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ACCURACY OF NEW SONOGRAPHIC MARKERS IN THE DIAGNOSIS OF ADENOMYOSIS

Presentata dalla Dott.ssa

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Abstract

Objective: to evaluate the diagnostic accuracies of well-known sonographic markers of adenomyosis and of two innovative ones, the *question mark sign* and the *transvaginal ultrasound uterine tenderness*.

Methods: 78 patients scheduled for hysterectomy for uterine benign diseases underwent preoperative transvaginal ultrasonography to evaluate the criteria of sonographic diagnosis of adenomyosis as reported by consensus statement MUSA. Adenomyosis was diagnosed in presence of two or more of the following parameters: asymmetry of the uterine walls, hyperechoic striae, anechoic myometrial cysts, hyperechogenic islands, echogenic subendometrial lines and buds, interruption/irregularities of the junctional zone and translesional vascular flow. In addition the *question mark sign* and the *transvaginal ultrasound uterine tenderness* were evaluated, the first being the longitudinal section of the uterus with a morphology similar to a question mark and the other being the dynamic ultrasound evaluation of uterine tenderness by the pressure of the transvaginal probe. Sonographic features were compared with histological examination.

Results: the prevalence of adenomyosis in the sample is 33.3%. Sensitivity, specificity, positive and negative predictive values and accuracy of transvaginal ultrasound in the diagnosis of adenomyosis are 83%, 96%, 91%, 89% and 92%. Asymmetry, hyperechoic striae and interruption of the junctional zone were the

most accurate markers for the diagnosis of adenomyosis. Myometrial heterogeneity was the most frequently encountered feature (100%), but showed a low specificity (7%). The *question mark sign* and the *transvaginal ultrasound uterine tenderness* showed sensitivity, specificity, positive and negative predictive values and accuracy of 41%, 96%, 83%, 77%, and 69% and 69%, 65%, 66%, 81% and 67% respectively.

Conclusions: the sonographic markers proposed by consensus statement MUSA were confirmed accurate in the diagnosis of adenomyosis in our sample. The *question mark sign* and the *transvaginal ultrasound uterine tenderness* showed good diagnostic capacities and may be a useful complement in the sonographic diagnosis of adenomyosis.

Introduction

Adenomyosis is a benign condition of the uterus defined by the presence of endometrial glands and stroma within the myometrium. Adenomyosis affects around 20% of women during their fertile age and may be associated to dysmenorrhea, menorrhagia and infertility[1].

A diagnosis of certainty can be posed only by histological examination. Several studies showed that transvaginal sonography (TVS) can be considered the first-line imaging modality for studying adenomyosis, because it is as sensitive and as specific as magnetic resonance,[2–6] nevertheless univocal ultrasound parameters for the diagnosis of adenomyosis are still lacking.[7] Recently the MUSA (Morphological Uterus Sonographic Assessment) consensus statement proposed terms, definitions and measurements that may be used to describe and report the sonographic features of the myometrium using gray-scale sonography, color/power Doppler and three-dimensional ultrasound imaging, with particular regard to two conditions: adenomyosis and fibroids.[8] Even if many ultrasound features are supposed to be associated with adenomyosis, the diagnostic weight of each one is not clear and some features may be more relevant than others in order to formulate a diagnosis.[8] A particular shape of the uterine rime, called the *question mark sign*, has been recently described as a typical sign of adenomyosis associated with deep

infiltrating posterior endometriosis[9,10] and it was deliberately not included in the MUSA statement.[8] Transvaginal ultrasound is a dynamic examination[11], permitting to the operator to evaluate the tenderness of an examined anatomical structures by a gentle pressure of the probe. An enlarged and tender uterus, painful at mobilization may suggest adenomyosis[12]. The *TVS uterine tenderness*, that is the tenderness of the uterus during the gentle pressure with the transvaginal probe, could be useful to rule out the presence of adenomyosis, often associated to painful uterine mobilization.

The aim of our study is to establish the diagnostic accuracy of the ultrasound features associated to adenomyosis according to the MUSA statement and of two new markers, the *question mark sign*, evaluated independently from the presence of endometriosis, and the *TVS uterine tenderness*.

Patients and methods

This is a cross-sectional observational study enrolling all consecutive premenopausal women with a diagnosis of a benign uterine pathology, diagnosed by ultrasound or by hysteroscopy, and scheduled for hysterectomy from November 2014 to June 2016 in the Department of Gynaecology and Human Reproduction Pathophysiology, Sant'Orsola Hospital, University of Bologna. Postmenopausal women and those with a pre-surgical diagnosis of a reproductive tract cancer were excluded.

A data sheet with most relevant information on each patient's medical history was filled in: age, BMI, last menstrual period, gravidity and parity, previous pelvic surgery, previous diagnosis of endometriosis, presence of dysmenorrhea, menorrhagia and dyspareunia.

Transvaginal ultrasound examination was carried out using a 4–9-MHz probe with a three-dimensional (3D) facility (Voluson E8, GE Medical Systems, Zipf, Austria). All transvaginal ultrasound scans were performed in a standardized fashion by a single operator with more than 7 years of experience (L.Z.). Photos, clips and 3D scans were saved and stored for further examinations. The study of the uterine corpus was carried out as indicated by the MUSA statement.[8] In addition the sonographer evaluated subjectively the globular-

shape of the uterus, the tenderness of the uterus at the gentle pressure of the transvaginal probe and the presence of the *question mark sign* (Figure 1).[9, 10] A 3D volume of each uterus was stored following the method of Exacoustos[13] in order to evaluate the *junctional zone* (JZ).[8] The diagnosis of adenomyosis was posed when at least two of the ultrasound features studied were present.

Each patient underwent laparoscopic, laparotomic or vaginal hysterectomy according to her clinical condition within one month from the ultrasound examination. In each case the whole uterus was sent to histological examination, except one for which morcellation was needed.

All histopathological examinations were performed by the same pathologist, skilled in gynaecologic pathology and blinded to the ultrasound findings. For each uterus a series of samples were taken, including all the wall from the serosa to the endometrium. Of these, at least three samples were taken both from the posterior and from the anterior wall. The diagnosis of adenomyosis was posed if endometrial stroma and glands were present into the myometrial layer. Adenomyosis was reported as diffuse or focal and evaluated by the grade of invasion: limited to the internal half of the myometrium (M1) or full-thickness (M2). In case of doubt, an immunochemical test with CD10 antibodies was performed in order to highlight the ectopical endometrium.[14] For the purpose of this study, only the presence or absence of adenomyosis was considered, but not the depth of infiltration.

All the data were recorded in an electronic database. Patients were divided into two groups, according to the presence or absence of adenomyosis at the histological examination. Means and standard deviations were calculated for the continuous variables, using the Student's T-test. Relative frequencies were calculated for the categorical variables using the chi-squared test or Fisher's exact test. P value less than .05 was considered statistically significant. In order to compare gravidity and parity of the two groups, each one was divided into three classes, considering the number of pregnancies and deliveries. Agreement between TVS and histological diagnosis was measured with Cohen's Kappa coefficient. Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, positive (LH+) and negative (LH-) likelihood ratios and accuracy (area under curve ROC) of each TVS variable were calculated. Analyses were performed by using the Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA).

All the patients signed an informed consent and the study was approved by our local ethics committee (clinical trial ARC-ENDO n. 149/2014/O/Oss).

Results

Seventynine patients were enrolled in this study and one was excluded because the uterus was morcellated during laparoscopic hysterectomy, due to its great size. The total number of patients considered in the statistical analysis is 78. Forty/78 (51.2%) patients were operated on for leyomyomatosis, 24/78 (30.8%) for adenomyosis, 10/78 (12.8%) for uterine prolapse, 4/78 (0.5%) for fibroids with atypical ultrasound appearance. Hysterectomy was performed through laparoscopy in 62/78 (79.5%) patients, laparotomy in 6/78 (7.7%) and vaginal approach in 10/78 (12.8%) cases.

Histology showed adenomyosis in 26/78 (33.3%) patients, among them 16/26 (61.5%) presented fibroids and 6/26 (23.1%) presented adenomyomas. Among the 52/78 (66.6%) patients without adenomyosis 41/78 (52.5%) had fibroids, 1/78 (1.2%) had a spindle-like cells neoplasia with myogenic differentiation and mitotic index <4 M/10 HPF, and 10/78 (12.8%) showed hysterocele not associated to myometrial pathology.

Clinical features of patients are shown in Table 1. There are no statistically significant differences between the two groups with and without adenomyosis for age, BMI, gravidity and parity. Student's T-test and linear regression shows an inverse correlation between the uterine volume calculated by ultrasound and the diagnosis of adenomyosis. Nevertheless, by dividing the

two populations into quartiles of volumes, no statistically significant difference between the two groups can be found.

Percentage frequencies of symptoms and association with previous pelvic surgeries or with endometriosis are summarized in Table 2. All the diagnosis of endometriosis nodules suspected by TVS were confirmed by histology. Menorrhagia was significantly more frequent in patients with adenomyosis.

TVS diagnosed adenomyosis in 22/78 (28.2%) patients: in 20/22 (90.9%) cases ultrasound diagnosis was confirmed by the pathologist, while 2/22 (9.1%) cases were false positives. Among the 56/78 (71.8%) patients without ultrasound features of adenomyosis, 4/56 (7.1%) were false negatives, while 52/56 (92.9%) were true negatives. TVS diagnosed adenomyosis in 20/26 (76.9%) patients positives at histological examination, with sensitivity, specificity, PPV, NPV 83%, 96%, 91% e 89% respectively. Positive and negative likelihood ratio were 20 and 0.24. Global accuracy of TVS is 92.3%. Kappa analysis showed a good accordance between histology and TVS (kappa=0.760).

Table 3 shows the statistical significance of each ultrasound feature included in the study according to presence/absence of adenomyosis at histology. Hyperechoic islands and subendometrial lines and buds were not present in the examined sample. Table 4 shows diagnostic capacities of each ultrasound marker. Heterogeneous myometrium showed the highest sensitivity and PPV (both 100%). Most specific markers were JZmax≥8mm, fan-shaped

striations and *question-mark sign* (respectively 99%, 96% and 96%), with PPV respectively 100%, 88% and 83%.

Discussion

This is the first study strictly applying the MUSA indications[8] to prospectively validate the importance of each of these ultrasound features in the diagnosis of adenomyosis in a sample of 78 hysterectomies. In addition it showed two new ultrasound markers for the diagnosis of adenomyosis: the *question mark sign* and the *TVS uterine tenderness*, showing an accuracy of 69% and 67% respectively.

In this study 2D, 3D and power Doppler ultrasound features were associated in order to diagnose adenomyosis, obtaining diagnostic capacities superior than Kepkep *et al.*[15] and similar to those obtained in other studies, which report sensitivity up to 89% and specificity up to 100%.[16-18] In accordance with Bazot *et al.*[18] TVS is very specific, but prone to produce false negatives, which is the best condition for a test aiming to diagnose a benign pathology. In addition, our data show that TVS diagnostic capacity is reduced in the presence of comorbidities, as it was already demonstrated [3,18]: all the diagnostic mistakes (4 false negatives, 2 false positives) were made in patients affected by fibroids. A recent meta-analysis of 14 trials and 1985 participants reported sensitivity and specificity of ultrasound-diagnosed adenomyosis to be as high as 82.5 and 84.6%, respectively[19]. Our data showed a similar sensitivity but a greater

specificity, maybe due to the presence of two new sonographic markers of endometriosis.

The q*uestion mark sign* has been recently proposed by our group as a marker of adenomyosis strongly associated with deep infiltrating posterior endometriosis.[10] In the present study the *question mark sign* showed to be a marker of adenomyosis independent from the presence of endometriosis. Its strong association with adenomyosis is in contrast with MUSA consensus statement.[8] The question mark sign showed also great specificity (96%) and PPV (83%) with the best positive likelihood ratio among 2D ultrasound features. In this sample, only 4 out of the ten patients affected by deep infiltrating posterior endometriosis showed an associated *question mark sign* and Fisher's exact test excluded a correlation (p=0.245). These results suggest that *question mark sign* might have a wider application in diagnosing adenomyosis than previously thought.

As far as we are aware this is the first prospective study proposing *TVS uterine tenderness* as a marker of adenomyosis, showing a NPV of 81% and an accuracy of 67.3%. Original descriptions of adenomyosis reported an association between the disease and "a great deal of pain"[20]. Several later studies reported similar findings[21-23], but others have not shown significant differences in the prevalence of adenomyosis in women with and without a history of pain[24-26]. One possible confounder in the interpretation of pain could be the coexisting presence of endometriosis, which is a common cause of pain in women of reproductive age. We believe that the use of TVS, as a dynamic

examination[11], permits to show if the pain is related to the gentle pressure and mobilization of the uterus and permits as well to find the tenderness related to other location of endometriosis, if present.

Among the ultrasound features of adenomyosis the most specific were JZmax≥8mm, fan-shaped striations and *question-marked sign*. Our results are comparable to previous studies for the high specificity of fan-shaped striation[15-18] and of myometrial cysts[27], while Jzmax in our study showed better values than previously shown[27]. Heterogeneous myometrium is once again the most sensitive marker, but with very low specificity[18]. The main problem with the use of histology for the diagnosis of adenomyosis is the heavy selection bias incurred[28], indeed we had a very high percentage of leyomyomatosis, typically associated to heterogeneous myometrium at TVS.

Prevalence of adenomyosis in the sample is 33.3%, which is consistent with the Literature, where a mean value of 20-30% is reported in patients undergoing hysterectomy for various indications[29-31].

Differently from Literature[5,6,8] an association between increased uterine volume and adenomyosis was not found. Regarding this, it should be taken into account that patients without adenomyosis were often affected by leyomyomatosis, which also increases uterine volume. Nevertheless, Exacoustos *et al.* demonstrated a significant correlation between decreased uterine volume and adenomyosis, in comparison with uterus without fibroids[13].

Several strengths add power to this study: the use of histological confirmation of the diagnosis, the fact that all ultrasound scans were performed

using top-of-the-range equipment by a single operator, thereby minimizing interobserver variability and the fact that all the demographic, ultrasound and anamnestic data were collected prospectively. In particular it is remarkable that all the diagnosis of endometriotic nodules suspected by TVS were confirmed by histology. Moreover the choice of using wide inclusion criteria reduced the selection bias and allowed to evaluate adenomyosis in presence of numerous potentially confounding variables, as fibroids, that often reduce diagnostic accuracy[3,18]. Wide inclusion criteria are also a potential weakness of this study, as confounding factors, such as fibroids and hormonal treatments prevented some features from being detectable in several patients. Another main limitation of this study is the only inclusion of patients undergoing hysterectomy, creating a selection bias, as patients who chose surgery are more symptomatic than those who do not.

This study confirms TVS diagnostic accuracy in diagnosing adenomyosis, reinforcing TVS role as a first-line exam for its reliability, safety and cheapness. Comparing the ultrasound features considered currently to be typical of adenomyosis in a sample full of confounding factors demonstrates their validity even in less selected patients, providing an updated and realistic idea of TVS diagnostic capacities that could be applied in everyday clinical practice. The two new proposed features, the *question mark sign* and the *TVS uterine tenderness*, showed promising results and might prove to be useful for the diagnosis of adenomyosis. Further prospective studies are needed in order to prove their efficacy in wider samples.

Table 1. Population characteristic of 78 premenopausal patients according to presence/absence of adenomyosis at histology. Data are presented as mean ± standard deviation, or as % frequencies.

		Adenomyosis at histology		
Characteristic		Yes (*n*=26)	No (*n*=52)	P
Age (years)		48.2 ± 3.9	47.1 ± 3.9	n.s.
BMI		24.6 ± 1.1	25.5 ± 1.0	n.s.
Parity				
	0	38.5%	23.1%	n.s.
	1	23.1%	30.8%	n.s.
	>1	38.4%	46.1%	n.s.
Gravidity				
	0	30.8%	19.2%	n.s.
	1	23.1%	26.9%	n.s.
	>1	46.1%	53.9%	n.s.
Uterine volume		230 ± 189	295 ± 306	n.s.

Table 2. Clinical symptoms and association with previous pelvic surgeries and endometriosis according to presence/absence of adenomyosis at histology. Data are presented as frequencies.

	Adenomyosis at histology		
	Yes (n=26)	No (n=52)	P
Dysmenorrhea	77%	50%	n.s.
Dyspareunia	46%	31%	n.s.
Menorrhagia	85%	50%	0.045
Previous pelvic surgery	46%	46%	n.s.
Presence of endometriosis	30%	15%	n.s.

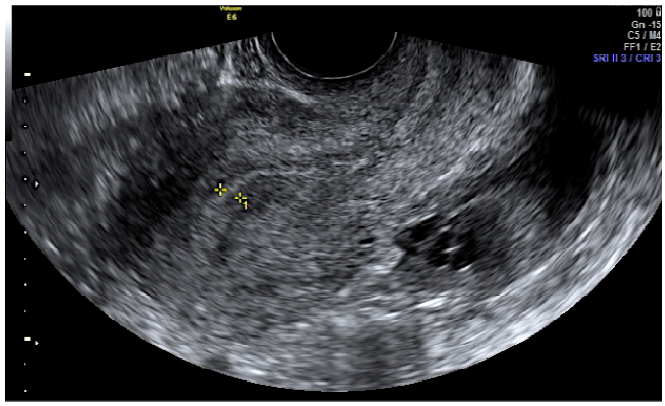
Table 3. Ultrasound features according to presence/absence of adenomyosis at histology. Data are presented as n(%). N.e. not evaluable.

	Adenomyosis at histology			
Features	Yes (n=26)	No (n=52)	N.e.	P
Globular shape	10 (77%)	14 (54%)	0	n.s.
Heterogeneus myometrium	13 (100%)	24 (92%)	0	n.s.
Fan-shaped striations	7 (54%)	1 (4%)	0	0.001
Myometrial cysts	4 (31%)	2 (8%)	0	n.s.
Ill-defined interface	11 (85%)	11 (42%)	1	0.037
Question mark sign	5 (38%)	1 (4%)	2	0.005
Walls asymmetry	8 (62%)	2 (8%)	20	0.001
TVS uterine tenderness	9 (69%)	9 (35%)	0	0.044
Doppler	4 (31%)	2 (8%)	2	0.011
JZ max $\geq$ 8 mm	4 (31%)	0 (0%)	13	0.015
Δ JZ $\geq$ 4 mm	6 (46%)	2 (8%)	13	0.027
JZ interruption	7 (54%)	2 (8%)	13	0.008

Table 4. Diagnostic capacities of each ultrasound features associated to the diagnosis of adenomyosis.

Feature	Sensibility	Specificity	PPV	NPV	LR+	LR-	Accuracy
Globular shape	77%	46%	42%	80%	1.43	0.5	61.5%
Asimmetry	80%	70%	72%	78%	2.67	0.29	78.9%
Heterogeneous myometrium	100%	7%	35%	100%	1.08	0	53.9%
Ill-defined interface	85%	56%	50%	88%	1.9	0.27	70.3%
Fan-shaped striations	54%	96%	88%	81%	14	0.48	75.0%
Myometrial cysts	30%	92%	67%	73%	4	0.75	61.5%
Question-mark sign	41%	96%	83%	77%	10.42	0.61	68.8%
TVS uterine tenderness	69%	65%	66%	81%	2	0.47	67.3%
JZ max	40%	99%	100%	73%	4	0.60	70.0%
JZ interruption	70%	88%	78%	82%	5.64	0.35	78.8%
∂JZ	60%	87%	75%	76%	4.5	0.46	73.8%
Doppler	55%	88%	66%	82%	4.72	0.51	71.5%

Figure 1. Transvaginal sonography longitudinal section of a uterus showing the *question mark sign* which is described when the corpus uteri is flexed backwards, the fundus uteri is facing the posterior pelvic compartment and the cervix is directed frontally towards the urinary bladder with the endometrial rhim resembling a question mark sign (a, b). Schematic drawing of the *question mark sign* (c).



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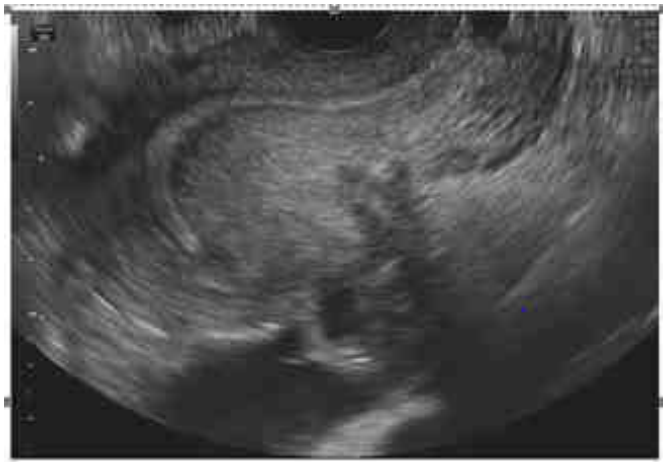

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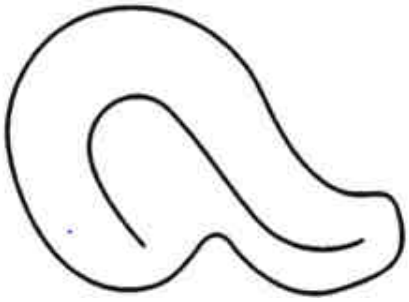

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