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HISTOLOGIC AND IMMUNOHISTOCHEMICAL STUDY OF THE INVASIVE PROCESS IN EXTRAGENITAL ENDOMETRIOSIS

311.02 – ANATOMATOMICAL PATHOLOGY

Summary of PhD thesis in medical sciences

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The thesis was developed at the Department of Pathology, Discipline of Mortopathology of Nicolae Testemitanu State University of Medicine and Pharmacy and the Discipline of Mortopathology of the University of Medicine and Pharmacy of Craiova, Romania

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CONTENTS

CONCEPTUAL MILESTONES OF THE THESIS

The topicality and importance of the problem.

Endometriosis is a chronic gynecological condition characterized by the presence of functional endometrial tissue outside the uterine cavity. Although the typical location of this disease is in the pelvic area - ovaries, fallopian tubes, peritoneum - in some cases, endometrial tissue can be found outside the genital sphere, causing what is called extragenital endometriosis [1, 10].

This rarer form of endometriosis can affect a variety of structures and organs outside the reproductive tract, including the urinary tract, digestive tract, diaphragm, lungs and even the nervous system. Symptoms are often nonspecific and can vary depending on location, making diagnosis difficult [2].

Endometriosis remains a controversial disease with multiple symptoms (chronic pelvic pain, dysmenorrhea, profound dyspareunia, infertility, dysuria, dyschezia), with significant negative effects on social, occupational and psychological activity, substantially affecting the quality of life of patients [3,8].

According to the latest World Health Organization data from 2023, endometriosis globally affects 10% (190 million) of women of reproductive age and girls causing pain and/or infertility [10].

Histologic confirmation is essential as in many cases the origin of endometriosis lesions is not confirmed.

Extragenital endometriosis involves an abnormal ability of endometrial cells to migrate, adhere and invade tissues distant from the uterine cavity. A central role in these processes is played by the EMT – *epithelial-mesenchymal transition* and MMP – *matrix metalloproteinases* [5].

The epithelial-mesenchymal transition is an essential biological process during embryonic development, but also in the pathogenesis of diseases such as cancer and endometriosis. In EMT, epithelial cells lose their cohesive and polarity characteristics and acquire mesenchymal properties such as migration and tissue invasion. In extragenital endometriosis, EMT facilitates the dissemination of endometrial cells through blood or lymphatic vessels to ectopic sites, where they can form viable implants [6].

On the other hand, matrix metalloproteinases play a crucial role in the degradation of the extracellular matrix, allowing endometrial cells to penetrate and settle in new tissues. MMP-2 and MMP-9, in particular, have been implicated in the pathogenesis of endometriosis by facilitating the invasion of endometrial cells into non-genital structures. Increased activity of these enzymes has been correlated with a more aggressive and extensive form of the disease [4].

The interaction between EMT and MMP is essential in the formation of extragenital lesions. Proinflammatory factors, such as TNF-α and IL-1β, as well as TGF-β signaling, stimulate EMT and In conclusion, the epithelial-mesenchymal transition and activation of matrix metalloproteinases are key mechanisms that explain the invasive behavior of endometrial cells and the development of extragenital endometrio-genesis, supporting the idea that this condition has features similar to those of tumor processes.

Research aim: Immuno-morphologic evaluation of extragenital endometriosis foci in order to determine potential invasiveness and diagnostic criteria.

Research objectives:

1. Histopathologic identification of lesions of extragenital endometriosis with the outlining of a macroscopic and microscopic picture particular to these lesions;
2. Immunohistochemical evaluation of endometriosis lesions using markers for ER, PR and CD10;
3. Immunohistochemical evaluation of the invasiveness potential of extra-genital endometriosis le-

sions by investigating MMP-1 (interstitial collagenase), MMP-2 (gelatinase-A), MMP-9 (gelatinase-B) and MMP-14 (transmembrane protease type 1) markers;

4. Determination of changes in the SDF-1/CXCR-4 axis in ectopic endometrial invasion and metastasis processes;
5. Evaluation of the expression of specific markers of the epithelial-mesenchymal transition process (E-cadherin/Vimentin) in extragenital endometriosis.

Methodology of scientific research. The positive opinion of the Research Ethics Committee for carrying out the study was obtained on March 16, 2017, verbal report no. 63.

The doctoral studies were conducted in the period 2015-2020 at the Department of Pathology, Discipline of Morphopathology, Faculty of Medicine No. 1 of the Public Institution *Nicolae Testemitanu* State University of Medicine and Pharmacy, the Republic of Moldova and the Discipline of Morphopathology, University of Medicine and Pharmacy of Craiova, Romania.

Scientific novelty of research. For the first time, prognostic markers have been identified with the highest predictive capacity of local aggressive and recurrent potential of these lesions. The MMP study, although not new, provides original data on their involvement in extracellular matrix modeling and local invasiveness of endometriosis lesions. An element of novelty is the panel of MMPs selected by us (MMP1 - interstitial collagenase, MMP-2 - gelatinase-A, MMP-9 - gelatinase-B, MMP-14 - type 1 transmembrane protease), which has not been used in any of the accessible studies to date. Previous research has only considered MMP-1 and MMP-2 or MMP-9 and MMP-14 in genital endometriosis and not in lesions with extragenital localization. In addition, MMP expression was determined not only in glandular and stromal elements of endometriosis lesions, but also in extragenital tissues to assess the correlation between the disease lesions and the tissues in which they develop. For the first time, SDF-1 and CXCR-4 expression were simultaneously investigated in 3 compartments: endometrial glandular cells, endometrial stromal cells and stroma adjacent to extragenital implants. In this study we focused on the concomitant expression of E-cadherin/Vimentin markers in extragenital endometriosis lesions. For the first time, the lesions in which the epithelial-mesenchymal transition (EMT) process is the most active and with the highest potential for loco-regional invasiveness were determined. **The scientific problem solved** was to determine the degree of expression of aggressiveness markers such as E-cadherin, Vimentin, N-cadherin and Twist in the progression of endometriosis.

Theoretical significance and application value of the thesis. The study revealed new data on the origin and development of extragenital endometriosis lesions of various localizations and the involvement of molecular mechanisms in the pathogenesis of this condition. The determination of the most useful biomarkers of the prognosis of extragenital endometriosis contributes to the correct stratification of patients for a targeted and effective treatment, assessment of the evolution, invasiveness and risk of recurrence of the disease.

Approval of thesis results. The results of the study have been presented and discussed at the following national and international scientific forums: *14th National Symposium on Microscopic Morphology with international participation,* Tirgu Mures, Romania, 2016; *Annual scientific conference of the scientific-teaching staff, doctoral students, master's students, residents and students of Nicolae Testemitanu University,* Chisinau, the Republic of Moldova, 2016; *Annual scientific conference of the scientific-teaching staff, doctoral students, master's students, residents and students of Nicolae Testemitanu University,* Chisinau, the Republic of Moldova, 2017; *The 7th International Medical Congress for*

Students and Young Doctors "MedEspera" Chisinau, the Republic of Moldova, 2018; *Annual scientific conference of the scientific-teaching staff, doctoral students, master's students, residents and students of Nicolae Testemitanu University,* Chisinau, the Republic of Moldova, 2018; *The USCAP 108 The Annual Meeting, National Harbor,* Maryland, USA, 2019; *The Congress dedicated to the 75th anniversary of the foundation of Nicolae Testemitanu University*, Chisinau, the Republic of Moldova, 2020; *The 8th International Medical Congress for Students and Young Doctors "MedEspera"* Chisinau, the Republic of Moldova, 2020; *17th National Symposium of the Romanian Society of Morphology*, Craiova, Romania, 2021; *18th National Symposium of the Romanian Society of Morphology,* Craiova, Romania, 2022; *Al 19th National Symposium of the Romanian Society of Morphology*, Craiova, Romania, 2024; *National* ***scientific-practical*** *conference with international participation „History, actuality and perspectives of pathologic anatomy service*", Cisinau, the Republic of Moldova, 2025.

Publications on the thesis topic. In the subject of the thesis 27 scientific papers were published, Including 1 articles in SCOPUS Journals, 1 articles in foreign journals, 3 articles in national reviewed journals. At the same time 1 certificate of innovator and 1 act of implementation of innovation in the scientific-practical process were obtained.

Volume and structure of the thesis. The work is presented on 124 pages, which includes all the mandatory elements stipulated by the Guide in force (2017), iconographically represented by 5 tables, 1 statistical formula and 45 figures, included in the text, 231 bibliographical sources.

Keywords: extragenital endometriosis, immunohistochemical study, invasiveness potential, extracellular matrix, epithelial-mesenchymal transition.

1. ONTOGENETIC MILESTONES OF EMBRYOLOGY AND ANATOMY IN THE EVOLUTION OF ENDOMETRIAL ECTOPY

It represents a detailed synthesis of the data highlighted in the scientific and practical sources in the history of the problem and in recent years in etiologic, pathogenetic, economic aspects of the diagnosis of extragenital endometriosis. Conceptual options regarding the peculiarities of extragenital endometriosis in the context of contemporary embryologic, anatomic, histologic and pathophysiologic aspects of local diagnostic and treatment tactics for the resolution of the pathology in question before the onset of complications are presented. The need for in-depth studies of the invasiveness processes in the development of extragenital endometriosis, as well as the development of an algorithm for early diagnosis in this pathology is argued. The literature review was carried out on the basis of scientific databases: PubMed, NCBI, Scopus, Medline, Cochrane Lybrary.

2. STUDY MATERIAL AND METHODS

2.1. General characteristics of the research methodology

The study was a retrospective descriptive study based on histopathologic reports and microscopic examination of 43 cases of extragenital endometriosis. The cases included in the study were selected in the period 2011 - 2018 for which data were extracted from the records and blocks from the archive of the hospitals: *Gheorghe Palade* Municipal Clinical Hospital, the State Chancellery Hospital, *Sfintul Arhanghelul Mihail* Municipal Clinical Hospital, *Timofei Mosneaga* Republican Clinical Hospital and County Emergency

Clinical Hospital of Craiova, Romania. The research was carried out according to the V-stage study design, developed questionnaire and biochemical, histologic and immunohistochemical investigations.

The chapter covers the general characterization of the research, the main directions and stages of the study and the design of the study group.

The study material consisted of biopsy fragments that were obtained following excision of endometriosis foci from patients aged 19-56 years, average age was 39.7±9.9 years and median Md - 38.0 years.

In order to improve the accuracy of the research, a number of inclusion and exclusion criteria were followed, thus making the study more focused and centered on a specific representative group.

Criteria for inclusion in the study:

- progressive stage of moderate and severe forms of extragenital endometriosis;
- female gender;
- age between 19 and 56 years;
- duration of disease (years);
- informed consent to participate in the study.

Criteria for exclusion from the study:

the last treatment given is up to 6 months;
occurrence of side effects of the therapy administered;
presence of decompensated chronic pathologies;
complicated allergic history;
alcoholism, drug addiction.

2.2. Investigation methods and diagnostic criteria

I. Clinical method:

- Retrospective study of the observation records of 43 patients

II. Biochemical method in blood serum:

- Biochemical exploration of the CA - 125 marker

III. Macroscopic method

- Description of postoperative material

IV. Methods of morphologic explorations of extragenital endometriosis:

- Conventional hematoxylin-eosin histologic method;
- Special histologic method, MASSON trichrome staining in GOLDNER modification
- Immunohistochemical method using antibody set: ER, PR, CD10, MMP1, MMP2, MMP9, MMP9, MMP14, SDF-1, CXCR4, E-cadherin/Vimentin, N-cadherin, Twist.

V. Methods of statistical processing of results

- SPSS program version 16.0 for Windows (SPSS Inc., Belmont, CA, USA, 2008) and Microsoft Office Excel 2019 on the personal computer, applying descriptive and inferential statistical procedures.

The diagnosis of endometriosis of the postoperative scar was based on the presence of obstetric-gynecological interventions in the anamnesis, objective clinical examination (presence of tumor formation in the postoperative scar projection), cyclic character of the dolor dolor syndrome, imaging and laboratory data.

The treatment of patients diagnosed with extragenital endometriosis has been carried out surgically.

Determination of CA-125. This is a cell surface antigen expressed in derivatives of the celomic epithelium, peritoneum and endocervix. This test uses monoclonal antibodies OC 125 and M11 that can recognize an OC 125 antigenic determinant. The investigation was performed automatically on Vidas analyzer, Biomerieux, France.

Sampling procedure. The patients were investigated clinically and imaging, the processes were certified by ultrasonographic, laparoscopic, computed tomographic and magnetic resonance imaging examinations, the data of these investigations were selected from the patients' observation charts. Surgical treatment was performed in all patients with extragenital endometriosis included in the study.

Macroscopic method. The postoperative material was fixed in 10% formalin solution and sent to the morphopathology department for examination.

Histologic method. Histological staining with hematoxylin - eosin was performed for each individual case, and suitable sections were selected for Masson Tricrom staining (modified after GOLDNER). Immunohistochemical tests were performed for differential diagnosis.

Immunohistochemical methods allowed the detection and visualization of antigens in tissues or cells. The actual immunohistochemical study was performed by an enzymatic method with chromogenic detection using the MACH 4 MICRO-POLYMER-HRP kit (Biocare Medical; M4U534), which is a uni- versal detection system based on HRP-polymer enzyme complexes that can detect both anti-mouse and anti-rabbit antibodies. Visualization of immunohistochemical reactions was performed with DAB chromogen (3,3'-diami- nobenzidine, Dako- 3467) and counterstaining with Harris hematoxylin.

As a first step, we were interested in confirming the endometrial origin of the lesions by detecting the immunoreactivity for ER and PR in glandular structures and for CD10 in the stroma between these glands in the extragenital endometriosis lesions.

In order to estimate the prevalence and intensity of PR and ER expression we used the Allred score.

Dual immunohistochemical reactions were used to evaluate the epithelial-mesenchymal transition process and the expression of E-cadherin and Vimentin markers. The result of these reactions consisted in visualization of the desired targets as brown for E-cadherin and red for Vimentin.

The microscopic images were obtained using the *Leica ECC50W* microscope and the *Leica application Suite* program. For the proposed study, the sections were photographed in the most informative foci containing the main components characteristic of endometriosis with 100×, 200× objectives, and for quantification 4 images were taken with 200× objective for each case. The endometrial glands, stromal cells and cells of the inflammatory system were counted for each individual image and then averaged for density and/or cell density of the case.

2.3. Methods for statistical processing of results

The primary survey data were entered into a database and processed using the functions and modules of the SPSS program version 16.0 for Windows (SPSS Inc., Belmont, CA, USA, 2008) and Microsoft Office Excel 2019 on the personal computer, applying descriptive and inferential statistical procedures. Pearson's χ^2, χ^2 with Yates' correction or Fisher's exact method were used to compare discrete variables; t-test or non-parametric statistical tests to determine the statistical difference in mean values between groups; correlation analysis to assess the degree of strength and direction of statistical relationships. Statistically significant were considered to be differences with a two-sided $p \leq 0.05$.

3. SOCIO-DEMOGRAPHIC, HISTOLOGIC FEATURES AND IMMUNOHISTOCHEMICAL PROFILE OF INVASIVENESS OF EXTRAGENITAL ENDOMETRIOSIS

3.1. Clinico-epidemiologic evaluation of patients with extragenital endometriosis

Socio-demographic data. The study included 43 patients with extragenital endometriosis aged between 19 and 56 years.

Table 1. **Caseload distribution by age groups**

Age groups	19-20	21-30	31-40	41-50	51-60	Total
No. of cases	1	8	13	14	7	43
%	2,33%	18,60%	30,23%	32,56%	16,28%	100%

The caseload distribution was done from the 2nd to the 5th decade, the earliest case of endometriosis being diagnosed in a 19-year-old patient, while the oldest patient was 56 years old. The highest number of cases was recorded in patients aged 41-50 years, followed by 31-40 years which included one less case, accounting for more than half of the cases (63.79%). 2nd and 5th decades with 35% and 1st decade with 2.33 of the cases examined (Table 1).

3.2. Clinical-topographic study of patients with endometriosis

In relation to the anatomic localization of extragenital endometriosis lesions, the results of our study have been summarized in Figure1. The most common localizations of extragenital endometriosis were the scar after cesarean section - 9 (20.9%; 95% IÎ: 10.9-34.7) cases, appendix - 6 (14.0%; 95% IÎ: 6.0-26.5) cases, inguinal hernia - 4 (9.3%; 95% CI: 3.2-20.6) cases, cecum - 3 (7.0%; 95% IÎ: 2.0-17.5) cases, diaphragm - 3 (7.0%; 95% IÎ: 2.0-17.5) cases, jejunum - 3 (7.0%; 95% IÎ: 2.0-17.5) cases, ombilicul - 3 (7.0%; 95% IÎ: 2.0-17.5) cases, rectum - 3 (7.0%; 95% IÎ: 2.0-17.5) cases, bladder - 2 (4.7%; 95% IÎ: 1.0-14.1) cases. The other localizations (ascending colon, descending colon, sigmoid colon, transverse colon, ileum, anterior abdominal wall and retro-peritoneal space) were found in 1 (2.3%; 95% CI: 0.3-10.4) patient each (Figure 1).

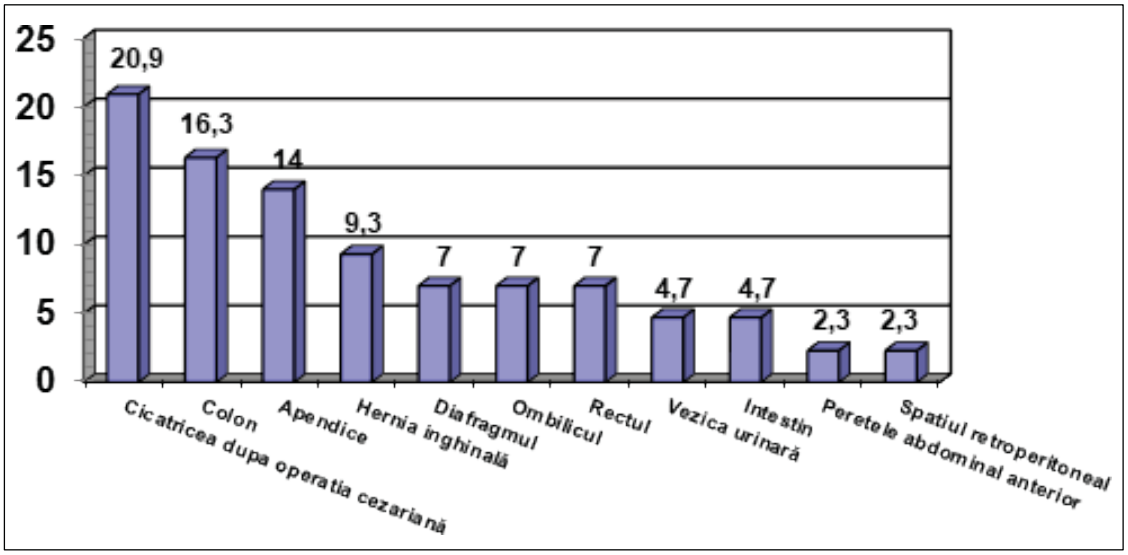

Figure 1. **Distribution of patients with extragenital endometriosis by localization of the disease (%)**

According to the data presented in the figure above, endometriosis of the gastrointestinal tract was diagnosed in 20 cases, which represented 46.5% of the cases investigated. Endometriosis in the cicatrices after cesarean section was detected in 9 cases, the most common in patients in the study group examined. The rarest localization was in the anterior abdominal wall and the retroperitoneal space, one case each, which constituted 2.3% of the total number of cases investigated.

Laboratory examination included evaluation of serum tumor antigen CA-125, the average value in the patients in our study was 48.37±32.9 U/mL (from 3.0 U/mL to 123.6 U/mL).

Macroscopic characteristic of the material. The disease usually presented as nodules or implants of a dense-elastic consistency, whitish-silvery in color (Figure 2 A). Small, brownish foci, ranging in size from microscopic to 1-2 cm in diameter, were found on section, localized on or just below the serous membranes.

Endometrial implants in the form of red, brown or black spots that are described as *„powder burn”*, but red, white or non-pigmented lesions were also found. Adipose and fibrous connective tissue with old hemorrhages were visualized on section (Figure 2 B).

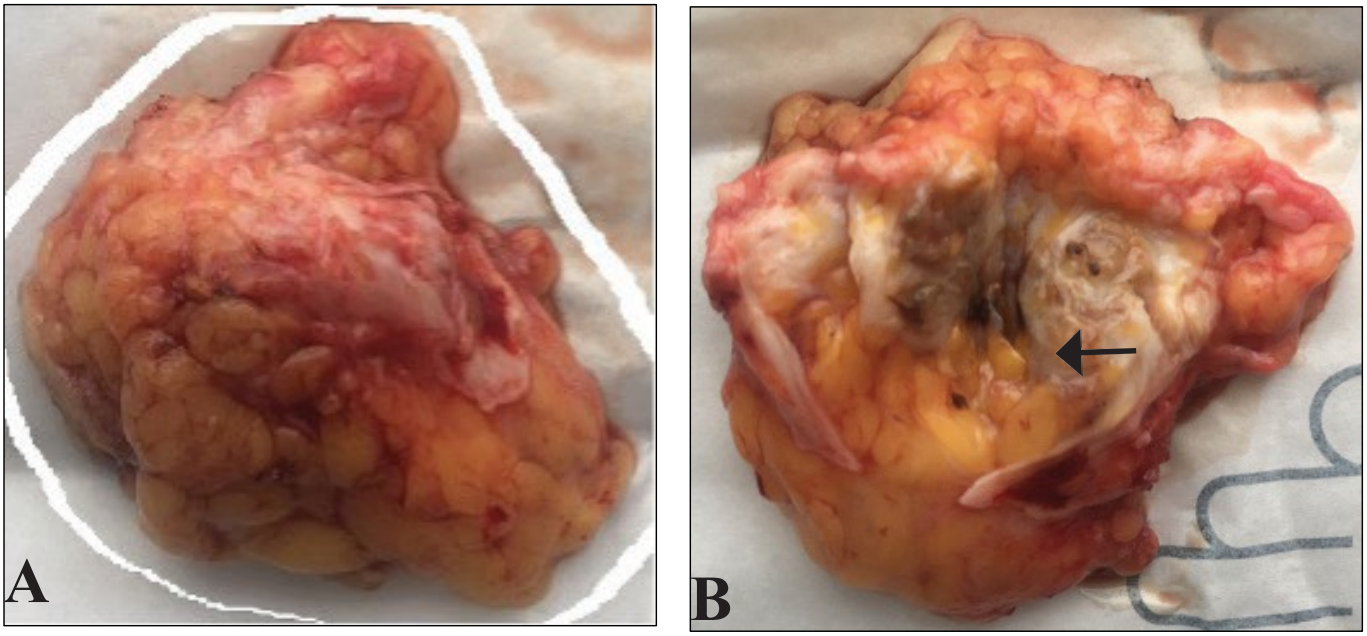

Figure 2. **A - Nodular formation consisting of adipose tissue and fibro-connective tissue. B - Postoperative image on section with „powder burn” lesion. Figures A, B published previously [17].**

Histopathologic features of extragenital endometriosis lesions

The diagnosis of endometriosis is dictated by histopathologic examination. By classic hematoxylin and eosin staining, glandular structures and adjacent stroma with ectopic localization externally are identified. The epithelial component was represented by an epithelium varying in structure and shape from simple columnar to columnar stratified. The stromal component was represented by young cells: fibroblasts, lymphocytes and macrophages, involved in triggering perileional inflammatory processes. Endometriotic implants consisted microscopically of endometrial glands and stroma, with or without hemosiderin-loaded macrophages.

From a histopathologic point of view, the analysis of lesions of extragenital endometriosis focused on several specific features of the affected tissues. Of primary interest was the identification of endometrial-type glands and stroma, which are essential components in confirming the diagnosis of endometriosis. Their presence indicates the persistence of ectopic endometrial tissue, which may undergo cyclical changes similar to normal uterine endometrium.

Another element analyzed was chronic inflammation, which may be present either as a diffuse inflammatory infiltrate or associated with granulomatous reactions including Langhans-type giant cells. This inflammatory component reflects the body's immune response to the abnormal presence of endometrial tissue in unusual locations.

We also aimed to detect bleeding in endometriotic lesions, which occurs as a result of vascular fragility and cyclic bleeding processes. Bleeding can contribute to inflammation, pain and fibrotic adhesion formation.

In addition, fibrosis was an important aspect of the assessment, representing an advanced stage of the pathological process in which repeated inflammatory and hemorrhagic episodes lead to the accumulation of fibrous connective tissue. This may be responsible for stiffening of the affected structures, contributing to symptoms such as chronic pain and dysfunction of the involved organs.

The histopathologic and histochemical study of extragenital endometriosis included 43 cases, revealing various morphologic features and associated tissue reactions. Endometrial glands were identified in 32 cases (74.4%; 95% IÎ: 60.1-85.6), while stromal presence was confirmed in all 43 cases (100.0%).

The chronic inflammatory process was noted in 42 cases (97.7%; 95% IÎ: 89.6-99.7), indicating a persistent immune response in the affected tissues. Langhans-type giant cells were also observed in 13 cases (30.2%; 95% IÎ: 18.1-44.9), suggesting a possible granulomatous mechanism associated with ectopic endometrial lesions.

Another frequently encountered feature was the presence of bleeding, detected in 38 cases (88.4%; 95% IÎ: 76.4-95.4), reflecting the vascular fragility and hemorrhagic activity of endometriotic lesions. These findings contribute to our understanding of the pathogenetic mechanisms of extragenital endometriosis and may have implications for diagnosis and treatment (Figure 3).

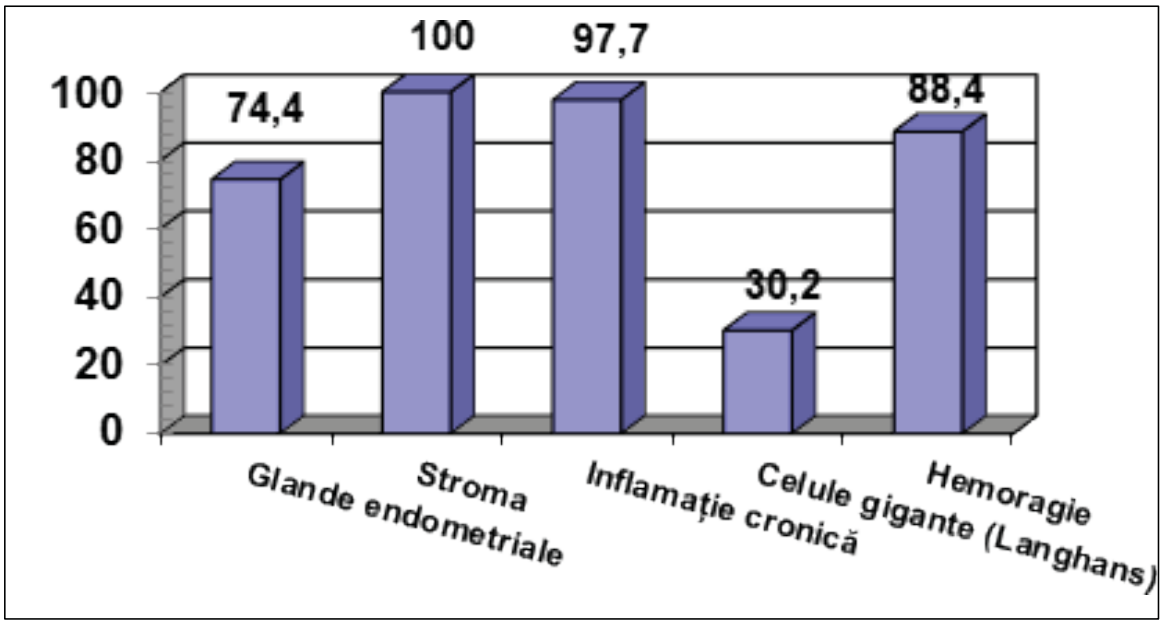

Figure 3. **Distribution of patients with extragenital endometriosis according to histopathologic and histochemical findings (%).**

Endometriosis of the gastrointestinal tract, particularly in the colon, is a form of extragenital endo-metriosis that can have a significant impact on the functioning of the digestive tract. This form of endometriosis is often found in areas such as the tunica muscularis and submucosa of the colon, and the lesions are frequently multifocal, i.e. present as multiple foci scattered in different segments of the colon (Figure 4).

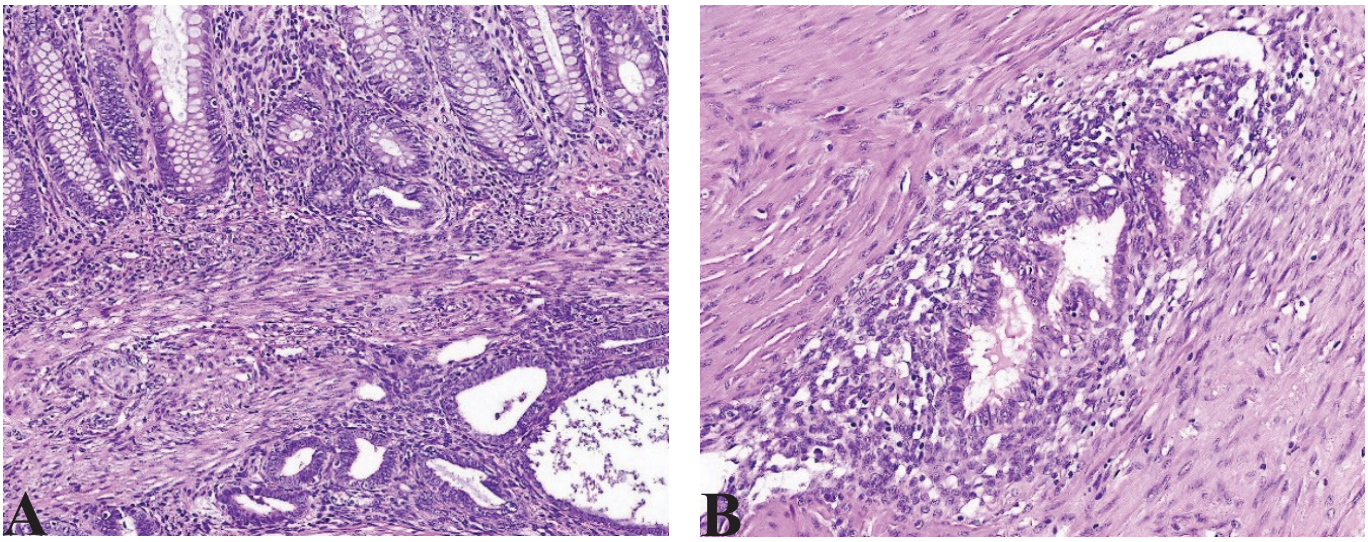

Figure 4. **Endometriosis of the colon and rectum.** A - Endometriosis in the submucosa. B - Focus of intramural endometriosis, intramuscular mixed form with predominantly glandular appearance. H-E staining, images A, B × 200

Histopathologically, lesions of colonic endometriosis were characterized by the presence of dilated endometrial glands within the foci. These glands were similar to normal glands in the uterus, albeit in an ectopic environment. Morphologically, the glands were dilated due to the accumulation of secretions, with a characteristic appearance in the form of chocolate cysts when they contained old blood from cyclic menstrual bleeding.

Endometrial glands and an abundant stroma, similar to that of the uterine endometrium, are present in the submucosa. The stroma is often rich in fibroblastic cells and endometrial stromal cells, which can contribute to fibrosis processes and adhesion formation, leading to colonic wall stenosis.

Another key aspect of endometriosis lesions in the colon is the presence of bleeding both within the foci and in the periphery of the colon. The bleeding is due to the vascular fragility of the endometriotic lesions, which are susceptible to bleeding with each menstrual cycle. These hemorrhages lead to the formation of hemorrhagic cysts and to the deposition of hemosiderin, which is visible in the images above. This process of repeated bleeding is one of the factors contributing to the chronic inflammation seen around endometriosis.

The chronic inflammation in our cases was present in all cases of endometriosis of the digestive tract and is characterized by a predominantly lymphocytic and plasmacytic cellular infiltrate, as well as the presence of macrophages that may contain hemosiderin. These inflammatory reactions are a manifestation of the body's immune response to the presence of ectopic endometrial tissue and contribute to the chronic pelvic pain associated with endometriosis. Also, in colonic endometriosis, chronic inflammation can lead to fibrosis around the foci, which can cause strictures (narrowing of the intestinal lumen), impacting on intestinal motility.

Endometriosis of the abdominal wall after caesarean section (Figure 5) was present in 9 cases, characterized by dilated endometrial glands containing secretions. The glands are irregularly distributed within the scar. Stroma was abundantly present in all cases, composed of endometrial stromal cells and fibroblasts, accompanying the endometrial glands. It find abundant and fibrous, with the appearance of collagen and elastic fibers, characteristic of tissue repair processes. The lesions of scar endometriosis were associated with a chronic inflammatory infiltrate, predominantly lymphocytic, plasmacytic and macrophage, which are visible at the periphery of the endometriotic foci. In 8 cases endometriosis foci showed hemosid-

erin deposits in the inflammatory cells. Around the endometriosis lesions, significant fibrosis resulting from chronic inflammation was observed. This fibrous tissue surrounds the endometriotic foci and later leads to the formation of adhesions and structural changes of scar tissue.

Endometriosis of the bladder is characterized by the presence of abundant endometrial glands and stroma, similar to those in the endometrium at the level in the bladder wall. These lesions were localized in the muscular layer in both cases and were accompanied by cyclic hemorrhages, which form hemorrhagic cysts or blood collections. Chronic inflammation is an essential feature, with infiltrates of lymphocytes, plasma cells and macrophages containing hemosiderin. In some 3 cases, Langhans-type giant cells were present in the inflammatory response (Figure 6).

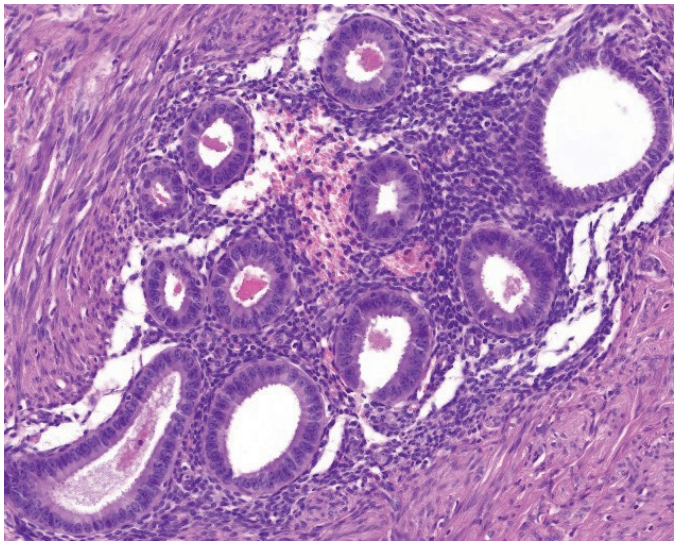

Figure 5. **Endometriosis of the abdominal wall after caesarean section.** Intramuscular endometriotic endometriotic focus with reactive changes, hemosiderin deposits in the stroma. H-E staining, image ×200

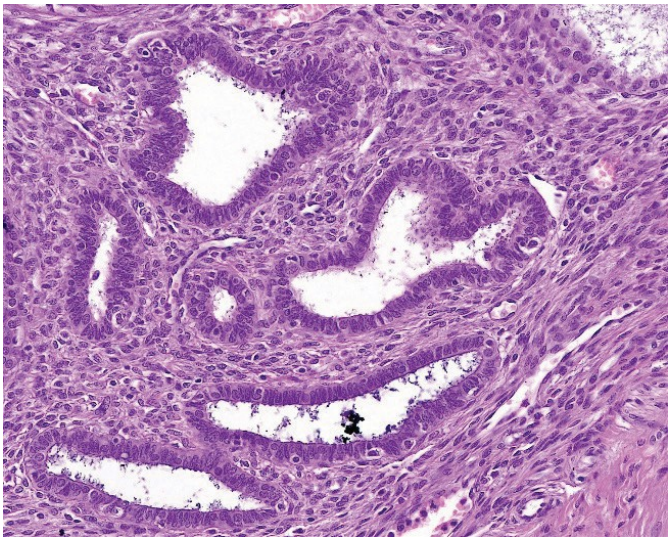

Figure 6. **Bladder endometriosis.** Focus of cystic glandular endometriosis in the tunica muscularis with development of a discrete stromal component. H-E staining, image ×200

The histologic picture of inguinal hernial sac endometriosis (Figure 7) included dilated endometrial glands present within the hernial sac in all cases of endoemetriosis. The glands are dilated and contain specific endometrial mucus-like secretions. Along with the endometrial glands, an abundant stroma, consisting of endometrial stromal cells and fibroblasts, is present. Endometriosis foci in inguinal hernia were accompanied by chronic inflammatory infiltrates, predominantly consisting of lymphocytes, plasma cells and macrophages being present in 3 cases. Presence of hemosiderin within endometriotic foci was present in all cases. Fibrosis around endometriosis foci was also present in 2 of the cases.

The histologic examination of endometriosis in post-cesarean scar cases shows the presence of structures characteristic of ectopic endometrial tissue, arranged in an intense fibrous background. There are endometrial glands lined by simple columnar epithelium, sometimes pseudostratified, which vary in size and shape and may show secretory changes depending on the phase of the menstrual cycle. The glands are surrounded by a densely cellularized, endometrial-like stroma composed of spindle-shaped cells with oval nuclei and pale cytoplasm, with rich vascularization, which gives a sharp contrast enhancement to the adjacent fibrous tissue.

In cases of extragenital endometriosis, the main histologic feature identified was the presence of endometrial stroma, which was observed in all cases examined. Endometrial stroma is an essential com-

ponent of endometriotic lesions and plays an important role in the survival and development of ectopic endometrial foci.

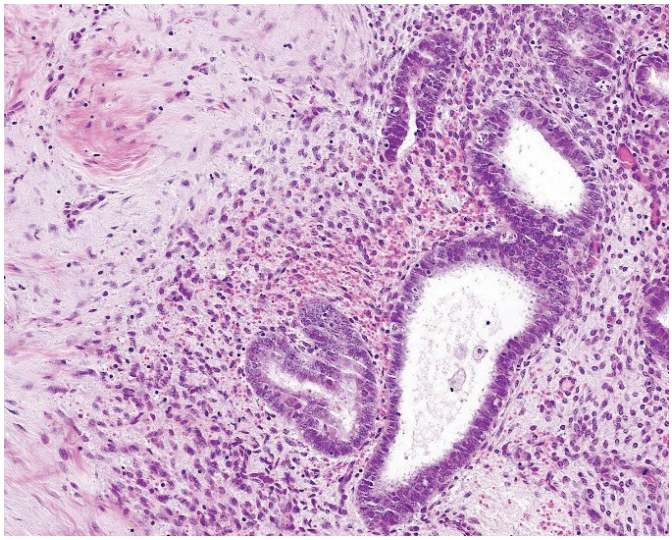

Figure 7. **Endometriosis of the inguinal hernia sac.** Cystic endometriosis with perifocal hemosiderotic deposits in the conjunctiva component of the hernia sac. H-E staining, image ×200

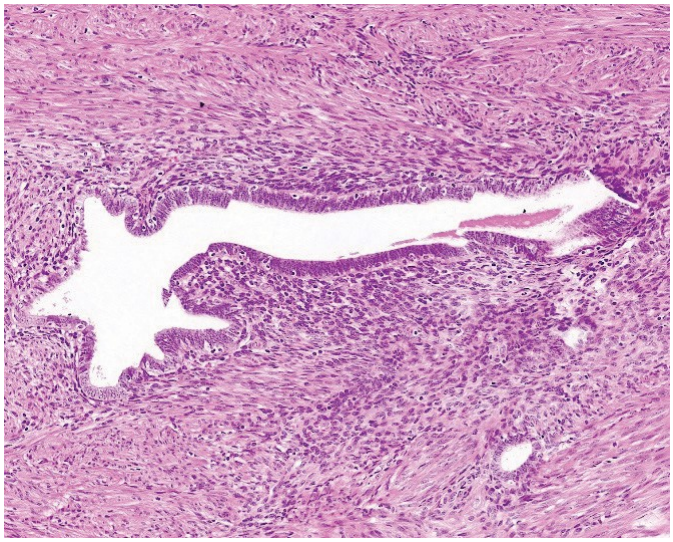

Figure 8. **Endometriosis of the abdominal wall scar after cesarean section.** Endometriotic focus with dilated cystic glands. H-E staining, image ×200

Another frequently encountered feature was chronic inflammation, characterized by infiltrates of lymphocytes, plasma cells and macrophages, reflecting an active immune response to ectopic tissue.

Hemosiderin deposits were also present in a significant number of cases, representing a histologic indicator of recurrent bleeding from endometriotic foci.

Endometrial glands were observed in the majority of cases, constituting a fundamental element for the histologic diagnosis of endometriosis. These glands showed a variable appearance, sometimes dilated and filled with secretions, surrounded by typical endometrial stroma.

The rarest histologic component identified in the cases analyzed was the presence of Langhans-type polynucleated giant Langhans cells, observed only in some lesions. These cells are characteristic of a granulomatous inflammatory process and may occur in the context of a sustained immune reaction, but their presence is not a defining feature of endometriosis.

Endometrial stroma, chronic inflammation and hemosiderin deposits are common and constant features of extragenital endometriosis, while endometrial glands remain an essential criterion for diagnosis. The presence of Langhans giant cells was a rare phenomenon, but may indicate a more complex inflammatory process in some cases.

Foci of endometriosis with different localization The Trichrome Masson staining modified by GOLD- NER was useful to highlight the relationship between the stroma, the glandular component and the fibrosis that develops in these lesions. Foci of endometriosis with a high degree of fibrosis suggest a long duration of disease which may form adhesions between organs and tissues.

The endometrial glands were well visualized by contrast with the stroma and showed that endometriosis involves a proliferation of endometrial tissues. The stroma was in contrast to the surrounding tissues due to the fibrous structures constituting it.

Therefore, histopathologic diagnosis is mandatory and informative, but sometimes needs to be confirmed by immunohistochemical tests.

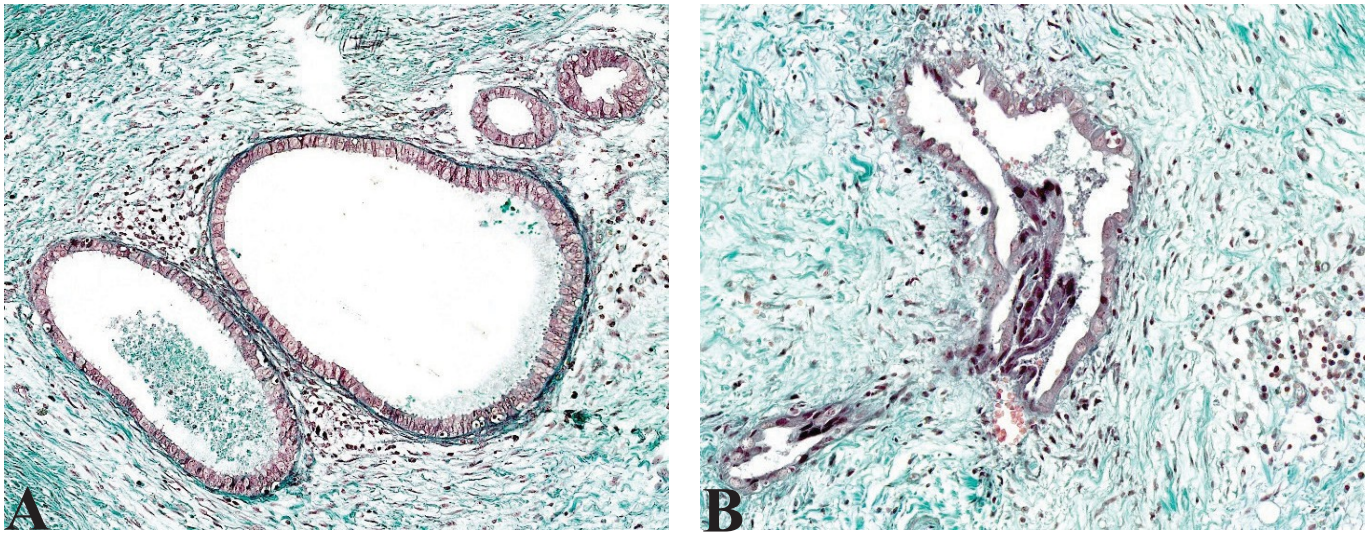

Figure 9. **Trichrome Masson staining in GOLDNER modification - intensification of collagen component in endometriotic stroma.** A - endometriosis glandular form with reduced periglandular connective component. B - ratty endometriotic focus with marked fibrillar conjunctival abundance and reduced endometrial stromal activity. Images A, B ×200

3.4. Immunohistochemical evaluation of endometriosis of different localization

It is now well known that histopathologic evaluation is no longer considered the only diagnostic method for extragenital endometriosis. The initial evaluation, based on morphologic criteria, must necessarily be complemented by immunohistochemical profile analysis.

The immunohistochemical study of invasiveness in extragenital endometriosis was carried out in three major directions, following:

- Determination of definite cases of extragenital endometriosis by applying ER, PR and CD10 markers;
- Investigation of the intrinsic potential for local invasiveness of endometrial cells and endometriosis component stroma by assessment of MMP-1, MMP-2, MMP-9, MMP-14 and SDF-1/CXCR4 markers;
- Investigation of the degree of involvement of mesenchymal epithelial-mesenchymal transition (EMT) factors in the epithelial-mesenchymal transition (EMT) process by immunoexpression of E-cadherin/Vimentin, N-cadherin, Twist

This immunohistochemical investigation was carried out in correlation with the main variables of morpho-clinical interest of the investigated patients, namely age, lesional topography.

Immunohistochemical expression of markers for estrogen (ER), progesterone (PR) and CD10. Markers for ER and PR showed intense nuclear staining in epithelial cells of the endometrial glands and stroma in extragenital endometriosis foci with various localization, as well as in cases with normal endometrium. In cases of endometriosis of the digestive tract, hormone receptors were present in 39 cases of glandular epithelial cells as well as in the stroma in approximately 80-90% of cases. In cases of retroperitoneal, diaphragmatic and umbilical endometriosis there was a poor response with an Allred score of 3. ER receptor expression correlated r=0.05 directly proportional to PR receptor expression in glandular and stromal epithelial cells that showed an Allred score of 8 in both endometriosis foci and normal endometrium.

Immunohistochemical confirmation of endometriosis lesions was performed by applying a panel of specific markers, including estrogen hormone receptor (ER), progesterone hormone receptor (PR) and

the stromal marker CD10. The results showed a significant expression of all three markers, both in terms of the proportion of positive cells and the intensity of the immunohistochemical reaction.

For the estrogen receptor (ER), the mean proportional score was 3.88 ± 1.2, indicating a moderate to high proportion of positive cells, while the intensity score was 2.65 ± 0.6, reflecting moderate to intense staining. The mean total score for ER was 6.53 ± 1.6, indicating a high overall expression of this marker. The results were similar for the progesterone receptor (PR), where the proportional score had a mean value of 3.95 ± 1.1 and the intensity score was 2.63 ± 0.6. The mean total score for PR was 6.58 ± 1.3, suggesting a sustained expression of the receptor in the cells examined.

For the endometrial stromal-specific marker CD10, the mean proportional score was 3.88 ± 1.1 and the mean staining intensity was 2.56 ± 0.6. The mean total score of 6.44 ± 1.5 indicates a clearly positive expression in the stroma of the lesions, confirming the endometrial origin of the connective tissue present (Figure 10).

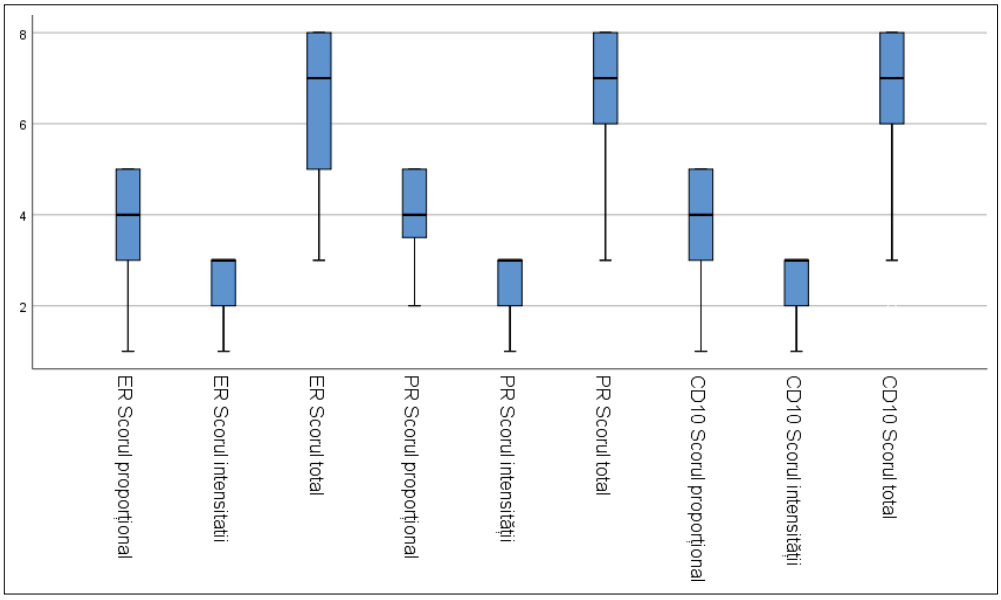

Figure 10. **Mean values of ER antibody, PR and stromal marker CD10 in patients with extragenital endometriosis. All these markers showed a total reaction score of 6.50**

All three markers - ER, PR and CD10 - were positively expressed, with high mean scores, both in proportion of positive cells and intensity. This confirms the endometrial character of the lesions in the post-cesarean scar, which supports the diagnosis of extragenital endometriosis.

These immunohistochemical results strongly support the diagnosis of scar endometriosis by demonstrating expression of endometrial-specific hormone receptors and the stromal marker CD10, all with high scores reflecting a typical immunohistochemical profile for endometriosis lesions.

Endometrial cells exhibited intense nuclear staining in endometrial and stromal cells from extragenital endometriosis foci with diverse localization (Figure 11). Correlation analysis between ER expression at the sites with extragenital endometriosis as well as normal endometrium along with clinico-pathologic features revealed no statistically significant differences (p=0.003).

The PR marker showed intense nuclear staining both in endometrial cells and in cases with normal endometrium. Positive PR expression and clinicopathologic features did not show statistically significant differences in relation to endometriosis localization. Endometriosis of the anterior abdominal wall, the Allred PR score was 5 in more than one third of cases (36.36%), and in 88.88% of cases of endometriosis with other localizations such as digestive tract and bladder, a high score was found (8). Statistical analysis

between ER receptor-positive endometrial cells and the proportion of PR receptor-positive endometrial cells showed no statistically significant differences (total score - 6.58±1.3). PR receptors showed the lowest immunoreactivity in diaphragm endometriosis.

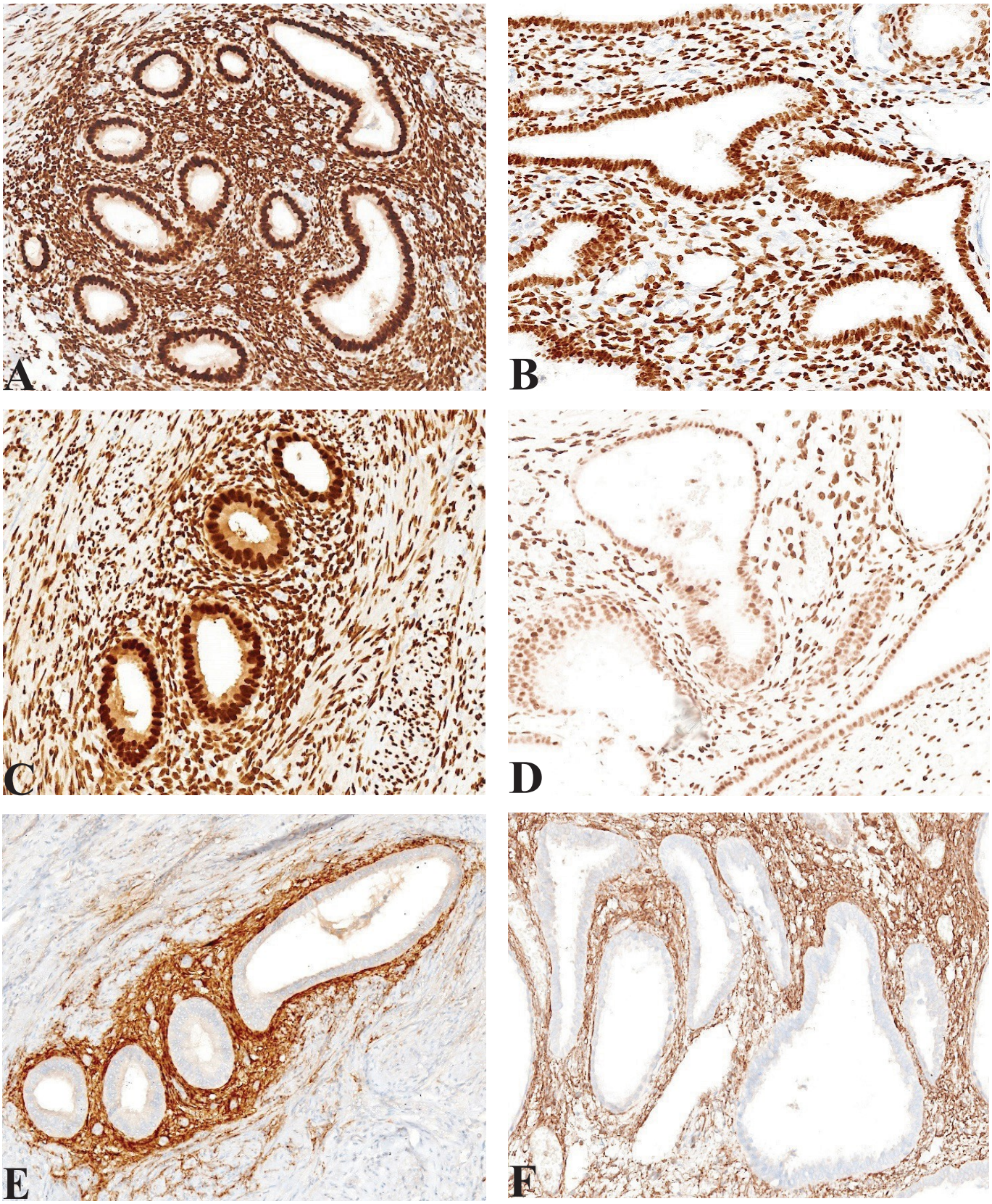

Figure 11. **Expression of ER, PR and CD10 markers. Endometriosis in inguinal hernia, images A, C, E. Endometriosis of the bladder, images B, D, F.** A, B - nuclear reaction intensely positive (score 3+) for ER marker in endometrial glands. C, D - PR marker nuclear reactivity (score 3+) in endometrial glands and stroma. E, F - immunoexpression of the stromal marker CD10, intensely expressed (score 3+) in the stroma of the endometriosis focus. IHC-DAB staining ×200

The immunoexpression of ER and PR markers in endometriosis showed a clear immunolabeling (3+) in a large number of 40 cases, being positive at nuclear level in both endometrial glands and stroma.

In our study, the ER score in extragenital endometriosis was high in most cases (78.94%), with strong nuclear expression in endometrial cells, supporting the role of hormone mediation in the pathogenesis of endometriosis. The high estrogen receptor (ER) expression in endometriosis and its large variations of 3.88±1.2 suggest a complexity in how these receptors are expressed in different disease contexts. One of the possible causes of this variability could be the involvement of estrogen receptor isoforms, which could influence how the ER behaves in endometriosis-affected cells.

Reporting high levels of ER, in parallel with low ER expression, could indicate a compensation or adaptation of the body to physiologic changes in the eutopic endometrium (the healthy tissue lining the uterus), especially in the context of hormonal changes associated with endometriosis. These changes could be a mechanism by which endometrial cells in ectopic foci respond to the abnormal hormonal conditions that characterize this condition.

The epithelial expression of receptors for PR in endometriosis, which was not significantly lower than that in tumor stroma ($p=0.807$), suggests a relatively uniform component of the progesterone response in these two regions, despite the pathological features of the disease. This could be interpreted as a sign of reduced sensitivity or decreased responsiveness to progesterone stimulation in both epithelial tissue and tumor stroma.

In the normal endometrium and in endometriosis we found reactivity for CD 10, which is present cytoplasmically in stromal cells.

CD10 marker immunoexpression was intense in endometriotic stroma in the digestive tract and bladder, which showed a total IHC score of 8 compared to that in the inguinal, retroperitoneal hernia where a mean score of 4 was recorded. It should however be noted that the patients were in menopause.

The CD10 marker revealed endometrial stromal cells that remained undetected on sections.

hematoxylin-eosin sections in 3 of the cases. Staining was cytoplasmic, strong and diffuse. The reactive cells formed thin sleeves around the glands or were diffusely arranged among macrophages and inflammatory cells. The marker in question showed no reactivity in endometrial glands or other tissues. In 2 cases (1 diaphragm, 1 retroperitoneal) no CD10 reactive cells were present.

Immunohistochemical assessment of the invasiveness potential involved in extracellular matrix patterning. Metalloproteinases represent a huge subclass of proteases, very similar structurally but varied in cellular and tissue localization.

In this study we analyzed immunohistochemically 43 cases with a clinical diagnosis of extragenital endometriosis and 6 cases of normal endometrium from patients undergoing surgery. For all cases included in the study we studied the expression of:

- collagenases: MMP-1
- gelatinases: MMP-2, MMP-9
- membrane: MMP-14.

The immunohistochemical expression of MMP-1, MMP-2, MMP-9 and MMP-14 was different. The markers MMP-2, MMP-9, MMP-14 showed variable expression not only from case to case but also within the same case. Importantly, glandular cells in the center of the ectopic foci showed an increased

intensity response compared to a weak expression in the peripheral focus of endometriosis.

The reactivity for MMP-1 in extragenital endometriosis foci was present in the stroma, the reaction pattern being predominantly membranous.

Immunoreactivity for MMP-1 was identified in 21 of the cases with endometriosis (48.83%), the mean IS score recorded was 0.58±0.5 and in only 1 of the cases with normal endometrium. Staining for MMP-1 was present in the stroma with predominantly low intensity (Figure 12). Analysis of MMP-1 intensity in relation to composite histopathologic composite score level showed significant differences (p=0.003), with increased staining intensity being associated with low (ST) IHC.

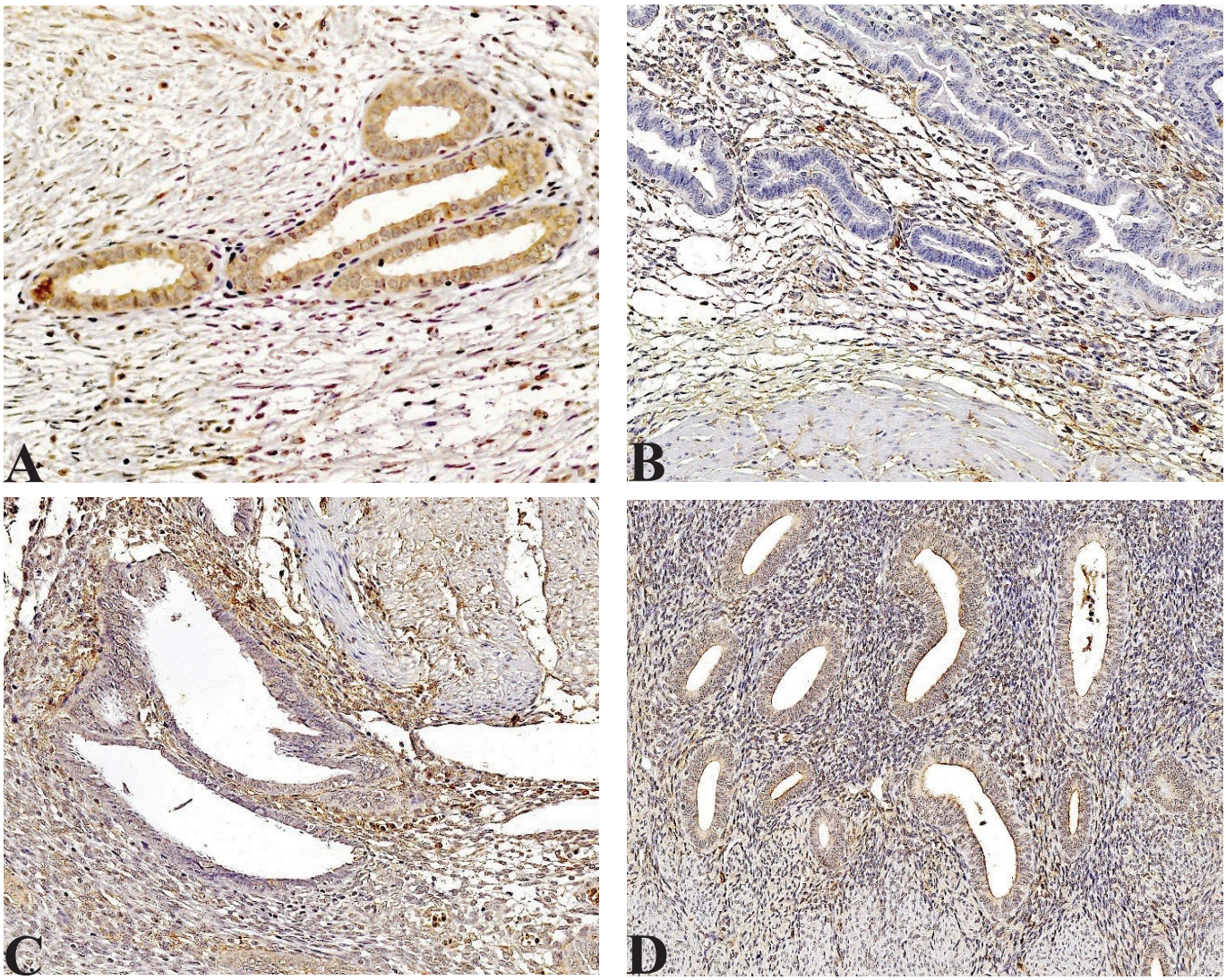

Figure 12. **MMP-1 marker expression in extragenital endometriosis.** A - scar endometriosis after cesarean section. Negative reaction (score 0) in the stroma. B - endometriosis in the colon. Weak positive reaction (score 1+) in the stroma. C - endometriosis of the intestine. Low positive reaction (score 1+) in the stroma. D - normal endometrium. Low positive reaction (score 1+) in the stroma. Col. IHC-DAB, ×200

In endometriosis foci, reactivity for MMP-2 was present in both stroma and glands, with a predominantly membranous reaction pattern. Cytoplasmic reactivity for MMP-2 was also observed in blood vessel endothelial cells, striated muscle fibers, stromal fibroblasts and macrophages (Figure 13).

Immunoreactivity for MMP-2 was identified in 31 of the cases with endometriosis (72.09%), the mean IS score recorded was 0.97±0.8 and in all cases of normal endometrium. Staining for MMP-2 was determined in glands and stroma with predominantly moderate intensity.

Analysis of MMP-2 intensity in relation to composite histopathologic composite score level showed significant differences (p=0.002), with increased marker intensity being associated with low (ST) IHC.

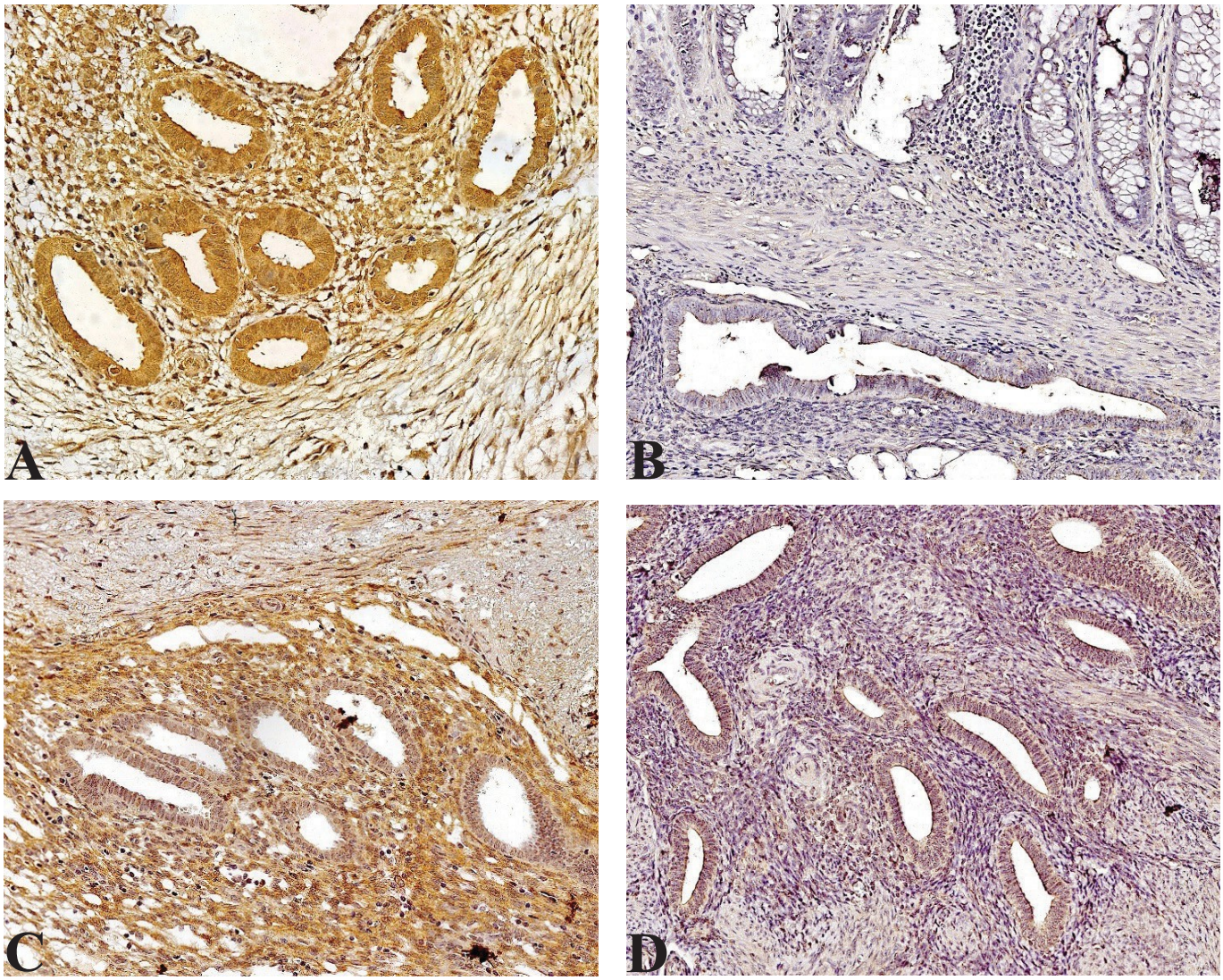

Figure 13. **MMP-2 marker expression in extragenital endometriosis.** A - abdominal wall after cesarean section. Positive reaction in the endometrial stroma (score 2+). B - endometriosis in the colon. Negative reaction (score 0). C - endometriosis of the bowel. Intensely positive reaction (score 3+) in endometrial stroma. D - normal endometrium. Weak positive reaction (score 1+) for MMP-2 at the endometrial stromal level. IHC-DAB staining, ×200

In endometriotic foci, reactivity for MMP9 was present in the stroma and glands, the pattern of reaction was membranous and cytoplasmic (Figure 14). The latter, cytoplasmic pattern was more evident in the endometrial granules. Moreover, reactivity for MMP-9 was additionally evidenced also in blood vessel endothelial cells, fibroblasts and inflammatory cells. Immunoreactivity for MMP-9 was identified in 37 cases of endometriosis (92.5%) and in all control cases, with a mean IS score of 2.21±0.7 out of the total cases examined.

Immunoreactivity for MMP-14 was identified in 33 cases of endometriosis (82.5%) and absent in 2 cases of unaffected endometrium. MMP-14 staining was present only in endometrial glands with predominantly moderate intensity. Analysis of MMP-14 intensity in relation to total IHC score level showed significant differences (p=0.005), with low or moderate intensity of staining being associated with low (ST) IHC.

The expression of MMP-2 and MMP-14 was significant (2+) in glandular cells from endometriotic

lesions, whereas MMP-9 was evident (2+) in both stromal and glandular cells. MMP-1 expression was absent in abdominal wall endometriosis and normal tissue. Unaffected endometrium showed high reactivity for MMP-14 and MMP-9 and low affinity for MMP-2.

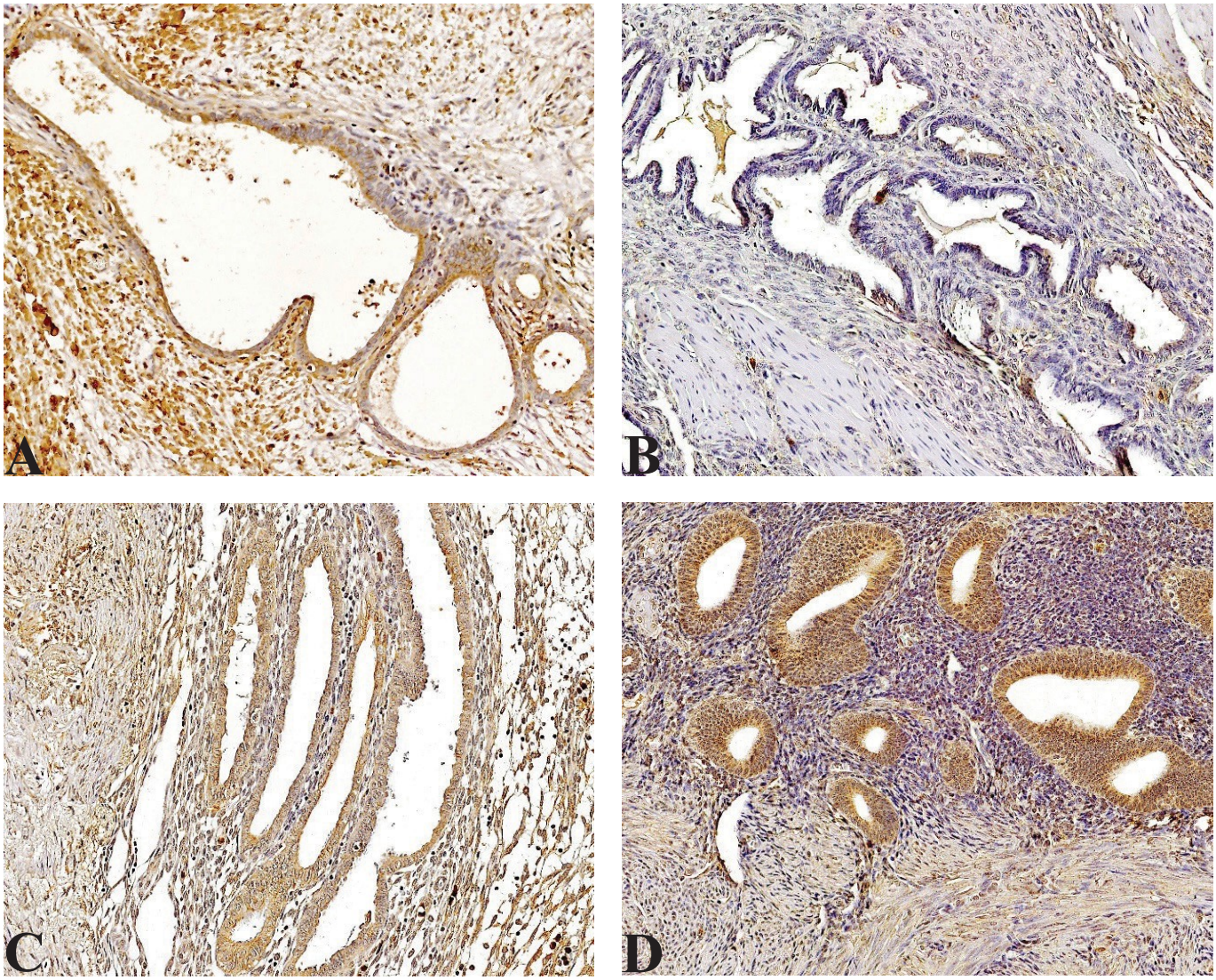

Figure 14. **MMP-9 marker expression in extragenital endometriosis.** A - abdominal wall endometriosis after cesarean section. Moderate intensity reaction (score 2+) in the stroma. B - endometriosis of the colon. Negative reaction (score 0) in the stroma and glands. C - endometriosis of the intestine. Moderate reaction (score 2+) in the stroma and endo- metrial glands. D - normal endometrium. Moderate reaction (score 2+) in the stroma and endometrial glands. IHC-DAB staining, ×200

MMP-2, MMP-9 and MMP-14 reactivity in endometriotic lesions was higher than in eutopic endometrium. Immunohistochemical staining for MMP-2 and MMP-9 was performed in endometriosis, assessing the degree of fibrosis and lymphocytic infiltrate. Nuclear staining of MMP-2 in glands, membranous and cytoplasmic staining of MMP-9 in stroma were considered positive.

The staining of the intestinal foci was diffuse and the intensity was strong (3+). In the abdominal wall the intensity of endometriosis was moderate (2+), while the staining of eutopic endometriosis was diffuse and the intensity was weak to moderate (1-2+) in all cases. MMP-14 was positive and the intensity was moderate in both cases. The expression of MMP-1 on the examined tissues was absent in endometriosis and eutopic tissue cases.

Immunohistochemical analysis demonstrated a significant increase in MMP-9 and MMP-14 expression in endometriosis and endometrium. The expression of MMP-9 and MMP-14 was more intense at the interface of the endometriotic lesion in the peritoneum.

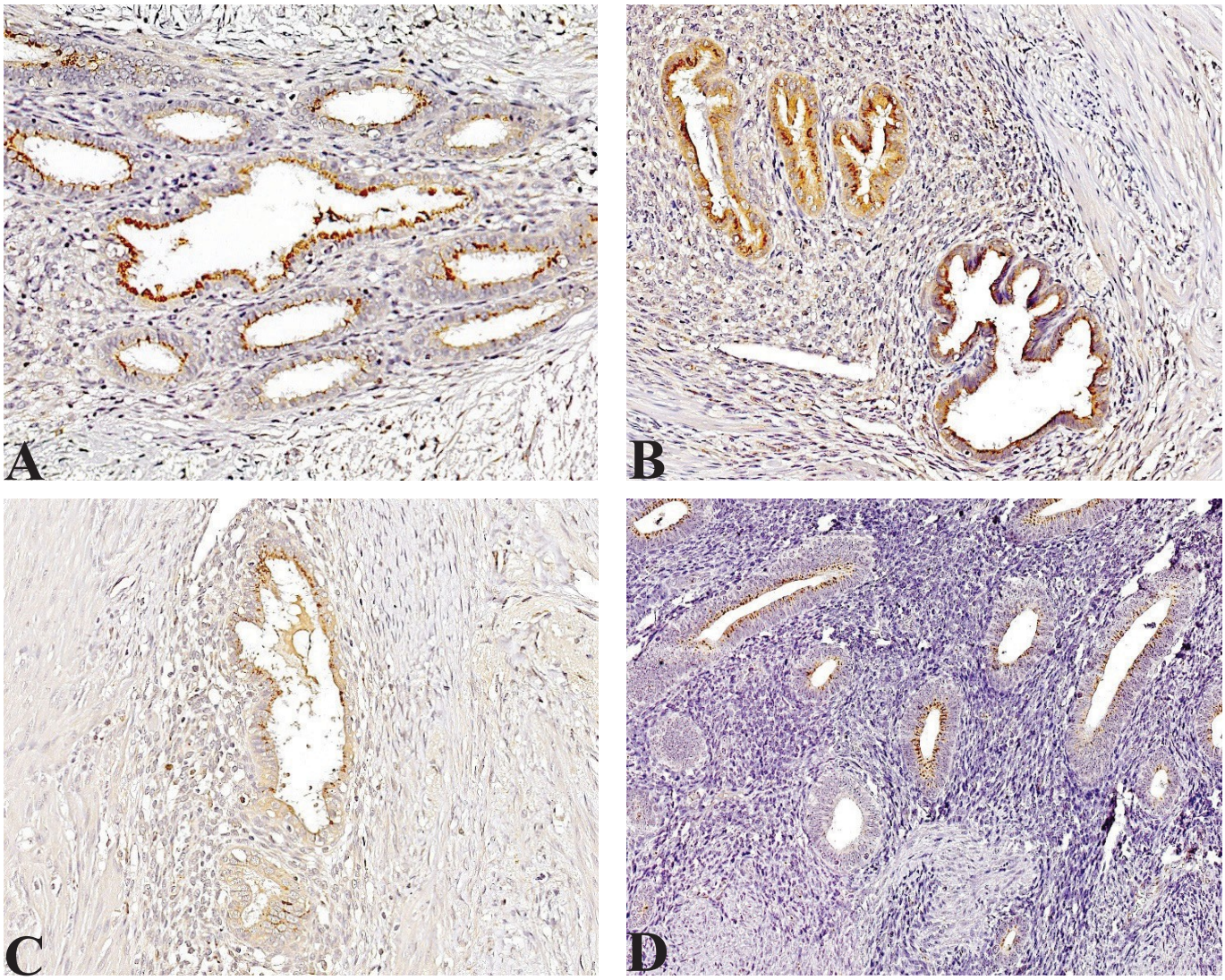

Figure 15. **MMP-14 marker expression in extragenital endometriosis.** A - abdominal wall after cesarean section. Weak positive reaction (score 1+) in the endometrial glands. B - endometriosis in the colon. Moderate reaction (score 2+) in the endometrial glands. C - endometriosis of the bowel. Weak positive reaction (score 1+) in the endometrial glands. D - normal endometrium. Weak positive reaction (score 1+) in the endometrial glands. IHC-DAB staining, ×200

Increased MMP-9 and MMP-14 activity was also observed in the stroma of the ectopic endometrium demarcated from tissue adjacent to the endometriotic lesion. Increased MMP-9 activity in the stroma of the ectopic endometrium demarcated from the underlying stroma was accompanied by the formation of macrophage-lymphocyte infiltrates.

Thus, the obtained research results suggest that increased MMP-9 activity in the endometriotic lesion at the border of the underlying stroma promotes invasiveness of the ectopic endometrium by remodeling the underlying stroma and infiltration of endometrial cells into the peritoneum. Increased MMP-2 activity was also observed at the border of the underlying stroma of the ectopic endometrium. Enhanced MMP-9 expression in the normal endometrium was found to predominate in the outer membranes of endometrial cells, whereas MMP-14 expression was determined in the inner. Thus, our results indicate increased MMP-9 and MMP-14 activity at sites of endometriotic lesion.

Analysis of MMP-1 expression revealed low values, with a mean proportional score of 0.58 ± 0.5 and an intensity score of 0.70 ± 0.7, resulting in a mean total score of only 0.98 ± 0.9. Median values were generally low (Md = 1.0), consistent with low interquartile ranges (IIQ: 0-2.0), suggesting a poor expression of this enzyme in the lesions studied.

Similarly, MMP-2 expression was low to moderate, with a mean proportional score of 0.85 ± 0.7, intensity score of 0.97 ± 0.8 and mean total score of 1.42 ± 1.4. Although some cases achieved higher scores (maximum total score of 4), median values and interquartile ranges (IIQ: 0-3.0) indicate high variability and limited expression in most cases.

In contrast to MMP-1 and MMP-2, MMP-9 expression was significantly increased. The mean proportional score was 3.63 ± 0.8, with a mean staining intensity of 2.21 ± 0.7, resulting in a mean total score of 5.84 ± 1.2. The high median values (Md = 6.0) and interquartile range (IIQ: 5.0-7.0) suggest a constant and strong expression of this metalloproteinase, which may reflect an active role of MMP-9 in tissue invasion processes associated with extragenital endometriosis

MMP-14 also showed comparable expression levels to MMP-9. The mean proportional score was 3.49 ± 0.8, the mean intensity score was 2.19 ± 0.8, and the mean total score reached 5.67 ± 1.2. The median total scores were 6.0, and the IIQ (5.0-7.0) indicated a robust and constant expression in the lesions analyzed. These data support the involvement of MMP-14 in the mechanisms of invasion and tissue remodeling of endometriosis outside the genital system (Figure 16).

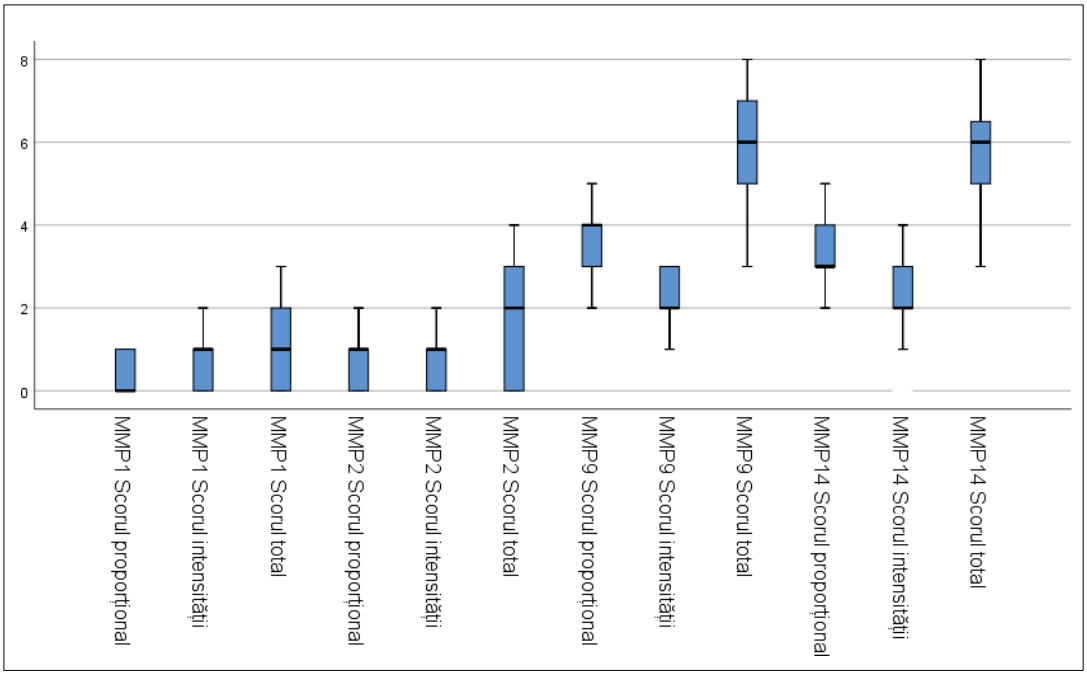

Figure 16. **Mean expression values of markers involved in extracellular matrix remodeling in patients with extragenital endometriosis**

The high expression of MMP-9 and MMP-14, in contrast to low levels of MMP-1 and MMP-2, outlines a selective invasive profile specific to extragenital endometriosis lesions, with preferential activation of certain metalloproteinases. This immunohistochemical pattern could have predictive value in assessing the biological behavior of endometriotic lesions.

Immunoreactivity for MMP-1 was weakly positive (1+) in all cases of normal endometrium. We found that increased intensity of the reaction for MMP-1 was present only in the stromal elements. Analysis of MMP-1 intensity in relation to the total immunohistochemical score showed significant differences (p=0.006). However we found an association of increased intensity of the markers with low total IHC score.

The expression of MMP-2, MMP-9, MMP-14 was identified in all cases investigated in both endometriosis and normal endometrium. Staining for MMP-2 had predominantly low intensity in stromal cells at the cytoplasmic level in endometriotic lesions and in eutopic endometrium. We found increased

intensity of the reaction for MMP-9 at the cytoplasmic stromal level in endometriosis, whereas it was absent in the normal endometrium. The intensity of MMP-14 immunoexpression was increased cytoplasmically at the stromal cytoplasmic level in endometriosis and moderate in normal endometrium. MMP-1 expression was absent in both extragenital endometriosis specimens and weakly positive (1+) in normal endometrium.

It is important to mention **the role of the SDF-1/CXCR-4 axis**, given its clear involvement in the invasion and metastasis processes of many human malignancies.

In endometriosis foci as well as in normal endometrium, reactivity for CXCR-4 was absent in glands and low in inflammatory cells in the endometriosis stroma. In cases of endometriosis of the colon, rectum, inguinal hernia, anterior abdominal wall, low reactivity was found in inflammatory infiltrates. In the endometriosis foci, in the stroma we recorded reactivity for CXCR-4 in all these investigated cases which constitutes (27.90%), but the IS intensity score was low (1+), the mean IS score was 1 (Figure 17).

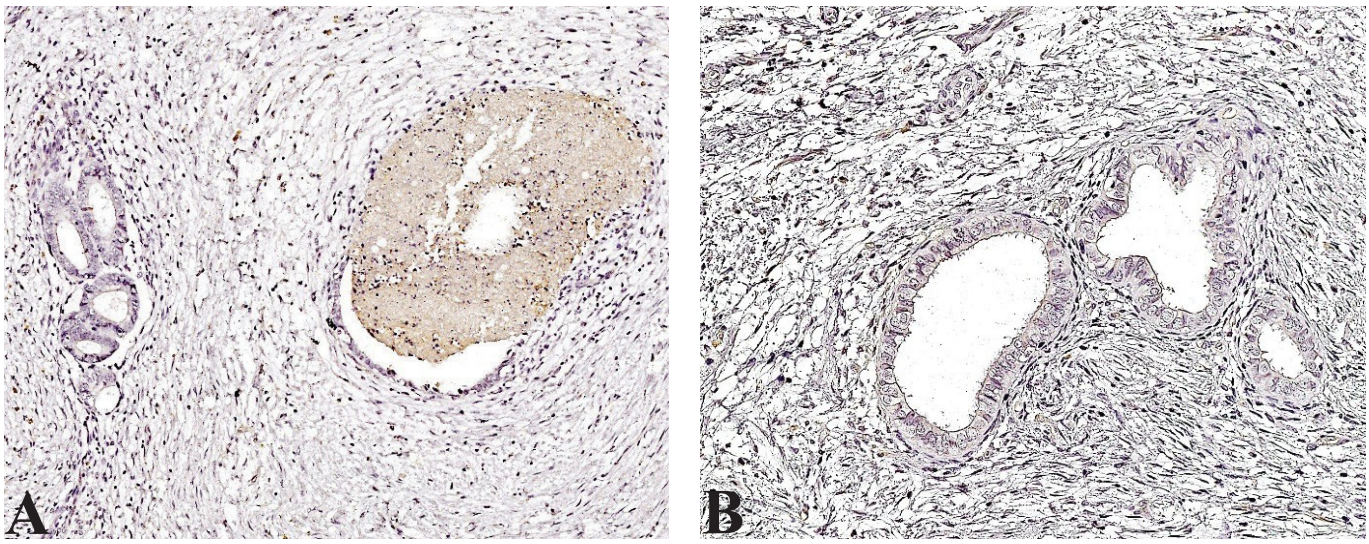

Figure 17. **CXCR-4 receptor expression in extragenital endometriosis and normal endometrium.** A - scar after cesarean section. B - colonic endometriosis. Negative expression (score 0). IHC-DAB staining, ×200

The reactivity for CXCR-4 was more pronounced in cases with chronic inflammation, being present at this level, while in the other cases of endometriosis, the reaction was absent.

Reactivity for SDF-1 was present in stromal cells from endometriosis lesions with cytoplasmic and less frequently membranous immunostaining pattern. In normal endometrium the reaction was absent. In 40 cases we recorded a rectivity for SDF-1 which constitutes (93.02%), but the IS intensity scores ranged from 1 to 3 and the mean IS score was 1.37±0.6. The expression of SDF-1 marker in endometriosis foci were significantly higher than in normal endometrial tissues (p=0.005) and the differences were statistically significant (p=0.021) (Figure 18).

Immunohistochemical evaluation of the expression of the SDF-1/CXCR-4 chemokine axis, known for its role in cell migration, angiogenesis and metastatic potential of cells in various pathologies, including endometriosis (Figure 19).

The SDF-1 marker showed low to moderate expression. The mean proportional score was 1.56 ± 0.9, with a median (Md) value of 1.0 and an interquartile range (IIQ) between 1.0 and 2.0. The mean immunohistochemical reaction intensity was 1.37 ± 0.6, indicating weak to moderate staining in most cases. The mean total score for SDF-1 was 2.93 ± 1.0, with a median of 3.0 and IIQ of 2.0-4.0.

These data suggest a relatively modest presence of SDF-1 in the lesions investigated, with variations between cases, but without indicating an intense and generalized activation of this chemotactic factor.

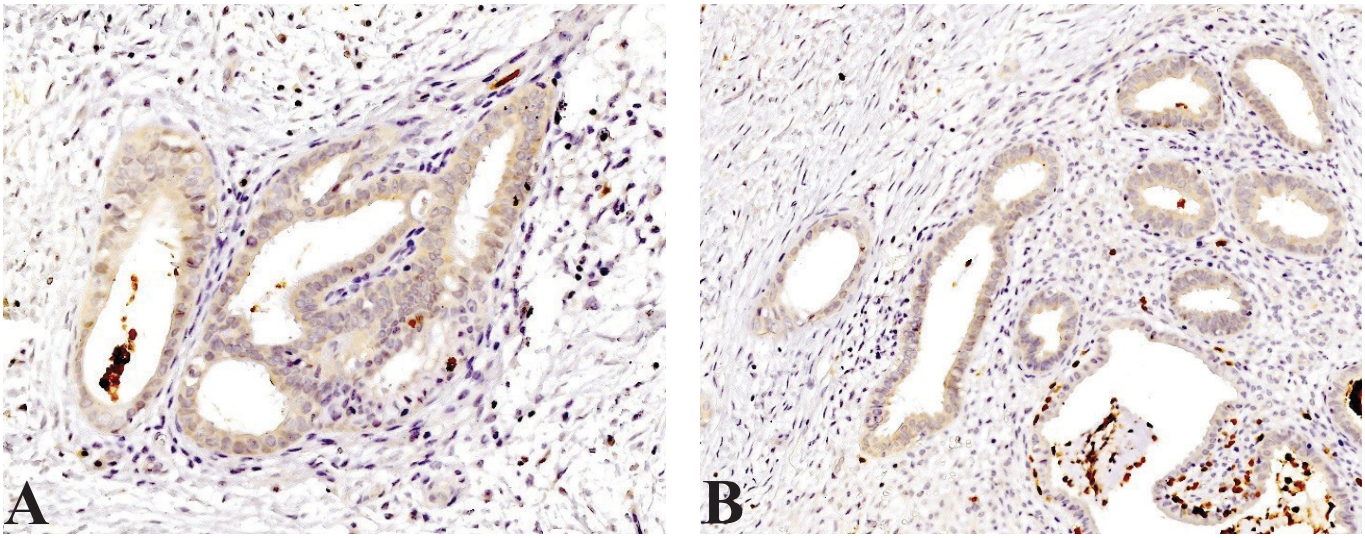

Figure 18. **Stromal cell-derived factor SDF-1 expression in extragenital endometriosis and normal endometrium.** A - post-caesarean scar endometriosis. Low intensity reaction (score 1+) in the stroma. IHC-DAB staining, ×200

In contrast, the expression of its specific receptor, CXCR-4, was more pronounced, which may indicate an increased sensitivity of endometriotic cells to signaling through this axis. The mean proportional mean score for CXCR-4 was 2.58 ± 1.1, with a median of 2.0 and IIQ between 2.0-3.0, suggesting that a sem- nificant proportion of cells express this receptor. The mean staining intensity was 1.95 ± 0.8, and the mean total score was 4.56 ± 1.6, with a median of 4.0 and IIQ between 3.0-6.0. These results indicate a medium to high activation of the CXCR-4 receptor in extragenital endometriosis lesions, which may reflect an increased potential for cell migration and possible mechanisms similar to those involved in tumor invasion.

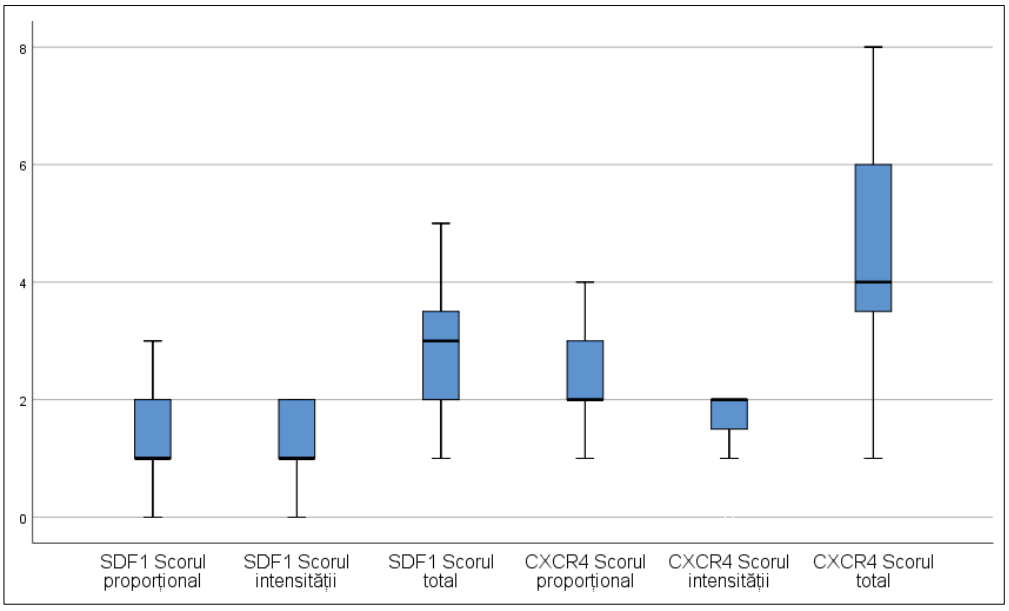

Figure 19. **Mean values of SDF-1 and CXCR-4 expression scores in patients with extragenital endometriosis**

Thus, on the one hand, we can confirm the mechanism of implantation in ectopic sites of endometrial cells for high levels of SDF-1 expression in these tissues, and on the other hand, we can support the low invasive potential of endometriosis lesions in the retroperitoneal region and bladder.

Both markers were more intensely expressed in extragenital endometriosis lesions showing a chronic

inflammatory response. The factor SDF-1 and its receptor CXCR-4 have a major role in the pathogenesis of endometriosis with a moderate potential in the process of local invasiveness by promoting the proliferation of endometrial stromal cells.

Immunohistochemical evaluation of specific markers of epithelial-mesenchymal transition (E-cadherin/Vimentin panel, N-cadherin, TWIST). In endometriosis foci, the reactivity for E-cadherin/Vimentin was heterogeneous, with both cytoplasmic and membrane patterns. Overall, the reactivity (IS= 6.44±0.9) was somewhat lower. Differences in reactivity were also observed according to the localization of the process. Endometriosis of the anterior abdominal wall showed maximum reactivity (IS=9±1.6), the pattern of reactions being both membranous and cytoplasmic. E-cadherin showed a membranous staining in epithelial cells, while Vimentin showed a cytoplasmic staining.

The intensity of staining was moderate in 58.14% constituting 25 cases and strong in 18 cases 41.86%. The percentage of positive cells was ≥50% in the majority of cases 34 (79.06%). In the investigated batch, all cases showed positive immunoexpression for E-cadherin/Vimentin, with score values ranging from 6 to 9.

E-cadherin showed high-intensity membrane staining of glandular epithelial cells in both extragenital endometriosis and normal endometrium.

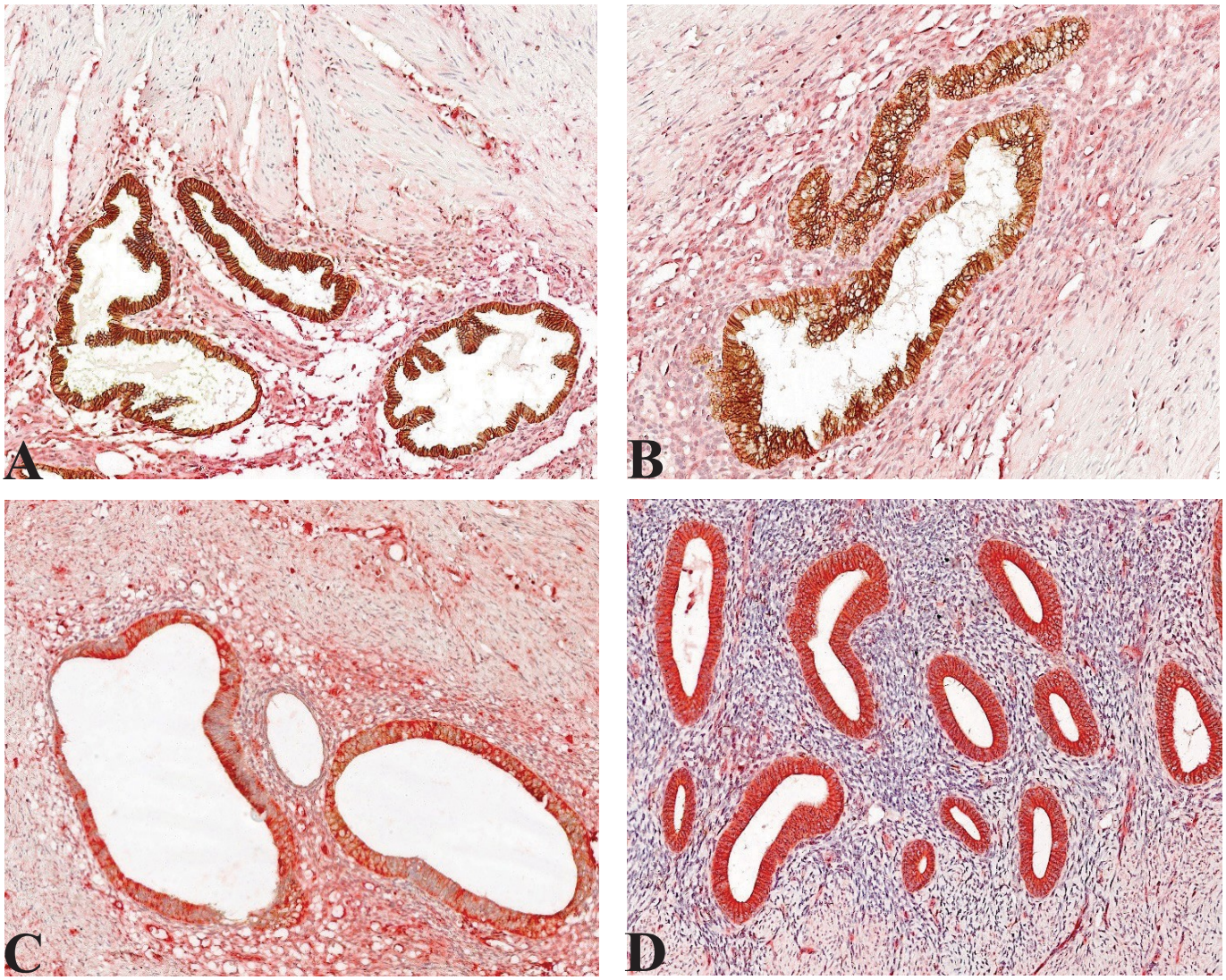

Figure 20. **Immunoexpression of E-cadherin/Vimentin markers in extragenital endometriosis and normal endometrium.** A - in the colon. High intensity reaction (score 3+) in endometrial glands. B - in the rectum. High intensity reaction (score 3+) in the endometrial glands. C - anterior abdominal wall after cesarean section. Moderate reaction (score 2+) in the endometrial glands and stroma. D - normal endometrium. Moderate reaction (score 2+) in the endometrial glands. IHC-DAB staining, images A, B, C, D ×200

In the cases with normal endometrium, all cases had score values between 2 and 6, with E-cadherin/Vimentin expression considered negative. Staining intensity was mild in 30 (69.76%) cases and moderate in 9 (20.93%) cases. The percentage of positive cells was <40% in the majority of cases 25 (58.13%). Six cases showed a very low percentage (<10%) of E-cadherin/ Vimentin positive tumor cells (Figure 20).

Immunohistochemical tests for the identification of glandular epithelium, hormone receptors, tissue proliferation, as well as for the identification of tumor proteins, vascularization and inflammatory cells have been the basis for the diagnosis of endometriosis in different locations.

Intensity for N-cadherin was observed in only 11 (25.0%) of the total cases investigated. Semi-quantitative immunolabeling analysis of endometriosis cases showed the following scores: 0.65±0.5 for the proportional score, 0.73±0.5 for the intensity score and 0.98±0.7 for the total score (Figure 21).

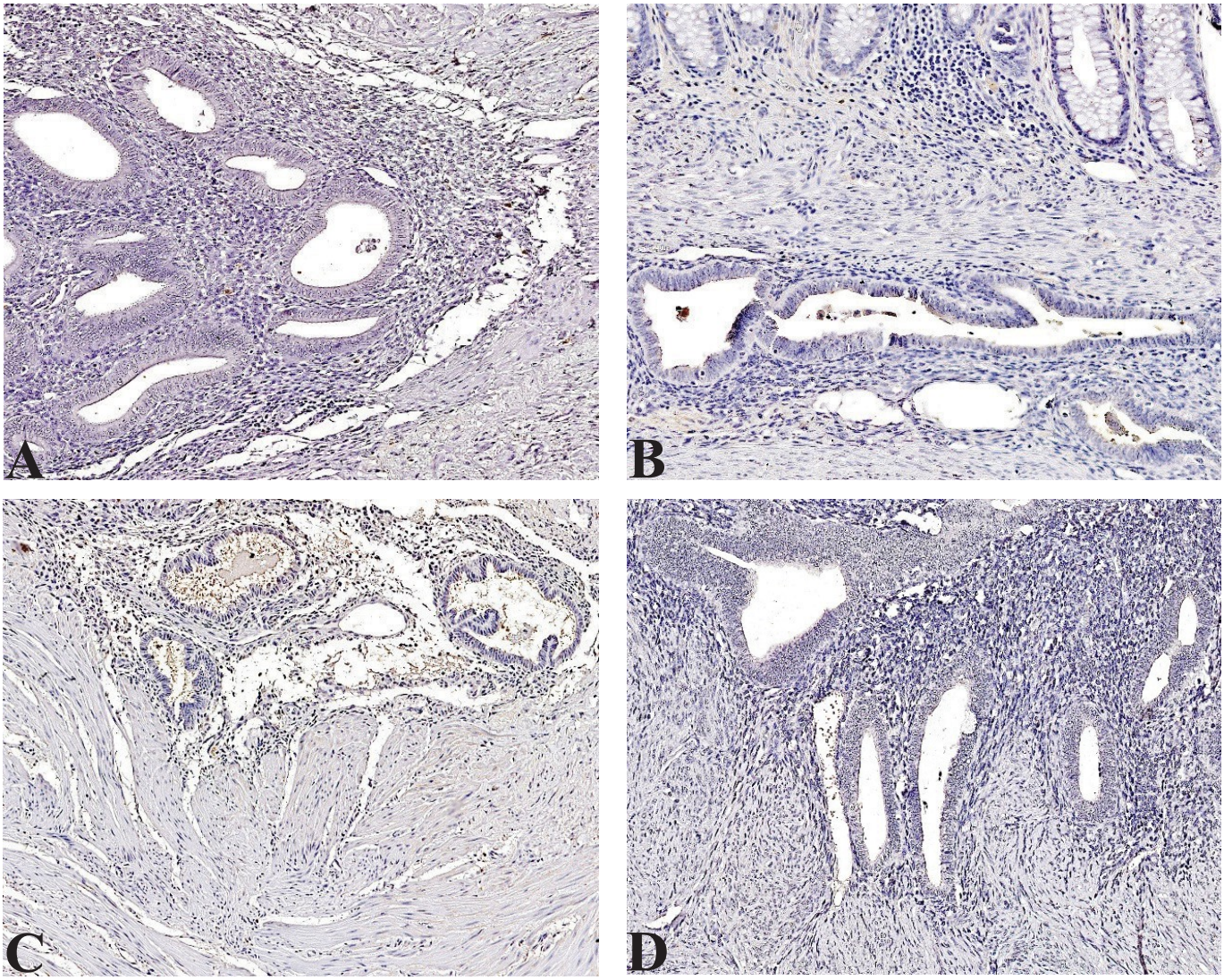

Figure 21. **Immunoexpression of N-cadherin markers in endometriosis and normal endometrium.** A - endometriosis in the colon. B - endometriosis in the rectum. C - endometriosis of the anterior abdominal wall after cesarean section. D - normal endometrium. Negative reactions (score 0) in endometrial glands and stroma. IHC-DAB staining, images A, B, C, D ×200

Of all the transcriptional factors inducing epithelial-mesenchymal transition that we studied, TWIST was the most poorly expressed in endometrial tissue, with a mean immunoreactivity score across all cases investigated of 0.86±0.7. The reactivity was more evident in glands in scar endometriosis after cesarean section.

Regardless of topography, cases with endometriosis of the anterior abdominal wall were the most reactive for TWIST, with mixed subcellular, cytoplasmic and nuclear patterns, with nuclear predominating.

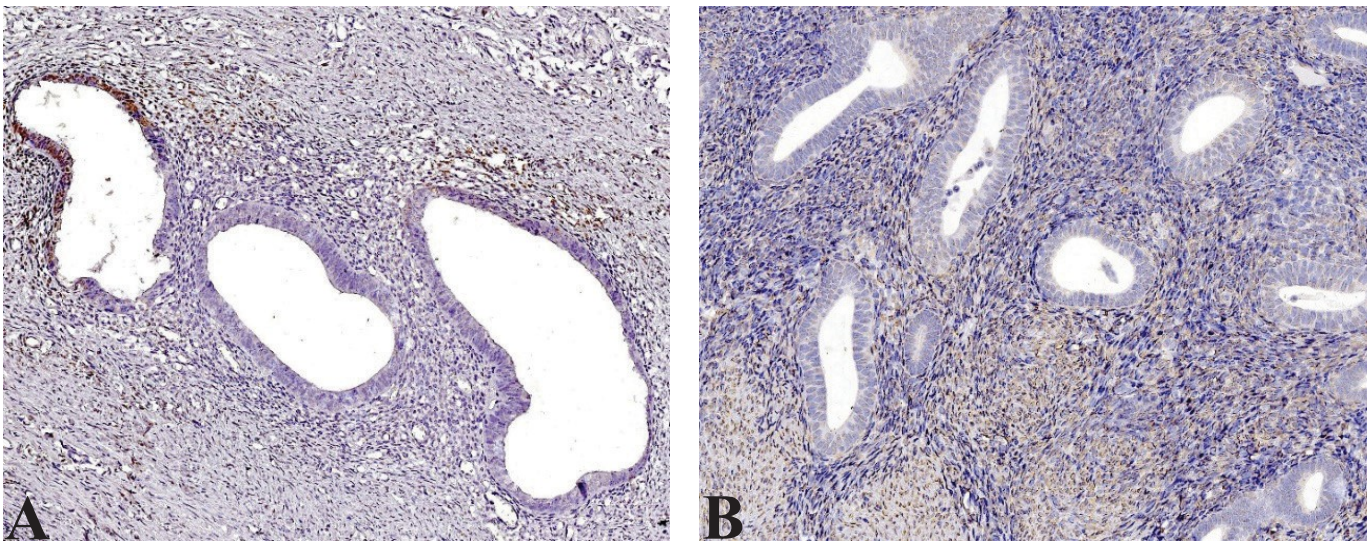

Figure 22. **TWIST marker immunoexpression in endometriosis and normal endometrium.** A - suture granuloma endometriosis. Low intensity reaction (1+). B - normal endometrium. The reaction was negative (score 0). IHC-DAB staining, images A, B ×400

To evaluate the cellular processes associated with epithelial-mesenchymal transition (EMT) in extragenital endometriosis lesions, immunohistochemical analysis of a specific panel of markers, including E-cadherin/Vimentin, N-cadherin and TWIST, known for their role in epithelial character loss and acquisition of an invasive, mesenchymal-like phenotype, was performed.

The combined expression of E-cadherin and Vimentin was high, with a mean proportional score of 3.95

± 0.8 and a median (Md) value of 4.0, in the context of an interquartile range (IIQ) between 3.0 and 5.0, reflecting a high proportion of cells positive for these markers. The staining intensity was moderate to intense (2.49 ± 0.6), and the mean total score reached 6.44 ± 0.9, with the majority of cases falling within the range 6.0-7.0. This sustained expression indicates partial maintenance of epithelial characters (through E-cadherin), but also an activation of the mesenchymal program (through Vimentin), reflecting a partial epithelial-mesenchymal transition profile often seen in potentially invasive endometriotic lesions.

In contrast, the expression of N-cadherin, a specific marker of the mesenchymal phenotype, was significantly lower. The mean proportional score was 0.65 ± 0.5, with a median of 1.0, and the mean intensity was 0.73 ± 0.5. The mean total score of 0.98 ± 0.7 confirms a weak expression of N-cadherin in the majority of cases, suggesting that the complete transition to a mesenchymal phenotype is not fully activated in these lesions.

Similarly, expression of the transcription factor TWIST, a key regulator of EMT, was minimal. The mean proportional score was 0.46 ± 0.4 and the intensity score had a mean value of 0.60 ± 0.5. The mean total score was 0.86 ± 0.7, with most values concentrated in the range 0-1.0. These results indicate a weak activation of the EMT molecular program at the transcriptional level, suggesting that epithelial-mesenchymal transition mechanisms in extragenital endometriosis may be present in an incompletely or partially regulated form.

Taken together, these data outline a partial EMT profile characterized by coexpression of E-cadherin and Vimentin, but in the absence of significant overexpression of the canonical mesenchymal markers N-cadherin and TWIST. This pattern may reflect a limited capacity for invasiveness and migration, specific to extragenitally localized endometriosis.

GENERAL CONCLUSIONS

1. Histopathologic examination of biopsies and postoperative material is considered the most appropriate method for the diagnosis of endometriosis. This pathology is characterized by the presence of endometrial glands and endometrial stroma, accompanied by chronic inflammation, fibrosis, old or recent hemorrhage and hemosiderophages.
2. Immunohistochemically, endometriotic endometriotic foci are characterized by an increased expression of estrogen receptors (ER) in the stromal cells, more intense compared to intact endometrium, which potentiates the extension of the process.
3. Zinc-dependent metalloproteinase (CD10) is highly expressed by stromal cells in ectopic foci, indicating its use as a specific diagnostic marker of endometriosis.
4. Decreased expression of E-cadherin and increased expression of N-cadherin indicate the presence of the epithelial-mesenchymal transition process in the foci of endo-metriosis. Its existence is also supported by overexpression of the TWIST marker in stromal cells of ectopic foci.
5. In endometriosis, regardless of the localization of the process, overexpression of matrix metalloproteinases (MMp2, MMP9 and MMp14) is found, which may facilitate invasion and extension of the process.

PRACTICAL RECOMMENDATIONS

1. For the diagnosis of extragenital endometriosis we recommend immunohistochemical evaluation of the expression of the stromal marker CD10 and hormone receptors (ER, PR) in the ectopic endometrium.
2. To assess the potential invasiveness of the ectopic process we recommend double immunostaining for E-cadherin/Vimentin.
3. As therapeutic targets in the treatment of endometriosis we recommend the evaluation of CXCR-4 receptor and TWIST transcription factor expression.
4. We recommend the use of the following additional diagnostic tests for endometriosis: assessment of serum levels of ER, matrix metalloproteinases and CXCR-4.

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