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Deep endometriosis. Clinical, histopathological and confocal microscopy correlations in intestinal sites

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Abstract

Intestinal endometriosis (IE), a chronic condition affecting a notable percentage of women with endometriosis (estimates varying from roughly 4% to 37%) and can impact any part of the intestine, but it most frequently involves the rectum and sigmoid colon. This is a retrospective study that included 178 women diagnosed with this condition that were investigated taking into consideration the symptoms, diagnostic approaches, surgical treatments, and detailed features of the intestinal wall, including the behavior and quantity of interstitial cells of Cajal (ICC) correlated with the symptomatology experienced. We were able to highlight the most common symptoms such as pelvic pain and bowel disorders. The rectum was identified as the most commonly affected intestinal segment. Transvaginal ultrasound can be valuable in assessing IE, improving preoperative diagnosis and treatment strategies. Laparoscopic surgery remains the definitive diagnostic method, allowing direct visualization and excision of lesions. Surgical technique selections are complex and require careful consideration tailored to each patient. A reduction in ICC numbers may disrupt gut motility, emphasizing their importance in maintaining normal intestinal function, a factor particularly relevant in endometriosis where disruption of ICC networks can contribute to gastrointestinal dysfunction.

Keywords: intestinal endometriosis, ultrasound in endometriosis, interstitial cells of Cajal, confocal microscopy.

Introduction

Endometriosis is a gynecological disease characterized by the presence of endometrial cells outside of the uterine cavity [1]. This chronic condition affects up to 5–10% of all reproductive age women [2]. This abnormal growth of endometrial tissue can occur in various locations within the abdominal cavity and pelvis, including the ovaries and fallopian tubes, parietal peritoneum and surrounding organs such as bladder, rectum, colon, appendix and small bowel [3, 4]. The extent of the disease can vary from minimal lesions on the pelvic peritoneum to deep infiltrating endometriosis (DIE) invading the surrounding organs, such as the bowel, bladder, and ureter [5].

Intestinal endometriosis (IE) is a challenging manifestation of this disorder [5], affecting between 3.8% and 37% of the patients diagnosed with endometriosis [6]. IE can affect all the segments of the intestine, but the rectal and sigmoid lesions are present in up to 90% of the patients [7]. Other sites of IE are the appendix affected by up to 13.2% of the patients [8]. Small bowel affected in 3–18% and the cecum in 2–5% of cases [9].

The pathogenesis is similar to all endometriosis lesion involving multiple factors, including retrograde menstruation, coelomic metaplasia, and lymphatic or hematogenous spread of endometrial cells [5].

IE has a wide range of symptoms, that can be non-specific such as dysmenorrhea and dyspareunia or intestine-related symptoms such as dysphasia, diarrhea, constipation, and rectal bleeding or rarely bowel obstruction, significantly impacting the women's quality of life (QoL) [5, 10, 11]. Studies have shown that obesity can be a risk factor for severe dysmenorrhea, increasing the risk of developing endometriosis, as well as other obstetric problems, including consequences for labor [12, 13].

IE lesions can be found at any level of the small and large intestine. The most common localization described is

rectosigmoid in up to 90% of patients with IE [6, 14, 15], but intestinal DIE can also be found in the other segments of the colon, appendix or small bowel, either isolated as a single lesion or with multiple localizations [16].

The symptomatology encountered in IE is often non-specific and is included in the clinical appearance of pelvic endometriosis, while the symptoms specific to the digestive tract are variable and can be frequently encountered in multiple pathologies involving the digestive tract [7, 17].

The most common symptoms are chronic pelvic pain associated with dysmenorrhea, constipation or diarrhea, abdominal bloating and distension, rectal bleeding, rectal tenesmus, dyschezia and nausea and vomiting [17].

These broad symptoms may be related to a variety of digestive conditions and do not correlate with the location or the size of the lesions [18].

The diagnosis is challenging because of the unspecific symptoms presented in many gastrointestinal (GI) disorders. The most used techniques for diagnosis are transvaginal ultrasonography (TVUS) and pelvic magnetic resonance imaging (MRI) [9]. Other useful investigations are computed tomography (CT) scan colography, water enema CT (WE–CT) and endoscopic ultrasonography (EUS) [16].

The choice of imaging technique depends on the location of the endometriosis and the specific clinical question being addressed.

The ability of this non-invasive technique to accurately detect and localize endometriotic lesions, including those involving the rectosigmoid colon, has been the subject of several studies. One multicenter study, for instance, found that ultrasound (US) mapping of pelvic endometriosis had a high diagnostic accuracy, with the location and number of lesions not significantly affecting the diagnostic rate [19].

Similarly, another study comparing the accuracy of WE–CT and laparoscopy in the detection of rectosigmoid endometriosis reported that WE–CT had a sensitivity of 92.3% and a specificity of 87.5% in identifying these lesions [20].

In contrast to US, MRI imaging has the advantage of providing a comprehensive assessment of the pelvis, including the visualization of DIE and the involvement of surrounding structures. A recent study investigating the diagnostic accuracy of MRI and US in mapping deep pelvic endometriosis found that both modalities had high sensitivity and specificity, with MRI performing slightly better in the assessment of rectosigmoid and bladder involvement [20].

TVUS is well tolerated and widely available, less time consuming and with reduced cost compared to the others diagnostic methods described [21].

US, particularly TVUS, has emerged as a valuable tool in the assessment of pelvic endometriosis, including intestinal involvement. Endometriotic lesions located in the intestines can present with a range of sonographic features, which can aid in the diagnosis and management of this condition [19]. One of the key US findings associated with IE is the presence of hypoechoic or mixed echogenicity lesions within the bowel wall. These lesions may appear as hypoechoic lesions attached to the intestinal wall [17] with irregular shape sometimes surrounded by a hyperechoic rim [22]. There are some specific appearances described such as comet sign, a solid, focal, and tubular lesion with slightly irregular margins and a "tail" [23], sometimes the normal layer of the *muscularis propria* is replaced by a nodule and can cause a retraction and possible adhesions, giving an US image of "Indian headdress" or "moose antler" sign [24].

Furthermore, the use of Doppler imaging can provide additional information, as these endometriotic lesions may demonstrate increased vascularity, a characteristic that can lend further support to the identification of this pathology [19, 25, 26].

In addition to the assessment of the intestinal lesions themselves, the use of US can also aid in the evaluation of associated features, such as the involvement of other pelvic structures. For example, the presence of endometriomas or DIE in other locations, such as the rectovaginal septum or bladder, may suggest a higher likelihood of concomitant intestinal involvement [21].

The accurate interpretation of these US findings, however, requires a thorough understanding of the potential pitfalls and limitations of this imaging modality [21].

While US can be a valuable tool in the diagnosis of IE, it is important to recognize that the sensitivity and specificity of this technique may vary depending on the location and extent of the lesions, as well as the skill and experience of the examiner [27].

The use of US, particularly TVUS, can provide valuable insights into the characteristics of IE, potentially aiding in the preoperative diagnosis and management of this complex condition [19]. A literature review on the accuracy of TVUS in searching for rectosigmoid endometriosis showed an overall and specificity of 91% and 97% [21].

CT enterography assessment can be used to find the lesions affecting the ileal segment and the ileocecal valve by offering a better luminal distention with improved visualization of the intestinal wall layers, highlighting the annular thickening and plaque-like lesions [28, 29]. Compared to CT enterography, MRI provides an improved image of the small bowel wall without radiation. The MRI lesions of the small bowel appear as a loss of the normal T2-hypointense signal of the bowel wall, with nodular and irregular thickening, that can be associated with adherences of an adjacent intestinal segment. Intralesional hemorrhage, although rare, can be observed on MRI as a hyperintense punctate foci on precontrast images without T1 fat, enhancing specificity of the diagnosis [30].

The selection of the appropriate imaging modality for diagnosing IE depends on a variety of factors, including the symptoms, the availability of specialized expertise, and the necessity for a comprehensive assessment of the pelvic anatomy. Both US and MRI have demonstrated utility in the diagnosis of this condition. The integration of US and MRI, combined with a comprehensive clinical evaluation, can significantly enhance preoperative planning and management for patients with suspected or confirmed IE [31].

Accurate preoperative diagnosis of IE is crucial, as it can guide the appropriate treatment approach and potentially avoid complications.

Laparoscopic surgery remains the "gold standard" for the final diagnosis in endometriosis, as it allows for direct visualization and biopsy of the lesions. Additionally, when GI or urinary structures are involved, the presence of symptoms such as dyschezia, dysuria, or hematuria may

provide further evidence of the disease [26]. The treatment of IE involves a multidisciplinary approach, with both medical and surgical interventions. In patient without obstructive symptoms or a pregnancy wish a hormonal therapy, can help to manage the symptoms and potentially slow the progression of the disease. The surgical treatment is reserved for those patients who do not respond to conservative treatment or whose symptoms significantly affect their QoL. The primary goal of surgery is the complete removal of all visible endometriosis lesions [32]. The excision of the affected segments of the intestine is recommended for patients with DIE. However, it is important to carefully weigh the potential benefits and risks of such a procedure, as it can be associated with significant complications, such as anastomotic leak, rectovaginal fistula, and bowel obstruction [3, 26, 33–35].

The choice of surgical technique is a complex decision and should be carefully tailored to each patient based on several factors such as size and location of the nodules, the depth of the lesions into the bowel wall, the symptoms experienced, and the desire for future fertility [16, 32, 36]. Given the potential for extensive surgery and the need to balance complete lesion removal with functional outcomes, a multidisciplinary approach involving both gynecological and colorectal surgeons is often recommended [32].

Surgical management of IE includes techniques like shaving, disc excision, segmental resection, and strictureplasty. Shaving technique involves the superficial removal (or dissection) of endometriotic nodules from the bowel surface [32]. While it's generally considered appropriate for superficial lesions that primarily involve the outer layers of the rectum, without deep infiltration into the *muscularis propria* [27], it can also effectively alleviate pain and GI symptoms, even in cases where DIE is present in the lower rectum [32].

Discoidal resection technique involves removing a disc-shaped section of the bowel wall that contains the endometriotic nodule [37]. It is considered a step up from shaving and is employed when the lesion extends more deeply into the bowel wall. Malzoni *et al.* [37] suggest that discoid resection may be suitable in those with nodules that are smaller or equal with 3 cm in size and deeper than 7 mm.

Segmental resection is typically reserved for cases with large or multiple nodules, when the endometriosis has caused significant bowel stenosis [38], or when conservative methods, such as shaving or disc excision, are not feasible or have failed [39].

Alboni *et al.* [40] propose using strictureplasty for ileal endometriosis to address fibro stenotic lesions, avoiding the removal of large sections of the intestine, which could lead to adverse events. This is particularly relevant in cases with multiple ileal localizations [40].

The involvement of the interstitial cells of Cajal (ICC), a specialized type of cells found into the GI system, has been increasingly recognized in the pathogenesis of IE. ICC are responsible for the initiation and coordination of the movement of the intestinal smooth muscle, and their survival, development, and proliferation are heavily dependent on the activation of the KIT receptor by its unique ligand, membrane-bound stem cell factor [41]. Disruptions in the function or distribution of ICC have been associated with many GI conditions, including irritable bowel syndrome, Crohn's disease, and now, IE. Existing literature suggests that changes in intestinal motility can affect the intestinal microbiome dynamic balance, with alterations in the composition and diversity of the gut flora potentially contributing to the development and progression of IE [41]. Moreover, Rho family of guanosine triphosphatases (GTPases), a family of small signaling proteins, have been shown to have an important role in the cytoskeletal function of various cell types within the gut mucosa, including ICC, and their dysregulation has been linked to chronic intestinal inflammation and the pathogenesis of colorectal cancer. The immune system had also a significant role in the pathogenesis of endometriosis, with natural killer (NK) cells being one of the main immune cells involved. Studies have found a decline in the activity and cytotoxicity of NK cells in the peritoneal fluid in patients with endometriosis, which may influence the survival and proliferation rate of ectopic endometrial cells [42]. The role played by the ICC in the pathogenesis of IE represents an emerging area of research, with potential implications for the development of novel diagnostic and therapeutic approaches.

Aim

The aim of this study was to investigate the ultrastructural imaging and morphopathological features of the intestinal wall in patients with IE and to show how the number of ICC varies and their involvement in the development of associated symptoms.

Patients, Materials and Methods

This is a retrospective study involving 341 patients diagnosed with DIE and treated for one year (2023) in Prof. Dr. Panait Sîrbu Clinical Hospital of Obstetrics and Gynecology, Bucharest and in the Emergency County Clinical Hospital, Craiova, Romania. We also included a control group who did not have DIE. The control group was represented by 20 women deceased in road traffic accidents. A written consent was obtained from the families of all the victims.

The 361 patients were divided according to the localization of the endometriosis sites. The control group included 20 (5.54%) patients who died in road traffic accidents and from whom uninfected intestinal fragments were taken for a microscopic comparative study. All patients and/or their relatives signed a written consent form for their participation in the study. The study was conducted with the written consent of all participants and/or their relative and according to the Guidelines of the Declaration of Helsinki and approved by the Committee of Ethics and Academic and Scientific Deontology of the University of Medicine and Pharmacy of Craiova (UMPhCv) (Approval No. 88/ 13 September 2018 and Approval No. 343/17.09.2024).

The diagnosis of the patients was guided both by the presenting symptoms and the imaging findings and was confirmed by histopathological (HP) examination. In order to find out the patients' symptoms, questionnaires were carried out and the results were converted into percentages using Microsoft Excel 2010.

The US scans were performed transabdominal and/or transvaginal in each patient using two-dimensional (2D) and color Doppler gray scale. The US machines used for the scans were equipped with both transabdominal 3–5 MHz

and transvaginal 3–9 MHz linear transducers (Voluson E10). MRI was also used to visualize the extension of the lesions in the pelvic cavity and at distance. All patients included in the study underwent a surgical procedure and the removed tissue specimens were submitted to a HP examination which confirmed the presence of ectopic foci of endometriosis.

For our microscopic study, we selected 20 patients with intestinal DIE with rectal involvement and 20 control patients, deceased in road traffic accidents, from whom non-lesional rectal tissue fragments were taken. Postoperatively, the tissue was harvested for HP and classical immunohistochemical (IHC) examination. These fragments were processed at the Research Center for Microscopic Morphology and Immunology, UMPhCv, and paraffin embedded, sectioned, stained and analyzed. Tissue fragments of interest were fixed using 10% neutral buffered formalin at room temperature. A classical Hematoxylin–Eosin (HE) staining was used for microscopic study. For the IHC study, we used anti-cytokeratin 7 (CK7) antibody to label ectopically localized endometrial epithelium, anti-cytokeratin 20 (CK20) antibody to label intestinal gland epithelium, anti-alpha-smooth muscle actin (α-SMA) antibody to label the actin in smooth muscles, anti-cluster of differentiation 117 (CD117) antibody to immunolabel ICC and anti-neurofilament protein (NFP) antibody to immunolabel NFPs.

Briefly, for enzymatic immunohistochemistry, the slides were subjected to antigen retrieval by microwaving in a 0.1 M citrate buffer solution of pH 6, according to the manufacturers' specifications (Table 1). Subsequently, the slides were incubated in a 3% hydrogen peroxide solution for 30 minutes to block endogenous peroxidase that might interfere with signal detection and then in 3% nonfat milk in saline to block nonspecific antibody binding sites. The primary antibodies were then incubated for 18 hours, on the slides, at 4°C, diluted as optimized (Table 1; Dako, Glostrup, Denmark and Abcam, Cambridge, UK). The next day, after thorough washing of the primary antibody in phosphate-buffered saline (PBS), the slides were incubated with Horseradish peroxidase (HRP)-labeled secondary antibodies specific for the primary antibody species (Nichirei-Bioscience, Tokyo, Japan). Lastly, the signal was detected with 3,3'-Diaminobenzidine (DAB, Nichirei-Bioscience). Afterwards each section was counterstained with Mayer's Hematoxylin, dehydrated, clarified in xylene, and coated with Canada balsam for imaging and analysis.

Table 1 – ***List of antibodies utilized for immunohistochemistry***

Antibody	Producer	Species, clone	Antigen retrieval	Dilution	Target label
Anti-CK7	Dako	Mouse, QBEnd/10	Citrate, pH 6	1:50	Endometrial epithelium
Anti-CK20	Dako	Mouse, Ks20.8	Citrate, pH 6	1:25	Intestinal gland epithelium
Anti-α-SMA	Dako	Mouse, 1A4	Citrate, pH 6	1:100	SMA
Anti-CD177	Dako	Rabbit, polyclonal	Citrate, pH 6	1:400	ICC
Anti-NFP	Dako	Mouse, 2F11	Citrate, pH 6	1:100	Neurofilaments

α-SMA: Alpha-smooth muscle actin; CD117: Cluster of differentiation 117; CK7/20: Cytokeratin 7/20; ICC: Interstitial cells of Cajal; NFP: Neurofilament protein.

For double immunofluorescence, 7 µm-thick slides were first processed for antigen retrieval, incubated in skimmed milk, and then incubated overnight at 4°C with either a mix of rabbit anti-CD117 and mouse anti-CK7, or with a mix of rabbit anti-CD117 and mouse anti-NFP. Next day, the slides were incubated for one hour in a mix of goat-anti-mouse Alexa 488 and goat-anti-rabbit Alexa 596 (Invitrogen), then further washed in saline and distilled water, then finally coverslip with a 4',6-diamidino-2-phenylindole (DAPI)-containing antifade medium (Vecta Shield, Vector Laboratories).

Images were acquired utilizing a Nikon AX-R inverted confocal microscope built on the Eclipse Ti2 platform, equipped with galvanometric and resonant scanners, a Nikon SPatial ARray Confocal (NSPARC) super-resolution module, a four wavelengths laser source (405 nm/488 nm/561 nm/640 nm), and detectors with freely tunable emission bands with ± 1 nm accuracy (Nikon Europe BV, Amsterdam, The Netherlands). Captured confocal *z*-stacks have been utilized to manually assess the minimum distance between the two fluorescence signal targets.

In order to estimate the number of ICC based on enzymatic immunohistochemistry, we took four pictures for each area and for each case included in the HP analysis with the 20× objective from each specimen using constant manual exposure and illumination settings. Thus, we analyzed eight areas of interest: intestinal mucosa in cases associated with intestinal DIE and in control cases; rectal submucosa in cases associated with intestinal DIE and in control cases; rectal serosa in cases associated with intestinal DIE and in control cases; and in the muscular layer in cases associated with intestinal DIE and in control cases. The count was done manually using the "manual tag" feature in Image ProPlus, averaged on each slide, and then for each subgroup. We counted the total number of cells and expressed them into percentages (%) using Microsoft Excel. All data was plotted in Microsoft Excel sheets and statistically analyzed using two-sample *t*-test with equal variance (significance set at $p<0.05$). The figures display the mean value and standard deviations (SDs).

Results

Localization of DIE

Of the 341 patients affected by DIE, 163 (45.16%) patients had deep pelvic endometriosis without intestinal involvement and 178 (49.30%) patients had intestinal DIE lesions. All 178 patients with bowel DIE had at least one lesion on the rectum. Of this group of patients with intestinal DIE, 115 patients had only rectal involvement (31.85% of all patients), eight patients had appendiceal lesions (2.21% of all patients), 19 patients had ileal involvement (5.26% of all patients), and 36 patients had sigmoid involvement (9.97% of all patients).

Assessment of patient symptoms

One of the points of interest of this study was on the symptoms associated with intestinal DIE. A total of 178 patients presented with intestinal DIE were included. The symptoms encountered were bowel disturbance, such as constipation or diarrhea (bowel disorders – BD), bloating (B), pelvic pain (PP) and catamenial rectorrhagia (R). Out of the total, 115 patients had only rectal involvement while the other had at least one other lesion: 74 (64%) cases presented with BD, 35 (30%) patients had B, 103 (90%) patients had PP, and five (4%) patients had R (Figure 1). Of the eight patients with appendiceal involvement, three (38%) patients had BD, five (63%) patients had B, six (75%) patients had PP, and no patient had R (Figure 1). Of the 19 patients with ileum involvement, 15 (79%) patients had BD, 13 (68%) patients had B, 15 (79%) patients had PP, and no patients had R (Figure 1). Out of the total of 36 patients with sigmoid involvement, 24 (67%) patients had BD, 17 (47%) patients had B, 31 (86%) patients had PP, and four (11%) patients had R.

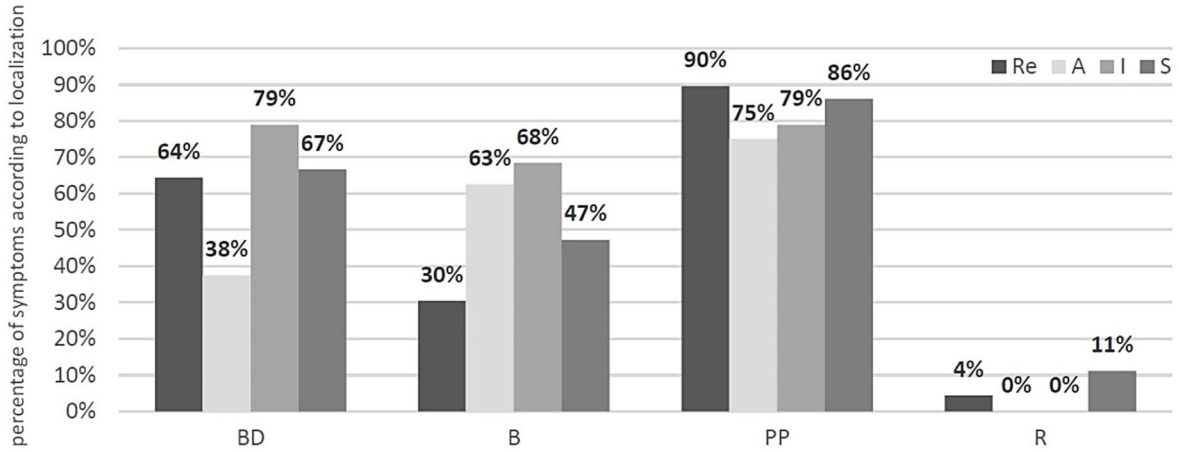

Figure 1 – ***Assessment of patients' symptoms. A: Rectum and appendix DIE; B: Bloating; BD: Bowel disorders; DIE: Deep infiltrating endometriosis; I: Rectum and ileum DIE; PP: Pelvic pain; R: Catamenial rectorrhagia; Re: Only rectal DIE; S: Rectum and sigmoid DIE.***

Imaging features of intestinal DIE

All 178 patients diagnosed with DIE with bowel involvement were evaluated both by US and by MRI.

A systematic pelvic evaluation was performed by TVUS according to *International Deep Endometriosis Analysis* (IDEA) Group recommendations.

During the assessment of the pouch of Douglas, we found the absence of sliding between the anterior rectal wall and the retrocervical region and posterior vaginal wall in 193 (58.59%) of the total of 341 patients studied. In 178 (92.22%) of the 193 patients with absent sliding signs were identified as endometriosis bowel nodules both by US and MRI and the lesions were confirmed intraoperatively and anatomopathological. Thus, for 15 (7.77%) of the patients, the absence of sliding sign was not correlated with imaging or intraoperative identification of endometriosis nodules.

The absence of the normal hyperechoic layer between the vagina and rectum was observed in all 178 diagnosed with intestinal DIE. Hourglass-shaped or "diabolo-like" nodules were visualized in 83 (46.62%) patients, "comet sign" nodules were found in 28 (15.73%) patients, "Indian headdresses" were found in 32 (17.98%) patients, "pulling sleeve" sign were found in 35 (19.66%) patients (Figure 2, A–C).

Pelvic MRI was performed in all 178 patients with US lesions consistent with bowel endometriosis, to assess the presence of multicentric intestinal DIE lesions in anatomical areas not reachable by the US probe.

MRI evaluation confirmed the presence of lesions of intestinal DIE in 178 of the patients included in the study (Figure 2D), for 112 (62.92%) of them only lesions in the rectum were identified and for 63 (35.39%) of them, following the MRI evaluation, multiple localizations in the intestine were found, so that the association of an endometriosis lesion in the sigmoid colon was identified in 36 (20.22%) of the scanned patients, endometriosis lesions in the ileal segment were identified in 19 (10.67%) patients, and the presence of appendiceal lesions was found in eight (4.49%) of the investigated patients.

Therefore, in three (1.68%) of the patients in whom single intestinal lesions in the rectum were identified by imaging, the intraoperative exploration revealed the presence of several additional lesions of IE. Of the 36 patients in whom endometriosis lesions in the sigmoid colon were confirmed intraoperatively, only one (2.77%) was not diagnosed prior to surgery. Of the 19 patients with endometriosis lesions in the ileal segment of the small bowel, 16 (84.21%) of them had a confirmed imaging diagnosis before surgery, and for two (10.52%) of them the diagnosis of endometriosis lesions was made during intraoperative exploration of the bowel segments. 100% of the patients with endometriosis lesions in the appendix were diagnosed preoperatively.

Surgical treatment of intestinal DIE

All patients included in this study underwent surgical treatment of endometriosis.

The colorectal surgical techniques used for these patients were as follows: rectal shaving was used in 10 (5.61%) patients of the patients diagnosed with endometriosis of the rectum among the 178 patients with IE included in the study, for 17 of the 178 (9.55%) patients with IE discoid

resection techniques were used, and for the other 151 (84.83%) patients a segmental resection of the affected region was performed of the 178 patients with bowel endometriosis lesions. In all 63 (35.39%) patients with multiple bowel lesions only segmental resection techniques were used (Figure 3, A–F).

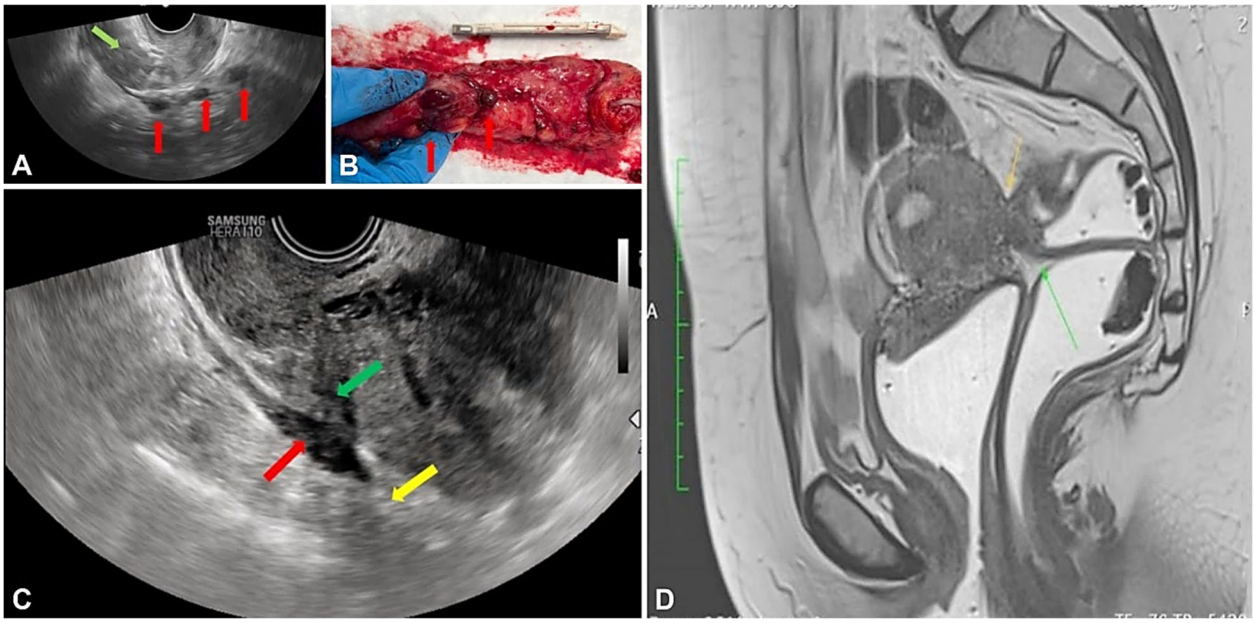

Figure 2 – ***Imaging and macroscopic features of endometriotic nodules: (A and B) Multiple nodules of IE; (A) Multiple nodules of IE US appearance; red arrow: rectosigmoid endometriosis nodules and green arrow: uterus; (B) macroscopic view of a recto sigmoid resection with the presence of deep endometriosis nodules pulling the intestinal wall (red arrow); (C) US appearance of a "comet-like" endometriosis nodule (red arrow) between the uterine torus (green arrow) and the rectum (yellow arrow); (D) MRI appearance of a deep endometriosis nodule localized between the uterine torus and rectum (yellow arrow), rectal wall and mucosa (green arrow). IE: Intestinal endometriosis; MRI: Magnetic resonance imaging; US: Ultrasound.***

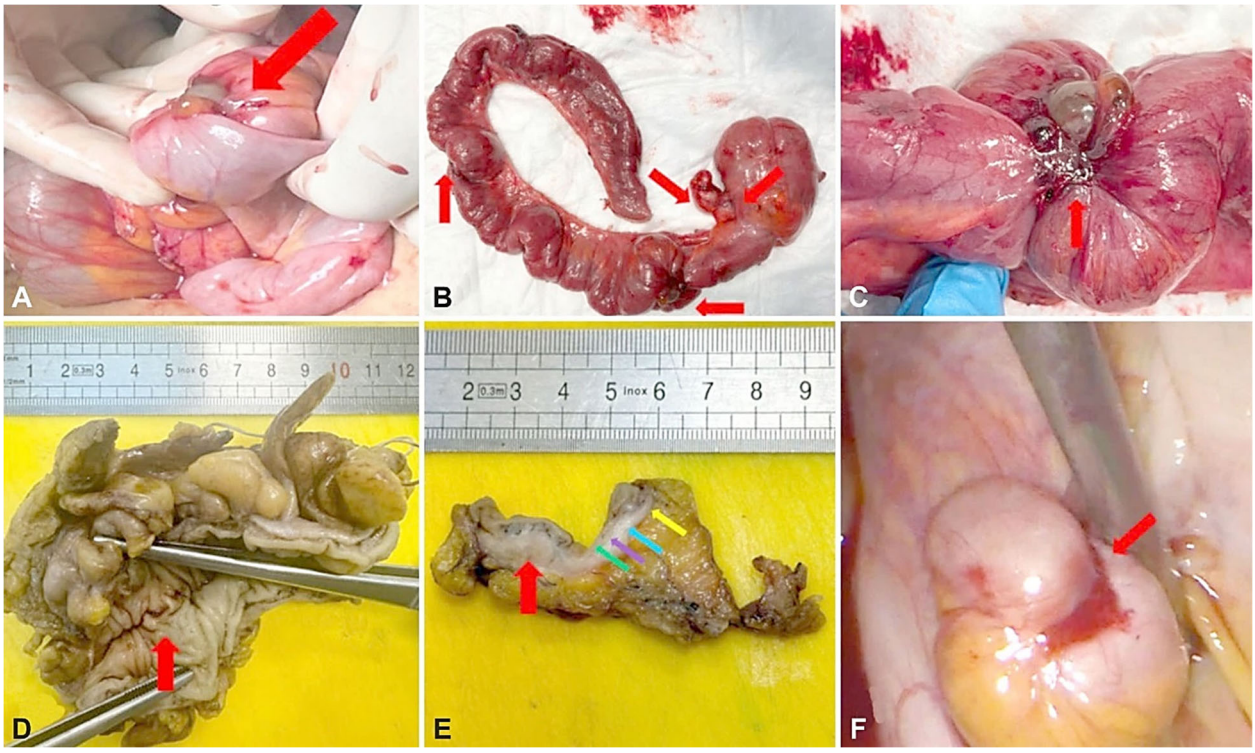

Figure 3 – ***Macroscopic feature of intestinal endometriosis: (A) Intraoperative macroscopic appearance of a nodule of deep endometriosis of the small bowel (red arrow); (B and C) Piece of colectomy, cecum and appendix affected by deep endometriosis (red arrow); (D) 12 cm rectal surgical specimen, showing the rectal mucosa pulled by the endometriosis nodule (red arrow); (E) 8 cm rectal resection surgical specimen, longitudinal section allowing visualization of the layers of the rectal wall: serosa (yellow arrow), muscular (blue arrow), submucosa (purple arrow) and mucosa (green arrow), the presence of the endometriosis nodule (red arrow) infiltrating rectal wall up to the level of the submucosa, pulling it without infiltrating; (F) Endometriosis node localized at the level of the appendix (red arrow).***

Histopathological characterization

The classical HE staining identified endometrial glands and chorion that protruded beyond the serosa, muscle (Figure 4A) and reached the intestinal wall down to the submucosa (Figure 4B; Figure 5, A and B).

We also identified by HE staining the nerve plexuses localized both between the muscle layers – the Auerbach plexus (Figure 5, A and B; Figure 6, A and B), which controls intestinal motility, and in the lax connective tissue in the submucosa – the Meissner plexus (Figure 5, A and B; Figure 6B).

Using IHC reactions, we identified and differentiated between CK20-positive immunolabeled (Figure 7A) and CK7-negative intestinal glands and CK7-positive immunolabeled and CK20-negative endometrial glands (Figure 7B).

Nerve plexuses located between muscle layers of the *muscularis propria* and in the submucosa were immunolabeled with anti-NFP antibody (Figure 8, A and B).

We immunolabeled smooth muscle cells in the *tunica muscularis* with the anti-*α*-SMA antibody, and ICC immunolabeled with the anti-CD117 antibody were found in all layers of the intestinal wall (serosa, muscle, submucosa and mucosa), varying in number according to the location and the group analyzed (control or endometriosis affected cases) (Figure 9, A–D).

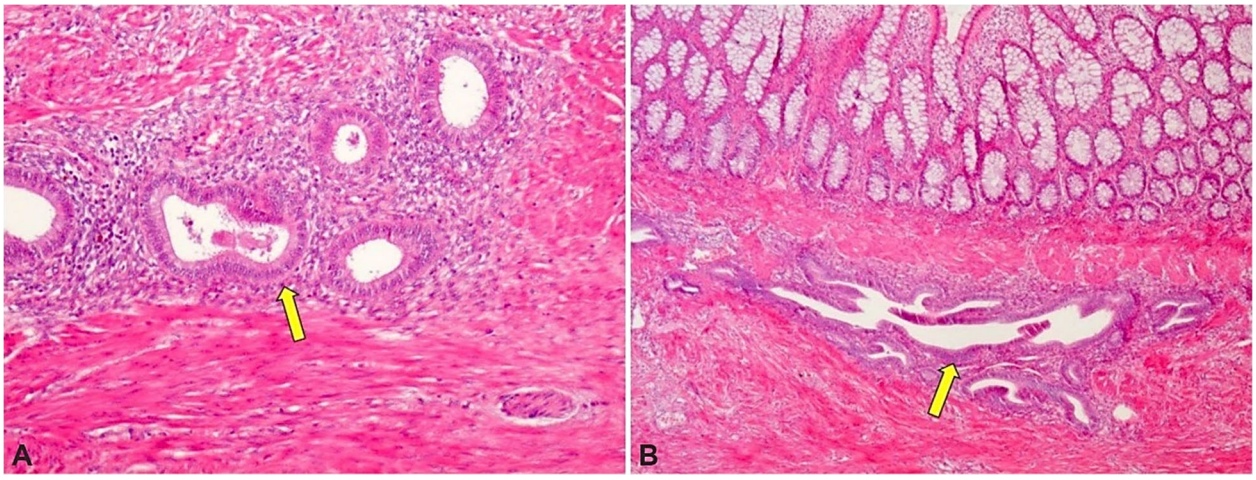

Figure 4 – ***Intestinal endometriosis: (A) The endometrial glands with ectopic localization and the chorion (yellow arrow) present among the smooth muscle fibers of the intestinal wall; (B) The endometrial glands with ectopic localization and the chorion (yellow arrow) present in the intestinal submucosa. Images from the collection of Dr. Anca-Maria Istrate-Ofiţeru. Hematoxylin–Eosin (HE) staining: (A) ×200; (B) ×100.***

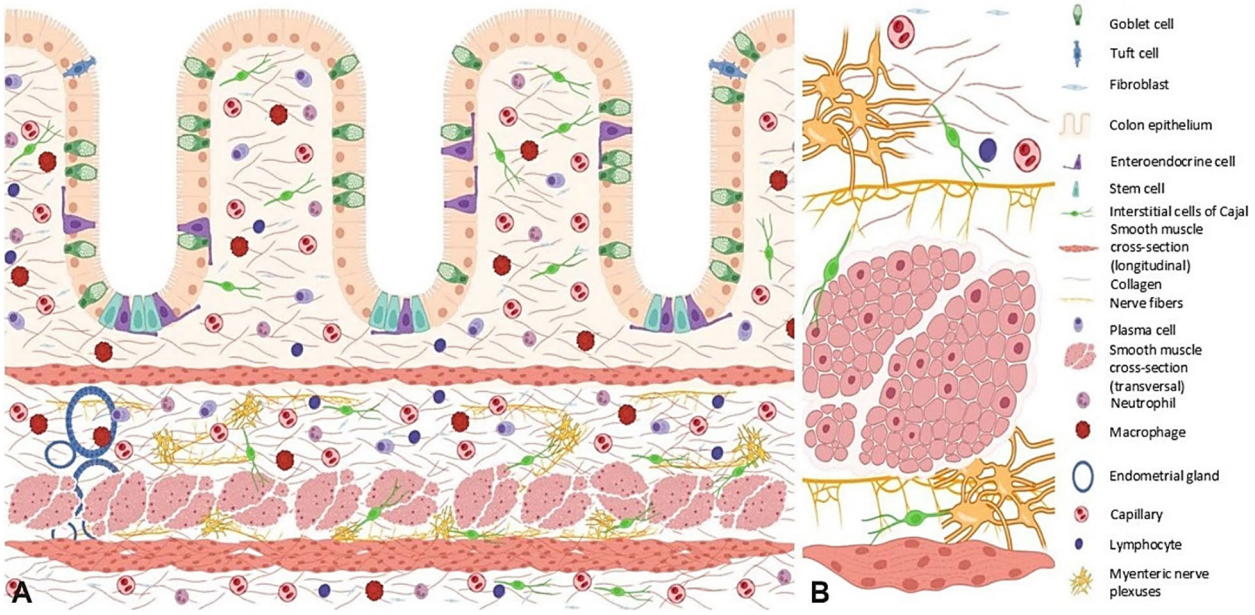

Figure 5 – ***Schematic image of the colonic wall: (A) Overview of the entire intestinal wall highlighting the mucosa with simple cylindrical epithelium with goblet cells, tuft cells, enteroendocrine cells, stem cells, intestinal submucosa with collagen fibers, blood capillaries, ICC and cells of the inflammatory system: neutrophils, macrophages, plasma cells, lymphocytes, but also Meissner nerve plexus, smooth muscle tunica, with inner circular muscle layer, outer longitudinal muscle layer, Auerbach nerve plexus, ICC and peritoneal serosa with lax connective tissue showing conjunctival elements, as well as ICC; (B) Image capturing the proximity of nerve plexuses, with ICC and myocytes. Created in BioRender. Anca-Maria Istrate-Ofiţeru (2025): https://BioRender.com/eur0v96. ICC: Interstitial cells of Cajal.***

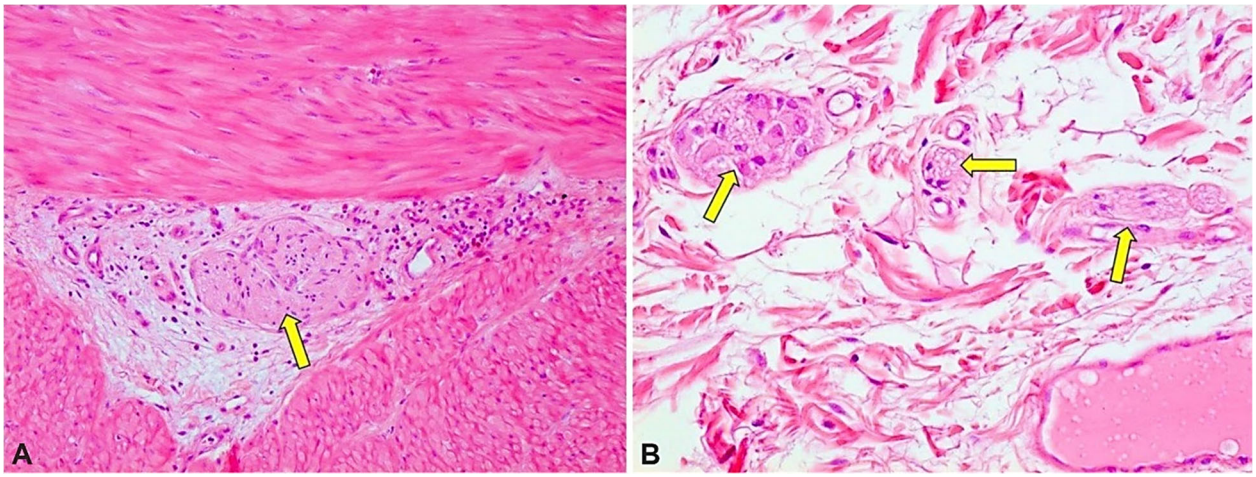

Figure 6 – ***Nerve plexuses in the intestinal wall structure: (A) The Auerbach nerve plexus (yellow arrow) is identified, localized between the layers of muscularis propria of the intestinal wall; (B) Meisser nerve plexus (yellow arrow), localized in the intestinal submucosa (yellow arrows), is observed. Images from the collection of Dr. Anca-Maria Istrate-Ofiţeru. Hematoxylin–Eosin (HE) staining: (A) ×100; (B) ×200.***

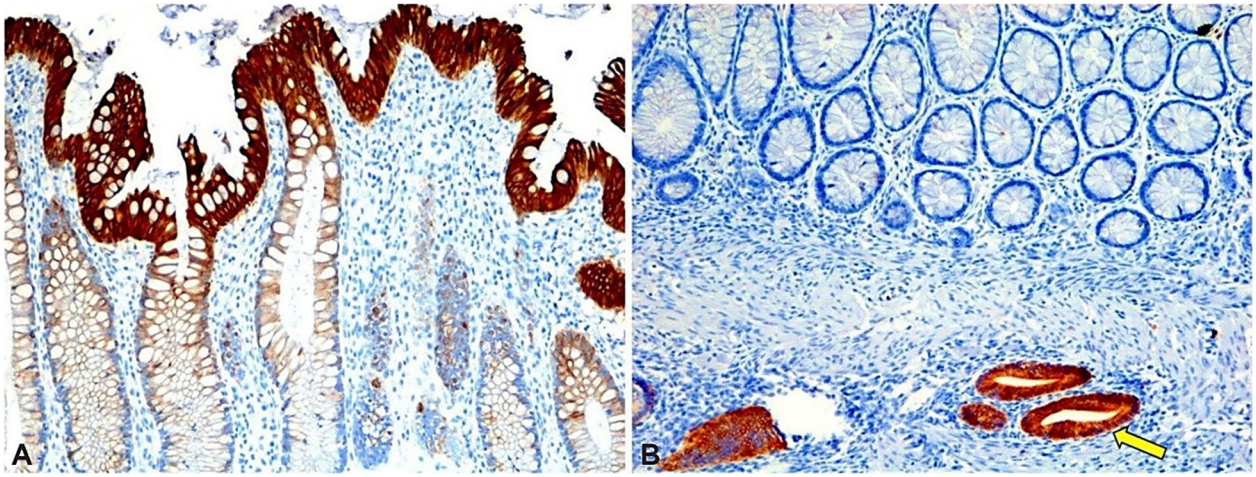

Figure 7 – ***Glands identified in the structure of the intestinal mucosa and submucosa: (A) Lieberkühn intestinal glands immunolabeled with anti-CK20 antibody, ×200; (B) Endometrial glands immunolabeled with anti-CK7 antibody, localized in the submucosa, ×200. Images from the collection of Dr. Anca-Maria Istrate-Ofiţeru.CK7/20: Cytokeratin 7/20.***

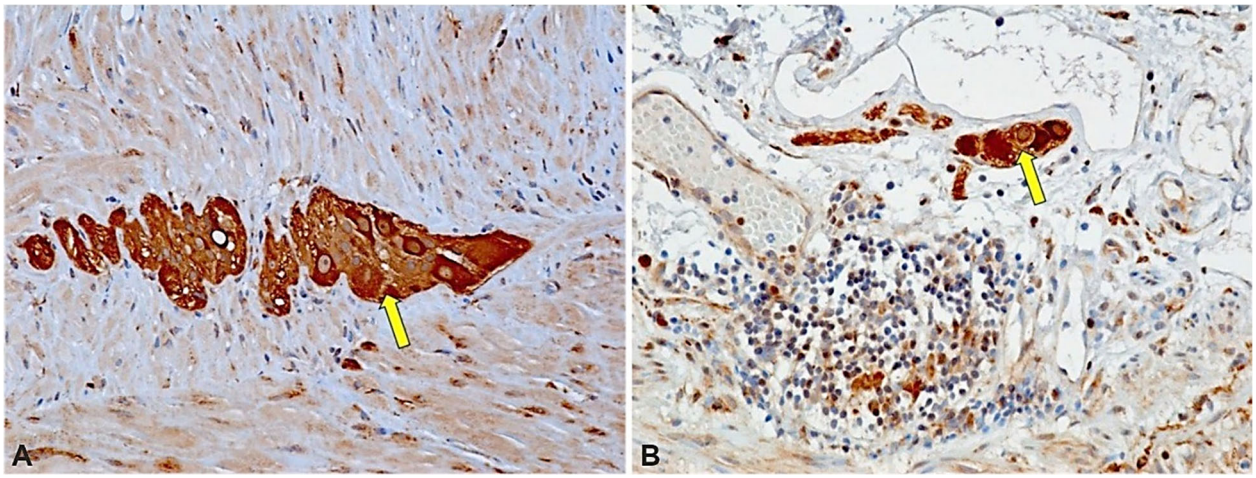

Figure 8 – ***Nerve plexuses in the intestinal wall structure: (A) Auerbach nerve plexus immunolabeled with anti-NFP antibody, localized between the smooth muscle layers of the intestinal wall (yellow arrow), ×200; (B) Meissner nerve plexus immunolabeled with anti-NFP antibody localized in the intestinal submucosa structure, ×100. Images from the collection of Dr. Anca-Maria Istrate-Ofiţeru. NFP: Neurofilament protein.***

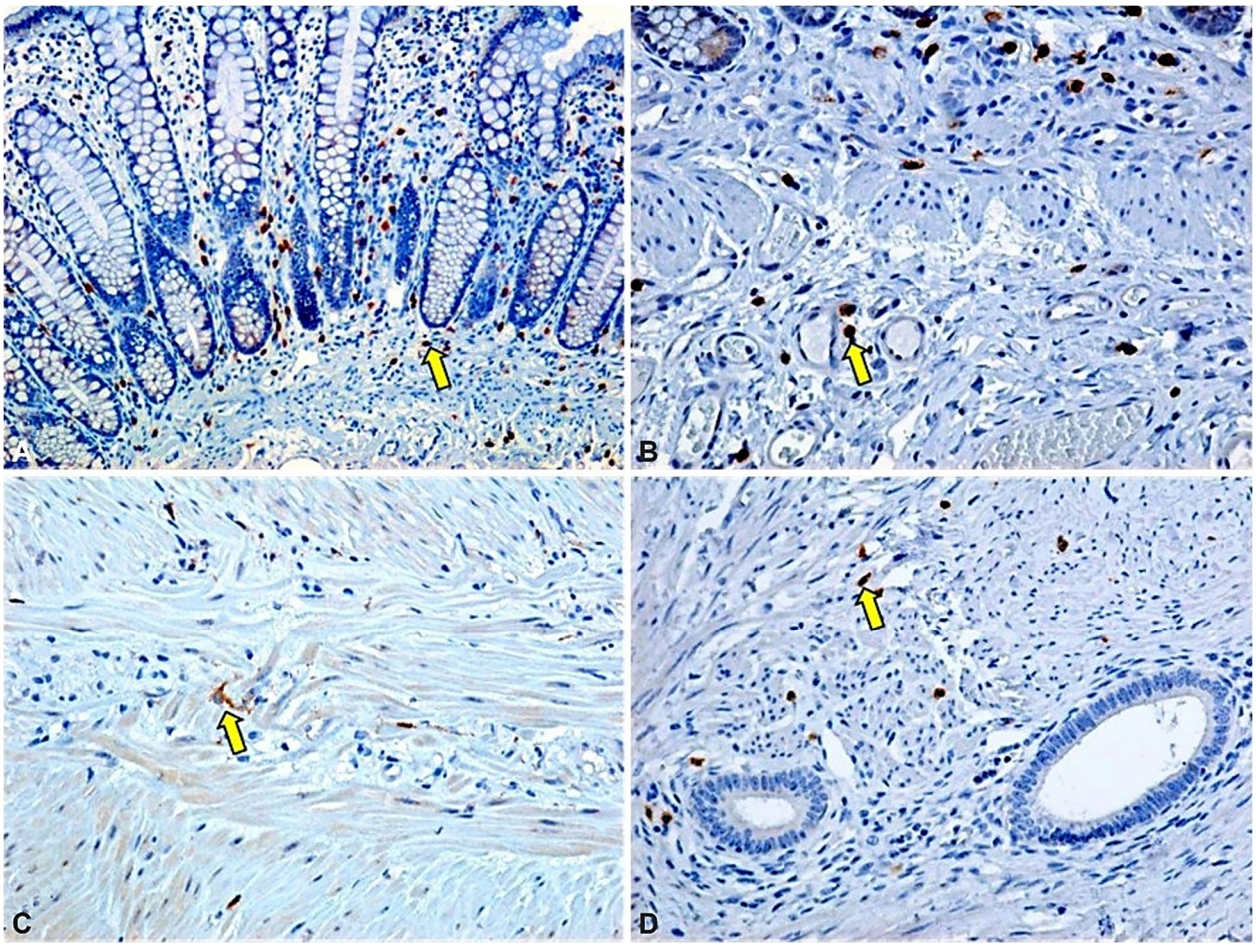

Figure 9 – ***Distribution of ICC (yellow arrow) in the intestinal wall structure: (A) ICC are seen among the Lieberkühn intestinal glands; (B) ICC (yellow arrow) distributed in the lax connective tissue in the submucosa of non-affected cases of IE; (C) ICC (yellow arrow) distributed among smooth muscle cells in cases unaffected by IE; (D) ICC (yellow arrow) distributed among smooth muscle cells in cases affected by IE, also identifying endometrial glands among the smooth muscle cells. Immunostaining with anti-CD177 antibody: (A–C) ×100; (D) ×200. Images from the collection of Dr. Anca-Maria Istrate-Ofițeru. CD117: Cluster of differentiation 117; ICC: Interstitial cells of Cajal; IE: Intestinal endometriosis.***

Also, in order to see how the number of ICC varied depending on the tissue layer analyzed, we counted them and observed that at the level of the intestinal mucosa in cases affected by DIE (M-I-DIE) the mean value per case ranged between 90.25 and 106.75 cells and the overall mean was equal to 97.09 cells (±4.47 cells), and in the intestinal mucosa control group (M-I-CC), the mean value per case of ICC ranged between 95.25 and 108.75 cells and the global mean was equal to 104.41 cells (±4.16 cells), thus there were significant differences between the pathological and the control group, $t(20) = -5.367$, $p<0.005$ (Figure 10).

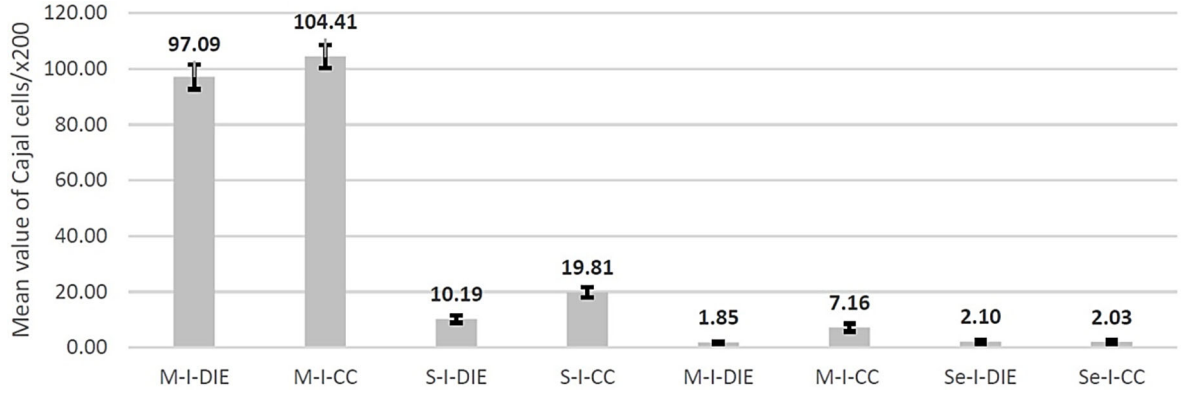

Figure 10 – ***Mean value of interstitial cells of Cajal distributed in the intestinal wall. There are significant differences between M-I-DIE and M-I-CC, t(20)=-5.367, p<0.005, and between S-I-DIE and S-I-CC, t(20)=-18.860, p<0.005, and also between M-I-DIE and M-I-CC, t(20)=-17.092, p<0.005, but no significant differences were noticed between Se-I-DIE and Se-I-CC t(20)=-0.329, p>0.05. DIE: Deep infiltrating endometriosis; M-I-CC: Intestinal mucosa in control cases; M-I-DIE: Intestinal mucosa in intestinal DIE; S-I-CC: Intestinal submucosa in control cases; S-I-DIE: Intestinal submucosa in intestinal DIE; Se-I-CC: Intestinal serosa in control cases; Se-I-DIE: Intestinal serosa in intestinal DIE.***

At the level of the intestinal submucosa in cases affected by DIE (S-I-DIE), the mean value per case ranged between 8.25 and 13 cells and the overall mean was equal to 10.19 cells (±1.29 cells), while in the control group of cases with intestinal submucosa (S-I-CC), the mean value per case of ICC ranged between 17 and 23.25 cells and the global mean was equal to 19.81 cells (±1.88 cells), thus there were significant differences between the pathological and control groups, $t(20)=-18.860$, $p<0.005$ (Figure 10).

At the level of the intestinal muscle in cases affected by DIE (M-I-DIE), the mean value per case ranged between 1.25 and 2.5 cells and the overall mean was equal to 1.85 cells (±0.4 cells), and in the cases of the control group in the intestinal muscularis (M-I-CC), the mean value per case of ICC ranged between 3.75 and 8.75 cells and the global mean was equal to 7.16 cells (±1.33 cells), thus there were significant differences between the pathological and control groups, $t(20)=-17.092$, $p<0.005$ (Figure 10).

At the level of intestinal serosa in cases affected by DIE (Se-I-DIE), the mean value per case ranged between 1 and 2.75 cells and the overall mean was equal to 2.1 cells (±0.71 cells), and in the intestinal serosa control group (Se-I-CC), the mean value per case of ICC cells ranged from 1 to 3.25 cells and the overall mean was equal to 2.03 cells (±0.73 cells), there were no significant differences between the pathological and control groups, $t(20)=-0.329$, $p>0.05$ (Figure 10).

Immunofluorescence and confocal microscopy

On confocal microscopy and three-dimensional (3D) renderings, we observed that there is a close morphological association between both CD177-labeled ICC and NFP-labeled nerve fibers (Figure 11, A–E), as well as between CD177-labeled ICC and α-SMA-labeled smooth muscle cells, demonstrating intercellular close proximity and thus the involvement of the enteric nervous system (ENS) in intestinal motility (Figure 12, A and B). Considering that CD117, α-SMA and NFP label cytoskeletal proteins and not the cellular membranes, it is most probable that in fact the two pairs of cellular partners are in fact in direct contact with each other. However, the two markers did not show a direct colocalization on NSPARC confocal microscopy, although at 200 nm lateral resolution no separating distance could be distinguished between them, judging that the two cells' domains must be in close vicinity or even direct membrane contact but without actually an overlap of their cytoplasm.

Discussions

Localization of intestinal DIE

The incidence of intestinal DIE in our patients was 49.30%, bigger than the one presented in the literature. That might be because our study included a referral Center for endometriosis [6]. Of all our patients, 100% of them had a rectal lesion of endometriosis, in concordance with the studies that report the rectum as being the most common localization of intestinal DIE [7]. We found also endometriosis lesions on the sigmoid, the ileum and the appendix.

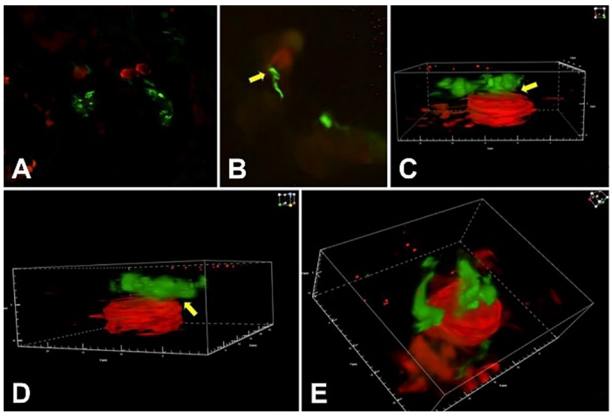

Figure 11 – ***Confocal microscopy imaging of CD117-labeled ICC (red) and NFP-positive nerve fibers (green) (A and B) and 3D renderings (C–E) in the thickness of the specimen, reveal an almost direct contact between the two cells, with indistinguishable interface space between them (arrow). 3D: Three-dimensional; CD117: Cluster of differentiation 117; ICC: Interstitial cells of Cajal; NFP: Neurofilament protein.***

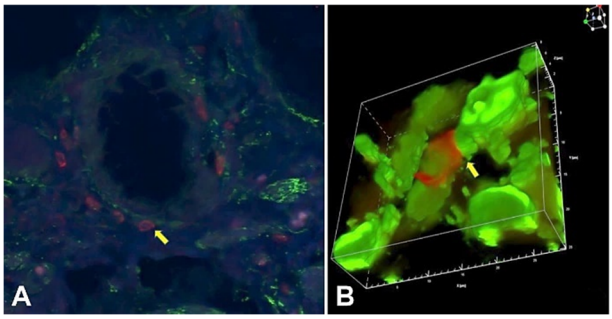

Figure 12 – ***Confocal microscopy imaging of CD117-labeled ICC (red) and α-SMA-labeled myofibroblasts (green) (A) and 3D rendering (B) reveal again no gaps between the two cells (arrow). 3D: Three-dimensional; α-SMA: Alpha-smooth muscle actin; CD117: Cluster of differentiation 117; ICC: Interstitial cells of Cajal.***

Assessment of patient symptoms

The study highlights that pelvic pain is the most common symptom in patients with intestinal DIE, regardless of the specific location of the lesion (rectum, appendix, ileum, or sigmoid) [43]. Bowel disturbance (constipation or diarrhea) is also highly prevalent, followed by bloating. Catamenial rectorrhagia (rectal bleeding during menstruation) is the least common symptom [15].

There are some variations in symptom presentation depending on the location of the DIE lesion. For example, bowel disturbance appears to be more prevalent in patients with ileum involvement compared to those with rectal involvement. Bloating is more prominent in appendiceal and ileal involvement, compared to rectal DIE [17]. But we can't make a correlation between the symptoms found in intestinal DIE and the location of the lesions [18].

It's important to acknowledge this study's limitations, including the absence of detailed information regarding symptom severity and the extent of bowel involvement, factors which could significantly influence symptom presentation. Additionally, the relatively small sample sizes for appendiceal and ileal involvement may limit the generalizability of these findings.

Imaging features of intestinal DIE

The study uses the *IDEA* Consensus for US. In this study, the absence of the sliding sign is emphasized as a key indicator of DIE in the pouch of Douglas [44]. The high correlation (92.22%) between the absence of this sign and confirmed bowel nodules suggests it's a reliable marker [45]. However, 7.77% of patients without correlation highlight the importance of considering other factors and imaging findings [43].

The description of specific US features like "hourglass-shaped" nodules, "comet sign" nodules, "Indian headdresses", and the "pulling sleeve" sign adds to the understanding of how DIE manifests on US [27].

The study appropriately utilizes MRI to assess areas beyond the reach of TVUS, particularly for identifying multicentric intestinal DIE lesions [43]. This highlights the complementary role of both imaging modalities in comprehensively mapping the extent of the disease [21].

The study acknowledges discrepancies between imaging and intraoperative findings in a small percentage of patients. This underscores the limitations of imaging and the importance of surgical exploration for a complete assessment [46]. The fact that a small percentage of sigmoid and ileal lesions were only diagnosed intraoperatively suggests that even with advanced imaging, some lesions can be missed [47].

The 100% preoperative diagnosis rate of appendiceal lesions is a notable finding. This could be due to the relatively accessible location of the appendix and the specific imaging characteristics of endometriotic lesions in this area [43].

Surgical treatment of intestinal DIE

Our study demonstrates the use of a range of surgical techniques, from conservative approaches like rectal shaving and discoid resection to more radical segmental resections. This highlights the importance of tailoring the surgical approach to the individual patient and the specific characteristics of their disease [32, 33].

The proportions of rectal shaving, discoid resection, and segmental resection can vary significantly across different studies [32]. Some studies may report a higher prevalence of conservative techniques like rectal shaving or discoid resection, while others may favor segmental resection, similar to our study [37]. The fact that segmental resection was the most common technique (84.83%) suggests that, in our study population, a significant proportion of patients had more extensive or deeply infiltrating disease requiring more aggressive surgical management because sometimes the patients treated were redirected to our Centers because of the severity of intestinal DIE.

The choice of surgical technique depends on several factors, including the size, location, and depth of infiltration of the endometriotic lesions, as well as the presence of multiple lesions [27]. For example, rectal shaving might be suitable for superficial lesions, while discoid resection could be used for larger, but still relatively localized, nodules [37]. Segmental resection was typically reserved for cases with extensive bowel involvement or when other techniques are not feasible [48].

The study's finding that only segmental resection techniques were used in patients with multiple bowel lesions (35.39%) is logical [40]. In these cases, a more extensive surgical approach is often necessary to remove all affected areas and minimize the risk of recurrence [49].

Histopathological characterization

With all these imaging features, sometimes, the correct assessment of the extent of pelvic endometriosis lesions can be performed only with laparoscopic surgery, and the definite diagnosis can be established only after anatomo-pathological examination of the excised tissue. With this in mind, in this study, we emphasized the HP diagnosis of colorectal lesions and the involvement of certain cells in the development of associated symptomatology.

Under microscopic examination using classical HE staining, we were able to identify endometrial glands in the colonic wall structure, as well as peri-glandular stroma, which infiltrated the wall down to the submucosa, similar to other published studies [50]. Immunohistochemistry helps establish the diagnosis of endometriosis by targeting the glandular epithelium. The endometrial glandular epithelium shows a positive reaction to CK7 and a negative one for CK20 ascertained the endometrial origin of the glandular entities, as CK20 is positive in intestinal glands [51–55].

The ENS is divided into three nerve plexuses: the subserosa or perivisceral plexus, the intramuscular or Auerbach's plexus and the submucosal or Meissner's plexus and is responsible for intestinal contractility and the occurrence of pain [55]. In addition, specialized myofibroblast cells called ICC are present in the intestinal structure. These cells are found in all the GI tract, from the esophagus to the anus, functioning as GI pacemakers generating and propagating slow electrical waves [56, 57]. ICC act as intermediary in the neural control of intestinal muscle activity, as spatial coordinators of intestinal motility and as stretch receptors playing important roles in the control of intestinal motor activity [58].

The lesions of ICC were associated with functional loss of spontaneous slow electrical waves and contractile activity [59]. Research on the distribution and function of ICC gained momentum after they were found to express c-Kit (CD117). ENS and ICC have an essential role in intestinal motility, along with other molecules such as progesterone, luteinizing hormone (LH), human chorionic gonadotropin (hCG) and relaxin [60]. These interactions suggest that ovarian hormones have a significant impact on bowel function, influencing how the bowel contracts and reacts to stimuli. This hormonal influence may contribute to the GI manifestations observed under various conditions, even in the context of the monthly menstrual cycle or other hormonal disturbances [32]. Previous studies were able to evaluate the relationship between endometriosis and GI involvement, reporting an ENS dysfunction, such as spasms of the wall of the Vater-duodenal ampulla, in cases with secondary lesions or absence of inhibitory control of the ENS. These findings suggest that present endometriosis may influence GI function by impairing nerve control mechanisms, causing symptoms such as PP, GI discomfort and BD. This link between endometriosis and ENS dysfunction emphasizes the complexity of the interactions between the reproductive and digestive systems, highlighting the need for careful evaluation of patients with endometriosis presenting with GI symptoms [61]. The group led by Anaf *et al.* was the first to highlight a close connection between ENS and endometriosis foci through perineural and endoneural invasion. In their studies, they found that endometriotic

lesions appear to infiltrate the colonic wall, preferring the nerve tracts [62]. They also found that in all resected areas of large bowel, that a peritoneal lesion was always in continuous histological contact with the deep endometrioid nodule underneath. This observation suggests that infiltration of the intestinal wall by endometriosis may be a progressive phenomenon, emphasizing the complexity of the interactions between endometriosis and the adjacent nervous structures [61–63].

Our study was based on research from other studies and was based on the numerical analysis of ICC according to the tissue layer involved in colorectal endometriosis patients as well as in the colonic wall structure unaffected by endometriosis and on the evaluation of the connection of these cells with nerve plexuses and nearby smooth muscle cells. For the selective identification of ICC, a polyclonal anti-c-Kit antibody (Dako, Japan) was used. The *c-Kit* gene encodes a growth factor receptor containing an internal component tyrosine kinase, called stem cell factor; this receptor is essential for normal ICC development and rhythmic activity of the GI tract [64–68]. ICC reactivity to c-Kit was assessed both in endometriosis-affected structures and in the normal intestinal wall. Thus, we observed that the density of ICC was lower in the mucosa, submucosa and colonic muscularis in endometriosis-affected cases, while in the serosa their number was slightly higher in the pathological cases. In order to highlight the presence of neovessel plexuses and nerve threads present in the intestinal wall structure, we used anti-NFP antibody (Dako, Japan). These highlight the cytoplasmic intermediate filament protein intermediate filament cytoplasm from the neuronal level [69]. To label myocytes in the intestinal mucosa and muscle structure, we used the anti-α-SMA antibody. α-SMA is one of the six different actin isoforms involved in the contractile function of smooth muscle [70].

Of interest for us was to highlight the link between myocytes, ICC and NFP. Thus, with confocal microscopy, we showed that no separation distance could be distinguished between them, suggesting that the domains of these cells could be in close proximity or even a direct contact between their membranes, but without a real overlap of their cytoplasm. Some studies have shown that there are gap junctions (GJs) between ICC and intestinal muscle cells, and other studies have argued with transmission electron microscopy (TEM) that GJs are nexus-like junctions that link the cells. At first, it was not clear whether they were composed of five or seven linear structures when visualized by TEM in cross-section. The discrepancy was resolved by recognizing the dependence of their appearance on fixation, incorporation and staining procedures [58, 71].

The very small and close approximations between some fibroblast-like ICC in the rodent myenteric plexus and adjacent smooth muscle cells may not be GJs, as has been claimed [72–75]. They do not have the typical structure of GJs as observed by TEM. That GJs are composed of connexon subunits is clear from TEM studies. These are connexons composed of six subunits (connexins) assembled to form hemichannels, which are joined end to end to form the channel. Connexons are spaced about 9–10 nm apart center to center in a regular arrangement in GJs, and within a GJ there can be from one to several thousand connexons. However, the studies failed to definitively identify the GJs [76, 77]. However, it is possible that in some cases there may be currents that generate slow waves or inhibitory junction potentials (IJPs) in intestinal muscle cells. Structurally, ICC networks in the colonic submuscular plexus or deep intestinal muscle plexus appear to have sufficient coupling to allow currents to passively generate slow waves, if the network can generate them. The currents underlying the slow waves of ICC in the colon are not canceled by blockers of L-type calcium channels, whereas currents generated in isolated circular muscle after potassium channel blockade are abolished. However, slow waves recorded from the circular muscle with attached submuscular ICC also resist cancellation by blockade of the L-type calcium channels. This suggests that when submuscular ICC are attached to the circular muscle, they either provide the current necessary to induce slow waves or alter the function of the circular muscle so that slow waves occur by another mechanism.

In the case of the deep muscle plexus, there is evidence only for the canine intestine that the ICC network conducts/initiates the slow waves of circular muscle. In other species, there is no evidence of this capability, but the viability of this network could be lost *in vitro*. Interestingly, we were able to observe by confocal microscopy that there is probably no separation distance between ICC and myocytes and that there may be intercellular GJs, and that when the number of ICC decreases, gut motility may be altered. Based on the basis of their anatomic locations several morphological types of ICC were described. In the GI tract, most ICC are found around the circumference of the myenteric plexus (Auerbach's plexus), and these are referred to as ICC of the myenteric plexus (ICC-MY or ICC-MP) or ICC of Auerbach's plexus (ICC-AP). These cells are multipolar, connecting to each other to form a network around the myenteric plexus, in the space between the circular and longitudinal muscle layers. This ICC-MY network is essential for the coordination of intestinal motility and communication between the different muscle layers. ICC of circular muscle (ICC-CM) are the cell types found in circular musculature. These cells are mainly bipolar or spindle-shaped cells that are not able to a network of their own, but can be found in connective tissue septs, where they are called ICC of connective tissue septa (ICC-SEP). ICC of longitudinal muscle (ICC-LM) are found in the longitudinal muscle. They are less numerous and similar in function to ICC-CM. These cells are called the intramuscular ICC (ICC-IM). In the deep muscle plexus is located the ICC of the deep muscle plexus (ICC-DMP). These multipolar cells connected closely to the nerve fascicles of the deep muscle plexus. ICC of the submucosa (ICC-SM) and those of the submucosal plexus (ICC-SMP) are found in the submucosa and submucosal plexus at the interface between the submucosal connective tissue and the innermost circular layer of muscle. These cells form a loose network between them. Finally, the ICCs of the subserous are essential for the functioning and coordination of intestinal motility [78]. Confocal microscopy used in this study showed a link between the nerve threads of the myenteric plexuses and the ICC, thus we can argue that they may be involved in the transmission of nerve impulses, their connection with the intestinal musculature and their damage may lead to influence intestinal motility.

Study limitations

This study has certain limitations that are important to be acknowledged. The study does not provide information on the severity of symptoms or the degree of bowel involvement, which could influence the symptom presentation. The sample sizes for appendiceal and ileal involvement are relatively small, limiting the generalizability of the findings.

Conclusions

This research provides significant insights into the diagnostic value of US and MRI in identifying intestinal DIE and offers a detailed analysis of specific imaging findings, contributing to a refined understanding of the disease's characteristic imaging features. These data offer valuable perspectives on the surgical management of intestinal DIE. By examining the distribution of surgical techniques and the variables guiding their selection, clinicians can better optimize surgical strategies, ultimately leading to improved patient outcomes when dealing with this complex condition.

Future research should try to confirm these findings in larger and more diverse cohorts and explore the mechanisms underlying the observed variations in symptoms associated with intestinal DIE. This will allow for more personalized and effective treatment strategies. Further studies could help to identify potential variations in surgical practice and patient selection criteria.

In colorectal endometriosis, the number of ICC in the colonic mucosa, submucosa and muscularis mucosa decreases and increases in the peritoneal serosa, and confocal microscopy revealed the proximity, if not direct contact, between myocytes, ICCs and nerve fibers and their close relationship between them and their role in nerve impulse transmission and intestinal motility. These findings contribute to our understanding of the perturbation of intestinal motility in this pathology. Thus, ICC are essential for intestinal motility through their connections with nerve fibers and muscle tissue. This is particularly relevant in endometriosis, where disruption of ICC networks may contribute to GI dysfunction.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Authors' contribution

Elena Iuliana Anamaria Berbecaru and George-Lucian Zorilă equally contributed to this article.

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