THE UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA" BUCHAREST
DOCTORAL SCHOOL
MEDICINE

THE EVALUATION OF DIAGNOSTIC AND PROGNOSTIC MARKERS IN ENDOMETRIOSIS

PhD THESIS SUMMARY

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Introduction

Endometriosis is defined as the presence of endometrial tissue (glands and stroma) outside the uterine cavity, producing a chronic inflammatory reaction. This condition is associated with infertility, painful symptoms (dysmenorrhea, dyspareunia, ovulation pain, dysuria, dyschezia) and affecting the quality of life [1]. The most common locations are the ovaries, the peritoneal cavity, the uterine ligaments (round, utero-sacral), caesarean or episiotomy scars [2].

One of the goals of this research is to provide an overview of endometriosis diagnosis and therapy, as this condition is a current topic of significant scientific interest. This doctoral work will discuss the factors that contribute to the pathogenesis of endometriosis, propose a preoperative non-invasive diagnostic protocol based on the symptoms and location of endometriosis lesions, and determine when a clinical examination and transvaginal ultrasound is enough and when an examination requires magnetic resonance imaging (MRI) to diagnose deep infiltrating endometriosis (DIE). Study I (chapter 6) will cover all of these topics.

Study II (chapter 7) focuses on the rate of successful reproduction following endometriosis surgery, which is important for managing symptoms, enhancing quality of life, and, in certain situations, increasing fertility. The patient's age, the desire to become pregnant, the anti-Mullerian hormone (AMH) levels, the endometriosis fertility index (EFI) score, the stage of endometriosis and the location of the lesions are some of the variables that may affect the chances of becoming pregnant following the intervention.

Current scientific research is investigating novel associations and non-invasive diagnostic techniques for the identification of endometriosis. The current work examines the relationship between gut microbiota and endometriosis in study III (chapter 8), assessing the interaction between microbiota and inflammation linked to endometriosis within the framework of gut dysbiosis. Research on this bidirectional interaction is promising because it combines the study of the immune system's response, changes in hormone metabolism, and the microbiome's impact on inflammation associated with endometriosis. An individualised treatment strategy that can enhance the prognosis and quality of life for people suspected of having endometriosis can be developed by conducting tests to identify the components of the intestinal flora.

I. GENERAL PART

1. The current status of research

1.2. Definition and prevalence

The presence of glandular tissue and endometrial stroma outside the uterine cavity (ovaries, peritoneum, Douglas pouch, post-operative scars), which causes a persistent inflammatory response, is a characteristic of endometriosis [2]. Infertility and painful symptoms such as dysmenorrhea, dyspareunia, discomfort during ovulation, dysuria, dyschezia, and intestinal pain are linked to this illness [2]. Ten percent of women who are of reproductive age and 25–35% of women who are infertile are affected by endometriosis [2].

The condition known as deep infiltrating endometriosis, or DIE, is characterised by the development of endometriotic implants that penetrate the pelvic organs at least 5 mm below the peritoneal surface [2, 3]. The most prevalent sites of DIE, which primarily occur below the rectosigmoid junction, are the uterosacral ligaments (USL), the fundus of the pouch of Douglas, and the intestine [4,5,6].

1.3. Pathogenesis

1.3.1. Theories of the origin of endometriosis

The initially proposed and best-known theory is Sampson's theory of retrograde menstruation (1921), which postulates that the condition is caused by the retrograde menstrual flow [7]. During the menstrual cycle, endometrial tissue extends into the peritoneal cavity in this manner [7].

The appearance of endometriosis in young patients, before menarche, contradicts the theory of retrograde menstrual flow. Other theories have been proposed for the etiopathogenesis of endometriosis, the most important of which are: the theory of Mullerian embryonic remains and the theory of induction (the development of endometriosis based on primordial cells of coelomic mesothelial origin, under the action of internal, biological or hormonal factors) [8].

1.3.2. Chronic inflammatory status

In the peritoneal fluid, a specialised microenvironment including estrogens, progestins, cytokines, growth factors, growth hormones, and angiogenic factors, endometrial cells that reach the superficial peritoneum multiply and develop [9]. Endometriosis lesions should therefore be histologically different and uneven with respect to the endometrium. Nonetheless, endometriosis lesions have been shown to be resistant to progesterone as seen by their active proliferation even in environments with comparatively high progesterone concentrations [9, 10].

1.3.3. Estrogen metabolism

Endometriosis is an estrogen-dependent pathology [11]. Estrogen and progesterone receptors have been identified in endometriotic tissue; their levels being significantly altered compared to normal endometrium [11]. Estrogen secreted in endometrial implants does not respond cyclically to hormonal variations [11]. Endometriotic tissue has significantly higher levels of estrogen receptors and progesterone receptors than normal endometrium [12]. Increased expression of estrogen receptors α and β in endometriotic stromal cells leads to the binding of progesterone receptor precursors by estrogen receptors β, thus leading to decreased levels of progesterone receptors [12].

1.4. The clinical symptoms

The clinical manifestations of endometriosis are highly variable. Endometriosis is most commonly characterised by chronic pelvic pain, which may or may not be associated with the menstrual cycle [6]. Dysmenorrhea (pain during menstruation), dyspareunia (pain during sexual activity), and dysuria (pain while urinating) are among ways in which this can show up. The visual analogue scale (VAS), which is a patient's subjective measure of the intensity of their pain, can be used to evaluate dysmenorrhea resulting from endometriosis. Patients are asked to rate their overall level of pain in a straightforward manner, typically on a scale of 0 to 5, or 0 to 10, where 0 indicates no pain and 5 or 10 indicates the highest level of pain experienced [13].

Infertility associated with endometriosis is related to the patient's age (fertility decreases from 35 years), ovarian reserve (AMH value), severity of clinical signs, endometriosis stage and depth extension, number of previous surgical interventions [14].

2. The diagnosis of endometriosis

Selecting the surgical indication and determining the treatment method need careful consideration of the extent and precise location of endometriotic implants. The most common techniques for diagnosing endometriosis are clinical examination, transvaginal ultrasound, magnetic resonance imaging (MRI), diagnostic laparoscopy, and computed tomography (CT) examination [12].

Correctly established preoperative diagnosis can have a decisive impact on the evolution of the patient, making a preoperative balance as complete as possible, so that the surgical intervention is curative, excises all lesions and thus prevents a subsequent reintervention [15].

Transvaginal ultrasonography (TVS) is the most commonly used diagnostic modality for pelvic endometriosis, being useful in distinguishing between endometriosis lesions, ovarian endometriomas and other causes of pelvic pain [7]. Transvaginal ultrasound is useful for examining the uterus and the adnexae, the anterior and the posterior compartments, as well as for evaluating other factors (sensitivity and mobility of certain structures) [7].

The MRI examination is important in the identification of small endometriotic nodules and in the evaluation of patients with deep infiltrative endometriosis, having a superior accuracy in the diagnosis and establishing the limits of the extension of the disease [8]. The diagnosis of endometriosis using MRI with endometriosis protocol is based on the detection of signal and morphological atypia in the anterior and posterior pelvic compartments, as well as the presence of adhesions [8].

3. Classification systems in endometriosis

3.1. The revised American Society for Reproductive Medicine score

In 1979, the American Society for Reproductive Medicine formulated the first endometriosis classification system, revised in 1985 and in 1997 [16]. According to the revised classification system of the American Society for Reproductive Medicine (rASRM), endometriosis is classified into four stages: minimal, mild, moderate and severe endometriosis [16].

3.2. The #ENZIAN classification of endometriosis

The #Enzian classification is based on the original Enzian classification [17]. The Enzian score classifies endometriosis lesions identified intraoperatively at the level of specific compartments: A-vagina, rectovaginal space (RVS); B-uterosacral ligaments (LUS) / cardinal ligaments/pelvic lateral wall and C – rectum and F (distant locations) such as urinary bladder (FB), ureters (FU) and other extragenital lesions (FO) [17]. The #ENZIAN classification additionally adds peritoneal (P), ovarian (O) lesions, other intestinal locations (sigmoid colon, small intestine; FI), as well as pelvic adhesions, involving the tubo-ovarian complex (T) and, optionally, tubal patency [17].

3.3. The Endometriosis fertility index

The Endometriosis fertility index (EFI) system developed in 2010 by Adamson and Pasta reflects anamnestic factors such as age, duration of infertility and previous pregnancies [18]. The EFI score is calculated by adding the anamnestic and surgical scores and ranges from 0 to 10 points, with 10 indicating the best prognosis and 0 the worst prognosis. The EFI system has a clear advantage in predicting pregnancy outcome and reflects the pregnancy rate better than the rASRM classification does [18].

4. New perspectives: The use of intestinal microbiota as a diagnostic and treatment method in endometriosis

The microbiota has a role in the immune regulation and the occurrence of numerous inflammatory pathologies [19,20]. The gut microbiota prevents bacterial translocation, which can lead to low-grade systemic inflammation and compromise the integrity of the gastrointestinal epithelial barrier as well as immunological homeostasis [19,20]. The intestinal, vaginal and uterine dysbiosis of patients with endometriosis have a role in the modulation of the immune response, in the alterations of estrogen metabolism and in the exacerbation of the disease [21].

The relationship between endometriosis and microbiota is bidirectional. When the intestinal dysbiosis is present, immunological function can be inhibited, the permeability of the intestinal barrier increases and the protection against pathogens decreases [21,22]. The microbiota contributes to the development of endometriosis by promoting a long-lasting inflammatory and hyperestrogenic environment [23].

II. Personal contributions

II.1. Objectives

The general aim of the PhD thesis is to analyze the diagnostic imaging methods used for patients with endometriosis and to evaluate the impact of these methods on the management and prognosis of the patients, including fertility.

The objectives of the studies contained in the doctoral thesis are represented by:

1. Synthesizing the current information about endometriosis by evaluating the specialized literature in relation to the etiology, pathogenesis, clinical manifestations, diagnostic methods and treatment of this disease
2. Examining different diagnostic techniques in endometriosis: transvaginal ultrasound and MRI and determining the sensitivity and specificity of these methods by comparing imaging results with intraoperative diagnosis
3. Comparison of the sensitivity, specificity and accuracy of the imaging methods included in the study in order to identify the most effective tools for the diagnosis of endometriosis and for the mapping of deeply infiltrative endometriotic lesions (DIE) located in the pelvic area
4. Choosing the ideal preoperative diagnosis method between transvaginal ultrasound or MRI depending on the location of the lesions and the characteristics of the patients
5. Investigating the effects of endometriosis on fertility prognosis and quality of life, as well as the correlation of AMH values and EFI score with the pregnancy rate achieved following surgery
6. Evaluation of current treatments and ongoing research on innovative and promising therapies for the management of endometriosis - we study the intestinal microbiome of the patients included in the study and we aim to identify a correlation between its components and the development of endometriosis
7. Proposing specific recommendations for optimizing the diagnostic process of endometriosis and improving prognosis, including identifying possible limitations and suggestions for future research

As part of my doctoral research, I conducted two retrospective, observational studies, which carried out between June 2018 and May 2021, in two specialized medical centers (Clinical Obstetrics and Gynecology Hospital "Prof. Dr. Panait Sârbu" Bucharest and Monza Hospital Bucharest) and a third retrospective, observational study, carried out during February 2021- May 2023 within the center of the Obstetrics and Gynecology Clinical Hospital "Prof. Dr. Panait Sârbu" Bucharest. Both the research methodology and the informed consent of the included patients in the doctoral research studies, were approved by the Ethics Committees of the two specialized medical centers mentioned above.

The three studies formulated as part of the doctoral research are:

1. Analysis of the sensitivity and the specificity of TVS and MRI examination compared with intraoperative appearance for the diagnosis of endometriosis
2. Analysis of the pregnancy rate obtained after surgery according to the patient's age, AMH value and EFI score
3. Analysis of the intestinal microbiota of patients with endometriosis

II.2. The research methodology

We performed three retrospective observational studies in which we analyzed the medical records of patients diagnosed with endometriosis. We considered an extended period of time to ensure adequate collection of relevant data and to assess long-term prognosis.

To fulfill the goals proposed by the doctoral thesis, data were collected about the socio-demographic characteristics of the patients included in the studies, such as age, the year of inclusion in the study, the department where the monitoring and therapeutic conduct of the patient was carried out, information about the clinical examination of the patient, the preoperative value of AMH, imaging investigations performed, the surgical intervention performed, the intraoperative appearance, the staging of endometriosis, the results of histopathological examinations and the postoperative complications that occurred, as well as the presence or absence of infertility and the patient's desire to conceive postoperatively and the success rate.

The selection of patients respected the inclusion and exclusion criteria established from the beginning of the studies. The criteria for the inclusion of patients in the 3 doctoral studies were:

- clinical aspect suggestive of endometriosis (presence of endometriosis cysts or lesions at vaginal level, utero-sacral ligaments, parameters, rectal nodules)
- pelvic ultrasound examination/MRI highly suggestive of endometriosis; signed informed consent for pelvic ultrasound imaging examination/MRI;
- signed informed consent for surgical management and for intraoperative histopathological samples.

The criteria for excluding patients in the 3 doctoral studies were:
- the patient' desire expressed at any stage of the research;
- failure to grant signed informed consent for TVS/MRI;
- failure to grant signed informed consent for surgical procedures;
- the absence of a diagnosis of endometriosis.

In order to respect the ethical principles, the study was carried out in accordance with the relevant guidelines on medical research and obtained the approval of the ethics committee of the basic institution, the Clinical Hospital of Obstetrics and Gynecology "Prof. Dr. Panait Sârbu" Bucharest. All patients provided signed informed consent for imaging examinations and surgical treatment of endometriosis. 256 patients with endometriosis were selected for study I, 250 of all 256 patients with endometriosis for study II and 9 patients with endometriosis for study III of the doctoral thesis.

Collected data were analyzed using appropriate statistical methods to identify trends, correlations, and association between diagnostic methods and intraoperative outcomes. The statistical analysis was performed using SAS 9.4 and Microsoft Office Excel 16.4 2023. The statistical results were interpreted at the level of significance p=0.05, except in cases where another value is specified.

Descriptive statistics for quantitative variables were expressed as means with standard deviations, minimum, median, and maximum values, interpercentile intervals, and confidence intervals. Statistical hypotheses for quantitative variables were examined using the t-test. The normal distribution of quantitative variables was examined using the Shapiro-Wilk test and/or the Smirnov-Kolmogorov test.

Descriptive statistics for categorical variables were expressed as frequencies and percentages. Associations between categorical variables were tested using Chi-square test, Chi-square exact test and Fisher's Exact test. Independence and level of accuracy/concordance/agreement of classifications for categorical variables were examined using Cohen's Kappa Coefficient and McNemar's test. Linear regression models were used to examine relationships between quantitative variables.

6. Study I: Analysis of the sensitivity and the specificity of TVS and MRI examination compared with intraoperative appearance for the diagnosis of endometriosis

6.1. Objectives

Main objective

Comparison of the sensitivity and specificity of TVS and MRI examination in detecting the presence of endometriosis compared with intraoperative diagnosis (considered the standard of reference). The aim is to evaluate the accuracy of these imaging methods in the correct diagnosis of endometriosis.

Secondary objectives

1. Description of the accuracy of the imaging methods included in the study for assessing deeply infiltrative endometriosis lesions located in the pelvis to identify which of the two imaging methods provides results closer to reality.
2. Choosing the ideal preoperative diagnostic method between TVS or MRI depending on the location of the lesions and the individuality of the patients.
3. Identification and analysis of possible errors or difficulties associated with the use of TVS and MRI examination in the diagnosis of endometriosis, with an emphasis on factors that may influence false-positive or false-negative results.
4. Formulation of recommendations for clinical practice based on the results of the study, with the aim of guiding doctors in the correct and effective use of TVS and MRI examination in the diagnosis of endometriosis.

6.2. Patients and methods

The present study is a retrospective, observational study, which included a total number of 256 patients with endometriosis according to the inclusion and exclusion criteria presented in chapter 5.

All patients enrolled in the study were examined using TVS and 177 of them were also examined using a 3.0 Tesla nuclear magnetic resonance system and underwent surgical

treatment of endometriosis, with the final diagnosis of endometriosis being established by histopathological examination.

177 patients out of 256 underwent an MRI examination, the exclusion criteria being: the high cost of this investigation and the financial limitation of certain patients, this investigation not being settled by the services offered by the insurance company; personal reasons (claustrophobia, anxiety, obesity, lack of time, geographical location), lack of need to perform an MRI examination due to the establishment of a clear diagnosis of endometriosis by TVS.

Descriptive statistics for categorical variables were expressed as frequencies and percentages. Associations between categorical variables were tested using Chi-square test, Chi-square exact test and Fisher's Exact test. Independence and level of accuracy/concordance/agreement of classifications for categorical variables were examined using Cohen's Kappa Coefficient and McNemar's test.

Results are expressed as mean, standard deviation or percentages. The definitive diagnosis of endometriosis was made based on intraoperative findings and histopathological examination. The results obtained from the preoperative imaging examinations were compared with the intraoperative results (standard diagnostic criteria). Each modality was evaluated for sensitivity, specificity, and diagnostic accuracy.

6.3. Results

6.3.1. Socio-demographic and clinical characteristics of the group

The average age of the patients was 33.94 years with a median of 34 years. The distribution by age categories was relatively balanced – they follow a normal distribution, according to the Shapiro Wilk test, p =0.336.

The symptoms of the patients consisted of dysmenorrhea (100%), dyspareunia (71.09%) and gastrointestinal disorders (50.78%).

Dysmenorrhea reported by the endometriosis patients in the study was assessed anamnestically using the visual analog scale (VAS). Most patients reported dysmenorrhea VAS=9 (37.11%), while only 0.78% reported the lowest value included in our study, VAS=5. No patient reported values below VAS=5 or VAS=6.

6.3.2. Analysis of the sensitivity and specificity of transvaginal ultrasonography compared with the intraoperative aspect in the diagnosis of endometriosis

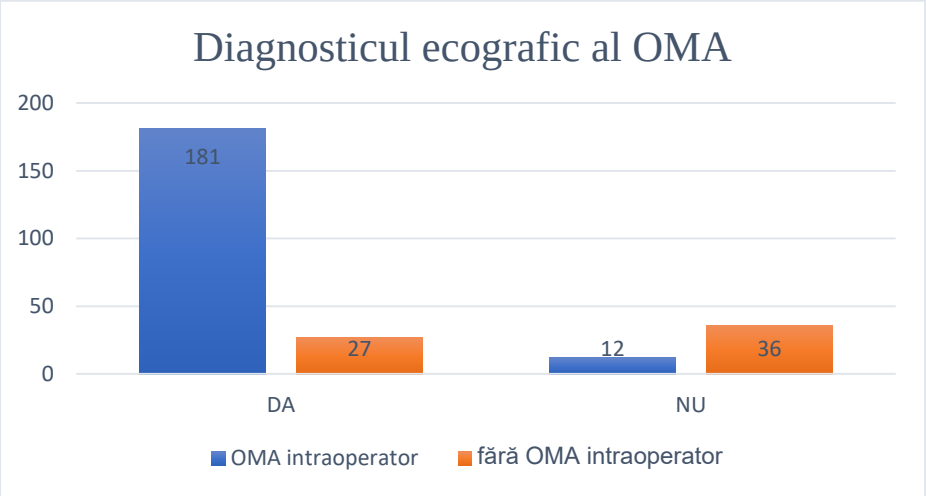

Fig. 6.1. Comparison of ultrasound diagnosis of endometriosis cysts with intraoperative diagnosis

The data presented in Fig. 6.1. represents the comparison of ultrasound diagnosis of endometriosis cysts with intraoperative diagnosis. The observed association is statistically significant according to the statistical tests Chi-square $\chi^2(df = 1) = 80{,}85, p < 0{,}001$ and Fisher's exact test p<0,001, so in this group, the ultrasound diagnosis is consistent with the intraoperative diagnosis for pelvic endometriomas. (Specificity = 57.14%; Sensitivity = 93.78%, Diagnostic accuracy (significant) = 84.47%). Cohen's κ=0.55, p<0,001.

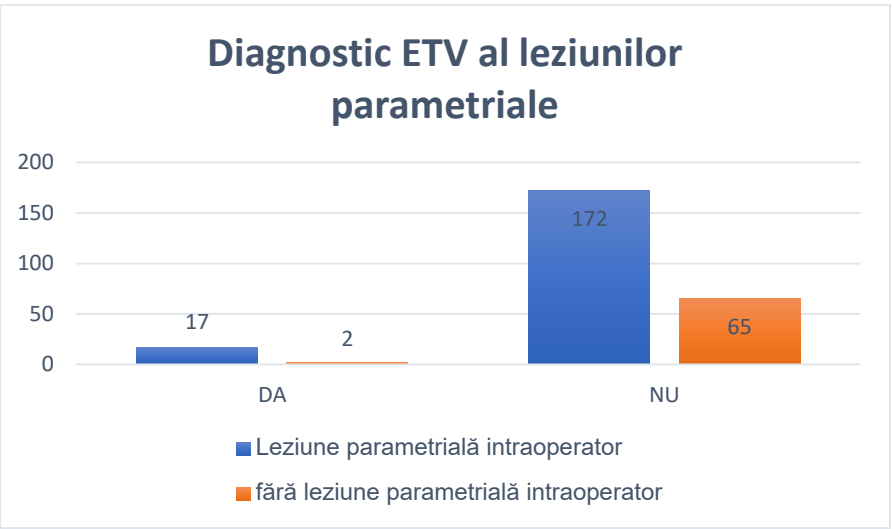

Fig. 6.2. Comparison of ultrasound diagnosis of parametrial lesions with intraoperative diagnosis

The data from Fig.6.2. represents the comparison of the ultrasound diagnosis of endometriosis lesions located at the parametrial level with the intraoperative diagnosis. The observed association is not statistically significant according to the exact statistical tests Chi-square $\chi^2(df = 1) = 2{,}60, p = 0{,}172$ and Fisher's exact test p=0.172, so in this group the ultrasound diagnosis is not consistent with the intraoperative diagnosis for parametrial lesions of endometriosis. (Specificity = 97%; Sensitivity = 9%, Diagnostic accuracy (not significant) = 32%). Cohen's κ=0.03, p =0.172.

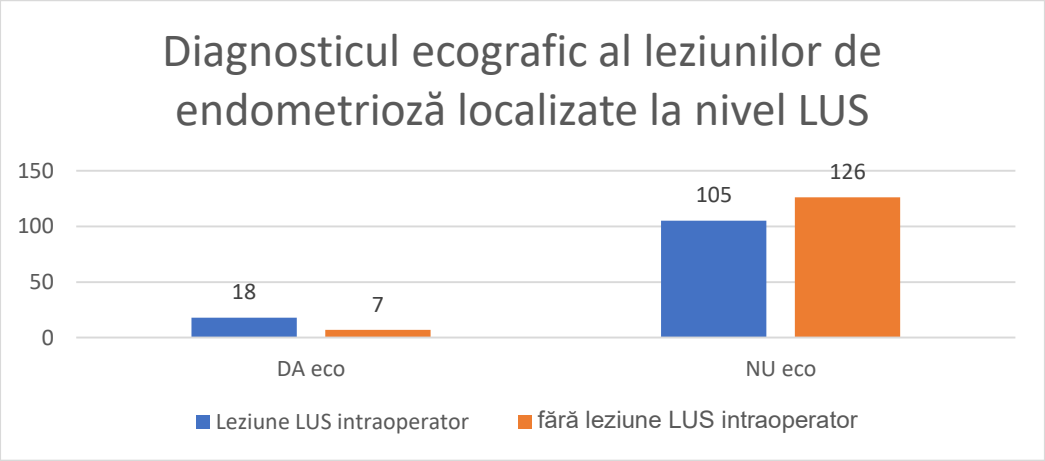

Fig. 6.3. Comparison of ultrasound diagnosis of USL lesions with intraoperative diagnosis

The data in Fig. 6.3. represents the comparison of the ultrasound diagnosis of endometriosis lesions located at the level of the uterosacral ligaments with the intraoperative diagnosis. The observed association is statistically significant according to the statistical tests Chi-square $\chi^2(df = 1) = 6{,}37, p = 0{,}012$ and Fisher's exact test, p=0.019, so that in this group the ultrasound diagnosis is consistent with the intraoperative diagnosis for the USL lesions. (Specificity = 94.74%; Sensitivity = 14.63%, Diagnostic accuracy (not significant) = 55%). Cohen's κ=0.09, p =0.019.

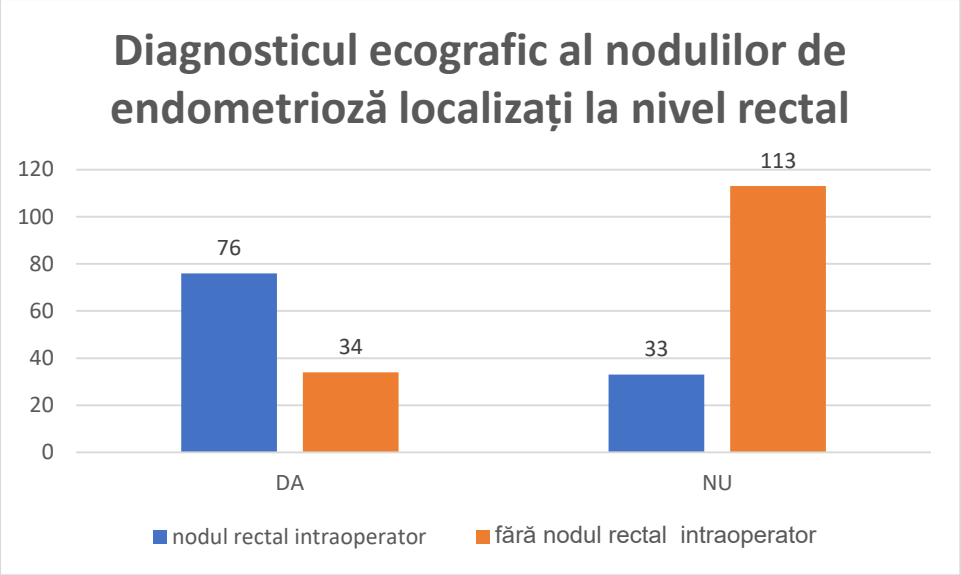

Fig. 6.4. Comparison of ultrasound diagnosis of rectal nodules with intraoperative diagnosis

The data in Fig. 6.4. represents the comparison of the ultrasound diagnosis of endometriosis nodules located at the rectal level with the intraoperative diagnosis. The observed association is statistically significant according to Chi-square statistical tests $\chi^2(df = 1) = 55{,}45, p<0{,}001$and Fisher's exact test, p<0,001, so in this group the ultrasound diagnosis is consistent with the intraoperative diagnosis for rectal endometriotic nodules. (Specificity = 76.87%; Sensitivity = 69.72%, Diagnostic accuracy (significant) = 73.82%). Cohen κ=0.46, p <0.001.

6.3.3. Analysis of sensitivity and specificity of MRI compared with intraoperative appearance in the diagnosis of endometriosis

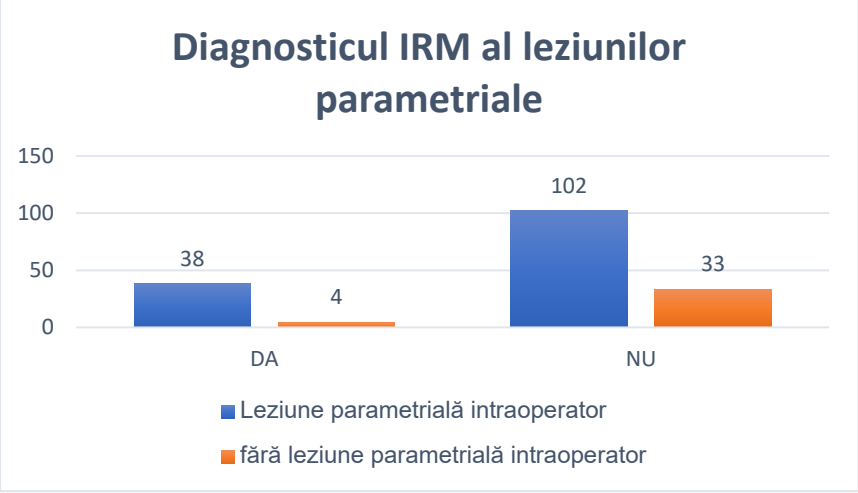

Fig. 6.5. Comparison of MRI diagnosis of parametrial lesions with intraoperative diagnosis

The data in Figure 6.5. represents the comparison of the MRI diagnosis of endometriosis lesions located at the parametrial level with the intraoperative diagnosis. The observed association is statistically significant $\chi^2(df = 1) = 4{,}31, p$=0,049, according to the Chi-square exact statistical test, and Fisher's exact test, p =0.049, so in this subgroup the MRI diagnosis is concordant with the diagnosis intraoperatively for parametrial lesions of endometriosis. (Specificity = 89.19%; Sensitivity =27.14%, Diagnostic accuracy (not significant) = 40.11%), Cohen κ=0.08, p =0.049.

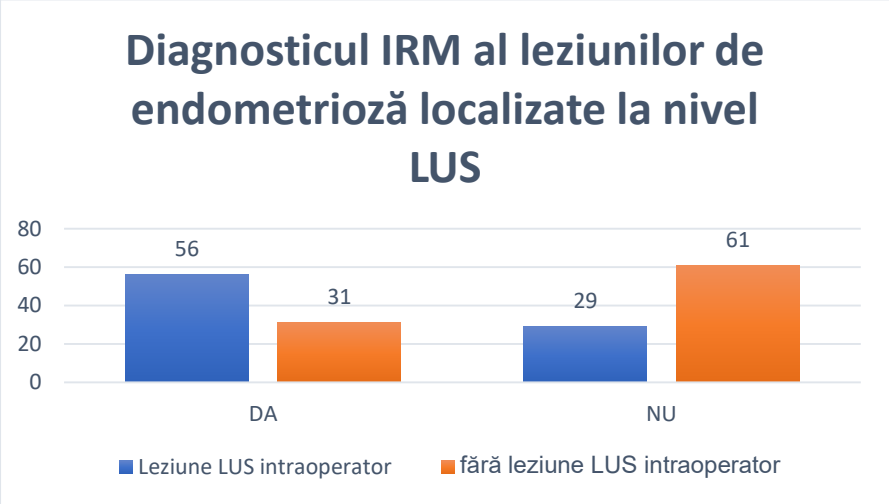

Fig. 6.6. Comparison of MRI diagnosis of USL lesions with intraoperative diagnosis

The data in Fig. 6.6. represents the comparison of the MRI diagnosis of endometriosis lesions located at the USL level with the intraoperative diagnosis. The observed association is statistically significant according to the statistical tests Chi-square $\chi^2(df = 1) = 18{,}31, p$<0,001 and Fisher's exact test, p<0,001, so that in this subgroup the MRI diagnosis is consistent with the intraoperative diagnosis for the lesions LUS of endometriosis. (Specificity = 66.30%; Sensitivity = 65.88%, Diagnostic accuracy (significant) = 66.1%). Cohen's κ=0.32, p<0,001.

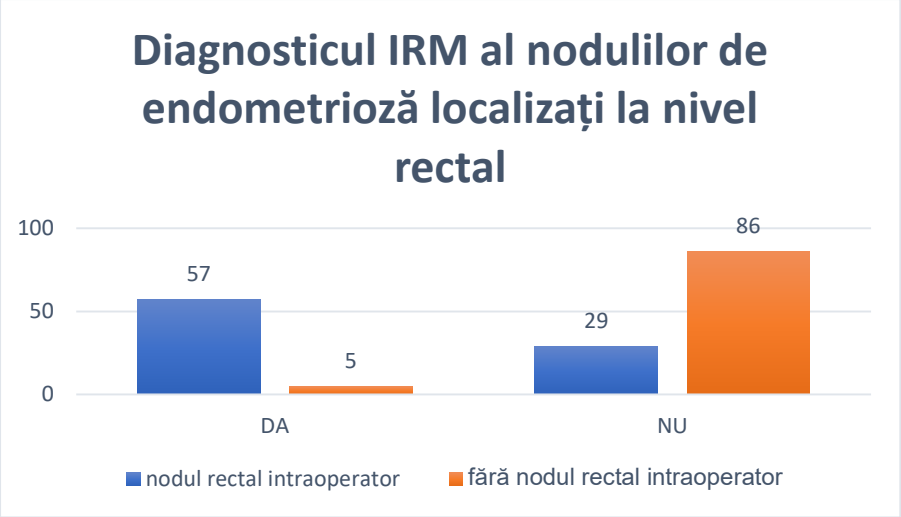

Fig. 6.7. Comparison of MRI diagnosis of rectal nodules with intraoperative diagnosis

The data in Fig. 6.7. represents the comparison of the MRI diagnosis of endometriosis nodules located at the rectal level with the intraoperative diagnosis. The observed association is statistically significant according to the statistical tests Chi-square $\chi^2(df = 1) = 71{,}78, p<0{,}001$ and Fisher's exact test, $p<0{,}001$, so in this subgroup the MRI diagnosis is consistent with the intraoperative diagnosis for the endometriosis rectal nodules. (Specificity = 94.51 %; Sensitivity = 66.28%, Diagnostic accuracy (significant) = 80.79%). Cohen's κ=0.61, $p<0{,}001$.

6.4. Discussions

The diagnostic accuracy for identifying DIE lesions was examined in the current study between two non-invasive techniques: TVS and MRI. Even though laparoscopy and histopathological examination have been considered the gold standard for the diagnosis of DIE, they are invasive and require preoperative preparation [15]. Both TVS and MRI are noninvasive, practical, and affordable techniques. Prognosis, quality of life and reproductive function can all be predicted with some accuracy by an early DIE diagnosis [24].

Specialized studies reported similar values of sensitivity and specificity for the two imaging techniques in the diagnosis of endometriomas, respectively a sensitivity of 70.86–96% for TVS and 63.5–92.6% for MRI and a specificity of 71–96% for TVS and 71–93.9 % for MRI [25-28]. Our study reported a sensitivity of 93.78% and a specificity of 57.14% with a diagnostic accuracy of 84.47%. Regarding the imaging diagnosis of ovarian endometriosis cysts, we consider transvaginal ultrasound the most suitable diagnostic method, being

accessible and reliable, the specific ultrasound appearance of endometriomas being sufficient for diagnosis.

For parametrial endometriosis lesions, specialist studies report a specificity of 98.6%, sensitivity of 83.3% and a high accuracy of 96.4% for MRI diagnosis [29]. And our study demonstrates a statistically significant association between MRI diagnosis and intraoperative appearance for parametrial endometriosis lesions, with a diagnostic accuracy of 40.11%, a sensitivity of 27.14%, and a specificity of 89.19%. In the case of ultrasound diagnosis of parametrial lesions, following our study, it is not concordant with the intraoperative diagnosis of parametrial lesions of endometriosis, obtaining a specificity of 97%, a sensitivity of 9% and a non-significant diagnostic accuracy of 32%.

Regarding endometriotic lesions of the uterosacral ligaments, the specialized literature reports a higher sensitivity of the MRI examination compared to the diagnosis of TVS: 63.5–95.6% for MRI compared to 55.6–78.3% for TVS. The specificity of the two techniques is similar, 66.7–98% for TVS and 60–93.9% for MRI [25-28]. Our study reported a sensitivity of 14.63% and a specificity of 94.74%, respectively a diagnostic accuracy of 55% for TVS diagnosis of endometriotic lesions of the uterosacral ligaments, data relatively similar to those found in the literature. Regarding MRI diagnosis, our study reported a sensitivity of 65.88% and a specificity of 66.30%, respectively a diagnostic accuracy of 66.1%. The sensitivity obtained by our study is significantly higher with regard to the MRI examination than the TVS examination, which suggests the need for an MRI examination when deep infiltrative endometriosis is suspected.

Regarding recto-sigmoid intestinal endometriosis lesions, according to data from the specialized literature, MRI diagnosis has a sensitivity of 76.9–94% and a specificity of 50–96.6% [25-28]. Our study reports a sensitivity of 66.28%, a specificity of 94.51% and a significant diagnostic accuracy of 80.79%, which demonstrates the concordance of the MRI diagnosis of rectal nodules with the intraoperative diagnosis. Regarding sigmoid nodules, our study reports a sensitivity of 58.33%, a specificity of 96.73% and a significant diagnostic accuracy of 93.22%, demonstrating the statistically significant association between MRI diagnosis and intraoperative diagnosis.

Regarding ultrasound diagnosis, through our study we identified the presence of rectosigmoid nodules sonographically with a specificity of 76.87%, sensitivity of 69.72% and

a significant diagnostic accuracy of 73.82%, there being a statistically significant association between ultrasound diagnosis and the intraoperative one. A limitation of ultrasound in the diagnosis of rectal endometriosis may be that sigmoid nodules may be misdiagnosed as rectal nodules.

7. STUDY II: Analysis of the pregnancy rate obtained after surgery according to the patient's age, AMH value and EFI score

7.1. Objectives

Main objective

Analysis of the pregnancy prognosis after endometriosis surgery using patient' age, preoperative AMH value, EFI score and the type of ovarian surgery.

Secondary objectives

1. Determining the existence of an association between AMH values and the rate of obtaining a pregnancy following surgery for endometriosis, spontaneously or through assisted reproductive techniques.

2. Studying the association between the EFI score and the pregnancy rate obtained after surgery for endometriosis, spontaneously or through assisted reproduction techniques, with the decision of the optimal moment to send patients to an assisted human reproduction department

3. Studying associations between the EFI score and the AMH value and the influence on future pregnancies

7.2. Patients and methods

The present study is a retrospective, observational study, which included a total number of

250 patients from the initial group of 256 patients from study I, diagnosed with endometriosis according to the inclusion and exclusion criteria presented in chapter 5. The exclusion of 6 patients from the initial group was based on the EFI fertility score criterion, the 6 excluded patients not having the EFI score at the end of surgery because they underwent total hysterectomy.

All patients enrolled in the study were examined using TVS and underwent surgical treatment of endometriosis, the final diagnosis of endometriosis being established by the histopathological examination. For the 250 patients with endometriosis included in the study,

the following quantitative variables obtained from the anamnesis and clinical examinations were studied: age, year of inclusion in the study, medical unit, symptoms, clinical appearance, preoperative AMH value (for 134 of them), EFI score and achieving a pregnancy after the intervention (spontaneous or by IVF).

The 250 patients were divided into 2 groups according to the results of the infertility treatment. Patients who achieved a pregnancy were classified in the pregnant group and the others were classified in the non-pregnant group. We also created a subgroup of 134 patients (group A) in which we measured the AMH value preoperatively, this group being divided in into the group of those who got pregnant (group G) spontaneously or through IVF and the group of those who did not achieve pregnancy postoperatively (NG group).

7.3. Results

7.3.1. The EFI score

The EFI score was calculated after the surgery for the 250 patients, with its mean value 5.38, the standard deviation 1.91, with a median of 5.00 (value range 1-9). The data in Fig. 7.1. represents the distribution of the EFI score according to the number of patients.

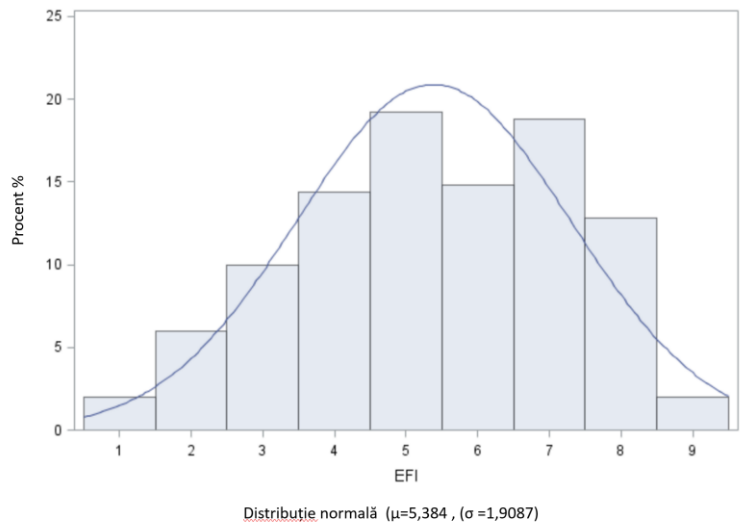

Fig. 7.1. EFI score distribution for the 250 patients

The linear regression of the EFI score with age produces the regression line with the ordinate at the origin 9.33 (standard error =0.72) and slope -0.12 (standard error =0.02) with the probability p<=0,001. It is observed that the EFI value decreases with the age. EFI is expected to decrease by an average of 0.12 with increasing the age by one year.

The mean value of the EFI score is significantly higher in the group of patients who did not achieve pregnancy after surgical treatment compared to the group of patients who achieved pregnancy, according to the statistical test Two sided two sample T t(df=248)= 2.44, p=0.015.

Of the 141 patients who got pregnant, 58 (41.13%) got pregnant spontaneously and 83 (58.87%) got pregnant through in-vitro fertilization (IVF). Of those who conceived spontaneously, the mean EFI value is 5.59, standard deviation 1.70, median 5, and range 1-9. Of those who achieved pregnancy through IVF, the mean EFI value is 4.81, standard deviation 2.1, median 5, and range 1-9. In the group of patients with IVF pregnancy, the average value of the EFI score is significantly lower by 0.78 than in the group of patients with spontaneous pregnancy, according to the Two sided two sample test t(df=139)=2.34,p=0.021.

7.3.2. The AMH value

The preoperative AMH was performed for a subgroup of 134 patients (group A), and its average value was 2.5 ng/mL, the standard deviation being 2.01, with a median of 2 ng/mL, and the range of values being 0.01-10.20 ng/dl. The data in Fig. 7.2. represents the distribution of preoperative AMH values for the 134 patients.

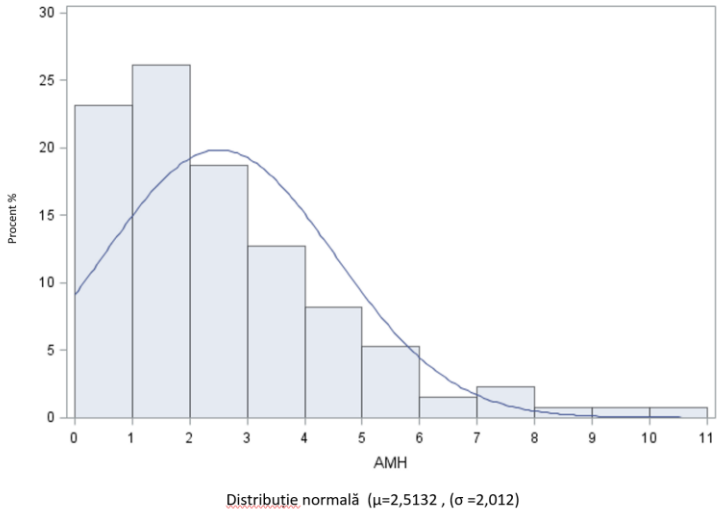

Fig. 7.2. Preoperative AMH distribution for the group of 134 patients

The linear regression of the preoperative AMH value with the patients' age produces the regression line with the ordinate at the origin 6.66 (standard error =1.20) and slope -0.13

(standard error =0.04) with the probability $p<=0,001$. The decrease of the preoperative AMH value with advancing age is statistically significant.

For patients who achieved pregnancy after surgery, group G, the mean value of preoperative AMH was 2.59 ng/mL, the standard deviation was 2.10, with a median of 2.00 ng/mL, and the range of reference being 0.02-10.20 ng/mL.

For patients who did not achieve pregnancy after surgery, the NG group, the mean preoperative AMH value was 2.29 ng/mL, the standard deviation was 1.74, with a median of 1.94 ng/mL, and the reference range being 0.01-7.24 ng/mL.

It is noted that the mean preoperative AMH value was higher in the group of patients who got pregnant, but it is not statistically significant, according to the statistical test Two sided two sample t(df=132) =0.75, p=0.454.

The preoperative AMH value decreases significantly with each year of advancing age, for each of the 2 groups of patients. The preoperative AMH value decreases more rapidly with age in the NG group than in the G group. It is expected that the rate of decrease of the preoperative AMH value with each year of advancing age is greater by 0.01 (standard error=0.08, p=0.87) in the NG group, but it is not statistically significant.

In the group of patients with IVF pregnancy, the mean preoperative AMH value is significantly lower by 1.60 than in the group of patients with spontaneous pregnancy, according to the Two-sided two sample test t(df=97) =-4.04, $p<=0,001$.

7.3.3. The EFI score in group A

The EFI score in group A has a mean value of 5.20, a standard deviation of 1.97, with a median of 5.00 (range 1-9). Of the 134 patients, 35 did not get pregnant after surgery, and 99 got pregnant naturally or through assisted human reproduction techniques.

In the group of patients who got pregnant at least 1 year postoperatively, the mean value of the EFI score was 5.10, the standard deviation was 1.91, with a median of 5.00, and the value range was 1-9.

In the group of patients who did not achieve pregnancy at least 1 year postoperatively, the mean value of the EFI score was 5.49, the standard deviation was 2.12, with a median of 5.00, and the range of values was 2-9.

The mean value of the EFI score is higher in the NG group compared to the G group, according to the statistical test Two-sided two sample T t(df=132) =0.99, p=0.322, not statistically significant.

7.3.4. The association between EFI and AMH

We investigate the association between the EFI score and preoperative AMH value for the 134 patients in group A who had AMH measured before surgery. The correlation between the EFI score and the preoperative AMH value is 0.29 (p=0.001), being statistically significant.

The linear regression of the EFI score with the preoperative AMH value produces the regression line with the ordinate at the origin 4.49 (standard error =0.26) and slope 0.28 (standard error =0.08) with the probability p=0.001, statistically significant result – Fig. 7.3.

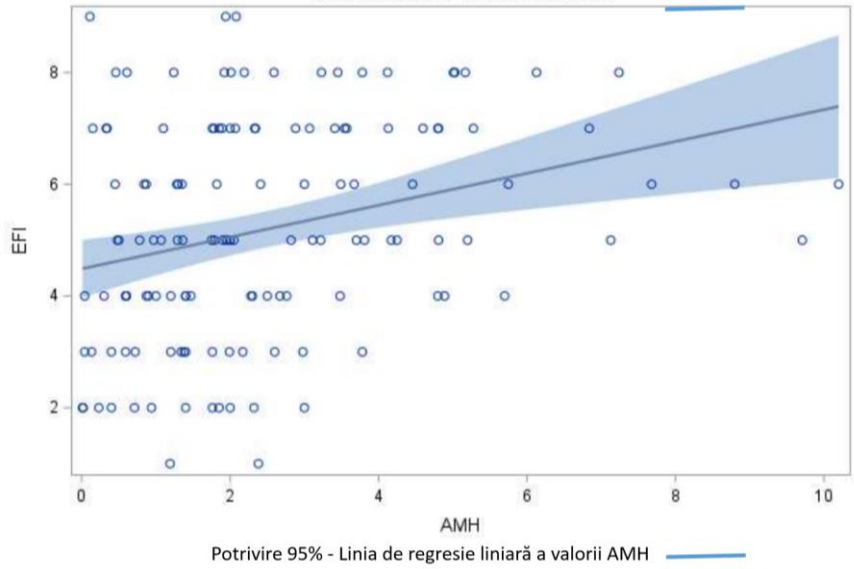

Fig. 7.3. Association of EFI score with preoperative AMH value – Regression line and 95% confidence levels

7.3.5. Ovarian cystectomy in group A

The type of ovarian surgery for patients with endometriosis is being studied. Of the 134 patients in group A, 23 did not undergo ovarian cystectomy, 42 had left ovarian cystectomy performed, 27 had right ovarian cystectomy performed and 42 had bilateral ovarian cystectomy performed - Table 7.1

Table 7.1. Distribution of patients in group A according to type of ovarian surgery and pregnancy

Pregnancy	Ovarian cystectomy				Total
	No	Left	Right	Bilateral	
YES	14	33	19	33	99
NO	9	9	8	9	35
Total	23	42	27	42	134

The association between the type of ovarian surgery and pregnancy is not significant according to Chi Square test χ^2 (df=3) = 3.15, *p*=0.369.

We investigate the value of the EFI score following surgery according to the type of ovarian surgery (no intervention, left ovarian cystectomy, right ovarian cystectomy, and bilateral ovarian cystectomy). The 4-category ANOVA test (no cystectomy, left cystectomy, right cystectomy, and bilateral cystectomy) for the EFI value after surgery fails to reject the null hypothesis - the EFI value does not differ on average among all 4 groups: F (3,130) =1, 26, *p*=0.293. The ANOVA test shows that the EFI value is not significantly associated with ovarian surgery.

We investigate the association of preoperative AMH value in the 4 ovarian surgery groups (no intervention, left ovarian cystectomy, right ovarian cystectomy, and bilateral ovarian cystectomy). The 4-category ANOVA test (no cystectomy, left cystectomy, right cystectomy, and bilateral cystectomy) for the preoperative AMH value fails to reject the null hypothesis - the AMH value does not differ on average among all 4 groups: F (3,130)=0.24, p =0.866, indicating that the preoperative AMH value is significantly associated with ovarian surgery, from a statistical point of view.

We investigate the age of patients in the 4 groups of ovarian surgical intervention (no intervention, left ovarian cystectomy, right ovarian cystectomy and bilateral ovarian cystectomy). The ANOVA test with 4 categories (no intervention, left ovarian cystectomy, right ovarian cystectomy and bilateral ovarian cystectomy) for age rejects the null hypothesis - the age of the patients does not differ in all 4 groups: F (3,130) =3.12, p=0.028, which indicates that age is significantly associated with ovarian surgery, from a statistical point of view – Fig. 7.4.

Two sample two sided T tests for pairs of ovarian surgery categories indicate that, on average, the age in the group without cystectomy is significantly higher than in each of the other 3 groups: the group with bilateral cystectomy (t(df= 63)=2.84,p=0.006), the group with right cystectomy (t(df=48)=2.70,p=0.010) and in the group with left cystectomy (t(df=63)=2, 77, p=0.007).

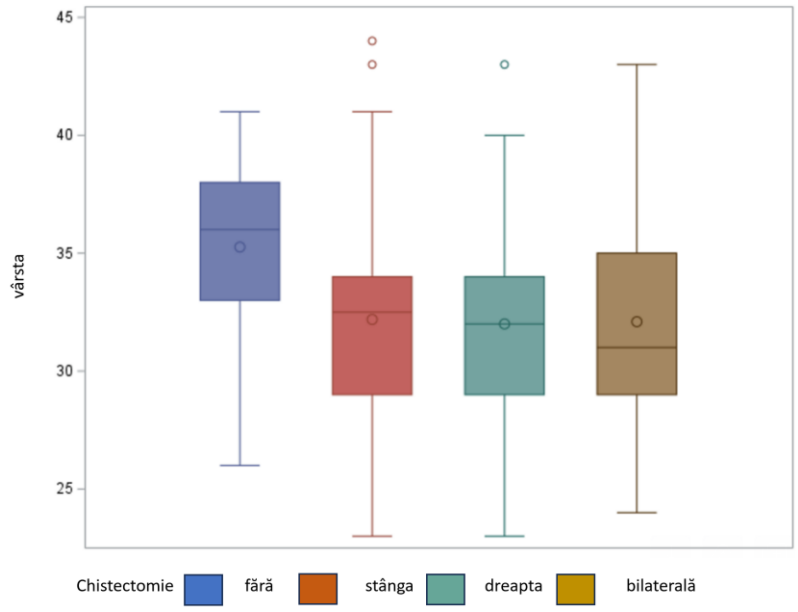

Fig. 7.4. Age distribution of patients in the 4 groups of ovarian surgery

7.3.6. Fertility prognosis in group A

The mean preoperative AMH value was higher in group G, statistically insignificant (p= 0.454). The mean value of the EFI score is higher in the NG group compared to the G group, statistically insignificant (p =0.322). The mean value of the patients' age is higher in the NG group compared to the G group, statistically insignificant (p=0.908). The association between the type of ovarian surgery and pregnancy is not significant (p=0.369).

Therefore, none of the 4 factors (AMH, EFI, type of ovarian surgery and age) is directly associated with pregnancy rate after surgery. It is possible that the 4 predictors taken together as a single group influence the chances of pregnancy.

The results of the logistic regression indicate that for patients with a mean age of 32.65 years, performing ovarian cystectomy for the diagnosis of ovarian endometrioma in patients diagnosed with endometriosis significantly increases the chances of obtaining pregnancy postoperatively in patients who want compared to patients without surgical

treatment. Ovarian cystectomy is essential for young patients (under 32.65 years) diagnosed with endometriosis (at the ovarian level).

The mean values of the predictors used to determine the fertility prognosis of patients diagnosed with endometriosis who wish to achieve pregnancy (spontaneous or through IVF) after surgical treatment are as follows: AMH =2.51 ng/dl, EFI =5.20 and age = 32.65 years. Thus, it is expected that patients with values equal to or greater than these results will have a significantly improved chance of becoming pregnant after surgery (in this case, ovarian cystectomy). Fig. 7.5. illustrates and summarizes the results of the logistic regression analysis.

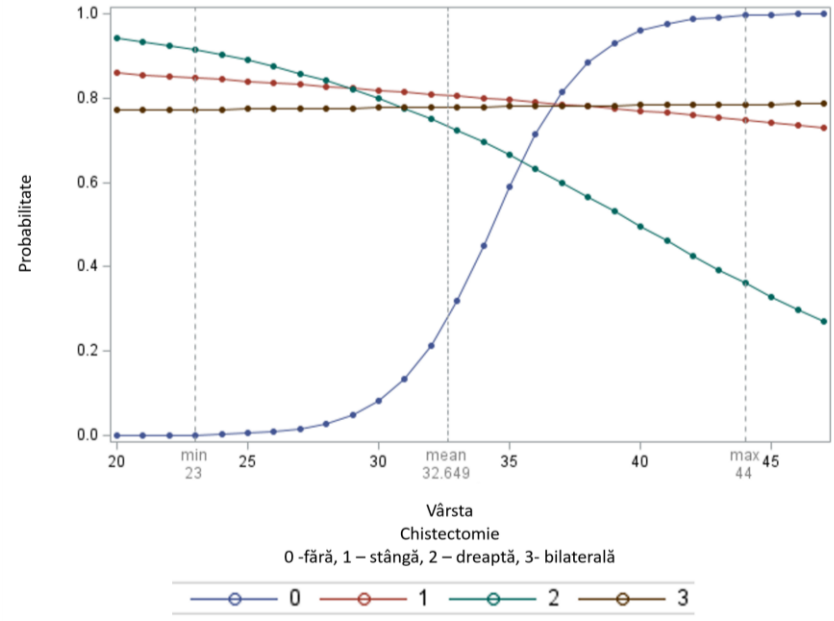

Fig. 7.5. Estimated probabilities of achieving pregnancy in the ovarian cystectomy groups reduced logistic regression

7.4. Discussions

The surgical treatment of endometriosis aimed to improve spontaneous fertility in addition to the complete excision of the lesions, improving the quality of life and preventing the recurrence of endometriosis. The EFI score is the most commonly used predictor to prognosticate the rate of achieving a spontaneous pregnancy within three years after surgery for endometriosis [18].

The existence of a system for quantifying the chances of achieving a natural pregnancy after a complex surgical intervention, with the excision of all endometriotic implants, the restoration of the anatomy of the pelvis and the elimination of the factors that

maintain a chronic inflammatory status is important, and it should be based in addition to the EFI score and the age of the patients and the ovarian reserve (AMH) and the type of ovarian surgery. Although the EFI score is calculated based on surgical results and anamnestic factors (age, infertility), combining preoperative AMH with surgical results, particularly ovarian, has been shown to be useful in studying the chances of to get pregnant postoperatively.

Although the focus in this study was on the impact of ovarian cystectomy, we did not eliminate the importance of excision of endometriotic lesions at the pelvic level. Endometriomas characteristic of advanced stages of endometriosis can contribute to the formation of a pelvic adhesion syndrome, to the torsion of the fallopian tubes and to the difficulty of the physiological processes of ovulation, fertilization and nidation [29]. All these aspects are included in the EFI score, by calculating the functional postoperative score.

Preoperative AMH levels can be used to assess the likelihood of a poor postoperative ovarian response and to identify patients with low ovarian reserve prior to surgery. The role of laparoscopic excision of endometriotic lesions in improving pregnancy rates in infertile women with endometriosis is controversial. A minimal decrease or eventual increase in postoperative AMH is due to compensatory mechanisms that occur following ovarian damage, such as recruitment and proliferation of primordial follicles and hyperactivation of granulosa cells. This causes the reorganization of the cohort of follicles, including those producing AMH, which represents the "recovery" of the ovarian reserve [30].

Our study showed that the mean preoperative AMH value was higher in the group of patients who got pregnant at least one year after the operation, 2.59 ng/mL vs. of 2.29 ng/mL, for those who did not become pregnant. Mean AMH values were higher in patients who got pregnant spontaneously 3.48 ng/mL vs. patients who underwent an IVF procedure: 1.88 ng/mL, the values being significantly different (lower by 1.60 ng/mL, $p<0.001$).

The preoperative AMH level may be a useful indicator for predicting the likelihood of a spontaneous pregnancy and may be provided as a component of the preoperative patient evaluation.

The EFI score is a proven element used to predict the incidence of spontaneous conception after surgery for endometriosis. It is generally accepted in the literature that a decision threshold of 5 indicates a good chance of spontaneous pregnancy [31-33].

In the present study, the mean value of the EFI score is significantly higher in the group of patients who did not achieve pregnancy after surgical treatment compared to the group of patients who achieved pregnancy (5.72 vs. 5.13, $p=0.015$), results that do not

support the null hypothesis (the EFI score is lower for patients who do not achieve pregnancy).

Conversely, the EFI score is lower in patients who achieved pregnancy through IVF compared to those who conceived spontaneously after surgery. Also, the present study demonstrates that the EFI value decreases with advancing age.

The management of patients with infertility associated with endometriosis is different depending on age, the stage of endometriosis and the desire to regain fertility, each case being treated appropriately to individual needs and conditions.

8. Study III: The analysis of the intestinal microbiota of patients with endometriosis

8.1. Objectives

Main Objective

Establishing a correlation between the intestinal microbiota and the occurrence/development of endometriosis.

Secondary objectives

1. Identifying a diagnosis/prevention strategy - gut microbiota assessment can be used as a screening test in the diagnosis of endometriosis, combined with clinical and imaging examination.

2. Studying a therapeutic method – antibiotics, probiotics, prebiotics combined with hormonal and surgical treatment.

8.2. Patients and methods

The present study is a retrospective, observational study that included a total number of 9 patients with endometriosis according to the inclusion and exclusion criteria presented in chapter 5.

All patients enrolled in the study were examined with TVS and with a 3.0 Tesla nuclear magnetic resonance system and underwent surgical treatment of endometriosis, with the final diagnosis of endometriosis established by histopathological examination.

Stool samples were analyzed to assess microbiome status using the GI Effects® Comprehensive Stool Profile test (proprietary of Genova Diagnostics). With the help of this test panel, we obtained information regarding several inflammatory markers - calprotectin, β-glucuronidase, eosinophil cationic protein A, fecal secretory IgA and 8 commensal microbial species detected microscopically and by PCR. Descriptive statistics for categorical variables were expressed as frequencies and percentages of abnormal tests.

8.3. Results

8.3.1. Socio-demographic and clinical characteristics of the group

We included in this study nine patients diagnosed with endometriosis, and the following quantitative variables obtained from the anamnesis and clinical and paraclinical examinations were studied: age, year of inclusion in the study, medical unit where the study was carried out, symptoms, imaging aspects of endometriosis lesions, surgical intervention, inflammatory markers - calprotectin, β-glucuronidase, eosinophil cationic protein A, fecal secretory IgA and 7 commensal microbial species detected microscopically and by PCR. The small number of patients enrolled in the study is due to the extremely high cost of the intestinal microbiota test.

The average age of the patients was 33.55 years with a median of 34 years. The distribution by age category was relatively balanced - they follow a potentially symmetrical distribution, according to the Shapiro Wilk test, p =0.42.

The patients were investigated in two centers: the Obstetrics and Gynecology Clinical Hospital "Prof. Dr. Panait Sârbu" Bucharest and Monza Hospital, Bucharest. No study participant was under oral contraceptive treatment or had an intrauterine device inserted. No patient reported sexual intercourse in the three days prior to sample collection. All patients were diagnosed with deeply infiltrative endometriosis with the help of TVS and MRI, presenting endometriomas (77.77%), recto-sigmoid endometriosis nodules (66.66%) and lesions at the level of the uterosacral ligaments (55.55%).

The symptoms of the patients consisted of dysmenorrhea (100%, on various VAS values), dyspareunia (55.55%) and gastrointestinal disorders (88.88%). Regarding the existence of gastrointestinal symptoms (transit disorders – constipation/diarrhea, dyskesia, abdominal flatulence) and the imaging identification of endometriosis lesions at the recto-sigmoid level, 6 out of 9 patients showed images suggestive of nodules on pelvic MRI images recto-sigmoid endometriosis.

8.3.2. Intestinal microbiota test

The intestinal microbiota test confirms the presence of a commensal intestinal flora that is rich in species reported in the literature. We identified a pattern of inflammation-associated dysbiosis as follows in the results presented. We did not create a control lot to

compare the results because the GI Effects® Comprehensive Stool Profile test used compares the abundance and diversity of the seven major bacterial phyla in commensal species with values considered normal in the standard population, which could indicate more significant differences in gut microbiota composition of patients with endometriosis [34].

7 commensal microbial species: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Euryarcaeota, Fusobacteria, Verrucomicrobia were investigated in the stool sample of the 9 patients, together with the detection of Clostridium spp (Fig.8.1.).

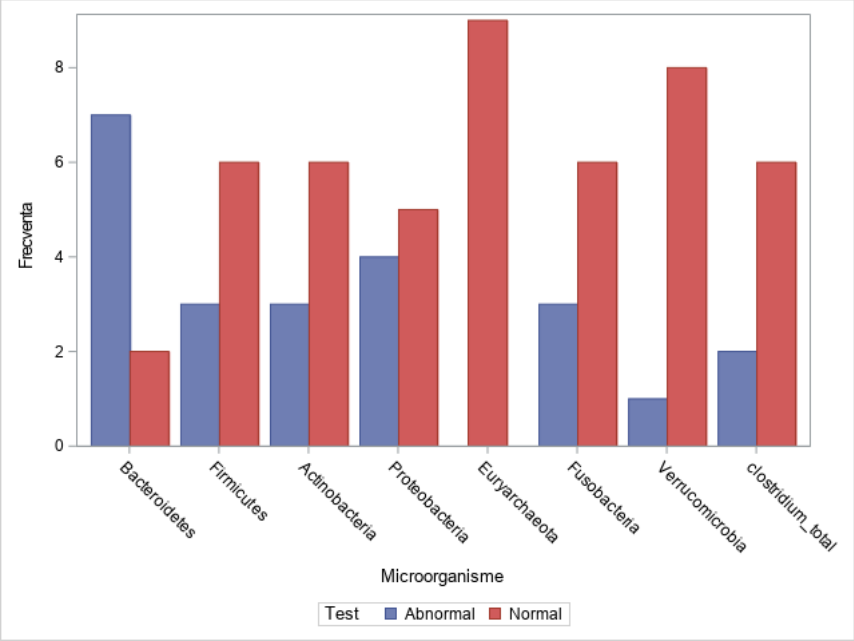

Fig.8.2. Frequency of abnormal and normal clinical tests for microorganisms in the intestinal microbiota of patients

The Firmicutes/Bacteroidetes ratio (FB ratio) is mentioned in the literature as an important marker of intestinal dysbiosis, its increase or decrease indicating an imbalance of the intestinal microbiota [34]. The value of this ratio is normally <1.5. The ratio was calculated for 7 of the 9 patients, and the results were between 0.5 and 35, with 5 abnormal values vs 2 normal values.

We analyzed the Genova Diagnostics proprietary inflammation-associated dysbiosis (IAD) score for all 9 patients. Mean IAD score was negatively associated with commensal abundance and positively associated with faecal calprotectin, EPX and secretory IgA [35].

In the test performed, the considered normal level of the IAD score is 60. All values <60 are considered high, associated with inflammation, and values below 60 are considered to have a low risk of inflammatory status. We obtained values for the 9 patients, 6 with

normal results, and the other 3 abnormal, higher than the upper limit of normal. Their average was 28.44, with a median of 10 (values being between 0 and 74).

There is sufficient evidence in our cohort of 9 patients to suggest that the Bacteroidetes test is more likely to be abnormal than normal in the population of women with endometriosis. Clopper-Pearson 90% one-tailed test confidence intervals are (0.51 - 1.00). It is completely to the right of 0.5 (chance), which also indicates that the Bacteroidetes test is more likely to be abnormal than normal in the population of women with endometriosis. This suggests that there is sufficient evidence in our sample that the proportion of abnormal clinical tests is greater than pure chance (1/2).

For the other 7 commensal microbial species (Firmicutes, Actinobacteria, Proteobacteria, Euryarcaeota, Fusobacteria, Verrucomicrobia, Clostridium spp), for the Firmicutes/Bacteroidetes ratio and for inflammatory markers (calprotectin, β-glucuronidase, eosinophil cationic protein A, secretory IgA in faeces), the right-sided exact binomial test gives a p-value ≥ 0.10, so we fail to reject the null hypothesis that a "clinical test" is less than or at least as likely to be abnormal in the population of women with endometriosis.

We failed to reject the null hypothesis, suggesting that there is insufficient evidence in our cohort to reject that the proportion of abnormal test equals ½ (pure chance). With only 9 patients enrolled in the study, the results cannot be transferred to the population level, which is a limitation of our analysis. Continuing the study by enrolling more patients with endometriosis may lead to more accurate results, comparable to those studied in the specialized literature.

8.4. Discussions

The literature related to a possible correlation between endometriosis and intestinal dysbiosis supports the theory of an altered immune response through dysbiotic changes, which promote and maintain a chronic inflammatory response, altering the population of immune cells and favoring the growth of endometriotic implants [36].

The relationship between endometriosis and gut microbiota is bidirectional, endometriotic implants promote the maintenance of bacterial pathogens through changes in estrogen metabolism [23].

Endometriosis appears to be associated with increased levels of various microorganisms in the gut microbiome: Clostridia, Prevotella, Proteobacteria, Firmicutes,

Bacteroidetes, Enterobacteriaceae, Brucellacea, Klebsiella, Veillonella, Ruminococcus [37,38,39].

The intestinal microbiota of the patients enrolled in the study shows high levels of bacteria from the Bacteroidetes phylum, 7 patients associating abnormal values, above the threshold value considered to be normal, findings supported by literature studies [37-40].

In the case of the presence of beta-glucuronidase activity, Dabek performed a study on 40 different bacterial strains, which are part of the dominant bacterial groups in the intestinal flora, identifying nine strains with beta-glucuronidase activity [41]. The phylum Firmicutes appears to be most associated with a high level of beta-glucuronidase [41].

The 3 studied inflammatory markers (secretory IgA in faecal matter, faecal calprotectin, EPX) demonstrate the activation of different immune cells in the immune response pathways. The IAD score investigates whether inflammation-associated dysbiosis is a cause and/or an effect of inflammation [35]. This score could be used to diagnose intestinal inflammation associated with endometriosis and especially intestinal endometriosis, it being possible to hypothesize that an inflammatory intestinal microbiome pattern precedes the increase of inflammatory markers and favors the development of endometriotic implants.

A strength of the current study is the investigation of associations between gut microbiome profile and stool biomarkers and the presence of endometriosis lesions, using biomarkers and the IAD score currently used specifically for inflammatory bowel disease. These three inflammatory biomarkers could also be used for the diagnosis of deeply infiltrative endometriosis, especially endometriosis with rectosigmoid infiltration.

Not enough comprehensive studies have been performed to include the 3 biomarkers as well as the IAD score in the noninvasive diagnosis of deeply infiltrative endometriosis.

9. Final conclusions and personal contributions

9.1. Final conclusions

The doctoral research led to conclusions supported by statistical processing. The applied statistical methods allowed the formulation of conclusions based on reasoned evidence, the etiopathology of which is scientifically confirmed in this doctoral thesis.

Final conclusions – study I:

1. TVS is the first line of diagnostic imaging, with a high accuracy in detecting ovarian endometriomas (84.47%) and intestinal endometriotic nodules (73.82%).
2. MRI has superior accuracy to TVS examination for parametrial endometriosis lesions (89.19% vs. 32%) and uterosacral ligament endometriosis lesions (66.1% vs. 55%)
3. Rectosigmoid endometriosis lesions are diagnosed in a similar way by the two methods, the diagnostic accuracy of TVS (73.82%) being comparable to the diagnostic accuracy of MRI examination (80.79%).
4. Advantages of imaging methods: TVS and MRI examination are noninvasive, accessible and specific techniques. Prognosis, quality of life and fertility can be improved and controlled by an early DIE diagnosis.
5. The MRI examination complements and improves the diagnosis provided by TVS in the correct establishment of a diagnosis of deep infiltrative endometriosis
6. The management of patients with endometriosis is done in a multidisciplinary team: gynecologists, radiologists, surgeons (colorectal, thoracic surgery), urologists, nutritionists, psychologists and specialists in physical therapy or pain management.
7. Benefits: high accuracy of preoperative evaluations in patients with endometriosis may reduce the number of incomplete or unnecessary surgeries. An early diagnosis of DIE prevents chronicity, progression of lesions, decrease in ovarian reserve and fertility, and promotes improvement of quality of life through early and individualized treatment.

Final conclusions – study II:

1. Fertility prognosis of endometriosis patients after surgery can be evaluated based on several markers: EFI score, preoperative AMH value, type of ovarian surgery and patient's age.
2. All the analyzed parameters formulate an algorithm for predicting the chances of obtaining a spontaneous pregnancy postoperatively, with the decision of the optimal moment to send patients to an assisted human reproduction department.
3. The serum level of AMH dosed preoperatively (2.51 ng/ml) and the threshold value of the EFI score ≥5.2 in patients with a mean age of 32.65 years diagnosed with endometriosis are useful markers for evaluating the fertile prognosis related to achieving a spontaneous pregnancy following surgical treatment for DIE.
4. Surgical treatment of endometriosis significantly increases the chances of getting pregnant postoperatively compared to patients without surgical treatment.

Final conclusions – study III:

1. Gut microbiota can be used as a screening test in the diagnosis of endometriosis, combined with clinical and imaging examination.
2. 2. Gut microbiota influences the development and progression of endometriosis lesions
3. 3. Marked intestinal dysbiosis is associated with the existence of intestinal endometriosis lesions
4. 4. The phylum Bacteroidetes is found in the intestinal microbiota of patients with endometriosis.
5. 5. The intestinal microbiome precedes the increase of inflammatory markers (secretory IgA in faecal matter, faecal calprotectin, eosinophil cationic protein A), exacerbating endometriosis lesions.

9.2. Personal contributions

Personal contributions start with the conception of the study design and the emphasis in turn of the two directions: diagnosis and prognosis; creating a database with 265 (256 + 9) patients with obtaining information through anamnesis, clinical and imaging examination and studying the medical records, after obtaining the written informed consent of each patient;

interpreting the statistical results in a way that follow the objectives of the 3 studies; comparing the results obtained with data reported in the specialized literature; creating the graphics, figures and tables presented in the doctoral thesis.

Elements of innovation can be found in each of the 3 studies in the doctoral thesis. The first study emphasizes the importance of the certification of gynecologists and radiologists in the imaging evaluation of patients suspected of endometriosis. The high accuracy of the preoperative evaluations of these patients by means of the two imaging methods (TVS and MRI) depends on the experience of the examiner and leads the therapeutic management towards the eradication of endometriosis lesions and the improvement of fertility, by establishing an early and individualized treatment.

The second study supports the establishment of a surgical treatment of endometriosis after its correct diagnosis, demonstrating the statistically significant association of performing curative surgical intervention with the concurrent object of preserving and improving spontaneous conception. Another innovative element is the association of several fertility prognostic markers, in addition to the EFI score, used as the gold standard: preoperative AMH value, age, type of ovarian surgery ore. All these parameters strengthen the ability to correctly predict the chance of obtaining a spontaneous pregnancy postoperatively.

The third study proposes adding a non-invasive diagnosis through the determination of the intestinal microbiota component and inflammatory markers (secretory IgA in faeces, faecal calprotectin, cationic protein of eosinophils A) together with the IAD score, addressed to patients suspected of endometriosis who present gastrointestinal symptoms (excluding inflammatory bowel disease) associated with symptoms of chronic pelvic pain, dysmenorrhea or dyspareunia. Research on these diagnostic markers is at an early stage, and their association is not reported in the literature in studies of human subjects diagnosed with endometriosis.

The three studies that make up this doctoral thesis integrate the information and results in the notion of a diagnostic panel, and the weight in establishing the diagnosis and prognosis increases by using all these previously presented elements.

In this way, the present thesis, through the accumulation of obtained results, contributes to raising the level of knowledge in the field and raises new research approaches, in which the deepening and reporting of the results to the population can improve the diagnostic and therapeutic management of patients with endometriosis, considering their prognosis.

Selective Bibliography

1. Berkley KJ, Stratton P. Mechanisms: lessons from translational studies of endometriosis. In: Giamberardino MA, editor. Visceral Pain: Clinical, Pathophysiological and Therapeutic Aspects. Oxford: Oxford University Press; 2009. pp. 39–50.
2. Speroff, L., Glass R. H., Kase, N. G. (1999). Clinical gynecologic endocrinology and infertility. Lippincott Williams & Wilkins, p 1057
3. R.E. Batt ”A History of Endometriosis”, 2011, Springer, pg 50-52, 118-119
4. Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. 2016 Feb 26;2(2):CD009591. doi: 10.1002/14651858.CD009591.pub2. PMID: 26919512; PMCID: PMC7100540.
5. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. Fertil Steril. 1991 Apr;55(4):759-65. doi: 10.1016/s0015-0282(16)54244-7. PMID: 2010001.
6. Missmer, S.A.; Hankinson, S.E.; Spiegelman, D.; Barbieri, R.L.; Marshall, L.M.; Hunter, D.J. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. *Am. J.Epidemiol.* **2004**, *160*, 784–796. [CrossRef] [PubMed]
7. Hansen KE, Kesmodel US, Baldursson EB, et al. Visceral syndrome in endometriosis patients. Eur J Obstet Gynecol Reprod Biol 2014; 179:198.
8. Mark D Hornstein, MD, William E Gibbons, MD - Treatment of infertility in women with endometriosis, 2017 [uptodate]
9. Koninckx PR, Heyns W, Verhoeven G, Van Baelen H, Lissens WD, De Moor P, et al. . Biochemical Characterization of Peritoneal Fluid in Women During the Menstrual Cycle. *J Clin Endocrinol Metab* (1980) 51(6):1239–44. doi: 10.1210/jcem-51-6-1239 [PubMed] [CrossRef] [Google Scholar] [Ref list]
10. Vallvé-Juanico, J.; Santamaria, X.; Vo, K.C.; Houshdaran, S.; Giudice, L.C. Macrophages Display Proinflammatory Phenotypes in the Eutopic Endometrium of

Women with Endometriosis with Relevance to an Infectious Etiology of the Disease. *Fertil. Steril.* 2019, *112*, 1118–1128. [CrossRef] [PubMed]

11. Hesla JS., Rock J.A., 2003; Endometriosis. In: Rock JA, Jones HW., Te Linde's Operative Gynecology, Ninth Edition, Lippincott Williams & Wilkins, Philadelphia; 593-638.
12. Xue Q, Lin Z, Cheng YH, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod 2007;77:681-687
13. Guilherme Tavares de Arruda, Patricia Driusso, Jéssica Cordeiro Rodrigues, Amanda Garcia de Godoy, Mariana Arias Avila. (2022) Numerical rating scale for dysmenorrhea-related pain: a clinimetric study. *Gynecological Endocrinology* 38:8, pages 661-665.
14. Hendarto H. (2012). Pathomechanism of Infertility in Endometriosis, Endometriosis - Basic Concepts and Current Research Trends, Prof. Koel Chaudhury (Ed.), pp 344-350, ISBN: 978-953-51-0524-4
15. **Baușic, A.I.G.;** Coroleucă, C.; Coroleucă, C.; Comandașu, D.; Matasariu, R.; Manu, A.; Frîncu, F.; Mehedințu, C.; Brătilă, E. Transvaginal Ultrasound vs. Magnetic Resonance Imaging (MRI) Value in Endometriosis Diagnosis. Diagnostics 2022, 12, 1767. https://doi.org/10.3390/diagnostics12071767 (**IF=3,992** :2022)
16. American society for reproductive medicine(ASRM)(1997)Revised American society for reproductive medicine classification of endometriosis.Fertil Steril. 1996;67:817–821.[PubMed]
17. Keckstein, J, Saridogan, E, Ulrich, U A, et al. The #Enzian classification: A comprehensive non-invasive and surgical description system for endometriosis. Acta Obstet Gynecol Scand. 2021; 100: 1165– 1175. https://doi.org/10.1111/aogs.14099
18. G.D. Adamson et al. "Endometriosis fertility index: the new, validated endometriosis staging system." Fertil Steril 2010;94:1609–15.
19. Ogunrinola, G.A.; Oyewale, J.O.; Oshamika, O.O.; Olasehinde, G.I. The Human Microbiome and Its Impacts on Health. Available online: https://doaj.org
20. Cho, I.; Blaser, M.J. The Human Microbiome: At the Interface of Health and Disease. *Nat. Rev. Genet.* 2012, *13*, 260–270. [CrossRef] [PubMed]
21. Benner, M.; Ferwerda, G.; Joosten, I.; van der Molen, R.G. How Uterine Microbiota Might Be Responsible for a Receptive, Fertile Endometrium. *Hum. Reprod. Update* 2018, *24*, 393–415. [CrossRef]

22. Baker, J.M.; Chase, D.M.; Herbst-Kralovetz, M.M. Uterine Microbiota: Residents, Tourists, or Invaders? *Front. Immunol.* 2018. [CrossRef]
23. Bulun, S.E., et al., Role of estrogen receptor-beta in endometriosis. *Semin Reprod Med*, **2012**. 30(1): p. 39-45.
24. **Bauşic, A.I.G**.; Matasariu, D.R.; Manu, A.; Brătilă, E. Transvaginal Ultrasound vs. Magnetic Resonance Imaging: What Is the Optimal Imaging Modality for the Diagnosis of Endometriosis? *Biomedicines* **2023**, *11*,2609.https://doi.org/10.3390/biomedicines11102609
25. Hudelist, G.; Oberwinkler, K.H.; Singer, C.F.; Tuttlies, F.; Rauter, G.; Ritter, O.; Keckstein, J. Combination of transvaginal sonography and clinical examination for preoperative diagnosis of pelvic endometriosis.*Hum.Reprod.* **2009**, *24*, 1018–1024. [CrossRef] [PubMed]
26. Cazalis, C.G.; Koskas, M.; Martin, B.; Palazzo, L.; Madelenat, P.; Yazbeck, C. Imagerie préopératoire dans l'endométriose profonde: Échographie pelvienne, écho-endoscopie rectale et IRM. *Gynécologie Obs. Fertil.* **2012**, *40*, 634–641. [CrossRef]
27. Alborzi, S.; Rasekhi, A.; Shomali, Z.; Madadi, G.; Alborzi, M.; Kazemi, M.; Nohandani, A.H. Diagnostic accuracy of magnetic resonance imaging, transvaginal, and transrectal ultrasonography in deep infiltrating endometriosis.*Medicine* **2018**, *97*, e9536. [CrossRef] [PubMed]
28. Saba, L.; Guerriero, S.; Sulis, R.; Pilloni, M.; Ajossa, S.; Melis, G.; Mallarini, G. Learning curve in the detection of ovarian and deep endometriosis by using Magnetic Resonance: Comparison with surgical results. *Eur. J. Radiol.* **2011**, *79*, 237–244. [CrossRef]
29. Barnhart, K.M., Dunsmoor, R., Su, M.S., & Coutifaris, C. (2002). Effect of endometriosis on in vitro fertilization. Fertil. Steril., Vol. 77, pp. 1148-1155, ISSN 1556-5653.
30. Ozaki R., Kumakiri J., Tinelli A., Grimbizis G.F., Kitade M., Takeda S. Evaluation of factors predicting diminished ovarian reserve before and after laparoscopic cystectomy for ovarian endometriomas: A prospective cohort study. *J. Ovarian Res.* 2016;9:37. doi: 10.1186/s13048-016-0241-z. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

31. Benoit L, Boujenah J, Poncelet C, Grynberg M, Carbillon L, Nyangoh Timoh K, et al. Predicting the likelihood of a live birth for women with endometriosis-related infertility. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. November 2019;242:56–62. 10.1016/j.ejogrb.2019.09.011 [PubMed] [CrossRef] [Google Scholar]
32. Li X, Zeng C, Zhou Y-F, Yang H-X, Shang J, Zhu S-N, et al. Endometriosis Fertility Index for Predicting Pregnancy after Endometriosis Surgery. *Chin Med J*. 2017;130(16):1932. 10.4103/0366-6999.211892 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
33. Zhou Y, Lin L, Chen Z, Wang Y, Chen C, Li E, et al. Fertility performance and the predictive value of the endometriosis fertility index staging system in women with recurrent endometriosis: A retrospective study. *Medicine*. September 2019;98(39):e16965. 10.1097/MD.0000000000016965 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
34. **Bauşic, A.I.G**, Cretoiu S.M., Bauşic V., Matasariu D.R., Stănculescu R.V., Bratilă E. ”The role of gut dysbiosis in endometriosis diagnosis and treatment approaches - case report”, Vol. 64 No. 2, 2023, ROMANIAN JOURNAL of MORPHOLOGY and EMBRYOLOGY DOI: 10.47162/RJME.64.2.17
35. Chen L, Reynolds C, David R, Peace Brewer A. Development of an Index Score for Intestinal Inflammation-Associated Dysbiosis Using Real-World Stool Test Results. Dig Dis Sci. 2020 Apr;65(4):1111-1124. doi: 10.1007/s10620-019-05828-8. Epub 2019 Sep 16. PMID: 31529411; PMCID: PMC7069909.
36. Soo Hyun Ahn, Kasra Khalaj, Steven L. Young, Bruce A. Lessey, Madhuri Koti, Chandrakant Tayade,Immune-inflammation gene signatures in endometriosis patients,Fertility and Sterility,Volume 106, Issue 6,2016,Pages 1420-1431.e7,ISSN 0015-0282, https://doi.org/10.1016/j.fertnstert.2016.07.005.
37. Ata, B., et al., The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls. *Sci Rep*, **2019**. 9(1): p. 2204.
38. Chadchan, S.B., et al., Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. *Hum Reprod*, **2019**. 34(6): p. 1106-1116.
39. Svensson, A., et al., Associations Between Endometriosis and Gut Microbiota. *Reprod Sci*, **2021**. 28(8): p. 2367-2377.

40. Yuan, M., et al., Endometriosis induces gut microbiota alterations in mice. *Hum Reprod*, **2018**. 33(4): p. 607-616.
41. Dabek, M., et al., Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria. *FEMS Microbiol Ecol*, **2008**. 66(3): p. 487-95.

- ## List of published scientific papers

- **Bauşic, A.I.G.;** Coroleucă, C.; Coroleucă, C.; Comandaşu, D.; Matasariu, R.; Manu, A.; Frîncu, F.; Mehedinţu, C.; Brătilă, E. Transvaginal Ultrasound vs. Magnetic Resonance Imaging (MRI) Value in Endometriosis Diagnosis. Diagnostics 2022, 12, 1767. https://doi.org/10.3390/diagnostics12071767 (**IF=3,992** :2022) - chapter 6

- **Bauşic, A.I.G**, Cretoiu S.M., Bauşic V., Matasariu D.R., Stănculescu R.V., Bratilă E. "The role of gut dysbiosis in endometriosis diagnosis and treatment approaches - case report", Vol. 64 No. 2, 2023, ROMANIAN JOURNAL of MORPHOLOGY and EMBRYOLOGY DOI: 10.47162/RJME.64.2.17 (**IF=1** , 2023) – chapter 6

- **Bauşic, A.I.G**.; Matasariu, D.R.; Manu, A.; Brătilă, E. Transvaginal Ultrasound vs. Magnetic Resonance Imaging: What Is the Optimal Imaging Modality for the Diagnosis of Endometriosis? *Biomedicines* **2023**, *11*, 2609. https://doi.org/10.3390/biomedicines11102609 (**IF= 4,7**, 2023) - chapter 6