Dissertation for the Degree of Ph.D.

Adenomyosis.

Diagnosis and steps towards a molecular analysis.

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“Nur zwei Dinge: Du musst sie lieben und darfst nicht faul sein.»

Das Motto meiner Mutter zur Kindererziehung.

Gilt auch für Forschungsprojekte.

"Only two things: you must love them and not be lazy."

My mother's motto for parenting.

Also applies to research projects.

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LIST OF PAPERS

Paper I:

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Paper II:

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Paper III:

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ABBREVIATIONS

MRI	Magnetic resonance imaging
TVUS	Transvaginal ultrasound
JZ	Junctional zone
TIAR	Tissue injury and repair
MUSA	Morphological Uterus Sonographic Assessment
ART	Assisted reproduction treatment
OR	Odds Ratio
Lng-IUD	Levonorgestrel intrauterine device
GnRHa	Gonadotropin Releasing Hormone agonist
T1W/T2W	T1/T2 weighted
JZmax	Maximum junctional zone thickness
2D	Two dimensional
3D	Three dimensional
MMP	Matrix metalloproteinases ,
EMT	Epithelial-to-mesenchymal transition
NSAID	Non-steroidal anti-inflammatory drugs
UAE	Uterine artery embolization
HIFU	High intensity focused ultrasound
VCI	Volume contrast imaging
G	Gauge
O.C.T	Optimal cutting temperature compound
HE	Hematoxylin/eosine
RNA	Ribonucleic acid
CI	Confidence interval
NPV	Negative predictive value
PPV	positive predictive value
ROC	Receiver operating curve
AUC	Area under the curve
VNRS	Verbal numerical rating scale
ICC	intraclass correlation coefficient
IOTA	International ovarian tumor analysis
LASSO	least absolute shrinkage and selection operator
TRIPOD	Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis
R1, R2	Reader 1, Reader 2

BACKGROUND FOR THIS THESIS

Adenomyosis is a disease where ectopic endometrial glands affect the muscular wall of the uterus. The gold standard of diagnosing adenomyosis is histopathology. But a large proportion of women that suffer from dysmenorrhea or infertility need to confirm or rule out adenomyosis without removing their uterus, and therefore tools for non-histologic confirmation of the diagnosis are indubitably required. Magnetic resonance imaging (MRI) provides a good visualization of the uterus and was therefore for many years the tool that seemed to be superior to transvaginal ultrasound (TVUS) in diagnosing adenomyosis. But MRI is costly, not widely available and the performing and interpretation need expertise. With the technical development in ultrasound, providing a higher resolution and helpful software features that support automated image quality improvement, it became easier to find signs of adenomyosis. Ultrasound is almost universally available, cheap and well tolerated by almost all women. Also, many studies showed that it has good accuracy for diagnosing adenomyosis. However, less experienced clinicians find it difficult to reproduce the same level of confidence in the ultrasound-based diagnosis, as there are many diagnostic signs and they have a heterogeneous sensitivity and specificity, in addition to being based on subjective pattern recognition. When 3D TVUS was developed, the junctional zone (JZ) and the coronal plane of the uterus could be better depicted, which was only possible with MRI before. In MRI, the measurement of the JZ is used to diagnose adenomyosis, and it is a less subjective, metric marker. A study performed by Exacoustos et al. in 2011, was the first and by the time only (1) to show that the measurement of the maximal JZ thickness gave an excellent diagnostic accuracy also in 3D TVUS. Those findings were remarkable, as it opened for a more objective, accurate and easier way of diagnosing adenomyosis with ultrasound. We found it therefore relevant to validate those findings in a prospective study.

As there was almost no knowledge on what JZ-findings in ultrasound represent, we wanted to compare the results of the JZ findings in 3D ultrasound to the more established findings in MRI, to evaluate the significance of the changes seen by 3D ultrasound. All aspects regarding imaging were performed in study 1.

Besides the diagnosis, another aspect of adenomyosis seemed to be highly under-investigated: the pathogenesis of adenomyosis is largely unknown, and there are only few treatment options for the condition. Imaging studies do unfortunately not provide information on neither pathogenesis or treatment possibilities and that led to our hypothesis that it would be relevant to investigate molecular processes in adenomyosis cells. As we had access to tissue samples through the

hysterectomy specimens, we decided to build up a uterine tissue biobank.
At the same time, the use of hysterectomy specimens limits the gathered information to those women usually having advanced disease, being parous and refractory to medical treatment. Therefore we tried to find an alternative way to gain adenomyosis tissue, in vivo and without the need for hysterectomy. The solution we came up with, was to take in-vivo core-biopsies of adenomyosis. This could be performed ultrasound-guided, transvaginally and in this study under controlled circumstances: with full anesthesia and before the planned hysterectomy, so that possible complications could be seen and controlled at once. The aspects of biopsy taking and biobanking were investigated in study 2.
In summary, the overall aim with this work was to improve the diagnosis of adenomyosis using ultrasound and prepare the possibility to investigate adenomyosis on a molecular level.

INTRODUCTION

1. Definition and prevalence

Adenomyosis is defined as ectopic endometrial glands within the muscular wall of the uterus (2).

The reported prevalence of adenomyosis found in hysterectomy specimen shows a considerable variation, of between 20 -70% (3). This is most likely due to patient selection in studies and also variation in histologic definitions and variation in the number of microscopic sections obtained during histopathology. The limitations of histopathology are discussed more extensively in the chapter “Diagnosis”. However, results based on histopathology are not representative of the prevalence in a normal population, as hysterectomy represents an obvious selection bias. With the development of imaging modalities like magnetic resonance imaging (MRI) and ultrasound, it was possible to estimate the prevalence in a normal population. Naftalin et al. found sonographic signs of adenomyosis in about 20% of women attending an outpatient clinic (4), while Choi reported the prevalence of adenomyosis to be 13% in a register study including 61.516 patients (5).

2. Pathophysiology

Several theories are described that could explain the development of adenomyosis and the most acknowledged are described below. It is likely that all described processes are contributing to the development of adenomyosis, though not necessarily in the same patient. The described pathways share many common features with pathological processes in endometriosis, explaining why the two entities often co-exist. It is evident that molecular and genetic studies are essential tools to identify the mechanisms behind adenomyosis pathophysiology.

a. Tissue injury and repair (TIAR)

This theory was published first by Leyendecker et al. (6) and describes a repeated micro traumatization of the inner layer of the uterus (junctional zone, JZ), that leads to local repair mediated by estrogen. Local estrogen again causes more uterine peristalsis by stimulating myocytes, and subsequent further micro traumatization at the JZ (7). Hyperestrogenism is found in the menstrual blood of women with adenomyosis (8), and

both local estrogen production and aberrations in estrogen receptors are described in adenomyotic foci (9). This theory is consistent with the fact that worsening of adenomyosis is associated with number of pregnancies and birth, which cause damage to the JZ; and is also associated with damages to the JZ by curettage (10). Also, adenomyosis is typically progressing with time (4). JZ-injury by hyperperistalsis and extreme intrauterine pressure during menstruation is a likely cause for this progression (11). The TIAR theory is supported by electron microscope investigations on the ultrastructural features of the JZ (12) and by more recent MRI studies (13).

b. Invasion from the endometrium

Several authors describe down growth and invagination of endometrium through the junctional zone into the myometrium. Various factors might contribute to this event, such as an altered or even absent JZ (3, 14), or a cell type called “pale cell” that possibly initiates the process (12). Dysregulation of genes in the endometrium and myometrium and various local factors seem to play a role in this pathogenesis, as well (15). Matrix metalloproteinases (MMP), mitochondrial dysfunction, mTOR, and estrogen receptor signaling are such local factors previously described (16). The TIAR-theory might not necessarily be an exclusive alternative to this invasion-theory, but supplementary, as many mutual features are present.

c. Stem cell theory

The existence of adult stem cell populations in the endometrium was proven in 2004 by Chan et al. and they play a central role in the regeneration of the endometrium as a part of the menstrual cycle (17). Some authors describe that adenomyosis might origin from circulating multipotent stem cells (17, 18). Those adult stem cells can originate from bone marrow and other sources (18-20). Tissue injury can activate adult stem cells and cause ectopic endometrial implants by disruption of endometrial stem/progenitor cells niches (21, 22). The stem cell theory might explain cases of adenomyoma that are found in the outer myometrium without any connection to the JZ or signs of myometrial invasion (see picture 4 in Figure 1).

d. Epithelial-to-mesenchymal transition (EMT)

The EMT is an embryonic program that contributes to the development of various tissues in embryo growth but also wound healing and other important processes in the uterus and ovaries in adult life. It is characterized by loss of cell adhesion and increased cell mobility (23). It plays, therefore, an essential role in cancer development and a dysfunctional EMT may also play a role in the evolution of adenomyosis (24, 25). Oh et al. identified a possible pathway (26), showing that elevated levels of β-catenin in adenomyosis resulted in the activation of the Wnt pathway, which can ultimately lead to aberrant activation of EMT in the uterus (24). EMT can also be induced by estrogen (through upregulation of the transcription factors Snail or Slug), and markers of EMT could be seen in response to 17β-estradiol in human tissue samples (7).

3. Symptoms

About 70–95% of affected women show signs of adenomyosis and the main symptoms are dysmenorrhea and menorrhagia (27, 28). Age, parity and prior uterine surgical procedures are associated with the occurrence and intensity of symptoms (27-29). The number of ultrasonographic features of adenomyosis is also correlated to the severity of symptoms, indicating that a progression of morphologic changes correlates with a progression of symptoms (30).

Adenomyosis often co-exists with endometriosis and uterine fibroids. Dysmenorrhea is closest associated with adenomyosis, and chronic pelvic pain with endometriosis. Fibroids, on the other hand, do not seem to cause dysmenorrhea (27-29, 31). Pressure symptoms and bulk-related discomfort are equally frequent in women with fibroids as adenomyosis, even if women with fibroids have larger uteri (28, 29). Menorrhagia is associated equally with fibroids and adenomyosis (31).

Uncoordinated uterine contractions, showing a higher frequency and altitude as well as impaired directionality of the peristaltic waves, contribute to pain and exaggerated blood loss during the menstrual period in women with adenomyosis (32-34). The pressure inside the uterine cavity during menstruation is significantly higher in women with dysmenorrhea, contributing to ischemia and pain and probably also auto traumatization of the JZ, see TIAR above (11).

Quinn and Kirk postulated that birth or other trauma to the uterus leads to denervation and

then impaired re-innervation of the uterine isthmus, being the cause of pain for adenomyosis (35, 36). Some studies see a link between a higher presence of nerve fibers in uterine lesions and pain (37, 38), but Choi et al. could not find a statistically significant association of nerve fibers to pain and no significant difference in women with adenomyosis vs. fibroids (39).

Other molecular factors associated with dysmenorrhea and pain in women suffering from adenomyosis are a higher density of oxytocin receptors (33, 40), proangiogenic features (41), and CD56. CD56 is a protein expressed in neural tissues, neuroendocrine tissues and tumors and plays an important role in the growth and aggregation of nerve fibers and neuroendocrine tumor metastases (42).

About a third of women with adenomyosis exhibit lower urinary tract symptoms, like pollakiuria, nocturia or dysuria, and those symptoms are positively correlated to an enlarged uterus and menorrhagia (43, 44). Urinary incontinence (both urge and stress incontinence) are also associated with adenomyosis, with a prevalence of 26% amongst women with adenomyosis (44). Dyspareunia, dyschezia, chronic pelvic pain, and lower back pain are associated with adenomyosis, independently of the presence of endometriosis (27, 29, 31).

More diffuse symptoms that are not described in detail for adenomyosis, but acknowledged among experts are IBS-like symptoms and radiating pain in the lower extremities. Those symptoms can be explained by the inflammatory processes caused by adenomyosis, affecting the surrounding organs (45) and are extensively described for endometriosis, a similar condition to adenomyosis (46).

Patients with symptomatic adenomyosis exhibit lower quality of life scores than control groups (44, 47, 48) and they have a higher risk of depression (49).

Non-gynecological co-morbidity associated with adenomyosis is anemia, hyperlipidemia, thyroid cancer, and endometrial cancer (50), but there is a significant lack of research on this field (5). As endometriosis and adenomyosis share many commonalities, it is likely that similar comorbidity is to be found in those conditions (51).

4. Impact on fertility, on the outcome of fertility treatment and pregnancy/labor

Both the lack of reliable non-histological confirmation of the diagnosis and good studies on this topic, as well as many possible confounders, lead to claims that adenomyosis has no

impact on fertility (52). This is problematic, as there is both clinical and epidemiological evidence for the negative impact of adenomyosis on fertility (53, 54). The overrepresentation of women with adenomyosis seeking assisted reproduction treatment (ART) is also a clear indication for adenomyosis having a negative impact on fertility (55). Optimally, a large prospective cohort study that follows women from adolescence to menopause should be performed to study in which way adenomyosis evolves and how it will affect fertility and childbirth.

The negative impact of adenomyosis on ART-outcome is established, though. A recent meta-analysis concluded that the presence of adenomyosis more than doubles the risk for miscarriage (OR 2.2) and reduces the chance of live birth (OR 0.59) in women undergoing ART (56). The implantation rate and clinical pregnancy rate was also negatively affected (OR 0.73 and 0.66), confirming the results of an earlier review (57). The authors of both publications recommend screening women undergoing ART for the presence of adenomyosis, as it was also shown that long pituitary downregulation and frozen embryo transfer might improve ART outcome in the affected women (55, 56, 58). A matched-pairs study on women undergoing ART showed that asymptomatic adenomyosis diagnosed by ultrasound did not have a negative effect on live birth rate (59). But there are some major limitations to this study and those results have to be interpreted with caution: Women suffering from abnormal uterine bleeding, which is a main symptom of adenomyosis and almost always present, were excluded. Also, the authors used only one ultrasound criterion to verify the diagnosis, which can lead to overdiagnosis as some signs have a very low specificity (see above). Therefore, it is very likely that a large proportion of the women in this study might not have had adenomyosis at all. Furthermore, they used ART-protocols that were described to be preferable in adenomyosis and by that they might have “treated” some adenomyosis-related issues already.

Different possible mechanisms on how adenomyosis affects infertility are discussed in the literature. Uterine hyperperistalsis can cause impaired transport of sperm and also the embryo, leading to both lower rates of fertilization and implantation (60, 61). Furthermore, inflammation in the endometrium can create a hostile intrauterine environment and impair endometrial receptivity (62).

More recently published studies confirm that adenomyosis could pose a more significant risk factor during pregnancy and labor than anticipated before. Hormonal and inflammatory

factors affecting the endometrium, myometrium, the placenta, and fetal membranes elevate the risk of preeclampsia, preterm birth, premature rupture of membranes, antepartum and postpartum bleeding and placenta previa (63). Bruun et al. performed a meta-analysis and identified 21 studies with a total of 2 517 516 women that could be included. The risk for both preterm delivery (OR: 3.09, 95% CI; 1.88—5.09) and small for gestational age (OR: 3.23, 95% CI; 1.71—6.09) was more than tripled on women with adenomyosis, compared to women without adenomyosis. The authors suggest closer monitoring of pregnant women suffering from adenomyosis (64). In a case-control study, Hashimoto et al. found an increased risk for preeclampsia OR 21.0 (95%CI 4.8—124.5), placental malposition OR 4.9 (95%CI 1.4–16.3) and preterm delivery OR 3.1 (95% 1.2–7.2) (65).

5. Diagnosis

a. Histopathology

So far, microscopic vizualization of ectopic glands within the uterine wall is the most certain way to confirm the presence of adenomyosis. Histology is therefore considered to be the gold standard of the diagnosis. However, there are certain relevant limitations for histopathology. As the endometrial-myometrial junction is not straightly demarked and the endometrium lacks a basal lamina, it is not always obvious what is a part of the normal (eutopic) endometrium and what is ectopic. It is widely accepted that ectopic glands have to be located deeper than the lowest glands of the basalis in order to be defined as adenomyosis; but how deep is not unanimously defined and a range from 1—2.5mm is found. Also, some define it as “more than a microscopic field at 10x magnification” (2, 66). Also, even if the cells of adenomyosis and endometriosis have different molecular features and habits compared to eutopic endometrium, they cannot be discriminated by microscopy. Therefore, endometrial glands that are found only subserous in the outer layer of the uterus are defined as invading endometriosis by some, and as adenomyosis by others. The same problem is encountered when dealing with cervical adenomyosis, which might represent deep infiltrating endometriosis and not adenomyosis. Also, endocervicosis might be misdiagnosed as adenomyosis (67).

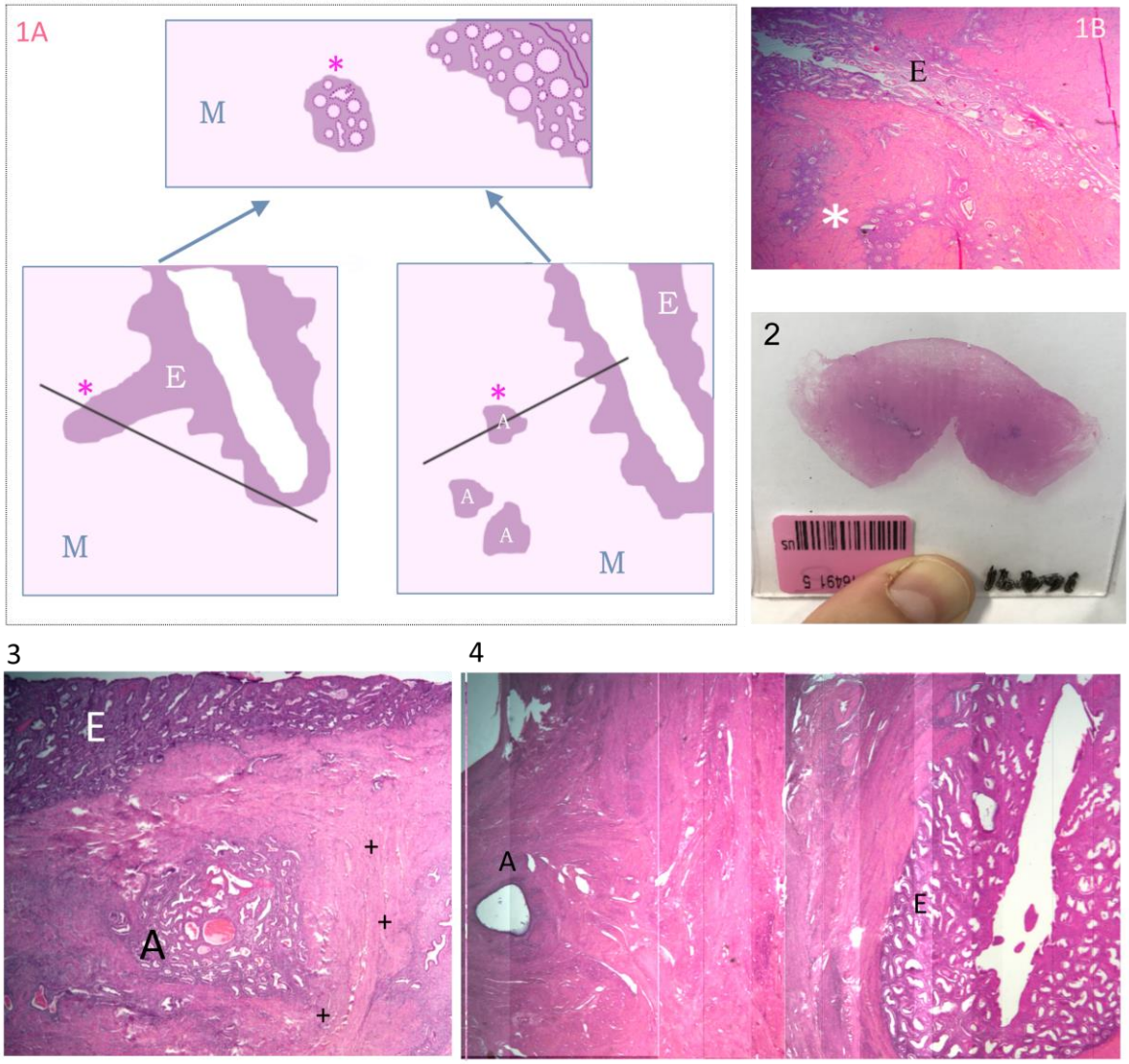


Figure 1: *Adenomyosis in histology with pitfalls***.** 1A) Schematic presentation of tangential vs. orthogonal sections for histopathology: The cartoon illustrates a histological slide, with eutopic endometrium (E) visible on the right upper corner and glandular formation (*) to the left. Without exact knowledge of the orientation of the section through the specimen, it cannot be determined if the glandular focus (*) represents for example eutopic glands from a section tangential to the cavity (lower left cartoon), or a real ectopic focus. The black line illustrates the direction of a section. 1B) Histopathological image analog to the cartoon of 1A. Uterine cavity in the upper part of the image (E), the area marked with (*) could look like ectopic endometrial tissue, but represented the fallopian tube. 2) Axial whole-organ section of the fundal part of the uterus, the dark violet areas represent the internal os of the fallopian tubes on both sides. The anterior wall of the uterus was incised to allow formalin-fixation of the inner parts. 3) Eutopic endometrium (E) with a regular endometrial-myometrial boarder. In-depth a focus of adenomyosis is visible, containing stroma and glands of various size that is surrounded by circular muscular hypertrophy (+). 4) Eutopic endometrium (E) with a regular boarder. An adenomyotic cyst (A) with very thin glandular epithelium, stroma, and surrounding, circular muscular layers is visible. The eutopic and ectopic glands show a very different morphology in this case. M; myometrium. E; endometrium. A; adenomyosis. All are in x2 magnification and hematoxylin/eosin stained.

The sensitivity and specificity of a histopathological examination do not only depend on which definition one uses, but also on how thorough the specimen is examined. The higher the number of microscopic sections taken from the corpus uteri is, the more sensitive the diagnosis will be. Furthermore, is also essential to have a full orientation of the section and the specimen when performing a histological investigation, to avoid false positive cases caused by tangential sections through the irregular layer of the basal endometrium or the intramural part of the fallopian tubes, as illustrated in Figure 1.

b. Magnetic resonance imaging (MRI)

Diagnostic features of adenomyosis on MRI can be classified into direct or indirect signs. Direct signs are high-intensity signal areas in the myometrium or the JZ, representing ectopic adenomyosis glands (myometrial cysts) or irregularities of the JZ on the inner border caused by invasion of adenomyosis ("finger-like signs"). Irregularities of the outer border of the JZ (focal thickening of the JZ) can be caused directly by invasion of adenomyosis, but sometimes også represent indirect reactions to adenomyosis (68). Diffuse or circumscribed low-signal areas in the myometrium (with or without cystic foci) describe adenomyoma, with hypertrophy of the muscular layers surrounding adenomyosis tissue (that might be microscopic in size). Indirect signs, such as a globular enlarged uterus (not due to fibroids) or asymmetric thickening of the uterine walls, are consequences of reactive muscular hypertrophy (68-72). Direct visualization of adenomyosis in MRI shows the best specificity and a high image resolution improves sensitivity (73). Pitfalls in the MRI diagnosis of adenomyosis are physiological contractions mimicking irregularities of the JZ, or changes to the JZ due to cyclic variations or hormone therapy, as well as fibroids (74).

The pooled diagnostic accuracy for MRI was calculated in a recent meta-analysis, resulting in a sensitivity of 77%, specificity of 89%, the positive likelihood ratio of 6.5, and negative likelihood ratio of 0.2 (73).

MRI has the advantage compared to ultrasound that it can depict the uterus and especially the JZ in detail, even in the presence of fibroids. With the new MRI systems, a very thin slice thickness with no intersection gap (or even overlap of sections) results in an extremely high resolution, making it possible to identify very small foci of adenomyosis. A precondition for optimal results is still that suitable protocols are applied, especially providing oblique planes. Even with appropriate sequences being present, the interpretation of the images should be

performed by an expert in gynecological imaging to obtain reliable results (73). Also, the high cost of MRI and restricted availability, are limitations for this modality in diagnosing adenomyosis.

Like in TVUS, diagnostic criteria for the diagnosis of adenomyosis in MRI differ in previously published studies, and also the diagnostic accuracy of the same evaluated features shows a variation (68-70, 73, 75-78). Many published studies have clear limitations due to selection bias or lack of histopathology to confirm the findings of adenomyosis in MRI, and there are only three studies that investigate the diagnostic accuracy of MRI prospectively and confirm the diagnosis with histopathology (70-72). Only those fulfilled the standards required to be included in two published meta-analyses (73, 79).

The sign that is described to be the most established for diagnosing adenomyosis with MRI is a maximal JZ thickness of ≥12mm (JZ_{max}≥12mm). It is widely used by clinicians as the primary criterion to diagnose adenomyosis (68, 69). However, the universal validity of this feature for all patient populations is not yet established. JZ_{max}≥12mm is described in the three studies mentioned above (70-72) and is neither prospectively validated nor tested in younger women, and the validity of this criterion as a diagnostic marker for adenomyosis may be questioned, as discussed in paper II. Furthermore, the JZ seems to be measured in different ways by various research groups and clinical departments, which also limits the reliability of this sign.

c. Ultrasound

Transvaginal (TVUS) and transabdominal ultrasound (TAUS) are both suited for the diagnosis of adenomyosis, but TAUS has definite limitations in resolution, resulting in a good specificity, but low sensitivity, and therefore TVUS should always be performed if possible (73).

A systematic meta-analysis including high-quality studies calculated the pooled sensitivity for 2D TVUS to be 83.8%, and the specificity 63.9%. For 3D TVUS the pooled sensitivity and specificity for all combined imaging characteristics were 88.9% and 56.0% (80). This meta-analysis included only studies from the last ten years, which might be a limitation. However, the development of ultrasound machines that are more powerful and give a more detailed and clear visualization than before might justify a limitation to newer studies. Diagnostic signs for adenomyosis in ultrasound include a globular uterus (not due to fibroids),

myometrial anechoic areas (cysts), fan-shaped echoing, wall-asymmetry, invasion and irregularities of the JZ, thickening of the JZ, the “question mark sign” (a distortion of the uterus), diffuse vascularization, and hyperechoic myometrial islets (81). The challenge with ultrasound imaging of adenomyosis is that the most specific signs are not so prevalent, while the most common signs are not very specific, leaving the examiner with the difficult task to balance each finding. Experience in ultrasound assessment of adenomyosis is therefore beneficial when diagnosing adenomyosis (82). At the same time, there is no consensus regarding how many ultrasound signs need to be present to diagnosis adenomyosis. There is no unanimous classification or agreement on the terms or description for adenomyosis, either, even if there are several propositions made over the last decades (73, 83-87). Two publications that suggest a consensus on the terms and definitions for diagnosing adenomyosis by TVUS were recently published by the Morphological Uterus Sonographic Assessment (MUSA)-group and comprehensively described (81, 85), and is the closest one has come to find a classification for adenomyosis, yet.

d. Hysteroscopy, laparoscopy, and biopsies

As adenomyosis is located intramurally in the uterus, direct visualization of adenomyosis from inside the uterine or abdominal cavity is not possible (83). Changes caused by adenomyosis can be observed though, but both the specificity and sensitivity is very low (88, 89). During hysteroscopy, endometrial changes like hyper-vascularization, strawberry pattern, endometrial defects, and submucosal hemorrhagic cysts are suggestive of adenomyosis, but also for a variety of other conditions (88). Hysteroscopy is better suited as a therapeutic tool to treat cystic adenomyosis, rather than to diagnose (90).

During laparoscopy, the uterus can appear enlarged and soft when adenomyosis is present, but this is a very unspecific and subjective sign.

However, clinicians should preferably aim to diagnose adenomyosis before laparoscopy, as the diagnosis might influence the decision for surgery.

Biopsies of the myometrium have shown a lower diagnostic quality than both MRI and TVUS. Previous studies published on diagnostic biopsies for adenomyosis have inhomogeneous study populations, unclear selection criteria and describe different ways to obtain the biopsies (hysteroscopic, transabdominal, laparoscopic or from the specimen after hysterectomy) so that it is difficult to determine their overall accuracy (89, 91-97).

Surprisingly, none of the authors of these previous studies discusses possible adverse side effects of obtaining the biopsies, like complications or effect on uterine function. We addressed this issue in paper III.

e. Elastography

Elastography can visualize the stiffness of tissue. Pressure is applied to the organ of interest with the ultrasound probe and the change in deformation is calculated and color-coded by the machine and visualized on the corresponding B-mode image. It is described to be an easy-to-perform and little time-consuming procedure, with a good inter-observer agreement that can supplement regular TVUS examinations (98). The results of the existing studies are promising. Acar et al. found a sensitivity of 90% and a specificity of 93% for identifying adenomyosis with strain wave elastography in a study including 109 women, but the study was retrospective and has most likely a selection bias (99). Stoelinga et al. showed good discrimination between fibroids and adenomyosis using shear wave elastography in a prospective study but found it challenging to find standard values for adenomyosis that could be applied by other examiners (98).

There are two forms of elastography, strain and shear wave elastography, using different outputs and the two previous studies are therefore not comparable. A reported limitation of elastography is that the setup and standard values are different from different manufacturers, and software for the system has to be bought. Furthermore, strain wave elastography can only evaluate tissue up til 3cm in depth, which excludes axial orientated or larger uteri (100). Overall, elastography seems to be a promising, supplementary ultrasound tool for diagnosing adenomyosis.

6. Treatment

The type of treatment that should be proposed to women presenting with adenomyosis will depend on the women`s life situation and kind of problem they face at time of consultation: pain, heavy bleeding, infertility, bulk-related problems or a combination of those. The wish for preservation of the uterus and/or future childbearing is also essential for a treatment recommendation.

a. Hysterectomy

The most effective and definite treatment of adenomyosis is a hysterectomy, and it will cure dysmenorrhoea, heavy bleeding/anemia, dyspareunia and most often also lower back pain, radiating pain and lower urinary tract symptoms that are caused by adenomyosis. Some authors raised concerns about the recurrence of adenomyosis and endometriosis in the cervical stump or rectovaginal septum when performing a subtotal hysterectomy (101). There is no definite evidence for or against the removal of the cervix when adenomyosis is present, but the risk of cyclic bleeding from the cervical stump, the presence of pain associated with cervical palpation and presence of deep infiltrating endometriosis should be taken into consideration when recommending the type of hysterectomy procedure (87, 102).

b. Conservative surgery (adenomyomectomy)

Patients who are refractory or unsuitable for long-term medical treatment and those with focal adenomyoma are best suited for conservative surgery. It was shown that removal of adenomyotic tissue could improve dysmenorrhoea in up to 75% of women, but the recurrence rate might be up to 50%, depending on the interval of follow up (103). Both Dueholm and Younes et al. concluded in two different meta-analyses, that there is no evidence of improvement of fertility after conservative surgery, and that adenomyomectomy should primarily be reserved for symptom relief and only performed in controlled studies (103, 104).

Various surgical techniques for laparoscopic or open removal of adenomyosis are described, all of them requiring extensive surgical skills and experience (105, 106). The challenge of conservative surgery of adenomyosis compared to myomectomy is that adenomyosis has no clearly defined borders or capsule, making it difficult to know how much tissue to remove and keep the orientation in the organ during resection. Also, increased vascularization of adenomyosis compared to normal myometrium or fibroids result in a higher blood loss and grade of difficulty of the surgery. Myometrial tissue that is left behind might still contain adenomyosis, making it more difficult to suture and the scar to be strong enough to endure labor. All authors recommend therefore elective cesarian section at various weeks of gestation for women with prior conservative surgery for adenomyosis, in contrast to myomectomy, which has a much lower risk for such a complication (107, 108). Spontaneous

uterine rupture following adenomyomectomy is reported already from the second trimester on and seems to be associated with uterine wall thickness after adenomyomectomy (109). The absolute risk of uterine rupture after adenomyomectomy is not determined for sure, as studies are inhomogenous and small and most surgeons recommend elective cesarian section after previous adenomyomectomy.

c. Medical treatment

Interestingly enough, no drug is currently labeled for the treatment of adenomyosis, and there are no specific guidelines for the best medical management, either. Anyhow, previous studies have shown that medical treatment might improve symptoms like pain, abnormal uterine bleeding, and infertility (110). None of the available medical options are compatible with conceiving during treatment, though and women trying to become pregant can only be treated with pain killers.

The various treatments angle at different pathogenetic mechanisms of adenomyosis: aberrations in sex steroid hormone receptors and function, impaired apoptosis, and increased inflammation. The levonorgestrel intrauterine device (Lng-IUD) contributes to the reduction of pain, menorrhagia, urinary symptoms, and dyspareunia, and reduces the uterine volume and JZ thickness in women with adenomyosis (111-114). The effect of levonorgestrel is through shrinkage of adenomyotic lesions caused by downregulation of estrogen receptors, preventing further stimulation by estrogens (115). The Lng-IUD shows little side effects and is well tolerated, though younger nulliparous women might experience problems if the uterine cavity is too small (116). Combined oral contraceptives can relieve pain and improve menorrhagia, but it is discussed if the estrogen-component contributes to the proliferation of adenomyosis in some women and should, therefore, be the second choice after the Lng-IUD (117). Even if it is used off-label for the treatment of adenomyosis, the Lng-IUD is recommended if the woman tolerates it and it usually offers good symptom control (110).

The antiproliferative and anti-inflammatory effect of progestins, such as dienogest, danazol, and norethindrone acetate, suggest their use in the medical management of adenomyosis mainly to control pain (118-121). This is also the case with non-steroidal anti-inflammatory drugs (NSAIDs), that control inflammation, dysmenorrhea, and menorrhagia and do not contain hormones, and are possible options for women experiencing adverse side effects of

hormonal therapy (122, 123).

Gonadotropin-releasing hormone (GnRH)-analogs effectively reduces pain and menorrhagia in patients with adenomyosis by inducing apoptosis, inhibiting neoangiogenesis and lowering inflammatory processes in the eutopic endometrium (124, 125). The uterine volume can be reduced and GnRHa also positively effects fertility (58, 126, 127). Also, local progestin resistance in the ectopic endometrial lesions can be reduced (15). At the same time, GnRHa-therapy exhibits significant side effects, such as heat flushes, loss of bone mineral density, headache and mood swings, that make it little feasible as a long-time therapy for most women, even if addback (estrogen supplement) is given.

Other drugs are tested in pilot studies or other minor trials for the treatment of adenomyosis. Those are for example selective progesterone receptor modulators (128), aromatase inhibitors (129), valproic acid (130, 131), bromocriptine (132-134) and antiplatelet therapy (135). All of those drugs have a positive effect on symptoms but seem not to work too well in the majority of patients.

d. Interventional treatment options

Uterine artery embolization (UAE), radiofrequency ablation and high focused ultrasound (HIFU) are described as uterus sparing, interventional treatment options in women suffering from adenomyosis. All reduce pain and menorrhagia, but the effect on fertility is not ultimately determined, yet (104, 136). HIFU-treated women show high conception and live birth rates, indicating that improvement of fertility could be achieved (136). Serious complications in pregnancy are reported for UAE and radiofrequency ablation (137, 138), but not for HiFU. HIFU treatment has a very good safety profile, with only 0.3% reported complications that needed observation or shorter hospitalization, for example due to leg pain or numbness, or skin burns. Bowel injuries are described with an incidence of 0.05% (136). Lower abdominal pain, vaginal discharge or lower back pain are common side effects of HIFU and are experienced by at least 40% of women, but the effective reduction of dysmenorrhoea can be observed for several years (139).

UAE decreases the uterine volume with about 25% on average and has a good initial effect on dysmenorrhea and menorrhagia in women with adenomyosis, though the recurrence of symptoms occurs in almost half of the women within 12-40 months. Younger age and

extensive disease as the main risk factors for treatment failure (138, 140). It is not recommended to treat women with adenomyosis who seek pregnancy with UAE, due to lack of documentation that it improves fertility, and because adverse effects on pregnancy and labor were documented (138).

Radiofrequency ablation of adenomyosis seems to relieve symptoms, but the need for reintervention in about 20% of the treated women and the risk of intrauterine adhesions represent limitations (141, 142). Also, there only very few studies and therefore the evidence for the effectiveness of this intervention as well as long-term effects are missing.

KNOWLEDGE GAP

The role of the JZ in diagnosing adenomyosis with ultrasound was only investigated in one study when the present research was developed. Also, there was no defined and unanimous non-histological criteria for adenomyosis. Furthermore, little is known about the pathophysiology of adenomyosis, and wich aberrant pathways lead to the development of the disease. Limitations in present studies were that molecular investigations were performed on hysterectomy specimens only.

AIMS OF THE THESIS

The overall aim of our studies was to improve the diagnosis of adenomyosis using ultrasound and prepare the possibility to investigate adenomyosis on a molecular level.

The primary outcome of study 1 (resulting in paper I and II) was the overall diagnostic accuracy of the JZ thickness in diagnosing adenomyosis with 3D TVUS, compared to conventional 2D TVUS and MRI. The secondary outcome was the diagnostic accuracy of other predefined diagnostic predictors for adenomyosis in 3D TVUS and MRI, as described in the material and method section.

The primary outcome of study 2 (resulting in paper III) was to assess the safety of transvaginal, ultrasound-guided uterine biopsies, defined as no occurrence of major complications. The secondary outcome of the study was the sensitivity of those biopsies in gaining adenomyosis tissue.

Both studies enabled us to build up a biobank that contained tissue samples, parallel with imaging and clinical data.

MATERIALS AND METHODS

1. Ethical approval and inclusion

All collected data was under the scope of the Norwegian Adenomyosis Study, a prospective observational study performed at the Department of Gynecology, Oslo University Hospital, Oslo (OUS), Norway. The inclusion took place from September 2014 to August 2016, and the last surgery was performed in March 2017. The Scientific Advisory Board at OUS, the Advisory Committee on the Protection of Patient Records at OUS and the Regional Committee for Medical Research Ethics in Eastern and Southern Norway (Approval Number 2014/637) approved the trial, and the study was registered in ClinicalTrials.gov (protocol number NCT02201719 and NCT02197923) before recruiting study participants. Written consent was obtained from all study participants before inclusion.

2. Patient selection

Premenopausal women aged 30–50 years and needing a hysterectomy, who were not taking any hormonal contraceptives or receiving hormonal treatment or GnRHa therapy at least three months before inclusion, or suffering from a malignant condition were eligible for inclusion in the study. Exclusion criteria were: the need for tissue morcellation of the uterus, use of hormones three months or less before the ultrasound and hysterectomy, and malignancy. We assessed eligibility based on referral letters received from referring gynecologists, and those with no obvious exclusion criteria were scheduled for a clinical examination performed by the first investigator. When a hysterectomy was the concluded therapeutic advice and eligibility criteria were fulfilled, the woman was invited to participate in the study, written as well as oral information was given and written consent was obtained. The patient information and inclusion form is attached as Appendix 1. To secure consecutive recruitment, all eligible women that were already scheduled for surgery by another gynecologist, were contacted by phone and asked if they would like to participate, and also scheduled for an investigation with the first investigator.

3. Clinical examination and Questionnaire

All study participants underwent the same history taking and clinical examination, including a gynecological exam. The findings were documented in the patient record, according to the department's practice. After the consultation they were asked to fill out a questionnaire,

assessing baseline data and symptoms (Appendix 2). This questionnaire was designed for this study, as there is no validated tool available for the evaluation of adenomyosis related symptoms. Symptoms were evaluated using a verbal numerical rating scale (VNRS) ranging from 0-10 and frequency of symptoms were documented on a 5-point Likert-scale (143, 144).

4. Ultrasound imaging and reading

All study participants underwent a clinical and gynecological examination that included 2D and 3D TVUS (5–9 MHz endovaginal probe; Voluson S8, GE Kretz, Austria). The 2D TVUS findings were documented in images and video recordings, while 3D volumes were acquired and stored in a standardized manner as described in the literature previously, and analyzed clinically during the consultation and later again using the software 4D View (GE Healthcare) (1, 145).

The correlation of the JZ thickness with the diagnosis of adenomyosis as first described for 3D by Exacoustos et al. was the primary feature we investigated (1). The JZ was measured in the coronal and sagittal plane, using VCI or rendering.

An assessment of the JZ morphology, with smaller modifications to the suggested description by the MUSA group in their first consensus-paper, was also performed (81). We categorized the JZ appearance into regular, irregular, interrupted, irregular and interrupted, not visible and not assessable, in the sagittal and coronal plane using 3D TVUS.

The presence or absence of the following other criteria for adenomyosis was also evaluated: the uterine shape (globular or normal), and presence of myometrial alterations (hyperechoic islets, fan-shaped echo, subendometrial buds and lines, anechoic areas, and myometrial cysts) (Figure 2). The thickness of both uterine walls, uterine size, and volume of the corpus uteri and the endometrial thickness were additionally documented. All documented parameters were chosen based on previous publications. All images were obtained and assessed by the same gynecologist (T.T.) who was blinded to the histopathological and MRI results. A visual synopsis of the various parameters is given in Figure 2.

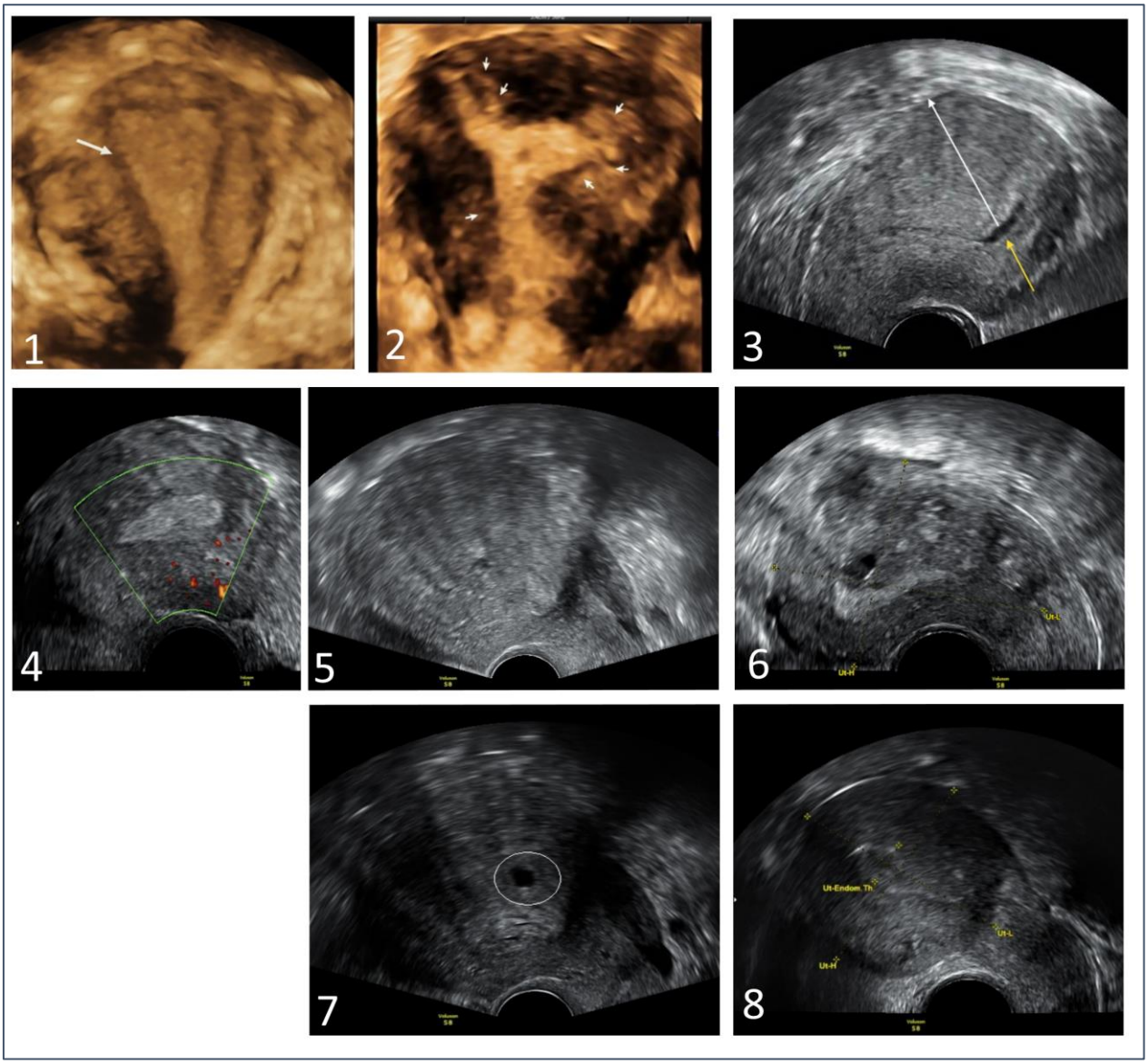


Figure 2: *Diagnostic signs of adenomyosis in ultrasound*. 1: A regular JZ (arrow). 2: Invaded junctional zone (JZ), the arrows indicate adenomyosis tissue invading the myometrium. 3: Asymmetry of the anterior and posterior uterine wall. 4: Translesional vascularization. 5: Fan-shaped (radiating) echo with echo-enhancement (isthmic region). 6: Hyperechoic myometrial islets representing adenomyosis. 7: Anechoic myometrial cyst. 8: A globular shaped corpus uteri.

5. Magnetic resonance imaging and reading

MRI was performed with a 3-Tesla (T) Philips Ingenia with dStream anterior and posterior coils, or 1.5-T Philips Achieva device with a 32-channel cardiac coil (Philips Medical Systems, Eindhoven, The Netherlands). The acquisition parameters are shown in Supplementary Table 1 of paper II. Examinations were performed regardless of the menstrual cycle phase. Patient preparation included fasting for 4 hours before the examination, emptying of the bladder, and administration of 20 mg of butylscopolamine (Buscopan, sanofi-aventis Norge, Lysaker, Norway) intravenously and 1 mg of glucagon intramuscularly. A contrast agent was given when

the clinical examination had reviled signs of deep infiltrating endometriosis. In cases where MRI had already been performed at another institution, the acquired images were retrieved and reassessed. All images were stored anonymously on the Syngo Imaging picture archiving and communication system (Siemens Healthcare). G.M. (Reader 1, R1), with 14 years of body-MRI experience and who was blinded for both the sonographic and histopathological data performed the reading of all images. All of the evaluated features are listed and defined in Table 1. We defined adenomyosis as being present if one or more of JZ_{max} ≥12 mm, myometrial cysts or adenomyoma which are comprehensively described elsewhere, were present (70, 71, 146-149). Other features that have been described less comprehensively previously were also documented and tested for their diagnostic accuracy (70, 72).

Signs used for diagnosing adenomyosis	**Definition**
JZ_{max} ≥12 mm [a]	**JZ is a low-intensity band in T2W MRI of the inner myometrium, lining the endometrial cavity. JZ ≥12 mm, measured in any plane, including focal enlargement, and not including adjacent focal adenomyoma (definition see below) or diffuse adenomyosis.**
Myometrial cysts	High-intensity foci in the myometrium or subendometrial area, as seen in T2W or T1W imaging (hemorrhagic content).
Adenomyoma	Ill-defined, focal low-intensity areas with or without high-intensity foci.
Other documented features	
JZ_{max}	Thickest part of the JZ, measured in the midsagittal and axial plane perpendicular to the endometrial cavity, in millimeters.
JZ_{min}	Thinnest part of the visible JZ, measured in the midsagittal and axial plane perpendicular to the endometrial cavity, in millimeters
JZ_{diff}	JZ_{diff} is calculated as $JZ_{max\ (all\ planes)} - JZ_{min\ (all\ planes)}$ and represents irregularities of the JZ.
$JZ_{max\text{-}A}$	JZ measurement including all low-intensity signal areas representing diffuse or circumscribed adenomyosis, attached to the JZ (see also Fig. 3).
Appearance of the JZ[b]	Subjective impression of the JZ morphology being regular or irregular, not assessable, or not visible (see Fig. 2).
JZ-to-myometrial thickness ratio	Using JZ_{max} in the midcorporal area (sagittal and axial) and the corresponding thickness of the myometrium obtained at the same measurement level. Only assessable when no fibroids distort the wall.
Globular uterine shape	Subjective impression of the corpus uteri being round, and caused by smooth muscle hypertrophy resulting in a globular uterine shape, not due to fibroids.
Number of fibroids	Fibroids, which appear as well-circumscribed uterine masses.
Size of largest fibroid	Largest diameter (in millimeters).

Table 1: *Definition of predictors for the diagnosis of adenomyosis and other documented features.* [a]Primary outcome measure; JZ, junctional zone; MRI, magnetic resonance imaging; T1W, T1-weighted; T2W, T2-weighted.

One of the less described features is the morphological classification of the JZ that is introduced in this work. It is based on previously described features and modified for MRI (Figure 3) (70, 81). There is no unanimous definition of the JZ in MRI, and it is measured in different ways amongst radiologists and research groups. In order to reflect that variation, we introduce different terms of JZ measurements (JZ_{max} and $JZ_{max\text{-}A}$), that reflect different measurement practices that we used in our clinical work and found in the literature (68, 69, 75). Those are comprehensively explained in Table 2 and Figure 3 of paper II. R1 repeated the reading of the predictors JZ_{max}, JZ_{min} and JZ_{diff} 6 month after the first reading, to enable testing of the intra-reader agreement of those signs and confirm the reliability of the results. A second reader (E.V., here R2, with 20 years of body MRI experience) also assessed the main outcomes (JZ_{max}, JZ_{min}, JZ_{diff}, $JZ_{max\text{-}A}$, morphological JZ classification) in order to allow the evaluation of the inter-reader agreement of those signs. The readings were performed independently on two different image sets, blinded to the clinical, sonographic and histopathological data.

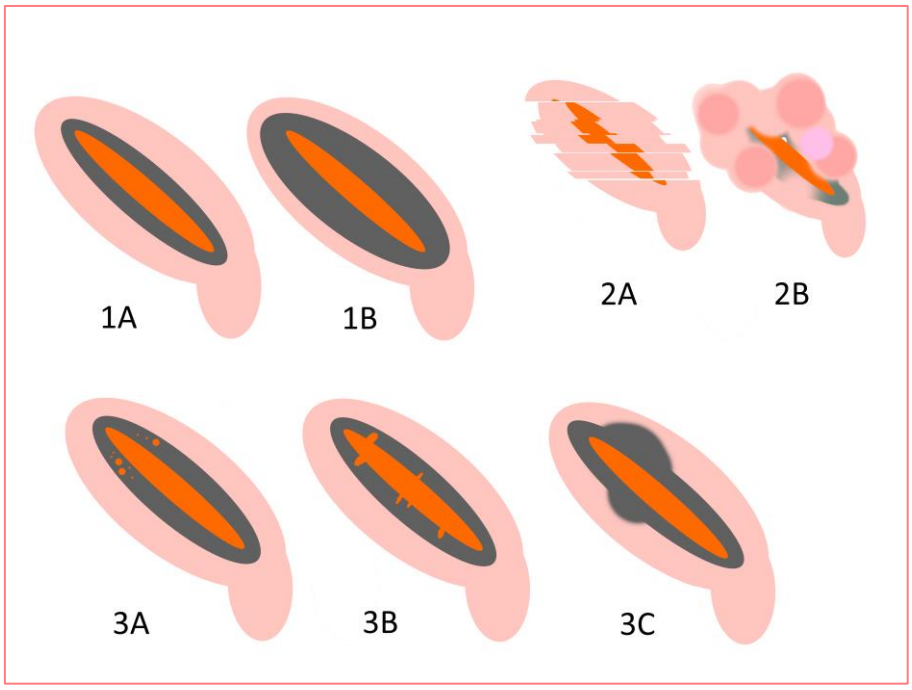


Figure 3: *Classification of the junctional zone (JZ)* morphology, *as proposed by the author* **1: Normal JZ.** The inner and outer borders of the JZ are smooth and satisfyingly defined. 1A) thin JZ 1B) regularly enlarged JZ. **2: JZ not visible or not assessable.** 2A) Due to motion artifacts 2B) Due to fibroids or large areas of adenomyosis. **3: Irregular JZ** If one or multiple of the following findings are present, and not caused by fibroids: 3A) JZ shows disruption by high-intensity foci (cysts) (3B) fingerlike indentations at the endometrial-myometrial junction (3C) focal thickening of the JZ, not representing a contraction.

6. Hysterectomies and histopathology

The hysterectomies were performed according to the standard clinical practice in the department with regards to both indication and surgical method. Only women with a uterus that did not require laparoscopic morcellation were included, and any presence and extent of endometriosis were registered perioperatively. Biopsy cores were obtained before the hysterectomy, fixated in 10% buffered formalin and sent to analysis to the pathologist. The fresh hysterectomy specimen was cut open, to allow optimal fixation with formalin throughout the whole specimen. The sectioning and gross examination of the specimen were standardized and performed by laboratory staff or a pathologist together with the first investigator.

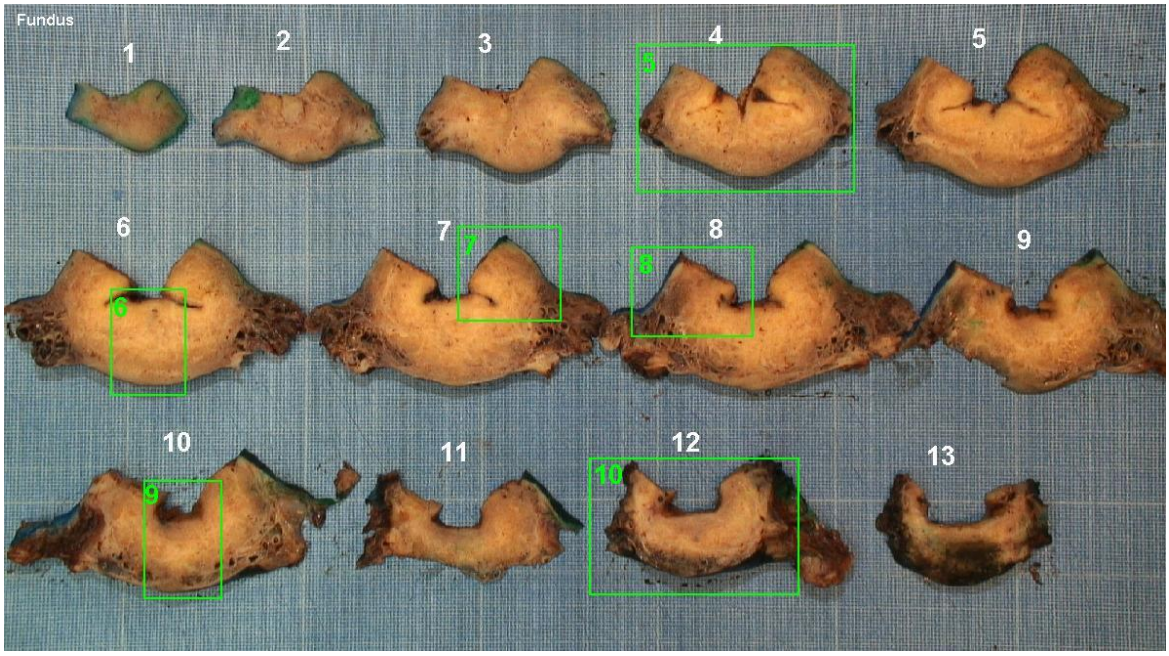


Figure 4: *Standardized gross examination of the hysterectomy specimen*. The uterus was cut into 5-10mm, axial slices. The green squares mark the areas where the microscopic sections were taken. The white numbers indicate the slice number, the green numbers the microscopic section number. Sections were also routinely taken from the fallopian tubes, cervix and other structures (not shown here).

The uterus specimens were cut axially into 5-mm-thick slices and photo documented. Microscopic sections were obtained from areas macroscopically suspicious of adenomyosis, areas where ultrasonography imaging had indicated signs of adenomyosis, and/or random sections (Figure 4). This protocol aimed to maximize diagnostic sensitivity (2). Two senior

pathologists, who were blinded to the ultrasonography and MRI results, performed the microscopic histopathological analysis and made the final diagnosis. The presence of ectopic endometrial glands and stroma at ≥2.5mm below the endometrial-myometrial junction was defined as adenomyosis. Endometriosis was diagnosed if glands were found on the serosa of the uterus or immediately in a subserosal location and not deeper in the myometrium. The biopsies were initially examined independently from the hysterectomy specimens, but an indication of presence of adenomyosis in the biopsies was compared to and verified with findings in the hysterectomy specimen.

7. Biopsy-taking

The primary investigator (T.T) obtained uterine biopsies before hysterectomy when the woman already was under general anesthesia. The woman was placed into a lithotomy position and biopsies were taken transvaginal and ultrasound-guided, using a 2D 5-9Hz transvaginal ultrasound probe (Voluson S8, GE Healthcare, Austria) with a reusable needle guide provided by the ultrasound manufacturer. The tru-cut core biopsies (BIP-HistoCore®, BIP Biomed. Instrumente & Produkte GmbH, Türkenfeld, Germany) devices had needles of 14-20 gauge (G) in diameter. Using 2D TVUS, the uterus and surrounding organs were scanned and checked for structures that could be damaged during the procedure, like adherent intestines or large vessels. Then, possible direct signs of adenomyosis were identified, as described above. If no direct signs of adenomyosis were visible, random biopsies were obtained throughout the myometrium. A total of four biopsies were taken from each woman. Two biopsies were fixated in 10% buffered formalin and sent for histopathological analysis, and two were capped into vials (CryoTube™Vials, Thermo Fischer Scientific, Roskilde, Denmark) and snap frozen on liquid nitrogen, without adding any buffer (19). An endometrial biopsy was finally taken (Pipelle de Cornier, Laboratoire C.C.D., Paris, France) and also snap frozen in liquid nitrogen, and all samples were later transferred to -70°C for storage. The woman was then re-positioned for hysterectomy. When entering the abdominal cavity, the status in the peritoneal cavity was assessed and checked for signs related to the biopsies. Any changes were registered, using a standardized form, see Appendix 3.

In addition to the needle-biopsies, we sampled larger biopsies that were embedded in O.C.T. compound (Tissue-Tek™, Sakura Finetek, Tokyo, Japan) before freezing in liquid

nitrogen (Figure 5). Those were also used to test microdissection and RNA-isolation as described in the next section.

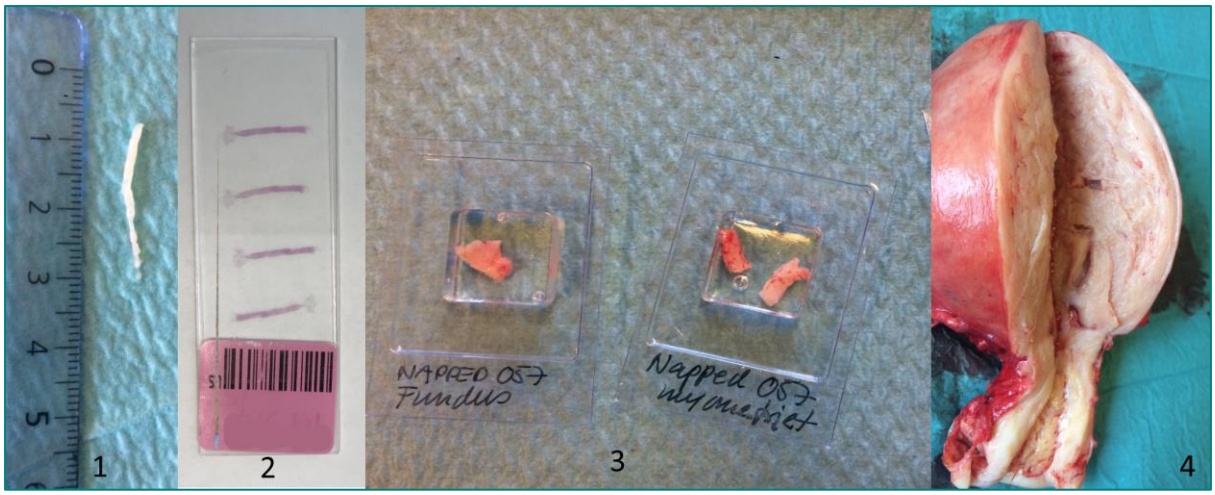


Figure 5: *Illustration of biopsies.* From left to right: 1. Fresh tru-cut biopsies. 2. Sections of paraffin embedded and hematoxylin/eosin-stained tru-cut biopsy. 3. OCT-embedded, fresh biopsies obtained from the hysterectomy specimen after hysterectomy. 4. Hysterectomy specimen opened in the anterior wall with marks where the biopsies shown in 3. are taken.

8. Preparing of frozen sections, staining, and RNA isolation

After snap freezing and storage on -70˚ C, the biopsy specimens were mounted on a chilled pin and cut into 2µm thin slices on a microtome (Leica CM350S, Leica Microsystems GmbH, Wetzlar, Germany) at -40˚ C. Test sections from the frozen sections were first hematoxylin/eosin (HE) stained on glass plates in order to see if they contained the cell-entities that were the target of investigation, and, if yes, further sections were applied onto membrane slides (mmi Slides RNAse free, MMI AG, Glattbrugg, Switzerland). They were then stored in concealed RNAse free plastic bags at -70°C until further use. Total RNA extraction from the tissue was performed using the ARCTURUS® PicoPure® RNA Isolation Kit (Applied Biosystems™, California, USA) and RNA analysis was performed on the Agilent 2100 bioanalyzer (Agilent Technologies, California, USA) following the protocols provided by the manufacturers. HE staining was performed according to the manufacturer`s instructions (H&E Staining Kit Plus, MMI AG, Glattbrugg, Switzerland) and endometrial and muscular tissue was separated with a laser dissection system (MMI CellCut, Molecular Machines and Industry, Glattbrugg, Switzerland), as illustrated in Figure 6.

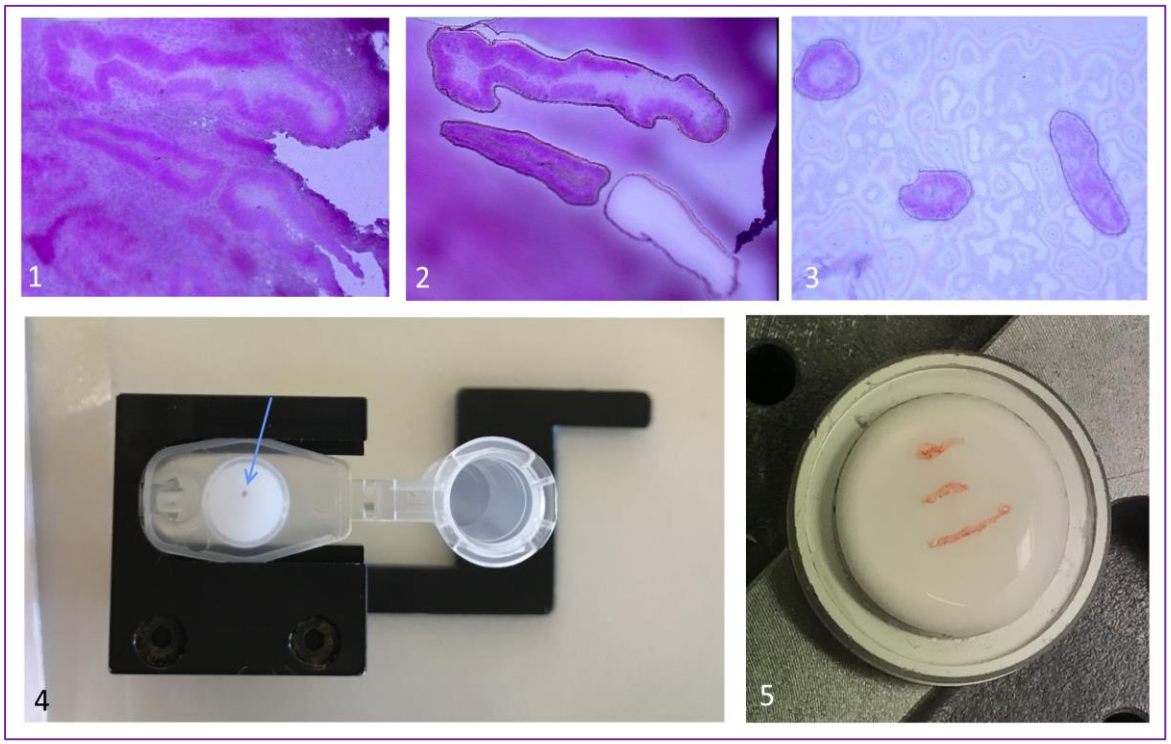


Figure 6: *Illustration of microscopic laser capture dissection*. 1. Sections from the eutopic endometrium, hematoxylin/eosin (HE) stained, x20 magnification. 2. Endometrial glands are dissected and two out of three attached to the collection cap of the tube. 3. Adenomyosis glands are dissected from the myometrium and isolated to the cap (x20 magnification, HE staining). 4. The silicone collection cap with a dissected cell entity (blue arrow). 5. Tru-cut biopsies that are embedded in O.C.T. and mounted on a pin before preparation of frozen sections.

9. Statistics

a. Power calculation

We calculated the power for the study using two different methods to secure sufficient study power for different outcomes. Firstly, we used the R pwr library (pwr.2p.test, Champely, 2012) to calculated the power based on sensitivity and specificity of 3D TVUS as described by Exacoustos et al. (1), the prevalence of adenomyosis in our institution and a significance level of 0.05. This yielded n=82 as a minimal number of women to include. Secondly, we calculated the required sample size based on the concept for range of confidence interval (CI) for specificity and sensitivity for the main predictor [maximum junctional zone thickness (JZ_{max})]. Using a CI of 95% with a width of 0.2 and test sensitivity and specificity of 75%, the nomogram showed that at least 73 study participants were required (150). Finally, we decided to include 100 women to guarantee power for our study

in case of drop out.

The most frequently applied determination of power for prediction models is that every event (in this case: presence of a diagnostic predictor) should appear at least ten times (151). This was verified post hoc when developing the present model.

b. General statistics

The proportions for categorical variables were compared with the chi-square test or Fisher's exact test. The sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) and their 95% CI were calculated using the MedCalc software (https://www.medcalc.org). Numerical variables were compared using Student's *t*-test (normally distributed samples) or the Mann-Whitney *U*-test (nonnormally distributed samples). Normality of the samples was determined by Shapiro-Wilk test. Variables conforming to a normal distribution were reported as mean±SD values, while nonnormally distributed variables were reported as median and range values. The receiver operating characteristics (ROC) curve and the area under the ROC curve (AUC) were used to determine significant cutoffs for linear variables. Each cutoff value was then used to create two new categorical variables within that variable, and they were tested again with the chi-square test, and the sensitivity, specificity, accuracy, PPV, and NPV were calculated. The score of the VNRS was considered to be a continuous variable, in accordance with previous publications (30). A probability value of $p < 0.05$ was considered statistically significant.

The simple pairwise Cohen κ statistic was used to measure the inter-reader agreement for categorical response of imaging features, whereas the intraclass correlation coefficient (ICC) was used to assess the level of agreement for numerical response features. The κ and ICC values were categorized as follows: 0–0.20: slight agreement, 0.21–0.40: fair agreement, 0.41–0.60: moderate agreement, 0.61–0.80: substantial agreement, and 0.81–1: almost perfect agreement (152).

Statistical analysis was performed using IBM SPSS Statistics (version 25, IBM Corporation, New York, USA).

c. Prediction model

The diagnostic prediction model was developed in a three-step procedure. In the first step, before data collection, candidate predictors were defined based on previously published

studies. In the next step, the individual diagnostic performance of each predictor was tested. In the third step, the individual association of the best performing predictors with the outcome (histopathologically confirmed adenomyosis), was determined. The development of the prediction model is described in detail in Paper I.

RESULTS

1. Patient flow

The patient flow for all three studies is illustrated in Figure 7. The indications for hysterectomy were at least one of menorrhagia, dysmenorrhea, bulk-related symptoms, dyspareunia or pelvic pain, mostly a combination of those. “Other therapy” included Lng-IUD (*n*=21), embolization (*n*=2), or a need for laparoscopic subtotal hysterectomy with morcellation (*n*=2). The main exclusion criteria were the use of hormone therapy, wanting a laparoscopic subtotal hysterectomy, or not wanting any treatment. The two women that refused inclusion had claustrophobia and were therefore not willing to take an MRI.

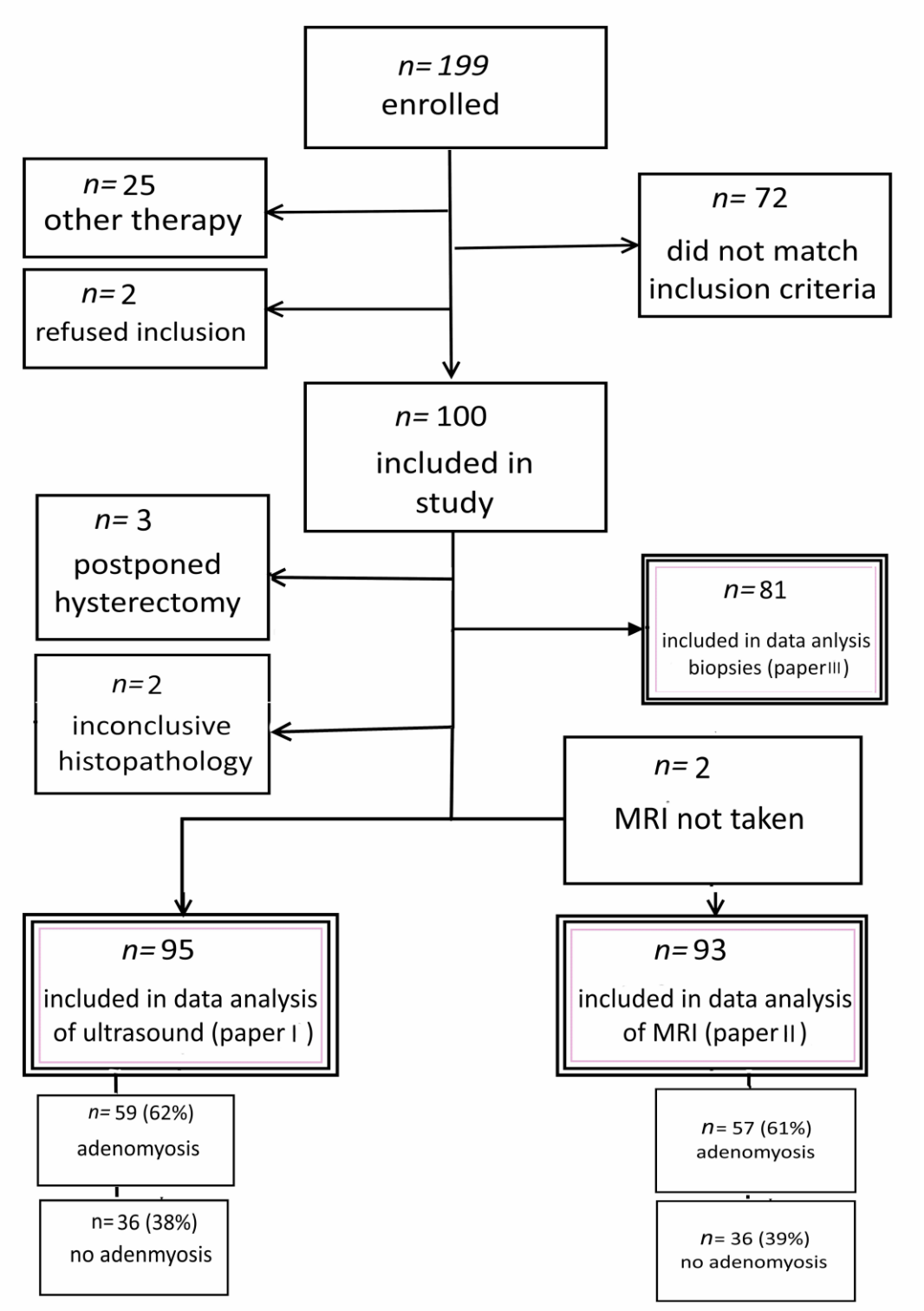


Figure 7: *Patient flow through the study.* MRI; magnetic resonance imaging. For paper III, n=2 declined inclusion, n=13 no biopsy was taken due to organizational causes, n=1 no biopsy was taken due to equipment failure, leaving n=81 included.

2. The baseline characteristics of the study population

Clinical features of the study population are presented in Table 2. The numbers are based on the largest included study group, which corresponds to paper I.

	Adenomyosis *n*=59	No adenomyosis *n*=36	*p*
Age, years	43.5±4.9	41.2±4.2	0.01*
BMI, kg/m^2	25.9 (16–44)	25.6 (19–34)	0.73
Number of pregnancies	3 [0–9]	2 [0–5]	0.16
Parity	1.4±1.4	1.5±1.2	0.66
Previous use of oral contraceptives	49 (83)	28 (78)	0.53
Duration of oral contraceptive use, *years*	4 (0-23)	2.5 (0-25)	0.80
Previous curettage, *n*	29 (49)	9 (25)	0.02*
History of infertility, *n*	27 (46)	15 (43)	0.91
Age at menarche, *years*	13 (10-18)	13 (10-16)	0.74
Any complications in pregnancy or labor, *n*	19 (32)	12 (33)	0.85
Weight of uterus, *gram*	153 (72-1709)	123 (55-3100)	0.86
Uterus retroverted, *n*	20 (34)	12 (33)	0.94
Presence of endometriosis, *n*	28 (48)	8 (22)	0.01*
Presence of fibroids, *n*	33 (56)	18 (50)	0.46
Regular menstrual cycle, *n*	34 (59)	28 (80)	0.03*
Duration of menstrual bleeding, days	7 (1-28)	7 (2-30)	0.96
Occurrence of intermenstrual bleedings, *n*	22 (37)	16 (44)	0.49
Premenstrual pain (VNRS 0-10)	6.2 (SD±2.7)	4.9 (SD±2.9)	0.03*
Dysmenorrhoea (VNRS 0-10)	7.7 (SD±2.3)	6.4 (SD±3.2)	0.03*
Dyspareunia (VNRS 0-10)	4.8 (SD±3.2)	3.9 (SD±3.4)	0.22

Table 2: *Baseline characteristics of the study population.* Data are mean±SD, median [range], or *n* (%) values. Presence of endometriosis confirmed by laparoscopy. *Statistically significant difference (p<0.05).

3. Histological results

The number of cases with and without adenomyosis is displayed in Figure 7. In two cases, the final diagnosis could not be made due to uncertainty if endometrial glands in one of the microscopic slides were possible tangential sections of a deeper layer of the uterine cavity or might have represented a fallopian tube (as illustrated in Figure 1). In one case, subserous endometrial glands were interpreted as endometriosis infiltrating the uterus as this woman had no other signs of adenomyosis in the corpus uteri, but extensive deep infiltrating endometriosis was present.

4. Imaging Results

a. JZ measurements

We investigated the significance of the JZ thickness for the diagnosis of adenomyosis in both TVUS and MRI.

In TVUS, the JZ was visible in the coronal plane in 64 (68%) and in the longitudinal plane in 43 (46%) cases. The overall JZ_{max} difference was not statistically significant when comparing women with and without adenomyosis, 5.6mm (95%CI 4.9-6.4mm) vs. 4.7mm (95%CI 3.9-5.4mm), p=0.07. Only JZ_{max} in the sagittal plane ($JZ_{max(sag)}$) showed a statistically significant difference, with a mean of 5.7mm vs. 3.9mm (p=0.03), respectively. $JZ_{max(sag)}$ also showed a fair test-quality (AUC=0.71, p=0.02), with a cut-off of JZ_{max}>5.3mm yielding a specificity of 78% and sensitivity of 58%. $JZ_{diff,}$ as an expression for irregularities of the JZ, did not correlate with the diagnosis of adenomyosis (AUC 0.52, p=0.80), and also not if calculated for each plane separately ($JZ_{diff\text{-}sagittal}$ AUC 0.63, p=0.16, $JZ_{diff\text{-}coronal}$ AUC 0.63, p= 0.16).

In MRI, we examined JZ_{max} for each plane separately (sagittal, axial and coronal) and the maximum value for all planes combined. Further, for each reader and the mean of the two readers. None of those measures exhibited a statistically significant correlation to the diagnosis of adenomyosis. JZ_{diff} was significantly higher in the group with adenomyosis (8.4mm±9.2 vs 4.5±3.1, p=0.02), but with a poor test quality (AUC = 0.68, 95%CI 0.56–0.80; p=0.006). The measurement of $JZ_{max\text{-}A}$ showed a weak, but statistically significant correlation with adenomyosis (AUC=0.68, 95% CI=0.57–0.80, p<0.001). Also, a cut off of JZ_{max} ≥12mm did not correlate with the diagnosis of adenomyosis, in none of the readings or with any reader. Using JZ_{max} ≥12mm as a diagnostic marker led to a high number of false negative and positive diagnosis. The criterion of JZ_{max} ≥12 mm was present in all false-positive cases, and in 7/12 (58%) as

the sole predictor. Examples of false positive cases based on JZ_{max} is shown in Figure 8.

The detailed results of the JZ measurements are displayed in Table 3 and 4 of paper II.

The inter-observer agreements for the values of $JZ_{max,}$ as measured with MRI were almost perfect (ICC=0.81, 95% CI: 0.70–0.87, p<.001), as they were for $JZ_{max\text{-}A}$ (ICC=0.95, 95% CI: 0.93–0.97, p<.001), and substantial for JZ_{diff} (ICC=0.73, 95% CI: 0.59–0.83, p<.001). There was also substantial intra-observer agreement in the measured JZ_{max} values (first and second readings of G.M.), with an ICC of 0.75 (95% CI: 0.59–0.84, p<.001).

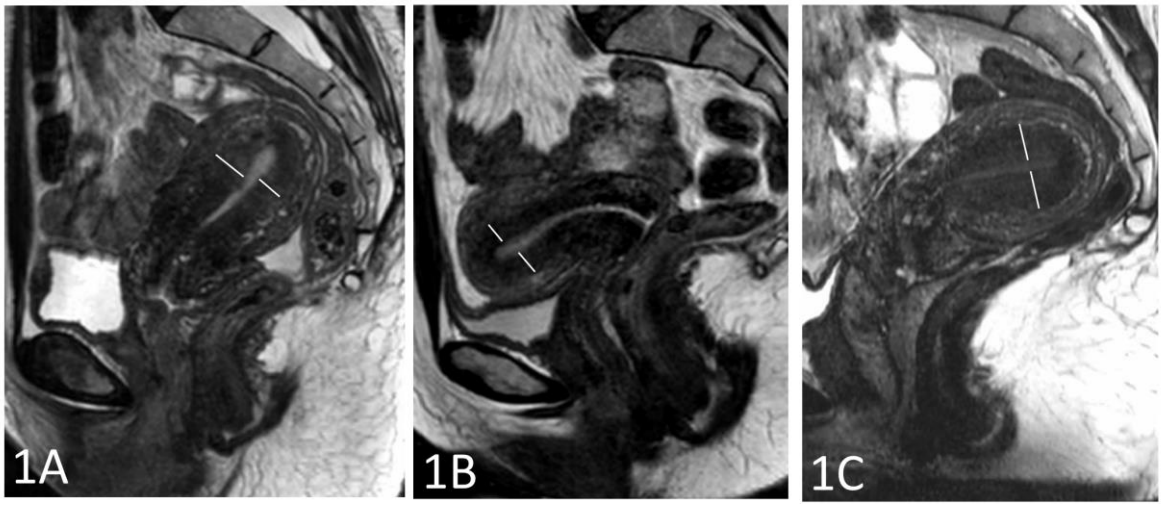


Figure 8: *Cases with a regular junctional zone (JZ) and ≥12mm thickness, none with adenomyosis.* All magnetic resonance images of the uterus with T2 weighted sequences and turbo spin echo, the JZ measurement indicated with a white line. Presence of adenomyosis was determined by histopathology. Cases 1A-C are false positive when using a cut off of JZ_{max}≥12mm as diagnostic marker, but true negative with pattern recognition of the JZ morphology (regular JZ).

b. JZ appearance

We classified the appearance of the JZ into various groups, as described in the method section (see also Figure 3 for MRI). The frequency of JZ appearance as seen in TVUS and MRI, are shown in table 3.

In TVUS, an irregular JZ was associated with having adenomyosis (p=0.04) and a regular JZ was significantly associated with not having adenomyosis (p=0.002). The groups of interrupted JZ [irregular & interrupted JZ (p=0.21) and not visualized JZ (p=0.17)] were not statistically significantly associated with having adenomyosis. In 30 (32%) of all cases, the JZ was not visualized or assessable on TVUS.

In MRI, the JZ was assessable in almost all cases (91, 98%). In the remaining two cases, it was

not assessable due to motion artifacts. The presence of an irregular JZ was strongly correlated with having adenomyosis, with a sensitivity of 74% and a specificity of 83% ($p<0.001$). Examples of an irregular JZ are shown in Figure 9.

Accordingly, a regular JZ showed a strong statistical association with not having adenomyosis, with a sensitivity of 81% and a specificity of 75%, as shown in detail in Table 3 in paper II, and as illustrated in Figure 8. We found that there was no association with adenomyosis if the JZ was regular, independently of the JZ thickness.

The inter-observer agreement for the classification of the JZ was almost perfect ($\kappa=0.89$, 95% CI: 0.78—0.97). Amongst the cases with an irregular JZ, when comparing women with and without adenomyosis, 15 (14 vs 1) were classified irregular due to small cysts in the JZ, 15 (10 vs 5) due to a focal enlargement of the JZ, 11 (10 vs 1) due to interrupted appearance of the JZ, and 3 (3 vs 0) due to fingerlike indentations at the endometrial-myometrial boarder. The results, as well as further characteristics of the different appearances of JZ, are presented in detail in Table 3 and 4 in paper II. Examples of an irregular JZ in MRI are presented in Figure 9.

	TVUS		MRI	
	Adenomyosis	*No adenomyosis*	*Adenomyosis*	*No adenomyosis*
JZ morphology	*n (%)*	*n (%)*	*n (%)*	*n (%)*
regular	4 (7)	11 (31)	15 (26)	28 (80)
irregular	10 (17)	2 (6)	41 (72)	7 (17)
interrupted	15 (25)	8 (22)		
irregular & interrupted	7 (12)	8 (22)		
not visualized	13 (22)	6 (17)	0	0
not assessable	10 (17)	1 (3)	1(2)	1 (2)

Table 3: *Frequency of junctional zone (JZ) appearance.* The numbers for transvaginal ultrasound (TVUS) are based on 3D imaging in the coronal plane. The number for magnetic resonance imaging (MRI) is based on a consensus of the two readers, using all three imaging planes. The categories irregular and interrupted were merged into one category "irregular" for MRI.

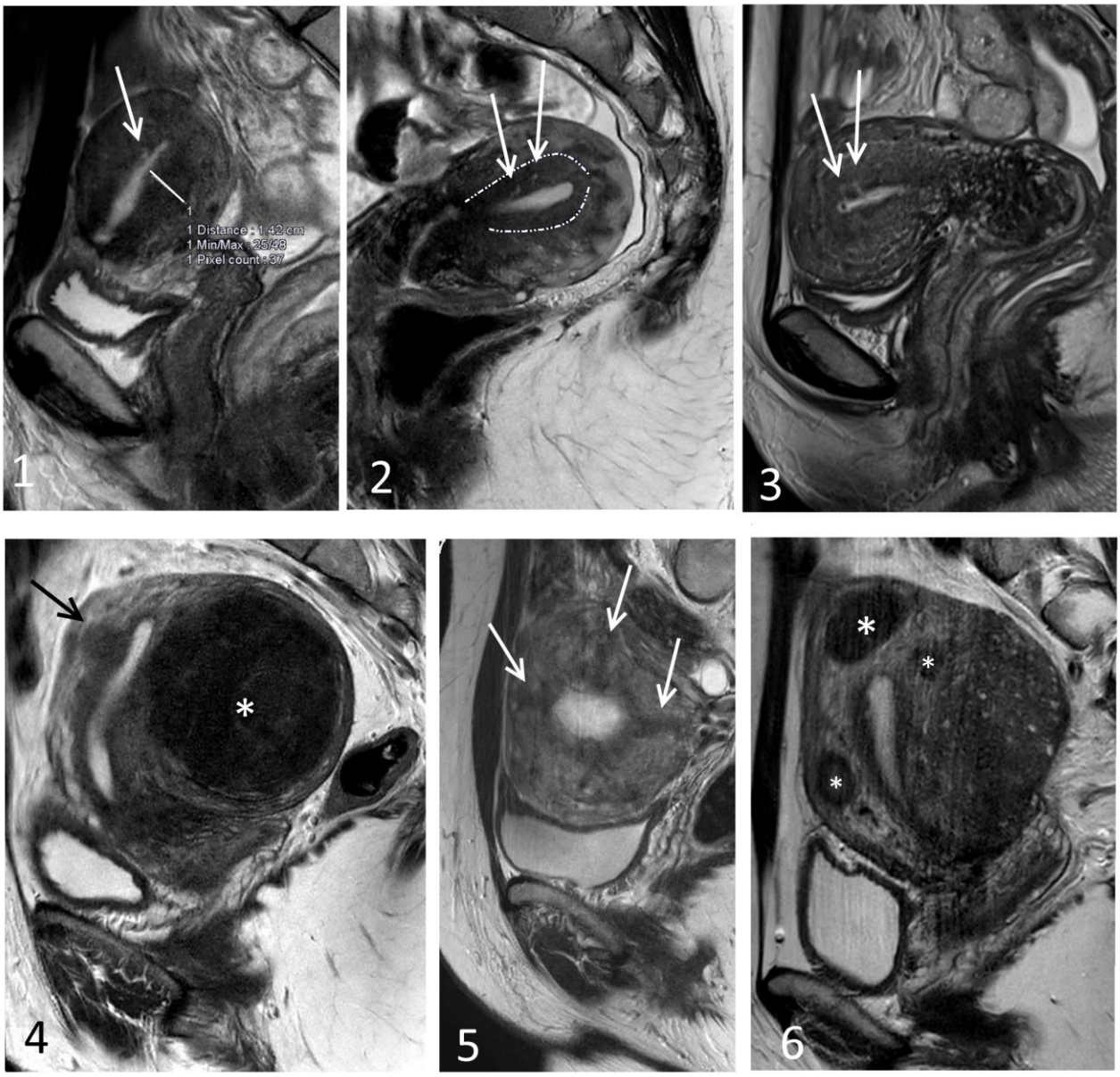


Figure 9: *Cases with an irregular junctional zone (JZ), true positive and false positive.* Magnetic resonance images of the uterus, all in sagittal plane, with T2 weighted sequences and turbo spin echo. Presence of adenomyosis was determined by histopathology. The white lines indicate measures of the JZ. **1:** Retroverted uterus. The JZ is thickened (14mm) in the posterior wall and thin in the anterior wall (arrow). True positive diagnosis. **2.** Retroverted uterus. High-intensity signals on the inner border (arrows) representing infiltration of adenomyosis that interrupts the JZ. The outer border of the JZ (stippled line) should not be confused with the low-intensity, starfish-like signal from the stratum vasculare. True positive diagnosis. **3.** Anteverted uterus, The JZ is not visible in the fundus and thin (max. 6mm) in the visible parts. Finger-like invasion of adenomyosis to the myometrium (arrows) visible. True positive diagnosis. **4.** Retroverted uterus, containing a large fibroid in the posterior wall (marked with *). Focal thickening to 14mm of the JZ in the anterior wall represented most likely a change due to a very small fibroid. False positive diagnosis. **5.** Anteverted uterus, the cervix is not visible on this image. The arrows indicate areas that we interpreted as an irregular JZ or invasion of adenomyosis, but represented vessels. This was the only histopathological correlate that was found. False positive diagnosis. **6.** Anteverted uterus with a thin, almost regular JZ, but a large adenomyoma in the posterior wall. Three well demarked fibroids (*).

c. Other diagnostic parameters

In TVUS, the diagnostic sign that exhibited the best sensitivity was the globular formation of the uterus (sensitivity 61%, 95% CI 46—75%) and the signs with the best specificity were a fan-shaped echo (specificity 92%, 95% CI 78—98%), followed by the presence of myometrial cysts (sensitivity 86%, 95% CI 71—95%). Other diagnostic signs that had a statistically significant association with the diagnosis of adenomyosis were hyperechoic myometrial islets, wall thickness over 24mm and wall asymmetry, expressed by the ratio of the thickest to the thinnest wall. We could not find a statistically significant association for subendometrial buds, internal shadows or the volume of the corpus uteri. All values (sensitivity, specificity, PPV, NNP, accuracy, the 95% CIs) are shown in Table 2 in paper I.

In MRI, the sign with the best sensitivity for adenomyosis was the presence of an irregular JZ (sensitivity 74%, 95% CI 60—85%), and presence of cysts in the JZ exhibited the best specificity (specificity 97%, 95% CI 86—100%).

The results of the diagnostic performance of all assessed signs listed in table 1 are shown in table 3 and 4 in paper II.

5. Clinical symptoms and signs

The clinical symptoms that were evaluated based on the questionnaire (appendix 2) were assessed for a statistically significant association with adenomyosis. The VNRS-score of dysmenorrhea was almost statistical significantly linear associated with adenomyosis (AUC 0.61, p=0.06). When treated as a categorical variable with a cut off ≥ 8, and compared those with values of 8 or greater with those under 8, we found that it was significantly associated with adenomyosis [sensitivity 66% (95% CI 53—78%), specificity 56% (95% CI 38—72%), p=0.04]. In addition, the frequency “always present” of urinary irritation was statistically significant for the adenomyosis group, compared to the group with no adenomyosis (p=0.04). All evaluated symptoms, signs and anamnestic characteristics of study participants with and without adenomyosis are shown in Table 2.

6. The development of the prediction model

The prediction model for TVUS was based on the individual performance of all parameters that were described in the previous chapters. The following 13 parameters were tested for inclusion in the prediction model: presence of a globular enlarged uterus, myometrial cysts, fan-shaped

echo, wall size, wall asymmetry (expressed by the ratio of the thickest/thinnest wall), hyperechoic islets, maximum width of the junctional zone in the sagittal plane, a regular, irregular, or interrupted appearance of the junctional zone, VNRS score for dysmenorrhea, and frequency of urinary symptoms. The following predictors were considered to be obligatory because they are extensively described in the literature, even if not all performed too well in our data set: globular uterus, myometrial cysts, fan-shaped echo, and asymmetrical walls. We additionally chose predictors that were highly significant in our own data, including those that have not been described in previous publications (all others mentioned above). LASSO analysis using these 13 variables yielded nine variables and (unstandardized) β values, expressing the weight of each variable. The β intercept value was -1.11.

The ROC curve of the model is illustrated in Figure 10. The AUC of this model was 0.86 (95% CI 0.79–0.94). The optimal cutoff for predicting the probability of adenomyosis was 0.56, which gave a sensitivity of 85% and a specificity of 78%.

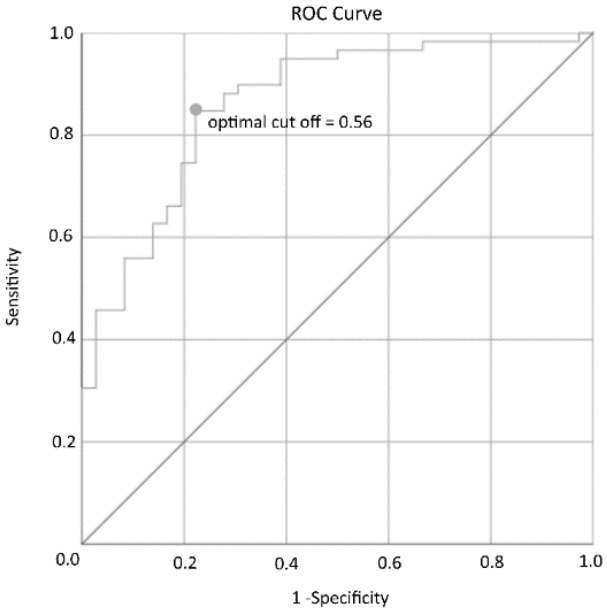
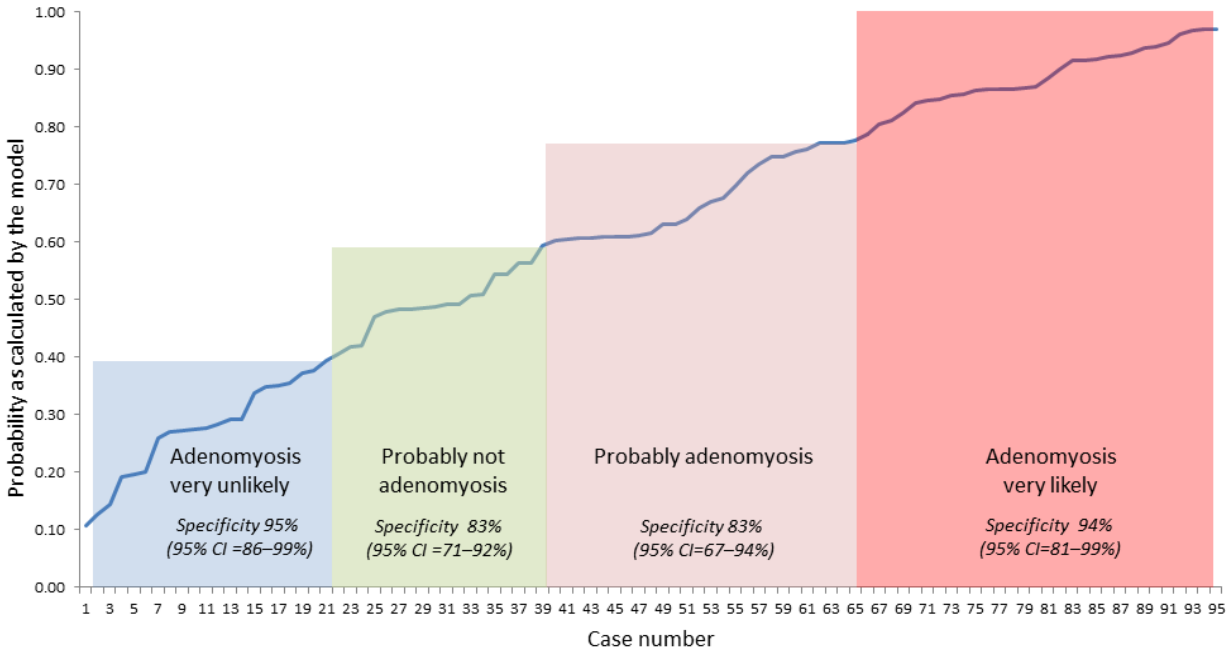


Figure 10: *ROC curve of the prediction model.* The optimal cutoff for predicting the probability of adenomyosis was 0.56, for which the sensitivity was 85%, and the specificity was 78%.

The leave-one-out cross-validated AUC was 0.75 (95% CI 0.79–0.94). We tested how the model would perform in subgroups with and without the presence of fibroids, and found no statistically significant difference, with AUCs of 0.78 (95% CI=0.65–0.94) and 0.92 (95% CI 0.85–1.0) (p=0.14), respectively. We analyzed if the size of fibroids (large, >50mm; small, <50mm) would influence the model and compared the presence in each group of probability, but could not see a difference (Figure 11). We found that there was a similar number of

fibroids in all groups of probability, and they did not seem to influence the results as the model predicted the presence or absence of adenomyosis correctly; even when large fibroids were present.

Figure 11 illustrates further a proposed interpretation of the calculated probabilities into clinically useful categories. We see that the specificity for the diagnosis of the outer ranges (predicted probability of adenomyosis <40% or >86%) is very high and adenomyosis can be diagnosed or ruled out with satisfying confidence. In the middle range, the diagnosis is not as specific. Therefore, we suggest using further clinical and anamnestic information to strengthen the diagnostic prediction.



Fibroid size	A. Unlikely	Probably not	Probably yes	A. Very likely
no fibroid	n=10 (48%)	n=12 (67%)	n=12 (44%)	n=13 (45%)
<50mm	n=6 (29%)	n=3 (17%)	n=11 (41%)	n=15 (52%)
>50mm	n=5 (24%)[1]	n=3 (17%)[2]	n=4 (15%)[3]	n=1 (3%)[4]

Figure 11: *Predicted probabilities by case.* The outer ranges (blue, probability <40% and red, probability >78%) show a high specificity with only one false positive/negative in each category. The middle range has a lower certainty of diagnosis. The presence of fibroids and their size was the same in each group and seemed not to influence the prediction. [1] All five cases were true negative for adenomyosis. [2] Two cases had adenomyosis, one did not. [3] Two cases had adenomyosis; two had not. [4]True positive case.

7. Biopsy taking

It was possible to perform biopsy taking in all but one case, and no major complications occurred during the procedure or postoperatively. The outcomes of this procedure are presented in table 5. Perioperative inspections of the abdominal cavity showed no visible damage in 20 (25%) of the cases, puncturing of the serosa in 56 (70%), and minor ongoing bleeding from the small subperitoneal vessels in 4 (5%), all of which stopped spontaneously during the subsequent surgery. In all cases, the amount of bleeding was minor, with a median of 2 ml (range 0–200 ml) and with no significant difference between the groups with and without adenomyosis (p=0.68). We identified biopsy needles with a length of 25cm and 18G to be best fitted for this purpose. Of the 81 cases where a biopsy was taken, 46 (57%) had adenomyosis, and 33 (41%) had not. The other two cases had inconclusive histological results, as described above. Adenomyosis tissue could be obtained in only 10 (22%) of the adenomyosis cases.

Results of technical details are comprehensively described in paper III. It was possible to produce frozen sections, staining of those sections, laser dissection and RNA isolation. The detailed description of the procedure, as well as illustrating figures, are presented in paper III.

Outcome	**All cases**	**Adenomyosis**	**No adenomyosis**	***P***
Procedure classified as “easy”	68 (84%)	36	30	0.25
No visible damage	20 (25%)	10	10	0.70
Puncturing of the serosa	56 (70%)	33	22	0.70
Ongoing bleeding	4 (5%)	2	2	0.25
Median amount of bleeding, ml	2 (0—200)	5 (0—160)	1 (0—200)	0.68
Time consumption, min	6.1 (SD±1.9)	6.1 (SD±1.4)	6.2 (SD±2.4)	0.79

Table 5: Outcome of evaluated parameters of transvaginal biopsy taking.

DISCUSSION

1. Discussion of results

a. JZ thickness

The primary interest in study 1 (paper I and II) was the significance of JZ measurements for the diagnosis of adenomyosis. In 3D TVUS, we only found a significant association with JZ_{max} measured in the sagittal plane, not in the coronal plane. This was somewhat surprising, as all published 3D images of the JZ depict the coronal plane and measurements taken there. At the same time, it is known that adenomyosis primarily affects the anterior and posterior walls, not the lateral, which is also reflected in the presence of wall-asymmetry in adenomyosis, and round shape of the corpus uteri. In the published MRI-based studies, radiologists only report JZ measurements from the sagittal and axial plane (69, 70, 75, 147, 153-155). Our MRI data also confirm that the JZ is quite thin in the coronal plane, independently of the thickness in the other planes. The relevance of the sagittal plane in diagnosing adenomyosis seems to be under-communicated amongst gynecologists, and we suggest not measuring the JZ in the coronal plane.

The test quality for $JZ_{max\text{-}sag}$ in TVUS was fair in our study, with an AUC=0.71. A cut off of $JZ_{max} \geq 5.3$mm yielded a sensitivity of 58% and specificity of 78%. Exacoustos et al. (1) found a significant association with both JZ_{diff} and JZ_{max} (all planes), but the AUC is not reported. They determined a cut off of 8mm for JZ_{max} to result in a sensitivity of 84% and specificity of 75%, and $JZ_{diff} \geq 4$mm to yield a sensitivity of 88% and specificity of 83% in their study. The reported mean JZ_{max} values for the adenomyosis group were 15.4mm in their study, compared to 5.6mm in our data, which seems to be a significant difference. At the same time, the JZ was only measurable with 3D TVUS in 44% of our cases, and it was reported that the evaluation of the JZ has a low inter-reader reliability (82), which represents a limitation to this as a diagnostic sign. In MRI, we could not reproduce that JZ_{max} or a $JZ_{max} \geq 12$mm was associated with adenomyosis and a diagnosis of adenomyosis based on these JZ measurements contributed to a high number of false positive and false negative diagnoses in our study population. Those results were unexpected. Especially in MRI, the JZ thickness and the cut off of ≥12mm seemed to be a fondly established diagnostic predictor for adenomyosis, and it was also routinely used in our department for clinical readings.

There are several possible explanations for why our results differ from those of previous studies. Firstly, the mean age of our study participants was lower (42 years vs. 51 years), and it

is known that adenomyosis progresses over time and hence could have been more extensive in the previous studies. Secondly, Reinhold and Bazot also included a large proportion of postmenopausal women in their study (31–55%), and it is questionable whether diagnostic characteristics of the hormone-dependent JZ are transferable between pre- and postmenopausal populations (71, 72).

Thirdly, it is not ultimately clear how the JZ was defined in other studies. We measured the JZ in accordance with our usual clinical practices, since there is neither a unanimous classification of adenomyosis nor a clear definition of the JZ; the main reason for that is most likely that the JZ has the same signal intensity as adenomyosis, and is not visible in histopathology (73, 156). We found using JZ measurements that included all low-intensity areas, also those representing diffuse or circumscribed adenomyosis that is in connection with the JZ ($JZ_{max\text{-}A}$) problematic for several reasons. In those cases, the presence of adenomyosis is usually apparent and therefore performing a measurement does not add any diagnostic value. If $JZ_{max\text{-}A}$ is measured in a study population with extensive disease, the average JZ thickness will be much higher, and a statistically significant association with adenomyosis in a ROC-curve is more likely to be found. However, this association might not be meaningful for individual evaluation and in clinical practice, especially not in younger women of childbearing age and less extensive disease (157). The interest in adenomyosis has shifted toward younger, infertile women and defining dedicated diagnostic markers for this group is of great importance. Bazot and Darai have recently stated that “In our experience, the JZ_{max} alone should be used with caution to diagnose internal adenomyosis.” This is in line with the conclusion of all the authors of comparable studies, who all state that other signs in addition to JZ measurements have to be considered (70-73).

The advantage of JZ measurement for both 3D TVUS and MRI seemed to be that it is an objective parameter, while other parameters (for example the globular shape of the uterus) are subjective and based on pattern recognition. But is shown that measurements of the JZ in 3D TVUS are not too well reproducible (82), and it still seems to be that the JZ is measured differently in-between different research groups or hospitals, as it is not a well-established and defined structure, yet. Further studies of the JZ in 3D TVUS and MRI are needed to determine how exactly the JZ should be defined and measured.

b. JZ appearance

It was possible to classify the JZ based on morphology in both MRI and TVUS in our study, but MRI was superior to depict the JZ, to detect smaller changes and discriminate better between artifacts and real changes. In TVUS, the sub-classification of interrupted or irregular JZ, buds or stripes, as suggested by the MUSA group, seemed to be difficult to apprehend and apply. It is therefore not sure the detailed results from our TVUS, especially from the 3D investigations, are reproducible. A recent study of Rasmussen et al. showed that the reliability and reproducibility of 3D TVUS depend on image quality and ultimately on the experience of the examiner (82).

Also, in MRI, the sub-classification into interrupted and irregular JZ as proposed by the MUSA group was not feasible for the radiologists. The two readers identified the same structures as pathological, but often classified the same findings into different groups. A collective group that contained all kinds of irregularities under one, called $JZ_{irregular}$, was, therefore, more meaningful in our opinion and did not alter the overall results. The radiologists showed an almost perfect inter-observer agreement when classifying the JZ into regular, irregular or not visible/assessable cases.

The classification of the JZ that we present in this work is not described in the radiological literature and is, therefore, a novelty. At the same time, many authors describe various irregularities of the JZ to for the diagnosis of adenomyosis, so that the acknowledgment of irregularities as an indication for adenomyosis is no novelty (72, 158).

With the limitations described above, in TVUS, it might be a better approach to identify a regular JZ, rather than to classify irregularities. This might be especially the case with less experienced investigators, as 3D TVUS can be challenging to interpret and artifacts are common. As we found a strong statistical association with a normal JZ and not having adenomyosis, we found this sign to be a good predictor and included it in our prediction model. When a regular JZ is seen we would like to suggest that direct signs of adenomyosis should be mandatory for a positive diagnosis, not only indirect signs.

In conclusion, we found that the appearance of the JZ has a good predictive value for the presence or absence of adenomyosis in both MRI and TVUS.

c. Prediction model

We developed a prediction model based on nine ultrasound criteria and one clinical feature.

We found the diagnostic accuracy of the single diagnostic markers to be in line with previous studies, although there is a variation of the reported prevalence and diagnostic accuracy throughout these studies (1, 70, 159, 160). Especially the sensitivity of findings will depend on the prevalence of the sign in the study population and therefore be influenced by selection criteria in each study. The specificity of the diagnosis will mainly rely on the examiner's skills and subjective interpretation of findings. To combine various findings into one model and provide different weights according to their reliability, will, therefore, be helpful to obtain a diagnosis independent of the examiners level of expertise. As adenomyosis has a very heterogeneous appearance in imaging, such a model is therefore especially valuable for this condition. We found that the performance of the prediction model [AUC=0.86 (95% CI=0.79–0.94), optimal cutoff 0.56, sensitivity of 85%, specificity 78%] was better than the performance of the best single parameter, and we therefore assume that combining all parameters in that way is a meaningful approach.

Also, the model was not disturbed by the presence of fibroids, which is an indication for the good robustness of the model. The leave one out cross-validation showed good results, but it is necessary to prospectively validate the model on a new dataset before it can be taken in clinical use.

d. Biopsies

The biopsy-taking could be performed according to our initial work hypothesis, gaining adenomyosis tissue without performing a hysterectomy and without any serious complications. The percentage of adenomyosis cases were we could obtain adenomyosis tissue was rather low (22%). There are no directly comparable studies with regard to effectiveness of adenomyosis tissue retrieval, as none describes the transvaginal route; instead a hysteroscopic, laparoscopic, abdominal or post-hysterectomy approach (89, 91, 92, 94-96, 161). We think that the rather low yield of positive tissue samples in our study is due to the lack of direct visualization of adenomyosis. We experienced satisfying access to the region of interest with the biopsies but struggled with direct visualization of adenomyosis. In cases with extensive disease, it was unproblematic to obtain adenomyosis, as also shown in a study by Nam et al. (92). We assume that a more powerful ultrasound system with a higher resolution or even MRI-ultrasound fusion imaging would yield a higher rate of adenomyosis positive tissue samples.

It seems that the biopsy taking is not associated with the risk of serious complications, which is in line with several individual studies and a meta-analysis, evaluating the biopsies of pelvic organs (162-169). Two studies reported the mean blood loss in normal oocyte pick up procedures to be 232ml and 72ml, which is significantly higher than the observed amount of bleeding in our study, which was 2ml (170, 171). By isolating RNA, we could further proof our hypothesis that those biopsies could be used for molecular investigations, opening up for the possibility to investigate adenomyosis at an early stage as well as the progress of the disease.

2. Methodological considerations

a. Study design

The prospective design with a consecutive inclusion of women is a strength of this study. The confirmation of the diagnosis with histopathology is also a strength, as it is still the gold standard. At the same time, it is a limitation, as it requires a hysterectomy. It is discussed extensively below how this introduces a selection bias. The test power calculation was performed a priori for the main outcome, which is a strength of the study. However, there is no formula to calculate the power of a prediction model; this is why we had to control the included variables post-hoc for their power.

b. Study participant selection

As discussed previously, the need for histopathological confirmation of adenomyosis represents the most significant selection bias in this study and limits the generalizability of our findings. Women undergoing a hysterectomy usually have more advanced stages of adenomyosis or other gynecological conditions, like fibroids or endometriosis. They are consequently more likely to represent cases that do not respond to medical or interventional treatment, which could also pose a selection bias.

The lack of alternative treatment options to hysterectomy for women with pelvic pain introduces another selection bias. In general, the main benign indications for hysterectomy are heavy menstrual bleeding, fibroids or pain (or a combination of those). At our institution, when medical treatment fails, we would usually treat women suffering from heavy menstrual bleeding with transcervical endometrial resection and women with symptomatic fibroids with

transcervical resection of the fibroid (if submucuous), laparoscopic myomectomy or laparoscopic hysterectomy. However, we do not have good alternative treatment options for women suffering from adenomyosis. This probably gives a selection towards a higher number of women with adenomyosis in the hysterectomy group and hence a higher prevalence of the disease than in a normal population. The clinical profile of our study group is therefore not representative of a general population.

As we excluded women who used hormones or GnRHa, as those can alter both the JZ appearance and expression profiles, we introduced another selection bias. Many women suffering from menorrhagia and dysmenorrhea are treated with hormones, most commonly with combined oral contraceptives or Lng-IUD. Women with endometriosis that undergo hysterectomy often receive GnRHa preoperatively, and those patients were consequently not available for inclusion.

A strength of our study was the consecutive inclusion of the study participants. Also, all women were very positive to be included when asked, and with only two women rejecting inclusion (due to claustrophobia), we assume that refusal to participate in the study did not introduce a bias.

Another strength of our study is that we included women with fibroids of any size, as those commonly co-exist with adenomyosis and it reflects a clinical reality. The performance of the prediction model could have been exaggerated when excluding factors that create artifacts such as fibroids.

c. Questionnaire

We used a self-designed questionnaire to evaluate various symptoms and their frequency, as there is no validated tool for the evaluation of adenomyosis-associated symptoms. As this was not a central research question in our study, we assume that using standard tools, such as VNRS and Likert-scales, is methodologically satisfying. We also registered historical data, for example, complications in pregnancy and various surgical procedures, but due to the retrospective nature of this approach and the relatively low number of women included, it does not allow any conclusions on the association of adenomyosis with those events.

d. Histopathology

The histopathological examination of the hysterectomy specimen was performed very

thoroughly, with analysis of a high number of sections from the corpus uteri and a large number of whole-organ sections. Both false-negative and false-positive results are less likely to occur when a high number of microscopic section is taken from each uterus, as doubtable cases can be confirmed with several other sections. At the same time, the meticulous approach might have resulted in a higher sensitivity for adenomyosis, compared to the findings in a regular histopathological examination following hysterectomy, and hence a higher prevalence of adenomyosis. Consequently, our results might not be comparable to other studies that perform regular histopathological examinations that might only detect adenomyosis that affects large parts of the uterus. Furthermore, in our study, some cases showed a very limited amount of adenomyosis, and it is not clear to what extent those minor findings are clinically relevant.

e. Image interpretation

Only one reader performed the image interpretation of the ultrasound images. This might have introduced a bias in the analysis of the results, and might also exaggerate the performance of the prediction model.

In MRI, two readers read the images blinded, independently, and on different data sets. Reader 1 performed two reads on the images, with an extensive first read, containing a considerable number of features. Due to capacity problems, not all features could be evaluated in the second reading and we focused on the main outcomes, the JZ measures and the new classification of the JZ morphology. There was a time gap of at six months or more from the last included patient to the second reading, which is an accepted reading interval for radiologists. The excellent inter- and intra-observer correlation strengthen the reliability of our outcome.

f. Prediction model development

Diagnostic prediction models calculate the probability of a disease being present based on different features, also called predictors. Those can be imaging features, but also blood pressure values or other clinical information that is shown to be related to the disease of interest. Known prediction models in gynecology are the IOTA adnex model and the Bishop-score for evaluating the cervical ripening (172-174).

When a model is fitted on the dataset that is used to develop it, there is a risk of “overfitting,” meaning it might perform very well with the population of the study, but it might not work

equally well with another patient population. Creating universally valid and reliable prediction models that work for all kind of patient populations, demand not only a substantial number of included cases, but is also not feasible when having histopathology as an outcome measure. A way to avoid overfitting is to apply the "last shrinkage and selection operator," also called LASSO. This regression analysis method was described by Robert Tibshirani in 1996 (175) and selects and regulates those variables in a model, that seem to exaggerate the real effect. LASSO was applied in the development of our dataset.

Another challenge in developing prediction models is that of missing values. Some values might not be available, such as the evaluation of the uterine shape, when fibroids are present. In order to still get reliable probabilities, it is possible to "impute" those missing values. Based on the values that are present for the other cases in the dataset, the imputation formula will "guess" what the respective value would most likely be. This function is called k-nearest neighbor (K-NN) imputation, and various free imputation formulas and software are available.

To secure a valid functioning of the prediction model, it should be validated on a dataset that is independent of the dataset it was built on (ref. overfitting). If one has a large dataset, it is, for example, possible to split it randomly, develop the model with one half, and then validate it with the other half. We chose to use our whole dataset for development and to validate and adjust it in a second study. However, a simulation of a validation can be performed by a step called "leave-one-out cross-validation," that mimics an independent data set and we applied that to our model (176).

ETHICAL CONSIDERATIONS

With any clinical study, there is a risk that patients feel obligated to participate in a study when asked by the consulting physician, even if they might not feel comfortable with participation. We tried to prevent this conflict by ensuring the women that their choice to participate or not would not affect the treatment they receive. Furthermore by giving all women the possibility to withdraw easily from the trial by oral or written notice. In addition, all invitation to the study was performed in good time before the hysterectomy.

Another ethical problem might be that the primary investigator is tempted to recommend a hysterectomy, rather than conservative treatment options when consulting with the patients in order to include into the study. All patients were therefore asked to participate in the study after

all possible treatment options were presented, and the decision for hysterectomy was already made. In our study, we excluded a quite significant number of women because the primary investigator recommended a different treatment than hysterectomy, and we think this reflects our conscious approach to this problem. The data collected for this study was not considered ethically problematic by our group, as it contains information that is normally received during a regular gynecological consultation.

The result of our research poses another ethical dilemma that we often face in clinical practice. There are limited treatment options and a lack of understanding of the extent to which adenomyosis contributes to infertility. The knowledge of having a condition that might affect fertility, but not sure if or to which degree, and not having many valid treatment options, can inflict stress on a woman. Our research provides a diagnostic tool that diagnoses a disease, without providing a proper therapeutic algorithm or treatment options for all women. This dilemma is even more significant when the woman does not seek consultation for a problem associated with adenomyosis, and the condition is found coincidentally.

On the other hand, not to pursue the development of diagnostic tools because the clinical consequence is limited is also ethically doubtful. Today's doctor-patient relationship is based on that "patient participation in decision-making is justified on humane grounds alone" (177), which also implies that the patient should have the possibility to get all possible information. In Norway, the patient`s right on health-related information is statutory and regulated in § 3-2 of the law on patient and user rights (Lov om pasient- og brukerrettigheter). The law specifically states that information has to be given in a balanced and appropriate manner, something that is considered to be good practice. When diagnosing adenomyosis in younger patients, it is essential to communicate the diagnosis, it`s significance, and implications in a very balanced manner and be aware that it can inflict stress. Using a diagnostic algorithm like ours demands therefore that the consultant is aware and open about its limitations.

A further ethical aspect of this research is the possible harm of the biopsy taking. This was discussed extensively within the group, before and during the study. Given that biopsies of the pelvic organs are taken routinely by gynecologists, without good and structured documentation on safety collected in prospective studies, we found that the setting of our study with its safety precautions was a reasonable approach. The primary investigator had experience in both oocyte pickup and other transvaginal surgery so that the procedure as performed in the study was considered to be responsible.

CONCLUSIONS AND PERSPECTIVES

1. Conclusions

- The prediction model for diagnosing adenomyosis with ultrasound appears to be a robust and useful tool for clinicians who have to interpret the very heterogeneous appearance of adenomyosis.
- It is necessary to validate the prediction model before releasing it for clinical use.
- Measurements of the JZ are of limited value, both in TVUS and MRI. They should be used with caution and preferably only together with other signs to diagnose adenomyosis.
- There is a need for an international consensus of the detailed definition for measuring the JZ, both in MRI and TVUS
- The JZ is better depicted in MRI than 2D and 3D ultrasound, but this does not affect the overall diagnostic performance, and both modalities have comparable diagnostic results.
- Molecular investigation of adenomyosis and the JZ are possible to perform without the need of a hysterectomy, using uterine biopsies obtained in vivo.

2. Perspectives

- The diagnostic prediction model needs to be validated, preferably in a multi-center study.
- In order to find the true association of adenomyosis with infertility and pregnancy/birth-related complications, a prospective study with a large cohort should be performed. Such a study would also provide information on the natural history of adenomyosis, early diagnostic signs of adenomyosis and their relevance.
- A detailed histopathological confirmation of changes in the JZ found in MRI and TVUS, and the detailed comparison of findings between MRI and ultrasound should be performed to strengthen the reliability of various signs.
- Molecular investigations on adenomyosis tissue and endometrial biopsies could reveal potential therapeutic targets and should be pursued. This could also lead to the development of biomarkers and the possibility for a non-histologic diagnosis.

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I

ORIGINAL ARTICLE: GYNECOLOGY

Development of a clinical prediction model for diagnosing adenomyosis

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Objective: To develop a multivariate prediction model for diagnosing adenomyosis using predictors available through transvaginal ultrasonography and clinical examinations.
Design: Prospective observational single-center study.
Setting: Teaching university hospital.
Patient(s): One hundred consecutively enrolled premenopausal women aged 30–50 years, undergoing hysterectomy due to a benign condition and not using hormonal treatment.
Intervention(s): Preoperative 2-D and 3-D transvaginal ultrasonography investigations were performed, and the results were documented in a standardized form. Clinical information was collected using a questionnaire. Histopathology confirmed the outcome.
Main Outcome Measure(s): Diagnostic performance (sensitivity, specificity, area under the curve (AUC)) of a multivariate prediction model for adenomyosis. Independent diagnostic performance of single predictors and their quantitative effect (β) in the final model.
Result(s): The final model showed a good test quality (area under the curve [AUC] = 0.86, [95% confidence interval = 0.79–0.94], optimal cutoff 0.56, sensitivity of 85%, specificity 78%). The following nine predictors were included ([sensitivity, specificity, β] or [AUC, β]): presence of myometrial cysts (51%, 86%, β = 0.86), fan-shaped echo (36%, 92%, β = 0.54), hyperechoic islets (51%, 78%, β = 0.62), globular uterus (61%, 83%, β = 0.2), normal uterine shape (83%, 61%, β = −0.75), thickest/thinnest ratio for uterine wall (0.61, β = 0.26), maximum width of the junctional zone in sagittal plane (0.71, β = 0.1), regular appearance of junctional zone (31%, 92%, β = −1.0), and grade of dysmenorrhea measured on a verbal numerical rating scale (0.61, β = 0.08).
Conclusion(s): We have presented a multivariate model for diagnosing adenomyosis that weights predictors based on their diagnostic significance. The reported findings could aid clinicians who are interpreting the heterogeneous appearance of adenomyosis in ultrasonography.
Clinical trial registration number: NCT02201719. (Fertil Steril® 2018;110:957–64. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Junctional zone, prediction model, adenomyosis, 3-D ultrasound

Adenomyosis is a common condition that affects about 20% of women in a typical gynecological population (1, 2). The prevalence of adenomyosis is reportedly higher among women seeking assisted reproduction, at 30% to 40%, and adenomyosis has a detrimental effect on the outcome of in vitro fertilization (3, 4). Adenomyosis causes pain, menorrhagia and genitourinary symptoms, and it affects fertility (5). The symptoms of adenomyosis overlap those of other common gynecological conditions such as fibroids and endometriosis, but their treatments differ (6). Early diagnosis and treatment in women suffering from adenomyosis can help to preserve their fertility by interrupting the vicious circle of tissue injury and repair that drives the development of adenomyosis (7–9). It is therefore imperative to diagnose adenomyosis correctly and choose appropriate treatment options.

Advances in ultrasonography have made it possible to accurately diagnose adenomyosis using transvaginal ultrasonography (TVUS), which has led to descriptions of multiple sonographic features of adenomyosis (10, 11). A common challenge for clinicians is to interpret those findings properly, since they show great variation in specificity and many of them depend on subjective pattern recognition rather than on variables that can be

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measured objectively. Also, the most-specific TVUS findings are usually those that are less prevalent, thus resulting in a lower sensitivity. The diagnosis of adenomyosis based on ultrasonography will therefore involve the evaluation of many different predictors while simultaneously weighting their individual significances.

Combining clinical symptoms and ultrasonographic features in a diagnostic algorithm could improve the diagnostic sensitivity without any clinically relevant loss of specificity. This has already been demonstrated for other conditions (12), but only individual sonographic markers have been described for adenomyosis. Some authors consider that at least two sonographic signs need to be present before diagnosing adenomyosis, but there is no consensus on how many markers should be found to ensure the optimal sensitivity and specificity when diagnosing adenomyosis (10, 13).

The aim of our study was to develop a diagnostic prediction model incorporating various elements available through clinical investigations and TVUS to predict the probability of adenomyosis in women presenting with dysmenorrhea and menorrhagia. Such a model would be a useful tool for gynecologists diagnosing adenomyosis and making therapeutic decisions.

METHODS

Source of Data

We used data collected for the Norwegian Adenomyosis Study, which is a prospective observational study evaluating diagnostic markers for adenomyosis amongst women referred for hysterectomy to the Department of Gynecology, Oslo University Hospital, Oslo, Norway. Subjects were recruited from September 2014 to August 2016. The trial was approved by the Institutional Review Board as well as the Regional Committee for Medical Research Ethics in Eastern and Southern Norway (approval number 2014/637), and the study was registered at clinicaltrials.gov (protocol number NCT02201719) before recruiting participants. None of the authors reported a conflict of interest for this study.

Study Participants

Premenopausal women aged 30–50 years who were not taking any hormonal contraceptives or receiving hormonal treatment or gonadotropin-releasing hormone analogues therapy or suffering from a malignant condition and needing a hysterectomy were eligible for inclusion in the study. We assessed their eligibility based on referral letters received from referring gynecologists, and those with no exclusion criteria were scheduled for a clinical examination performed by the first investigator (T.T.). When a hysterectomy was the concluded therapeutic advice and eligibility criteria were fulfilled, the woman was invited to participate in the study and written consent was obtained.

Transvaginal Ultrasonography and clinical Data

All study participants underwent a clinical and gynecological examination that included 2-D and 3-D TVUS (5–9 MHz endovaginal probe; Voluson S8, GE Kretz). The 2-D TVUS findings were documented in images and video recordings, while 3-D volumes were acquired and stored in a standardized manner as described previously (11, 13, 14).

The presence or absence of the following criteria for adenomyosis were evaluated: the uterine shape (globular or normal), asymmetry of anterior and posterior walls, and presence of myometrial alterations (hyperechoic islets, fan-shaped echo, subendometrial buds and lines, anechoic areas, and myometrial cysts) were assessed using 2-D TVUS; while the maximum and minimum widths of the junctional zone and the appearance of the JZ (categorized into regular, irregular, interrupted, irregular and interrupted, not visible and not assessable) in the sagittal and coronal planes were assessed using 3-D TVUS (Supplemental Figs. 1 and 2). The size and volume of the corpus uteri and the endometrial thickness were additionally documented. All images were obtained and assessed by the same experienced gynecologist (T.T.) who was blinded to the histopathological results.

The medical history, demographic data and information on symptoms were gathered using a questionnaire that was handed out to the study participants during the clinical consultation. The frequency of premenstrual pain, dysmenorrhea, dyspareunia, menorrhagia, urinary tract symptoms (irritation and pollakisuria), bulk-related symptoms and chronic pelvic pain were quantified using a 5-point Likert scale (15). The average peak intensity of symptoms experienced over the previous year was quantified using a verbal numerical rating scale (VNRS) ranging from 0 to 10 (16, 17).

The hysterectomies were performed according to the standard clinical practice in the department with regards to both method and indication. Only women with a uterus that did not require laparoscopic morcellation were included, and the presence and extent of endometriosis were registered perioperatively. A histopathology specimen was obtained after performing the hysterectomy. The sectioning and gross examination of the specimen was standardized and performed by laboratory staff or a pathologist together with the first investigator. The uterus specimens were cut axially into 5-mm-thick slices. Microscopic sections were obtained from macroscopically suspicious areas, areas where ultrasonography imaging had indicated signs of adenomyosis, and/or random sections from at least every second slice; this protocol maximized the diagnostic sensitivity (18). Two senior pathologists who were blinded to the ultrasonography results performed the microscopic histopathological analysis and made the final diagnosis. The presence of ectopic endometrial glands and stroma at 2.5mm below the endometrial-myometrial junction was defined as adenomyosis. Endometriosis was diagnosed if glands were found on the serosa of the uterus or immediately in a subserosal location and not deeper in the myometrium.

Development and Validation of the Prediction Model

A positive outcome was defined as a histopathological diagnosis of adenomyosis. We choose candidate predictors based on previous high-quality studies (19–23). In addition, we tested the significance of symptoms associated with

adenomyosis (e.g., dysmenorrhea, menorrhagia, and genitourinary symptoms) based both on our own clinical experience and on previous reports.

The required sample size was derived based on the 95% confidence interval (CI) for specificity and sensitivity. Using a CI of 95% with a width of 0.2 and a test sensitivity and specificity of 75%, the nomogram showed that at least 73 women were required. We therefore planned to include 100 women in order to increase the statistical power of our study (24).

Cases where hysterectomy was not performed, or a definitive histological diagnosis was not possible were excluded. Where nominal variables were used, the predictor was categorized into "present," "not present," or "not assessable." There were no missing data in any of the categories.

For scale variables that could not be evaluated due to artifacts or distortion of the uterus due to fibroids, we used the *k*-nearest-neighbor imputation function in the impute package of the R/Bioconductor library with default parameters (25). Missing values were replaced in order to avoid selection bias and statistically inaccurate results (26).

Predictors for the model were chosen using a three-step approach: In the first step, the study design included collecting all predictors that have been described in multiple high-quality studies, as described in the section entitled "Candidate predictor selection." In the second step we tested all candidate predictors individually. The proportions for categorical variables were compared with the chi-square test or Fisher's exact test. The sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) were calculated. Numerical variables were analyzed using Student's *t*-test or the Mann-Whitney *U*-test. Variables conforming to a normal distribution are reported as mean ± standard deviation values, while nonnormally distributed variables are reported as median and range values. The receiver operating characteristics (ROC) curve and the area under the ROC curve (AUC) were used to determine significant cutoffs for linear variables. Each cutoff value was then used to create two new categorical variables within that variable, and this was tested again with the chi-square test, and the sensitivity, specificity, accuracy, PPV, and NPV were calculated. The score on the VNRS was a continuous variable, in accordance with previous publications (27). A probability value of $P<.05$ was considered statistically significant.

The first and second steps yielded a list of variables that were individually associated with adenomyosis. In the third step, these variables were combined into a logistic regression model, where the β values quantify the effects of the variables on the probability of adenomyosis. In order to avoid overfitting due to random associations between the predictors and the outcome, we applied LASSO (least absolute shrinkage and selection operator) using the penalized R library (28, 29).

The three-step procedure described above predicted the probability of adenomyosis in each individual. The performance of the predictions was evaluated by performing ROC analysis (R package pROC and Youden index) to find the optimal threshold value based on the ROC curve (30, 31). A more-detailed description of the statistical procedure is provided in Supplemental Appendix. We performed leave-one-out cross-validation to mimic an independent data set (32).

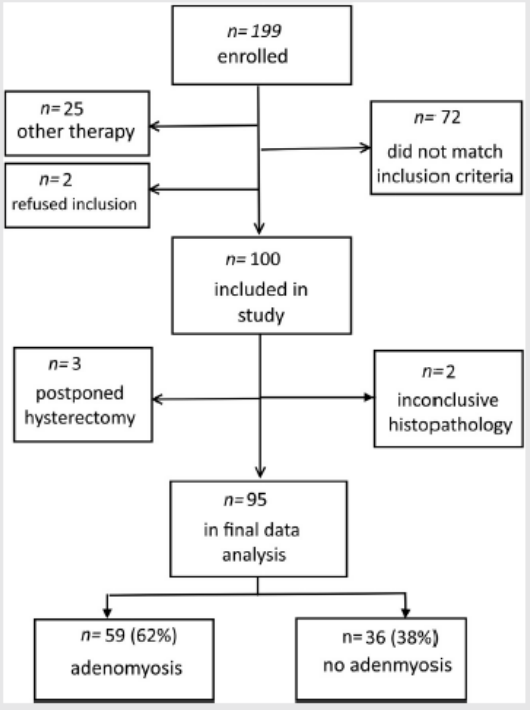
RESULTS

Figure 1 shows the flowchart for the women included in the study. The indications for hysterectomy were menorrhagia, dysmenorrhea, bulk-related symptoms, dyspareunia or pelvic pain. "Other therapy" included a levonorgestrel intrauterine device (n = 21), embolization (n = 2), or a need for laparoscopic subtotal hysterectomy with morcellation (n = 2). The main exclusion criteria were the use of hormone therapy, wanting laparoscopic subtotal hysterectomy, or not wanting any therapy. The clinical baseline characteristics were not statistically different in women with and without adenomyosis; the only exception was that the women with adenomyosis were older (Table 1).

The prevalence of fibroids did not differ significant between the adenomyosis and control groups (33 [56%] vs. 18 [50%], $P=.57$), whereas endometriosis was significantly more common in women with adenomyosis (28 [48%] vs. 8 [22%], $P=.01$)]. The extent of endometriosis, if present, did not differ significantly with the exception of peritoneal endometriosis being more common in the adenomyosis group (15 [25%] vs. 1 [3%], $P<.01$].

For the histopathological examinations, 8.6 ± 2.6 microscopic sections were taken from the corpus uteri, with equal numbers in the adenomyosis and control groups (8.6 vs. 8.7, $P=.68$). The final diagnosis could not be made

FIGURE 1



Study flowchart.

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

TABLE 1

Clinical characteristics of the study population.

Characteristic	Adenomyosis (n = 59)	No adenomyosis (n = 36)	*P* value
Age (y), mean ± SD	43.5 ± 4.9	41.2 ± 4.2	.01[a]
Pregnancies (n), median (range)	3 (0–9)	2 (0–5)	.16
BMI (kg/m^2), mean (95% CI)	25.9 (16–44)	25.6 (19–34)	.73
Parity, mean ± SD	1.4 ± 1.4	1.5 ± 1.2	.66
Previous curettage, n (%)	29 (49)	9 (25)	.02[a]

Note: BMI = body mass index; CI = confidence interval; SD = standard deviation.
[a] Statistically significant difference ($P < .05$).

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

in two cases due to uncertainty if the ectopic endometrial glands seen in one slide were tangential sections of a deeper layer of the uterine cavity or possibly represented a fallopian tube.

Model Development

Table 2 presents the unadjusted relationships of the candidate predictors with the outcome, as well as the number of outcome events. Based on these results, the following 13 parameters were tested for inclusion in the prediction model: presence of a globular enlarged uterus, myometrial cysts, fan-shaped echo, wall size, wall asymmetry (expressed by the ratio of the thickest/thinnest wall), hyperechoic islets, maximum width of the junctional zone in the sagittal plane, a regular, irregular, or interrupted appearance of the junctional zone, VNRS score for dysmenorrhea, and frequency of urinary symptoms. The following predictors were obligatory because they are extensively described in the literature, even though they did not perform well for our data set: globular uterus, myometrial cysts, fan-shaped echo, and asymmetrical walls. We additionally chose predictors that were highly significant in our own data, including those that have not been described previously. LASSO analysis using these 13 variables yielded 9 variables and (unstandardized) β values (Table 2). The β intercept value was −1.11.

The ROC curve is illustrated in Figure 2. The AUC of this model was 0.86 (95% confidence interval [CI] 0.79–0.94). The optimal cutoff for predicting the probability of adenomyosis was 0.56, which gave a sensitivity of 85% and a specificity of 78% (Fig. 2). The leave-one-out cross-validated AUC was 0.75 (95% CI 0.79–0.94). We tested how the model would perform in subgroups with and without the presence of fibroids, and found no significant difference, with AUCs of 0.78 (95% CI 0.65–0.94) versus 0.92 (95% CI 0.85–1.0) (P=.14). Supplemental Figure 3 illustrates a proposed interpretation of the calculated probabilities in clinical useful categories.

DISCUSSION

We have presented a clinical diagnostic prediction model that calculates the probability of adenomyosis being present in a population of women suffering from menorrhagia and dysmenorrhea. The prediction model comprises nine predictors and showed a sensitivity of 85% and a specificity of 78%. The test quality of our model is very good, with an AUC of 0.86. A recent meta-analysis found pooled sensitivity and specificity values of 83% and 64%, respectively (10)—our model exhibits comparable sensitivity but improved specificity. Although the test quality of single diagnostic parameters was consistent with previous findings, the improved specificity might reflect greater robustness of the model, in terms of not being easily affected by patient or examiner factors.

There is no consensus on which and how many imaging criteria should be used for the nonhistological confirmation of adenomyosis. For example, some authors have used only one sign while others used a minimum of two signs, which could explain the variations in diagnostic accuracy in previous studies (19, 22, 33–35). The weakness of this approach is that all signs will be weighted equally, including those that do not have the same diagnostic relevance. Applying a two-sign rule as proposed by other authors to our data set decreased the sensitivity to 71% and the specificity to 74%.

Another advantage in our proposed model is that negative predictive signs are also considered, which better reflects normal findings. Where only low-specificity positive predictors are present, this is very useful, and we think this contributes to the superior specificity of our model.

Another indication of a greater robustness of the present model is that it works equally well among groups of women with and without fibroids. Some of the previous studies applied inclusion criteria that restricted the number and size of fibroids, since they create sonographic artifacts (19, 22). We did not exclude women with fibroids, as they commonly co-existent with adenomyosis and we aimed to reflect clinical reality as best as possible in the study group.

The inclusion of as many measurable objective parameters as possible (in contrast to subjective pattern recognition), such as the ratio of thickest to the thinnest wall, instead of a subjective consideration of asymmetry, will theoretically help to make a model more robust against variations in the experience and skill of the examiners.

Weighting the importance and specificity of each single finding can be challenging for a clinician who has little experience of diagnosing women with adenomyosis, our model could be helpful to such inexperienced clinicians. The alternative approach of restricting an evaluation to only a handful of

TABLE 2

Unadjusted relationships between candidate predictors and the outcome, as well as the number of outcome events.

Predictor	Adenomyosis (n = 59), n (%)	No adenomyosis (n = 36), n (%)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)	*P* value	β
Hyperechoic islets	30 (51)	8 (22)	51 (38–64)	78 (61–90)	79 (66–88)	49 (42–57)	61 (51–71)	.01	0.62
Fan-shaped echo	21 (35)	3 (8)	36 (24–49)	92 (78–98)	88 (69–96)	47 (42–57)	57 (46–67)	<.001	0.54
Subendometrial buds	7 (12)	4 (11)	12 (5–23)	89 (74–97)	64 (36–85)	38 (35–42)	41 (31–52)	.91	
Internal shadows	6 (10)	1 (3)	10 (4–21)	97 (86–100)	86 (43–98)	40 (37–42)	43 (33–54)	.18	
Myometrial cysts	30 (51)	5 (14)	51 (38–64)	86 (71–95)	86 (72–93)	75 (44–59)	64 (54–74)	<.001	0.86
Myometrial alterations overall (≥ 1)	47 (80)	15 (42)	78 (67–89)	58 (41–75)	76 (68–83)	64 (50–76)	72 (61–80)	<.001	
Globular formation	30 (51); $n_A = 49$[a]	5 (14); $n_{NA} = 30$[a]	61 (46–75)	83 (65–94)	86 (72–93)	57 (47–65)	70 (58–80)	<.001	0.20
No globular formation[b]	19 (32); $n_A = 30$[a]	25 (69); $n_{NA} = 30$[a]	83 (65–94)	61 (46–75)	57 (47–66)	86 (72–93)	70 (58–80)	<.001	−0.75
Wall asymmetry, thickest/thinnest (mm), mean ± SD	1.52 ± 0.67; $n_A = 47$[a]	1.27 ± 0.22; $n_{NA} = 31$[a]	*P*=.02			AUC = 0.61 (0.48–0.73, *P*=.11)			0.26
Wall asymmetry, thickest/thinnest ratio ≥1.5 (mm), mean ± SD	20 (43); $n_A = 47$[a]	5 (16); $n_{NA} = 31$[a]	43 (28–58)	84 (66–95)	80 (63–91)	49 (42–56)		.01	
Thickest wall (mm), mean ± SD	28.2 ± 15.6; $n_A = 49$[a]	20.5 ± 5.5; $n_{NA} = 32$[a]	*P*=.01			AUC = 0.67 (0.56–0.79, *P*=.01)			
Any wall ≥25.5 (mm)	17 (29); $n_A = 49$[a]	6 (17); $n_{NA} = 32$[a]	35 (22–50)	81 (64–93)	74 (56–87)	45 (38–51)	53 (42–64)	.12	
Uterine volume (ml), mean ± SD	149 ± 204; $n_A = 50$[a]	74 ± 37; $n_{NA} = 29$[a]	*P*=.02			AUC = 0.61 (0.49–0.74, *P*=.96)			
Irregular or interrupted JZ	32 (54); $n_A = 51$[a]	22 (61); $n_{NA} = 35$[a]	53 (41–67)	39 (23–57)	64 (51–67)	39 (24–46)	48 (38–59)	.74	
Regular JZ[b]	4 (8); $n_A = 51$[a]	11 (31); $n_{NA} = 35$[a]	31 (17–49)	92 (81–98)	73 (49–89)	66 (61–71)	67 (56–77)	.01	−1.0
Maximum JZ width in sagittal plane	6.2 (2–11); $n_A = 19$[a]	4.0 (1.3–9.7); $n_{NA} = 23$[a]				AUC = 0.71 (0.55–0.87, *P*=.02)			0.10
Maximum JZ width in sagittal plane ≥5.1 mm	11 (19); $n_A = 19$[a]	5 (14); $n_{NA} = 23$[a]	58 (34–80)	78 (56–93)	69 (48–84)	69 (56–80)	69 (53–82)	.02	
Maximum JZ width in coronal plane, mean ± SD	5.1 ± 1.7; $n_A = 35$[a]	4.3 ± 2.1; $n_{NA} = 30$[a]	*P*=.09			AUC = 0.63 (0.49–0.77, *P*=.07)			
Dysmenorrhea VNRS score, mean ± SD	7.7 ± 2.3	6.4 ± 3.2	*P*=.03			AUC = 0.61 (0.5–0.73, *P*=.06)			
Dysmenorrhea, VNRS score ≥8	39 (66)	16 (44)	66 (53–78)	56 (38–72)	71 (62–79)	50 (39–61)	62 (52–72)	.04	0.08
Always genitourinary irritation, 5 points on Likert scale	17 (29)	4 (11)	29 (18–42)	89 (75–97)	81 (61–92)	43 (38–48)	52 (41–62)	.04	
Two-sign rule[c]	42 (86)	9 (39)	71 (58–82)	74 (56–87)	82 (72–89)	60 (48–70)	72 (62–81)	.00	
Two-sign rule[c] extended with dysmenorrhea VNRS score >8	52 (88)	20 (56)	88 (77–95)	44 (28–62)	72 (66–78)	70 (51–84)	72 (61–80)	.00	

Note: AUC = area under the curve; CI = confidence interval; JZ = junctional zone; NPV = negative predictive value; PPV = positive predictive value; SD = standard deviation; VNRS = verbal numerical rating scale.

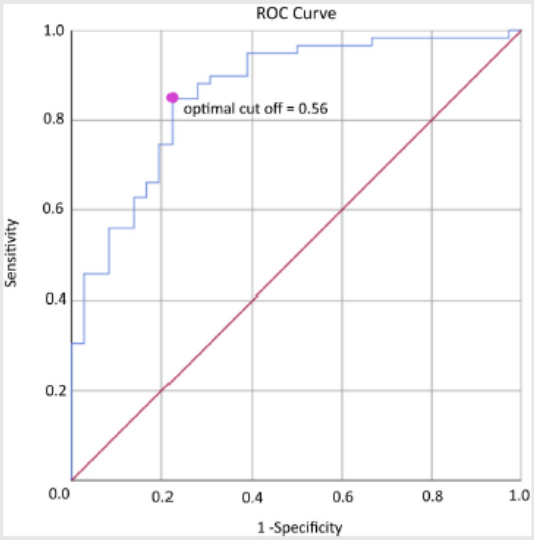
[a] *n* differs from the total when predictors were not assessable in all individuals. The available number of outcomes is then indicated as "n_A" for number within group with, and "n_{NA}" within group without adenomyosis. Percentage values are relative to the total.

[b] Diagnostic value for not having adenomyosis displayed.

[c] Performance of ultrasonography when using a simple rule consisting at least two out of the following signs: globular uterus, irregular or interrupted junctional zone (in at least one plane), presence of myometrial cysts, presence of echogenic striations, asymmetry (expressed by thickest/thinnest uterine wall ratio ≥1.5), and wall thickness >25 mm (at least one wall).

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

FIGURE 2



Receiver operating curve of the prediction model. The optimal cutoff for predicting the probability of adenomyosis was 0.56, for which the sensitivity was 85% and the specificity was 78%.

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

easy-to-assess signs, to avoid a complicated assessment, would be problematic given that adenomyosis shows great heterogeneity in ultrasonography findings and the need to always consider multiple parameters.

Our study was subject to some limitations. Firstly, the model still must be validated using an independent data set and preferably also with different study populations. This might lead to adjustments both to the selection of predictors and their β values. However, we did internally cross-validate the model to mimic the performance when using an independent data set, which produced satisfying and promising results.

Secondly, it is likely that selection bias was present. To obtain histopathological confirmation of adenomyosis, we included women requiring hysterectomy, and it could be argued that this population presents with more-severe or more-advanced disease. Differences in their diagnostic appearance relative to other patient populations could compromise the generalizability of the study. However, histopathology still represents the gold standard for a diagnosis of adenomyosis, and so hysterectomy with histopathology was necessary to confirm the outcome when developing a model such as the present one. We attempted to mitigate the possible effect of such selection bias by using predictors that we assumed are relevant to earlier stages of the disease, such as the appearance of the junctional zone, which is hypothesized to show early structural changes induced by adenomyosis (36). Another type of selection bias could derive from the exclusion of women receiving hormonal treatment. Hormones can reportedly change the appearance of the junctional zone and their inclusion might therefore have affected the model performance (37, 38). Thirdly, the obtained imaging data were assessed by a single experienced examiner, which might have exaggerated the performance of the model.

Clinical Implications

Since our model has not been externally validated, we suggest that in its present form it should only be used for research purposes. In the future the model might be useful in clinical settings where therapeutic choices will be influenced by the presence of adenomyosis, for example when choosing a protocol in artificial reproductive technology or deciding about interventions such as hysterectomy versus transcervical resection of the endometrium or endometrial ablation. Therapeutic interventions should not only depend on the presence or absence of adenomyosis, but also on presented clinical challenges such as infertility, menorrhagia, or pain, as well as the woman's individual treatment preferences.

We included one clinical symptom into the model, as women with adenomyosis show a characteristic clinical profile (39). Especially where the model is less accurate (probability of 40%–79%) the medical history of the patient and use of a standardized questionnaire in addition to TVUS can be very useful in order to strengthen the accuracy of the diagnosis (39, 40).

Our model might also be useful in clinical studies where the gold standard for a diagnosis of adenomyosis (histopathology) is not available. However, it remains necessary to achieve a consensus on the nonhistological confirmation of an adenomyosis diagnosis.

CONCLUSION

We have developed a clinical prediction model for diagnosing adenomyosis based on a prospective observational study. The model shows a good test quality (AUC=0.86) and a high sensitivity (85%) and specificity (78%), but still requires validation.

ADDITIONAL RESOURCES

We have constructed a free app for mobile devices called "Adenomyosis Calculator" that could be used by clinicians. We plan to publicly release this app once the model has been validated.

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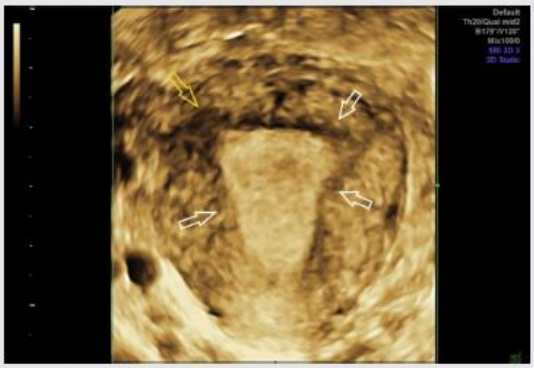
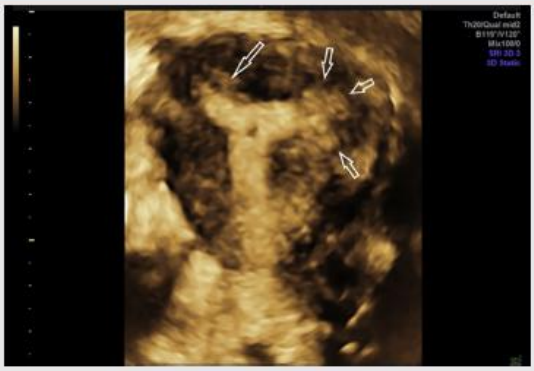
SUPPLEMENTAL FIGURE 1



Coronal plane of the uterus, render mode. The *arrows* indicate the outer boarder of a regular junctional zone (JZ), surrounding the endometrial cavity. The *yellow arrow marks* an area where the JZ could seem to appear widened or irregular with variable echogenicity, but this is a common artifact in the isthmus of the fallopian tube and careful evaluation of the whole 3-D volume is recommended.

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

SUPPLEMENTAL FIGURE 2



Coronal plane of the uterus, render mode. The junctional zone (JZ) is irregular in width and *arrows* indicate hyperechoic areas that represent adenomyotic invasion through the JZ (arrows; histologic correlation confirmed). An evaluation of the whole 3-D volume will reveal the total extend of the invasion.

Tellum. Prediction model for adenomyosis. Fertil Steril 2018.

II

Diagnosing adenomyosis with MRI: a prospective study revisiting the junctional zone thickness cutoff of 12 mm as a diagnostic marker

ABSTRACT

Objectives: To assess the diagnostic accuracy of a junctional zone (JZ) thickness of ≥12 mm and morphological features of the JZ in MRI in diagnosing adenomyosis in a premenopausal study population.

Methods: This single-center, prospective observational study consecutively enrolled 93 premenopausal women suffering from a benign gynecological condition, from September 2014 to August 2016. Institutional review board approval and written consent were obtained. All participants underwent MRI and hysterectomy with a histopathological examination. MR images were evaluated in a blinded fashion by two independent readers. The maximum junctional zone thickness (JZ_{max}), presence of JZ_{max} ≥12 mm, and any irregular appearance of the JZ (defined as irregular outer or inner borders, focal thickening, presence of high-intensity signal foci or fingerlike indentations at the inner border) was documented, and the diagnostic performance was evaluated with the AUC, chi-square test and multiple regression.

Results: Adenomyosis was histopathologically confirmed in 57 (61%) of the women. JZ_{max} was not positively correlated with adenomyosis diagnosis (AUC=0.57, *P*=0.26) and did not differ significantly between those with and without adenomyosis (10.3 vs 10.1 mm, *P*=0.88), nor was a cutoff of JZ_{max} ≥12 mm [*n*=30/57 (53%) vs *n*=16/36 (44%), *P*=.29]. The presence of an irregular JZ showed the best association with adenomyosis among the evaluated signs [sensitivity 74% (95% CI: 60, 85); specificity 83% (95% CI: 67, 94) (*P*<0.001)].

Conclusions: JZ_{max} was not correlated with adenomyosis in the present study population, but direct signs of adenomyosis such as irregularities of the JZ provided a good diagnostic accuracy.

Key Points:

- Measuring the junctional zone thickness is of limited value for diagnosing adenomyosis with MRI and should not be used for diagnosing adenomyosis in premenopausal women with moderate disease severity.
- An irregular appearance of the junctional zone, the presence of myometrial cysts and adenomyoma appears to provide the highest specificity for diagnosing adenomyosis.
- A consensus for the definition and reading of the junctional zone is needed.

Key words (MeSH): adenomyosis; magnetic resonance imaging; hysterectomy; prospective studies; Genital Diseases, Female

Abbreviations:

JZ	junctional zone
MRI	magnetic resonance imaging
T1W	T1-weighted
T2W	T2-weighted
TSE	turbo spin echo
R1	Reader 1
R2	Reader 2
AUC	area under the receiver operating characteristics curve
ROC	receiver operating characteristics
PPV	positive predictive value
NPV	negative predictive value
ICC	intraclass correlation coefficient

Introduction

Adenomyosis is a common condition whose prevalence is described to be about 20% amongst women attending a general gynecological clinic [1]. It is defined by the presence of ectopic endometrial tissue located in the muscular wall of the uterus [2]. The predominant symptoms of adenomyosis are severe dysmenorrhea and heavy menstrual bleeding, which cause concomitant disease such as anemia, and reduce the quality of life [3; 4]. Adenomyosis has been receiving more attention from clinicians due to several recent studies showing the wider implications of this condition. They could establish the negative impact of adenomyosis on fertility, as well as its relationship to complications in pregnancy and during labor, such as having preeclampsia and small-for-gestational-age children [5]. In contrast to adenomyosis previously being characterized as a disease of older and multiparous woman, it is now also described in younger women and

even in adolescence, which indicates the importance of diagnosing this condition early [1; 6]. For decades, hysterectomy was regarded as the only treatment of adenomyosis, but several new treatment options are now available, including different hormonal treatments, high-intensity focused ultrasound, and uterine artery embolization, and it is therefore important that a correct diagnosis is established [7; 8].

Magnetic resonance imaging (MRI) and transvaginal ultrasound play the most important roles in diagnosing adenomyosis, and various imaging features of adenomyosis have been described, such as increased thickness of the junctional zone (JZ), ill-defined areas of low signal intensity or bright foci on T2-weighted images, which represent foci of heterotopic endometrial tissue [9]. A cutoff of ≥12 mm for the JZ thickness has been previously described as a key marker of adenomyosis [10-12]. Although adenomyosis is a frequent clinical challenge, only three studies have investigated the diagnostic accuracy of MRI compared to the gold standard which is histopathology; those studies were performed 17-22 years ago [10-12]. Over the last decades there has been a continuous evolvement of MRI techniques on the female pelvis resulting in faster acquisition with fewer artifacts and higher image resolution. The main objective of the present study was therefore to prospectively determine the diagnostic accuracy of the JZ thickness in a premenopausal study population, along with other diagnostic markers as secondary objectives, using MRI.

Material and methods

This prospective observational study was approved by the institutional review board and the regional committee for medical research ethics and is registered at www.clinicaltrials.gov. All study participants provided written consent prior to their inclusion.

Study participants

Women that were referred to the Department of Gynecology due to a benign condition requiring hysterectomy (symptomatic fibroids, heavy menstrual bleeding, pain, or a combination of these) were consecutively enrolled in the study. All women were examined clinically for inclusion by the first investigator (T.T.) from September 2014 to August 2016 and a transvaginal ultrasound was performed. The results from the ultrasound examination are reported elsewhere. Inclusion criteria were being aged 30–50 years, having a

benign condition, and hysterectomy being recommended as the appropriate treatment by a gynecologist. Exclusion criteria were presence of malignancy, use of any hormonal medication 3 month prior to the ultrasound examination and hysterectomy, or the need to morcellate the uterus during the hysterectomy. Figure 1 shows the study flowchart. The baseline characteristics did not differ significantly between the two study groups, except the mean age being higher in the group with adenomyosis (Table 1). The indications for hysterectomy were the following for women with and without adenomyosis, and more than one indication was often present at the same time: chronic pelvic pain 25(44%) and 16(44%), dysmenorrhea 46(81%) and 22(61%), bulk-related symptoms 8(14%) and 16 (50%) and heavy menstrual bleeding 46 (81%) and 23 (64%). "Other therapy" included a levonorgestrel intrauterine device (*n*=21), embolization (*n*=2), or a need for laparoscopic subtotal hysterectomy with morcellation (*n*=2). The main exclusion criteria were the use of hormone therapy, wanting laparoscopic subtotal hysterectomy, or not wanting any therapy.

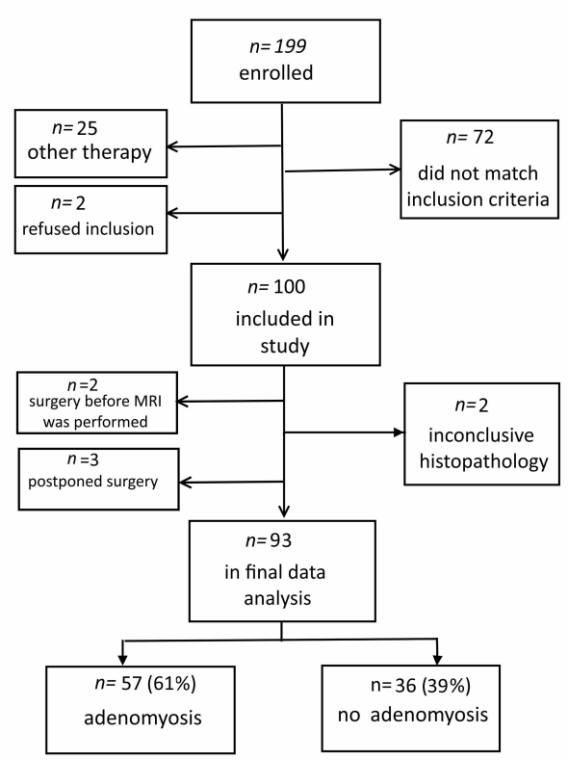


Figure 1: *Study flowchart.* MRI, magnetic resonance imaging.

Magnetic resonance imaging

MRI was performed with a 3-tesla (T) Philips Ingenia with dStream anterior and posterior coils, or 1.5-T Philips Achiva device with a 32-channel cardiac coil (Philips Medical Systems). On the 3.0-T system T2 weighted (T2W) turbo spin echo (TSE) images were acquired in the sagittal plane, oblique axial plane perpendicular to the long axis of the uterine cavity and oblique coronal plane parallel to the long axis of the uterine cavity. T1 weighted (T1W) TSE and T1W fat-suppressed images were acquired in the oblique axial plane. On the 1.5 T system 3D balanced turbo field echo and 3D T2W were acquired in the axial plane with sagittal and coronal reformates, T1W TSE with and without fat suppression and T2W TSE in the oblique axial plane. The acquisition parameters are provided in Supplementary Table 1 (online). Examinations were performed regardless of the menstrual cycle phase. Patient preparation included fasting for 4 hours before the examination, voiding of the bladder, and administration of 20 mg of butylscopolamine (Buscopan, sanofi-aventis) intravenously and 1 mg of glucagon intramuscularly. In seven cases, the MRI had already been performed at another institution and the acquired images were retrieved and reassessed. When the quality was not satisfying, the MRI was performed again (two cases). The median time interval between the MRI was performed and the surgery was 41 days (range 1–308 days).

Image interpretation

All images were stored anonymously on the Syngo Imaging picture archiving and communication system (Siemens Healthcare). G.M. (Reader 1, R1), with 14 years of body-MRI experience and who was blinded for both the sonographic and histopathological data performed the reading of all images. All of the evaluated features are listed and defined in Table 2. We defined adenomyosis as being present if one or more of JZ_{max} ≥12 mm, myometrial cysts or adenomyoma which are comprehensively described elsewhere) were present, [10; 11; 13-16]. Other features that have been described less comprehensively previously were also documented, and tested for their diagnostic accuracy [11; 12]. One of the less described features is the morphological classification of the JZ that is introduced here. It is based on previously described features and modified for MRI (Figure 2) [11; 17].

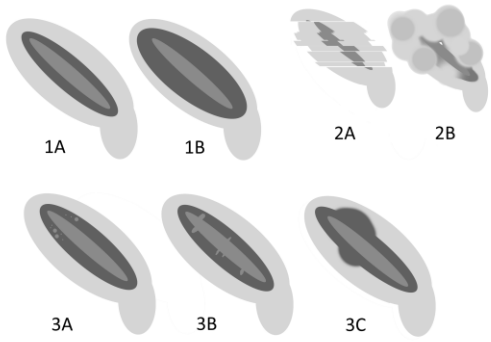


Figure 2: *Classification of the junctional zone (JZ)* **1: Normal JZ.** The inner and outer borders of the JZ are smooth and satisfyingly defined. 1A) thin JZ 1B) regularly enlarged JZ. **2: JZ not visible or not assessable.** 2A) Due to motion artifacts 2B) Due to fibroids or large areas of adenomyosis. **3: Irregular JZ** If one or multiple of the following findings are present, and not caused by fibroids: 3A) JZ shows disruption by high intensity foci (cysts) (3B) fingerlike indentations at the endometrial-myometrial junction (3C) focal thickening of the JZ, not representing a contraction.

There is no unanimous definition of the JZ in MRI and it is measured in different ways amongst radiologists and research groups. In order to reflect that variation, we therefore introduce different terms of JZ measurements (JZ_{max} and $JZ_{max\text{-}A}$), that reflect different measurement practices that we used in our clinical work and found in the literature [9; 18; 19]. Those are comprehensively explained in Table 2 and Figure 3. R1 repeated the reading of the predictors JZ_{max}, JZ_{min} and JZ_{diff} 6 month after the first reading, to enable testing of the intra-reader agreement of those signs and confirm the reliability of the results. A second reader (E.V., here R2, with 20 years of body MRI experience) also assessed the main outcomes (JZ_{max}, JZ_{min}, JZ_{diff}, $JZ_{max\text{-}A}$, morphological JZ classification) in order to allow the evaluation of the inter-reader agreement of those signs. The readings were performed independently on two different image sets, blinded to the clinical, sonographic and histopathological data.

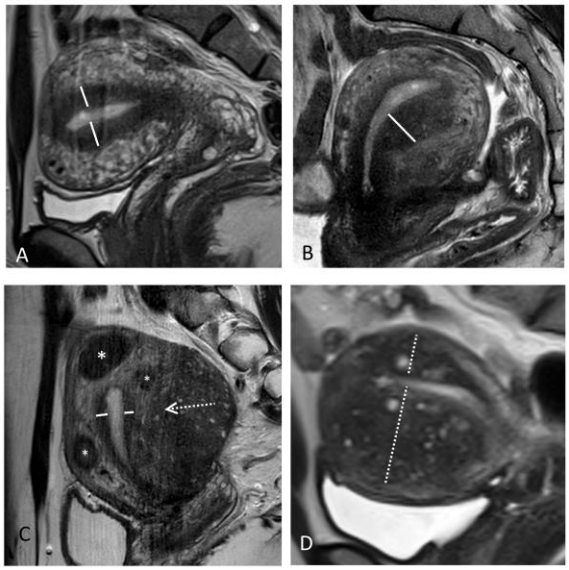


Figure 3: *Different methods used to define and measure the maximal thickness of the junctional zone (JZ).* Magnetic resonance images of the uterus, all with T2 weighted sequences and turbo spin echo. In our clinical practice and in this study, we defined the JZ thickness as in panels A and B (solid lines). In C, the JZ is thin and still visible, but an area of adenomyosis seems to grow towards the JZ (dotted arrow); * indicates fibroids. In D, the JZ is no visible anymore and seems to be replaced by adenomyosis. Some authors interpret the whole area as an enlarged JZ (dotted line) and measure it accordingly. We introduce the term "$JZ_{max\text{-}A}$" to discriminate this way of measuring from our definition of the JZ (A, B).

Reference standard

A positive outcome was defined as histopathologically confirmed adenomyosis. The pathological examination was performed in a standardized manner, cutting the fixated uterus in axial sections of 5-10 mm thick slices. Microscopic sections were obtained based on instructions from the first investigator, covering areas of the hysterectomy specimen that appeared suspicious in the gross examination, where MRI had shown signs of adenomyosis, and/or randomly from at least every second slice in order to include all areas of the corpus [2]. The pathologist, had no access to the imaging data. Two senior pathologists performed the microscopic histopathological analysis and made the final diagnosis. The presence of ectopic endometrial glands and stroma at 2.5 mm below the endometrial-myometrial junction was defined as adenomyosis [20].

Sample size and statistical analysis

The required sample size was derived based on the concept for range of confidence interval (CI) for specificity and sensitivity for the main predictor [maximum junctional zone thickness (JZ_{max}) ≥12 mm]. Using

a CI of 95% with a width of 0.2 and a test sensitivity and specificity of 75%, the nomogram showed that at least 73 study participants were required [21].

We used the Shapiro-Wilk test to test normality of our samples. The proportions for categorical variables were compared with the chi-square test or Fisher's exact test. The sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) were calculated. Numerical variables were analyzed using Student's *t*-test or the Mann-Whitney *U*-test. The receiver operating characteristics (ROC) curve and the area under the receiver operating characteristics curve (AUC) were used to identify linear variables that were significantly associated with the analyzed outcome. Multivariate linear regression was used to identify independent imaging predictors of adenomyosis. The simple pairwise Cohen κ statistic was used to measure the inter-reader agreement for categorical response imaging features, whereas the intraclass correlation coefficient (ICC) was used to assess the level of agreement for numerical response features. The κ and ICC values were categorized as follows: 0–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1, almost perfect agreement [22]. Statistical analysis was performed using IBM SPSS Statistics (version 25, IBM Corporation), and a probability value of $P \leq 0.05$ was considered statistically significant.

Results

JZ_{max} ≥12 mm was not significantly associated with having adenomyosis, and the frequency of a JZ_{max}≥12mm was similar in the groups with and without adenomyosis [*n*=30/57 (53%) vs *n*=16/36 (44%), *P*=0.29]. This was the case for both readers and each reading (individual results in table 3 and 4). Myometrial cysts and adenomyoma were the signs with the highest specificities, the detailed results of their diagnostic performance are listed in Table 3.

Combining the primary diagnostic markers $JZ_{max} \geq 12$ mm, myometrial cysts and adenomyoma, resulted in 41/57 (72%) true-positive, 12/36 (33%) false-positive, 19/36 (53%) true-negative, and 13/57 (23%) false-negative cases, and 8/93 (9%) cases being undetermined. The combined test quality when accounting for the undetermined cases as being respectively positive or negative could be quantified as follows (with 95% CI values in brackets): sensitivity of 77% (64, 87%) and 72% (59, 83%), specificity of 53% (36, 70%) and 67% (49,

81%), PPV of 72% (64, 79%) and 77% (68, 81%), NPV of 59% (45, 72%) and 60% (48, 70%), and accuracy of 68% (57, 77%) and 70% (60, 79%) (all *P*<0.001).

The criterion of JZ_{max} ≥12 mm was present in all false-positive cases, and in 7/12 (58%) as the sole predictor. Figure 4 illustrates examples of false positive cases with JZ_{max} ≥12 mm. In 3/12 (25%) of the false-positive cases, a single myometrial cyst was seen and interpreted as adenomyosis, and in 2/12 (17%) of the false-positive cases, fibroids with diffuse borders were interpreted as adenomyoma. Six of the seven false-negative cases (86%) showed JZ_{max} <12 mm as a predictor. JZ_{max} was not correlated with the diagnosis of adenomyosis (AUC=0.6, 95% CI: 0.48, 0.72, *P*=0.11). JZ_{diff} showed an almost statistically significant association in this reading (AUC=0.62, 95% CI: 0.50, 0.74, *P*=0.06). With a cutoff of JZ_{diff} ≥5.5 mm, a sensitivity of 53% and specificity of 75% was reached when using this as a categorical variable. We found a weak correlation with a positive outcome for the JZ_{max-A} and adenomyosis (AUC=0.68, 95% CI=0.57, 0.80; *P*<0.001). The JZ-to-myometrial thickness ratio was not statistically significant associated with adenomyosis (AUC=0.54, 95% CI: 0.40, 0.69%, *P*=0.54). The diagnostic performance of the other documented features is presented in Table 3, and the number and size of fibroids are presented in Table 1.

JZ morphology

The presence of an interrupted and/or irregular JZ was strongly correlated with having adenomyosis, while a regular JZ was strongly correlated with not having adenomyosis (both *P*<0.001, detailed diagnostic performance in Table 3). Figure 2 illustrates the categories of JZ, while Figure 4 and Figure 5 depict MR images of regular and irregular JZ. The JZ was sufficiently well depicted in 91/93 (98%) of cases; in the remaining 2 cases it was not assessable due to motion artifacts.

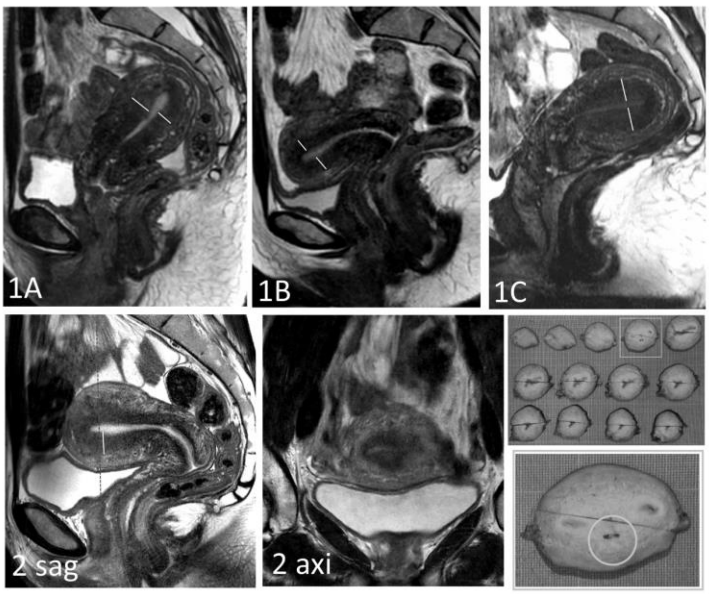


Figure 4: *False positive and negative cases with a regular junctional zone (JZ) and ≥12mm thickness.* All magnetic resonance images of the uterus with T2 weighted sequences and turbo spin echo. Presence of adenomyosis was determined by histopathology. Cases 1A-C are false positive when using a cut off of JZ≥ 12mm as diagnostic marker, but true negative with pattern recognition of the JZ morphology (regular JZ). Case 2 shown in all images below with a JZ of 11.5 mm, represents a false negative diagnosis. The JZ was interpreted as regular because irregularity in the axial plane was interpreted as a partial volume effect (image cross reference is indicated with the red, stippled line in the sagittal plane of the uterus). Histopathology showed adenomyosis in that area, which was also visible in the gross examination (illustrated on the lower right pictures). 5-7mm thick axial sections of the formalin fixed hysterectomy specimen were taken, slice 4 (blue box, enlarged below) contained a focus of adenomyosis (blue ring), that corresponds to the irregularity of the JZ as seen on the axial image in MRI.

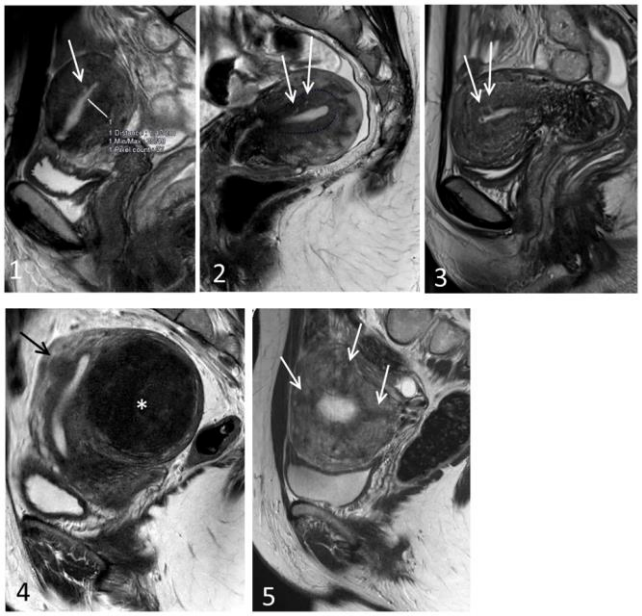


Figure 5: *Cases with an irregular junctional zone (JZ), true positive and false positive.* Magnetic resonance images of the uterus, all in sagittal plane, with T2 weighted sequences and turbo spin echo. Presence of adenomyosis was determined by histopathology. The white lines indicate measures of the JZ. **1:** Retroverted uterus. The JZ is thickened (14mm) in the posterior wall and thin in the anterior wall (arrow). True positive diagnosis. **2.** Retroverted uterus. High-intensity signals on the inner border (arrows) representing infiltration

of adenomyosis that interrupts the JZ. The outer border of the JZ (stippled line) should not be confused with the low-intensity, starfish-like signal from the stratum vasculare. True positive diagnosis. **3.** Anteverted uterus, The JZ is not visible in the fundus and thin (max. 6mm) in the visible parts. Finger-like invasion of adenomyosis to the myometrium (arrows) visible. True positive diagnosis. **4.** Retroverted uterus, containing a large fibroid in the posterior wall (marked with *). Focal thickening to 14mm of the JZ in the anterior wall represented most likely a change due to a very small fibroid. False positive diagnosis. **5.** Anteverted uterus, the cervix is not visible on this image. The arrows indicate areas that we interpreted as an irregular JZ or invasion of adenomyosis, but represented vessels. This was the only histopathological correlate that was found. False positive diagnosis.

In the multiple linear regression analysis only the presence of an irregular JZ (β=0.16, *P*=0.006) and myometrial cysts (β=0.18, *P*= 0.005) showed an independent association with having adenomyosis. The choice of MRI system did not influence the results.

Intra- and inter-reader agreement

There was a substantial intra-reader agreement in the measured JZ_{max} values (first and second readings performed by R1), with an ICC of 0.75 (95% CI: 0.59, 0.84, *P*<0.001). The inter-reader agreements were almost perfect for the measured values of JZ_{max} (ICC=0.81, 95% CI: 0.70, 0.87, *P*<0.001) and $JZ_{max\text{-}A}$ (ICC=0.95, 95% CI: 0.93, 0.97, *P*<0.001), and substantial for JZ_{diff} (ICC=0.73, 95% CI: 0.59, 0.83, *P*<0.001). The inter-reader agreement for the classification of the JZ was almost perfect (κ=0.89, 95% CI: 0.78, 0.97).

Discussion

In this prospective, single-center study, adenomyosis was not correlated with JZ_{max} or the previously proposed JZ_{max} cutoff of 12 mm, which is contrary to previously published prospective studies [10-12]. A diagnose of adenomyosis based on these JZ measurements contributed to a high number of false positive and false negative diagnoses in our study population.

There are several possible explanations for why our results differ from those of previous studies. Firstly, the mean age of our study participants was lower (42 years vs 51 years), and it is known that adenomyosis progresses over time and hence could have been more extensive in the previous studies. Secondly, Reinhold and Bazot also included a large proportion of postmenopausal women in their study (31–55%), and it is questionable whether diagnostic characteristics of the hormone-dependent JZ are transferable between pre-

and postmenopausal populations [10; 12].

Thirdly, we measured the JZ in accordance with our usual clinical practices, since there is neither a unanimous classification of adenomyosis nor a clear definition of the JZ; the main reason for that is most likely that the JZ has the same signal intensity as adenomyosis, and that it is not visible in histopathology [23; 24]. It is not ultimately clear how the JZ was defined in the other studies.

We find using JZ thickness measurements that included all low-intensity areas, also those representing diffuse or circumscribed adenomyosis that are in connection with the JZ (JZ_{max-A}) problematic for several reasons. In those cases, the presence of adenomyosis is usually obvious and therefore performing a measurement does not add any diagnostic value. If JZ_{max-A} is measured in a study population with extensive disease, a statistically significant association with adenomyosis in a ROC-curve is found. However, this association might not be equally meaningful for individual evaluation and in clinical practice, especially not in younger women of childbearing age and less extensive disease [25]. The interest in adenomyosis has shifted toward younger, infertile women and defining dedicated diagnostic markers for this group is of great importance. Bazot and Darai have recently stated that “In our experience, the JZ_{max} alone should be used with caution to diagnose internal adenomyosis”. This is in line with the conclusion of all the authors of comparable studies, who all state that other signs in addition to JZ measurements have to be considered [10-12; 23].

We introduce a classification of the JZ that reflects different kinds of JZ irregularities based on pattern recognition. This classification showed an almost perfect inter-reader agreement. Combined with signs of adenomyosis of the outer myometrium (adenomyoma and myometrial cysts) we yielded a sensitivity of 81% and specificity of 81%, which is comparable to the performance of various combined markers in previous studies with sensitivities of 86%, 77%, and 64%, and specificities of 86%, 93%, and 88% [10-12]. The sensitivity in our study was higher than in two of the others, probably because our MR images were obtained from thinner slices (1 to 3 mm thick, with a gap of 0.5 to 0.3 mm, vs 4 mm and a gap of 2 mm).

Our study was subject to some limitations. The post-hoc decision for the second read might have influenced the results of the second reading, though a high intra- and inter-reader correlation shows the consistency of

the readings. Like all studies involving hysterectomy with histopathology as the gold standard in diagnosing adenomyosis as an outcome, a selection bias is likely to have been present. Women that undergo hysterectomy might have more-severe disease and possibly different phenotypes of adenomyosis than women receiving conservative treatment. Furthermore, the MRI images were obtained during random phases of the menstrual cycle. There is conflicting evidence on the extent to which this is relevant, but it might have affected the measurements [23; 26]. Also, we did not exclude cases due to a certain time interval between MRI and surgery. One might argue that progression of the disease might influences the results, but that would result in false negative cases amongst those with a long time-gap between imaging and surgery. In our data, the false negative cases all show a time interval of under 3 month, with one exception, where a well-circumscribed adenomyoma was interpreted as a fibroid. As this study does not quantify the amount of adenomyosis found, we consider also longer time-gaps as acceptable.

One major strength of our study is the performing of very thorough histopathological examinations, which aimed at achieving a high diagnostic sensitivity and also most likely resulted in the prevalence of adenomyosis being much higher (61%) than in the other studies (21–33%). The clinical implications of very small adenomyosis-foci that might be detected only by a thorough histological is not clear. However, in a study of diagnostic accuracy like the present we think that a very thorough diagnosis is imperative and the clinical relevance of small findings needs to be determined in other studies. Another strength is that we used two independent readers who exhibited extremely high inter-reader agreement. Furthermore, we did not exclude patients with fibroids, since fibroids often coexist with adenomyosis and the exclusion might lead to an exaggeration of diagnostic performance of some predictors.

Conclusions

The irregular appearance of the junctional zone and the presence of myometrial cysts are independent predictors of adenomyosis. Measurements of the JZ had no statistically significant association with the presence of adenomyosis in our study population. JZ measurements are not validated for a young patient population with moderate disease and should therefore be used with caution.

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TABLES

	Adenomyosis *n*= 57	No adenomyosis *n*= 36	*P*
Age, years	43.5±4.9	41.2±4.2	0.01*
Body mass index, kg/m^2	25.9 (16–44)	25.6 (19–34)	0.73
Parity	1.4±1.4	1.5±1.2	0.66
Presence of fibroids	33 (58%)	18 (50%)	0.46
Presence of fibroids >50 mm	4 (7%)	10 (27%)	0.03*
Number of histopathological sections obtained from the corpus uteri	8.6±2.5	8.6±2.7	0.68

Table 1: *Baseline characteristics of the study population*. Adenomyosis/no adenomyosis confirmed by histopathology. Data are mean±standard-deviation, *n* (%), or median (range) values. **P* was determined using Student's *t*-test or the Mann-Whitney *U*-test, a value ≤0.05 was considered statistically significant.

Signs used for diagnosing adenomyosis	Definition
JZ_{max} ≥12 mm [a]	**JZ is a low-intensity band in T2W MRI of the inner myometrium, lining the endometrial cavity. JZ ≥12 mm, measured in any plane, including focal enlargement, and not including adjacent focal adenomyoma (definition see below) or diffuse adenomyosis[b].**
Myometrial cysts	High-intensity foci in the myometrium or subendometrial area, as seen in T2W or T1W imaging (hemorrhagic content).
Adenomyoma	Ill-defined, focal low-intensity areas with or without high-intensity foci.
Other documented features	
JZ_{max}	Thickest part of the JZ, measured in the midsagittal and axial plane perpendicular to the endometrial cavity, in millimeters.
JZ_{min}	Thinnest part of the visible JZ, measured in the midsagittal and axial plane perpendicular to the endometrial cavity, in millimeters
JZ_{diff}	JZ_{diff} is calculated as $JZ_{max\ (all\ planes)} - JZ_{min\ (all\ planes)}$, and represents irregularities of the JZ.
$JZ_{max\text{-}A}$	JZ measurement including all low-intensity signal areas representing diffuse or circumscribed adenomyosis, attached to the JZ (see also Fig. 3).
Appearance of the JZ[b]	Subjective impression of the JZ morphology being regular or irregular, not assessable, or not visible (see Fig. 2).
JZ-to-myometrial thickness ratio	Using JZ_{max} in the midcorporal area (sagittal and axial) and the corresponding thickness of the myometrium obtained at the same measurement level. Only assessable when no fibroids distort the wall.
Globular uterine shape	Subjective impression of the corpus uteri being round, and caused by smooth muscular hypertrophy resulting in a globular uterine shape, not due to fibroids.
Number of fibroids	Fibroids, which appear as well-circumscribed uterine masses.
Size of largest fibroid	Largest diameter (in millimeters).

Table 2: *Definition of predictors for the diagnosis of adenomyosis and other documented features.* [a]Primary outcome measure; JZ, junctional zone; MRI, magnetic resonance imaging; T1W, T1-weighted; T2W, T2-weighted.

Predictor	Adenomyosis *n*=57 (%)	No adenomyosis *n*=36 (%)					*P*
Categorical variables			**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Accuracy (95% CI)**	
JZ_{max}≥12 mm	30 (53)	16 (50)	53% (39, 66%)	56% (38,72%)	65% (55, 74%)	43% (33, 53%)	54% (43, 64%)	0.44
Presence of myometrial cysts	40 (70)	4 (11)	70% (57, 82%)	89% (74, 97%)	91% (80, 96%)	65% (55, 74%)	77% (68, 86%)	<0.001
Presence of adenomyoma	18 (32)	2 (6)	32% (20, 45%)	94% (81, 99%)	90% (69, 97%)	47% (42, 51%)	56% (45, 66%)	<0.001
JZ_{diff} ≥5.5 mm (optimum cutoff)	30 (53)	9 (25)	53% (39, 66%)	75% (58, 88%)	77% (64, 86%)	50% (42, 58%)	61% (51, 71%)	0.01
Presence of irregular JZ[a]	42/56 [a] (74)	6/35 [a] (22)	74% (60, 85%)	83% (67, 94%)	88% (77, 94%)	67% (51, 80%)	77% (68, 86)	<0.001
Regular JZ as negative predictive sign [b]	14 (26)	29 (81)	81% (64, 92%)	75% (62, 86%)	67% (56, 77%)	86% (76, 92%)	77% (68, 86%)	<0.001
Cysts and/or fingerlike indentations in the JZ	22 (39)	2 (6)	39% (26, 52%)	94% (81, 99%)	92% (73, 98%)	49% (44, 55%)	60% (50, 70%)	<0.001
JZ-to-wall-thickness ratio ≥50%[b]	24/39[a] (62)	15/28[a] (54)	42% (29, 56%)	58% (41, 75%)	50% (50, 72%)	39% (31, 48%)	48% (38, 60%)	0.51
Globular corpus uteri [b]	29/44[a] (66)	13/23[a] (57)	51% (37, 64%)	64% (46, 79%)	69% (57, 79%)	45% (36, 54%)	56% (45, 66%)	0.16
Numerical variables	**Mean ± SD (mm)**		***P***	**AUC (95% CI)**				***P***
JZ_{max} (mm)	11.1±3.3	10.4±3.9	0.37	0.57 (0.44, 0.70)				0.26
JZ_{diff} (mm)	8.4±9.2	4.5±3.1	0.02	0.62 (0.50, 0.74)				0.06
JZ_{max-A} (mm)	15.8±11.9	10.4±3.9,	0.01	0.68 (0.57, 0.80)				<0.001

Table 3: *Diagnostic performance of diagnostic predictors, Reader 1.* Adenomyosis/no adenomyosis confirmed by histopathology. [a]*n* differs from the total if the feature was not assessable due to motion artifacts. [b]not assessable cases (due to distortion of the uterine shape by fibroids) were counted as negative for this sign. AUC, area under the receiver operating characteristics curve; CI, confidence interval; NPV, negative predictive value; PV, positive predictive value. *P* was determined using Chi-square test or Fisher's exact test, *P*≤0.05 was considered statistically significant.

Predictor	Adenomyosis *n*=57 (%)	No adenomyosis *n*=36 (%)					*P*
Categorical variables			**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Accuracy (95% CI)**	
JZ_{max}≥12 mm	30 (53)	16 (50)	53% (39, 66%)	56% (38,72%)	65% (55, 74%)	43% (33, 53%)	54% (43, 64%)	0.44
JZmax≥12mm	19 (33)	14 (39)	33% (21, 47%)	61% (44, 77%)	58% (44, 70%)	37% (30,44%)	44% (34,55%)	0.59
JZ_{diff} ≥6.5 mm (optimum cutoff for R2)	28 (49)	7 (19)	49% (36, 63%)	81% (64, 92%)	80% (66, 89%)	50% (43, 58%)	62% (51, 71%)	0.01
Presence of irregular JZ	39/56 [a] (68)	6/35 [a] (22)	68% (56, 80%)	83% (67, 94%)	87% (75, 93%)	63% (53, 72%)	74% (64, 83%)	<0.001
Regular JZ as negative predictive sign [b]	14 (26)	28 (78)	78% (61, 90%)	75% (62, 86%)	67% (55, 77%)	84% (74, 91%)	76% (66, 85%)	<0.001
Presence of cysts in the JZ	16 (28)	1 (3)	33% (20, 47%)	97% (86, 100%)	94% (69, 99%)	52% (47, 57%)	60% (49, 71%)	<0.001
Cysts and/or fingerlike indentations in the JZ	22 (39)	2 (6)	39% (26, 52%)	94% (81, 99%)	92% (73, 98%)	49% (44, 55%)	60% (50, 70%)	<0.001

Numerical variables	**Mean ± SD (mm)**		***P***	**AUC (95% CI)**	***P***
JZ_{diff}	10.5±12.4	5.2±2.8	0.02	0.65 (0.53, 0.77)	0.06
JZ_{max}	10.3±3.7	10.1±3.7	0.85	0.50 (0.37, 0.62)	0.97
JZ_{max-A}	15.7±12.6	10.3±3.7	0.02	0.64 (0.53, 0.76)	0.02

Table 4: *Diagnostic performance of diagnostic predictors, Reader 2.* Adenomyosis/no adenomyosis confirmed by histopathology. [a]*n* differs from the total if the feature was not assessable due to motion artifacts. [b]not assessable cases (due to distortion of the uterine shape by fibroids) were counted as negative for this sign. AUC, area under the receiver operating characteristics curve; CI, confidence interval; NPV, negative predictive value; PV, positive predictive value. *P* was determined using Chi-square test or Fisher's exact test, *P*≤0.05 was considered statistically significant.

Supplementary Table 1 (online only):

	3.0-tesla (Phillips Ingenia)					1.5-tesla (Phillips Achiva)				
		T2W TSE		T1W TSE with fat suppression	T1W TSE	T2W TSE	T2 3D VISTA	3D B-TFE[2] with fat suppression	T1W TSE with fat suppression	T1W TSE
Imaging planes	Oblique axial[a]	Sagittal	Oblique coronal[b]	Oblique axial	Oblique axial	Oblique axial	Axial with sagittal and coronal reformates	Axial with sagittal and coronal reformates	Oblique axial	Oblique axial
Repetition time /Echo time (msec)	Range 3000-5000/80	Range 3000-5000/80	Range 3000-5000/80	Range 500-700/20	700/20	1175/85	5200/100	5.0/2.5	500/10	500/10
Bandwidth (Hz/pixel)	290	290	290	356	437	198	144	732	144	144
Flip angle	90	90	90	90	90	90	90	90	90	90
Field of view (mm)	220x220	240x240	240x240	240x240	240x240	190x288	160x160	200x290	146x185	150x190
Acquisition Matrix	480x478	480x478	440x431	344x336	376x390	195x252	256x225	244x356	132x161	168x161
Section thickness/ gap (mm)	3/0.3	3/0.3	3/0.3	3/0.3	3/0.3	3/0.3	1/-0.5	2/0	3/0.3	3/0,3
Number of signals aquired	1	1	1	2	2	4	1	3	3	3

Supplementary Table 1: *Protocol for magnetic resonance imaging.* [a]Perpendicular to the long axis of the uterine cavity. [b]Parallel to the long axis of the uterine cavity. T1W, T1-weighted; T2W, T2-weighted; B-TFE, balanced turbo field echo; TSE, turbo spin echo; VISTA, Volumetric Isotropic TSE Acquisition

III

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JMIG The Journal of Minimally Invasive Gynecology

Original Article

In Vivo Adenomyosis Tissue Sampling Using a Transvaginal Ultrasound–guided Core Biopsy Technique for Research Purposes: Safety, Feasibility, and Effectiveness

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ABSTRACT **Study Objective:** To determine if it is possible and safe to obtain adenomyosis tissue in vivo without removing the uterus in order to use it for further molecular investigations of adenomyosis, which would allow investigating the pathogenesis of the disease.

Design: A prospective cohort study.

Setting: A university hospital.

Patients: Eighty-one premenopausal women scheduled for a hysterectomy because of various benign indications were included.

Interventions: Ultrasound-guided, transvaginal uterine core biopsy samples were obtained, and the required time was registered. Any trauma to the pelvic organs, blood loss, and other complications were documented during the subsequent hysterectomy. Two biopsy samples were analyzed histopathologically to confirm the presence of adenomyosis, and another 2 were snap frozen using liquid nitrogen for use in further research. Laser microscopic dissection and RNA extraction were performed on the collected samples.

Measurements and Main Results: Biopsy specimens could be obtained in 80 (99%) of the 81 cases. There was no visible trace of the biopsy retrieval in 20 women (25%), perforation of uterine serosa or peritoneum was present in 56 (70%), and ongoing minor bleeding occurred in 4 (5%). The median amount of bleeding was 2 mL (range, 0–200 mL). No serious complications were observed. The procedure took 6.1 ± 1.9 minutes (mean ± standard deviation). Adenomyosis tissue was obtained in 10 (22%) of the 45 cases with adenomyosis. The inner myometrium with the junctional zone was accessible in all cases. It was possible to produce frozen sections, extract RNA, and dissect single adenomyosis glands with laser microscopic dissection.

Conclusions: No serious complications caused by the uterine biopsies were observed. This technique opens up the possibility of investigating early stages of adenomyosis and the inner myometrium containing the junctional zone independent of hysterectomy specimens. Journal of Minimally Invasive Gynecology (2019) 00, 1–6. © 2019 Published by Elsevier Inc. on behalf of AAGL.

Keywords: Adenomyosis; Biopsy; Junctional zone; Pathophysiology; Ultrasound guidance

Adenomyosis is a common condition affecting about 15% to 20% of women [1], and the interest in adenomyosis has been growing among clinicians. Adenomyosis causes debilitating symptoms, infertility, and major complications in pregnancy and labor, such as miscarriage, giving birth to a small-for-gestational-age child, premature rupture of membranes, and preeclampsia [2–7]. We understand little about the pathogenesis of adenomyosis, which is also reflected in the limited amount of treatment options we can offer young and infertile women [8]. Imaging modalities such as ultrasound and magnetic resonance imaging are useful tools for diagnosing adenomyosis but have not clarified the mechanisms underlying adenomyosis [9].

The authors declare that they have no conflict of interest.

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Preliminary data of the safety of the biopsy taking was presented as an oral presentation at the 25th Annual Meeting of the European Society of Gynaecological Endoscopy (ESGE), Brussels, Belgium, October 2-5, 2016.

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Experimental studies are better suited to reveal the pathways involved in the development of adenomyosis. Previous reports on the molecular implications of adenomyosis have come from studies that used tissue obtained from hysterectomy specimens, which are more likely to represent more advanced stages of adenomyosis [10−12]. Another major challenge in molecular research into adenomyosis is the location of the ectopic glands within the muscular layers of the myometrium, which can make analyzing the genomic (DNA/RNA) or proteomic expression of adenomyosis in biopsy specimens difficult or even impossible because they contain different tissue types.

We performed the present prospective study to determine if an ultrasound-guided, transvaginal in vivo biopsy technique is safe in women with adenomyosis and if adenomyosis glands in small samples can be isolated from the myometrial fractions by laser capture microdissection (LCM). The biopsy samples were obtained with regard to molecular investigations, not diagnostic purposes.

Materials and Methods

Study Setting and Patients

We performed this prospective, interventional cohort study between October 2014 and May 2017. The study formed part of the Norwegian Adenomyosis Study, which investigated the diagnostic accuracy of ultrasound and magnetic resonance imaging for adenomyosis. The results from those studies are reported elsewhere [13]. Women included in this study were scheduled for hysterectomy for benign conditions (menorrhagia, dysmenorrhea, or bulk-related symptoms) and were asked to participate in the present study. Inclusion criteria were women 30 to 50 years of age, premenopausal status, not having received any hormonal or gonadotropin-releasing hormone agonist therapy within the 6 months before inclusion, not suffering from a malignant condition, and needing a hysterectomy that could be performed without tissue morcellation.

Obtaining Biopsy Specimens

Biopsy specimens were obtained by the primary investigator before the hysterectomy with the woman under general anesthesia. The woman was placed in a lithotomy position, and biopsy specimens were obtained transvaginally under ultrasound guidance using a 2-dimensional 5- to 9-Hz transvaginal ultrasound probe (Voluson S8; GE Healthcare, Kretz, Austria) with a reusable needle guide provided by the ultrasonography manufacturer. The core biopsy device (BIP-HistoCore; BIP Biomed Instrumente & Produkte, Türkenfeld, Germany) had needles with 14- to 20-G diameters and lengths of 25 to 30 cm. Needles of different diameters were chosen in a random order to determine the optimal size for the biopsy specimens. Longer needles were chosen with large uteri, or they were picked in a nonsystematic way.

Two-dimensional transvaginal ultrasound was used to scan the uterus and surrounding organs and check whether they obstructed the planned biopsy route. Any possible direct signs of adenomyosis such as anechoic intramyometrial cysts or hyperechoic islets were identified and targeted [14]. If no adenomyosis was visible, random biopsy specimens were obtained from throughout the myometrium.

Four biopsy specimens were obtained from each woman; 2 of these specimens were fixed in 10% buffered formalin and analyzed histopathologically. The other 2 were capped into vials (CryoTube Vials; Thermo Fischer Scientific, Waltham, MA), snap frozen on liquid nitrogen without adding any buffer [15], and later transferred to −70°C for storage. The woman was then repositioned for hysterectomy. The status of the peritoneal cavity was assessed while entering the abdominal cavity by laparoscopy or laparotomy.

The time taken to perform the biopsy procedure (measured from the point of inserting the vaginal ultrasound probe until the biopsy specimens were obtained) was registered. The following parameters were documented after the biopsy procedure and during the subsequent surgical procedure: subjective impression of the procedure being easy, difficult, or impossible to perform; any direct signs (e.g., adenomyoma, myometrial cysts, or hyperechoic areas) or indirect signs of adenomyosis (e.g., diffuse thickening of 1 uterine wall or globular formation of the corpus uteri) on transvaginal ultrasound [14]; and the status of the peritoneal cavity as seen during surgery, such as the presence or absence of visible damage to the uterus or other organs, puncturing of the uterine serosa or peritoneum, active bleeding, and the amount of free blood.

All histopathological examinations of the biopsy specimens were performed by the same pathologist who was blinded to the findings of the ultrasound examinations. The specimens were examined independently from the hysterectomy specimens, but suspicion of the presence of adenomyosis in the biopsy specimens was compared with and verified with findings for the hysterectomy specimens. Only in 1 case was a retrospective change of the biopsy analysis made, so we considered that this approach did not introduce significant bias to the results. Adenomyosis was considered to be present when ectopic endometrial tissue was seen 2.5 mm under the endometrial-myometrial border [16].

LCM, Staining, and RNA Isolation

We performed LCM to test if it would be possible to isolate adenomyosis cells from the surrounding myometrial tissue. Random samples were embedded in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Tokyo, Japan) and cut into 2-μm-thick slices using a microtome (Leica CM350S; Leica Microsystems, Wetzlar, Germany) at −40°C. The frozen sections were mounted on membrane slides (MMI Slides RNAse free; Molecular Machines and Industry, Glattbrugg, Switzerland) and stored at −70°C until further use. Hematoxylin-eosin staining was

performed with a dedicated kit in accordance with the manufacturer's instructions (H&E Staining Kit Plus, Molecular Machines and Industry), and endometrial tissue and muscular tissue were separated with an LCM system (MMI CellCut, Molecular Machines and Industry). The dissected tissue was sampled using diffusor MMI Isolation Caps (Molecular Machines and Industry).

We also tested the RNA content of the biopsy samples. After producing frozen sections, the tissue was stained as described previously, and RNA extraction was performed using the ARCTURUS PicoPure RNA Isolation Kit (Arcturus Tissue scrape Protocol #1; Applied Biosystems, Foster City, CA) and RNA analysis was performed on a bioanalyzer (Agilent 2100; Agilent Technologies, Santa Clara, CA) following the protocols provided by the manufacturers.

Statistical Analysis

Data were described as the mean ± standard deviation, median and range, or frequency and percentage values as appropriate. Numeric variables were compared using the Student *t* test for 2 independent samples or the Kruskal-Wallis test for more than 2 samples with nonnormal distribution. The chi-square or Fisher exact test was performed to compare categoric data where applicable. Statistical analysis was performed using IBM SPSS Statistics (Version 25; IBM Corp., Armonk, NY), and a probability value of $p < .05$ was considered statistically significant.

Ethical Approval

The study was approved by the institutional review board and the Regional Committee for Medical Research Ethics in Eastern and Southern Norway (Approval Number 2014/637). The study was registered at ClinicalTrials.gov (protocol number NCT02197923, release date July 17, 2014) before recruiting study participants. Written consent was obtained from all of the participants.

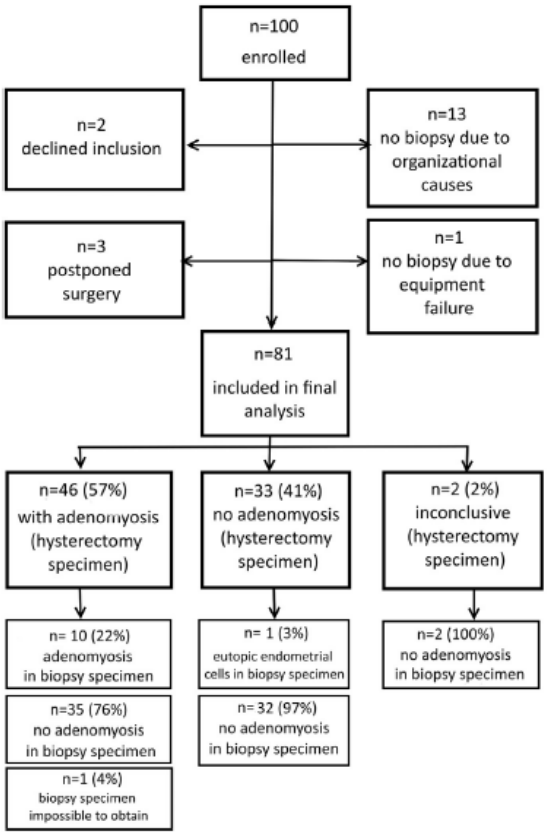
Results

The final analysis included 81 women. Figure 1 presents the study flowchart, including the results of the histopathological examinations of the biopsy specimens. In 1 case, a technical failure occurred during the biopsy procedure, and in another case the bowels were adherent to the uterus and bladder in a manner that prevented obtaining a biopsy sample without the risk of bowel perforation, so the procedure was aborted. The baseline characteristics of the study population are presented in Table 1. Figure 2 shows a biopsy sample containing adenomyosis.

Based on the subjective impression of the primary investigator, the biopsy procedure was classified as easy in 68 (84%) cases and difficult in 12 (15%). Difficulties were mainly related to a hypermobile or straight uterus where it was problematic to reach the region of interest and the cervix needed to be punctured.

Fig. 1

A study flowchart of the study population and findings of the histopathological analyses.



It was possible to visualize either direct or indirect signs of adenomyosis using transvaginal ultrasound before the surgery in 29 of 46 (63%) and 17 of 33 (52%) of the adenomyosis and no adenomyosis cases, respectively, resulting in no statistically significant difference ($p = .31$). An enlarged uterine wall, a low-specific sign of adenomyosis, was the most frequent finding ($n = 13$ vs $n = 8$) followed by myometrial cysts and/or hyperechoic myometrial islets ($n = 13$ vs $n = 3$).

Perioperative inspections of the abdominal cavity showed no visible damage in 20 (25%) of the cases,

Table 1

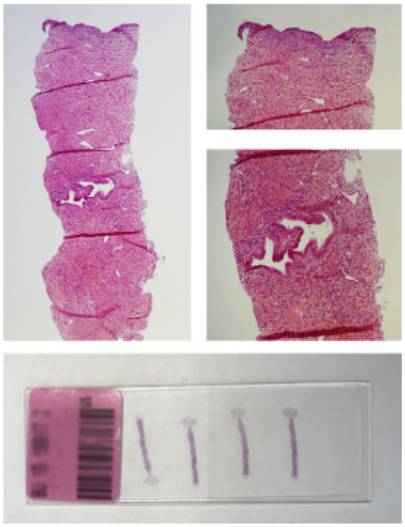
Characteristics of the Study Population

Age, years	42.4 ± 4.5
Body mass index, kg/m^2	26 (16–28)
Weight of the hysterectomy specimen, g	154 (55–1709)
Presence of myoma(s)	43 (53%)
Presence of myoma(s) ≥50 mm	11 (14%)

Data are mean ± standard deviation, median (range), or n (%) values.

Fig. 2

A histologic image of a biopsy sample obtained with an 18-G needle and stained with hematoxylin-eosin. The endometrial cavity is visible in the upper part of the specimen (top right). The ectopic endometrial glands (adenomyosis) are visible in the middle of the biopsy specimen (middle right). The bottom image displays a glass slide (75 mm × 26 mm) with 4 sections from a biopsy sample.



puncturing of the serosa in 56 (70%), and minor ongoing bleeding from the small subperitoneal vessels in 4 (5%), all of which stopped spontaneously during the subsequent surgery. In all cases, the amount of bleeding was minor, with a median of 2 mL (range, 0−200 mL) and with no significant difference between the groups with and without adenomyosis (p = .68). Six women (7.4%) had a blood loss of 50 to 100 mL, whereas 3 women had a blood loss of 150 mL, 160 mL, and 200 mL, respectively. Of those 3, 1 had adenomyosis, and the others did not. A retrospective analysis of the ultrasound images showed that all 3 had a highly vascularized myometrium. This might have been the reason for the more extensive bleeding. The woman that bled 200 mL reported during the clinical consultation that she had previously experienced heavy bleeding during surgical/dental procedures. Therefore, we checked preoperatively if she had coagulopathy, which she did not have. After the biopsy taking, she also bled extensively during the surgery without a clear explanation. We conclude from this experience that patient selection before biopsy taking is important.

The vesicouterine space was sometimes punctured during the procedure in women with an anteverted uterus, leaving a small subperitoneal hematoma in the area. However, we observed no perforation of the bowel, ureters, or larger vessels. We did not identify any postoperative complications, such as abscess, fistulas, sepsis, or rebleeding, that could be related to the obtaining of biopsy specimens.

The mean biopsy procedure time was 6.1 ± 1.9 minutes, with no significant difference between the groups with (6.1 ± 1.4 minutes) and without (6.2 ± 2.4 minutes) adenomyosis (p = .79). We tested the use of biopsy needles of different sizes randomly, both in terms of their diameter and length. The needle diameter was 16 G in 31 (38%) cases, 18 G in 42 (52%) cases, and 20 G in 8 (10%) cases. Needles with a length of 20 cm were used in 41 (51%) of the cases, and 25-cm length needles were used in the other 40 (49%). The median amount of bleeding did not differ between the groups with different needle diameters (3.5 mL [range, 0−160 mL], 4.0 mL [range, 0−200 mL], and 0.0 mL [range, 0−70 mL] for 16-G, 18-G, and 20-G needles, respectively; p = .81). We found that biopsy specimens obtained using 20-G needles were too thin so the uterine tissue fell apart and a reliable histopathological analysis with the correct orientation of the sample could not be appropriately performed. There was considerable resistance when inserting 16-G needles into the myometrial wall because of their diameter, which resulted in the uterus being pushed away rather than penetrating it smoothly, making it difficult to reach the targeted region. Therefore, we considered the 18-G needles to be optimal. The longest needles (25 cm) were easy to handle and worked well in all cases, whereas the 20-cm-long needles were too short in some patients with an enlarged uterus. However, we considered the direct visualization of adenomyosis, rather than the choice of needle size, to be the main factor influencing the sensitivity of the method. It was possible to gain access to the inner myometrium, containing the junctional zone, in all cases.

LCM and RNA Isolation

We were able to produce frozen sections from the biopsy specimens and performed LCM with isolation of single adenomyosis glands (Fig. 3). We noted that the myometrial structures appeared to be intact and undamaged by the freezing process. We used the tissue scrape protocol for verifying RNA to isolate RNA (best gain obtained = 472 pg/μL, RNA integrity number = 7.9), showing that the further use of the samples was possible.

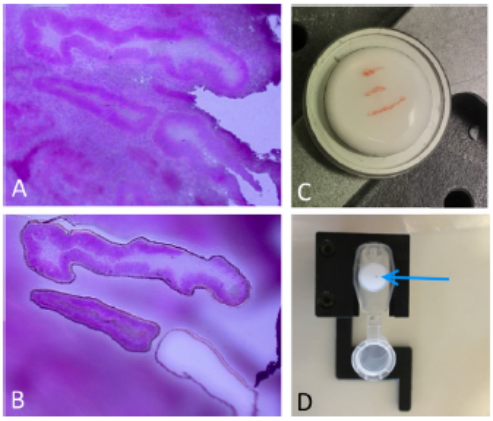
Discussion

This prospective study has shown that in vivo biopsy specimens of adenomyosis can be obtained from the uterus rapidly, and we provide evidence on the safety of this technique. The biopsy specimen can be used for a range of research purposes. Because ultrasound and magnetic resonance imaging provide a good diagnostic quality [13,17], we propose this technique for tissue retrieval and not for diagnostic purposes.

To the best of our knowledge, this is the first study to investigate the safety of transvaginal myometrial biopsies, and there is a lack of comparable reports. On the other hand, a transvaginal approach of biopsy taking in order to gain access to pelvic tumors is extensively described in the

Fig. 3

(A and B) Frozen sections stained with hematoxylin-eosin and mounted on membrane slides (× 20 magnification). (A) A membrane slide before laser dissection, showing the endometrial glands and stroma. (B) The glands were dissected with a laser beam and captured in the cap of the extraction tube (shown in panel D). One gland (lower right corner) did not attach properly to the silicone cap. (C) Fractions of a biopsy sample embedded in frozen optimal cutting temperature compound and mounted on a pin ready for the preparation of frozen sections. (D) Dissected tissue sample (*arrow*) attached to the silicone cap of the extraction tube.



literature, and all studies report good safety [15,18–24]. Two studies reported the mean blood loss in normal oocyte pickup procedures for in vitro fertilization to be 232 mL and 72 mL, which is significantly higher than the observed amount of bleeding in our study, which was 2 mL [25,26]. Puncturing of the peritoneum will lead to injury of the network of the finest peritoneal capillaries that perfuse the peritoneum, but the forming of a minor subperitoneal hematoma seems to prevent major bleeding. A systematic review including 8 studies performing transvaginal drainage of endometrioma, a procedure that also leads to inevitable puncturing of the peritoneal cavity, concludes that this is a safe procedure [27].

Although our data suggest that myometrial biopsies are without serious complications, the limitations of the present study need to be considered. First, our study contains only a limited number of cases, and larger prospective studies should be performed to confirm the safety of this approach. Second, we did not perform a long-term follow-up in order to rule out late complications, such as fistula of the bladder. Based on relatable studies and clinical 'experience, we would consider this to be a negligible scenario [28]. Third, the risk of infection and the issue of antibiotic administration have to be addressed. Because the vagina is colonized by microbes, the procedure is not sterile, and seeding of bacteria to the peritoneal cavity is possible. At present, there are no guidelines for or against antibiotic prophylaxis being given, and even if some authors consider the risk of infection as minor, this needs to be considered [18].

Another important issue to consider is that the biopsy taking of the myometrium might have a negative effect on the uterine function, such as uterine peristalsis [29,30]. However, myometrial damage caused by displaced intrauterine devices is quite common, and it seems that the myometrial tissue has a good healing ability, as also observed in myomectomies that are more extensive than the present biopsies [31,32]. Finally, it was not clear how painful the procedure is because we performed it under full anesthesia, but a similar hysteroscopic biopsy technique described by al-Azzawi et al [33] was well tolerated with local anesthesia only. We would suggest considering taking the biopsies during another medical procedure that requires sedation or anesthesia, such as oocyte pickup, hysteroscopy, or laparoscopy.

The sensitivity of the present method for obtaining adenomyosis tissue was low in our study, with only 22% of the biopsy specimens being positive. Optimally, direct visualization of adenomyosis is needed in order to obtain tissue by biopsy, which was not the case in our study population. Also, our study population was not selected in order to have optimal conditions for the biopsy taking of adenomyosis but was a consecutive group of women undergoing hysterectomy for various reasons. In a research setting, where the primary focus is to take myometrial biopsies, the population can be more specifically selected and possibly a higher yield reached. Nam and Lyu [34] reported a sensitivity of 92% for abdominal ultrasound–guided biopsies in a highly selected population of women undergoing radiofrequency thermal ablation for suspected adenomyosis, which indicates that this is more feasible when extensive disease is present. However, we assume that the sensitivity of our method could also be increased by using a more powerful ultrasound system with a higher resolution or combined magnetic resonance and ultrasound imaging (fusion imaging) that is widely used in procedures such as diagnostic biopsies for prostate cancer. On the other hand, we found that the inner myometrium, where the junctional zone is located, was accessible in all cases in which biopsy specimens were obtained. The junctional zone plays a central role in uterine function, peristalsis, embryonic implantation, and placentation and is affected in women with adenomyosis and endometriosis [35–37]. Early signs of adenomyosis might be found here, making it the structure of main interest in both the diagnosis and pathophysiology of adenomyosis.

LCM made it possible to isolate adenomyosis cells from the surrounding myometrial tissue even in the biopsy specimens that were relatively small, which provides the possibility of highly specific analyses of the diseased cells. Furthermore, we found that RNA could be isolated from the biopsy specimens, which shows the possible exploitation potential of such biopsies in basic research. Providing clinical information on women in whom the tissue sample was gained could help understand the clinical relevance of the discovered altered molecular pathways. Consequently, we recommend the cooperation of clinicians with

researchers in order to produce results that patients will profit from, both related to therapy and prevention of the disease.

Conclusion

In vivo biopsy samples from the uterus and adenomyosis can be obtained and possibly used for various molecular studies of adenomyosis. The procedure seems to be safe, but larger studies are needed to confirm the safety. We recommend that their use should be performed within research studies only and that they are performed after thorough ethical evaluations of both the possible adverse effects and benefits.

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APPENDIX 1

Forespørsel om deltakelse i forskningsprosjektet

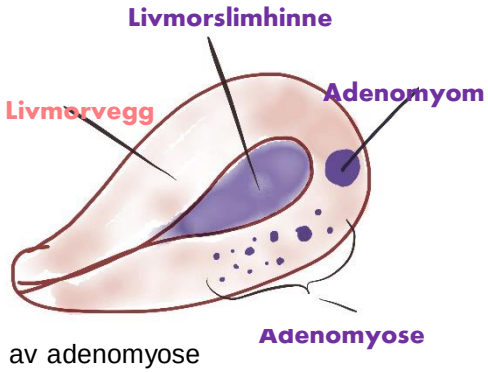
"Norsk Adenomyosestudie 1 og 2"

NAPPED *Norwegian Adenomyosis study: Pathophysiology, Peristalsis, Expressionprofiling and Diagnostics*

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke om tilstanden "adenomyose" kan diagnostiseres med ultralyd, om prøver (biopsier) tatt fra livmoren kan påvise adenomyose og om det er sammenheng mellom adenomyose og nivået av forskjellige hormoner i blodet. Du får forespørselen om å delta siden det er planlagt å fjerne livmoren din og du beskriver symptomer på adenomyose (se neste avsnitt). Ansvarlig for studien er Gynekologisk avdeling ved Oslo Universitetssykehus, Ullevål.

Hva er adenomyose?



Adenomyose er en tilstand hvor livmorslimhinnen, som vanligvis finnes kun i livmorhulen, befinner seg i livmorveggen. Symptomer på adenomyose kan være smerter under menstruasjon, sterke menstruasjonsblødninger eller andre blødningsforstyrrelser, kroniske bekkensmerter, nedsatt fruktbarhet og smerter ved samleie. Det er ikke kartlagt med sikkerhet hvorfor og hvordan adenomyose oppstår og det finnes dessverre svært lite forskning om behandlingsmuligheter. Man antar at cirka 8-20 % av alle kvinner har adenomyose (andelen øker med økende alder). Det finnes ingen studier som beskriver forekomsten av adenomyose blant norske kvinner.

Hva er formålet med denne studien?

Årsaken til at man vet så lite om en så forholdsvis hyppig tilstand, skyldes i stor grad at det lenge ikke var mulig å diagnostisere adenomyose på noen annen måte enn å fjerne livmoren og undersøke den mikroskopisk (histologisk undersøkelse). I tråd med utviklingen av MR og bedre ultralydapparater, har dette endret seg. Studier viser at man har muligheten til å diagnostisere adenomyose med ultralyd. Antageligvis vil en 3-dimensional ultralydundersøkelse være velegnet til å finne ut om det foreligger adenomyose hos en pasient, og det vil være en kortere og mer tilgjengelig metode enn for eksempel MR. Vi ønsker å sammenligne hvor nøye og korrekt en 3-dimensional ultralydundersøkelse via skjeden er - sammenlignet med MR-undersøkelse og med histologiundersøkelsen etter fjerning av livmor.
I tillegg ønsker vi å måle nivået av forskjellige hormoner i blodet for å finne ut om det er sammenheng mellom hormonnivåer i blodet og forekomst av adenomyose. For å kunne forstå prosesser som fører til at adenomyose oppstår, ønsker vi å ta biopsier fra livmoren mens du er i narkose, og foreta molekulærbiologiske undersøkelser av disse prøvene. Noen av disse prøvene kan også bli lagret i maks. 10 år til senere undersøkelser i forbindelse med adenomyoseforskning. Prøvene blir lagret uten navn i en så kallt biobank. For å kunne kartlegge adenomyose hos norske kvinner nærmere og for å finne ut om det finnes opplysninger i sykehistorien som kan stå i sammenheng med adenomyose, ber vi deg også om å fylle ut et spørreskjema. Opplysningene vi spør om gjelder gynekologiske sykdomer, forhold i forbindelser med svangerskap og fødsel og bruk av medisiner, som kan stå i sammenheng med utvikling av adenomyose. Dersom du ikke husker alle opplysningene, ber vi deg om å få lov å slå opp i journalen din for å finne disse opplysningene, men det innebærer ikke at vi leser hele journalen.
Vi håper at resultatene fra denne studien kan danne et viktig grunnlag for videre forskning på årsaker, forekomst og behandling av adenomyose.

Hva innebærer studien?

Når du samtykker i å delta, kommer vi til å gjennomføre en 3D-ultralyd av livmoren via skjeden. Du vil ikke merke noen forskjell på en 3D undersøkelse sammenliknet med den vanlige 2D ultralydundersøkelsen som uansett tas i utredningen før gynekologiske sykdommer. Du fyller også ut et spørreskjema hvor vi ber om informasjon om tidligere gynekologiske sykdommer og svangerskap/fødsler, samt generelle opplysninger som vekt, høyde, tidligere sykdommer og medisiner. Dersom du ikke husker alle opplysningene knyttet til dette, vil vi be deg om å få lov å målrettet slå disse informasjonene opp i journalen din. Mens vi tar de vanlige blodprøver som er påkrevd for operasjonen, vil det bli tatt noen ekstra prøver til hormonanalysen. Så vil vi planlegge en MR-undersøkelse av bekkenet, som du vil bli innkalt til i løpet av noen uker (gjennomføres på Ullevål). Det er ikke hos alle pasienter dette vanligvis ville bli gjort før fjerning av livmor, så for noen vil dette være en tilleggsundersøkelse. Under operasjonen, mens du ligger i narkose, vil vi ta vevsprøver (biopsier) fra livmoren. Disse prøvene tas ultralydveiledet via skjeden. Etter operasjonen vil livmoren bli sendt til vevsundersøkelse (histologisk undersøkelse), som hos alle pasienter.

Mulige fordeler og ulemper

Fordelen for deg som deltar i denne studien er at du får en grundigere kartlegging av din tilstand enn du ellers hadde fått. 3D-transvaginal ultralyd og MR av bekkenet er ikke standardundersøkelser og er ennå ikke tilgjengelig for alle. Du må ikke betale egenandel for disse undersøkelser. Det er vist at MR eller ultralyd IKKE utgjør noen helserisiko, du utsettes ikke for stråling og det brukes ikke kontrastmidler. Blodprøven tas samtidig med de påkrevde blodprøver før en operasjon, det er små volum av blod og du må ikke stikkes mer enn ellers.

Å ta biopsier fra livmoren under operasjonen vil ikke medføre smerter, siden du allerede har narkose. Det vil ikke ta lang tid og ikke forlenge narkosen på en uforsvarlig måte. Å ta ultralydveiledede prøver fra livmoren og eggstokkene via skjeden er en veletablert metode og er vurdert som trygg, men det er ikke ennå blitt forsøkt å ta biopsier av adenomyose. Tall for samenlignbare prosedyrer, som for eksempel ultralydveiledet stikking i eggstoker under kunstig befruktning, viser svært lave tall for komplikasjoner: Infeksjon 0,06-0,02%; blødning maks. 0,24%; tarmskade 0,04%. En ekstra trygghet for deg er at vi rett etter prøvetaking får full oversikt over bukorganene under operasjoen, og kan se om det ble skadet noe.
Deltagelse i studien vil ikke føre til at operasjonen din blir utsatt. Du vil motta den samme behandlingen og oppfølgingen som alle pasienter. Med deltagelse i denne studien vil du yte et viktig bidrag til klinsik forskning og kartlegging av adenomyose.

Hva skjer med prøvene og informasjonen om deg?

Prøvene tatt av deg og informasjonen som registreres om deg, skal kun brukes slik som beskrevet i hensikten med studien. Inntil du opereres og vevsprøvene er ferdig undersøkt vil våre funn følge journalen din, slik at de kan brukes av de behandlende legene. For studieformål, altså analyse av data, vil opplysningene og prøvene bli behandlet uten navn og fødselsnummer eller andre direkte, gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte lege Tina Tellum, epost tina.tellum@ous-hf.no, telefon 22 11 98 00.

Kapittel A- utdypende forklaring av hva studien innebærer

- *Følgende kvinner kan ikke delta i studien*: kvinner etter overgangsalderen, kvinner med mange myomer (muskelknuter) som ligger ugunstig til i forhold til livmorslimhinnen, kvinner med livmorkreft, kvinner som har fått anti-hormonell behandling de siste 3 måneder før undersøkelsen, kvinner hvor man ikke kan fjerne livmoren i et stykke under operasjonen.
- *Alternative prosedyrer eller behandling pasienten får dersom personen velger å ikke delta i studien:* Pasientene som ikke deltar vil vanligvis ikke få utført 3D-transvaginal ultralyd og kun i sjeldne tilfeller MR-undersøkelse. Operasjonen vil bli den samme.
- *Undersøkelser som skal gjennomføres*: Spørreskjema, 2D- og 3D-transvaginal ultralyd, blodprøver, MR-undersøkelse av bekken, biopsi av livmor.
- *Tidsskjema*: Når du har blitt inkludert i studien og 3D ultralyd ikke kan bli tatt med en gang, vil du få time til ny 3D-ultralyd undersøkelse. MR-undersøkelse vil man i de fleste tilfeller få i løpet av 2-3 uker. Blodprøvene vil bli tatt umiddelbart ved inkludering. Operasjonen gjennomføres i vår avdeling med en gjennomsnittlig ventetid på 12-17 uker, dette er uavhengig av studien.
- *Mulige fordeler*: Du får en nøyere kartlegging av din tilstand med MR og 3D-ultralyd, utover det som er vanligvis tilgjengelig.
- *Mulige ubehag/ulemper:* Noen opplever det som ubehagelig å måtte ligge stille under en MR-undersøkelse. MR-maskinen er et noe trangt apparat, men man blir ved undersøkelse av bekkenet ikke kjørt helt inn i maskinen. Undersøkelsen kan ta 15-30 minutter. MR-undersøkelsen innebærer at du må møte opp en gang ekstra på sykehuset. Ved biopsitaking kan det i svært sjeldne tilfeller oppstå komplikasjoner, de fleste av ikke-alvorlig art. Det er ikke forbundet smerter med selve biopsitaking.
- *Studiedeltakerens ansvar:* Det er viktig å gi korrekte opplysninger i spørreskjemaet. I tilleg er det viktig å møte opp til MR-undersøkelsen, eller å avlyse denne i god tid på forhånd, siden vi har svært begrenset kapasitet til denne.
- Du vil bli orientert så raskt som mulig dersom ny informasjon blir tilgjengelig som kan påvirke din villighet til å delta i studien.
- I tilfelle at studien blir avsluttet før den planlagte tiden, vil du bli informert umiddelbart. Det vil ikke føre til at din operasjon utsettes eller din behandling endres.
- Egenandel for MR-undersøkelsen og 3D-ultralydundersøkelsen vil bli dekket av sykehuset dersom du deltar i studien. Du får ingen økonomisk kompensasjon for studiedeltakelsen.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er kun de opplysninger som også ville stå i pasientjournalen din. Disse vil bli avidentifisert for studieformål. Det vil ikke bli innhentet informasjon om deg fra andre steder, som fødselsregister eller lignende.

Alle som får innsyn har taushetsplikt og er tilknyttet din behandling. I tilfelle andre forskere bruker datamaterialet, er dette avidentifisert.

Oslo universitetssykehus HF ved administrerende direktør Bjørn Erikstein er databehandlingsansvarlig.

Biobank

Vevsprøvene fra livmoren vil bli lagret i en forskningsbiobank ved oslo universitetssykehus, Ullevål. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Ansvarshavende for forskningsbiobanken er Bjørn Busund. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK). Prøvene destrueres etter 10 år.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi

Studien og biobanken er finansiert gjennom forskningsmidler fra Oslo Universitetssykehus. Det er ingen farmasøytiske firmaer eller produsenter av medisinskteknisk utstyr involvert som sponsorer i denne studien og det foreligger ingen interessekonflikter.

Forsikring

Som deltaker i den studien er du omfattet av de norske pasientrettighetslover og trygdelover, som alle pasienter. Det gjelder ingen særskilt forsikring.

Informasjon om utfallet av studien

Du har rett til informasjon om utfallet av studien. Dersom du ønsker å få tilsendt publikasjonen når den er ferdigstilt, skriv din e-post her:_____

Samtykke til deltakelse i studien

O Jeg er villig til å delta i studien

O Jeg samtykker i biopsitaking under operasjonen

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien:

NAPPED 1 og 2 -studie – Kapittel A og B - dato]

(Signert, rolle i studien, dato)

3

NAPPED 1
Norwegian Adenomyosis study:
Pathophysiology, Peristalsis, Expressionprofiling and Diagnostics

GENERELLE HELSEOPPLYSNINGER

Alder: _____ **Høyde:** _____cm **Vekt:** _____kg

1. Din menstruasjonssyklus nå er

☐ regelmessig ☐ uregelmessig

2. Hvor mange dager varer din menstruasjon? *Antall*: _____ dager
3. Hvor mange dager er det mellom hver gang du får menstruasjon (fra 1. dag til neste 1. dag)? *Antall*: minst _____ dager, maksimal _____ dager
4. Har du mellomblødninger? _____

Din gynekologiske sykehistorie

1. I hvilken alder hadde du din aller første menstruasjon? _____ år gammel
2. Har du noen gang brukt p-piller?

☐ ja ☐ nei

Hvis ja, hvor mange år sammenlagt har du brukt p-piller? ca ____ år

3. Antall svangerskap: ____ Antall fødsler: ____
4. Antall utskrapninger i forbindelse med svangerskap eller fødsel: ____
5. Antall utskrapninger uten graviditet eller fødsel: ____
6. Har du gjennomgått andre inngrep i underlivet (f. eks. fjerning av eggstokk/eggleder, endometriose, fjerning av muskelknute, kaisersnitt)? Hvis ja hvilke?

1._____ År: _____

2._____ År: _____

3._____ År: _____

7. Tok det mer enn 12 mnd å bli gravid, regnet fra da du aktiv prøvde å bli gravid (kryss av «ja» hvis det var tilfelle ved minst èn av gravidetene)?

☐ ja ☐ nei

1

Studie Nr.

NAPPED 1
Norwegian Adenomyosis study:
Pathophysiology, Peristalsis, Expressionprofiling and Diagnostics

7. Har du gjennomgått assistert befruktning (prøverør, pergotime eller lignende)?

☐ nei ☐ Ja, men husker ikke hvilken type ☐ Pergotime/Clomifen

☐ Ja, IVF eller ICSI („prøverør") ☐ Annen (f. eks. eggdonasjon)

Spørsmål om svangerskap og fødsel:

1. Har du hatt noen av de følgende komplikasjoner i svangerskap eller fødsel?

☐	Svangerskapsforgiftning (Preeklapsi, Eklampsi)	☐	Fastsittende morkake
☐	HELLP-syndrom	☐	Morkake foran mormunnen (Placenta prævia eller marginalis)
☐	For tidlig fødsel (hvis ja, hvor mange uker før terminen ble barnet født: ____ uker)	☐	Svært sterke blødninger under eller etter fødselen
☐	For tidlig vannavgang (før svangerskapsuke 37)	☐	Morkakeløsning

2. Hvis du har født barn, hva veide disse?

1. ____g 2. ____g 3. ____g 4. ____g

3. Har du ammet noen av barna dine? Hvis ja, hvor lenge?

1. barn ____ mnd 2. barn ____ mnd 3. barn ____ mnd 4. ____ mnd

EVT. KOMMENTAR

_____

_____

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Kartlegging av symptomer

VENNLIGST ANGI I NEDENSTÅENDE TABELL OM DU HAR NOEN AV FØLGENDE SYMPTOMER ELLER PLAGER. I SKALAEN BETYR 0 "INGEN PLAGER/SYMPTOMER" OG 10 ER "VERST TENKELIGE PLAGER/SVÆRT UTPREGETE SYMPTOMER".

Hvor ofte...

1. ... har du smerter **før** menstruasjon?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
	☺	0	1	2	3	4	5	6	7	8	9	10	☹
... og hvor utpreget er disse smertene?		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
2...har du smerter **under** menstruasjon?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
	☺	0	1	2	3	4	5	6	7	8	9	10	☹
...og hvor utpreget er disse smertene?		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
3. ...har du smerter under samleie?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
	☺	0	1	2	3	4	5	6	7	8	9	10	☹
... og hvor utpreget er disse smertene?		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
4. Har du kroniske smerter i bekkenet?	O ja			O nei									
	☺	0	1	2	3	4	5	6	7	8	9	10	☹
... og hvor utpreget er disse smertene?		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
5... menstruasjonssmerter som stråler ut, nedover eller til siden?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
6. ...opplever du nedtrykksfølelse i bekkenet/underlivet?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
	☺	0	1	2	3	4	5	6	7	8	9	10	☹
...og hvor utpreget er dette symptomet?		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
7. ...har du blødninger utenom menstruasjonen (mellomblødninger)?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
8. ...har du så sterke menstruasjonsblødninger slik at du opplever det som plagsomt?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	
9...opplever du tranglekkasje (urge-inkontinens)?	O Aldri		O Sjelden			O Noen ganger			O Ofte		O Alltid	

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Har du (hatt) noen av disse sykdommer?	ja	nei	Jeg vet ikke
1. Høyt blodtrykk	☐	☐	☐
2. Hjerte/kar-sykdommer	☐	☐	☐
3. Revmatisk- eller muskel/skjelett-sykdom	☐	☐	☐
4. Kreftsykdom	☐	☐	☐
5. Depresjon	☐	☐	☐
6. Kronisk luftveissykdom	☐	☐	☐
7. Autoimmun sykdom	☐	☐	☐

Medisinbruk

Noen medisiner kan øke nivået av hormonet "prolaktin" i blodet. Man diskuterer om prolaktin kan bidra til utvikling av adenomyose. Derfor spør vi her etter en rekke forskjellige medisiner som brukes f. eks. i behandlingen av høyt blodtrykk, depresjon, angst etc., og som øker hormonproduksjonen av prolaktin hos enkelte pasienter.

1. Kryss av hvis du har brukt noen av disse medisinene noen gang

☐	Klomipramin *(Anafranil Imipramin)*	☐	Risperidon *(Risperdal)*	☐	Verapamil *(Isoptin, Verakard)*	☐	Gabapentin *(Nevrontin)*
☐	Desipramin	☐	Olanzapin *(Zyprexa,Zyp4dhera)*	☐	Metyldopa *(Aldomet)*		
☐	Fluanxol	☐	Clorpromazin *(Largactil)*	☐	Haloperidol *(Haldol)*		

2. Dersom du har krysset av for en eller flere av medisinene i tabellen over, lurer vi på hvor lenge du har brukt den medisinen? Kryss av for den du har brukt lengst:

☐ Sjelden/ ved behov ☐ Fast, i noen uker ☐ Fast i noen måneder ☐ Fast, i noen år

Takk for din deltakelse i Norsk Adenomyosestudie!

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APPENDIX 3: Registration form for biopsy taking

Registreringsskjema biopsi

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Undersøker-ID:	Pasient-ID:

1. **Biopsitaking**

☐ enkel ☐ vanskelig

☐ Ikke mulig

2. **Var adenomyose synlig på ultralyd ved biopsitaking:**

☐ Adenomyom ☐ Diffus forstørrelse av en vegg

☐ Små cyster ☐ Hyperekkogen JZ

3. **Pipelleprøve tatt**

☐ ja ☐ nei

4. **Status under operasjonen:**

☐ Blødning ☐ Organskade

☐ Skade i serosa/perforasjon ☐ Ingen synlige

5. Tidsbruk hele prosedyren: _____ min

6. Kommentar (f. eks. komplikasjon, hvor blødning, hvilken biopsinål etc):

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