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ABSTRAT*

**CLINICAL, HISTOLOGICAL AND
IMMUNOHISTOCHEMICAL STUDY
IN ENDOMETRIOSIS**

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GENERAL PART

Introduction

Endometriosis is a fairly common benign pathology that affects most women and can have multiple locations: pelvis, peritoneum, rectovaginal septum, ovaries, abdominal wall after cesarean section, umbilicus and more less commonly in other organs: kidney, urinary tract, colon, lungs or even brain, and also, the eutopic location, in the myometrium structure, in which case it is called adenomyosis [*Shalin, S.C., Haws, A.L., și colab., 2012; Stevens, E.E., Pradhan, T.S., și colab., 2013; Efremidou, E.I., Kouklakis, G., și colab., 2012; Brătilă, E., Ionescu, O.M., și colab., 2016*].

The most common symptoms are pelvic pain, vaginal bleeding, infertility, dysmenorrhea, dysuria, dyspareunia. The severity and complexity of the pain involve major problems in managing the therapeutic possibilities [*Laux-Biehlmann, A., D'Hooghe, T., și colab., 2015; McKinnon BD, Bertschi D, și colab., 2015*].

The etiopathogenesis of this condition can be divided into five categories: the most accepted theory being that of retrograde menstruation, but also the coelomic metaplasia, the origin of the remaining embryonic cells, the theory of induction and the lymphatic or vascular dissemination are mentioned [*Berceanu, C., Ofițeru, A.M., și colab., 2018*].

It should be kept in mind that in addition to the symptomatology that may develop unfavorably, there may be associated risk factors that alter the structure of the endometrial glands, leading to premalignant or even malignant transformation of endometriosis outbreaks.

Studies have shown that genes, the immune system, environmental factors and hormonal influence can lead to endometrial hyperplasia, dysplasia and even malignant transformation of ectopic endometriosis outbreaks [*Berceanu, C., Ofițeru, A.M., și colab., 2018*].

From a microscopic point of view, this pathology is characterized by the presence of the endometrial glands and the endometrial stroma, accompanied by different degrees of fibrosis, old or recent hemorrhage and macrophages that have phagocytized hemosiderin [*Comănescu, M., Potecă, A., și colab., 2018*].

The epithelial component may have several aspects, may be unistratified, müllerian type or may have glands with different architectures, but which respect the characteristics of the normal endometrium [*Comănescu, M., Potecă, A., și colab., 2018*].

The stroma is represented by round ovary cells with spherical nuclei and reduced cytoplasm. Peristromal cells of the inflammatory system, T lymphocytes, rare B lymphocytes and numerous macrophages that have phagocytised hemosiderin are identified. This inflammatory microenvironment is associated with endothelial dysfunction and participates in the carcinogenetic transformation of the endometriosis islands [*Comănescu, M., Potecă, A., și colab., 2018*].

All these microscopic changes occurring in endometriosis / adenomyosis outbreaks, together with the symptomatology, clinical signs and possible tissue transformations, place this topic in a current field of research, based on interdisciplinary studies.

KEYWORDS: endometriosis, adenomyosis, symptomatology, microscopy.

CHAPTER I. Ontogeny of the female reproductive tract

Although the chromosomal sex or genetic sex of an embryo is determined at the time of fertilization by the type of sperm that fertilizes the egg, the male and female morphological characteristics begin to develop only in the seventh week of pregnancy. The early genital systems of the two sexes are similar; therefore, the initial period of genital development is referred to as the indifferent state of sexual development [*Moore, K.L. Persaud, T.V.N., 2003*].

Female gonads, ovaries, derive from three sources: the mesothelium (mesodermal epithelium) lining the posterior abdominal wall, the underlying mesenchyme (embryological connective tissue) and from the primordial germ cells [*Moore, K.L. Persaud, T.V.N., 2003*].

Both sexes initially show two pairs of genital ducts: the mesonephric duct or Wolffian and the paramesonephric duct or Müllerian. The second appears as a longitudinal invagination of the surface epithelium of the antero-lateral area of the urogenital ridge.

Initially, the two ducts are separated by a septum, later merging and giving rise to the uterine canal. The lower extremity of these two associated ducts is projected into the urogenital sinus, at the level of the posterior wall, where it forms a small protrusion, called the paramezonephric tubercle (Müller) [*Sadler, T. W., 2007*].

The SRY gene, via the SOX9 gene, participates in the development of male gonads, and the WNT gene induces ovarian development. WNT amplifies DAX1 expression belonging to the family of genes encoding nuclear hormone receptors, with the role in inhibiting SOX9 action. Also, WNT4 regulates the expression of genes responsible for the differentiation of female gonads - the ovaries, including the TAFII105 gene that belongs to the RNA polymerase of ovarian follicular cells [*Sadler, T.W., 2007*].

The main genital tract arises from the paramezonephric ducts in the female embryos. First, each duct is composed of 3 portions: 1) a vertical

upper segment, communicating with the abdominal cavity; 2) a segment placed horizontally that intersects with the mesonephric duct; 3) a lower vertical segment which merges with the opposite homologous segment. The first two enumerated (listed) segments will form the uterine tube, after the ovary descends, and the lower merged segments give rise to the uterine canal [Sadler, T.W., 2007].

After the fused extremity of the paramesonephric ducts reaches the urogenital sinus, two compact projections, the sinovaginal bulbs, begin to form in the pelvic portion, which will form a compact vaginal plate [Sadler, T.W., 2007].

In conclusion, the urinary and genital apparatus (systems) derive from the mesoderm. The 3 components of the genital system: the gonads, the genital tract and the external genital organs go through the indifferent state. Under the control of the WNT4 gene, the ovaries will develop, amplifying the expression of the DAX1 gene, leading to the inhibition of the SOX9 gene (involved in the development of male genital systems). Estrogens act on the paramesonephric system in females, directing the formation of the uterine tubes, uterus, cervix and upper portion of the vagina [Sadler, T.W., 2007].

CHAPTER II. Histophysiology of the uterus

The female genital tract is composed of female gonads (ovaries), with gametogenic and hormonogenic functions, female genital tract: Fallopian tubes, uterine and vaginal tracts, from external genital organs: labia majora, labia minora, clitoris and vaginal vestibule, but also from hormone-dependent structures such as: mammary gland and placenta.

The uterus is a pear-shaped organ, divided into: uterine body, isthmus and cervix; located between the bladder and rectum, superior to the vagina, normally in an anteverted and anteflexed position [Crăițoiu, Ș., 2003].

The uterine wall has 3 tunics: the perimeter (outer layer), myometrium (middle tunic) and endometrium (internal tunic). [Crăițoiu, Ș., 2003].

The uterine mucosa is composed of an epithelium and a chorion. In the fertile period of the woman (between puberty and menopause), the endometrium is made up of an epithelium and a lamina propria, which contains straight tubular glands. In the portion near the myometrium these glands sometimes have a branched appearance. The endometrial epithelium is located on a basement membrane with a continuous structure, which separates it from the cytogenetic chorion and presents a single row of few, secretory, ciliated cells, with short and scattered kinocilia and basal, proliferative, regenerating cells [Crăițoiu, Ș., 2003].

The epithelium of the endometrial glands resembles that of the superficial endometrium, but has fewer ciliated cells [Junqueira L.C., Carneiro, J., 2008].

Female hormones, estrogen and progesterone, control the activity of the female reproductive tract. Epithelial cells and associated connective tissue are proliferating and differentiating under hormonal control. Starting with puberty the pituitary hormones (follicle-stimulating-FSH and luteotropic-LTH) are triggering the ovarian secretion of estrogen and progesterone, which induce endometrial cyclic changes [Junqueira L.C., Carneiro, J., 2008].

The menstrual phase lasts for a few days, on average 3-4 and is followed by the proliferative phase and the secretory (luteal) phase. The proliferative phase varies with duration, averaging 10 days, and the secretory phase starts with ovulation and lasts 14 days (**Table 1**) [Junqueira L.C., Carneiro, J., 2008].

The uterine arteries, direct branches of the common iliac arteries, are the largest (main) blood supply to the uterus. The ovarian arteries and the round ligament arteries supply the uterus with a smaller quantity of blood.

From the uterine arteries emerge the arcuate arteries that branch in radial arteries, which send collaterals to the cervix and the uterine body. From the uterine arterial branches that penetrate into the myometrium will emerge branches that form the plexiform layer, a true arterial anastomotic plexus; the arteries that supply the 3 layers of the uterus arise from this plexus.

Two types of arteries supply the endometrium: short, straight, nutritive and long, functional, spiral. The long, functional, hormone-dependent, spiral arteries, also called helicine arterioles (spiral arteries), supply the superficial layers of the endometrium. During the proliferative phase, they increase in size, capillarize massively, form arterial-arterial anastomoses in the 2/3 endometrial surfaces and twist strongly during the secretory phase of the endometrial cycle. At the end of the progesterone phase, the wall of the functional arteries is strongly contracted due to the sudden decrease of the ovarian hormones and a process of local ischemia is triggered, followed by endometrial necrosis.

The venous blood flows in the opposite direction compared to arterial blood. And the veins form numerous venous plexuses in the myometrium that are better represented in pregnancy.

Lymphatic vessels have a particular importance in uterine malignant pathology. Each tunic of the uterus has an intrinsic lymphatic network: mucous, muscular and serous. These networks lead to the formation of collecting trunks which drain near the uterine margins and to the groin and iliac ganglia.

The uterine innervation is especially vegetative, parasympathetic and sympathetic, receiving branches from the inferior hypogastric plexus [*Bold, A., Mogoanta, L., și colab, 2011*].

CHAPTER III. Endometriosis

Endometriosis is a truly enigmatic disease, various hypothesis have been studied for nearly a century without being fully understood.

The histogenesis of this pathology is not known, but the obligatory feature in the definition of endometriosis is the presence of endometrial tissue in locations outside the uterine cavity or in the myometrium structure, also known as adenomyosis.

Morphologically, endometriosis and adenomyosis are represented by the existence of endometrial tissue and periglandular stroma in an ectopic region, but, from the anatomical and clinical point of view, the two pathologies are different. There are several theories regarding the pathogenesis of this condition (**Table 1**).

Phases of the menstrual cycle					
	Follicular phase (Proliferative)	Luteal phase (Secretory)			Menstrual phase
Pituitary hormones and their main actions	FSH (follicle-stimulating hormone) causes accelerated growth of ovarian follicles	LH (luteinizing hormone) reaches a peak that coincides with the onset of the secretory phase and occurs as a result of estrogen-stimulating action. It induces ovulation and the evolution of the yellow body			
Events occurring in ovaries	Pre-antral and antral ovarian follicles. The dominant follicle reaches the preovulatory stage	Ovulation	The evolution of the yellow body (corpus luteum)	The involution of the yellow body (corpus luteum)	

Ovarian hormones	Ovarian follicles secrete estrogen that influences (acts on) the uterus, vagina and fallopian tubes	The progesterone secreted by the yellow body has a main action on the uterus	The production of progesterone is stopped	
Main endometrial events	Regeneration of the mucosa after menstruation	Thickening of the mucosa, spiraling of the glands, occurrence of glandular secretion	Elimination of the superficial 2/3 of the endometrium, approximately 14 days after ovulation	

Table 1 – Events that occur during the menstrual cycle - synthesis.

<i>Current theories</i>	<i>Multifactorial etiology</i>	<i>Risk factors</i>
<i>Retrograde menstruation or transtubar regurgitation, tissue transplantation</i>	<p>INTERACTION</p> <p>Specific genes</p>  <p>Environmental factors</p>	<i>Family aggregation</i>
<i>Lymphatic dissemination</i>		<i>Genetic mutation and polymorphism</i>
<i>Hematogen / vascular dissemination</i>		<i>Anatomical defects</i>
<i>Metaplasia of the celomic epithelium</i>		<i>Toxins in the environment</i>
<i>Induction theory</i>		
<i>Hormonal dependence</i>		
<i>Immunological factors / The role of the immune system</i>		

Tabelul 2 - Table 2 - Theories regarding the pathogenesis of endometriosis) [after Berceanu, C., Brătilă, E., și colab. 2018].

Depending on the location of endometriosis, the associated risk factors various malignant transformations of this pathology have been studied (**Figure 1**).

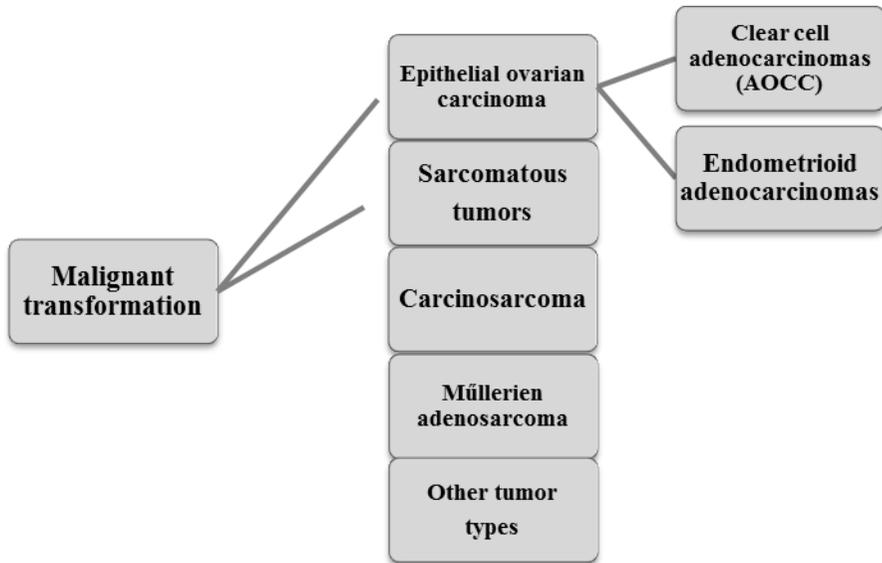


Figure 1 - Malignant tumors arising from endometriosis (endometriotic transformation).

SPECIAL PART

CHAPTER IV. Objectives

The purpose of this study is to evaluate and statistically analyze a group of patients with endometriosis/ adenomyosis in the purpose of fully grasping its clinical characteristics (symptomatology, personal antecedents both physiological and pathological, behavioral factors, laboratory analysis), diagnosis methods and the applicable treatments.

Also, another goal is to highlight certain histological, histopathological and immunohistochemical aspects of this pathology, depending on localization (ovarian, pelvic/ peritoneal, myometrial, parietal) and evaluating the endometrial focal abnormalities/ ectopic glandular abnormalities, in order to demonstrate the involvement of some risk factors in the evolution of this pathology.

Through immunohistochemistry, aided by specific markers of this pathology (cytokeratin 7, estrogen receptors and progesterone receptors) I wanted to highlight the presence of endometriosis/ adenomyosis in the structure of different organs and to establish a positive diagnosis regarding the tissue origin. With the aid of the differential marker (cytokeratin 20) I wanted to show eventual distinctive areas containing metastatic ectopic glandular proliferation with digestive origin.

Through numerical analysis, with the help of required immunohistochemical markers (cluster of cluster of differentiation 31/34, cluster of cluster of differentiation 3/20/68/79 α , tryptase, proliferation marker Ki67, tumor protein p53, regulator protein BCL-2 and PTEN) I aim to underline the involvement of increased peri-endometrial vascularization, inflammation, cell proliferation factors, the presence of regulator protein and

tumor suppressor protein, in the development of endometriosis/ adenomyosis and their eventual preneoplastic/neoplastic conversions.

CHAPTER V. Clinical-statistical study of endometriosis

Introduction: Endometriosis is a benign pathology that affects especially women of reproductive age. The most common symptoms encountered in this pathology are pain, subfebrility, bleeding, but also dysmenorrhea, dysuria, dyspareunia. The severity and complexity of the pain can cause major problems in the management of therapeutic resources. [*Laux-Biehlmann, A., D'Hooghe, T., și colab., 2015; McKinnon, B.D., Bertschi, D., și colab., 2015*].

Medication is the first-line therapy [*Walch, K., Unfried, G., și colab., 2009*], but the absence of a favorable response may resort to surgical treatment. The first intention in surgical treatment is laparoscopy. Depending on the location of the endometriosis outbreaks and the depth of the lesion, the surgical technique is chosen, which may represent the curative treatment of this pathology [*Crosignani, P.G., Vercellini, P., și colab., 1996*].

Material and methods: My study was carried out on 120 cases of endometriosis, 30 cases of ovarian endometriosis, 30 cases of pelvic endometriosis, 30 cases of adenomyosis (myometrial endometriosis) and 30 cases of abdominal wall endometriosis.

Patients were admitted and investigated in the Obstetrics-Gynecology Clinic II and the Surgery Clinic III of the Craiova County Emergency Clinical Hospital (SCJUC), between 2010-2018.

A clinical study and statistical analysis was performed using the Microsoft Excel 2010 program, based on: the year of hospitalization, localization of endometriosis (ovarian, pelvic, adenomyosis, endometriosis of the abdominal wall), age of patients, environment of origin, height, weight, body mass index (BMI), behaviors (consumption of coffee, alcohol, tobacco), living and working conditions (housewives / employees / student-

students), by symptomatology (bleeding, pain), personal pathological antecedents, personal physiological background: menarche, regular or irregular menstruation, spontaneous / on-demand abortions, number of natural or caesarian section deliveries, infertility, surgery, clinical laboratory data (leukocyte count, hemoglobin (Hb), hematocrit (Hct), 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 (CA-125 marker) and the medication used to alleviate symptomatology, through which all patient data were collected and compared.

Following the laboratory tests and the medical investigations, surgical intervention was carried for the excision of the endometriosis outbreaks. The surgical techniques were chosen according to the location of the endometriosis.

Results: Age at diagnosis of endometriosis varied according to location as follows: ovarian endometriosis (22-46 years old), pelvis (30-57 years old), adenomyosis (30-46 years), abdominal wall endometriosis (23-38 years old). Endometriosis of the abdominal wall occurs at younger age due to the increase of births by caesarean section.

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

Using the height and weight of each patient with endometriosis, we calculated the body mass index and observed that most normal weight patients had abdominal wall endometriosis (21 cases) and pelvic endometriosis (21 cases), followed by adenomyosis (20 cases) and the fewest normal-weight patients had ovarian endometriosis (16 cases). Underweight patients-9 with ovarian endometriosis, 8 patients with abdominal wall endometriosis and 4 patients with pelvic endometriosis. Overweight patients-

6 patients with adenomyosis, 2 patients with pelvic endometriosis and 2 patients with ovarian endometriosis. Class I obesity- a number of 4 patients with adenomyosis and 2 patients with ovarian and pelvic endometriosis. Grade II obesity- 1 patients with endometriosis of the abdominal, 1 patient with ovarian e3ndometriosis and 1 patient with pelvic endometriosis.

The most common symptoms related to hospitalization were: vaginal bleeding and pelvic pain.

Also according to the anatomical location, we compared the personal physiological history (the presence of regular / irregular menstruation, spontaneous / on-demand abortions, the number of eutocic births / by caesarean section) and the personal pathological background (infertility, surgery for the removal of endometriosis outbreaks: partial ovariectomy, hysterectomy, excision of endometriotic nodules from the post-caesarean scars), (**Figure 2**).

Studying the CBC (complete blood count), the erythrocyte sedimentation rate, CA-125 marker, I also noticed that there were changes in several cases. Behaviors of patients varied, being associated factors with the development of endometriosis.

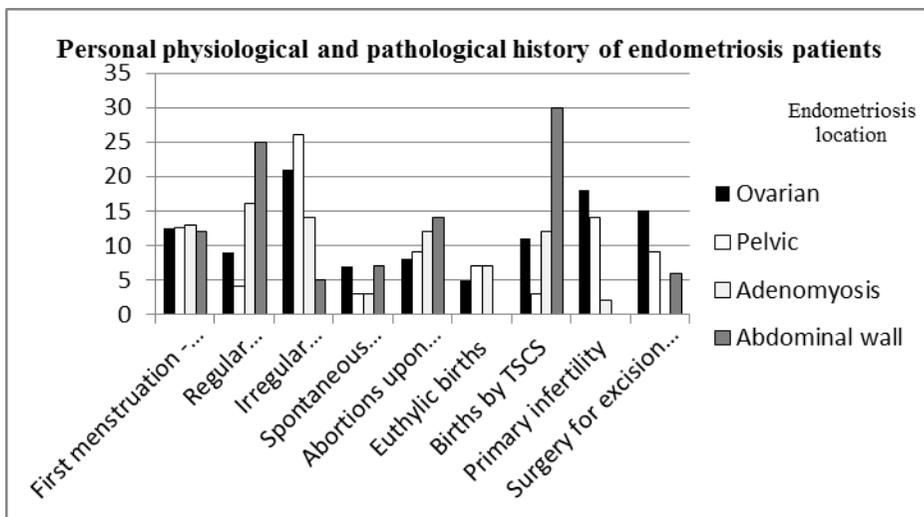


Figure 2 - Personal physiological and pathological history of patients with endometriosis

Conclusions: The extensive symptomatology associated with endometriosis can affect the physical as well as the mental state of the patient. Dysmenorrhea, chronic pelvic pain and infertility are most commonly associated with this benign pathology, which has the characteristics of malignancy: it spreads locally and remotely, damages adjacent tissues and causes cell invasion.

The treatment of endometriosis can be pharmaceutical, or, the second therapeutic option, surgical, the technique being chosen according to the location of the endometriosis and the status of the patient.

In the presence of risk factors: mechanical, chemical, genetic or inflammatory, endometriosis outbreaks can turn premalignant or even malignant.

The location of endometriosis varied according to the age of the patients, the youngest had ovarian or abdominal wall endometriosis, which appeared after caesarean section, and the oldest with pelvic endometriosis.

It has been studied that hormonal secretion is heavily influenced in overweight women and it can disrupt menstruation, changing its flow and implicitly rising the risk of endometriosis. In some women there is a directly proportional relationship between the two, and in other women an inversely proportional relationship.

CA-125 and ESR (1 hour) may be increased in patients with endometriosis and can be used as screening markers for this pathology.

The inversely proportional relationship between smoking and endometriosis was found by the interaction between cigarette smoke and the glutathione-S-transferase gene polymorphism as a possible risk factor for the development of endometriosis. It has been observed that patients consuming alcohol or coffee may be more frequently affected by this pathology, compared to women who do not consume these drinks.

CHAPTER VI. Histological study of endometriosis

Introduction: The positive diagnosis of endometriosis is supported by the histopathological examination. Through the use of classical histological techniques, through Hematoxylin-Eosin (HE) staining and Masson's Trichrome staining (TRI), we have highlighted the presence of eutopic / ectopic endometrial tissue.

From a microscopic point of view, endometriosis is a pathology characterized by the presence of the endometrial glands and the endometrial stroma, accompanied by different degrees of fibrosis, old or recent hemorrhage and macrophages that have phagocytosed hemosiderin. [Comănescu, M., Potecă, A, 2018].

Atypical endometriosis is characterized by the presence of cells with pleomorphism that vary from moderate to severe, arranged in several layers that can even form micro-papillae. This can potentially transform premalignant, due to cellular atypia and architectural changes [Comănescu, M., Potecă, A, 2018].

Malignant transformed endometriosis can be highlighted by identifying endometriotic outbreaks with malignant transformation. The diagnosis of malignancy associated with endometriosis pathology requires the presence of the same tissue structures of the benign lesion, confirmation of the primary origin and the histological continuity of the benign-malignant lesions.

Material and methods: Following surgical treatment for solving ovarian, pelvic (peritoneal) endometriosis, adenomyosis or endometriosis of the abdominal wall, tissue of interest was harvested (collected), the tissue samples were introduced in 10% neutral formalin and then embedded in paraffin blocks. The blocks thus obtained were sectioned using the HM350 microtome and the resulting slides were stained using the classical histological techniques Hematoxylin-Eosin and Masson's Trichrome

stainings, all of which were performed within the Histology department of the University of Medicine and Pharmacy of Craiova.

Conclusions: The diagnosis of endometriosis is dictated by the histopathological examination. The classic HE and TRI stains identify the glandular structures and the adjacent stroma with ectopic localization, internally: adenomyosis or externally: in the structure of the pelvic or abdominal organs. This histopathological diagnosis must sometimes be confirmed by IHC.

The identification of endometriosis is based on meeting the following criteria: the presence of the endometrial glands, the stroma and possibly the perilesional hemorrhagic effusion, which contains macrophages that have phagocytosed hemosiderin.

The epithelial component is represented by an epithelium that varies in form and structure from simple columnar, to pseudistratified columnar or pluristratified in hyperplastic premalignant transformations.

The stromal component is represented by young cells: fibroblasts, lymphocytes and macrophages, involved in the triggering of inflammatory processes and the eventual malignant transformation of endometriosis outbreaks.

The diagnosis of malignant transformed endometriosis requires immunohistochemical study and the fulfillment of obligatory histopathological criteria: the coexistence of both lesions in the structure of the same organ, with tissue continuity, but also the negation of the existence of another neoplastic lesion, in order to eliminate the possibility of confusion with a secondary determination.

CHAPTER VII. Immunohistochemical study in endometriosis

Introduction: Endometriosis can be identified using histological staining, but a better differential diagnosis can be made by

immunohistochemistry techniques. Therefore, the 120 cases studied in this thesis were also analyzed from an immunohistochemical point of view.

Using the anti-cytokeratin 7/20 antibodies (CK7, CK20), anti-Estrogen (ER) / Progesterone (PR) receptors we have demonstrated that the tissue areas we studied had endometrial origin.

Environmental, hormonal, inflammatory factors can influence these areas, so that the presence of ER / PR can be altered, the degree of cell proliferation may be increased (marking with anti-Ki67 antibody), the genetic structures of B-cell lymphoma 2 (BCL- 2+) and Phosphatase and tensin homolog (PTEN +) can be modified, tumor protein 53 (p53) may be positive in atypical cases, inflammatory cell density may be increased compared to the area adjacent to the normal endometrium: Cluster of differentiation 3/20/68 / 79 α (CD3 +, CD20 +, CD68 +, CD79 α +) and Triptase +, all of which may influence cell structure, histo-architecture of the surrounding microenvironment and cause premalignant or even malignant changes in endometriosis outbreaks.

Immunohistochemical tests confirm the histological suspicion of endometriosis. [*Istrate-Ofițeru, A.M., Pirici, D., și colab., 2018*].

Material and methods: The tissue sections were obtained similar to those for the histological study and went through a process of antigenic retrieval, blocking of endogenous peroxidase and blocking of non-specific binding sites, then the sections were incubated with the primary antibody for 18 hours at 4 ° C, and the next day the signal was amplified by using a peroxidase polymer system for 30 minutes (Nikirei-Bioscience, Tokyo, Japan). The signal was then detected with 3,3'-diaminobenzidine (DAB) (Dako, Glostrup, Denmark) and the slides were coated in a xylene-based mounting medium (DPX, Sigma-Aldrich, St. Louis, MO, USA), after staining with hematoxylin.

The microscopic images were photographed using a Nikon Eclipse 55i microscope, equipped with a 5 Mp color CCD camera, and analyzed with

the Image ProPlus 7 AMS software package (Media Cybernetics Inc., Buckinghamshire, UK).

For the proposed study, the sections were photographed in the regions of interest, with the objectives 100 ×, 200 ×, and for quantification, 4 images were produced with the objective 200 × for each case. The blood vessels and cells of the inflammatory system were counted for each image separately and then an average of the vascular / cellular density of the case was achieved. All values were graphically represented and interpreted using Microsoft Excel 2013. The ANOVA test (ANOVA - variation analysis) was used for multiple comparisons. In all cases, $p < 0.05$ was used to indicate statistical significance.

Results: With the help of immunohistochemical reactions, we demonstrated that from a microscopic point of view the ectopic tissue ,post-interventional, is endometrial.

With the help of the anti-CK7 antibody we showed that the areas of interest have positive glandular epithelium, and to make the differential diagnosis with a possible metastasis with digestive starting point, we performed the immunolabeling with the anti-CK20 antibody, which reacted negatively and showed that the tissue is not of digestive glandular type.

ER and PR are present in the endometrial cells, and the positive reaction to the immunolabeling once again demonstrates the endometrial origin.

For the study of peristromal vascularization we used the anti-CD34 antibody. The increased vascular density is observed periodically, especially in the hyperplastic transformed areas. For the numerical quantification of blood vessels, we took 4 photographs, with objective × 200 for each case, of the lesion areas - periglandular (at the same distance from the endometrial glands), depending on each location of the endometriosis and we observed that the average density the highest number was present in the case of abdominal wall endometriosis (23.69 CD34 + / × 200 vessels), followed by

the normal secretory-phase endometrium (26.06 CD34 + / × 200 vessels) and the normal, eutopic endometrium proliferative (23.69 CD34 + / × 200 vessels), adenomyosis (18.6 CD34 + / × 200 vessels), peritoneal / pelvic endometriosis (4.2 CD34 + / × 200 vessels) and ovarian endometriosis (2.7 CD34 + / × 200 vessels).).

To study the cell proliferation rate we used the anti-Ki67 antibody. We noticed that the marking for the cells going through cellular division is more pronounced in the areas with hyperplastic transformation, compared to the normal endometrium or endometriosis with different locations, but without hyperplastic transformation.

For the study of preneoplastic or neoplastic cell transformations we used the anti-p53 antibody to detect the cellular expression of a tumor suppressor protein and we observed that it is more intensely expressed in hyperplastic transformed than it is expressed in endometriotic lesions in cases with ovarian or abdominal wall endometriosis.

Also, using the anti-BCL-2 antibody, we observed the expression of a modified protein, which may have a regulatory role on programmed cell death, in cells that can potentially go through malignant transformation. The reaction is more intense if more genetic changes have occurred, especially in cases with hyperplastic transformation, but also in those with ovarian or abdominal wall endometriosis.

Similar to the anti-p53 antibody, the anti-PTEN antibody highlights altered cells, in which a tumor suppressor gene was activated, which is involved in regulating the cell cycle, preventing growth and over-accelerating cell division. We observed that in cases with hyperplastic transformed endometriosis, the reaction was more intense compared with the normal endometrium or with the ectopic lesions, but without structural changes.

Given that the inflammatory process may be involved in cellular structural and functional changes, we used several antibodies to determine the number of inflammatory cells around endometriosis outbreaks with

different locations and those adjacent to the normal endometrium, so we used: anti-CD3 antibody for T lymphocyte marking, anti-CD20 antibody for lymphocyte B marking, anti-CD68 antibody for macrophage marking, anti-Triptase antibody for mast cell marking, and anti-CD79 α antibody all for marking B lymphocytes.

We compared the results and observed that the highest average CD3 + T-type lymphocyte density was found in the case of abdominal wall endometriosis (116.19 cells / \times 200), followed by adenomyosis (66.27 cells / \times 200).), secretory phase eutopic endometrium (16.01 cells / \times 200), proliferative phase eutopic endometrium (13.56 cells / \times 200), peritoneal / pelvic endometriosis (12.93 cells / \times 200) and ovarian endometriosis (12.82 cells / \times 200).

The average CD20 + type B lymphocyte density varied as follows: abdominal wall endometriosis (34.2 cells / \times 200), adenomyosis (9.9 cells / \times 200), eutopic endometrium in the secretory phase (4.17 cells / \times 200) , peritoneal / pelvic endometriosis (3.63 cells / \times 200), proliferative phase eutopic endometrium (2.33 cells / \times 200) and ovarian endometriosis (1.99 cells / \times 200).

The average CD68 + macrophage cell density varied as follows: adenomyosis (65.6 cells / \times 200), abdominal wall endometriosis (57.66 cells / \times 200), eutopic endometrium in secretory phase (12.11 cells / \times 200) , peritoneal / pelvic endometriosis (11.96 cells / \times 200), ovarian endometriosis (11.94 cells / \times 200), and proliferative phase eutopic endometrium (10.01 cells / \times 200).

The average mast cell type Triptase + density varied as follows: adenomyosis (19.34 cells / \times 200), endometriosis of the abdominal wall (18.81 cells / \times 200), peritoneal / pelvic endometriosis (4.77 cells / \times 200), ovarian endometriosis (3.51 cells / \times 200), secretory phase eutopic endometrium (3.45 cells / \times 200) and proliferative phase eutopic endometrium (2.59 cells / \times 200). Also, the mean cell density of CD79 α +

lymphocyte type B varied as follows: abdominal wall endometriosis (29.05 cells / × 200), adenomyosis (12.45 cells / × 200), ovarian endometriosis (11.82 cells / × 200)), peritoneal / pelvic endometriosis (9.96 cells / × 200), eutopic endometrium in secretory phase (8.81 cells / × 200) and eutopic endometrium in proliferative phase (8.41 cells / × 200).

We compared the averages obtained for each category and acquired an overall result. There is a statistically significant difference, depending on the location of the endometriosis, for the values: CD34 - F (5,179) = 596,510, $p < 0.001$; CD3 - F (5,179) = 429,196, $p < 0.001$; CD20 - F (5,179) = 940.025, $p < 0.001$; CD68 - F (5,179) = 758,489, $p < 0.001$; Tryptase - F (5,179) = 808,694, $p < 0.001$; CD79 α - F (5,179) = 448,372, $p < 0.001$.

Conclusions: Immunohistochemical tests conducted for the identification of glandular epithelium, hormonal receptors, tissue proliferation, but also for the identification of tumor proteins, vascularization and inflammatory cells, were the basis for the positive diagnosing of endometriosis with different locations and to highlight the possibility of preneoplastic transformation.

The histopathological examination has a certain diagnostic value and dictates the choice of therapeutic behavior.

The higher the production of sex hormones, the higher the number of blood vessels and positive immunohistochemical reactions for the inflammatory cell line: CD3 +, CD20 +, CD68 +, Tryptase, CD79 α +, the higher the endometrial cell dissemination rate.

Disseminated cells and inflammatory response cause the most common symptoms of endometriosis: chronic pelvic pain and infertility.

The mediators secreted by the inflammatory cells cause important changes of the histoarchitecture of the microenvironment adjacent to endometriosis / adenomyosis outbreaks, but also to ectopically implanted endometrial cells, which may lead to the appearance of cellular atypia.

CHAPTER VIII: Final conclusions

Endometriosis / adenomyosis are benign pathologies of genital origin, which occur more frequently in women of childbearing (**reproductive**) age.

The extensive study to identify the ectopic endometrial structures and their importance in the prognosis of the disease were the targets pursued within this thesis.

Under the influence of certain stimuli, of the anatomy of the female reproductive organs and of the inflammatory processes, viable endometrial cells can migrate and can be implanted, ectopically, in the structure of the different organs.

The most common symptoms are chronic pelvic pain, vaginal bleeding and infertility.

The correct and rapid medical investigations, together with the application of the appropriate treatment substantially reduces the risk of infertility and reduces the symptomatology.

The current study methods are of major importance in the choice of therapeutic possibilities and in the prognosis of the disease.

A multitude of inflammatory, hormonal, mechanical factors are involved in the progression of this pathology.

Through the clinical study we have observed that the age of the patients can fluctuate, that the body mass index can influence the appearance of endometriosis / adenomyosis, thus, the normoponderal or underweight women are more predisposed to the development of this pathology, compared with the overweight ones, due to the decrease of the amount of the sex hormones. and most cases of primary infertility were observed in women with ovarian endometriosis.

With regard to laboratory tests, serum ESR, CA-125 may be elevated, and secondary anemia may occur in patients with heavy bleeding.

From a microscopic point of view, we observed the existence of glandular epithelium and periglandular stroma having a similar appearance to the normal endometrial tissue, in the structure of the different organs.

Using the immunohistochemical study with the help of anti-CK7 / CK20 antibodies we have shown that ectopic tissue has epithelial-endometrial origin and that these structures are not possible metastases from gastrointestinal tumors.

Marking with anti-ER and anti-PR antibodies has shown us that the areas studied are endometriosis / adenomyosis foci. Estrogen maintains the survival and dissemination of endometriotic cells, and the presence of ER in the structure of endometriosis / adenomyosis outbreaks shows an increased tissue responsivity to this hormone. Positive immunohistochemical staining with anti-ER and anti-PR antibodies may guide the physician in applying hormonal treatment to patients diagnosed with this pathology.

The presence of cell apoptosis inhibitory genes (BCL-2, PTEN) increases the rate of cell disruption (highlighted by the anti-Ki67 antibody), the determination of intracellular tumor protein 53 (evidenced by the anti-p53 antibody) and the strong immune response may influence the malignant transformation of endometriosis / adenomyosis outbreaks.

The periglandular inflammatory process highlighted with the help of anti-CD3 / CD20 / CD68 / Triptase / CD79 α antibodies demonstrates its involvement in the evolution and possibility of preneoplastic transformation through secreted mediators, which produce important changes in the histo-architecture of the microenvironment adjacent to the endometriosis / adenomyosis foci and ectopically implanted endometrial cells, which can lead to the appearance of cellular atypia.

The intense periglandular vascularization is involved in the processes of cell growth and proliferation, supports the hypothesis suggested by hematogenous dissemination, but may also influence the evolution of migrated cells.

The effective treatment of endometriosis remains the surgical intervention, thus suppressing the pain cause and preventing the preneoplastic / malignant transformation of the endometriosis / adenomyosis outbreaks.

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