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ORIGINAL ARTICLE *Gynaecology*

Relation between adenomyosis and elastographic characteristics of the cervix

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STUDY QUESTION: Is there a possible etiologic link between cervical stiffness and adenomyosis?

SUMMARY ANSWER: Women with adenomyosis have a stiffer internal cervical os than those without adenomyosis.

WHAT IS KNOWN ALREADY: An increased myometrial contractility during menses, leading to breaches in the endometrial basal lamina and subsequent infiltration of endometrial cells into the myometrium, has been proposed as a possible pathogenic mechanism for adenomyosis. Intense menstrual pain has already been shown to be associated with an increased stiffness, at elastography, of the internal cervical os.

STUDY DESIGN, SIZE, DURATION: A cross-sectional study on 275 women was performed between 1 February and 31 July 2022.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Among the participants, 103 were and 172 women were not affected by adenomyosis as evaluated by ultrasonography. General and clinical characteristics of the patients were collected. Strain elastography was used to document tissue stiffness at different regions of interest of the cervix, i.e. the internal cervical os, the middle cervical canal, the anterior and the posterior cervical compartment. Tissue stiffness was expressed as a colour score from 0.1 = blue/violet (high stiffness) to 3.0 = red (low stiffness). Simple and multiple logistic regression analyses were used to evaluate the relation between the presence of adenomyosis, as the dependent variable, and independent factors.

MAIN RESULTS AND THE ROLE OF CHANCE: Women with adenomyosis had a higher prevalence ($P = 0.0001$) and intensity ($P = 0.0001$) of pain during menses, between menses and at intercourse compared to control. The internal cervical os colour score was lower (higher stiffness) in women with adenomyosis (0.55 ± 0.29 versus 0.67 ± 0.26; $P = 0.001$) and the middle cervical canal/internal cervical os colour score ratio was greater (3.32 ± 4.36 versus 2.59 ± 4.99; $P = 0.008$), compared to controls. Upon logistic regression modelling ($R^2 = 0.077$), the internal cervical os stiffness was an independent factor related to adenomyosis (odds ratio (OR) 0.220, 95% CI 0.077, 0.627; $P = 0.005$) along with age ($P = 0.005$) and the use of gonadal steroid therapies ($P = 0.002$). We obtained the same results using a different logistic regression model ($R^2 = 0.069$), by substituting the internal cervical os stiffness with the ratio of the middle cervical canal/internal cervical os stiffness (OR 1.157, 95% CI 1.024, 1.309; $P = 0.019$).

LIMITATIONS, REASONS FOR CAUTION: Women did not undergo surgery therefore we have no histological confirmation of the adenomyosis diagnosis. Strain elastography is a semiquantitative analysis and can be conditioned by the force applied by the operator during the analysis. The data were obtained mainly in White women in a single centre.

WIDER IMPLICATIONS OF THE FINDINGS: To the best of our knowledge, this is the first study indicating that women with adenomyosis have an increased stiffness of the internal cervical os. The results indicate that a stiff internal cervical os, as determined by elastography, is a possible contributor to the development of adenomyosis. These findings may have clinical significance and should prompt further investigation.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: N/A.

Key words: internal cervical os / uterine cervix / elastography / adenomyosis pathogenesis / ultrasonography

Introduction

Adenomyosis is a chronic, inflammatory, and oestrogen-dependent gynaecological finding, characterized by endometrium-like glands and stroma within the myometrium, with surrounding myometrial hyperplasia and/or hypertrophy (Struble et al., 2016). It is a common finding in ~20% and 45% of asymptomatic and symptomatic women, respectively (Vercellini et al., 2006; Filip et al., 2019). Mechanisms involved in the development of adenomyosis are still uncertain and there is no unified concept of its pathogenesis (Zhai et al., 2020). One hypothesis is that during menses repeated microtraumas of the basal lamina of the endometrium, induced by intense uterine contractions, lead to microlesions (recently named endometrial–myometrial interface disruption), favouring invagination of the basalis endometrium, including stem cells, into the myometrium (Leyendecker et al., 2015). In such a circumstance, adenomyosis could then develop as the consequence of inflammation, local oestrogen production, epigenetic changes, and somatic mutation (Donnez et al., 2021; Khan et al., 2022). Metaplasia of embryonic Müllerian remnants, differentiation of embryonic stem/progenitor cells, and extension of peritoneal pelvic endometriosis into the myometrium have been also advocated to explain one type of adenomyosis (Donnez et al., 2021; Khan et al., 2022). Downstream absolute (reduction of orifice through which menstrual flow exits) or relative (a normal cervical orifice that becomes insufficient in cases of very heavy menstrual bleeding) obstacles to menstrual flow induced by cervical structures or excessive bleeding may cause intense uterine contractions and related menstrual pain, potentially favouring disruption of the endometrial–myometrial interface. An increased expression of oxytocin receptors in women with adenomyosis and dysmenorrhoea has also been described (Guo et al., 2013). Intense menstrual pain is perceived by women with an excessive angle of uterine retroflexion (Cagnacci et al., 2014), which seems to represent an independent risk factor for adenomyosis (Xholli et al., 2022). Intense menstrual pain is perceived by women with increased stiffness of tissue surrounding the internal cervical os (ICO) (Xholli et al., 2021). In this study, we evaluated whether ICO stiffness is also related to the presence of adenomyosis. Strain elastography (SE), along with shear wave elastography, is an ultrasound-based method used to evaluate tissue stiffness (Feltovich et al., 2012; Hee, 2014; Feltovich and Carslon, 2017). During SE analysis, the tissue deformation resulting from pressure applied to the tissue by the ultrasound transducer is transformed by software into a colour map depicting the tissue stiffness. Shear wave elastography evaluates the velocity of ultrasound waves and gives a more objective evaluation of tissue stiffness: it is not common in vaginal transducers, it requires dedicated software, and the results obtained are reliable only for homogenous tissue, such as the liver parenchyma, and not for non-homogeneous tissues, for example the cervix (Feltovich et al., 2012). In this study, the stiffness of different areas of the cervix was evaluated by SE.

Materials and methods

Design and ethical approval

A prospective observational study was performed between 1 February and 31 July 2022. All patients in our outpatient clinic routinely sign an informed consent for the anonymous use of their clinical data in scientific publications. The few women not giving their consent are identified and their data are not used for scientific purposes. Women were managed in accordance with standard clinical practice procedures, and diagnostic procedures were paid partially or completely by the Italian National Health System, according to national health authorities' indications. No incentive was given to any of the participants. All the data were recorded anonymously in an electronic database and then retrieved and analysed. The study was approved by the local ethical committee (CER Liguria 123/2022).

Data collection and patient characteristics

Data were collected from 300 patients who were referred to the outpatient services for 'endometriosis and chronic pelvic pain' or 'infertility' at our University Hospital. In 12 patients, the elastography evaluation was difficult and unreliable owing to the presence of organic alterations of the cervix, such as myomas close to or inside the cervix walls. These women were excluded from the study. Another 13 women were excluded because clinical data were incompletely collected, leaving 275 evaluable subjects. For each woman, we collected general characteristics and clinical data. The presence of menstrual pain, pain at intercourse, and intermenstrual pain were recorded by a physician, who also recorded the severity of each type of pain rated by the woman on a 100 mm visual analogue scale. Women were asked to place their mark anywhere on the line between 0 (no pain) and 100 mm (worst pain imaginable). The current history of heavy menstrual bleedings reported by the woman was also recorded. The presence of gynaecological disorders, such as uterine myomas, adenomyosis, and endometriosis, was evaluated by patient history, bimanual examination and ultrasonography. Ultrasound investigations were performed by an expert, trained practitioner (A.X.), who was blind to the woman's clinical history and pain quantification. The ultrasound investigation was performed with an empty bladder using a GE E6 (GE Medical Systems, Zipf, Austria) ultrasound machine, equipped with a wideband 5–9 MHz intravaginal transducer and appropriate software for elastography (Voluson E6 BT16). The longitudinal (L), transverse (T) and antero-posterior (AP) diameter of the uterus, length (CL) and transverse diameter (CT) of the cervix, double layer thickness of the endometrium, and elasticity of different cervical compartments were obtained during the ultrasound examination. The volume of the uterine corpus was measured by means of the ellipsoid formula ($L \times T \times AP \times 0.5223$), without considering the cervix. The volume of the cervix was calculated by the cylinder formula ($CL \times [CT/2]^2 \times 3.14$). Ovarian endometriosis was diagnosed following International Ovarian Tutor Analysis Consensus indications (Van Holsbeke et al., 2010).

According to Morphological Uterus Sonographic Assessment criteria (Van den Bosch et al., 2015; Xholli et al., 2021; Harmsen et al., 2022), the ultrasound diagnosis of adenomyosis was defined as the presence of two or more of the following indirect and direct indexes of adenomyosis: indirect indexes are: globular uterine configuration; asymmetrical myometrial thickening of the uterine wall; fan-shaped shadowing; trans-lesional vascularity; irregular junctional zone (JZ); and interrupted JZ. Direct indexes of adenomyosis are: myometrial anechoic lacunae

or cysts, seen as a round anechoic areas within the myometrium; hyper-echogenic islands into the myometrium; and echogenic sub-endometrial lines and buds. The direct signs of adenomyosis and their ultrasonographic assessment significantly increase the diagnostic specificity of the technique, up to 98% (Harmsen *et al.*, 2022). Diffuse adenomyosis was defined when lesions were distributed diffusely within the myometrium, and focal when circumscribed nodular aggregates were seen in the anterior or posterior wall of the uterus (Van den Bosch *et al.*, 2015).

Tissue elasticity was obtained by SE. During image acquisition, the vaginal probe was positioned in the anterior vaginal fornix and a B-Mode sagittal view of the cervix was obtained and displayed alongside to facilitate image interpretation (Gemici *et al.*, 2020; Xholli *et al.*, 2021). The operator performed a series of about five compression and decompression cycles, using sub-centimetric excursions perpendicular to the axis of the cervical canal (Hernandez-Andrade *et al.*, 2013; Hee *et al.*, 2014; Xholli *et al.*, 2021). A control bar of the ultrasound processing program indicated, in real time, optimal compression force. Regions of interest (ROIs) with a circular area of 19.6 mm^2 were placed in the middle of the anterior cervical compartment (ACC), in the middle of the posterior cervical compartment (PCC), in the middle portion of the cervical canal (MCC) and at the ICO (Fig. 1, Video 1). SE evaluations were recorded on clips and analysed off-line. Results were calculated at optimal compression force defined by the elastography software. Tissue elasticity coded with a scale ranging from violet/blue (low) to red (high), with yellow/green as intermediate (Fig. 2), was evaluated by three independent scorers, who had no access to the subject's clinical data except for the recorded SE analysis. A predefined value was assigned based on the colourimetric scale on the whole spectrum (from the value 0.1 = blue/violet to the value 3.0 = red) (Hee *et al.*, 2014; Feltovich and Carlson, 2017). The mean value of the three scorers was used.

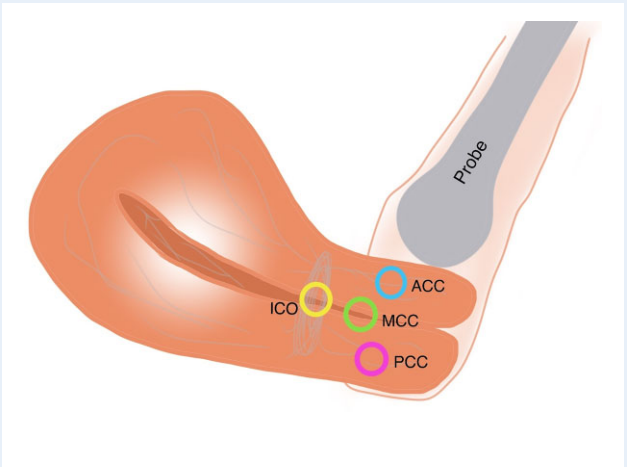


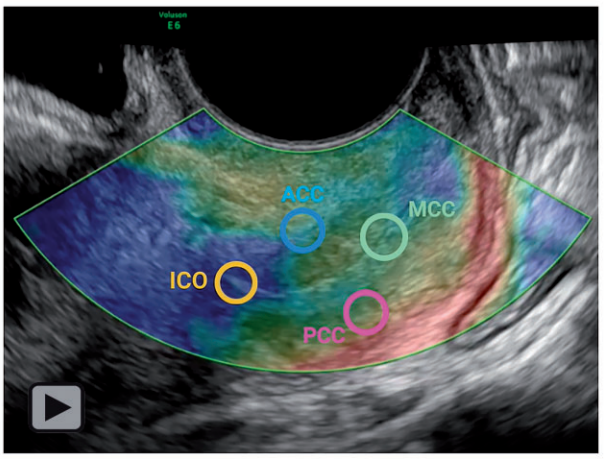
Figure 1. Schematic representation of the human cervix and the regions of interest for the evaluation of tissue stiffness by strain elastography. ICO: internal cervical os; MCC: middle cervical canal; ACC: anterior cervical compartment; PCC: posterior cervical compartment.

Statistical analyses

Simple and multiple logistic regression analyses were used to evaluate the relation between the presence of adenomyosis, as the dependent variable, and other factors (independent variables), expressed by continuous or categorical data. Age, age at menarche, BMI, volume of the corpus and cervix, ROIs stiffness, and the ratio between the stiffness of ROIs were considered as continuous independent variables. Term pregnancies, previous uterine surgery, gonadal steroid therapy at time of evaluation, and type of therapy, were considered as categorical (yes/no) variables. Only variables that, in simple logistic regression, were related to the dependent variable (up to a $P = 0.2$) were entered into the multiple logistic regression models. Only factors remaining independently related to the dependent variable were conserved in the multiple logistic regression model. Comparisons between means or frequencies were performed by the Student's *t*-test and the Chi-squared test, respectively. Statistical analysis was performed by the Statview program (SAS Institute Inc., Cary, NC, USA). The data are expressed as a mean with SD. A value of $P < 0.05$ is considered significant.

Results

Of the 275 total patients included in the study, 103 (37.5%) had ultrasound features of adenomyosis while 172 had not and were considered as the control group (Table I). Adenomyosis was classified as focal (n = 3) or diffuse (n = 100). Among the diffuse group, 58 patients presented diffuse adenomyosis in the whole uterine corpus; 19 women were affected by diffuse anterior wall adenomyosis; and 23 by diffuse posterior wall adenomyosis.



Video 1. Elastographic technique for the evaluation of the human cervix. Regions of interest have been positioned as shown in the still image. The control bar shows the optimal compression force to achieve during the examination (green bar). The colourimetric scale ranges from blue (stiff tissue) to red (soft tissue). ICO: internal cervical os; ACC: anterior cervical compartment; PCC: posterior cervical compartment; MCC: middle cervical canal.

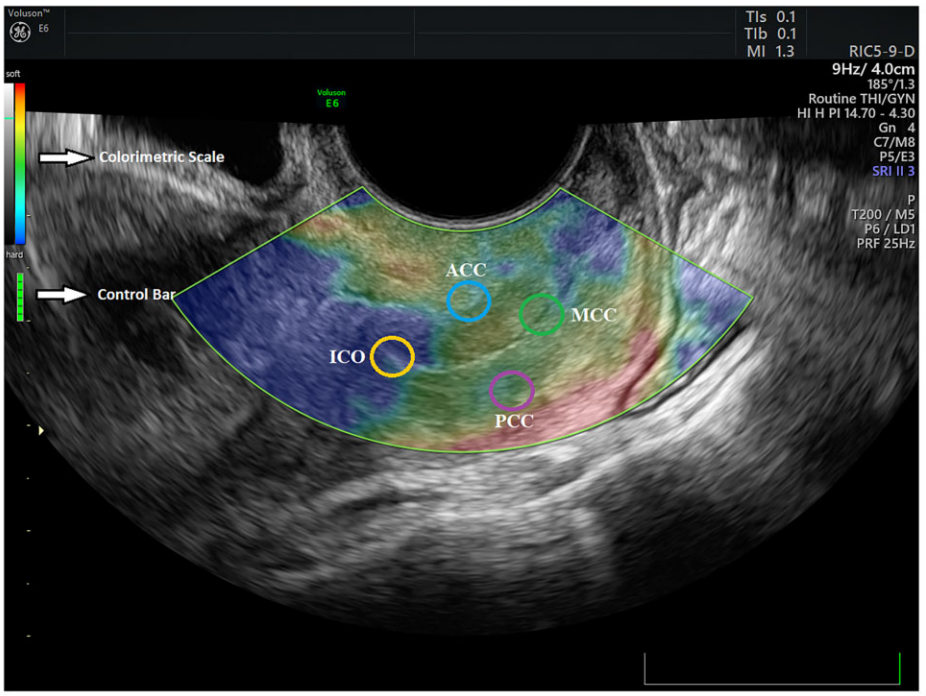


Figure 2. Elastography of the human uterine cervix. On the left, the two vertical bars indicate the colourimetric scale (upper bar) and the control bar (lower bar) that, when full green, indicates optimal compression force. Circles indicate regions of interest. ACC: anterior cervical compartment; PCC: posterior cervical compartment; ICO: internal cervical os; MCC: middle cervical canal.

Table I Clinical characteristics of women without and with a diagnosis of adenomyosis at ultrasound investigation.

	Control (n = 172)	Adenomyosis (n = 103)	*P*-value
Age (years)	34.9 ± 7.7	37.1 ± 6.8	0.014
Age at menarche (years)	12.8 ± 1.7	12.5 ± 1.6	0.416
BMI (kg/m^2)	22.9 ± 4.7	23.0 ± 5.0	0.807
Pregnancies at term (%)	22.6%	17.1%	0.330
Uterine surgery (%)	50.4%	44.4%	0.377
Hormone therapy (%)	30%	46%	0.011
Menstrual pain (VAS)	3.0 ± 3.5	5.5 ± 3.6	0.0001
Pain at intercourse (VAS)	2.0 ± 3.1	3.8 ± 3.7	0.0001
Intermenstrual pain (VAS)	1.0 ± 6.0	3.7 ± 14.1	0.0001
Heavy menstrual bleeding (%)	13%	16%	0.608
Uterine volume (cm^3)	58.3 ± 48.7	65.5 ± 38.8	0.208
Cervix volume (cm^3)	19.9 ± 5.9	19.6 ± 6.7	0.707
Cervix length (cm)	26.6 ± 5.3	26.7 ± 4.9	0.894
Endometriosis (%)	13.9%	31.0%	0.001
Myomas (%)	23.8%	9%	0.002

Data are reported as mean ± SD or percentage. Means were compared by the Student's *t*-test and percentages by the Chi-squared test. VAS: visual analogue scale.

The mean age was higher in cases than controls (37.1 ± 6.8 versus 34.9 ± 7.7 years; $P = 0.014$).

Menstrual pain was more frequent (75% versus 52%; $P = 0.0001$), and intense ($P = 0.0001$) in women with adenomyosis versus controls. Pain at intercourse was also more frequent (58% versus 32%; $P = 0.0001$), and intense ($P < 0.0001$) in women with adenomyosis. Similarly, intermenstrual pain was more frequent (58% versus 18%; $P = 0.0001$), and intense ($P = 0.0001$) in women with adenomyosis (Table I).

The frequency of term pregnancy and of previous uterine surgery was similar in cases and controls while the use of gonadal steroidal therapy was more prevalent in women with adenomyosis (46% versus 30%; $P = 0.011$) (Table I). Gonadal steroid therapies comprised either the intrauterine levonorgestrel system (5%), the oral progestin dienogest (5%), or combined oestrogen and progestin formulations including the vaginal ring (18%), dienogest combined with oestradiol valerate (24%), dienogest combined with ethynilestradiol (20%), nomegestrol acetate combined with oestradiol (15%), or drospirenone combined with ethynilestradiol (13%). Uterine corpus volume and cervix length and volume were similar in cases and controls (Table I). Ovarian endometriosis was more frequent in cases than controls (31.0% versus 13.0%; $P = 0.001$), while myomas were less frequent (9% versus 23.8%; $P = 0.002$) (Table I).

At SE, women with adenomyosis had a decreased colour score of the ICO (increased stiffness) (0.55 ± 0.29 versus 0.67 ± 0.26;

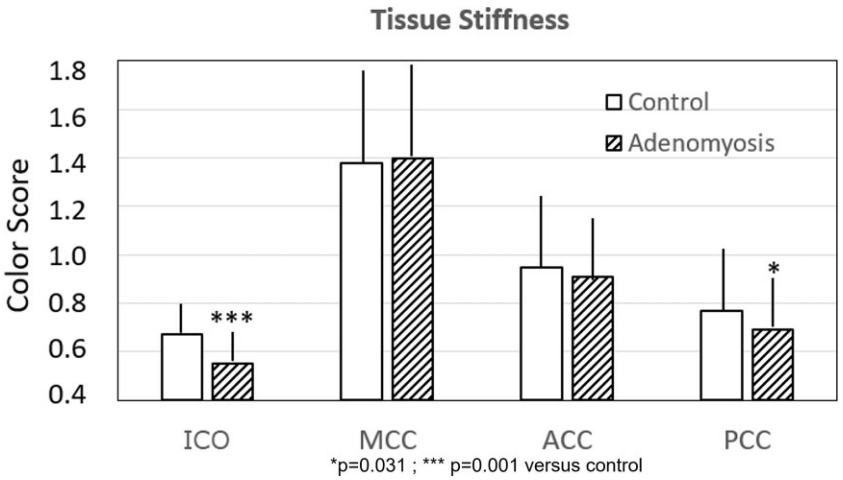


Figure 3. Tissue stiffness evaluated in women with and without (control) adenomyosis. Data are the mean colour scores determined at the internal cervical os (ICO), middle cervical canal (MCC), anterior cervical compartment (ACC), and posterior cervical compartment (PCC). Error bars are SD. *$P = 0.031$; ***$P = 0.001$ versus control by Student's *t*-test.

Table II Stiffness of different regions of interest of the cervix of women without and with a diagnosis of adenomyosis at ultrasound investigation.

	Control (n = 172)	Adenomyosis (n = 103)	*P*-value
ICO	0.67 ± 0.26	0.55 ± 0.29	0.001
MCC	1.38 ± 0.52	1.40 ± 0.49	0.807
ACC	0.95 ± 0.39	0.91 ± 0.31	0.396
PCC	0.77 ± 0.31	0.69 ± 0.24	0.031
MCC/ICO	2.59 ± 4.99	3.32 ± 4.36	0.008
ACC/ICO	1.83 ± 4.25	2.12 ± 1.66	0.208
PCC/ICO	1.39 ± 2.54	1.56 ± 0.95	0.341

ICO: internal cervical os; MCC: middle cervical canal; ACC: anterior cervical compartment; PCC: posterior cervical compartment. Data are reported as mean ± SD. Means were compared by the Student's *t*-test.

$P = 0.001$) and PCC (0.69 ± 0.24 versus 0.77 ± 0.31; $P = 0.031$) but not of the ACC ($P = 0.396$) or MCC ($P = 0.807$) (Fig. 3; Table II). The ROIs ratio showed a greater heterogeneity for cervical tissue in women with than without adenomyosis, and the MCC/ICO colour score ratio was greater in women with than without adenomyosis (3.32 ± 4.36 versus 2.59 ± 4.99; $P = 0.008$) (Table II). Gonadal steroid therapy did not affect SE results, independent of the formulation used. The ICO colour score was 0.64 ± 0.25 and 0.60 ± 0.25 ($P = 0.257$) in gonadal steroid therapy users and in non-users, respectively.

Logistic multiple regression analyses were carried out either including ICO stiffness (Model 1) or MCC/ICO ratio of stiffnesses (Model 2). In Model 1 ($R^2 = 0.077$), ICO stiffness was an independent determinant (odds ratio (OR) 0.220, 95% CI 0.077, 0.627; $P = 0.005$) of adenomyosis along with age and use of gonadal steroid therapy, independent of the type of formulation used (Table III). Similarly, in Model 2 ($R^2 = 0.069$), the MCC/ICO ratio was related to adenomyosis (OR 1.157, 95% CI 1.024, 1.309; $P = 0.019$), along with age and use of gonadal steroid therapy, independent of the type of formulation (Table III).

Discussion

To the best of our knowledge, this is the first study showing that women with adenomyosis have an increased ICO stiffness.

The regions of the cervical stroma vary in their relative composition of collagen and muscular fibres. The region around the ICO tends to be the stiffest of all these areas (Xholli *et al.*, 2021). Anatomical analyses show that the ICO region typically comprises radial collagen fibres with a bundle of circular collagen and muscular fibres, the latter being responsive to neurotransmitters and oxytocin (Vink *et al.*, 2016; Yao *et al.*, 2016). These characteristics make the ICO the main structure of the cervix able to counteract the dilatational forces during pregnancy. Stiffness evaluated by elastography reflects the deformability or elasticity of the tissue. Decreases in stiffness make the ICO more elastic and precede the period of labour and delivery.

In non-pregnant uteri, the force of ICO closure and, accordingly, its stiffness at elastography, may differ from one woman to another. This may have an influence on the intensity of myometrial contractions necessary to expel menstrual blood and, consequently, the intensity of menstrual pain. We previously showed that an increased ICO stiffness is associated with more intense menstrual pain (Xholli *et al.*, 2021). Herein, we show that an increased ICO stiffness is present in women with ultrasonographic findings suggesting the presence of adenomyosis. The combination of these data seems to support the idea that a stiff ICO represents an obstacle to menstrual flow, causing more intense uterine contractions. This circumstance may facilitate disruption of the

Table III Multiple logistic regression models on factors independently related to the presence of adenomyosis.

Model 1 ($R^2 = 0.077$)			
	Odds ratio	**95% CI**	***P*-value**
ICO	0.220	0.077; 0.627	0.005
Age (years)	1.058	1.018; 1.100	0.005
Hormone therapy (y/n)	2.573	1.448; 4.571	0.002
Model 2 ($R^2 = 0.069$)			
	Odds ratio	**95% CI**	***P*-value**
MCC/ICO	1.157	1.024; 1.309	0.019
Age (years)	1.062	1.021; 1.103	0.002
Hormone therapy (y/n)	2.448	1.386; 4.321	0.002

Model 1: include ICO as an independent factor; Model 2: includes MCC/ICO ratio of stiffness as an independent factor. ICO: internal cervical os; MCC: middle cervical canal.

endometrial–myometrial interface in a fashion that contributes to the pathogenesis of adenomyosis that involves the internal myometrium (Leyendecker *et al.*, 2015). A similar mechanism may explain more intense menstrual pain (Cagnacci *et al.*, 2014) and a higher prevalence of adenomyosis (Xholli *et al.*, 2022) in women with an increased angle of uterus retroflexion, which may also represent an obstacle to menstrual flow. The possibility that a stiffer ICO is secondary to the presence of adenomyosis seems to be less likely. A stiffer ICO was also observed in women with intense menstrual pain in the absence of sonographic features of adenomyosis (Xholli *et al.*, 2021). Adenomyosis has also been associated with a higher rate of caesarean section (Harada *et al.*, 2022). Consequently, future research should be designed to investigate the relation of ICO stiffness to the route of obstetrical delivery. The presence of adenomyosis increased with age, as previously reported (Vercellini *et al.*, 2006; Struble *et al.*, 2016; Filip *et al.*, 2019; Zhai *et al.*, 2020) and was more frequent in women taking gonadal steroid therapy, probably because women were under treatment to improve their symptoms (Xholli *et al.*, 2021). Pathology frequently associated with adenomyosis was not included in the logistic models because our aim was to investigate a potential mechanism of pathogenesis. A few studies suggest that endometriosis and adenomyosis may share mechanisms of pathogenesis (Donnez *et al.*, 2021; Khan *et al.*, 2022). Consequently, endometriosis was not included in our analysis. For the same reason, we excluded leiomyomas; the decreased prevalence in the adenomyosis group was probably due to a selection bias. In exploratory logistic analyses, endometriosis and myomas did not change the relation between ICO stiffness and adenomyosis (data not shown).

The number of women prospectively evaluated in this study is rather large, and adenomyosis was diagnosed by ultrasonography, not histopathology. Given that it is not possible to remove the uterus to diagnose adenomyosis, especially in those who wish to retain fertility, ultrasound and MRI-based imaging techniques have been developed and refined and have demonstrated relatively high sensitivity and specificity when compared to histopathological examination. The lack of standardized histopathological protocols for examination of the extirpated uterus must be considered when histopathology is used to diagnose adenomyosis (Khan *et al.*, 2022). However, several studies using well-defined and comprehensive protocols have demonstrated that transvaginal ultrasound has an accuracy exceeding 80%, with a specificity that may reach 98% (Bazot *et al.*, 2001; Naftalin *et al.*, 2012; Van den Bosch *et al.*, 2015; Exacoustos and Zupi, 2018; Zannoni *et al.*, 2020; Harmsen *et al.*, 2022). Studies are still needed that compare the accuracy of each ultrasound feature, of their different combinations and of prospectively defined, comprehensive histopathological evaluations. It should be considered that uterus removal is always inappropriate in women desiring future fertility.

SE is a semiquantitative analysis and can be conditioned by the force applied by the operator during the analysis. Exam variability was minimized by allowing only a single experienced sonographer to perform all the analyses. Elastography output was evaluated using optimal compression and decompression cycles, as indicated by the elastography software. For each analysis, adjudication of the colour score was performed by three independent readers. In addition, statistical analyses were performed not only considering the value of each single ROI but also the ratios of ROI stiffness. This is because, assuming that during the analysis each ROI receives the same force of compression, an ROI ratio becomes independent of the absolute force applied (Ozturk *et al.*, 2018). Analyses were not performed on a particular day of the cycle, but never during menstruation. At the time of writing, there are no data indicating that ICO stiffness changes with the hormonal milieu. In our analysis, ICO stiffness was similar in women regardless of exposure to gonadal steroid therapy; in our study population, this generally comprised oestrogen- and progestin-containing contraceptives. Still, more specific studies are necessary to evaluate variations of ICO stiffness across the menstrual cycle in women with and without menstrual disturbances or adenomyosis. Other factors may cause an absolute or relative downstream obstruction to menstrual flow, such as a narrow or long cervical canal or when there is a large volume of blood loss. We did not measure the diameter of the cervical canal, but the length of the cervix was similar in cases and controls. We did not measure menstrual blood loss; the symptom of heavy menstrual bleeding was not independently related to the presence of adenomyosis, as both cases and controls were frequently under the influence of gonadal steroid therapy.

The findings of this study need to be replicated in other settings, but they seem to reveal an important association between ICO stiffness and adenomyosis. The possibility that this association hints at a pathogenetic mechanism and its potential clinical implications needs to be further explored.

Data availability

The data that support the findings of this study are available, but restrictions apply to the divulgation of these data, which were used under licence for the current study and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Ethics Committee.

Authors' roles

A.X. and A.C. design of the study, execution, analysis, manuscript drafting, and critical discussion. F.M. execution, analysis, creation of figures, and video and manuscript drafting. F.O., U.S., I.V., M.G.S., and E.C. data collection.

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Conflict of interest

None of the authors declare any conflict of interest.

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