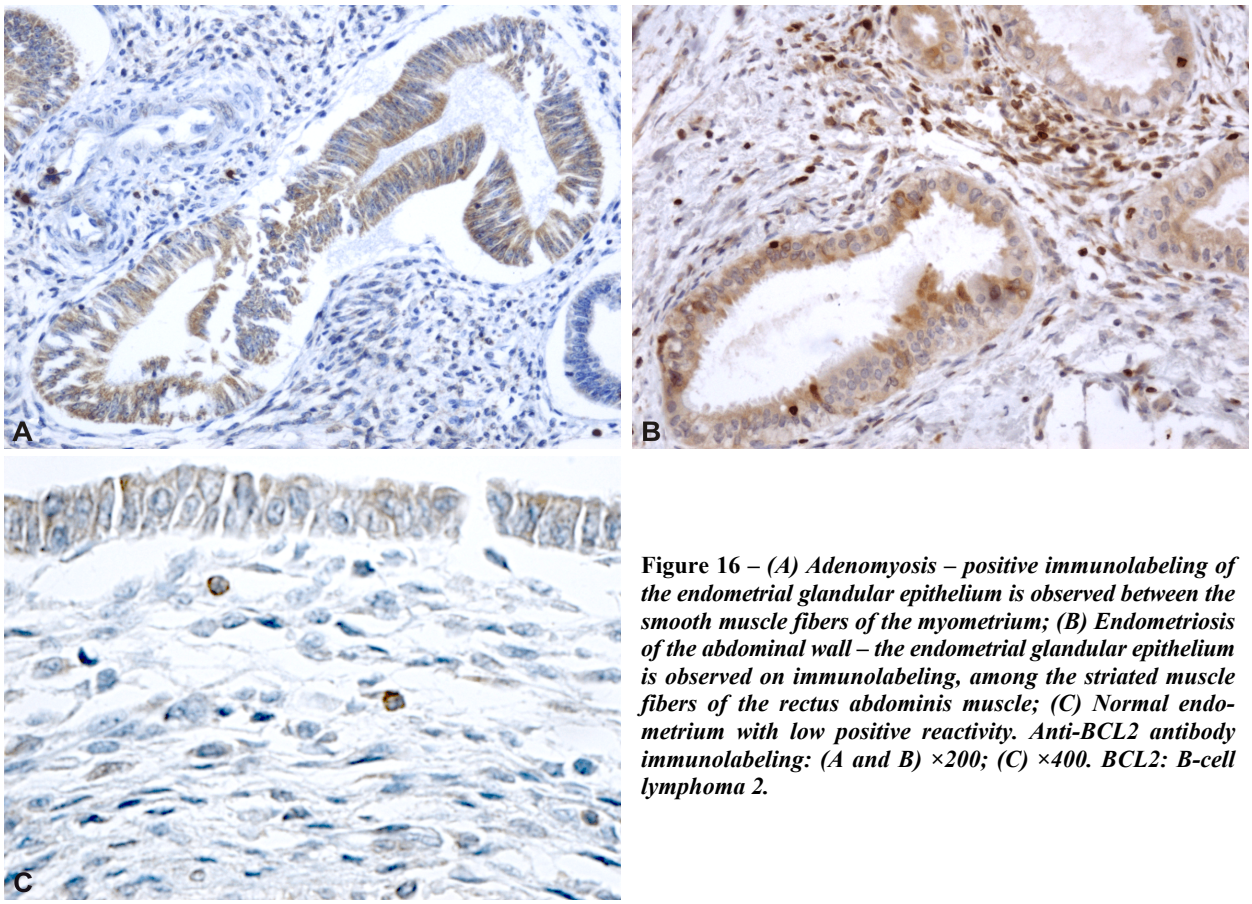

Figure 16 – *(A) Adenomyosis – positive immunolabeling of the endometrial glandular epithelium is observed between the smooth muscle fibers of the myometrium; (B) Endometriosis of the abdominal wall – the endometrial glandular epithelium is observed on immunolabeling, among the striated muscle fibers of the rectus abdominis muscle; (C) Normal endometrium with low positive reactivity. Anti-BCL2 antibody immunolabeling: (A and B) ×200; (C) ×400. BCL2: B-cell lymphoma 2.*

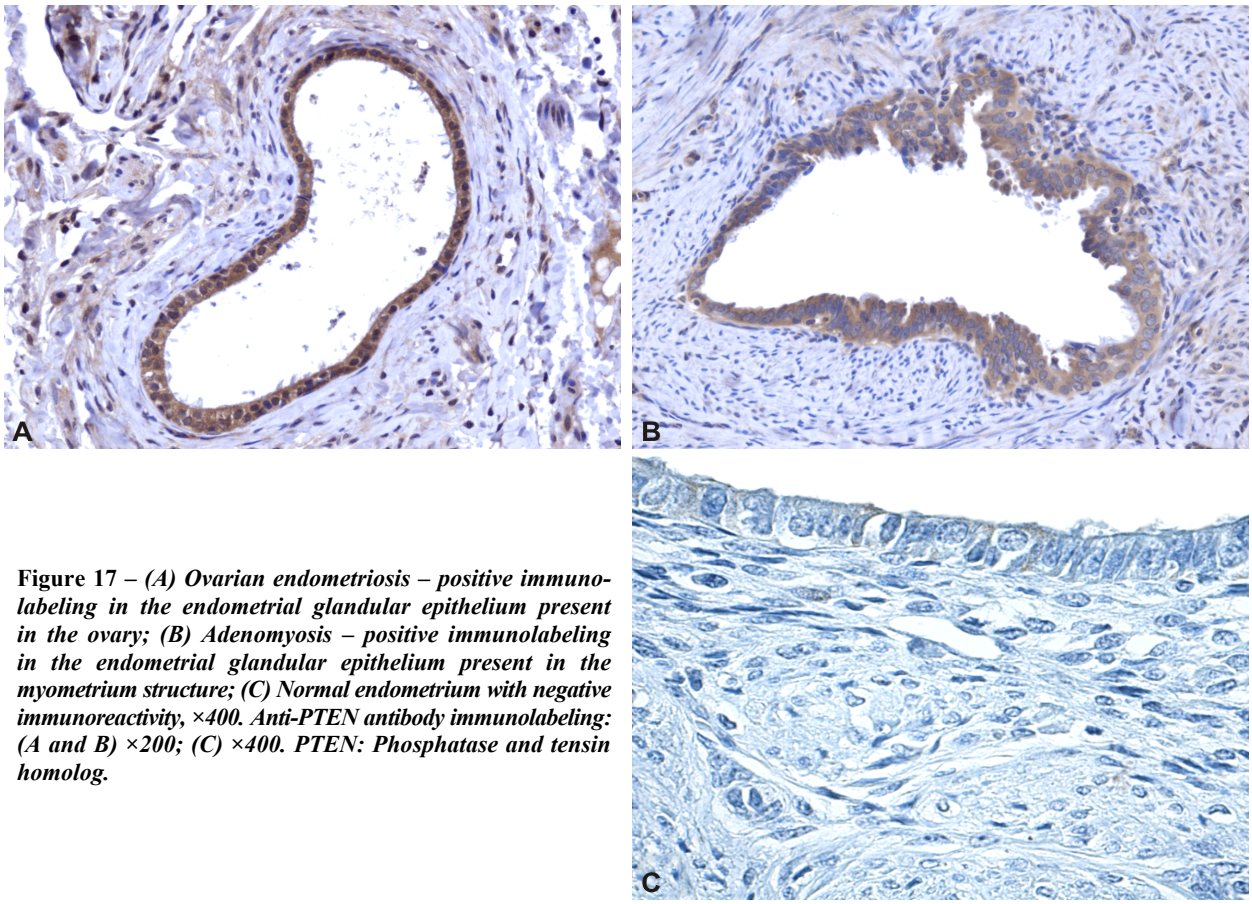

Figure 17 – *(A) Ovarian endometriosis – positive immunolabeling in the endometrial glandular epithelium present in the ovary; (B) Adenomyosis – positive immunolabeling in the endometrial glandular epithelium present in the myometrium structure; (C) Normal endometrium with negative immunoreactivity, ×400. Anti-PTEN antibody immunolabeling: (A and B) ×200; (C) ×400. PTEN: Phosphatase and tensin homolog.*

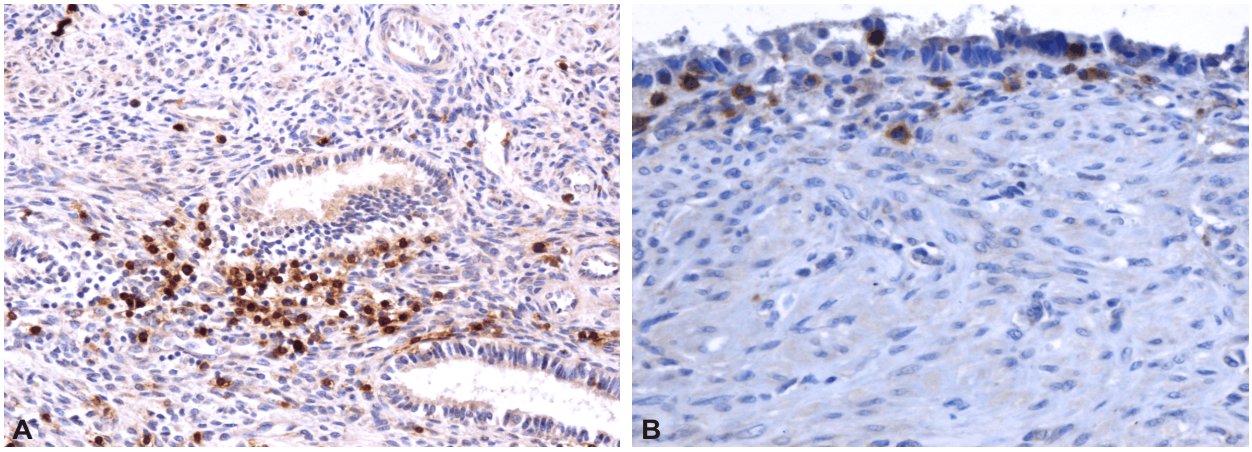

Figure 18 – ***(A) Adenomyosis – a large number of T-lymphocytes is observed around the endometrial glandular epithelium, ectopically located; (B) Normal endometrium – a smaller number of T-lymphocytes is observed around the eutopic endometrial glandular epithelium. Anti-CD3 antibody immunolabeling: (A and B) ×200. CD3: Cluster of differentiation 3.***

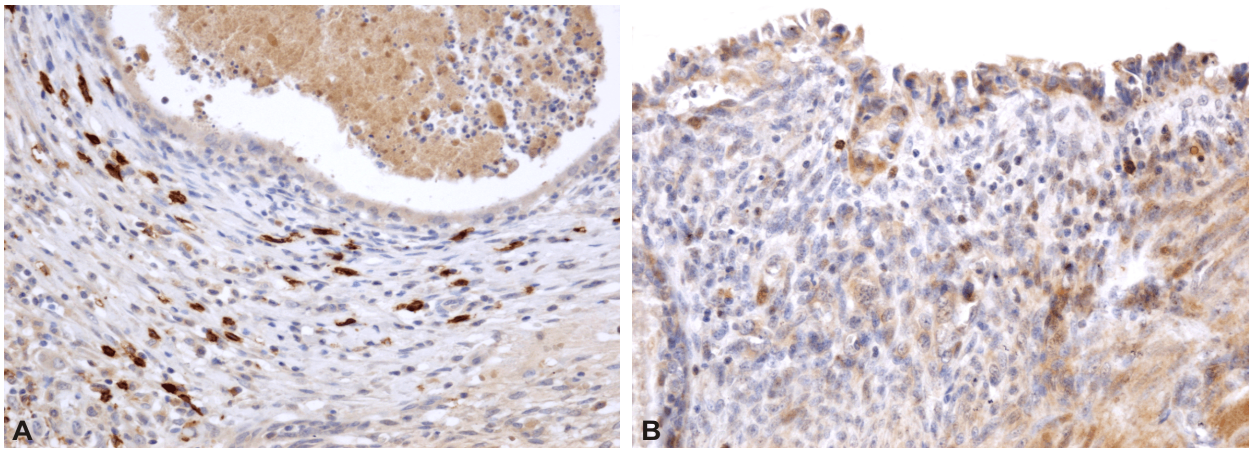

Figure 19 – ***(A) Endometriosis of the abdominal wall – there is a large number of B lymph cells around the endometriosis foci present in the rectus abdominis muscle; (B) Normal endometrium – a smaller number of B-lymphocytes is observed around the eutopic endometrial glandular epithelium. Anti-CD20 antibody immunolabeling: (A and B) ×200. CD20: Cluster of differentiation 20.***

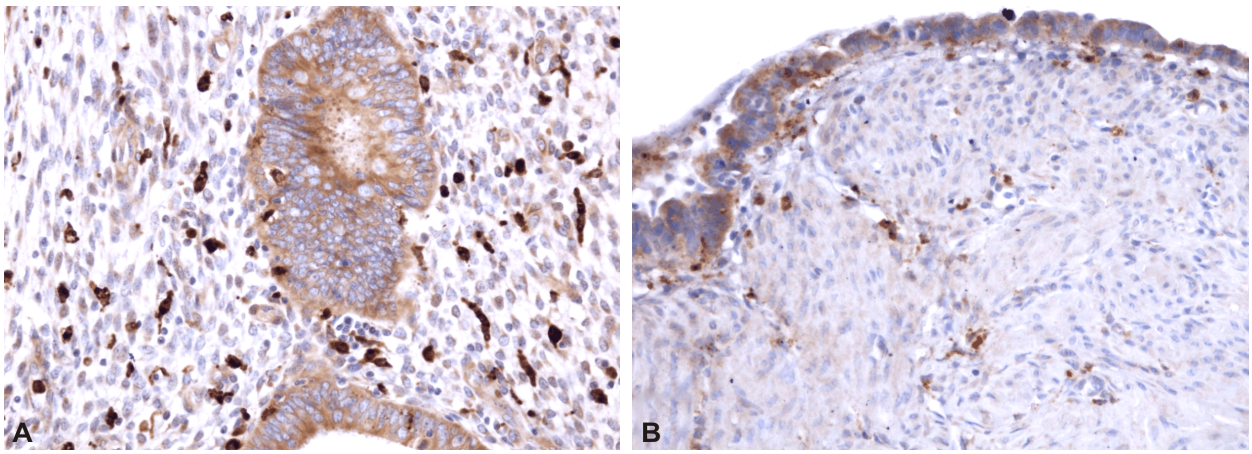

Figure 20 – ***(A) Endometriosis of the abdominal wall – there is a large number of macrophages around the endometriosis foci present in the rectus abdominis muscle; (B) Normal endometrium – there is a smaller number of macrophages around the eutopic endometrial glandular epithelium. Anti-CD68 antibody immunolabeling: (A) ×200; (B) ×100. CD68: Cluster of differentiation 68.***

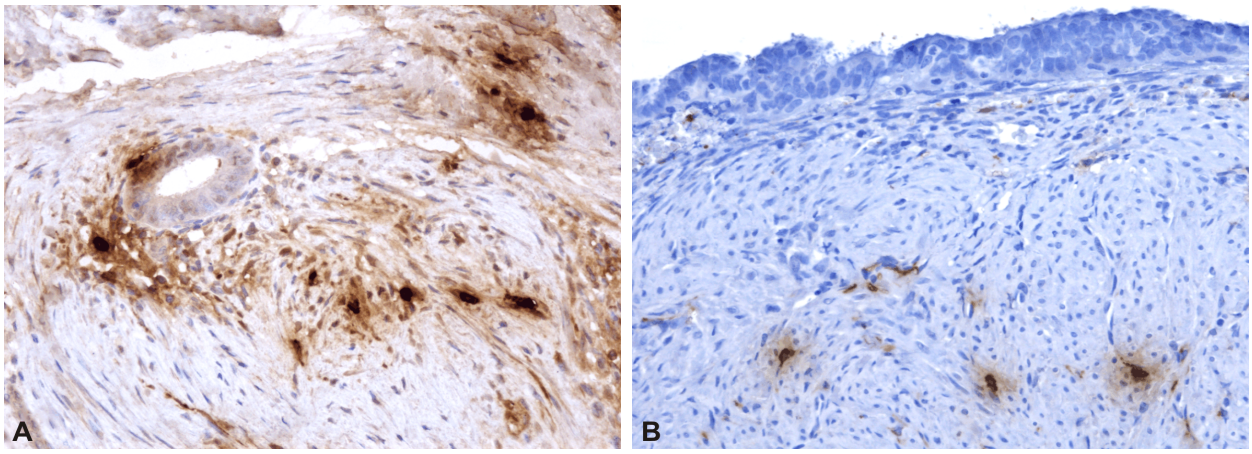

Figure 21 – *(A) Endometriosis of the abdominal wall – there is a large number of mast cells around the endometriosis foci present in the rectus abdominis muscle; (B) Normal endometrium – a smaller number of mast cells are observed around the eutopic endometrial glandular epithelium. Anti-tryptase antibody immunolabeling: (A and B) ×200.*

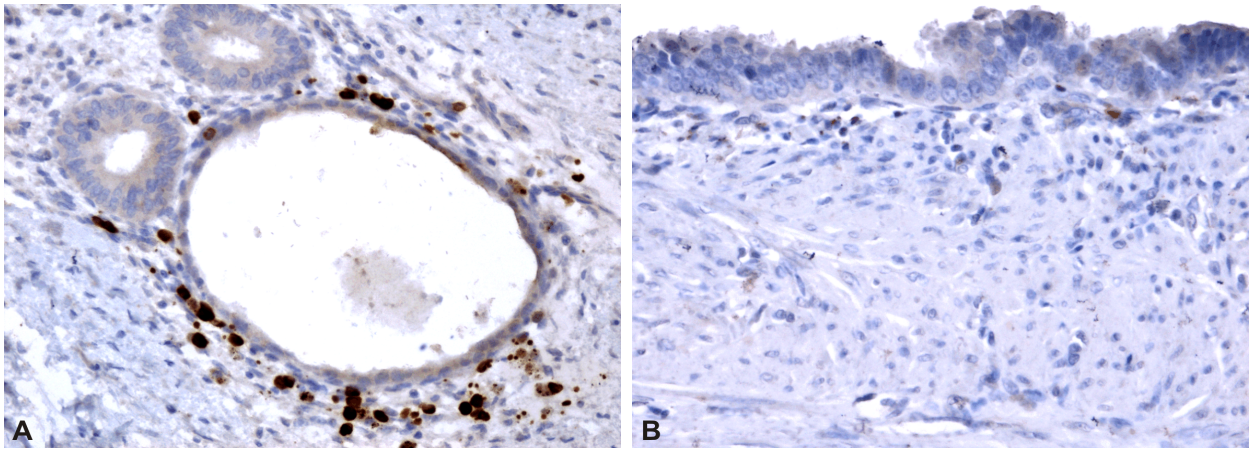

Figure 22 – *(A) Endometriosis of the abdominal wall – there is a large number of plasma cells around the endometriosis foci present in the rectus abdominis muscle; (B) Normal endometrium – there is a smaller number of plasma cells around the eutopic endometrial glandular epithelium. Anti-CD79α antibody immunolabeling: (A and B) ×200. CD79α: Cluster of differentiation 79 alpha.*

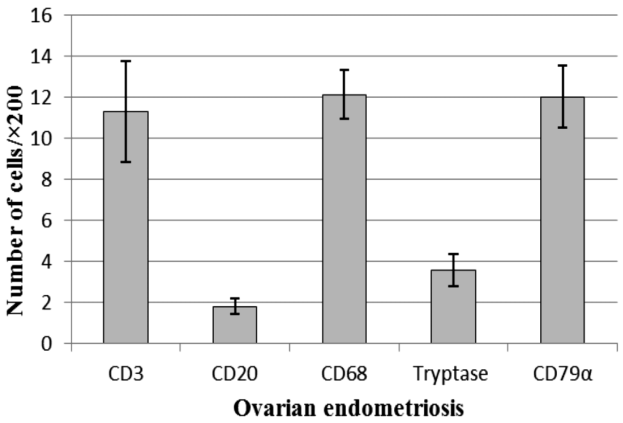

Figure 23 – *Ovarian endometriosis: number of cells/ ×200.*

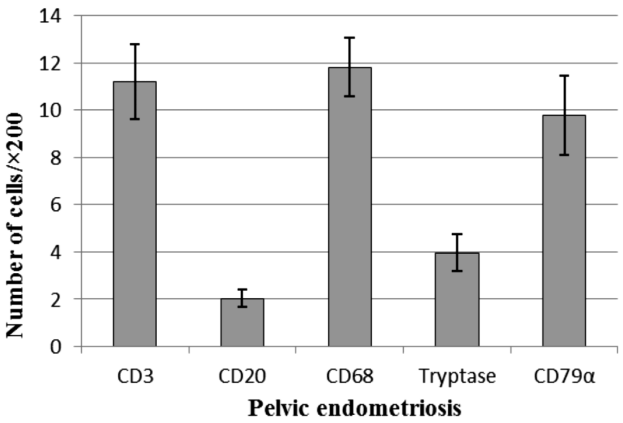

Figure 24 – *Pelvic endometriosis: number of cells/ ×200.*

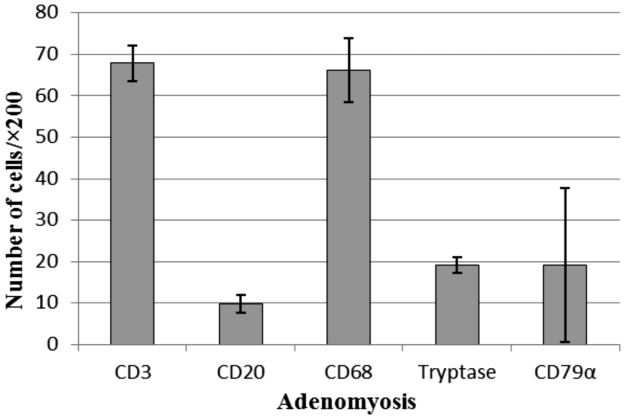

Figure 25 – ***Adenomyosis: number of cells/×200.***

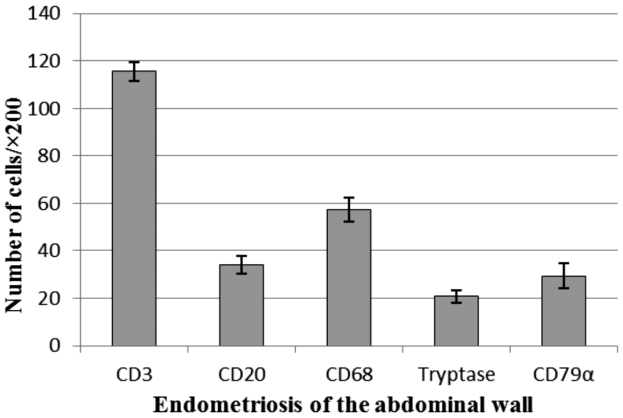

Figure 26 – ***Endometriosis of the abdominal wall: number of cells/×200.***

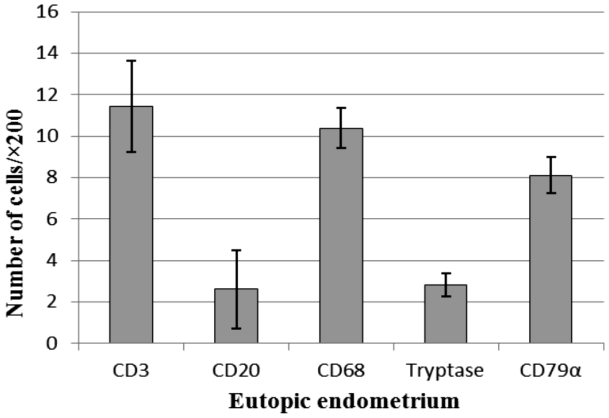

Figure 27 – ***Eutopic endometrium: number of cells/ ×200.***

Discussions

Clinical study, associated with imaging, surgical techniques and morphopathological examination can provide a diagnosis of certainty in endometriosis.

Endometriosis is estimated to occur in 6–10% of women especially in reproductive age. Studies have shown that it is commonly found between 30 and 40 years, and in our data, the age varied between 22 and 47 years old, depending on the location. The cause of occurrence is not clear, but genetic, environmental, hormonal factors, etc. may be involved. The ovaries, tubes and tissues around the uterus are most commonly affected, but in less frequent cases, other organs or anatomical structures may be involved. Endometriosis outbreaks can bleed under hormonal influences every month, leading to inflammatory, adherent and scarring processes [28].

Besides the clinical data related to the personal physiological history, it has been shown that obesity can greatly influence hormone secretion and disrupt menstruation, altering its flow and implicitly increasing the risk of endometriosis. Epidemiological studies [29–38] examined the link between the BMI and endometriosis, but with variable results. Some studies have argued that there is an inverse association between the two [31, 34–38], or that there is no association [29, 30, 36]. An increase in BMI of 5 kg/m^2 may reduce the risk of endometriosis by 33% [39]. It has been shown that women with lower BMI have been associated with more advanced stages of endometriosis, compared to fatter women [36]. Two studies reported an inverse association between low-weight women and the risk of developing endometriosis [40, 41]. In our study, 75% of patients were normo-ponderal, 14.28% underweight, 3.57% overweight and 7.14% with grade I obesity. Elucidating the link between the two, can help in the future to understand this pathology.

Serum CA125 may be increased in advanced cases of endometriosis. Drug treatments may lower the value of this marker, but with the cessation, it will resume the initial value by reactivating endometriosis. Bischof *et al.* [42] showed that CA125 is produced in human endometrial stroma under estrogenic influence, and Duk *et al.* [43] showed that it is a marker of endometrial carcinoma. Barbieri *et al.* [44] used IHC techniques and demonstrated the presence of CA125 on the surface of cells present in endometrial lesions. Decrease in serum CA125 in patients treated for endometriosis, occurs both by decreasing normal and ectopic endometrial growth and activity. Upon cessation of treatment, increase in serum CA125 above 35 IU/mL suggests a reactivation of endometriosis out-breaks, representing a screening marker for this pathology. In our study, three cases with serum CA125 values over the reference value were reported.

Endometriosis causes pain and infertility in 20–25% of patients. Associated symptoms as pelvic pain, dysmenorrhea, dyspareunia [45] may influence the psychological, mental, and social condition of those affected [46]. In general, endometriosis causes pain and infertility, and the relationship with infertility has been debated for years. In most couples, fecundity decreases with age, but in women with endometriosis the monthly fecundity is low [47, 48]. Endometriosis is associated with a lower rate of conception [47, 49]. Women with endometriosis are 6–8 times more prone to infertility [50], through several mechanisms, namely pelvic anatomy modification, endocrine and ovulatory abnormalities, alteration of peritoneal function, hormonal and endometrial cell function alterations. Using laparoscopy, pelvic anatomical changes called "pelvic factor", might explain infertility in advanced stages of endometriosis. The major adhesions in the pelvis and peritubal, disturb the connection and tubo-ovarian permeability, affecting oocyte release and transport [51].

An extensive association between hormonal and inflammatory cellular factors modulates the growth and behavior of endometriosis outbreaks, affecting embryonic implantation. Women with endometriosis have an increased fluid volume in the peritoneum, with an elevated concentration of activated macrophages, prostaglandins, interleukin 1 (IL-1), tumor necrosis factor (TNF) and proteases. These alterations may have negative effects on oocyte function, spermatic fluid, embryo on or uterine tube. With all these changes, the normal endometrium can be influenced, so abnormal uterine contractions can occur during the cascade of biochemical products, including prostaglandins in the pelvis, causing irritation and inflammation. This theory might explain the failure of implantation in patients with endometriosis. Abnormal uterine contractility may interfere with embryo adhesion and penetration into the pre-decidualized endometrium, representing a major factor in the pathophysiology of infertility associated with endometriosis [52, 53]. Increasing retrograde menstrual flow can be similar to peristaltic contractions with a "pumping-like effect", transporting and disseminating endometrial detritus into the abdominal cavity [54]. In our study, primary infertility was observed in 28.57% of patients.

Chronic pelvic pain, commonly seen in patients with endometriosis, may be associated with an increase in ESR, above the reference value [55]. In our study, we noticed that 32.14% of the patients had leukocytosis and 17.85% had ESR at one hour over the normal range, demonstrating the involvement of inflammatory factors in the evolution and eventual transformation of endometriosis.

Abnormal bleeding may be present in women with endometriosis, and if this loss is consistent, anemia may occur. In our study, we found 25% of patients with microcytic anemia, probably developed by constant blood loss. The effect of iron on the transformation of endometriosis has been studied, and it has been found that its increased value can be associated with cell death through pro-oxidative activity, which could lead to the destruction by oxidation of the deoxyribonucleic acid (DNA). This antioxidant has been described in association with malignant transformation of endometrial cells, on which destruction by oxidation of DNA persisted [56–58].

Several studies have focused on examining the role of smoking in the development of endometriosis. In a clinical trial based on the investigation of infertile Portuguese women, a low risk of developing endometriosis in smokers has been described [59]. Another study described the interaction between cigarette smoke and glutathione-S-transferase gene polymorphism, as a possible risk factor for the development of endometriosis, and an inversely proportional association between smoking and endometriosis was observed [60]. However, components of cigarette smoke may influence steroidogenesis, synthesis of estradiol and progesterone [61–65]. Furthermore, smoking has a strong effect on inflammation mediators in the lung and extra-pulmonary environment, and can be a powerful trigger of inflammation associated with endometriosis, causing overexpression of pro-inflammatory genes and increasing the risk of transformation of endometriosis [66]. By the anti-estrogenic effect of smoking, it has been suggested that this habit, may modulate the molecular systems involved in cellular adhesion and invasion. Also, a favorable smoking effect was observed in benign and malignant estrogen-dependent malignancies, such as endometrial cancer [67–69]. In our study, we identified 42.85% of smokers, and more studies are needed to determine whether they are better protected than non-smokers are.

According to a *Harvard School of Public Health* study, women consuming 2–3 cups of coffee/day are more likely to develop endometriosis, than women who do not consume coffee. The mechanism by which it occurs remains unknown [70], and in our study, we had 57.14% of patients consuming coffee.

Alcohol consumption might be a risk factor in endometriosis. By comparing healthy women with those diagnosed with other gynecological problems, alcohol-consuming patients have an increased risk of endometriosis [71].

In infertile, alcohol-consuming women, the risk of developing endometriosis is 50% higher, than in women who do not consume alcohol [72]. In our study, we found 3.57% of alcohol-consuming patients.

From the microscopic point of view, in the classical HE staining, the presence of ectopic endometrial glands in the in the myometrium, ovary, peritoneum or abdominal wall has been observed. The endometrial glands can be circular, with cylindrical cells, with hyperchromatic nuclei, or can be elongated. The endometrial stroma can be classically cellular and hyperchromatic, being similar to lymphocyte infiltrate, and the macrophages can be loaded with hemosiderin, having a granular appearance. The epithelium generally follows the aspects of the normal endometrium and may appear as a single cellular or glandular layer. In the stroma, there are round cells, with little cytoplasm and round nuclei. In the vicinity of the stroma, the cells of the inflammatory system may be present, which may influence the transformation of this benign pathology into malignancy [73].

CK7 belongs to the keratin family. Type II is specifically expressed in the simple epithelium, which lining cavities of internal organs. The genes encoding this keratin are located in chromosomal region 12q12-q13. Normally, CK7 occurs in the glandular epithelium, and pathologically it can be expressed in various tumor types [74]. The normal endometrium and endometriosis outbreaks are positive for the IHC marker achieved with the anti-CK7 antibody.

CK20 belongs to CK type I and represents a major cellular protein, present in mature enterocytes and in goblet cells, found in the gastric and intestinal mucosa [75]. From an IHC point of view, this CK is positive in epithelial adenocarcinomas present in the gastrointestinal tract, and negative in lung, prostate cancers and non-mucinous ovarian adenocarcinoma [74]. This is why it is used together with CK7, to make the differential diagnosis of different tumor types. In our study, CK7 was positive in all outbreaks of endometriosis, irrespective of localization, and negative CK20 in all of these areas, which demonstrates that the ectopically located epithelial glandular tissue is not a possible metastasis with a gastrointestinal origin.

ERα positive in the studied outbreaks, along with progesterone receptors, demonstrates once again that the studied areas are endometriosis outbreaks. ERα represents a nuclear receptor, activated by the estrogen sexual hormone, and in humans, it is encoded by the estrogen receptor 1 (*ESR1*) gene [76, 77]. It plays an important role in the physiological development of several organs, such as reproductive, central nervous, cardiovascular or skeletal [78]. ERα plays an essential role in maturation of the female reproductive system [79] and determines activation of cellular proliferation in the uterus [80]. Apostolou *et al.* [81] argue that ERα expression is present in the normal endometrium, and as cellular changes begin to appear locally or in the ectopic endometrial glands, epithelial receptors lose their ability to recognize sex hormones, and become independent of them [82, 83], and therefore, it can be seen that at the level of endometriosis outbreaks, the IHC expression has a lower intensity. PR belongs to the subfamily 3 of the nuclear receptors, being a protein activated by the progesterone sex hormone. In humans, it is encoded by the progesterone receptor (*PGR*) gene at 11q22 [84, 85]. Progesterone activates these receptors, making a structural change that removes inhibitory action, helping to build DNA. The presence of these steroid receptors in the areas of endometriosis, suggests that they are hormone dependent in various measures. This assertion is supported by the presence of the symptoms and cyclical signs of endometriosis [86], but also by the favorable response during hormonal treatment [87]. Comparing receptor distribution, in endometriosis and areas of normal endometrium, studies have shown that there is a major difference between them. In the normal endometrium, ER and PR are always present, and in endometriosis, these receptors are present in a much smaller percentage. This difference is much better observed in case of ER. There are situations where the PR is highly expressed, and ER is absent, which demonstrates that PR synthesis in the endometriosis outbreaks is independent of estrogen action [88]. By comparing these studies, endometriosis could be classified according to ER and PR concentration, respectively, with negative receptors, positive receptors, or PR positive only. This classification is extremely important in choosing treatment. Positive results were obtained with progestins [86] or Danazol [86, 87], which bind to progestin receptors [89, 90]. Exogenous progesterone has a strong inhibitory action on cellular proliferation, and at stromal level, there is a stagnation or even decrease of the areas with endometriosis [91].

Ki67 is a protein encoded by the human marker of proliferation Ki67 (*MKI67*) gene, antigen expressed by IHC reaction with the anti-Ki67 antibody [92, 93], and represents a nuclear protein associated with cells in the division, representing a cell proliferation marker [94], positive in the interphase at the nucleus, and in mitosis at the chromosome periphery domain [95]. It is present during all active phases of the cell cycle (G1, S, G2 and mitosis), absent in G0, being best represented in the S phase. At the level of endometriosis sites, positive cells were detected in the immunoassay with the anti-Ki67 antibody, and their numerical growth may represent a pronounced, uncontrolled cell division, being possible the premalignant or malignant transformation of the studied areas. The expression of this marker is low in cases of moderate endometriosis and increased in severe ones [96].

p53 is a protein that acts as a tumor suppressor [97], being described as the "guardian of the genome" because it retains stability and prevents genome mutations [98]. It acts by several mechanisms on anticancer function and plays a role in apoptosis, inhibiting angiogenesis and maintaining genome stability, enabling DNA repair, representing an important factor in the aging process, can block cells in G1/S phase to create sufficient time to repair DNA, or initiate apoptosis when DNA damage cannot be repaired [99]. In our study, we noticed a positive IHC marker with the anti-p53 antibody in endometriosis of the abdominal wall, in a slightly hyperplasic area, indicating that the area escaped from genomic control and may experience premalignant or malignant future changes.

BCL2 is a protein with role in regulating cell death (apoptosis), by inducing (pro-apoptotic effect) or inhibiting it (antiapoptotic effect) [100, 101]. It is located on the outside of the mitochondrial membrane, supporting cell survival and inhibiting the action of pro-apoptotic proteins (Bax/Bak), which increase membrane permeability, allowing cytochrome c and reactive oxygen species (ROS) to activate the apoptotic cascade. BCL2 protein alterations can be observed in the evolution of various cancers. In the case of endometriosis, there are changes in this protein, both in the epithelium and around it, which demonstrates that the cells can evolve chaotically, subsequently transforming malignant.

PTEN is a protein encoded by the *PTEN* gene [102], and its mutations represent a leap in the development of many cancers, by canceling its action of tumor suppressor gene. Normally, it exerts its function *via* a phosphatase protein, involved in cell cycle regulation, preventing increased cellular growth and multiplication [103]. During eventual tumor transformation, *PTEN* deletions or mutations block enzyme action, inducing cell proliferation and decreasing programmed death. These inactivations occur frequently in endometrial cancer, and in the ectopic endometrium sites, a positive IHC reaction to the anti-PTEN antibody has been obtained, indicating an increased malignancy predisposition.

The inflammatory process is intense around the endometriosis outbreaks, having a defensive role, but can also influence cellular transformation [27]. Leukocytes, lymphocytes, macrophages and plasma cells act, through synthesis products, on the normal endometrium, but especially on the outbreaks of the ectopic endometrium.

The CD3–T cell co-receptor helps to activate both, cytotoxic T-lymphocytes (CD8+, naïve T-cells) and T-helper (CD4+, naïve T-cells). From the IHC point of view, CD3 is initially expressed in the cytoplasm of prothymocytes and stem cells in the thymus. These cells subsequently transform into mature T-cells, identified on histological sections. Exaggerated numerical growth can demonstrate the existence of an important inflammatory process, or even a malignant pathology [104].

CD20 is expressed on the surface of B-lymphocyte cells, and in humans is encoded by the membrane spanning 4-domains A1 (*MS4A1*) gene [105]. CD20 has been shown

to play a role in the interaction of the B-cell microenvironment [106]. These cells are involved in various malignant pathologies. Around the endometriosis outbreaks, B-lymphocyte cells were present.

CD68 is a highly expressed protein in monocytic cell lines, circulating macrophages, or tissue macrophages. It is a transmembrane glycoprotein encoded by a gene located at chromosome 17 [107]. Numerous endometriosis-related studies have shown the presence of peritoneal macrophages [108–110], and have been said to play a role in mediating and exacerbating inflammation, supporting this pathology [111–115]. The higher the number of macrophages, the higher the secretion and synthesis of proinflammatory mediators, cytokines [112], TNF-α, IL-1, IL-2, IL-6, IL-8, IL-10 [110, 112, 114], T-cells [115], platelet activating factors, hepatocyte growth factor (HGF), macrophage-derived growth factor, fibroblast growth factor, angiogenesis factor and fibronectin, vascular endothelial growth factor [113, 114, 116, 117], in the ectopic endometrium outbreaks, compared to peritoneum or peritoneal fluid of the controls. All these changes facilitate persistence and maintain endometriosis [109, 113, 114, 116]. Macrophages play a crucial role in the destruction, repair and regeneration of endometrial tissue [118], during menstruation and the proliferative phase of the menstrual cycle. In endometriosis, some viable fragments of endometrial tissue can escape from inflammatory action, and may implant in ectopic areas. It has been shown that the number of macrophages can be increased [117], or may be decreased [119], in the proliferative phase, around the eutopic endometrium in women diagnosed with endometriosis. Also, various intrauterine changes have been observed in women with endometriosis, and eutopic endometrial glands and stromal cells may function differently [120]. In women without endometriosis, during menstruation, endometrial cells are subject to apoptosis and then phagocytized by the surrounding macrophages. Eutopic endometrial cells, in women diagnosed with endometriosis, are less susceptible to apoptosis [121, 122], increasing the number of viable cells capable of ectopic implantation [123, 124], proliferation and different reaction to the stimuli, present in the new environment [125]. These cells may have certain characteristics or constituents, which favor survival outside the uterine cavity. It is also known that eutopic endometrial cells in women with endometriosis are much more invasive, with a higher adhesive capacity and capable of triggering activation of immune system cells [126].

Tryptase is secreted by mast cells and can be used as a marker of cellular activation [127–130]. The mast cells are numerically elevated around the ectopic endometrium outbreaks, and the proteases secreted by them, play an important role in fibrogenesis [131, 132]. Tryptase is a serum protease representing about 20% of human mast cell proteins. The mast cells are essential in the allergic response, as well as in the initiation of the inflammatory response, by releasing several mediators such as proteases, histamine, cytokines (IL-1, IL-6, IL-8, TNF-α), transforming growth factor-beta (TGF-β), granulocyte macrophage-colony stimulating factor (GM-CSF). IL-1α, IL-6, IL-8, IL-18 and TNF-α cytokines are constantly elevated in endometriosis [114, 133, 134].

Through our study, we have demonstrated that many mast cells are present around the endometriosis outbreaks, compared to their number around the normal endometrium, which favors the maintenance of a powerful inflammatory process around the ectopically located endometrial outbreaks.

CD79α is a protein encoded by the *CD79α* gene and is present in the cell membrane, having an extracellular domain, represented by an immunoglobulin and a transmembrane region, having a short transmembrane domain [135]. It plays various roles in the development of B-cells and in their functioning, being present on their surface, throughout the entire life cycle. This protein remains present when B-cells turn into plasmocytes, and immunohistochemistry can identify plasma cells that play a role in antibody secretion, from the structure of different organs.

In this study, we noticed that these cells are present around endometriosis outbreaks and contribute to supporting the inflammatory process.

Conclusions

From a clinical point of view, we noticed that the age at diagnosis of endometriosis ranged in our group of patients from 22–47 years old, the youngest age in a case with endometriosis of the abdominal wall, and the oldest age in a case with pelvic endometriosis.

Normoponderal or underweight women are more likely to develop this pathology compared to overweight, because of the decrease in the amount of sex hormones in the latter. The most common symptoms were vaginal bleeding and pelvic pain.

Most women with endometriosis of the abdominal wall had regular menstruation, and those with pelvic or ovarian endometriosis had irregular menstruation, suggesting hormonal involvement in triggering this pathology. Most cases of primary infertility were in women with ovarian endometriosis.

ESR increase over normal values was present in five cases, two with ovarian endometriosis, two with pelvic endometriosis, one with adenomyosis.

Utilizing IHC with anti-CK7 and anti-CK20 antibodies, we have demonstrated that ectopic tissue has epithelial–endometrial origin and that these structures are not metastases of gastrointestinal tumors. Moreover, positivity for anti-ER and anti-PR antibodies has shown that these areas are endometriosis foci.

Positivity of the Ki67 proliferation marker and the marker that inhibits BCL2 cell apoptosis is directed toward increased aggressiveness of endometrial ectopic tissue. Anti-Ki67 antibody labeling was more intense in patients with advanced endometriosis, being directly proportional to the size of endometriosis foci. Positive immunoassay with the anti-p53 antibody revealed the proliferation process, important for tumor cell transformation.

Conflict of interests

The authors declare that they have no conflict of interests.

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Authors' contribution

Anca-Maria Istrate-Ofiţeru and Daniel Pirici equally contributed to this article.

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