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Endometriosis: A Retrospective Analysis on Diagnostic Data in a Cohort of 4,401 Patients

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Abstract. *Background/Aim: Endometriosis is a gynecological estrogen-dependent inflammatory disease due to ectopic endometrial tissue and often associated with pelvic pain. Despite its high prevalence, there are still uncertainties about its pathogenesis, diagnosis, and therapy. Patients and Methods: This study presents a retrospective study conducted on 4,401 endometriosis patients, 584 of which underwent laparoscopic procedures. The archived data about clinical signs, magnetic resonance imaging (MRI) results, topography of the endometriosis lesions (obtained via laparoscopy) associated diseases, sample analysis and histological findings were analyzed. Next, the statistical associations between the information for each case, provided by these diagnostic tools were determined. Results: MRI is the most sensitive and specific diagnostic system for ovarian lesions, but poor in sensitivity and specificity for deep endometriosis lesions and not indicated for peritoneal lesions which remain the exclusive prerogative of laparoscopy. Clinical signs are essential for diagnosing deep lesions. The Ca125 and Ca19.9 markers have a poor reliability and their negativity in symptomatic patients has no clinical value, while in positive cases it could probably be used as a monitoring parameter. Conclusion: The results generated will help provide an accurate picture of the topography and distribution of endometriotic lesions. Correlation analyses between the data generated by the clinical-instrumental examinations and those on the site of the disease identified by laparoscopy, allow to define the predictive value of the clinical-instrumental signs in the diagnosis and localization of endometriotic disease.*

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Endometriosis is a chronic inflammatory gynecological disease, characterized by the presence of endometrial tissue outside the uterine cavity. The most well-known sites of these endometriosis structures are the pelvic peritoneum and organs, as well as the ovaries; in such cases, these implants are strongly associated with pelvic pain symptoms (1). It is a common condition affecting up to 10% of all women in their reproductive years; this prevalence dramatically increases up to 30-50% in women suffering also from chronic pelvic pain and infertility (2). Endometriosis is considered an estrogen-dependent disorder; in fact, surgical or natural menopause can significantly ameliorate the clinical condition of patients (3). Moreover, several *in vitro* studies (4-8) have demonstrated that estrogens promote the growth of endometrial epithelial and stromal cells.

Nonetheless, endometriosis is still an enigmatic disease; the pathogenesis, diagnosis, and therapy are not completely defined. Its mode of development remains uncertain, but the occurrence of this condition is commonly attributed to the spreading of endometrium outside the uterine cavity and the consequent formation of ectopic endometrial implants (9, 10), which are responsible for the associated symptoms. The current debate is about the time and mechanism through which these implants are formed. The best known pathogenetic theory is the one proposed by Sampson about one century ago (11); it states that ectopic implants of menstrual shedding can reach the abdominal cavity through the Fallopian tubes and grow on the peritoneal surface. Brosens and Benagiano recently suggested a different mechanism, in which the primary phenomenon is the neonatal uterine bleeding, leading to hormonal deprivation, experienced by many female newborns in a retrograde fashion (12). This can consequently cause the formation of endometriosis implants, that would remain until puberty. Lately, various research groups have produced substantial

experimental data supporting a third theory, that claims the persistence of remnants of the embryonic Müllerian ducts in ectopic locations and its correlation with the endometriosis condition (13-16). Molecular evidence attributes the ectopic dislocation of these embryonic remnants to the perturbation in the fine-tuning of the female genital system development during a critical window of time in the fetal life; these remnants would remain silent until puberty when, stimulated by estrogens, they would grow into endometriosis lesions (17, 18). Lastly, the endometriosis cells have similar progesterone resistance like embryo-fetal endometrium cells (19).

This model is also reinforced by the rise in the incidence of endometriosis in patients presenting uterine malformations (17). Nonetheless, our research group has recently demonstrated an endometriosis-like phenotype in mice exposed *in utero* to the endocrine disruptor bisphenol-A (20). Undeniably, robust epidemiological studies link the *in utero* exposure to an endocrine disruptor and the uprising of endometriosis later in adult life (21). Lastly, we have been able to prove, by using a genomic method for the assessment of the transcriptional profiling of the ectopic endometrium with the corresponding eutopic one, that numerous genes implicated in embryogenesis are differentially expressed in the endometriosis tissues and that this expression pattern is independent of the menstrual and hormonal phase (22). This last observation further reinforces the theory that endometriosis might be caused by a modification in gene expression during embryogenesis.

Endometriosis has striking morbidity accompanied by injurious effects on the social, personal, and professional life of affected women, as well as their communication with physicians; the most common symptoms include dysmenorrhea (cyclical pain as-sociated with menstruation), dyspareunia (pain with or following sexual intercourse), and pelvic or abdominal pain. Besides, the endometriosis condition is associated with infertility, but only occasionally as its cause (23).

Due to the lack of knowledge about this disease, to date, endometriosis is an incredibly underdiagnosed and undertreated condition, with a disproportionately long interval (8-12 years) between the beginning of symptoms and the conclusive diagnosis. This happens because most of the symptoms are non-specific and, at present, there are no non-invasive diagnostic tests capable of providing a definitive diagnosis, even if our research group has recently proposed some interesting candidates as molecular diagnostic markers (24-26). Today, the final diagnosis of endometriosis can be attained only *via* the histological examination of ectopic implants, whose tissue samples must be collected through invasive surgical or laparoscopic procedures.

This article presents a retrospective study conducted on a significant cohort of endometriosis patients, referred to a period of over 10 years (from 2000 to 2010). The entire cohort consisted of 4.401 patients, 584 of which had also undergone laparoscopic surgery. We analyzed and compared the anamnestic and clinical data of these patients, along with the imaging, blood sample analyses, physical vaginal and rectal examination and histological identification of the endometriosis lesions and their anatomical locations. The results are discussed considering the existing literature. Finally, we determined the association between outcomes of the various diagnostic approaches, using the histological results as a reference to evaluate the efficacy of the non-invasive tools, in the selected cohort that underwent the surgical procedure.

Patients and Methods

Patients. The retrospective evaluation was performed on a cohort of 4,401 endometriosis patients that visited the Italian Endometriosis Center in the period 2000-2010.

The non-invasive diagnostic protocol includes the vaginal and rectal examination with accurate screening of the fornixes of the cervix and the virtual space of the rectal vaginal septum, followed by rectal exploration with screening of the perineum, of the deep rectal canal, of the posterior wall of the uterus, utero-sacral ligaments and inferior branch of Mackenrodt's ligament. MRI is then performed to diagnose any ovarian endometriosis and the upper part of the uterus that escapes the bimanual examinations. We also considered the levels of two serum markers, cancer antigen 125 (CA125) and carbohydrate antigen (CA19-9), which are commonly adopted as biomarkers for ovarian and pancreatic cancers, respectively.

Within the entire cohort, 584 patients had undergone laparoscopy for diagnostic and therapeutic purposes; all of these patients were stage AFS III and IV. Therefore, the topographical data of the endometriosis lesions, obtained during these laparoscopic surgical procedures, were available only for this subset. These data were registered for the lesions whose macroscopic appearance met at least one of the following criteria: a) palpable and visible nodule(s)-adenomyosis or peritoneum induration and retraction in the posterior and lateral areas of the cervix, at the level of the uterosacral and medial broad ligaments or the rectovaginal septum; b) dark-blue nodule(s)-adenomyosis in the posterior vaginal wall, visible *via* speculum examination. Only the cases where the presence of endometriotic glands was confirmed histologically were included in this study.

Statistical analysis. Descriptive analyses of the sites where signs of endometriosis were detected *via* MRI, clinical examination, and surgical interventions are presented with percentages; prevalence of the patients with different levels of the indicated biomarkers are also given.

In order to detect possible associations between the different distribution of variables between groups of patients, the Fisher Exact test was used. Where there was a valid 2×2 contingency table, the Haldane-Anscombe correction for small groups was ap-plied, as some of the frequencies in the contingency tables were zeros.

Moreover, the Mann-Whitney *U*-test for non-parametric variables was used to assess any possible significant difference between medians of the AFS score in patients, grouped according to the categories of the variable under exam (presence/absence of disease, or different levels of biomarkers) (27). All analyses were performed

Table I. *Characteristics of the patients included in the study.*

Variable	Categories	Patients (%)
Demografics (N=4,401)		
Age (years)	<20	4 (<1%)
	20-24	38 (1%)
	25-29	151 (3%)
	30-34	414 (9%)
	35-39	804 (18%)
	40-44	1.082 (25%)
	45-49	878 (20%)
	50-54	386 (9%)
	55-59	96 (2%)
	≥60	18 (<1%)
	Not known	530 (12%)
Job	Employee	1.800 (41%)
	Freelance	1.112 (25%)
	Housewife/Retired	570 (13%)
	Student	396 (9%)
	Business Owner	184 (4%)
	Unemployed	11 (<1%)
	Not known	328 (7%)
Medical history (N=4.311)		
Age at menarche	<10	454 (11%)
	<12	1.962 (46%)
	<14	1.517 (35%)
	>14	236 (5%)
	Not known	142 (3%)
Children	No children	199 (5%)
	One child	502 (12%)
	Two or more children	382 (9%)
	Not known	3.228 (75%)
Endometriosis surgery	No surgery	1.938 (45%)
	Already undergone surgery	2.373 (55%)
Immune disorders	No disorders	2.652 (62%)
	Disorders	1.659 (38%)
Period of treatment with contraceptive pill	Up to 60 months	2.111 (49%)
	From 61 to 120 months	937 (22%)
	More than 120 months	432 (10%)
	Not known	831 (19%)
Period of treatment with analogues	Not treated	3.253 (75%)
	Up to 6 months	730 (17%)
	More than 6 months	328 (8%)
Ovarian stimulations	No stimulation	2.658 (62%)
	One stimulation	56 (1%)
	Two or more stimulations	162 (4%)
	Not known	1.435 (33%)

using Python 3.7.10 libraries pandas, scipy.stats and statistics (www.python.org). A *p*-Value below 0,05 was associated with statistical significance.

Ethical approve and consent to participate. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000 and 2008. The study was approved by the Scientific Committee of Fondazione Italiana Endometriosi. All patients provided written informed consent before enrollment in the study to permit the use of the data generated in retrospective analyses.

Table II. *Distribution of clinical signs for 4,207 patients.*

Anatomical site	Present (%)	Absent (%)
Adenomyosis	2.187 (52%)	2.020 (48%)
Posterior fornix	1.384 (33%)	2.823 (67%)
Left uterosacral ligament	1.169 (28%)	3.038 (72%)
Recto-vaginal septum	977 (23%)	3.230 (77%)
Left fornix	721 (17%)	3.486 (83%)
Right uterosacral ligament	678 (16%)	3.529 (84%)
Anterior rectal wall	533 (13%)	3.674 (87%)
Right fornix	405 (10%)	3.802 (90%)
Pre-rectal fibers	368 (9%)	3.839 (91%)
Left parametrium	161 (4%)	4.046 (96%)
Right parametrium	111 (3%)	4.096 (97%)
Posterior vaginal wall	65 (2%)	4.142 (98%)
Anterior fornix	57 (1%)	4.150 (99%)
Central bladder wall	41 (1%)	4.166 (99%)
Right bladder wall	33 (1%)	4.174 (99%)
Left vaginal wall	49 (1%)	4.158 (99%)
Left bladder wall	45 (1%)	4.162 (98%)
Abdominal wall	3 (<1%)	4.204 (99%)
Anterior vaginal wall	5 (<1%)	4.202 (99%)
Right vaginal wall	19 (<1%)	4.188 (99%)
Right round ligament	8 (<1%)	4.199 (99%)
Left round ligament	7 (<1%)	4.200 (99%)

Results

Patients. Table I summarizes the anamnestic data of the whole patient cohort. In detail, data depicted in the table concern: age of the patients, age at menarche, the number of children, the job of patients, the presence of concomitant immune disorders, the period of treatment with contraceptive pill, the number of ovarian stimulations, the period of treatment with analogues.

Clinical topographic signs. Table II reports the prevalence of clinical signs for the various topographical areas usually interested by endometriosis. These data were available for a subset of 4,207 subjects. According to these results, the main areas showing clinical signs associated with endometriosis were the posterior wall of the uterus, the posterior fornix, the left uterosacral ligament, and the rectovaginal septum; however, none of them were detected in the majority of the patients (*i.e.,* prevalence ≥50%), showing poor predictive values.

Serum markers. The information about the CA125 and CA19-9 levels, reported in Table III, were partial (data available only for 2,373 patients). However, within the subset of available data, the majority of the patients exhibited normal values (<35 U/ml). These results were in line with many previous studies (28, 29). In fact, at present, there is still no serum marker that correlates significantly

Table III. *Biomarker levels of the 2.679 endometriosis patients (61% of entire cohort).*

Parameter	N° of patients (%)	% of cohort (N=4.401)
CA125 levels		
1	1,839 (69%)	42%
≥35 U/ml	702 (26%)	16%
Unknown	1,782 (5%)	42%
CA19-9 levels		
<35 U/ml	2,178 (81%)	50%
≥35 U/ml	236 (9%)	5%
Unknown	265 (10%)	45%

Table IV. *Detection of endometriosis implants/tissue via MRI for 2,072 patients.*

MRI site	Present (%)	Absent (%)
Left ovary	1,208 (58%)	864 (42%)
Right ovary	1,159 (56%)	913 (44%)
Uterus	747 (36%)	1.325 (64%)
Recto-vaginal septum	231 (11%)	1.841 (89%)
Bladder	145 (7%)	1.927 (93%)
Abdominal wall	137 (7%)	1.935 (93%)

Table V. *Distribution of endometriosis lesions according to the surgical results obtained via laparoscopy for 584 endometriosis patients III and IV AFS stage.*

Anatomical site	Present (%)	Absent (%)
Deep peritoneum	502 (86%)	82 (14%)
Pouch of Douglas	384 (66%)	200 (34%)
Dense adhesions left ovary	334 (57%)	250 (43%)
Dense adhesions right ovary	285 (49%)	299 (51%)
Deep left ovary	279 (48%)	305 (52%)
Deep right ovary	247 (42%)	337 (58%)
Dense adhesions left tube	240 (41%)	344 (59%)
Dense adhesions right tube	184 (32%)	400 (68%)
Superficial peritoneum	29 (5%)	555 (95%)
Superficial right ovary	30 (5%)	554 (95%)
Superficial left ovary	31 (5%)	553 (95%)
Filmy adhesions right ovary	21 (4%)	563 (96%)
Filmy adhesions left ovary	19 (3%)	565 (97%)
Filmy adhesions right tube	16 (3%)	568 (97%)
Filmy adhesions left tube	18 (3%)	566 (97%)

with endometriosis. Besides, this is in contrast with the few studies identifying CA125 as an endometriosis marker (30).

MRI results. Table IV illustrates the diagnosis outcomes based on MRI. These data were available for a subset of 2.072 subjects. With this diagnostic technique, the presence of possible endometriosis tissue was observed mainly in the ovaries, followed by the uterus, while deep sites of endometriosis were detected in a minority of cases.

Topographic laparoscopic data. Table V summarizes the topographical data about the endometriosis lesions, determined *via* laparoscopic procedures. These data were available for a subset of 584 subjects, that underwent surgical procedures. The highest incidence was found in the deep peritoneum, while the superficial peritoneum exhibited a very low incidence; a similar discrepancy between superficial and deep tissues was observed also for both the ovaries. Moreover, the occurrence of endometriosis lesions was significant in the pouch of Douglas as well.

Associations between different diagnostic tools. We investigated the statistical associations among the diagnostic results for biomarker levels, clinical signs, and MRI, and with respect to the topographical data obtained *via* laparoscopy, which served as a sort of reference. In detail, in Table VI the significant associations between the anatomical sites where endometriosic implants/tissue were detected *via* MRI, and those identified *via* other diagnostic tools are depicted. In Table VII the significant associations between biomarkers levels, and the anatomical sites where endometriosic implants/tissue were detected *via* anatomical examination and laparoscopy are indicated. In Table VIII, the significant associations between clinical signs, and the anatomical sites where endometriosic implants/tissue were detected *via* laparoscopy are reported. Finally, in Table IX the significant associations between the anatomical sites where endometriotic implants/tissue were detected *via* MRI, and those identified *via* other diagnostic tools are indicated. This analysis was performed only on the sub-sample of patients in which all analyses were executed (N=378).

Discussion

We conducted a retrospective study on a cohort of 4,401 endometriosis patients, which were assisted by the Italian Endometriosis Center in the period 2000-2010. In this article, we analysed separately the anamnestic data, the clinical signs, and MRI results, as well as the histologically determined topography of the endometriosis lesions, and compared them with what was reported in previous studies. Then, we determined the statistical associations between the outcomes of these diagnostic tools; the histological data, laparoscopically obtained for a subset of 584 patients, served as the reference to evaluate the effectiveness of the non-invasive techniques for the endometriosis diagnosis since such an approach is still the gold standard in this field.

Table VI. *Association between the anatomical sites where endometriosic implants/tissue were detected via MRI and those identified via other diagnostic tools.*

MRI sites (N=2,072)	Associated clinical signs (N=2,019)	Associated biomarkers (N=1,873)	Associated laparoscopy (N=584)
Right ovary	Left fornix, Recto-vaginal septum, Right uterosacral ligament, Left parametrium		Deep right ovary, Dense adhesions right ovary, Dense adhesions right tube
Left ovary	Recto-vaginal septum	CA125 levels ≥35 U/ml CA19-9 levels ≥35 U/ml	Deep left ovary, Dense adhesions left ovary, Dense adhesions left tube
Recto-vaginal septum	Left vaginal wall, Adenomyosis	CA125 levels ≥35 U/ml	
Bladder	Posterior fornix, Recto-vaginal septum, Pre-rectal fibers	CA125 levels ≥35 U/ml	Deep right ovary, Deep left ovary, Dense adhesions right ovary
Abdominal wall	Posterior fornix, Anterior rectal wall, Recto-vaginal septum, Right uterosacral ligament, Abdominal wall	CA125 levels ≥35 U/ml	Deep left ovary, Dense adhesions right ovary, Dense adhesions left ovary
Uterus	Right fornix, Left fornix, Anterior fornix, Pre-rectal fibers, Right uterosacral ligament		Deep right ovary, Deep left ovary, Pouch of Douglas, Dense adhesions left ovary

Associations were tested using the Fisher's Exact test (Fisher_exact function from the scipy.stats Python 3.7.10 module). A p-Value <0.05 was considered statistically significant.

Table VII. *Association between biomarkers levels, and the anatomical sites where endometriosic implants/tissue were detected via anatomical examination and laparoscopy.*

Biomarkers	Associated clinical signs (N=2,473)	Associated laparoscopy sites (N=509)
CA125 levels	Posterior fornix, Left fornix, Anterior rectal wall, Recto-vaginal septum, Right uterosacral ligament, Left uterosacral ligament, Left bladder wall, Adenomyosis	Deep right ovary, Deep left ovary, Pouch of Douglas, Dense adhesions right ovary, Dense adhesions left ovary, Dense adhesions right tube, Dense adhesions left tube
CA19-9 levels	Recto-vaginal septum	Deep left ovary, Dense adhesions left ovary, Dense adhesions left tube

Associations were tested using the Fisher's Exact test (fisher_exact function from the scipy.stats Python 3.7.10 module). A p-Value <0.05 was considered statistically significant.

Concerning the anamnestic data, the most important observations are the following. Regarding the relationship between endometriosis and infertility, unfortunately, the data about previous pregnancies were available only for one quarter of the group (1,083 patients). However, within this subset, 81.6% of patients had given birth to one or more children; this percentage is in agreement with the study of Sensky and Liu (31) and in contrast with that of Verkauf (32), which reported that 30-50% of endometriosis patients exhibit fertility issues. Thus, this partial result confirms that the endometriosis condition is associated with infertility, but does necessarily cause it (33). As for the age at menarche, the results are coherent with those of Parazzini *et al*. (34) and Hemmings *et al*. (35), which concluded that this parameter does not correlate with the endometriosis risk. Therefore, they also are in contrast with the claim of Missmer *et al*. (36) and Nnoaham *et al*. (37), which reported an increased risk associated with ear-ly menarche (before 11-12 years of age). However, a deeper evaluation of this relationship requires to consider also the age of the patients since the mean age at menarche has been changing (decreasing) during the last century in many developed countries, and it is being levelled-off only recently (38).

The majority of the patients did not suffer from any concurrent autoimmune disease; nonetheless, the incidence of such diseases in the entire cohort was still significant with respect to the average values for the whole female population worldwide, which ranges between 12% (39) and 32% (40). This confirmed the association between endometriosis and various autoimmune diseases reported by Shigesi *et al*. (41). A total of 71% of patients took the contraceptive pill for at least 5 years and up to more than 10 years, confirming the massive use of hormones based on ethiliestradiol. On the other hand, treatment with ovarian activity suppressants (GNRH analogues) was used little and for a short time. These data confirm the tendency to prescribe estrogen-

Table VIII. *Association between clinical signs detected by anatomical examination, and the anatomical sites where endometriosic implants/tissue were detected via laparoscopy.*

Clinical signs	Associated laparoscopy sites (N=560)
Posterior fornix	Superficial right ovary, Dense adhesions left ovary
Right fornix	Superficial peritoneum, Filmy adhesions right ovary, Filmy adhesions left ovary, Filmy adhesions right tube, Filmy adhesions left tube
Left fornix	Dense adhesions left tube
Anterior fornix	Superficial peritoneum, Superficial left ovary, Pouch of Douglas, Filmy adhesions right ovary, Filmy adhesions left ovary, Filmy adhesions right tube, Filmy adhesions left tube
Anterior rectal wall	Pouch of Douglas
Recto-vaginal septum	Superficial peritoneum, Deep peritoneum, Pouch of Douglas
Pre-rectal fibers	Pouch of Douglas, Dense adhesions left tube
Right uterosacral ligament	Superficial left ovary, Filmy adhesions right ovary, Filmy adhesions left ovary, Filmy adhesions right tube
Left uterosacral ligament	Deep peritoneum
Central bladder wall	Superficial peritoneum, Superficial right ovary, Pouch of Douglas, Filmy adhesions right ovary, Filmy adhesions left ovary, Filmy adhesions right tube, Filmy adhesions left tube
Right bladder wall	Superficial peritoneum, Pouch of Douglas, Filmy adhesions right ovary, Filmy adhesions left ovary, Filmy adhesions right tube, Filmy adhesions left tube
Anterior vaginal wall	Superficial peritoneum

Associations were tested using the Fisher's Exact test (fisher_exact function from the scipy.stats Python 3.7.10 module). $p<0.05$ was considered statistically significant.

containing drugs for estrogen-dependent disease with a strong stromal component such as profound disease and adenomyosis, which are prevalent in this cohort. Nonetheless, it must be considered that stromal disease is extremely rich in aromatase which allows a greater use of estrogen by this particular type of tissue (42). The clinical signs showed partial association with the histological data and poor association with the MRI results. The presence of endometriosis lesions was detected mainly in the ovaries and uterus *via* MRI. This diagnostic tool detected endometriosis implants in the bladder and rectovaginal septum only in a few cases. With regard to the bladder, since previous studies have shown the efficiency of this technique when analysing this area (1, 43, 44), we could assume that this result, for the analysed subset of patients, correctly reflected a low prevalence of endometriosis in this organ; in fact, the percentage of cases (7%) is compatible with the work of Buorgioti *et al*. (45), which reported involvement of the urinary tracts in 4% of endometriosis patients. As for the rectovaginal septum, previous reports (44) have highlighted the poor sensitivity of MRI for the rectal area and, thus, this result alone cannot be sufficient to exclude an underestimation of the endometriosis tissue incidence in this anatomical site.

Concerning the topographical data on the endometriosis lesions, as determined *via* laparoscopic procedures, the highest prevalence was found in the deep peritoneum, in agreement with what is generally known on the main locations of endometriosis implants (1), while the superficial peritoneum exhibited a very low prevalence; a similar discrepancy between superficial and deep tissues was also observed for both the ovaries. Moreover, the occurrence of endometriosis lesions was significant in the pouch of Douglas as well. The high prevalence in the ovaries and the pouch of Douglas agrees with what was reported by Munksgaard and Blaakaer (46) and Nezhat *et al*. (47). Furthermore, the results of this imaging technique showed a good association with the histological data for almost all the anatomical sites considered, except for the rectal area, confirming the poor sensitivity of MRI for this specific location. Finally, the histology-based topographical information revealed a difference in the occurrence of endometriosis lesions between deep and superficial tissues and organs, and confirmed their prevalence in the peritoneum ovaries and Douglas pouch. These results confirm the precious role of MRI for the diagnosis of endometriosis, except for the rectal area (44), especially in the preoperative phase (45, 48-50). Thus, although laparoscopy is required for a definite diagnosis, MRI can significantly help identify the endometriosis condition, excluding other diseases with similar symptomatology.

Finally, the information about the CA125 and CA19-9 levels were in line with many previous studies (1, 28, 29) since, within the subset of available data, the majority of patients exhibited normal values (<35 U/ml); at present, there is still no serum marker correlated significantly with endometriosis, also because they are usually associated with other conditions. Nevertheless, this is in contrast with the few studies identifying CA125 as an endometriosis marker (30). Interestingly, high levels of serum markers correlated with the presence of the disease at the level of the ovaries. The prevalence of Ca 19.9 marker positivity in the left ovary, given the higher statistical incidence of the risk of clear cell ovarian cancer or endometrioid carcinoma in ovaries that retain endometriosis in peri-post menopause, is in good agreement with the mild prevalence of ovarian carcinoma (51, 52) on the left, even if found on a small cohort of cases. Important correlations of topography and disease prediction are highlighted when we analysed the correlation between the clinical signs examined on the cohort of 560 who

Table IX. *Association between the anatomical sites where endometriotic implants/tissue were detected via MRI, and those identified via other diagnostic tools, only on the subsample of patients to whom all analyses were performed (N=378).*

MRI sites	Clinical signs	Biomarkers	Laparoscopy sites
Right ovary	Anterior rectal wall		Deep right ovary, Dense adhesions right ovary, Dense adhesions right tube
Left ovary		CA19-9 levels ≥35 U/ml	Deep left ovary, Dense adhesions left ovary, Dense adhesions left tube
Recto-vaginal septum	Right vaginal wall		
Bladder	Posterior fornix, Recto-vaginal septum, Pre-rectal fibers		Deep right ovary, Dense adhesions right ovary
Abdominal wall	Adenomyosis		Deep left ovary, Dense adhesions right ovary, Dense adhesions left ovary
Uterus	Pre-rectal fibers, Right uterosacral ligament		Deep right ovary, Deep left ovary, Pouch of Douglas

Associations were tested using the Fisher's Exact test (fisher's_exact function from the scipy.stats Python 3.7.10 module). $p<0.05$ was considered statistically significant. Note: Some tests could not be performed as the contingency tables were not 2×2; one or more of the groups were missing due to no patients being measured for that particular site and diagnostic tool.

underwent laparoscopy, as illustrated in tab 8. These data are able to determine a mapping of endometriosis in relation to the data found by the physical examination. A similar result is obtained when we analyse the association between the anatomical sites where endometriotic implants/tissue were detected *via* MRI, and those identified *via* other diagnostic tools, only on the subsample of patients to whom all analyses were performed.

It is possible to summarize the most important evidence that derives from the statistical analysis of this large amount of data. MRI is the most sensitive and specific diagnostic system for ovarian lesions, but is poor in sensitivity and specificity for deep endometriosis lesions and not indicated for peritoneal lesions which remain the exclusive prerogative of laparoscopy. Clinical signs (particularly vaginal and rectal examination) are essential for diagnosing deep lesions where both MRI and laparoscopy (when the lesions are retroperitoneal) do not give effective diagnostic results. The Ca125 and Ca19.9 markers have a poor reliability and their negativity in symptomatic patients has no clinical value, while in positive cases it could probably be used as a monitoring parameter. Laparoscopy correlates well with the distribution of endometriosis and adenomyosis. For the identification of extraperitoneal lesions, frequent in deep disease, the determination of clinical signs is essential, in particular the vaginal and rectal examination. This is essential in order to perform a correct intervention aimed at the complete removal of the lesions. Consequently, the produced data suggest that using diagnostic laparoscopy alone to detect all the patient's endometriotic lesions is insufficient, especially with regard to extra-peritoneal lesions.

Conclusion

In conclusion, this article describes and analyses a substantial and robust number of clinical and instrumental data obtained from a large cohort of patients with endometriosis. The results generated help provide an accurate picture of the topography and distribution of endometriotic lesions. The correlation analyses between the data generated by the clinical-instrumental examinations and those on the site of the disease identified by laparoscopy, allows to define the predictive value of the clinical-instrumental signs in the diagnosis and localization of endometriotic disease.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, P.G.S. and A.B; methodology, R.V., M.S. and V.M.; formal analysis, M.C.; writing—original draft preparation, P.G.S. and A.B; writing—review and editing, P-G.S. and A.B.; funding acquisition, P.G.S. All Authors have read and agreed to the accepted version of the manuscript. The Authors would also like to thank Dr. Lia Orfei for her contribution to the statistical analysis.

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