



# Relationship between endometriosis and uterine cervical elasticity assessed using ultrasound strain elastography

## ULTRA SONO GRAPHY

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### ORIGINAL ARTICLE

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**Purpose:** Internal cervical os (ICO) stiffness is related to menstrual pain, a key symptom of endometriosis. The study evaluated whether women with endometriosis have a stiffer ICO than unaffected women.

**Methods:** A retrospective cross-sectional analysis was conducted using prospectively collected data from women with and without endometriosis, spanning from June 2020 to September 2022. Endometriosis was diagnosed through clinical and ultrasound evaluations, with histological confirmation in a subset of participants. Strain elastography (SE) was employed to measure tissue elasticity in four cervical regions of interest: the ICO and the anterior, posterior, and middle cervical compartments (ACC, PCC, and MCC, respectively). Tissue elasticity was quantified using a color-based scoring system ranging from 0.1 (blue, indicating less elasticity) to 3.0 (red, indicating greater elasticity).

**Results:** Overall, 287 women were included, with 157 diagnosed with endometriosis and 130 controls. On SE, women with endometriosis exhibited a lower color score (mean±standard deviation), indicating lower elasticity, for the ICO ( $0.56\pm 0.28$  vs.  $0.70\pm 0.26$ ,  $P=0.001$ ) and PCC ( $0.69\pm 0.30$  vs.  $0.80\pm 0.27$ ,  $P=0.002$ ). Additionally, they had a lower ICO/MCC ratio ( $0.45\pm 0.28$  vs.  $0.60\pm 0.32$ ,  $P=0.001$ ) and ICO/ACC ratio ( $0.68\pm 0.42$  vs.  $0.85\pm 0.39$ ,  $P=0.001$ ). Multiple logistic regression analysis revealed that endometriosis was associated with the ICO color score (odds ratio, 0.053; 95% confidence interval, 0.014 to 0.202;  $R^2=0.358$ ;  $P=0.001$ ), even after adjusting for confounding factors like the presence of myomas ( $P=0.040$ ) and the use of hormonal therapy ( $P=0.001$ ). The results were corroborated in women with histologically confirmed endometriosis ( $n=71$ ).

**Conclusion:** The findings suggest a potential relationship between a stiffer ICO and endometriosis.

**Keywords:** Endometriosis; Dysmenorrhea; Elasticity imaging techniques; Cervix uteri; Menstruation disturbances; Pelvic pain

**Key points:** Intense menstrual pain, a key symptom of endometriosis, is likely promoted by mechanical impediments to the downstream flow of menstrual blood. Strain elastography is an emerging tool for assessing tissue stiffness and has revealed increased stiffness of the internal cervical os in women with intense menstrual pain. In this study, strain elastography revealed that the internal cervical os was stiffer in women with endometriosis than in those without the condition.

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## Introduction

Endometriosis is a chronic, estrogen-dependent inflammatory disease characterized by the presence of ectopic endometrial tissue outside the uterine cavity [1]. As one of the most common benign gynecological conditions, it affects approximately 10% of women of reproductive age [2] and often involves the uterus and pelvic organs [3]. The primary pathogenic mechanism has historically been identified as trans-tubal retrograde menstruation [4]. This leads to the dissemination of viable endometrial tissue within the peritoneal cavity, where various factors perpetuate a chronic inflammatory state [5,6]. However, since retrograde menstruation occurs in 90% of premenopausal women [7], additional mechanisms must also play a role in endometriosis [6]. These factors include a repetitive and excessive amount of retrograde menstrual flow. Downstream obstacles to menstrual blood, such as obstructive congenital Mullerian anomalies [8], cervical stenosis [9–12], and an unfavorable angle of uterine version or flexion [13], have been associated with endometriosis.

Intense menstrual pain is a key symptom of endometriosis. Such pain has also been linked to cervical stenosis [11] and an unfavorable uterine flexion angle [14–16], likely requiring stronger uterine contractions to overcome the obstruction of menstrual blood flow [17]. Elastography, a technique employed in obstetrics and gynecology to evaluate tissue stiffness [18], has shown that increased stiffness of the internal cervical os (ICO) can obstruct menstrual flow. This stiffness is associated with intense menstrual pain and adenomyosis [19,20].

The objective of this study was to assess whether women with endometriosis exhibit greater stiffness of the ICO as measured using strain elastography (SE).

## Materials and Methods

### Compliance with Ethical Standards

The study was conducted in accordance with recommendations from national health authorities and followed good clinical practices. Participants did not receive any incentives. All women provided their written informed consent for the anonymous use of their clinical data in scientific publications. Data were stored in an electronic database, from which they were later retrieved and analyzed anonymously. The local ethics committee (CER Liguria 11/04/22 registry number 123/2022-DB id 12185) approved the analysis and publication of the study.

### Patient

A retrospective cross-sectional analysis of prospectively collected data and ultrasound scans was conducted from June 2020 to September 2022 at a university department. This study focused on outpatient patients who were evaluated for either endocrine or benign gynecological disturbances (Fig. 1). The data collected for each woman included current age, age at menarche, body mass index (BMI; kg/m<sup>2</sup>), parity, history of uterine surgery (including caesarean section, dilation and curettage, hysteroscopy, and conization), presence of heavy menstrual bleeding, and use of hormonal therapy (including hormonal contraceptives). The presence and severity of menstrual pain, pain during intercourse, and intermenstrual pain were assessed using a 10-cm visual analogue scale (VAS). The presence of gynecological diseases, such as endometriosis, was evaluated based on the patient's history, a bimanual examination of the genitalia, and ultrasound, in accordance with current guidelines [3]. If surgical intervention was indicated, the diagnosis was confirmed through intraoperative visualization of endometriotic lesions and histological analysis.

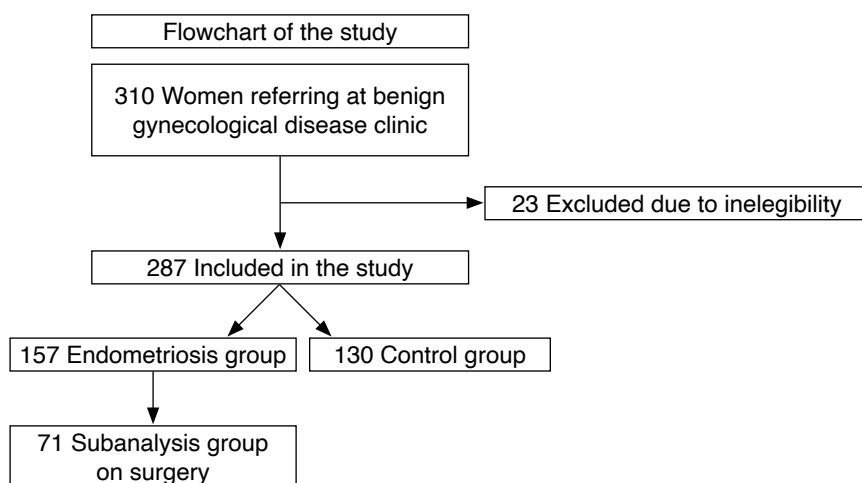


Fig. 1. Flowchart of the study.

Women diagnosed with endometriosis were considered the group under investigation. Control women were defined as those with no medical history, signs, or symptoms indicative of endometriosis.

The exclusion criteria for control women included uterine malformations and pathologies that could potentially impact the elasticity of the ICO. These pathologies include diffuse myomas, myomas situated in the upper cervix, a history of conization, and the presence of adenomyosis without any indication of endometriosis.

### Ultrasonographic Evaluation

Ultrasound examinations were conducted by a practitioner with expert training, who was blinded to the patient's symptoms and clinical history. The ultrasounds were performed on patients with an empty bladder using a GE machine (Voluson E6 BT16, GE Medical Systems, Zipf, Austria). This device was equipped with a wideband 5–9 MHz endocavitary transducer and specialized elastography software. Measurements taken during the ultrasound examination included the longitudinal (L), transverse (T), and anteroposterior (AP) diameters of the uterus; the length (CL) and transverse diameter (CT) of the cervix; the thickness of the endometrium; the position of the uterus as anteverted or retroverted (flexion); the degree of the anterior angle of flexion between the uterus and the cervix; and the elasticity of various cervical compartments. The uterine volume was calculated using the formula for the volume of an ellipsoid ( $L \times T \times AP \times 0.5223$ ), excluding the cervix. The cervical volume was determined using the formula for the volume of a cylinder ( $CL \times [CT/2]^2 \times 3.14$ ).

Standardized terminology established by the International Ovarian Tumor Analysis group was used to characterize ultrasound findings of the ovary, including endometriotic ovarian masses [21]. Similarly, the International Deep Endometriosis Analysis terms were employed to describe ultrasound findings in the anterior and posterior pelvic compartments, specifically for the detection of deep infiltrating endometriosis (DIE) [22]. Additionally, the Morphological Uterus Sonographic Assessment criteria from 2015 were applied in diagnosing myometrial pathologies [23]. The diagnosis of adenomyosis relied on identifying at least one direct indicator of endometrial tissue within the myometrium, such as intra-myometrial cysts, hyperechoic islands, or sub-endometrial lines or buds [24]. The presence of myomas not surrounding the cervix was also noted.

### Elastography Evaluation

The SE analysis was performed between the fifth and ninth days of spontaneous or hormonal contraceptive-induced menstruation. For those on continuous hormone treatment, the timing of the evaluation was based on the woman's preference. SE quantifies tissue deformation or displacement resulting from an applied force

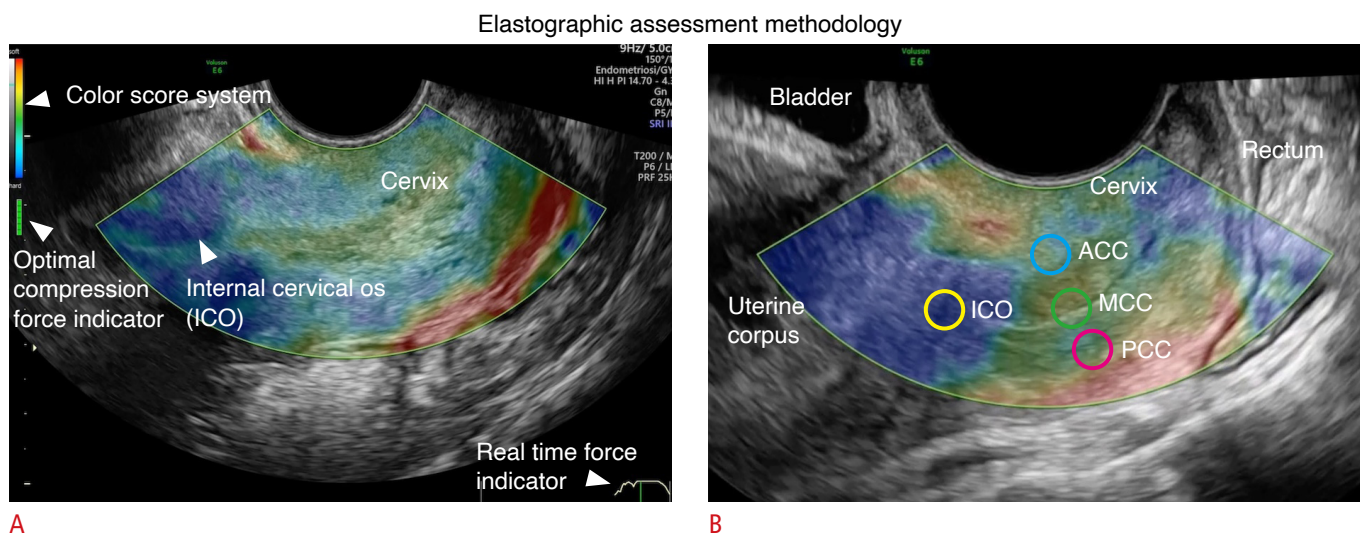
and appears to be a reliable diagnostic tool for assessing uterine disorders and DIE [25–27]. The method used to examine the cervix has been detailed previously [20,28].

The vaginal probe was placed in the anterior vaginal fornix, perpendicular to and approximately at the midpoint of the cervix, in the midsagittal plane. A series of compression and decompression cycles were generated using subcentimetric movements. A control bar within the ultrasound processing program provided real-time feedback on the optimal compression force (Fig. 2, Video clip 1). Circular regions of interest (ROIs) with an area of 19.6 mm<sup>2</sup> were positioned in the center of the anterior cervical compartment (ACC), the center of the posterior cervical compartment (PCC), the middle cervical canal (MCC), and at the ICO (Fig. 2). SE assessments were captured on video clips and later analyzed by three blinded independent scorers. The results were calculated using the optimal compression force as determined by the elastography software. Tissue elasticity was color-coded on a scale from violet/blue (indicating low elasticity) to red (indicating high elasticity), with yellow/green representing intermediate values. The three independent scorers, who were unaware of the patients' VAS scores for symptoms and pathology, evaluated the elasticity. A predefined value was assigned based on the colorimetric scale, ranging from 0.1 (blue/violet) to 3.0 (red) [29]. The mean of the values from the three scorers was used in the statistical analysis.

To evaluate the reproducibility of the color score analysis, each scorer independently assessed a random sequence of the same 20 ROIs three times at intervals of 3 days. The data were recorded, and the intra- and inter-rater interclass correlation coefficient (ICC) for the three operators was calculated. To minimize variability in SE results due to differences in the force applied during the examination, ratios of ROI elasticities assessed simultaneously in a single analysis were also calculated and compared.

### Statistical Analyses

Comparisons between means or frequencies were performed using the Student t-test and the chi-square test, respectively. To evaluate the association between endometriosis as the dependent variable and the elasticity of the ICO as the independent variable, simple and multiple logistic regression analyses were utilized. Potential confounders related to endometriosis or ICO stiffness were documented, including current age, age at menarche, BMI, uterine and cervical volumes, the angle of flexion between the uterine corpus and the cervix, the elasticity of other cervical areas, and the ratios of the ICO value to those of these areas. Term pregnancy, prior uterine surgery (including caesarean section, conization, hysteroscopy, and dilation and curettage), hormone therapy at the time of evaluation, uterine position (retroverted



**Fig. 2.** Illustration of strain elastography assessment and analysis.

**A.** Real-time examination during strain elastography, featuring a control bar on the left side of the screen that indicates the optimal force and a pressure indicator displayed as a continuous line on the bottom right. The color-coded elastography box reflects tissue stiffness, ranging from stiff (blue) to elastic (orange/red). **B.** Regions of interest during post-examination analysis: the internal cervical os (ICO), middle cervical canal (MCC), anterior cervical compartment (ACC), and posterior cervical compartment (PCC), are each marked by a circle.

as opposed to anteverted), presence of myomas, menstrual pain, intermenstrual pain, pain during intercourse, and heavy menstrual bleeding were all considered categorical variables (yes/no). The absence of these factors was used as the reference. Variables that showed an association with the dependent variable in the simple logistic regression analysis ( $P \leq 0.200$ ) were included in the multiple logistic regression models. Only factors that remained independently associated with the dependent variable were retained in the final model. Sub-analyses were conducted in women with only endometrioma, those with only DIE, and those with endometriosis but without adenomyosis. Additionally, a sensitivity analysis was performed that included only the subset of women with histologically confirmed endometriosis.

The study sample size exceeded those reported in the previous literature regarding the association between ICO elasticity and menstrual pain [20]. Statistical analyses were conducted using the Statview 5.01 statistical package (SAS Institute Inc., Cary, NC, USA). Data are presented as mean  $\pm$  standard deviation where applicable. A P-value of less than 0.05 was considered to indicate statistical significance.

The present work was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

## Results

### Clinical Characteristics

During the study period, 310 women were assessed at the authors' clinic (Fig. 1). Eleven patients were excluded from the study because a proper elastography evaluation could not be obtained due to the presence of endocervical polyps (six with endometriosis; five in the control group) or cervical myoma (one in the control group). An additional 11 were excluded due to incomplete clinical data collection, resulting in a total of 23 exclusions. The mean age of the excluded women was  $35.8 \pm 8.2$  years.

The study ultimately included 287 patients. Of these, 157 women (55%) were diagnosed with endometriosis, while the remaining 130 (45%) were not (Table 1, Fig. 1). Endometriomas were present in 81 women. DIE of the uterosacral ligaments and rectum was identified in 93 women, and 31 cases involved the parametrium.

The mean age was higher in women with endometriosis than in those without it ( $P=0.003$ ). Additionally, menstrual pain, pain during intercourse, and intermenstrual pain were more frequent ( $P=0.001$ ) and intense ( $P=0.001$ ) in patients with endometriosis (Table 1).

### SE Evaluation

For the SE analysis, the inter-rater ICC was 0.93 (95% confidence interval [CI], 0.89 to 0.96), while the intra-rater ICC was 0.95 (95% CI, 0.91 to 0.98).

Women with endometriosis exhibited a lower color score,

indicative of lower elasticity, in the ICO (P=0.001) and PCC (P=0.002), but not in the ACC or MCC (Table 2, Fig. 3). The ROI ratios revealed greater inhomogeneity of cervical tissue in women with endometriosis. The ICO/MCC and ICO/ACC ratio values were

lower in women with endometriosis compared to those without the condition (P=0.001) (Table 2).

Based on logistic multiple regression analysis, factors independently associated with endometriosis (R<sup>2</sup>=0.358) included ICO elasticity (P=0.001), the angle of uterine flexion (P=0.034), the presence of myomas (P=0.040), menstrual and intermenstrual pain (P=0.001), and the use of hormone therapy (P=0.001) (Table 3).

**Table 1.** Clinical characteristics of women with and without a diagnosis of endometriosis

	Non-endometriosis (n=130)	Endometriosis (n=157)	P-value
Age (year)	34.3±7.1	37.0±7.3	0.003
Age at menarche (year)	13.5±1.7	12.2±1.4	0.015
BMI (kg/m <sup>2</sup> )	22.7±4.8	23.0±4.7	0.633
Vaginal delivery	30 (23.1)	27 (17.2)	0.213
Previous uterine surgery	61 (46.9)	89 (56.7)	0.098
Hormone therapy	31 (23.8)	66 (42.1)	0.001
Menstrual pain	56 (43.1)	121 (79.0)	0.001
Menstrual pain (VAS)	2.3±3.2	5.5±3.6	0.001
Pain at intercourse	21 (16.1)	121 (77.1)	0.001
Pain at intercourse (VAS)	1.6±2.8	3.6±3.6	0.001
Intermenstrual pain	10 (7.7)	86 (54.7)	0.001
Intermenstrual pain (VAS)	0.4±1.6	3.7±3.7	0.001
Heavy menstrual bleeding	12 (9.2)	26 (16.6)	0.066
Presence of adenomyosis	0	103 (65.6)	0.001
Presence of myomas	32 (24.6)	23 (14.6)	0.032
Retroverted uterus	22 (16.9)	30 (19.1)	0.630
Uterine volume (cm <sup>3</sup> )	55.6±47.8	64.5±41.7	0.130
Cervix volume (cm <sup>3</sup> )	11.8±5.9	11.6±7.0	0.790
Angle of uterine flexion (°)	133.8±49.4	144.2±48.6	0.070

Values are presented as mean±SD or number (%). BMI, body mass index; VAS, visual analogue scale; SD, standard deviation.

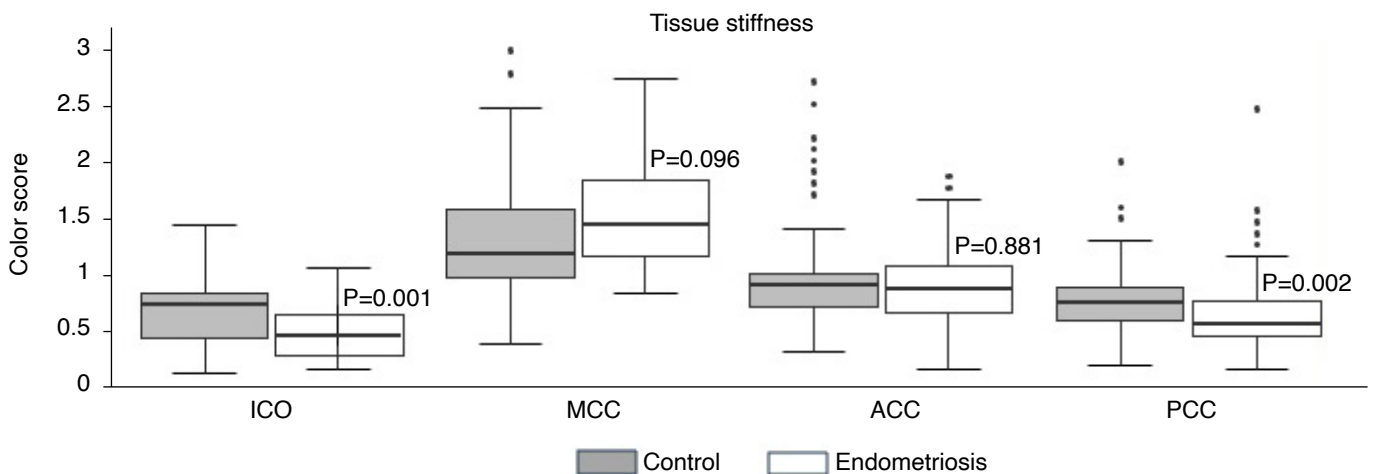
**Subgroup Analyses**

No significant differences were observed between women with ovarian endometriosis and those with DIE. The color score of the ICO was similarly associated with the presence of endometriomas alone (odds ratio [OR], 0.196; 95% CI, 0.061 to 0.631; P=0.006) and the presence of DIE without endometriomas (OR, 0.201; 95% CI, 0.071 to 0.569; P=0.003).

**Table 2.** Stiffness of various cervical regions of interest in women with and without a diagnosis of endometriosis

	Non-endometriosis (n=130)	Endometriosis (n=157)	P-value
ICO	0.70±0.26	0.56±0.28	0.001
MCC	1.33±0.50	1.43±0.51	0.096
ACC	0.93±0.38	0.94±0.35	0.881
PCC	0.80±0.27	0.69±0.30	0.002
ICO/MCC	0.60±0.32	0.45±0.28	0.001
ICO/ACC	0.85±0.39	0.68±0.42	0.001
ICO/PCC	0.96±0.46	0.91±0.53	0.380

Values are presented as mean±SD. ICO, internal cervical os; MCC, middle cervical canal; ACC, anterior cervical compartment; PCC, posterior cervical compartment; SD, standard deviation.



**Fig. 3.** Comparison of tissue stiffness at four landmarks within the cervix between women with endometriosis and the control group: the internal cervical os (ICO), middle cervical canal (MCC), anterior cervical compartment (ACC), and posterior cervical compartment (PCC).

**Table 3.** Multiple logistic regression assessment of factors independently associated with the presence of endometriosis

	Logistic model ( $R^2=0.358$ )		
	Odds ratio	95% Confidence interval	P-value
ICO	0.05	0.01–0.20	0.001
Myomas (y/n)	0.35	0.14–0.94	0.040
Angle of uterine flexion (°)	1.01	1.00–1.01	0.034
Intermenstrual pain (y/n)	10.60	4.41–25.92	0.001
Menstrual pain (y/n)	5.52	2.40–12.65	0.001
Hormone therapy (y/n)	6.47	2.67–15.60	0.001

The following variables were excluded from the model as they did not independently relate to the presence of endometriosis: uterine anteversion versus retroversion, vaginal delivery (yes/no), prior uterine surgery (yes/no), uterine volume (cm<sup>3</sup>), and cervical volume (cm<sup>3</sup>).

ICO, internal cervical os.

Adenomyosis was identified exclusively in women with endometriosis, reflecting the selection criteria of the population studied (Table 1). To determine whether adenomyosis acted as a confounding factor, women with adenomyosis were removed from the analysis. Even after this exclusion, the ICO color score remained significantly associated with the presence of endometriosis (OR, 0.102; 95% CI, 0.025 to 0.426;  $P=0.002$ ).

### Sensitivity Analysis

A sensitivity analysis was conducted on a subgroup of 71 women who had a histological diagnosis of endometriosis following surgery. This subgroup included 49 women with endometrioma alone and 22 with DIE, with or without endometrioma. The characteristics of these women were comparable to those of the overall endometriosis cohort. The mean age was  $36.5\pm 7.3$  years, the BMI was  $23.3\pm 4.9$  kg/m<sup>2</sup>, the VAS score for menstrual pain was  $5.3\pm 3.3$ , the score for pain during intercourse was  $3.4\pm 3.7$ , and the score for intermenstrual pain was  $4.0\pm 3.6$ . In this subgroup, the sensitivity of the anamnestic, clinical, and ultrasonographic investigation was approximately 100% for detecting both endometrioma and DIE. The combined sensitivity was 100% (95% CI, 84.5% to 100%), and the combined specificity was 95.2% (95% CI, 77.3% to 99.2%). Multiple logistic regression analysis in this subset identified ICO elasticity (OR, 0.217; 95% CI, 0.063 to 0.742;  $P=0.015$ ) and the presence of intermenstrual pain (OR, 2.90; 95% CI, 1.54 to 5.46;  $P=0.001$ ) as independent factors associated with endometriosis.

## Discussion

### Key Results

In this study, elevated ICO stiffness was associated with a higher

risk of pelvic endometriosis in patients presenting with clinical symptoms, such as various types of pelvic pain, defined as variables. Additional risk factors included a higher prevalence of pain-related symptoms, including dysmenorrhea, pain during intercourse, and chronic pelvic pain, as well as the use of hormone therapy. These findings contribute to the existing body of research investigating organic and mechanistic factors in the development of endometriosis and its symptoms.

### Interpretation

On SE, the cervix displays varying degrees of elasticity, reflecting its different tissue textures. Notably, the tissue around the ICO appears stiffest [14]. Anatomical studies have revealed that this region consists of radial and circular collagen fibers, as well as circular muscular fibers that respond to oxytocin [30]. The ICO serves as a barrier to fetal expulsion during pregnancy [30] and likely influences menstrual blood flow in a similar manner. Women with adenomyosis [19] and those who experience severe menstrual pain [20] have been found to have a stiffer ICO than those without these conditions. The present study also suggests a stiffer ICO in women with endometriosis. The question of whether this association is causal or consequential warrants further discussion. A stiffer ICO could pose an obstacle to the passage of menstrual flow, leading to intense, painful contractions. This may exacerbate retrograde menstruation, potentially contributing to the development of endometriosis [31,32]. Increased stiffness may also hinder the dynamic modification of the cervix reducing ICO diameter during menstruation. Interestingly, even a minimal reduction in ICO diameter (less than 0.5 mm) is considered sufficient to markedly increase retrograde menstrual blood flow into the peritoneal cavity, thereby raising the risk of endometriosis [11,12]. The same underlying mechanism—resistance to menstrual flow or increased retrograde menstruation—may also be present in uteri with congenital anomalies, which have been linked to a higher prevalence of the disease [9,10]. Contributing factors include cervical stenosis [10,11], variations in the intramural portion of the fallopian tubes [33], utero-tubal hypotonia [34], and relative cervical stenosis in the context of heavier and more forceful menstrual flow [6]. A similar etiopathogenetic model has been observed in baboons, where supracervical ligation, leading to increased retrograde menstruation, was found to induce endometriosis within 3 months [35,36].

The hypothesis that a softer ICO may offer protection against endometriosis remains unconfirmed. However, indirect evidence may support this notion. Research has suggested that a cervix that is more permissive to menstrual flow could be associated with a lower prevalence of endometriosis in multiparous than nulliparous women [9]. Additionally, the potential softening of the cervix by mifepristone

[37] has been linked to reduced pain symptoms in women with adenomyosis [38].

### Limitations and Generalizability

This study has several strengths. Despite being a retrospective analysis, it had well-defined inclusion criteria for the comparative groups and a strict protocol for analyzing elastographic data and endometriosis. Numerous factors that could potentially influence the results were considered, including cervical and uterine surgery; however, none of these factors significantly impacted the findings. One limitation of the study is the lack of data on possible cervical lacerations during delivery. Multiparity rates were similar in women with endometriosis and in controls, with no reason to expect differences in the incidence of cervical lacerations. Nonetheless, this omission represents a limitation of the present research. Furthermore, women with endometriosis were older than control participants. A previous study suggested that the stiffness of the cervical canal, as evaluated by shear wave elastography, varies with age, but it did not test the stiffness of the ICO [39]. Another recent report indicates that ICO stiffness on shear wave elastography is not affected by age, which aligns with the SE findings of the present study [40]. In previous research, uteri with varying angles of uterine flexion were evaluated; the angle of flexion did not influence ICO stiffness [28].

SE is a semi-quantitative analysis and can be influenced by the external force applied by the operator during the examination. To minimize this variability, a single experienced sonographer using consistent equipment performed all ultrasonographic evaluations in the present study. However, results might differ with different operators or equipment. According to the elastography software, SE data were analyzed at the optimal compression/decompression cycle. Subsequently, the color score of each ROI was evaluated by three independent readers to minimize individual bias. The inter- and intra-rater ICC of the three scorers exceeded 0.93. For each ROI, the mean of the SE values from these three individuals was used in statistical analyses. In addition to the absolute values of each individual ROI, data were also expressed as the ratio of ROI elasticity. If each ROI receives the same compression force during analysis, their ratio becomes independent of the absolute force applied. The distance between the ROI under investigation and the transducer can also affect elasticity, with ROIs further from the transducer appearing stiffer. However, the ICO was at a similar distance from the transducer in the women with and without endometriosis, with comparable cervical and uterine volumes in these groups. The elasticity ratios of the ICO were also measured with ROIs at various distances from the transducer, supporting the finding of a stiffer ICO in patients with endometriosis. The methodology applied to improve

the reliability and accuracy of SE is rather complex and not easily implemented in routine clinical practice. All analyses were performed by a single experienced sonographer, with multiple blinded scorers evaluating the outcomes; mathematical calculations, such as stiffness ratios, were also employed to obtain more reliable results.

Endometriosis was diagnosed using medical history, clinical manifestations, bimanual examination, and ultrasonography, with confirmation by post-surgical histology in a subset of 71 women included in the sensitivity analysis. Within this subset, the sensitivity and specificity of the diagnostic approach were found to be very high. Additionally, the sensitivity analysis corroborated the relationship between ICO elasticity and endometriosis. It is possible that some cases of superficial endometriosis were inadvertently categorized as non-endometriosis. Surgical investigation in these instances would have been unjustified and unethical. However, if a woman with endometriosis was mistakenly placed in the control group, it would mean that a woman with a stiffer ICO was included in the group generally characterized by more elastic ICOs, thereby reducing the observed difference between cases and controls. Thus, such potential misclassification does not compromise the validity of the evidence indicating a stiffer ICO in women with endometriosis compared to controls.

The results were obtained from a relatively large sample; however, the participants were predominantly white and from a single center. To validate the findings, replication in diverse centers and among women of various ethnicities is necessary.

This study suggests that women with endometriosis have a stiffer ICO, as measured by SE, compared to those without the condition. Despite the potential value of the results, the findings must be validated in larger cohorts and across different centers, and their clinical implications should be thoroughly investigated.

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Conceptualization: Xholli A, Molinari F, Londero AP, Cagnacci A. Data acquisition: Xholli A, Molinari F, Scovazzi U, Perugi I, Kratochwila C, Cremonini F. Data analysis or interpretation: Xholli A, Scovazzi U, Londero AP, Cagnacci A. Drafting of the manuscript: Xholli A, Molinari F, Scovazzi U, Cagnacci A. Critical revision of the manuscript: Xholli A, Molinari F, Londero AP, Perugi I, Kratochwila C, Cremonini F, Cagnacci A. Approval of the final version of the

manuscript: all authors.

### Conflict of Interest

No potential conflict of interest relevant to this article was reported.

### Supplementary Material

Video clip 1. Methodological assessment of the cervix using strain elastography (<https://doi.org/10.14366/usg.24117>).

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