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ORIGINAL ARTICLE

Anogenital distance in adult women is a strong marker of endometriosis: results of a prospective study with laparoscopic and histological findings

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STUDY QUESTION: Could anogenital distance (AGD) be a non-invasive marker of endometriosis and correlated to the American Society for Reproductive Medicine revised score (r-ASRM) and ENZIAN classifications?

SUMMARY ANSWER: Surgically and histologically proven endometriosis is associated with a short AGD in women of reproductive age but not correlated either to the severity or to the location of the disease.

WHAT IS KNOWN ALREADY: AGD is a marker of intrauterine androgen exposure and exposure to oestrogen-like chemicals such as phthalates. Moreover, exposure to endocrine disruptors, such as organochlorine chemicals, is associated with endometriosis. It has been suggested that a short AGD in women is associated with an increased risk of endometriosis based on clinical and ultrasound exams.

STUDY DESIGN, SIZE, DURATION: A prospective cohort study was conducted from January 2018 to June 2019 in a tertiary-care centre including 168 adult women undergoing pelvic surgery.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Of the 168 women included, 98 patients had endometriosis (endometriosis group) and 70 did not (non-endometriosis group). An operator (not the surgeon) measured the distance from the clitoral surface to the anus (AGD-AC) and from the posterior fourchette to the anus (AGD-AF) before surgery using a millimetre accuracy ruler. Endometriosis was diagnosed on exploration of the abdominopelvic cavity, and the r-ASRM and ENZIAN scores were calculated. All removed tissues underwent pathological examination.

MAIN RESULTS AND THE ROLE OF CHANCE: Mean (±SD) AGD-AF measurements were 21.5 mm (±6.4) and 32.3 mm (±8.1), and average AGD-AC measurements were 100.9 mm (±20.6) and 83.8 mm (±12.9) in the endometriosis and non-endometriosis groups ($P < 0.001$), respectively. Mean AGD-AF and AGD-AC measurements were not related to r-ASRM stage ($P = 0.73$ and 0.80, respectively) or ENZIAN score ($P = 0.62$ and 0.21, respectively). AGD-AF had a better predictive value than AGD-AC for discriminating the presence of endometriosis (AUC = 0.840 (95% CI 0.782–0.898) and 0.756 (95% CI 0.684–0.828)), respectively. For AGD-AF, an optimal cut-off of 20 mm had a specificity of 0.986 (95% CI 0.923–0.999), sensitivity of 0.306 (95% CI 26.1–31.6) and positive predictive value of 0.969 (95% CI 0.826–0.998). In multivariable analysis, the diagnosis of endometriosis was the only variable independently associated with the AGD-AF ($\beta = -9.66$ mm 95% CI −12.20–−7.12), $P < 0.001$).

LIMITATIONS, REASONS FOR CAUTION: The sample size was relatively small with a high proportion of patients with colorectal endometriosis reflecting the activity of an expert centre. Furthermore, we did not include adolescents and the AGD-AF measurement could be particularly relevant in this population.

WIDER IMPLICATIONS OF THE FINDINGS: The measurement of AGD could be a useful non-invasive tool to predict endometriosis. This could be especially relevant for adolescents and virgin women to avoid diagnostic laparoscopy and empiric treatment.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: Endometriosis / environmental effects / *in utero* exposure / surgery / anogenital distance

WHAT DOES THIS MEAN FOR PATIENTS?

The origin of endometriosis in women is unknown but may be linked to exposure of the embryo to chemicals called 'endocrine disruptors'. Exposure to endocrine disruptors can be assessed by measuring the anogenital distance (i.e. the distance between the clitoris and the anus), using a centimetre ruler with millimetre accuracy.

In this study, we compared the anogenital distance in patients with and without endometriosis. We showed that a short anogenital distance was strongly associated with the presence of endometriosis.

A short anogenital distance could therefore be used as a non-invasive marker, or predictor, of endometriosis.

Introduction

Endometriosis is a chronic disease characterised by the presence of glands and endometrial stroma outside the uterus (Giudice, 2010). It is an oestrogen-dependent disease affecting 10% of women of reproductive age (Eskenazi and Warner, 1997) with a negative impact on quality of life linked to chronic pelvic pain, dysmenorrhoea and/or infertility (Giudice, 2010). Three entities, which are often associated, have been identified: peritoneal endometriosis, ovarian endometriosis also called endometrioma and deep endometriosis (DE) defined by the infiltration of anatomical structures and organs (Nisolle and Donnez, 1997).

Numerous predictive factors have been explored to diagnose endometriosis non-invasively such as blood and urinary biomarkers (Nisenblat *et al*., 2016b) and endometrial features (Gupta *et al*., 2016). However, the accuracy of these approaches is poor, ranging between 0.56 and 0.74 (Nisenblat *et al*., 2016c). Female foetuses have a shorter anogenital distance (AGD) than males, and this is used as an early marker to determine gender during the first trimester (Ajay *et al*., 2009). In males, the AGD has been found to be a marker of intrauterine androgen exposure (Dean and Sharpe, 2013). High intrauterine androgen exposure results in a longer AGD. Conversely, exposure to oestrogen-like chemicals, such as some phthalates, reduces the AGD (Swan *et al*., 2015). Moreover, a recent meta-analysis (Cano-Sancho *et al*., 2019) has reported human epidemiological evidence of the association between exposure to endocrine disruptors, such as organochlorine chemicals, and endometriosis.

Clinical and ultrasound examinations suggest that a short AGD is associated with an increased risk of endometriosis in women (Sánchez–Ferrer *et al*., 2017; Sánchez-Ferrer *et al*., 2019). However, neither clinical nor imaging examinations can accurately diagnose peritoneal endometriosis, which is confirmed on biopsy following laparoscopy (Johnson and Hummelshoj, 2013). Diagnosis by transvaginal ultrasound (TVUS) is based on indirect signs, such as poor organ mobility, and is of low diagnostic value with an accuracy of 0.71 (Reid *et al*., 2019). MRI, on the other hand, using T1-weighted sequences with fat suppression can detect focused hyper-signs when the size of the implants exceeds 4 mm (Takahashi *et al*., 1994). Clinical and imaging techniques also have low accuracy for diagnosing DE (Bazot *et al*., 2009; Nisenblat *et al*., 2016a; Bazot and Daraï, 2017). In a meta-analysis, Nisenblat *et al.* (2016a) found that neither TVUS nor MRI can be used as a replacement, or even a triage, test to detect any type of pelvic endometriosis. Although TVUS and MRI have high accuracies to diagnose endometrioma and colorectal endometriosis, the sensitivities of these techniques for some DE locations, such as uterosacral endometriosis, the most common DE lesions, are 0.64 and 0.81, respectively.

The aim of this prospective study was therefore to compare the AGD in patients with endometriosis diagnosed on laparoscopic and histological findings with a non-endometriosis group and thus determine whether AGD could be a non-invasive marker of endometriosis. We also explored whether AGD was correlated with location and severity of endometriosis using both the revised American Society for Reproductive Medicine score (r-ASRM) and Enzian classifications, to identify an optimal cut-off for the clinical diagnosis of endometriosis.

Materials and Methods

Study population

We conducted a prospective cohort study from January 2018 to June 2019 in the tertiary-care Tenon University Hospital in Paris, France. All women over 18 years old who underwent scheduled or emergency pelvic surgery in the gynaecological department were included in the present analysis. Pregnant and menopausal women were excluded from the study. All the surgeons who performed the surgical procedure were skilled in the intraoperative diagnosis of endometriosis.

The following parameters were recorded: age at surgery, parity, history of vaginal delivery, BMI, smoking status, presence of endometrioma, superficial endometriosis and DE on imaging (MRI and TVUS), history of infertility before surgery, symptoms, previous surgery for endometriosis, type of surgery and surgical route (laparoscopy or laparotomy).

AGD was measured before the beginning of the surgery in the lithotomy position and thighs at a 45° angle to the examination table. Two measurements were performed using a centimetre ruler with millimetre accuracy (Fig. 1): from the clitoral surface to the anus (AGD-AC) and from the posterior fourchette to the anus (AGD-AF). The measurements were not carried out by the surgeon, who remained blinded to the AGD-AC and AGD-AF values.

Surgery consisted of exploring the abdominopelvic cavity to evaluate the diagnosis of endometriosis and to calculate the r-ASRM and surgical Enzian scores (Johnson *et al*., 2017). The Enzian score distributes the lesions into one of three compartments (A: the rectovaginal septum and vagina; B: the uterosacral ligament to the pelvic wall; and C: the rectum and sigmoid colon) and severity grades (Grade 1: when infiltration is <1 cm; Grade 2 when infiltration is between 1 and 3 cm; and Grade 3 when infiltration is >3 cm). Deep invasion beyond the lesser pelvis or invasion of organs is registered separately in the Enzian classification: FA (adenomyosis), FB (involvement of the bladder), FU (intrinsic involvement of the ureter), FI (bowel disease cranial to the sigmoid colon) and FO (other locations). In this study, only the highest grade of Enzian within the pelvis was considered. For patients with endometriosis, surgery was performed with a view to removing all visible lesions. All tissues removed during the surgery underwent anatomical pathology examination.

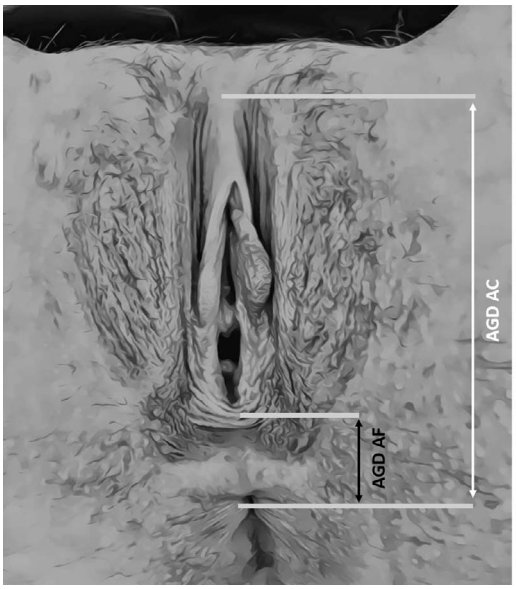

Figure 1 Measurement of anogenital distance. Landmarks for measuring the anogenital distance (AGD): from the clitoral surface to the anus (AGD-AC) and from the posterior fourchette to the anus (AGD-AF).

Patients with histological and/or laparoscopic findings of endometriosis were included in the endometriosis group. Patients without histological or laparoscopic findings of endometriosis were included in the non-endometriosis group.

Statistical analysis

Statistical analyses were carried out using Stata/IC 14.0 (StataCorp LLC4905, College Station, TX, USA), with significance value set at $P = 0.05$. Data are represented as mean ± SD for continuous variables or *n* (%) for categorical variables, where appropriate. To compare the variables across groups, Student's *t* test and ANOVA were used for normally distributed data, the Mann–Whitney *U* test for non-parametric data and the chi-square test for categorical data.

Simple and multiple linear regression models were conducted to search for a correlation between individual characteristics and both AGD-AC and AGD-AF. Variables correlated with the AGD (with a *P*-value <0.2) in the simple linear regression were incorporated in the multiple linear regression.

The AUC for the receiver operating characteristic curve measured the ability to discriminate the presence of endometriosis, with an AUC of 0.5 indicating no discrimination and a value of 1, perfect discrimination. We estimated the optimal cut-off to correlate both AGD and presence of endometriosis. The optimal cut-off was determined by a minimal *P* value approach. This involved dichotomizing the AGD into dummy variables with a cut-off every 5 mm. The cut-off with the lowest *P*-value was chosen as the optimal cut-off for this variable.

The protocol was approved by the 'Groupe Nantais d'Ethique dans le Domaine de la Santé' and registered under reference 02651077.

Results

Epidemiological and surgical characteristics of the population

Of the 168 patients eligible for inclusion during the study period, 98 had endometriosis (endometriosis group) and 70 did not (non-endometriosis group). The epidemiological characteristics of the study population are detailed in Table I. The patients in the endometriosis group were younger ($P < 0.001$) and had a lower parity rate ($P < 0.001$). Patients in the endometriosis group were more likely to smoke ($P = 0.017$). The BMI was similar in both groups with 17 (17%) obese patients in the endometriosis group versus 11 (16%) in the non-endometriosis group.

The surgical characteristics of the endometriosis group are summarised in Table II. All surgeries were performed by laparoscopy. Two women (2%) had endometrioma alone, and 56 (57%) had bowel endometriosis. Of the 98 women with endometriosis 6, 18, 15 and 61% had

Table I Characteristics of the study populations.

Patients	Endometriosis group (*N* = 98)	Non-endometriosis group (*N* = 70)	*P* value
Age mean (SD)	34.1 (6.6)	39.9 (9.3)	$< 10^{-5}$
BMI (kg m^{-2}) mean (SD)	24.9 (5.4)	26.2 (5.7)	0.15
Parity			0.001
0	62	25	
1	16	14	
≥2	20	31	
Prior vaginal delivery *N* (%)			$< 10^{-3}$
Yes	25 (25.5)	37 (52.9)	
No	73 (74.5)	33 (47.1)	
Smoking *N* (%)	22 (22)	6 (8.6)	0.017
Obese (BMI ≥ 30 $kg.m^{-2}$) *N* (%)	17 (17)	11 (16)	0.67

AGD-AF, anogenital distance from the anus to the posterior fourchette; AGD-AC, anogenital distance from the anus to the anterior clitoral surface.

Table II Distribution of endometriosis lesions and surgical procedures.

Characteristics	Number *N* (%) of patients in the endometriosis group (*N* = 98)
Bowel endometriosis	56 (57%)
Colorectal endometriosis	55 (98%)
Small bowel alone	1 (2%)
Colorectal and small bowel	7 (35%)
Colorectal and caecum	9 (16%)
Distribution of endometriosis lesions	
Endometrioma and deep endometriosis	34 (35%)
Endometrioma alone	2 (2%)
Torus uterinum	80 (82%)
Utero-sacral ligament endometriosis	81 (83%)
Vaginal endometriosis	20 (20%)
Bladder endometriosis	3 (3%)
Adenomyosis	21 (21%)
Distribution of patients according to r-ASRM and Enzian scores	
r-ASRM	
r-ASRM I	6 (6%)
r-ASRM II	17 (18%)
r-ASRM III	15 (15%)
r-ASRM IV	60 (61%)
Enzian	
Grade 1	16 (16%)
Grade 2	19 (20%)
Grade 3	63 (64%)
Type of colorectal surgery	
Shaving	14 (14%)
Discoid excision	16 (16%)
Segmental resection	25 (26%)

r-ASRM revised American Society for Reproductive Medicine score; DE deep endometriosis
Some patients had multiple synchronous locations.

an r-ASRM stage I, II, III and IV, respectively, and 16, 20 and 64% had an Enzian score I, II and III, respectively. All the cases of endometriosis diagnosed intraoperatively and by tissue resection were confirmed by histology. Adenomyosis was found in 21 patients with endometriosis (21%).

In the non-endometriosis group, 52 women (74%) underwent surgery by laparoscopy and 18 by laparotomy (26%). The distribution of surgical procedures is summarised in Table III. During the surgical procedure, tissue removal was required for 65 patients (93%), for whom no evidence of endometriosis was found on histological examination. Five patients (7%) had no histology as the surgery did not require tissue removal: one laparoscopic extraction of an intrauterine device, three methylene blue tube tests and one adnexal torsion. Adenomyosis was found in 18 patients (26%): 17 on histology and 1 on MRI.

Distribution of AGD measurements according to the groups

The average AGD measurements and their distribution within the groups are summarised in Table IV and Fig. 2. Average AGD-AF measurements were 21.5 mm (±6.4) and 32.3 mm (±8.1), respectively, in the endometriosis and non-endometriosis groups ($P < 10^{-5}$). Average AGD-AC measurements were 83.8 mm (±12.9) and 100.9 mm (±20.6) in the endometriosis and non-endometriosis groups, respectively ($P < 10^{-7}$).

In the endometriosis group, AGD-AF and AGD-AC measurements in patients with and without associated adenomyosis were respectively 21.1 mm (±6.9) and 21.6 mm (±6.3) ($P = 0.77$), and 87.1 mm (±16.5) and 82.9 mm (±11.8) ($P = 0.27$). In the non-endometriosis group, AGD-AF and AGD-AC measurements in patients with and without adenomyosis were respectively 31.9 mm (±7.3) and 32.2 mm (±8.9) ($P = 0.89$), and 98.1 mm (±22.5) and 101.6 mm (±20.0) ($P = 0.57$).

Distribution of AGD measurements according to the endometriosis phenotype and severity

In the endometriosis group, no difference in the average AGD-AF and AGD-AC was found between patients with and without endometrioma:

Table III Surgical indications in the non-endometriosis group.

Surgical procedures and indications	Number *N* (%) of patients in the non-endometriosis group
Laparoscopic:	**52 (74%)**
Hystectectomy for:	21 (30%)
Malignant lesions	3 (4%)
Benign lesions	18 (26%)
Myomas	11 (16%)
Myoma and adenomyosis	3 (4%)
Adenomyosis	4 (6%)
Myomectomy	2 (3%)
Bilateral salpingo-oophorectomy:	9 (13%)
Cancer and borderline	2 (3%)
Benign cyst	7 (10%)
Laparoscopic sacrocolpopexy	4 (6%)
Ovarian cystectomy	9 (13%)
Others:	7 (10%)
Infertility management	4 (6%)
Adnexal torsion	1 (1%)
Extrauterine pregnancy	1 (1%)
Extraction of intrauterine device	1 (1%)
Laparotomy:	**18 (26%)**
Hysterectomy	7 (10%)
Hysterectomy with adnexectomy	1 (1%)
Debulking: hysterectomy oophorectomy, omentectomy, lymphadenectomy	1 (1%)
Myomectomy	9 (13%)

83.1 mm (±13.1) and 84.1 mm (±12.9) ($P = 0.79$), and 21.3 mm (±5.9) and 21.1 mm (±6.6) ($P = 0.36$), respectively. Similarly, no difference in the average AGD-AF and AGD-AC was found between patients with and without bowel endometriosis: 21.8 mm (±6.6) and 21.1 mm (±6.1) ($P = 0.60$) and 85.3 mm (±14.1) and 81.7 mm (±11.1) ($P = 0.15$), respectively.

Mean AGD-AF and AGD-AC measurements were not statistically different between patients with an r-ASRM stage I, II, III and IV ($P = 0.76$ and 0.80, respectively). Average AGD-AF and AGD-AC measurements were not statistically different between patients with an Enzian scores I, II and III ($P = 0.62$ and 0.21, respectively).

Univariable and multivariable analysis and diagnostic relevance of AGD for endometriosis

Results for the univariable and multivariable linear regression on the AGD-AF and AGD-AC are presented in Tables V and VI, respectively. The diagnosis of endometriosis was negatively associated with both the AGD-AF ($\beta = -9.66$ mm (95% CI −12.20–−7.12), $P < 0.001$) and AGD-AC ($\beta = -13.75$ mm (95% CI −19.37–−8.12), $P < 0.001$) in multivariable analysis. Age ($\beta = +0.45$ mm (95% CI 0.062–0.83), $P = 0.023$) and BMI ($\beta = +0.63$ mm (95% CI 0.14–1.12), $P = 0.012$) were positively associated with the AGD-AC measurements in multivariable analysis.

The diagnostic relevance of AGD is represented by the ROC curves in Fig. 3. AGD-AF had a better predictive value than AGD-AC for discriminating the presence of endometriosis with an AUC of 0.840 (95% CI 0.782–0.898) and 0.756 (95% CI 0.684–0.828).

The definition of an optimal cut-off denoting the strongest correlation between AGD-AF and the presence of endometriosis selected with a *P* value approach is summarised in Fig. 4. With a cut-off of 20 mm, we obtained a specificity of 98.6% (95% CI 0.923–0.999), a sensitivity of 30.6% (95% CI 26.1–31.6) and a positive predictive value (PPV) of 0.969 (95% CI 0.826–0.998).

Discussion

This is the first study to demonstrate that surgically and histologically proven endometriosis is significantly associated with a short AGD, and especially AGD-AF, in women of reproductive age. AGD was not found to be correlated either to the severity or to the location of the disease.

The strength of our study is that the diagnosis of endometriosis was proved surgically and confirmed by histology as recommended by gynaecological societies ('Practice bulletin no. 114', 2010; Dunselman *et al.*, 2014; Collinet *et al.*, 2018). In previous studies reporting AGD measurements in women with endometriosis, endometriosis was diagnosed by clinical examination and TVUS (Sánchez-Ferrer *et al.*, 2017; Sánchez-Ferrer *et al.*, 2019): the control group in these studies

Table IV Evaluation of AGD according to histological findings.

Groups and *P* values.	AGD-AF mm (SD)	AGD-AC mm (SD)
All patients		
Endometriosis group ($n = 98$)	21.5 (6.4)	83.8 (12.9)
Non-endometriosis group ($n = 70$)	32.3 (8.1)	100.9 (20.6)
P value	$<10^{-5}$	$<10^{-7}$
In patients with prior vaginal delivery		
Endometriosis group ($n = 25$)	21.2 (7.4)	84.4 (17.1)
Non endometriosis group ($n = 37$)	33.1 (8.6)	101.6 (21.3)
P value	$<10^{-6}$	0.0008
In patients without prior vaginal delivery		
Endometriosis group ($n = 73$)	21.7 (6)	83.6 (11.8)
Non endometriosis group (n = 33)	31.4 (7.6)	100.1 (20)
P value	$<10^{-10}$	$<10^{-6}$
In obese patients (BMI $\geq$ 30 kg m^{-2})		
Endometriosis group ($n = 17$)	21.2 (6.5)	84.4 (17.8)
Non-endometriosis group ($n = 11$)	38.2 (9.6)	117.7 (26.6)
P value	$<10^{-4}$	0.002
In patients with adenomyosis ($n = 49$)		
Endometriosis group ($n = 21$)	21.1 (6.9)	87.1 (16.5)
Non-endometriosis group ($n = 18$)	21.1 (6.9)	87.1 (16.5)
P value	$<10^{-4}$	0.09
Endometriosis group ($n = 98$)		
Patients with adenomyosis ($n = 31$)	21.1 (6.9)	87.1 (16.5)
Patients without adenomyosis ($n = 67$)	21.6 (6.3)	82.9 (11.8)
P value	0.77	0.27
Non-endometriosis group ($n = 70$)		
Patients with adenomyosis ($n = 18$)	31.9 (7.3)	98.1 (22.5)
Patients without adenomyosis ($n = 38$)	32.2 (8.9)	101.6 (20.0)
P value	0.89	0.57
No information ($n = 14$)		

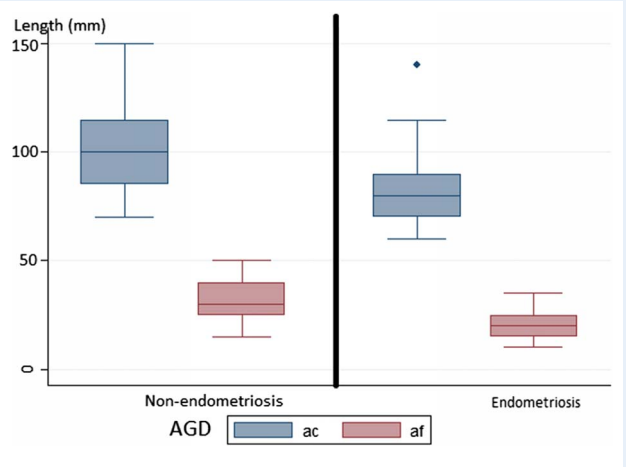

Figure 2 Distribution of the AGD-AC and AGD-AF values in the endometriosis and non-endometriosis group. Box-whisker plots for AGD in the endometriosis ($n = 98$) and non-endometriosis ($n = 70$) groups. The box represents the values for the median and the 25th and 75th percentiles. The whisker plots represent the upper and lower adjacent values. The outside points represent the outside values.

consisted of patients without suggestive symptoms of endometriosis and with normal physical examination and normal TVUS. This selection introduces a major bias as it has been demonstrated that 2–50% of patients with endometriosis are asymptomatic (Nisenblat *et al.*, 2016a). Moreover, physical examination, even performed by experts, can often misdiagnose endometriosis (Bazot *et al.*, 2009). Finally, a recent Cochrane review by (Nisenblat *et al.*, 2016a) on the accuracy of imaging techniques to assess the diagnosis of pelvic endometriosis, demonstrated the low accuracy of TVUS, even when performed by experienced physicians (Nisenblat *et al.*, 2016a): although the accuracy of TVUS was high for diagnosing endometriomas, it was only 65% for uterosacral ligament involvement and peritoneal endometriosis (Nisenblat *et al.*, 2016a).

Early, non-invasive diagnosis of patients with suspected endometriosis is crucial for optimal patient management. However, in the absence of pathognomonic clinical presentation or imaging techniques, diagnosis can take as long as 5–11 years (Soliman *et al.*, 2017). Moreover, diagnosis is particularly difficult in adolescents who have different symptoms from adults. For example, most adolescents experience non-cyclic pain. Furthermore, it is not possible to examine a virgin patient by the vaginal route or by TVUS (Laufer *et al.*, 2003; Shim and Laufer, 2019). Faced with this clinical dilemma, the current study is of interest as we demonstrate that the AGD measurement could constitute a non-invasive diagnostic alternative for endometriosis. We found that AGD-AF is a better marker than AGD-AC, with an AUC of 0.840 (95% CI 0.782–0.898) compared with 0.756 (95% CI 0.684–0.828), respectively. These data are in agreement with those of Sanchez-Ferrer *et al.* (2017) showing that, based on physical examination and TVUS, the AGD-AF but not AGD-AC was associated with the presence of endometriomas and DE ($P < 0.001$–0.02) or both (Sánchez-Ferrer *et al.*, 2017). Using multivariable analysis, the current study found that AGD-AF was independent of age, obesity, prior vaginal delivery and parity. Finally, a cut-off of 20 mm for AGD-AF had a specificity of 0.98 and a PPV of 0.969 (95% CI 0.826–0.998) supporting its use as a physical marker of endometriosis. This result is also in agreement with those of Sánchez-Ferrer *et al.*, (2017), who obtained a cut-off of 20.9 mm. For patients with endometriomas and DE, Mendiola *et al.*, (2016) observed that women in the lowest tertile of the AGD-AF distribution were 7.6-times (95% CI 2.8–21.0; *P* trend <0.001) more likely to have endometriosis compared with those in the upper tertile. Similarly, for women with DE they observed that an AGD-AF below the median were 41.6-times (95% CI 3.9–438; $P = 0.002$) more likely to have endometriosis than those with an AGD-AF above the median (Mendiola *et al.*, 2016). However, in contrast to the present study, no simple criterion was found to identify patients with a high risk of endometriosis.

Another crucial result of the current study, which included patients with early stages of the disease, is that a short AGD-AF is independent

Table V Univariable and multivariable linear regression on the AGD-AF.

	Univariable		Multivariable	
Variable	**Coef β (95% CI)**	***P* value**	**Coef β (95% CI)**	***P* value**
Age (years)	0.34 (0.18;0.49)	$<1.10^{-3}$	0.16 (−0.01;0.33)	0.07
BMI (kg m^{-2})	0.31 (0.06;0.56)	0.016	0.19 (−0.03;0.4)	0.09
Prior vaginal delivery	3.61 (0.84;6.38)	0.011	−0.09 (−4.08;3.89)	0.963
Parity				
0				
1	2.79 (−.87;6.45)	0.134	0.044 (−4.25;4.33)	0.984
≥2	4.30 (1.25 ;7.35)	0.006	0.458 (−4.88 ;5.80)	0.866
Endometriosis	−10.75 (−12.97; −8.54)	$<1.10^{-3}$	−9.66 (−12.20; −7.12)	$<$**1.10 $^{-3}$**

β = coefficient for the variable in the linear regression model (in millimetre)
Smoking, size, gravidity, Enzian grades, r-ASRM scores, bowel involvement and adenomyosis were also tested but non-significant in univariable analysis ($P > 0.2$)

Table VI Univariable and multivariable linear regression on the AGD-AC.

	Univariable		Multivariable	
Variable	**Coef β (95% CI)**	***P* value**	**Coef β (95% CI)**	***P* value**
Age (years)	0.75 (0.43;1.07)	$<1.10^{-3}$	0.45 (0.062;0.83)	**0.023**
BMI (kg m^{-2})	0.87 (0.37;1.38)	0.009	.63 (0.14;1.12)	**0.012**
Prior vaginal delivery	5.95 (0.14;11.76)	0.045	−4.07 (−12.92;4.78)	0.365
Parity				
0				
1	5.82 (−1.78 ;13.43)	0.133	1.65 (−7.9 ;11.17)	0.732
≥2	9.4 (3.12;15.79)	0.004	2.80 (−9.04 ;14.65)	0.641
Endometriosis	−17.13 (−22.27; −12.031)	$<1.10^{-3}$	−13.75 (−19.37;-8.12)	$<$**10 $^{-3}$**

β = coefficient for the variable in the linear regression model (in millimetre)
Smoking, size, gravidity, Enzian grades, r-ASRM scores, bowel involvement and adenomyosis were also tested but non-significant in univariable analysis ($P > 0.2$).

of r-ASRM and Enzian classification. Indeed, we observed that patients with an r-ASRM stages I–II (23 patients, 24%) had similar values of AGD-AF to those of advanced stages, confirming that AGD-AF can be used to diagnose patients with minor endometriosis lesions. Similarly, we did not find a relation between AGD-AF measurements and the presence of endometrioma. In the same way, our results support that AGD-AF measurements were independent of the extent of DE even in patients with colorectal infiltration, which represents one of the most severe forms of endometriosis. Our results are in contrast to those of previous studies (Mendiola *et al.*, 2016) suggesting a relation between AGD-AF and the presence of DE. Indeed, Sanchez-Ferrer *et al.* (2017) noted that the highest AUC (0.91; 95% CI 0.84 to 0.97) was obtained for the DE subgroup with a sensitivity and specificity of 84.4 and 91.4%, respectively (Sánchez-Ferrer *et al.*, 2017).

From a pathophysiological point of view, a short AGD-AF in women with endometriosis suggests that they were exposed to genetic and epigenetic factors and endocrine disruptors early during their intrauterine life. Using the cord blood transcriptome, Remy *et al.* found that the presence of dichlorodiphenyldichloroethylene, polychlorinated biphenyl-153, perfluorooctanoic acid and perfluorooctane sulfonate were potentially associated with metabolic disorders later in life (Remy *et al.*, 2016). Similarly, Solomon *et al.* (2017) identified genes with differentially methylated regions (DMRs) in a group of 336 Mexican–

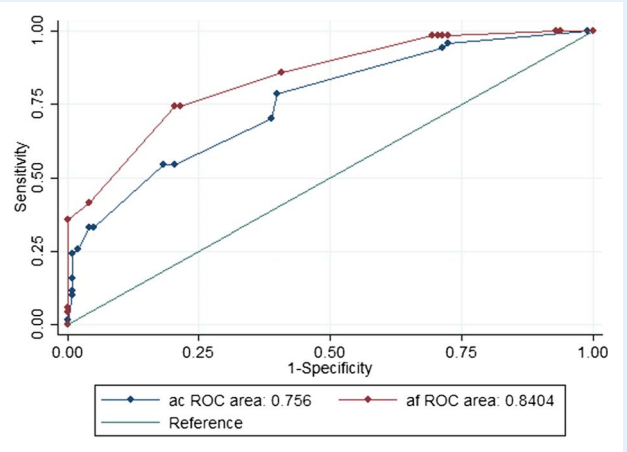

Figure 3 Receiver operating characteristic curves for AGD-AC and AGD-AF. ROC: receiver operating characteristic.

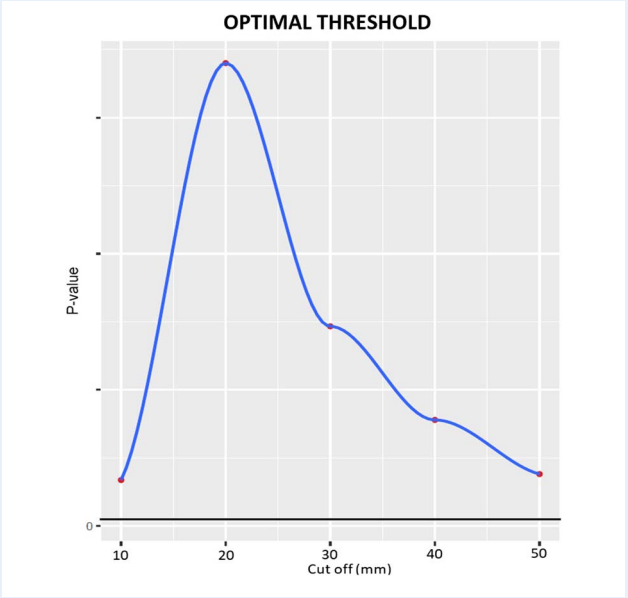

Figure 4 Optimal cut-off distribution for correlation between AGD AF and presence of endometriosis. The optimal cut-off for AGD-AF was determined using the minimal *P* value approach.

American newborns. These genes with DMRs are involved in the inflammatory response (interleukin receptor-associated kinase 4 and endothelial cell-specific molecule 1), cancer (breast cancer type 1 and LIM and sarcoma homolog 3 protein 1), endocrine function (canopy 1) and male fertility (intraflagellar transport 140 homolog, calcineurin B homologous protein 3 and pain receptor domain 8). In contrast, although some reports highlight similar pathways in the pathogenesis of endometriosis and adenomyosis, normal AGD length was found in women with adenomyosis. Thus, although our sample size of patients with adenomyosis was small, our study suggests a different pathogenesis between endometriosis with early exposure to endocrine disruptor and adenomyosis.

Some limits of the present study deserve to be underlined. First, the sample size was relatively small and there was a high proportion of patients with colorectal endometriosis, which reflects the activity of an expert centre. However, one-quarter of our population with endometriosis had r-ASRM stages I–II, which suggests the relevance of AGD-AF as a marker of endometriosis independent of the extent of the disease. Second, due to the low number of patients with adenomyosis in the endometriosis group, we could not evaluate the relevance of AGD-AF according to internal and external adenomyosis, which may have a different pathogenesis. Third, our population excluded adolescents whereas AGD-AF measurements in this demographic could be a particularly relevant non-invasive tool to be integrated in a first diagnostic approach. Finally, further studies are required to evaluate the use of AGD-AF measurements as a triage test to reduce the costs of diagnosis: MRI could potentially be reserved for patients with a short AGD-AF.

In conclusion, the present study supports the use of AGD-AF as a physical marker of endometriosis. The potential psychological impact and patient acceptance of performing this measurement during routine gynaecologic visits deserves to be evaluated.

Authors' roles

Study concept and design: A.A. and E.D. Acquisition of data: A.C., M.B., A.-S.B. Statistical analysis: C.F. and S.B. Interpretation and synthesis of data: A.A., S.P. and E.D. Drafting the manuscript: A.C. Supervision and critical revision of the manuscript for important intellectual content: A.A., E.D. and S.B. All authors have read, and confirmed that they meet, the authorship criteria.

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Conflict of interest

None.

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