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ARTICLE

Two- and three-dimensional transvaginal ultrasonography for diagnosis of adenomyosis of the inner myometrium

BIOGRAPHY

Christina Kjaergaard Rasmussen is a doctor and PhD student at the Department of Obstetrics and Gynecology, Aarhus University Hospital, Denmark. Her work emphasizes the potential role of three-dimensional imaging for the diagnosis of internal adenomyosis, focusing on the ultrasonographic assessment of the junctional zone in relation to histopathology.

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KEY MESSAGE

A combination of at least two two-dimensional features and two three-dimensional (junctional zone) features may be highly accurate for the diagnosis of adenomyosis of the inner myometrium. When the junctional zone cannot be visualized or measured, a diagnosis may rely on the presence of at least three two-dimensional features.

ABSTRACT

Research question: How diagnostically accurate is two-dimensional (2D-TVS) compared with three-dimensional transvaginal ultrasonography (3D-TVS) in diagnosing adenomyosis of the inner myometrium. What is the most accurate combination of ultrasonographic features?

Design: Premenopausal women (n = 110) scheduled for hysterectomy or transcervical resection of the endomyometrium owing to abnormal uterine bleeding were consecutively enrolled. All participants had real-time 2D-TVS and, later, blinded off-line 3D-TVS to diagnose adenomyosis. Results were compared with a detailed histopathological examination of the inner myometrium as gold standard.

Results: Prevalence of adenomyosis of the inner myometrium was 29%. For 2D-TVS and 3D-TVS, respectively, the diagnostic accuracy was sensitivity 72% (95% CI 53 to 86) and 69% (95% CI 50 to 84); specificity 76% (95% CI 65 to 85) and 86% (95% CI 76–93); and area under the curve (AUC) 0.74 (95% CI 0.7 to 0.8) and 0.77 (95% CI 0.7 to 0.9). Specificity of 3D-TVS was not statistically significantly better than 2D-TVS; the difference between them almost reached statistical significance (P = 0.06). The most accurate three-dimensional feature was junctional zone irregularity (JZ_{max}–JZ_{min} ≥5mm) (AUC: 0.78). A combination of two or more two-dimensional and two or more three-dimensional features was highly accurate (AUC: 0.77).

Conclusions: For diagnosing adenomyosis of the inner myometrium, 3D-TVS offers a high accuracy similar to 2D-TVS. Identification of junctional zone irregularity with 3D-TVS may be beneficial to diagnosis. Two or more two-dimensional features and two or more three-dimensional features combined may give a more objective diagnosis, and may be useful for clinical practice and future research.

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KEYWORDS

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Diagnostic accuracy
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Ultrasonography

INTRODUCTION

Adenomyosis is often referred to as a disease of the inner myometrium (Bird et al., 1972; Brosens et al., 1995; Leyendecker and Wildt; 2011; Dueholm, 2017), also known as the junctional zone. The inner myometrium or endomyometrial junctional zone differs from the outer myometrium in the architecture of the muscle cells causing its hypoechoic appearance on ultrasonography (Tetlow et al., 1999). Adenomyosis of the inner myometrium is adenomyosis. This is developed in direct connection to the endomyometrial junction (Kishi et al., 2012) rather than the extrinsic adenomyosis located in the outer shell of the uterus and intramural adenomyosis, where healthy muscular structures are preserved between the adenomyosis and the endomyometrial junction. Most studies suggest that adenomyosis develops as a direct invasion of the endometrium into the myometrium, causing junctional zone changes and expansion (Togashi et al., 1988). This may be regarded as intrinsic adenomyosis (Kishi et al., 2012). To improve the diagnosis of adenomyosis in early stages, special attention must be drawn to the inner myometrium (or junctional zone).

In clinical practice, two-dimensional transvaginal ultrasonography (2D-TVS) is often the preferred diagnostic tool owing to its high availability, low cost and low time consumption. Adenomyosis diagnosis with 2D-TVS relies on a subjective evaluation of several myometrial ultrasonographic two-dimensional features representing both ectopic endometrial tissue and muscular hypertrophia and hyperplasia (Bromley et al., 2000; Dueholm, 2006; Sakhel and Abuhamad, 2012). Although previous studies have shown that 2D-TVS is accurate in the diagnosis of adenomyosis (Meredith et al., 2009; Andres et al., 2018), three-dimensional transvaginal ultrasonography (3D-TVS) may hold much potential as it offers the advantage of detailed display of the junctional zone (Exacoustos et al., 2011; Naftalin et al., 2012). Moreover, 3D-TVS makes it possible to store three-dimensional volumes to be used for later off-line examination.

So far, 3D-TVS for the diagnosis of adenomyosis has been evaluated in three studies (Exacoustos et al., 2011; Luciano et al., 2013; Tellum et al., 2018). No blinded comparison between 2D-TVS and 3D-TVS has been conducted. One study showed that addition of 3D-TVS to 2D-TVS improved the diagnosis of adenomyosis (Exacoustos et al., 2011). They reported sensitivity and specificity with 3D-TVS based on the best cut-off values for combined three-dimensional features.

Existing studies on diagnostic accuracy for image diagnosis of adenomyosis are reliant on pathological examination of the uterus from patients having hysterectomy. The challenge lies mainly in evaluating the accuracy of an image diagnosis of adenomyosis among a broader group of patients (Naftalin et al., 2014; 2016). A widely used histopathological criteria for adenomyosis is the presence of endometrial glands within the myometrium in a distance of at least one field of view (about 2 mm) from the basal endometrium (Gompel, 1985). In patients undergoing transcervical resection of the endometrium (TCRE), a histopathologic reference standard based on parallel and deep endomyometrial biopsies representing the entire endomyometrial border may diagnose adenomyosis within the inner myometrium (McCausland, 1992; Wood et al., 1994; Goswami et al., 1998; Darwish et al., 1999).

Adenomyotic foci located solely within the outer myometrium may constitute another associated disease entity derived from deep infiltrating endometriosis (extrinsic or external adenomyosis) (Chapron et al., 2017). In this study, our focus was on intrinsic and internal adenomyosis (adenomyosis of the inner myometrium). We hypothesized that off-line 3D-TVS may improve the diagnosis compared with real-time 2D-TVS owing to improved visualization of the junctional zone with off-line image manipulation. To the best of our knowledge, this is the first study that evaluates patients scheduled for hysterectomy but also patients scheduled for TCRE using the same histopathological criteria for both methods.

The aim of this study was to evaluate and compare the diagnostic accuracy of real-time 2D-TVS and off-line 3D-TVS for diagnosing adenomyosis of the inner myometrium. It also aims to identify the most accurate combination of two-dimensional and three-dimensional ultrasonographic features.

MATERIALS AND METHODS

Study design

This blinded, prospective study was approved by the local ethics committee of the Central Denmark Region and Danish Data Protection Agency. The Standards for Reporting of Diagnostic Accuracy checklist was followed (Bossuyt et al., 2003). Written informed consent was obtained from all study participants. From October 2011 to early July 2013, a consecutive sample of premenopausal women (n = 110) scheduled for either hysterectomy (n = 46) or TCRE (n = 64) caused by heavy menstrual bleeding, menstrual pain, or both, was recruited at the Department of Gynecology and Obstetrics, Aarhus University Hospital, Denmark. Recruitment of participants was carried out by a medical student (CKR). Inclusion and exclusion criteria of study participants are presented in FIGURE 1.

Inner adenomyosis was defined when adenomyosis was located in connection with the endomyometrial junction, often with healthy muscular structures preserved outside the adenomyosis. In cases in which the whole uterine wall was affected, however, inner adenomyosis was defined when most of the adenomyosis was located in the inner half of the myometrium.

Transvaginal ultrasonography

Early on the day of surgery, all patients underwent 2D-TVS examination by the same gynaecologist (MD), who had more than 10 years' experience and was blinded to any previous findings or diagnoses. The 2D-TVS was an additional ultrasonographic examination carried out for this study only. The type of surgical procedure was determined several weeks before surgery, and all study participants had their preliminary examination carried out by other gynaecologists. The experienced gynaecologist had no access to the patient's medical record before the ultrasonographic examination and was blinded to any previous findings or diagnoses. Patients were informed about the blinding before the examination. All examinations were carried out in two perpendicular planes using a High-End Voluson E8 Expert machine (GE Healthcare Ultrasound, Milwaukee, WI, USA) equipped with a multifrequency (6–12 MHz) endovaginal probe. The diagnosis of adenomyosis of the inner myometrium was based

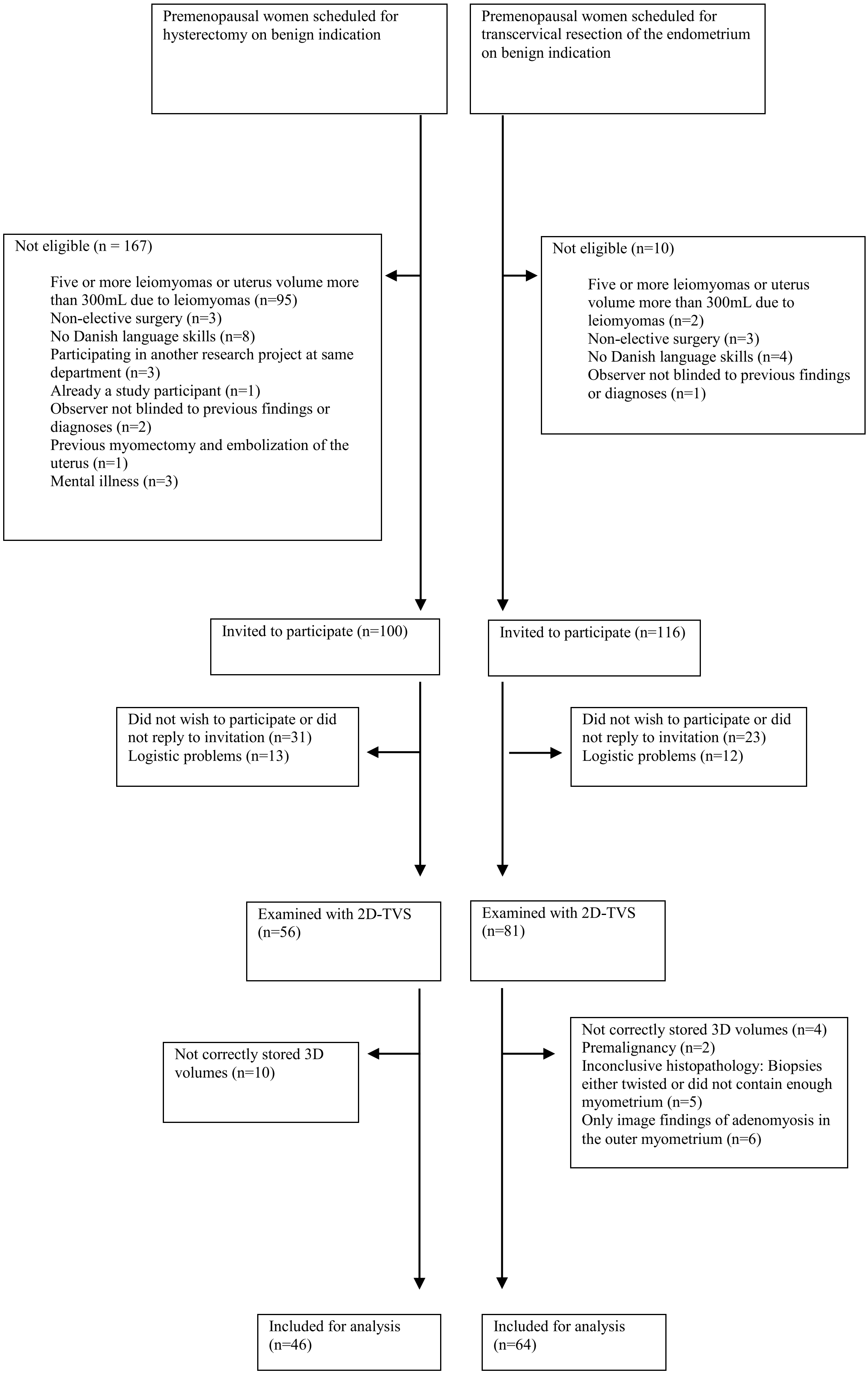

FIGURE 1 Participant selection.

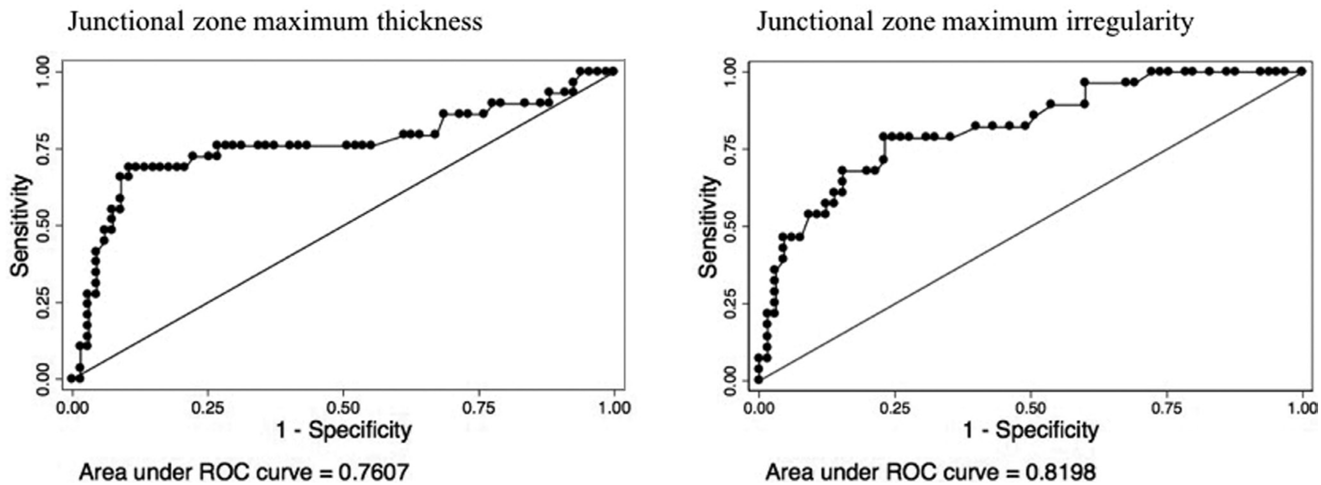

FIGURE 2 Receiver operating characteristic (ROC) curves for junctional zone measurements.

on a subjective evaluation of the characteristic two-dimensional features described in a consensus paper (*Van den Bosch et al., 2015*). The presence of five characteristic two-dimensional features were reported for each patient, including heterogeneity, anechoic lacunae, myometrial cysts, fanshaped shadowing and asymmetric corpus myometrium. An example is shown in FIGURE 2. At each examination, at least two three-dimensional volumes and two three-dimensional Doppler angiograms of the uterus were obtained and stored in a separate file. Off-line 3D-TVS took place at least 6 months after real-time 2D-TVS, over a time period of about 2 years, four-dimensional view programme and volume contrast imaging (VCI 2–4 mm) was used as described previously (*Rasmussen et al., 2016*). All volume-files were assigned a new identification number and date of examination, and patient identification was removed from the three-dimensional volumes. This was to ensure successful blinding between real-time 2D-TVS and off-line 3D-TVS and to avoid recall bias. The three-dimensional volume acquisition technique was standardized as follows: frequency 6 MHz, highest possible zoom, sweep angle 120°, sweep velocity maximum quality, and highest quality for Doppler angiograms. Besides an evaluation of the characteristic two-dimensional features, off-line 3D-TVS also included evaluation and measurements of characteristic features of the junctional zone (*Van den Bosch et al., 2015*). The presence of four characteristic three-dimensional features were reported for each patient, including junctional zone thickness, junctional zone irregularity, subendometrial lines and buds, and junctional zone interruption. An example is shown in FIGURE 3. The junctional zone was evaluated in all three planes (sagittal, transversal and coronal) and measured as maximum (JZ_{max}) and minimum (JZ_{min}) junctional zone in each wall. These measurements were later used to calculate junctional zone irregularity (JZ_{max}–JZ_{min} = JZ_{dif}) and to identify the most accurate measure of junctional zone thickening (JZ_{max}) and junctional zone irregularity.

Reference standard

For the purposes of this study, we developed a new method for detailed histopathological examination of the inner myometrium in uterus specimens and endomyometrial biopsies. The same experienced gynaecological pathologist carried out all histopathological examinations, blinded to the findings from real-time 2D-TVS and applied the same criteria to both type of specimens.

Before starting the study, the pathologist correlated histopathology of 10 hysteroscopic endomyometrial biopsies with hysterectomy specimens to identify the needed myometrial depth to detect adenomyosis of the inner myometrium. The biopsies proved sufficient with a myometrial depth of at least 5 mm given this was the depth needed in hysterectomy specimens for a diagnosis of adenomyosis of the inner myometrium. The diagnosis obtained by endomyometrial biopsies was confirmed in all but one patient who later had a hysterectomy (n = 15).

Deep endomyometrial biopsies were systematically obtained. To ensure correct orientation and to avoid twisting of the biopsies, each biopsy was fastened on separate polystyrene-coated plates with small needles. Uterine specimens had histopathological analysis with a slice thickness of 1–1.5 cm. The same diagnostic criteria for adenomyosis of the inner myometrium was used for uterus specimens and endomyometrial biopsies. A diagnosis of adenomyosis of the inner myometrium was positive when a focus of endometrial glands was situated at a distance of at least one field of view (using a 10 x objective) (2 mm) from the endometrium without contact to the basal endometrium (*Gompel, 1985*).

The histopathological evaluation took place within 1–3 weeks of surgery. The pathologist documented the findings from both uterus specimens and endomyometrial biopsies in the standardized form designed for this study. Results from the histopathological evaluation remained blinded to the observer until all off-line 3D-TVS evaluation was completed. Details of the histopathological examination are presented in the **Appendix**.

Statistical analysis

The sample size was calculated on the basis of a presumed prevalence of adenomyosis of the inner myometrium of 30% and a difference in area under the curve (AUC) of 15% between 2D-TVS and 3D-TVS (power 80%, type I error <0.05). Wilcoxon's signed rank test, McNemar's and the

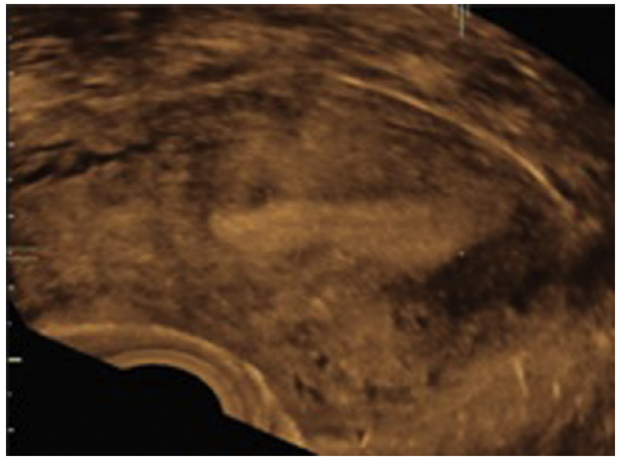

FIGURE 3 Two-dimensional transvaginal ultrasonography (2D-TVS) of the uterus in the sagittal plane, with the presence of heterogeneity, anechoic lacunae and asymmetric corpus myometrium associated with adenomyosis.

Pearson chi-squared tests were used for comparison of patients with or without a histopathological diagnosis of adenomyosis of the inner myometrium. The diagnostic accuracy was calculated and reported as sensitivity, specificity and AUC. Receiver operating characteristics (ROC) curves (*Richardson et al., 1985*) were used, with calculation of minimally important change thresholds to determine the best cut-off value for junctional zone maximum thickness and difference between maximum and minimum thickness in the present study (*Farrar et al., 2001*). The ROC analysis was used to compare AUC between 2D-TVS and 3D-TVS and between patients scheduled for either hysterectomy or TCRE. STATA 13.1, (StataCorp, Texas, USA) was used for all statistical analyses.

Ethical approval

Local Ethics Committee of the Central Denmark Region, journal number: 20010175, date of approval: 19/09-2011. Danish Data Protection Agency, journal number: 2007-58-0010, date of approval: 03/10-2011.

RESULTS

A total of 110 patients met the inclusion criteria: both real-time 2D-TVS and off-line 3D-TVS were conducted by the same observer and sufficient histopathology was obtained for the diagnosis of adenomyosis of the inner myometrium. Patient characteristics are presented in TABLE 1. Thirty-two patients (29%) had a histopathological diagnosis of adenomyosis of the inner myometrium of whom 13 (41%) had TCRE, and 19 (59%) had hysterectomy. Hysterectomy specimens with adenomyosis of the inner myometrium had changes within the junctional zone no matter the depth of myometrial invasion. Few women were taking hormonal treatment or had been fitted with an intrauterine device before surgery, and the endomyometrial biopsies had a median thickness (25th to 75th percentile interval) of 6.4 mm (6–8). Among all patients who had TCRE 59 (92%), all six biopsies had sufficient thickness for the histopathological examination. Two patients had two biopsies and three patients had one biopsy that were insufficient for histopathological examination; two patients were diagnosed with and three patients without adenomyosis of the inner myometrium from the remaining biopsies.

The diagnostic accuracy for adenomyosis of the inner myometrium is presented in TABLE 2. No difference was observed in the AUC between real-time 2D-TVS and off-line 3D-TVS between patients, although for specificity the difference almost reached statistical significance ($P = 0.06$). Furthermore, the AUC did not change if only patients in the luteal phase were included (data not shown). Moreover, no difference was found in diagnostic accuracy with either 2D-TVS or 3D-TVS between the two subgroups of patients scheduled for either hysterectomy or TCRE. The ROC curves for JZ_{max} and $JZ_{max}–JZ_{min}$ are presented in FIGURE 4. The most optimal cut-off value for junctional zone thickening (JZ_{max}) was greater than 10.5 mm, and the most optimal cut-off value for junctional zone irregularity ($JZ_{max}–JZ_{min}$) was 5.0 mm and over. In 14 patients, however, the three-dimensional features were not assessable. In another three patients, the junctional zone minimum thickness could not be measured. Most of these patients had leiomyomas (14/17 patients), some of them with coexisting adenomyosis of the inner myometrium ($n = 4$). The diagnostic accuracy of the individual ultrasonographic features associated with adenomyosis of the inner myometrium by 2D-TVS and 3D-TVS is presented in TABLE 3. No difference was observed in the AUC between the five characteristic two-dimensional features by either real-time 2D-TVS or off-line 3D-TVS (first five rows of TABLE 3). Among these individual two-dimensional features, asymmetric corpus myometrium and anechoic lacunae both had a sensitivity and specificity of over 60%, but the presence of three or more characteristic two-dimensional features had a higher AUC (0.72). Among the individual three-dimensional features, when excluding the patients that could not be assessed ($n = 17$), junctional zone irregularity had the highest AUC (0.78), but the presence of two or more three-dimensional features was a little more accurate (AUC: 0.80). When the two techniques were combined, two or more two-dimensional features combined with two or more three-dimensional features had better specificity (79%) and was highly accurate (AUC: 0.77).

DISCUSSION

In the present study, real-time 2D-TVS and off-line 3D-TVS had the same diagnostic accuracy, although the

TABLE 1 PATIENT CHARACTERISTICS

	With adenomyosis of the inner myometrium (n = 32)		Without adenomyosis of the inner myometrium (n = 78)	
Mean age at examination (years)	46	44–47	45	44–47
Median BMI at examination	25	23–27	26	23–30
Median number of pregnancies	3	2–4	3	2–4
Mean number of labours	2	2–2	2	2–2
Median uterine volume, ml	94	66–132	85	56–128
Previous hysteroscopy surgery or myomectomy	5	16%	20	26%
Previous evacuation	5	16%	8	10%
Intrauterine device[a]	5	16%	26	33%
Hormone therapy[a]	2	6%	11	14%
Scheduled for TCRE	13	41%	51	65%
Due to HMB	4	31%	13	25%
Due to menstrual pain	1	8%	1	2%
Due to a combination of HMB and menstrual pain	8	62%	37	73%
Scheduled for hysterectomy	19	59%	27	35%
Due to HMB	5	26%	7	26%
Due to menstrual pain	0	0%	1	4%
Due to a combination of HMB and menstrual pain	14	74%	19	70%
Menstrual cycle at the day of examination and surgery				
Follicular phase	7	22%	10	13%
Luteal phase	13	41%	21	27%
Irregular (menstrual phase unknown)	12	38%	47	60%
2D-TVS diagnosis of leiomyomas	10	31%	28	36%
2D-TVS diagnosis of polyps	0	0%	3	4%
2D-TVS quality				
Good	22	69%	39	50%
Medium	7	22%	25	32%
Poor	3	9%	14	18%
3D-TVS quality				
Good	19	59%	40	51%
Medium	8	25%	24	31%
Poor	5	16%	14	18%

[a] Three months or more before surgery.
Values presented as mean and 95% confidence interval, median and 25th to 75th percentile interval or number and %.
There was no statistically significant difference in patient characteristics between patients with and without adenomyosis of the inner myometrium ($P > 0.05$).
BMI, body mass index; HMB, heavy menstrual bleeding; TCRE, transcervical resection of the endometrium; 2D-TVS, two-dimensional transvaginal ultrasonography; 3D-TVS, three-dimensional transvaginal ultrasonography.

difference in specificity between them almost reached statistical significance ($P = 0.06$). Furthermore, no difference was observed in the AUC between patients scheduled for hysterectomy and patients scheduled for TCRE. The presence of at least two two-dimensional features had high sensitivity but low specificity but, when combined with the presence of two or more three-dimensional features, the specificity increased, and sensitivity remained the same. This suggests that characteristic three-dimensional features may help determine a diagnosis of adenomyosis of the inner myometrium in cases with non-specific myometrial findings at 2D-TVS. In cases in which the three-dimensional features could not be assessed, however, diagnosis of adenomyosis of the inner myometrium should instead rely on the presence of at least three characteristic two-dimensional features, as this would improve specificity.

Our results support recent findings from a systematic review and meta-analysis that an equally accurate diagnosis of adenomyosis can be made with 2D-TVS and 3D-TVS (Andres et al., 2018). One previous study evaluated the diagnostic accuracy of combined two-dimensional and three-dimensional features (Exacoustos et al., 2011) and demonstrated a higher diagnostic accuracy than with two-dimensional features alone. This study supports our

TABLE 2 DIAGNOSTIC ACCURACY OF AN ULTRASONOGRAPHIC DIAGNOSIS OF ADENOMYOSIS OF THE INNER MYOMETRIUM

	Real-time two-dimensional transvaginal ultrasonography						Offline three-dimensional transvaginal ultrasonography					
	Sensitivity (%)	95% CI	Specificity (%)	95% CI	AUC	95% CI	Sensitivity (%)	95% CI	Specificity (%)	95% CI	AUC	95% CI
All patients (n = 110)	72	53 to 86	76	65 to 85	0.74	0.7 to 0.8	69	50 to 84	86	76 to 93	0.77	0.7 to 0.9
Patients scheduled for hysterectomy (n = 46)	63	38 to 84	74	54 to 89	0.69	0.6 to 0.8	63	38 to 84	89	71 to 98	0.76	0.6 to 0.9
Patients scheduled for TCRE (n = 64)	85	55 to 98	77	63 to 87	0.81	0.7 to 0.9	77	46 to 95	84	71 to 93	0.81	0.7 to 0.9

Comparing real-time 2D-TVS with off-line 3D-TVS showed no statistically significant difference among all patients and the two groups (patients scheduled for hysterectomy or TCRE) ($P > 0.05$).

Comparing the two groups showed no statistically significant difference in the diagnostic accuracy with either 2D-TVS or 3D-TVS ($P > 0.05$).

AUC, area under the curve; TCRE, transcervical resection of the endometrium; 2D-TVS, two-dimensional transvaginal ultrasonography; 3D-TVS, three-dimensional transvaginal ultrasonography.

findings of a higher diagnostic value of three-dimensional features, junctional zone irregularity of 5 mm or over. Junctional zone irregularity may not be as influenced by hormonal status as junctional zone thickening (JZ_{max}), and it may also be more accurate (Dueholm et al., 2001). As previously observed (Novellas et al., 2011; Luciano et al., 2013), however, the junctional zone cannot be assessed in all patients, and concomitant leiomyomas seem to hamper ultrasonographic diagnosis of adenomyosis (Bazot et al., 2001). Magnetic resonance imaging has proven to be highly accurate for this diagnosis of intrinsic adenomyosis by focusing on the junctional zone, and may be preferred in cases with large intramural or submucous leiomyomas (Bazot and Darai, 2018). However, 2D-TVS and magnetic resonance imaging have similar efficiency (Dueholm and Lundorf, 2007; Champaneria et al., 2010) in other cases, and a combination of 2D-TVS and 3D-TVS may act as a cheaper and less time-consuming alternative.

The results of a recent study also support our findings that an image diagnosis of adenomyosis may be confirmed by the postoperative pathology after hysteroscopic excision (Xia et al., 2017). The biopsy method and efficiency of 2D-TVS and 3D-TVS, however, has to be confirmed in larger studies.

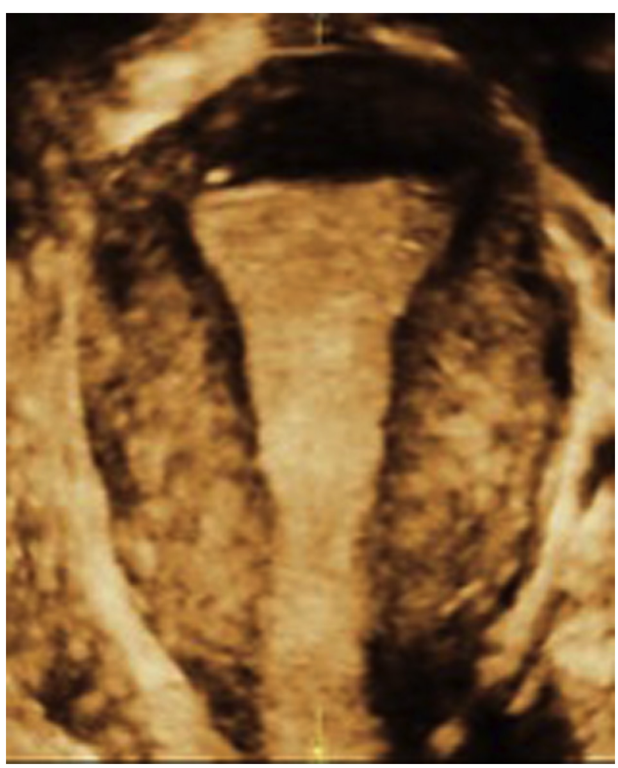

FIGURE 4 Three-dimensional transvaginal ultrasonography (3D-TVS) of the uterus in the coronal plane, with the presence of subendometrial buds and lines and a thickened and irregular junctional zone associated with adenomyosis.

TABLE 3 DIAGNOSTIC ACCURACY OF INDIVIDUAL AND COMBINATION OF ULTRASONOGRAPHIC FEATURES OF ADENOMYOSIS

	Real-time two-dimensional transvaginal ultrasonography						Offline three-dimensional transvaginal ultrasonography					
	Sensitivity (%)	95% CI	Specificity (%)	95% CI	AUC	95% CI	Sensitivity %	95% CI	Specificity (%)	95% CI	AUC	95% CI
Characteristic two-dimensional features												
Heterogeneity	84	67 to 95	31	21 to 42	0.58	0.5 to 0.7	81	64 to 93	60	49 to 71	0.71	0.6 to 0.8
Anechoic lacunae	69	50 to 84	64	52 to 75	0.66	0.6 to 0.8	75	57 to 89	69	58 to 79	0.72	0.6 to 0.8
Asymmetric corpus myometrium	72	53 to 86	64	52 to 75	0.68	0.6 to 0.8	53	35 to 71	90	81 to 96	0.71	0.6 to 0.8
Myometrial cysts	47	29 to 65	90	81 to 96	0.68	0.6 to 0.8	50	32 to 68	94	86 to 98	0.72	0.6 to 0.8
Fanshaped shadowing	41	24 to 59	85	75 to 92	0.63	0.5 to 0.7	63	44 to 79	77	66 to 86	0.70	0.6 to 0.8
Best combinations of two-dimensional features[a]												
Heterogeneity + anechoic lacunae	69	50 to 84	65	54 to 76	0.67	0.6 to 0.8						
Heterogeneity + asymmetric corpus myometrium	72	53 to 86	68	56 to 87	0.70	0.6 to 0.8						
Heterogeneity + anechoic lacunae + asymmetric corpus myometrium	69	50 to 84	76	65 to 85	0.72	0.6 to 0.8						
Anechoic lacunae + asymmetric corpus myometrium	69	50 to 84	76	65 to 85	0.72	0.6 to 0.8						
Anechoic lacunae + fanshaped shadowing							63	44 to 79	83	73 to 91	0.73	0.6 to 0.8
≥2/5 characteristic two-dimensional features	75	57 to 89	51	40 to 63	0.63	0.5 to 0.7	78	60 to 91	63	51 to 74	0.71	0.6 to 0.8
≥3/5 characteristic two-dimensional features	72	53 to 86	72	61 to 81	0.72	0.6 to 0.8	69	50 to 84	81	70 to 89	0.75	0.7 to 0.8
Characteristic three-dimensional features (with VCI)												
JZ_{max} >10.5mm							76	57 to 90	73	61 to 83	0.75	0.6 to 0.8
JZ_{dif} (JZ_{max}–JZ_{min}) ≥5 mm							79	59 to 92	77	65 to 87	0.78	0.7 to 0.9
JZ interruption							48	29 to 68	87	76 to 94	0.67	0.6 to 0.8
Echogenic subendometrial lines and buds							52	33 to 71	87	76 to 94	0.69	0.6 to 0.8
Best combinations of three-dimensional features[a+]												
JZ_{max} + JZ_{max}-JZ_{min}							68	48 to 84	79	67 to 88	0.73	0.6 to 0.8
≥2/4 characteristic three-dimensional features							86	67 to 96	74	62 to 84	0.80	0.7 to 0.9
Best combination both techniques[c]												
≥2/5 two-dimensional features + ≥2/4 three-dimensional features							75	55 to 89	79	67 to 87	0.77	0.7 to 0.9

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Table 3 – (continued)

	Real-time two-dimensional transvaginal ultrasonography						Offline three-dimensional transvaginal ultrasonography					
	Sensitivity (%)	**95% CI**	**Specificity (%)**	**95% CI**	**AUC**	**95% CI**	**Sensitivity %**	**95% CI**	**Specificity (%)**	**95% CI**	**AUC**	**95% CI**
≥3/5 two-dimensional features + ≥2/4 three-dimensional features							71	51 to 87	89	79 to 96	0.80	0.7 to 0.9
Heterogeneity + JZ_{dif} ≥5 mm							75	55 to 89	80	68 to 89	0.78	0.7 to 0.9

No statistically significant difference was found in the area under the curve of any myometrial ultrasonographic features between two-dimensional transvaginal ultrasonography (2D-TVS) and three-dimensional transvaginal ultrasonography (3D-TVS).

In 14 patients (13%), the three-dimensional features were not assessable in any of the five walls; three patients had adenomyosis of the inner myometrium (AMIM) and coexisting leiomyomas and nine patients had leiomyomas. In another three patients, the junctional zone minimum thickness could not be measured in any of the five walls; one patient had AMIM and coexisting leiomyomas and one had leiomyomas. Type of leiomyomas: six were intramural type 3 or 4 (>3 cm in diameter) and six were submucous type 1 or 2. Menstrual cycle: eight patients were in luteal phase and nine patients had irregular menstruation (menstrual phase unknown).

Among patients with AMIM, the median (75% percentile interval) junctional zone maximum thickness (mm) in any of the measured walls was 14.6 (11–22) and 8.9 (8–11) among patients without AMIM. The median (75% percentile interval) maximum junctional zone difference between maximum and minimum thickness (mm) in any of the measured walls was 9.2 mm (5–14) among patients with AMIM and 3.6 mm (3–5) among patients without AMIM.

[a] Only displayed if sensitivity was greater than 60%.[b]Most patients with AMIM had at least junctional zone thickening (JZ_{max} >10.5mm) and junctional zone irregularity (JZ_{max}–JZ_{min} ≥5mm). In only one patient with AMIM, subendometrial lines and buds + junctional zone interruption were present without junctional zone thickening or junctional zone irregularity.

[c] In four patients with AMIM, the only feature recorded was junctional zone irregularity at 3D-TVS. In three patients with AMIM, the only features recorded were heterogeneity, asymmetric corpus myometrium and anechoic lacunae at 2D-TVS.

AUC, area under the curve.; JZ, junctional zone; VCI, volume contrast imaging.

Women scheduled for TCRE represent a less selected group with milder symptomatic adenomyosis than women for hysterectomy, because endometrial ablation is the first surgical method of choice for abnormal uterine bleeding. The frequency of adenomyosis in women scheduled for TCRE match previously published studies of women with heavy menstrual bleeding (*Naftalin et al., 2014*). The finding of a comparable efficiency of TVS in the two groups (TCRE and hysterectomy) is important for the selection of image techniques in women with abnormal uterine bleeding. Unfortunately, we could not verify the endomyometrial biopsy method by later hysterectomy in all patients, and ideally the diagnosis of adenomyosis of the inner myometrium should be based on hysterectomy specimens, as there are concerns about the accuracy of using TCRE specimens. During follow-up, however, 15 patients had hysterectomy because of persisting symptoms after TCRE (eight with adenomyosis of the inner myometrium and seven without). Among these patients, the histopathological diagnosis with endomyometrial biopsies from TCRE equaled the histopathological diagnosis of uterine specimens from hysterectomy, except for one patient. This patient was diagnosed with adenomyosis of the inner myometrium but had a repeat TCRE before hysterectomy. Therefore, adenomyosis of the inner myometrium was most likely removed by the two preceding TCRE before hysterectomy. Therefore, a systematic evaluation of the entire endomyometrial surface seems to be an efficient reference standard for diagnosis of adenomyosis in patients not undergoing hysterectomy. Adenomyosis of the inner myometrium is initiated by an endometrial invasion through the junctional zone (*Kishi et al., 2012*); therefore, it is most likely detected with deep endomyometrial biopsies. This is also supported by the fact that all hysterectomy patients with adenomyosis had changes involving the junctional zone. The method, however, needs to be validated in larger studies.

The present study has some limitations. The study population was small and type II error cannot be excluded. We must emphasize that the diagnostic accuracy may apply specifically in the selected population focusing on adenomyosis of the inner myometrium and for the histopathological criteria used to identify adenomyosis of the inner myometrium. Ideally, the study population should only consist of patients with heavy menstrual bleeding, menstrual pain without leiomyomas or previous myomectomy, or both, as this may hamper both the two-dimensional and three-dimensional evaluation for adenomyosis of the inner myometrium. Localized adenomyosis, however, is often misinterpreted as small leiomyomas and adenomyosis and leiomyomas often coexist. Therefore, exclusion of these patients impedes the generalizability of the study. The luteal phase has been reported to be superior than follicular phase scan to detect subendometrial adenomyosis in a small study (n = 5) (*Abdel-Gadir et al., 2012*). Our results, however, do not indicate that luteal phase scan is more accurate than follicular phase scan. Fourteen patients were excluded because three-dimensional volumes were lost, and, in five patients, endomyometrial biopsies after surgery were incorrectly handled. Therefore, selection bias cannot be ruled out. Knowing the patients require surgery renders the observer more likely to make a diagnosis of adenomyosis of the inner myometrium so an unselected, symptomatic population may have been more appropriate. Only patients who had surgery within a short period after ultrasonography, however, could be included, otherwise the correlation between histopathology and ultrasonography would be questionable. We used strictly defined histopathological criteria for adenomyosis of the inner myometrium. Even though the surgical procedures were different, the histopathological diagnosis was based on the same criteria (endometrial glands at a distance of at least 2 mm from the basal endometrium). Unfortunately, we were unable to use established consensus criteria as this does not exist and the diagnostic criteria for adenomyosis range from a disrupted junctional zone to myometrial invasion greater than 4 mm (*Vercellini et al., 1993; Uduwela et al., 2000; Bazot et al., 2001; Struble et al., 2016*). Moreover, the observer variation of a histopathologic diagnosis is unknown.

Strengths of the present study were the prospective design, which included patients scheduled for both hysterectomy and TCRE, and the use of off-line 3D-TVS, which allowed blinded comparison between 2D-TVS and 3D-TVS. Furthermore, to the best of our knowledge, this is the first study that focuses on adenomyosis of the inner myometrium as a specific subtype of adenomyosis.

Adenomyosis of the inner myometrium or intrinsic adenomyosis is probably the most common subtype (*Bazot and Darai, 2018*). This subtype is seen in endometriosis (*Larsen et al., 2011*), but is commonly presented in women without endometriosis (*Chapron et al., 2017*). Adenomyosis of the outer myometrium is closely associated with deep invasive endometriosis and may be another disease (*Chapron et al., 2017*). Therefore, the effect of the different subtypes on pain, bleeding and reproduction remains unclear, and in future studies that evaluate the potential role of alternative treatment options, the correct diagnosis of adenomyosis subtypes may be crucial.

In conclusion, off-line 3D-TVS offers the same high diagnostic accuracy as real-time 2D-TVS for the diagnosis of adenomyosis of the inner myometrium. Identification of three-dimensional features, especially junctional zone irregularity, may be beneficial to the diagnosis and a more objective diagnosis may be based on a combination of two or more two-dimensional features and two or more three-dimensional features. These results may be useful for clinical practice and future research.

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SUPPLEMENTARY MATERIALS

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