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Adenomyosis
Imaging features for diagnosing the disease and treatment effects of bromocriptine

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Cover illustration: Transvaginal ultrasonographic image from one of the first women that was included in study I, with permission.

Adenomyosis- Imaging features for diagnosing the disease and treatment effects of bromocriptine

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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“The glory of medicine is that it is constantly moving forward, that there is always more to learn. The ills of today do not cloud the horizon of tomorrow, but act as a spur to greater effort.”

William Mayo 1926

To all women

POPULÄRVETENSKAPLIG SAMMANFATTNING

Första gången sjukdomen nämns är år 1860, då Rokitansky beskriver en växt inuti livmodervägg innehållande öar av slemhinna. I slutet av 1800-början av 1900-talet beskrevs flertal förändringar i livmoder och bukhinna. Man kallade förändringarna adenomyom, vilket var ett samlingsnamn för de 3 tillstånden muskelknutor, endometrios och adenomyos. Olika teorier framhölls om vad som orsakade fynden och forskare var inte ense. 1920 lyckades Dr Cullen, som var gynekolog, ena forskarna om att särskilja livmoderslemhinna som återfinns i bukhålan (endometrios) från slemhinna som återfinns i livmoderns muskelvägg (adenomyos) och fastställde att det rör sig om 2 olika sjukdomar. Dr Cullen beskrev symtomen vid adenomyos som ”långdragen extremt smärtsam menstruationsblödning”. Sjukdomen fick 1925 sitt nuvarande namn, adenomyos.

Trots att det har gått 100 år sedan forskare beskrev adenomyos och endometrios som 2 olika tillstånd, har inte det antagandet levt vidare. Vi tar till oss ny kunskap om endometrios och blir allt bättre på att ta hand om drabbade kvinnor. Forskning om adenomyos hade dock fallit i en nästan 100-årig sömn, för att sakta väckas till liv under de senaste 10-20 åren och ta fart på senare år.

De två tillstånden har gemensamt att de utgörs av livmoderslemhinna på avvikande ställen vilket ger smärta kopplat till menstruationsblödning. Förutom smärtsamma menstruationer har kvinnor med adenomyos även kraftiga blödningar. Många kvinnor lider av båda tillstånden samtidigt, vilket försvårar forskningen. Det är också vanligt att kvinnor med adenomyos även har muskelknutor, vilket ger liknande symtom.

Den snabba utvecklingen av teknik har möjliggjort framsteg inom forskning på kvinnans reproduktionsorgan. Tidigare gjordes så gott som all forskning på livmödrar som tagits bort i samband med kirurgiska ingrepp. Utvecklingen av röntgen såsom magnetkamera och ultraljud har lett till forskning som ej var möjlig tidigare. Nu behöver man inte operera bort en livmoder för att undersöka den, utan kan med radiologiska tekniker följa olika förlopp. Detta har gjort att kunskapen om olika symtom och diagnoser ökat och därmed möjlighet att behandla tillstånd med läkemedel.

Vi känner inte till den bakomliggande orsaken till varför en del kvinnor drabbas av adenomyos. Det finns olika teorier. På 90-talet gjordes en del studier på möss, vilket visade ett eventuellt samband med förhöjda nivåer av hypofyshormonet prolaktin i livmoderslemhinnan. Om detta samband gäller även hos människa har inte tidigare studerats. Det ville vi ta reda på genom att undersöka om förhöjda nivåer av prolaktin i livmodern är förknippat med adenomyos och om det leder till rikliga menstruationer. Om så är fallet borde man kunna minska kvinnors symtom genom att sänka prolaktinnivåerna.

För att undersöka det behandlade vi 23 kvinnor som lider av rikliga blödningar pga adenomyos, med bromokriptin, ett läkemedel som sänker nivåerna av prolaktin. Slutsatsen

var att kvinnorna fick mindre blödningsbesvär och mindre mensvärk under de 6 månader som de behandlades med läkemedlet. Kvinnorna genomgick ultraljuds och magnetkameraundersökning före och efter behandlingen. Vi kunde inte se någon röntgenologisk skillnad före och efter behandlingen, annat än hos ett fåtal kvinnor. Detta var ett förväntat resultat då 6 månaders behandling sannolikt är för kort tid att förvänta sig röntgenologiska förändringar. För att ytterligare undersöka läkemedlet bromokriptins effekt och verkningsmekanism togs slemhinneprover från livmodern före och efter behandlingen. Nivåerna av prolaktin i slemhinnan förändrades inte, men gener som styr celldöd ökade och gener som styr tillväxt av slemhinnan hämmades. Detta kan vara en del i förklaringen till varför kvinnorna fick mindre blödningar och mindre smärtor efter behandlingen.

Under studiernas gång blev det tydligt att det saknas klara röntgenologiska kriterier för när en kvinna har adenomyos, både vad gäller undersökning med magnetkamera och ultraljud. Gemensamma riktlinjer om vilka parametrar som krävs för diagnos saknas och uppfattningen om vad som är av vikt, skiljer sig mellan bedömare. Som patient är det viktigt att få samma diagnos oberoende av vem som har bedömt bilderna. För att undersöka hur samstämmiga olika bedömare är gjorde vi magnetkameraundersökning samt ultraljudsundersökning på 51 kvinnor med rikliga menstruationer där misstanke om adenomyos fanns. Fyra röntgenläkare bedömde magnetkamerabilderna och fem gynekologer som är specialiserade i ultraljudsundersökning bedömde ultraljudsbilderna. Vi ville ta reda på hur bedömningarna skildes åt och vilka tecken på sjukdom som flest bedömare var eniga om. Studien visade att röntgenologerna som bedömde magnetkamerabilderna var mer eniga i de enskilda tecknen på sjukdom som sågs, jämfört med ultraljudsbedömarna. Trots det var gynekologerna som bedömde ultraljudsbilderna mer eniga i sin slutgiltiga bedömning huruvida kvinnan hade adenomyos eller inte. Studien visar svårigheten att ställa diagnosen, även för erfarna läkare. Resultatet visar att fler röntgenologiska studier behövs. Kriterier för vad som krävs för att ställa diagnosen behöver fastslås, så att svaren inte skiljer sig beroende på vem som tolkar bilderna.

Den sammantagna slutsatsen av studierna, är att vi som ett första steg behöver fastslå riktlinjer för vad som krävs för att ställa diagnosen adenomyos via magnetröntgen och ultraljud samt öka den kunskapen bland gynekologer. När vi har lärt oss att ställa diagnosen systematiskt kan detta underlätta studier av symtom samt bakomliggande orsaker. Denna kunskap kan därefter leda till riktade läkemedelsbehandlingar mot adenomyos.

ABSTRACT

Background

Adenomyosis is a benign uterine disease, causing various symptoms including heavy menstrual bleeding (HMB) and pelvic pain. In affected women, endometrial glands and stroma are located in the myometrium surrounded by hypertrophied myometrial tissue. The eutopic endometrium is associated with increased proliferation, high migration and a high invasive capacity. The knowledge of the pathogenesis is largely unknown; however, mice models have shown a link between increased uterine concentration of prolactin (PRL) and the disease. The gold standard in the treatment for hyperprolactinemia is the dopamine agonist bromocriptine. Vaginal administration is effective in reducing serum PRL and has less gastrointestinal side effects than oral administration. Reducing uterine PRL may improve symptoms and could be a possible medical treatment in the future if PRL is associated with the disease. Whether bromocriptine reduce uterine PRL is not known.

In the presence of clinical symptoms adenomyosis diagnosis can be confirmed using Magnetic Resonance Imaging (MRI) or Transvaginal ultrasonography (TVS). The reproducibility has been reported high for both modalities, but consensus criteria for diagnosing the disease are still lacking in both MRI and TVS.

Aim

The overall aim was to examine the effects of the dopamine agonist bromocriptine in women with adenomyosis and to assess agreement between MRI and TVS for imaging features associated with the disease. The specific objectives were to assess symptoms before and after treatment with vaginal bromocriptine. Another objective was to assess changes in MRI and TVS during treatment and to analyze changes in the endometrium regarding protein biomarkers and differentially expressed genes. A further objective was to compare the inter-rater agreement between MRI and TVS for diagnosing adenomyosis and for various features in the same set of women.

Methods and results

In ***study I***, 18 women from Sweden and 1 woman from the USA with regular HMB and suspected adenomyosis were included. Women were treated with a daily dose of 5mg vaginal bromocriptine for 6 months. Self-administered questionnaires were used to assess symptoms at baseline, 3 months, 6 months, and 9 months (3 months after cessation of the study drug). The Pictorial Blood Assessment Chart (PBLAC) and the Aberdeen Menorrhagia Clinical Outcomes Questionnaire (AMCOQ) were used to assess the amount of bleeding. The Visual Analog Scale for pain (VAS) and the McGill Pain Questionnaire (MPQ) were used to assess pain. The Fibroid Symptom Quality of Life (UFS-QOL), the Endometriosis Health Profile (EHP-30) and the Female Sexual Function Index (FSFI) were used to assess quality of life. PBLAC, AMCOQ, VAS, and MPQ showed a significant reduction at 6 months, indicating an improvement in bleeding and pain severity. An

improvement in quality of life was seen with UFS-QOL. Total EHP and FSFI did not show any significant differences.

Study II was a secondary outcome of ***study I***. The 18 women at the Swedish site underwent MRI and TVS at baseline and after treatment with vaginal bromocriptine. The MRIs were assessed by one radiologist and the TVS were assessed by one gynecologist specialized in gynecologic ultrasound. For MRI, no significant differences were found in Junctional Zone (JZ) max, JZ differential, ratio JZ/myometrium or myometrial cysts. TVS showed a significant reduction in JZmax and in asymmetric wall thickness. No significant changes were seen in irregular JZ, fan shaped shadowing, striations, hyperechogenic islands, or cystic lesions.

In ***study III,*** MRI and TVS images from the same set of 51 women with HMB and suspected adenomyosis were assessed. MRIs were assessed by four radiologists and the TVS images were assessed by five gynecological ultrasonographers. For MRI, the inter-rater reliability for JZ measurements were 'moderate to good'. Inter-rater agreement for wall asymmetry and irregular JZ were 'moderate', while the inter-rater agreement for globular uterus shape was 'poor' and 'fair' for cysts. The overall subjective impression if adenomyosis was present or not was 'fair'. For TVS, the inter-rater agreement for globular uterus shape and wall asymmetry were 'moderate'. Irregular JZ, fan shaped shadowing and buds or striations were 'fair' and the inter-rater agreement for cysts were poor. Measurement of the JZ did not show any agreement between the raters. The overall subjective impression of whether adenomyosis was present or not was 'moderate'.

Study IV was a secondary outcome of ***study I***. Endometrial biopsies were taken at baseline and after treatment with vaginal bromocriptine. 12 paired (i.e 12x2) samples were included in the study. Analyses were carried out to evaluate PRL and differentially expressed genes before and after treatment were performed. A significant reduction in serum PRL was observed following bromocriptine treatment, but no changes in the eutopic endometrium. Gene expression analysis showed a significant upregulation of *BAX* (a marker of apoptosis) and downregulation of Ki67 (a marker of proliferation) and downregulation of genes associated with glucose metabolism.

Conclusion

A significant improvement in menstrual bleeding, pain, and quality of life was seen after 6 months of vaginal bromocriptine treatment. A significant decrease in JZmax and asymmetric myometrial wall thickness were demonstrated with TVS. An anti-proliferative effect by downregulating genes associated with glucose metabolism was seen in the eutopic endometrium after treatment.

The inter-rater agreement for diagnosis was higher for TVS than for MRI despite MRI manifesting higher agreement in most features associated with the disease.

LIST OF SCIENTIFIC PAPERS

I. **Vaginal bromocriptine improves pain, menstrual bleeding and quality of life in women with adenomyosis**

ANDERSSON JOHANNA KARIN, Khan Z, Weaver AL, Vaughan LE, Gemzell-Danielsson K, Stewart EA

Acta Obstet Gynecol Scand. 2019 Oct;98(10):1341-1350

II. **Vaginal bromocriptine for treatment of adenomyosis: Impact on magnetic resonance imaging and transvaginal ultrasound**

ANDERSSON JOHANNA KARIN, Pozzi Mucelli R, Epstein E, Stewart EA, Gemzell-Danielsson K

Eur J Obstet Gynecol Reprod Biol. 2020 Nov;254:38-43

III. **Inter-rater agreement for diagnosing adenomyosis using Magnetic Resonance Imaging and Transvaginal Ultrasonography**

ANDERSSON JOHANNA KARIN, Pozzi Mucelli R, Dueholm M, Fridsten S, Grigoriadis A, Guerrierro S, Leone F, Valentin L, Van den Bosch T, Voulgarakis N, Gemzell-Danielsson K, Epstein E

Manuscript

IV. **Bromocriptine reduces endometrial cell growth in women with adenomyosis by targeting glucose metabolism**

Ponandai-Srinivasan S, ANDERSSON JOHANNA KARIN, Frisendahl C, Korsching E, Tengelin J, Pavone D, Stewart E, Parameswaran Grace Luther L, Rao Bogavarappu N, Gemzell-Danielsson K

Manuscript

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LIST OF ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
AMCOQ	Aberdeen Menorraghia Clinical Outcomes Questionnaire
BMI	Body Mass Index
cDNA	Complementary DNA
COC	Combined Oral Contraceptives
DE	Differentially Expressed
DNA	Deoxyribonucleic acid
EHP-30	Endometriosis Health Profile Questionnaire
ELISA	Enzyme-Linked Immunosorbent Assay
FSFI	Female Sexual Function Index
GnRH	Gonadotropin Realizing Hormone
HMB	Heavy Menstrual Bleeding
HRQOL	Health Related Quality Of Life
HSIL	High grade Squamous Intraepithelial Lesion
ICC	Intraclass Correlation Coefficient
IUD	Intrauterine Device
IVF	In Vitro Fertilization
LNG-IUS	Levonorgestrel releasing intrauterine system
MD	Mean Difference
MPQ	McGill Pain Questionnaire
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
OAB	Overactive Bladder
PACS	Picture Archiving and Communication System
PBLAC	Pictorial Blood Loss Assessment Chart
PCR	Polymerase Chain Reaction
PRL	Prolactin
PRL-R	Prolactin Receptor
REDCap	Research Electronic Data Capture
RNA	Ribonucleic Acid

RNA-Seq	RNA Sequencing
SD	Standard Deviation
SPSS	Statistical Package for the Social science
SSS	Symptom Severity Subscore
TCRE	Trans Cervical Resection of the Endometrium
TF	Tissue Factor
TVS	Trans Vaginal ultrasonography
UAE	Uterine Artery Embolization
UFS-QOL	Uterine Fibroid Symptom Quality of Life Questionnaire
VAS	Visual Analogue Scale
VCI	Volume Contrast Imaging
WHO	World Health Organization
JZ	Junctional Zone
JZdiff	Junctional Zone differential
JZmin	Minimal Junctional Zone thickness
JZmax	Maximal Junctional Zone thickness
JZmax/myom	JZmax/myometrium

1 INTRODUCTION

The Latin word “Adenomyosis” stands for Adeno- glands, myo- muscle, and osis- derangement which describes the disease well. Adenomyosis is a benign disease where glands and stroma from the endometrium are found in the myometrium surrounded by hypertrophic myometrial cells (1, 2).

There are 3 different types of adenomyosis; The most common type is “diffuse”, where the adenomyotic tissue is scattered diffusely throughout the myometrium. In “focal adenomyosis”, the lesion is located in one restricted area in the myometrium. “Cystic adenomyosis” is rare and consists of hemorrhagic cystic structures.

As far as possible, this thesis focuses on diffuse adenomyosis.

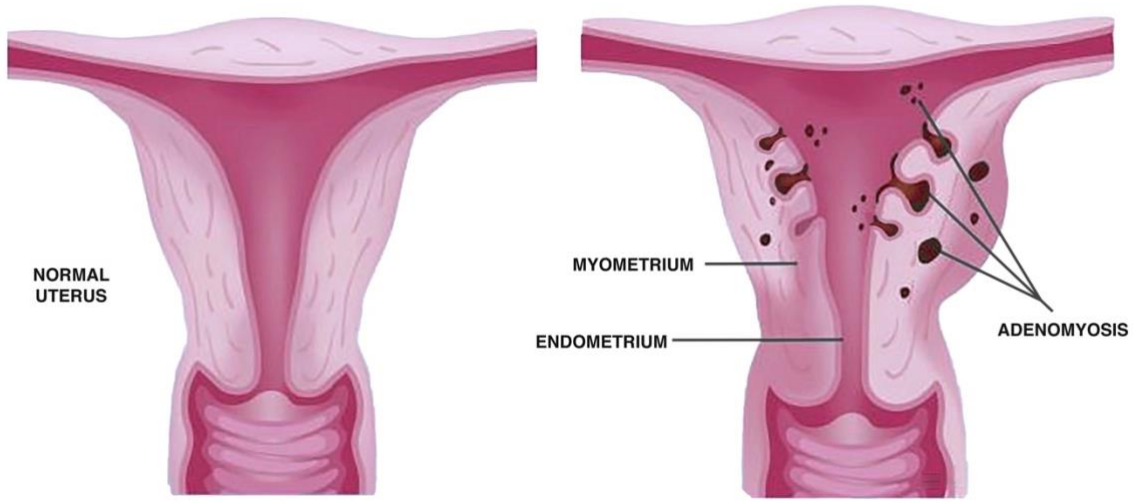

Figure 1. A normal uterus to the left and an uterus with diffuse adenomyosis to the right. The figure is adapted from Dr. Liang, *Sydney Fibroid Clinic website*

1.1 HISTORICAL BACKGROUND

The first time the disease was mentioned in the literature was in 1860, when Rokitansky described a growth inside the uterus, containing islands of endometrium. The discovery was followed by other similar findings in the uterus and peritoneal cavity. All different findings were called “adenomyoma”, a collective name for what is today known as “leiomyoma”, “endometriosis” and “adenomyosis”. In 1925, Dr. Cullen determined that the two different findings of conditions with ectopic endometrium were different diseases; “endometriosis” for endometrium growing in the peritoneal cavity and “adenomyosis” for endometrium inside the myometrium of the uterus. Dr. Cullen described the clinical condition of adenomyosis as “lengthened menstrual periods and a great deal of pain”.

The current definition of adenomyosis was suggested 1972 by the pathologist Bird; "Adenomyosis may be defined as the benign invasion of the endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium"(1).

It is striking, that we, 50 years later, still have limited knowledge about the disease, and that we are still debating if endometriosis and adenomyosis are one or two different diseases. It is common to be affected by both diseases at the same time and they have similar symptoms, which makes research in the field challenging.

1.2 PREVALENCE

Adenomyosis is a common disease, but the exact prevalence is not known. In studies conducted over the last 20 years, a prevalence of 23- 43 % (3-7) is reported among women who underwent a hysterectomy. The studies are retrospective and based on histological diagnosis. A limitation of the studies is that only women who need a surgical intervention are included, which may be more severe cases (Figure 2). Other limitations are that both pre- and postmenopausal women are included in most studies and that there are heterogenous indications for the surgery.

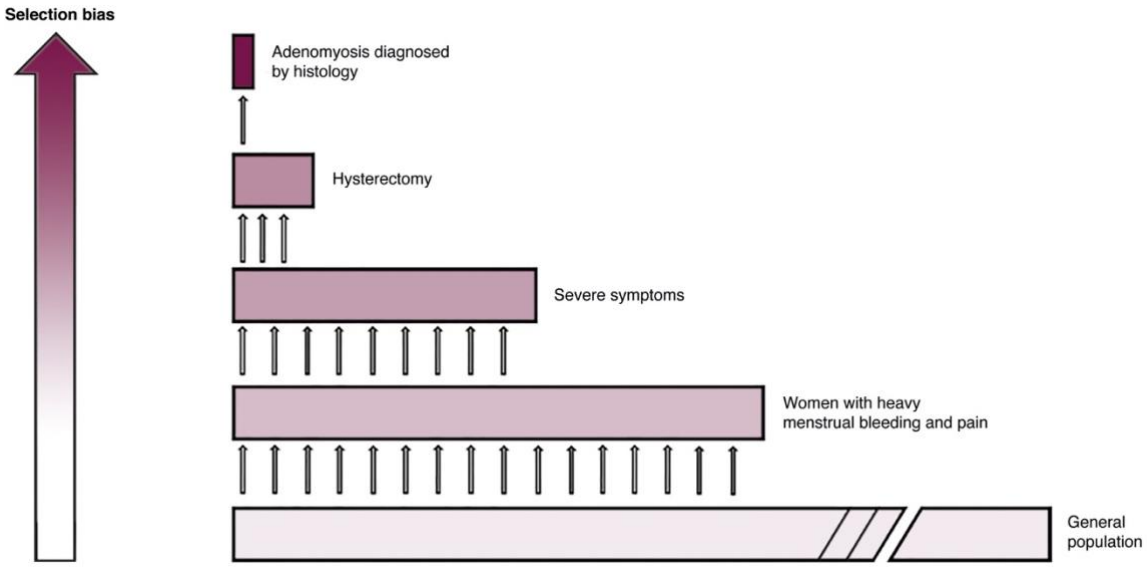

Figure 2: It is important to be aware of the selection bias in retrospective studies including women with a histological diagnose after hysterectomy. The figure is adapted from Upson et al, *Semin Reprod Med 2020*

Studies assessing prevalence among women with symptoms associated with the disease are lacking. However, in one study, from Nepal, where there is limited access to preoperative diagnostic tools such as Magnetic Resonance Imaging (MRI) and Transvaginal ultrasound (TVS), 45 % of women that underwent a hysterectomy on the indication heavy menstrual bleeding (HMB), were found to have adenomyosis (7). In studies utilizing TVS in women referred to university clinics, 21-34 % were diagnosed with adenomyosis (8, 9).

Diffuse adenomyosis is the most common form of adenomyosis. One study reported diffuse adenomyosis in 84 % of women with histologically confirmed adenomyosis (4).

Thus, adenomyosis is a common disease but the true prevalence among premenopausal women with HMB and pelvic pain needs to be further explored.

1.3 COMORBIDITY

As many as 80 % (10, 11) of women with adenomyosis and dysfunctional uterine bleeding may have additional pelvic pathology, such as endometriosis or leiomyomas that interfere with the symptoms and makes research in the field challenging.

In women with adenomyosis that underwent a hysterectomy, 40-46 % were reported to have comorbidity with endometriosis (4, 12). Especially deep infiltrating endometriosis seems to be associated with adenomyosis (10, 13, 14).

1.4 MOLECULAR MARKERS

Adenomyosis is suggested to be associated with altered apoptosis and proliferation (15-18).

The protein Ki67 is associated with cell proliferation. The presence of Ki67 was reported higher in the eutopic endometrium in women with adenomyosis than in healthy women (Figure 3) (16), indicating a more rapid proliferation. The same study also showed reduced apoptosis, but not related to the Bcl 2 gene, which is a common marker for apoptosis.

Tissue Factor (TF) is a cytokine that plays a role in angiogenesis and apoptosis. TF has shown to be elevated in both eutopic and ectopic endometrium in women with adenomyosis (18).

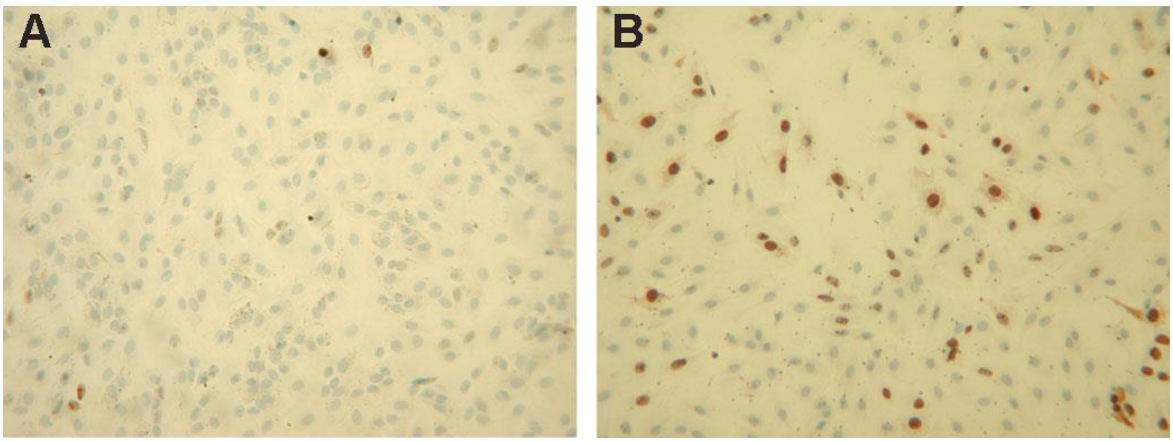

Figure 3: Immunocytochemical staining of Ki67 (brown color)in the endometrium from a woman without adenomyosis (to the left) and a woman with adenomyosis (to the right). The figure is reused from Yang et al 2007, with permission provided by Rights Link Copyright clearance center.

Differentially expressed (DE) genes were seen in adenomyotic mice compared with controls suggesting impaired differentiation of cells during fetal development of the myometrium (19). DE genes in the myometrium in women with adenomyosis compared with myometrium from healthy women are also reported (20). The enzyme aromatase cytochrome P450 catalyzes the conversion of androgens to estrogen and has been shown to be overexpressed in women with adenomyosis and endometriosis (21).

1.5 HISTOLOGY

The disease has, until recently, been a histological diagnosis, even though consensus for histological criterion is lacking. Adenomyosis is histologically defined as endometrial glands invading the basalis layer of the endometrium. Areas of endometrial glands and stroma are found in the myometrium, causing hyperplasia and hypertrophy of the surrounding myometrium (1). Different minimal depths are suggested (1, 2, 22). However, invasion of at least 2.5 mm below the basalis layer is used in most studies (23, 24). To make the diagnosis, hypertrophy of the surrounding myometrium must be present.

The histological diagnosis is dependent of how the biopsies are sampled and how well they represent the location of the disease. For example, in one study, 31% of the samples contained adenomyosis when three routine sections where taken, and 61% when six sections where taken (1). There is still no consensus on how the uterus should be sampled after a hysterectomy.

1.6 PATHOLOGY

The pathology of adenomyosis is unknown, but there are different theories;

1.6.1 Invasion of the endometrium

The most widespread theory proposes that the endometrium grows into the myometrium by invading a traumatized endometrial-myometrial border (25). The trauma is thought to be caused by surgical procedures, such as curettage for termination of a pregnancy or cesarean delivery. An increased incidence of adenomyosis after curettage for termination of a pregnancy has been reported (6, 26, 27). However, there are also studies where association with curettage or cesarean delivery has not be found (22, 27, 28). Therefore, it has been proposed that it may be the pregnancy itself that causes a disruption of the barrier between the endometrium and the myometrium, not the surgery. Parous women, particularly those with a first birth at an early age, are more likely to develop the disease than nulliparous women (7, 23, 28-32). Further, an increased risk has been shown with an increasing number of births (9, 23).

1.6.2 Müllerian remnants

A commonly proposed theory is that adenomyosis has a Müllerian origin, caused by altered development of the genital tract during embryonic life. The endometrium and the inner myometrium have different origins than the outer myometrium. Displaced embryonic pluripotent Müllerian remnants in the myometrium of the female fetus are thought to be able to differentiate into endometrial cells in the myometrium and cause adenomyosis.

1.6.3 Hyperperistalsis

Hyperperistalsis and increased intrauterine pressure might result in rupture of the basalis layer, leading to infiltration of the endometrium into the myometrium (33-35). Briefly, micro traumatization may lead to a cascade that increases the level of estradiol, which in turn leads to proliferation, angiogenesis and hyperperistalsis. The increased peristalsis would further exacerbate the process with increased injury and ultimately the formation of adenomyotic lesions (Figure 4).

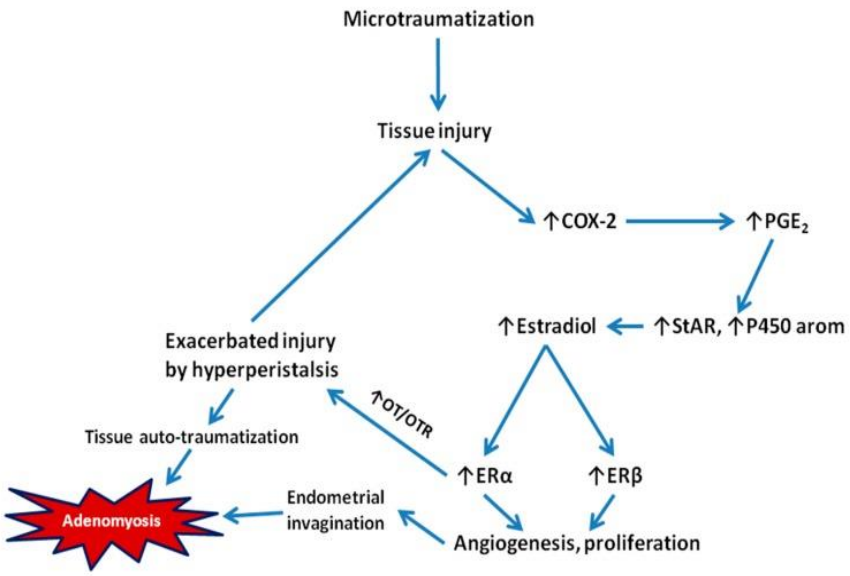

Figure 4: Model of tissue injury and repair that initiates the genesis of adenomyotic lesions. The hyperperistalsis induces increased production of Prostaglandins, which increase estrogen production. Estrogen activates the estrogen receptor α and β, leading to induction of Oxytocin (OT/OTR) that increases uterine contractions and exacerbate the injury. Reused from Guo et al, *Journal of Clinical Medicine*, with permission.

1.6.4 Immune system

A role of the immune system in adenomyosis has also been considered (36). Proliferation of specific lymphocytes and formation of antibodies may be involved in the tissue damage. Another reported risk factor are macrophages affecting the endometrial cells and causing subsequent increase in interleukin production (37).

1.6.5 Increased Estradiol exposure

Adenomyosis is shown to be associated with hyperestrogenism (38, 39). Increased estrogen exposure resulting from an early menarche, increasing age, short menstrual cycles and obesity are reported as risk factors (29, 40). Higher concentrations of estradiol in menstrual blood were detected in women with adenomyosis than in women without the disease, whereas the level in serum was equal (41). Furthermore, genes involved in estrogen synthesis are shown to be upregulated in women with adenomyosis (42, 43).

1.6.6 Prolactin

Animal models suggests that adenomyosis is related to increased uterine concentrations of Prolactin (PRL) (44, 45).

PRL is produced in the pituitary gland and in small amounts in the endometrium and the myometrium (46, 47). Prolactin acts as a smooth muscle cell mitogen in vitro (48, 49). The PRL receptor (PRL-R) has shown to be upregulated in adenomyotic uteri of mice and the degree of upregulation correlated to the histologic extent of the disease (50). There is evidence that minimal increase in the level of PRL in serum secondary to medication with

antidepressants is sufficient to cause adenomyosis both in murine and human uteri (51, 52). A retrospective human study observed a history of depression as the only factor associated with adenomyosis compared to women with leiomyoma (53).

Reducing the concentration of uterine PRL may be a possible future medical treatment option if PRL is central in the pathogenesis.

1.7 SYMPTOMATOLOGY

1.7.1 Heavy menstrual bleeding

The most common symptom is HMB (29, 32, 54, 55), reported by up to 80% of symptomatic women (4, 5, 32). The severity of HMB has been shown to correlate with the depth of glandular invasion (27, 56, 57).

1.7.2 Pelvic pain

Dysmenorrhea and other forms of pelvic pain are reported by 30 - 80 % (4-6, 8, 29, 30, 32, 53, 55) of women with adenomyosis. The severity of pain correlates to deeper foci (27, 58). In one study, where dysmenorrhea was reported by 79 % of women, the mean Visual Analogue Scale (VAS) score was as high as 8 (8). Another study reported that more than half of the women with dysmenorrhea associated with adenomyosis suffered from severe pain (6).

1.7.3 Urinary tract symptoms

A few studies have investigated bladder symptoms and concluded that overactive bladder symptoms (OAB) were significantly more frequent in women with adenomyosis than in healthy controls. Increased daytime frequency of voiding were common and 20-45 % of women with adenomyosis reported urge incontinence (59, 60). The women had TVS findings suggestive of adenomyosis as inclusion criteria. How many of them that had comorbidity with endometriosis were not determined. Thus, whether OAB is a symptom associated with adenomyosis or rather a symptom associated with endometriosis is not known. The underlying cause for OAB in women with adenomyosis needs to be further explored. One reason for the symptom could be pressure on the urinary bladder from an enlarged uterus. Further, chronic pelvic pain leads to nerve sensitization that may cause OAB.

1.7.4 Subfertility

Due to high comorbidity with endometriosis, it is a challenge to study whether adenomyosis is associated with impaired fertility. All existing studies have been conducted in fertility centers, thus in women with known subfertility. Adenomyosis may cause alterations in the endometrium and inner myometrium (Junctional Zone (JZ)) causing disturbed endometrial receptivity (61, 62). JZ thickness was shown to be the best predictive

factor for implantation failure (62, 63). Thus, further studies are required to determine the association with adenomyosis and fertility.

1.8 RADIOLOGICAL FINDINGS

1.8.1 Magnetic Resonance Imaging

The ability to make the diagnosis of adenomyosis by MRI has a long tradition and focuses on the inner part of the myometrium, the JZ. Adenomyosis is a disease in the JZ and changes its appearance. However, despite the knowledge of features associated with adenomyosis on MRI, no consensus exists regarding criteria for the diagnosis. The most widespread criteria for diagnosing adenomyosis with MRI is an increased thickness of the JZ $\geq$12mm (64).

MRI T2-weighted sequences are the key to diagnosing adenomyosis since the sequences highlight the JZ. The myocytes in the JZ have a different morphology from the myocytes in the outer myometrium, with a greater relative nuclear area, a looser extracellular matrix, and lower water content (65). Therefore, on T2-weighted images, the JZ appears darker (hypointense) than the outer myometrium (intermediate intensity) (66). The endometrium appears bright (hyperintense).

There are no guidelines suggesting a MRI protocol for the examination of adenomyosis. Bowel movements create artifacts that decrease the quality of the image of the uterus. Therefore, it is an advantage if the women fast before the MRI examination and if antispasmodic drugs are used. To obtain an optimal image of the JZ, an oblique axial sequence perpendicular to the uterine long axis should be taken (11).

The JZ of women with adenomyosis is thicker than in healthy women, due to the smooth muscle hyperplasia that surrounds the ectopic endometrial cells. However, a normal JZ can mimic that of adenomyosis. The JZ is thicker the first days of the menstrual cycle, and contractions can be mistaken for a focal thickening (67). Therefore, adding elements to the criteria for adenomyosis is suggested, such as an irregular appearance of the JZ and its relation to the thickness of the entire myometrium (Figure 5) (11, 68, 69).

The JZ border is often irregular to the myometrium, due to the different depth of the lesion. The level of irregularity can be measured by the difference between the maximal and minimal thickness of the JZ, the JZ differential (JZdiff). A JZ diff >5 mm may be more reliable for diagnosing adenomyosis than a JZ thickness of >12 mm (11, 68)

Since the JZ is part of the myometrium and adenomyosis is located in the JZ, the percentage of the JZ related to the total myometrium is of interest (JZ/myom). The thickness of the myometrium (healthy myometrium+JZ) is measured at the same level as JZmax. A ratio of 40% indicates adenomyosis (11).

Intramyometrial cysts are pathognomonic for the disease but are found in only one-third to half of the affected women (11, 64).

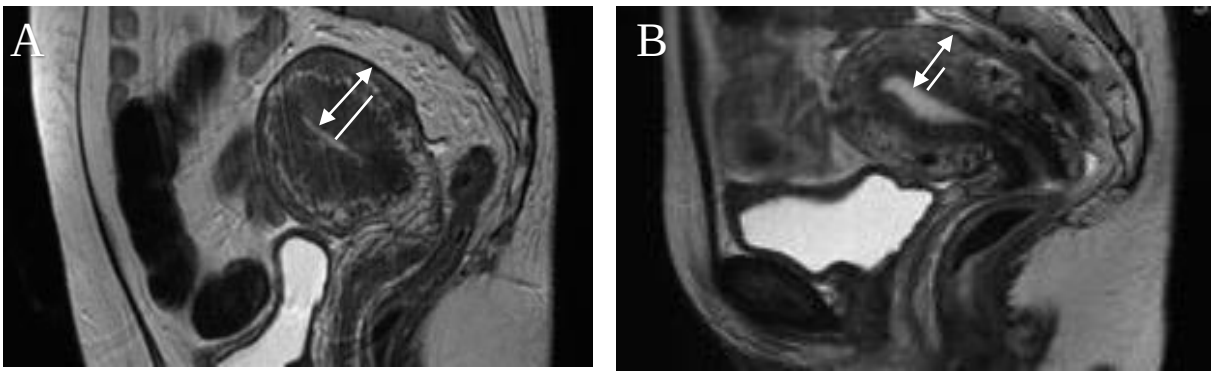

Figure 5A-B: T2-weighted MRIs showing the part of the myometrium (dubbel arrow) that consist of the JZ (line). A) The JZ >12mm and most of of the myometrium is involved B) The JZ >12mm and less than half of the myometrium is involved. The images are used with permission from women who participated in the study.

1.8.2 Transvaginal ultrasonography

The technique for TVS has developed over the last decade and the ability to visualize signs of adenomyosis has improved since heterogeneity of the myometrium and presence of myometrial cysts were proposed to indicate the disease (24, 64, 68).

Similar to MRI there is no consensus on which diagnosis criteria to include and require for a diagnosis of adenomyosis. Different features on 2-dimensional (2D) ultrasound are associated with the disease (Figure 6). Adenomyosis enlarges the corpus uteri and makes the shape globular (70). It is common that the lesions are more widespread in one of the walls leading to asymmetrical myometrial thickening (8, 71, 72). The endometrial junction appears irregular as the ectopic endometrium grows into the myometrium (70, 71). Hyperechogenic striations and areas corresponding to ectopic endometrium are found within the myometrium and fan shaped shadowing is present (70).

Myometrial cysts are seen as anechoic areas and are considered highly specific for the disease (70, 73-75).

It is difficult to visualize the JZ with 2D TVS. 3-dimensional (3D) TVS makes it possible to assess the JZ in a reconstructed coronal plane. To make the 3D-image, a series of 2D images are collected and converted into a volume that can be visualized from different angles. The JZ appears as a hypoechogenic zone around the hyperechogenic endometrium. The JZ can be measured at the 3D coronal view. JZmax ≥ 8mm and JZdiff ≥4mm is proposed as a cut-off for adenomyosis (Figure 7) (75).

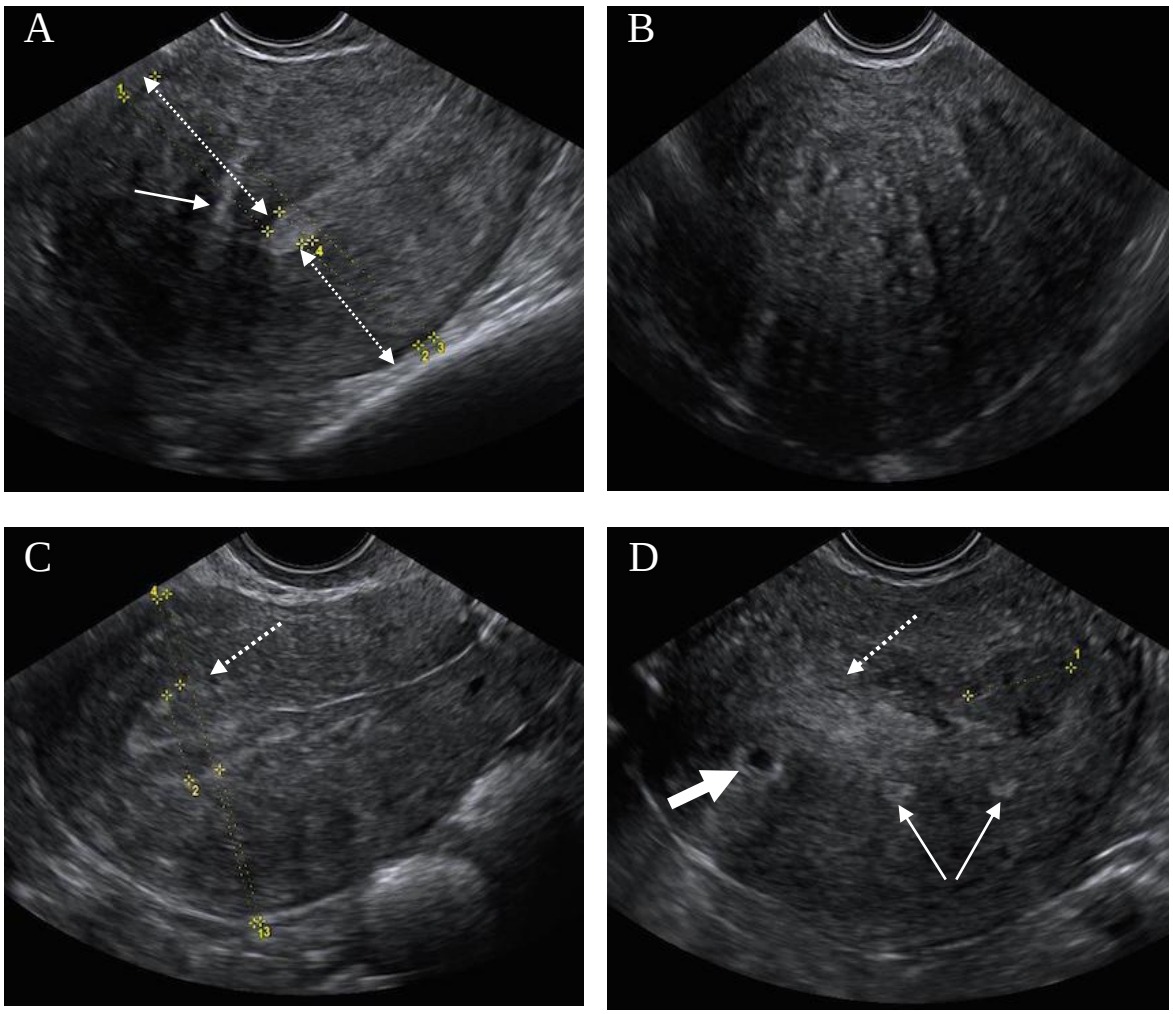

Figure 6 A-D: TVS images from women in the study. Figure D is reused from paper II. The images are used with permission from the study participants. Different TVS features associated with adenomyosis are shown. A) The uterus has a globular shape and the myometrial walls have asymmetric thickness (dashed dubbel arrow) and hyperechogenic areas in the myometrium are present (thin arrow). B) Fan shaped shadowing is present in the myometrium C) The endomyometrial junction is irregular and the border is difficult to follow (dashed arrow) D) The endomyometrial junction is irregular and the border is difficult to follow (dashed arrow). Hypoechogenic myometrial cysts (thick arrow) and hyperechogenic endometrial spots are found within the myometrium (thin arrow).

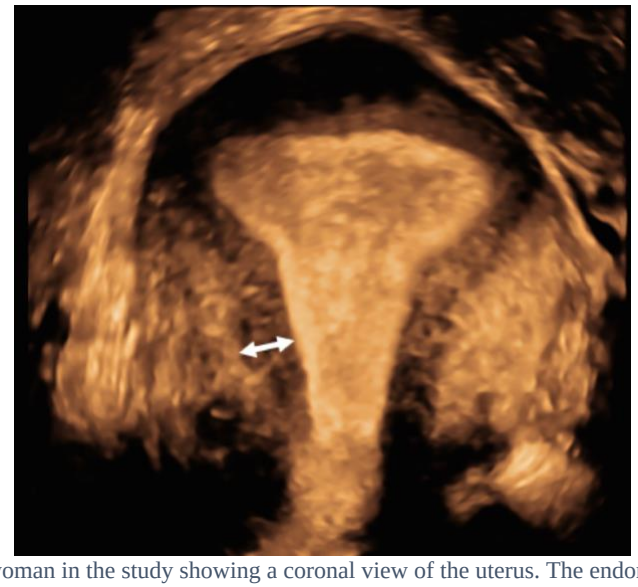

Figure 7: 3D TVS from a woman in the study showing a coronal view of the uterus. The endometrium appears bright. The JZ (dubbel arrow) appears darker than the rest of the myometrium. The image is reused from paper II, with permission.

1.9 TREATMENT

Traditionally, hysterectomy has been the only treatment option for women with adenomyosis and is still the only curative treatment. However, uterine sparing surgery is possible and medical treatments are available that reduce HMB and pain in women with adenomyosis.

1.9.1 Uterine sparing surgical interventions

1.9.1.1 Excision by laparoscopy or laparotomy

For diffuse adenomyosis, only part of the lesion will be removed, leaving a defect in the myometrium. Different techniques are used to close the defect in the myometrium. The surgery is only suitable in large centers, due to the high risk of complication.

When resecting diffuse adenomyosis in heavily enlarged uterii, improvement in bleeding and pain has been reported after laparotomy and laparoscopic surgery (76-79). However, most studies have a short follow-up time. Recurrency with regrowth of the adenomyosis is common (78, 79).

1.9.1.2 Trans Cervical Resection of the Endometrium and Ablation techniques

The aim of the treatment is to damage the basal layer of the endometrium to prevent regeneration and thus reduce the amount of menstrual bleeding. Endometrial resection is performed with a loop or rollerball and is operator-dependent while thermal ablation techniques use devices from different companies. Different inclusion criteria are used. However, the different techniques show similar results. Few studies distinguish women with adenomyosis from other diseases that also cause HMB (leiomyoma, polyps or unknown causes) thus making the evaluation of the results uncertain.

The short-term results for surgery in women with adenomyosis are equal to the results seen in women with other diseases. Improvement in bleeding and pain with a success rate of over 80% is reported (80-83). However, the treatment effect decreases over time and 30% of women with adenomyosis need further surgery within 3 years (83).

Depth of penetration is an important prognostic factor. Women with deep adenomyosis have an increased risk for treatment failure (80, 82, 84, 85).

1.9.1.3 Uterine artery embolization

Uterine artery embolization (UAE) is a radiological procedure using angiography. Synthetic embolic agents are inserted in the uterine arteries, leading to tissue ischemia and infarction. UAE is an established treatment for fibroids, but limited efficacy has been shown in adenomyosis. Promising short-term results (3-12 months) showing reduced bleeding and pain are reported, with success rates of 80-95 % (86, 87). However, the long-term results are insufficient with only 50-60 % of the women reporting symptom relief 3-5 years after the embolization (88-90).

1.9.2 Medical Treatment

Studies focusing on medical treatment for adenomyosis are lacking. Reasons for that may be the comorbidity with leiomyomas and endometriosis together with the fact that adenomyosis until recently has been a histological diagnosis. There is no available drug that is labeled for adenomyosis. However, most medical treatments for endometriosis and leiomyoma that improve the symptoms HMB and pain may also be effective for adenomyosis.

1.9.2.1 Levonorgestrel intrauterine system and other progestins

The levonorgestrel-releasing system (LNG-IUS), a progestin, is the most studied medical treatment for adenomyosis and the only drug that is labeled for idiopathic HMB. Progestins such as LNG induce downregulation of the estrogen receptor and prevent endometrial proliferation (91) leading to reduced HMB and pain (92). The LNG-IUS releases 20 microgram LNG over 24h. A 90 % reduction in Pictorial Bleeding Assessment Chart (PBLAC) (93, 94) and almost total pain relief (93, 95) at follow-up at 6 months to 5 years has been reported. Other studies report similar results (96, 97). Satisfactory rate is reported to be 70-90% at 3 years after insertion of an LNG-IUS (95, 96). Further, LNG-IUS improved OAB significantly 6 months after insertion (98).

Notable, in the majority of studies, the results are analyzed in women that continued the treatment for the entire study period. Women who discontinued the study are not included, which will bias the results. The most common reasons for dropping out of the studies are expulsion of the device, continuation of HMB, or pain. Women who are dissatisfied with the treatment frequently chose to remove the device and will thus be excluded from the study, which may give false positive results for PBLAC and VAS in the studies.

Other progestins have high efficacy for reducing pain in endometriosis and are commonly used for adenomyosis even though studies in women with adenomyosis are scarce. In two small pilot studies, Dienogest showed improved pain in women with adenomyosis, but with less improvement in HMB (99, 100).

1.9.2.2 Other hormonal treatments

Combined oral contraceptives (COCs) with estrogen and progestin induce decidualization and subsequent atrophy of the endometrium and reduces HMB and pain (101). COCs is commonly used for the treatment of adenomyosis with satisfactory pain and bleeding control but reported to be less effective than LNG-IUS (102).

Gonadotropin realizing hormone (GnRH) suppresses ovarian estrogen production. GnRH analogues have shown to be effective in the treatment of HMB and pain in women with adenomyosis (100, 103). GnRH also decreases the expression of aromatase P450 (104). Aromatase cytochrome P450, the enzyme that catalyzes the conversion of androgens to

estrogen, is overexpressed in women with adenomyosis (21). Thus, besides causing a hypoestrogenism in the endometrium, GnRH may have a specific effect on the disease.

Aromatase inhibitors are in clinical use for treating adenomyosis and are believed to be effective. However, supporting evidence from the scientific literature is scarce (105).

1.10 BROMOCRIPTINE

Bromocriptine is a dopamine receptor agonist that inhibits pituitary secretion of PRL. It is approved since 1978 and is the gold standard of treatment for hyperprolactinemia. Other indications include Parkinson´s disease, acromegaly and inhibition of lactation in breastfeeding women. In the USA the drug is also approved for the treatment of type 2 diabetes (106).

The most common side effects of bromocriptine are nausea, headache, tiredness, dizziness and vomiting . Uncommon side effects include low or high blood pressure, orthostasis, fainting, compulsive behavior such as an increased urge to spend money, gambling and a hypersexual behavior. Serious side effects are rare but include myocardial infarction, arrhythmia, pericarditis and changes in the cardiac valve. Contraindications includes hypersensitivity to ergot alkaloids, syncopial migraines and uncontrolled hypertension.

Vaginal administration is well tolerated and effective in reducing circulating PRL levels in women with hyperprolactinemia (107, 108) and has fewer gastrointestinal side effects than oral administration (107, 109).

Bromocriptine increases glucose tolerance and decreases insulin resistance, leading to improved glycemic control (110, 111). The underlying mechanism of action is not known in detail.

1.11 SUMMARY

Comorbidity with leiomyoma and endometriosis is very common. Until recently, adenomyosis has been a histological diagnosis only possible after a hysterectomy. Taken together this may have contributed to the lack of knowledge about the disease. Still, the pathogenesis remains to be elucidated. Further, there are no consensus neither when relying on histology nor on radiology on required diagnostic criteria.

With my thesis, I hope to fill in some of the knowledge gaps that are needed to learn more about adenomyosis. Increased knowledge about pathogenesis and radiological features is fundamental for the possibility to correctly make the diagnosis and for the development of future disease-specific medical treatment.

2 RESEARCH AIMS

The overall aims of this thesis was to examine the effects of the dopamine agonist bromocriptine in women with adenomyosis and to assess agreement between MRI and TVS for diagnosing the disease.

The specific aims of the different studies were:

Study I: To assess subject symptoms (bleeding and pain) and evaluate quality of life, in women diagnosed with adenomyosis before and after treatment with vaginal bromocriptine.

Study II: To assess changes in the uterus by MRI and TVS in women with adenomyosis before and after treatment with vaginal bromocriptine.

Study III: To compare the inter-rater agreement between MRI and TVS for diagnosing adenomyosis and for various features in the same set of women.

Study IV: To analyze endometrial biopsies from patients with adenomyosis before and after bromocriptine treatment, for RNA and protein biomarkers and to decipher the mechanism of action for bromocriptine.

3 MATERIAL AND METHODS

3.1 TABULATED OVERVIEW OF STUDIES

	Design and study subjects	Outcome	Subjects in final analyses	Method	Statistical analyses
Study I	Pilot study including women with adenomyosis treated with vaginal bromocriptine for 6 months.	Changes in bleeding, pelvic pain, and quality of life after treatment.	19	Study visit 1. Baseline 2. 3 months of treatment 3. 6 months of treatment 4. 9 months from baseline. 3months after cessation of study drug. Subjects filled in PBLAC, VAS, MPQ, UFS-QOL, EHP30, and FSFI each visit.	Descriptive statistics
Study II	Secondary outcome from study I. MRI and TVS images before and after treatment with bromocriptine.	Changes in radiological features associated with adenomyosis after treatment.	18	1 radiologist evaluated the MRI images and 1 gynecologic ultrasonographer evaluated the TVS images from the women before and after treatment.	Descriptive statistics
Study III	Reproducibility study including MRI and TVS images from women with HMB and suspect adenomyosis.	Agreement between raters evaluating MRI images compared with raters evaluating TVS images.	51	4 radiologists evaluated the MRI pictures and 5 gynecologic ultrasonographers evaluated the TVS images.	Inter-rater agreement, Fleiss kappa Interclass correlation, ICC
Study IV	Secondary outcome from study I. Endometrial Pipelle biopsies from study subjects in study I.	Alterations of genes following bromocriptine treatment.	12	Bioinformatic analyses PCR Immunohistochemistry ELISA	Descriptive statistics

3.2 STUDY DESIGN AND STUDY SUBJECTS. STUDY I, II AND IV

This thesis is mainly based on data from a prospective single-arm pilot study conducted in a collaboration between the Mayo Clinic, Rochester, USA, and Karolinska Institutet.

The protocol for ***study I*** was designed by the research team at the Mayo Clinic where the first woman was included in July 2013, before initiation of the Swedish site (autumn 2013). For the Swedish part of the trial TVS was added to conduct ***study II*** and endometrial biopsies for ***study IV***.

3.2.1 Tabulated study protocol

	Enrollment	Baseline	Variable time to reach 5mg bromocriptine	1 month	3 month	6 month	9 month
Phone call			X	X			
Visit	X	X			X	X	X
Blood Sample		X				X	
MRI	X					X	
TVS	X					X	
Informed consent	X						
Baseline characteristic questionnaire	X						
PBLAC[#]		X			X	X	X
AMCOQ[#]		X			X	X	X
VAS[#]		X			X	X	X
MPQ[#]		X			X	X	X
UFS-QOL[#]		X			X	X	X
EHP 30[#]		X			X	X	X
FSFI[#]		X			X	X	X
Endometrial biopsy		X				X	

[#] Abbreviation of questionnaire explained in Material and Method section

3.2.2 Study subjects

The study subjects at the Swedish site were recruited between January 2014 to April 2016. 54 women were screened for enrollment in Sweden, of which 34 were eligible, and 22 were included. Four women dropped out and 18 women completed the study in Sweden. In addition, one woman was recruited and completed the study in the USA. This, a total of 19 women completed the study and were included in the analyses.

Women 35-50 years with regular HMB, MRI or TVS highly suggestive of adenomyosis and not using antidepressants, hormonal contraceptives, or an intrauterine device (IUD), were recruited. Further, women with known or suspected endometriosis, leiomyomas >4cm, or multiple leiomyomas, were not considered for the study.

3.2.2.1 Baseline characteristics

Women enrolled in the study were 44.8±3.5 years, with an average body mass index (BMI) of 26.5±3.9. 84 % were parous and 63 % had no prior cesarean deliveries . At baseline, all women reported HMB, 78 % reported PBLAC > 250. 79 % used >10 tampons or pads on their heaviest day of menstrual bleeding, 79 % reported menses lasting >7 days and 68 % reported moderate to severe cramps with menses. The history of HMB and pain was long-

standing; 68 % reported painful menses and 47 % reported HMB before the age of 20. While few women had a history of blood transfusion (5 %), the majority had previously been on pharmacotherapy for anemia (63 %). The use of medication for painful periods was reported by 63 % of the women.

3.3 STUDY DESIGN AND STUDY SUBJECTS STUDY III

3.3.1 Study design

The study was a reproducibility study comparing agreement among raters of MRI and TVS images. Images from 51 women examined from January 2014 to December 2016 were collected.

3.3.2 Study subjects

Women aged 35-50 years with regular HMB and suspected adenomyosis on clinical examination (including TVS) were referred to MRI and expert TVS as part of the investigation. All women were clinically examined and recruited by the doctoral student. Women with adenomyoma, known endometriosis, uterine leiomyomas >4cm, multiple leiomyomas, or current use of an IUD or hormonal contraception were not eligible for the study.

In total 67 women were referred to MRI and TVS. MRI was missing in 3 women (declined examination (n=2); wrong identification number (n=1)) and TVS was missing in 13 women (images were not pseudonymized (n=2); 3D volumes (n=6) or 2D video sequences (n=5) were not recorded or of insufficient quality). Both MRI and TVS images were available in 51 women and included in the study.

3.4 METHODS STUDY I

Women meeting the inclusion criteria and no exclusion criteria were included in the study. Women with suspect adenomyosis were referred to the department of diagnostic imaging, Aleris Specialist care Sabbatsberg for MRI and to Karolinska University Hospital for expert TVS examination before inclusion.

The women sent an email or phone call to the study coordinator on day 1 of their menstruation and the visits were scheduled for the first possible day after the last day of bleeding. Women handed in their questionnaires at scheduled appointments. 200 bromocriptine 2,5 mg tablets were distributed at visit 1. Treatment with bromocriptine was started the same day. The women were told to insert 1 tablet deep intra-vaginally before bedtime every day for 1-2 weeks and then increase the dose to 2 tablets. If side effects were occurring, the woman was told to go on with 1 tablet per day one more week. Among the first 7 enrolled women, 3 dropped out due to side effects (fatigue, dizziness, nausea, and

headache). The protocol was therefore modified to a slower dosage increase. The women were told to start with ½ tablet (1,25mg) once daily for 1 week and then increasing with ½-1tablet a week until reaching the final dose of 5mg per day. After the modification of the protocol, no further women dropped out due to side effects.

The enrolled women received a phone call from the study coordinator every week during the first month, to check for compliance, side effects and decide when to increase the dosage. The first day the woman successfully took 5 mg bromocriptine was considered study day 1. The medication was then continued for 6 months with visits in the proliferative phase of the menstrual cycle after 3 and 6 months. At the 3 months visit, 200 tablets of bromocriptine were distributed. The 9 months visit was scheduled 3 months after cessation of the study drug. Subjects were instructed to complete the questionnaires close to their last day of menstrual bleeding during the proliferative phase of the cycle, at baseline and at 3, 6, and 9 months. Women were provided with tampons and sanitary pads for standardization of PBLAC reporting. Women were instructed to refrain from the use of nonsteroidal anti-inflammatory drugs or tranexamic acid therapy during the periods when bleeding was assessed (baseline, 3, 6, and 9 months) to avoid confounding. Scores obtained from these questionnaires were compared between baseline and, 3, 6, and 9 months.

3.4.1 MRI

Women were considered to have adenomyosis if on MRI the JZ ≥12 mm, JZ diff >5 mm and JZ/myom >40 % (11, 68). The presence of cystic changes was also considered.

3.4.2 TVS

Women were considered to have adenomyosis through pattern recognition; Prescence of globular uterus shape, asymmetric thickness of the myometrial walls, irregular endometrial myometrial junction, fan shaped shadowing in the myometrium and myometrial cysts.

3.4.3 Questionnaires

In 2014, when the study started, there were no quality of life questionnaires validated for adenomyosis or translated to the Swedish language. To measure blood loss, pain, and quality of life, a variety of questionnaires were used that are validated and frequently used in studies for diseases with similar symptoms, namely endometriosis, and leiomyomas. The questionnaires were translated from English to Swedish by the doctoral student and the main supervisor. The questionnaires were distributed to the women in advance, filled in at home by the woman at the end of the menstrual period, and handed in on study visits. The questionnaires were sent to the Mayo Clinic, Rochester, USA, and transferred into the Research Electronic Data Capture (REDCap®, Vanderbilt University) data entry and management program (112, 113) by statisticians.

3.4.3.1 Baseline characteristics questionnaire

Questions included baseline characteristics such as age, BMI, medical history, smoking, heredity, and physical habits. The questionnaire also consisted of gynecologic history and related questions such as gravidity, parity, cesarean section, fertility problems, use of contraceptives, age when the HMB and pain began, previous medication, and when the woman got the diagnosis of adenomyosis.

3.4.3.2 Pictorial Blood Loss Assessment Chart (PBLAC)

PBLAC is a subjective assessment of the volume of blood loss during each menstrual period, based on the degree of soiling of sanitary pads and tampons (Figure 8) (114). The degree of soiling is scored 1-20. Total scores >100 are considered HMB. PBLAC can be used as an evaluation tool for treatment outcomes (115).

	Pad			Tampon			Clots		Leakage	Score
							small	large		
	 1 point	 5 points	 20 points	 1 point	 5 points	 10 points	1 point	2 points	1 point	
1		IIIII								
2		IIIIII								
3		IIIII	IIIII						II	
4		IIIII	IIIII		IIII	III		IIIII	II	
5		IIIII	IIIIII		III	III		IIIII	III	
6										
7										Tot: 662
8										

Figure 8: PBLAC from one of the women that participated in study I.

3.4.3.3 Uterine Fibroid Symptom Quality of Life (UFS-QOL)

The UFS-QOL is a questionnaire that assesses symptom severity (SSS) and health-related quality of life (HRQOL) in women with leiomyomas over the preceding 3 months (116). The UFS-QOL has become a standard instrument in the USA. It is composed of 2 parts, the HRQOL, and the SSS. In total there are 37 questions. SSS consists of questions assessing ongoing symptoms and HRQOL consist of questions about anxiety for how the symptoms would interfere with daily life. The SSS was the primary measure in our study since it has been used as a primary outcome in previous studies at the Mayo Clinic (117, 118). The word “uterine fibroids” was changed to “adenomyosis” in the questionnaire. Higher SSS scores indicate worse symptom severity. A higher HRQOL score indicate a better health-related quality of life. Both subscores utilize a 100-point scale.

3.4.3.4 Aberdeen Menorrhagia Clinical Outcomes Questionnaire (AMCOQ)

The AMCOQ is an instrument measuring blood loss, specifically for women with HMB (119, 120). It is comprised of 13 questions to assess the amount of bleeding and impact on daily living over the previous three months. The total score ranges from 0-100, where a higher score indicates worse symptoms.

3.4.3.5 McGill Pain Questionnaire (MPQ) and Visual Analogue Scale (VAS)

Pain was assessed by MPQ (121) and VAS. We used the short form of MPQ that uses 15 words to describe three attributes of present pain (affective, evaluative and sensory) (122). The subjects assess how well the words describes the present pain the last week. The words "last week" were changed to "last menstruation" in this study. Each selected word was scored 0 (none) to 3 (severe). The total score is obtained by summing the item scores (range 0-45). A higher MPQ score indicates more severe pain.

VAS is a standard scale for pain assessment (scale 0-10) (123). A higher VAS score indicates more severe pain. The VAS scale is integrated into the MPQ-questionnaire as the last question " How do you evaluate the pain the worst day of your last menstruation?"

3.4.3.6 Endometriosis Health Profile (EHP30)

The EHP30 has become a standard instrument to assess symptoms of endometriosis over the preceding four weeks (124). EHP30 consists of two parts, the "core" questions and the "modular" questions. The core part consists of 30 questions in five core measures (pain, control and powerlessness, emotional well-being, social support and self-image), and the modular part consists of 23 questions (work, sexuality, fertility, relation with children, relation with medical profession and medical treatment). It is possible to answer "not applicable" in the modular part. The word "endometriosis" in the instruction was changed to "adenomyosis" in this study. Higher scores in the core questionnaire indicate severe symptoms.

3.4.3.7 Female Sexual Function Index

The FSFI is an instrument measuring sexual function in women over the preceding four weeks (125, 126). It consists of 19 questions in six relevant domains using a 5-point likert scale. Scores ranging from 2-36. A higher score indicates better sexual function.

3.5 METHODS STUDY II

Study II is a secondary outcome from study I. The 18 women from the Swedish site who were included in study I underwent MRI and TVS at baseline and after 6 months of treatment with bromocriptine. The images were assessed for evaluating differences in features before and after treatment.

3.5.1 MRI

MRI of the pelvis was performed on a 1,5T system (Optima MR450w, GE Healthcare, Waukesha, WI, USA or Siemens Magnetom Symphony Tim, Siemens-Healthineers, Erlangen, Germany). The minimum acquired protocol included the following sequences: a T2-weighted Fast Relaxation Fast Spin Echo or a Turbo Spin Echo on the axial, sagittal and coronal plane (slice thickness 4-5 mm; gap: 10-20 %); a T1-weighted Fast Spin Echo or a Gradient Echo on the axial and coronal plane (slice thickness 5 mm; gap 10-20 %). All the examinations were performed with a phase array coil. The participants were asked to fast four hours before the examination. No antispasmodic drugs were administrated to the patients.

All MRIs were evaluated on a Picture Archiving and Communication System (PACS) (Sectra AB, Linköping, Sweden), by the same dedicated radiologist working at the Karolinska University Hospital. The following parameters were recorded on T2-weighted images: length of the uterus excluding the cervix, antero-posterior and latero-lateral diameters; measurement of the thinner and thicker uterine wall excluding leiomyomas; JZmax and JZmin measured on a midsagittal image through the long uterine axis; The JZdiff correspond to the calculated difference between JZmax and JZmin. JZmax/myom is calculated by dividing the JZmax and the corresponding myometrial thickness measured at the same level. Further, the presence of cystic changes in the JZ (defined as foci of high signal intensity on T2-weighted and/or on fat-saturated T1-weighted images) was also recorded.

3.5.2 TVS

TVS was performed using a high-end ultrasound system Voluson E10, GE Healthcare (GE Medical Systems, Zipf, Austria) with a 5-9 MHz transvaginal probe. 2D grayscale volumes and video clips comprising the whole uterine body were collected. The software 4D View GE Healthcare (GE Medical Systems, Zipf, Austria) was used to process 3D volumes.

All examinations were performed by a single gynecologist specialized in gynecologic ultrasonography. The women were examined in the lithotomy position with an empty bladder. The uterus was scanned in the sagittal plane from cornu to cornu and in the transverse plane from the cervix to the fundus. The following parameters were recorded: anteroposterior diameter of the uterus; uterine length, excluding cervix; uterine anterior and posterior wall thickness, excluding leiomyomas, presented as the thickest and thinnest wall, in the sagittal plane; latero-lateral diameter of the uterus in the transverse plane. Further, sonographic findings according to the MUSA terms and definitions were assessed (70): absence or presence of fan shaped shadowing, asymmetrical wall thickness, irregular endometrial myometrial junction, myometrial cysts, myometrial hyperechoic islands, endometrial buds or striations. The JZ was measured in the reconstructed coronal plane from the 3D volume (75) .

3.6 METHODS STUDY III

MRI and TVS images from the same set of 51 women were assessed. MRIs were assessed by four experienced radiologists working at the Karolinska University Hospital, Stockholm. TVS were assessed by five experienced gynecological ultrasonographers from different centers in Europe. Assessment of the images was performed between December 2019 and February 2020. The raters were blinded to the clinical history, physical examination, and the evaluation of MRI and TVS images made by other raters. Each rater entered the assessments into the REDCap data entry and management program (112, 113) hosted at the Karolinska Institutet. The raters could save their assessments and resume later, to reduce the risk of fatigue.

3.6.1 MRI

MRI of the pelvis was performed on a 1.5T system (Optima MR450w, GE Healthcare, Waukesha, WI, USA or Siemens Magnetom Symphony Tim, Siemens Healthineers, Erlangen, Germany). The minimum protocol included the following sequences: T2-weighted Fast Relaxation Fast Spin Echo or Turbo Spin Echo in the axial, sagittal and coronal plane (slice thickness 4-5 mm; gap: 10-20 %); T1-weighted Fast Spin Echo or a Gradient Echo in the axial and coronal plane (slice thickness 5 mm; gap 10-20 %). All examinations were performed with a phased array coil. The women were asked to fast for 4 hours before the examination. Antispasmodic drugs were not administered.

The images were pseudonymized and evaluated on a PACS at the Karolinska University Hospital by four experienced radiologists. With regard to the presence or absence of adenomyosis, each rater based the assessment on their subjective evaluation of the radiological features. With regard to the presence or absence of adenomyosis, there were no standardized criteria given on when to make the diagnosis. The predetermined features assessed are listed in Table 1.

3.6.2 TVS

All women underwent ultrasound examination by a single expert examiner using a high-end ultrasound system Voluson E10 or E8, GE Healthcare (GE Medical Systems, Zipf, Austria) with a 5-9 MHz 3D transvaginal probe. 2D grayscale volumes and video clips, and 3D-VCI volumes including the whole uterine body were saved. The GE 4D View software (GE Healthcare, Wood Dale, IL, USA) was used to assess the 3D-VCI volumes. The 2D volumes and video clips and 3D volumes for each case were pseudonymized and downloaded to memory sticks and sent to five experienced ultrasonographers. The raters used their own personal computers to assess the volumes. The volumes could be modified during the analysis to optimize the assessment (remove the VCI function, change slice thickness or grey mix, rotate the volume in any plane). With regard to the presence or absence of adenomyosis, there were no standardized criteria given on when to make the

diagnosis. The JZ was assessed at the coronal plane in the 3D volumes. The predetermined TVS features assessed are listed in Table 1.

Table 1. Predetermined features that were assessed by MRI and TVS and entered into REDCap

Feature	**MRI**	**TVS**
Globular uterus shape	yes/no	yes/no
Asymmetric wall thickness	yes/no	yes/no
Irregular/interrupted JZ	yes/no	yes/no
Cysts in the JZ/myometrium	yes/no	yes/no
Fan shaped shadowing	-	yes/no
Buds or striations	-	yes/no
Hyperechogenic Islands	-	yes/no
JZ max	In millimeter	In millimeter
JZ min	In millimeter	In millimeter
Myometrial thickness at the same level as JZmax	In millimeter	-
Ratio JZmax/Myometrium	Automatically retrieved by REDCap	-
JZ differential (JZmax-JZmin)	Automatically retrieved by REDCap	-
Diagnosis: Does the subject have adenomyosis?	yes/no	yes/no

3.7 METHODS STUDY IV

3.7.1 Sample collection

Study IV is a secondary outcome from study I, analyzing endometrial biopsies from women participating in the clinical trial. Endometrial Pipelle biopsies were taken in the proliferative phase of the menstrual cycle at baseline and after 6 months of treatment with bromocriptine. The baseline and 6 months visits were scheduled as follows: The women sent an email or phone call to the study coordinator on day 1 of the menstruation. An appointment was scheduled for the first possible day after the last day of bleeding. The biopsies were transported to the research lab at Karolinska University Hospital on ice and stored at -80°C until further processing. A venous blood sample was taken at the baseline and at the 6 months visit for analysis of PRL at the Karolinska University Hospital clinical chemistry laboratory .

18 paired (i.e 18 x 2, baseline, and 6 months) endometrial biopsies were collected. Samples from women who reduced the PBLAC score by more than 50 % were classified as “good responders”. Six samples, whereof 3 from “good responders”, had to be excluded due to

poor RNA quality or unavailability of paired biopsies. Therefore, a total of 12 paired endometrial samples, of which 3 were classified as "good responders", were included in the study. Transcriptomic analysis to evaluate DE genes and small RNAs before and after bromocriptine treatment was performed. The protocol is briefly described in this section. Details can be found in paper IV.

3.7.1.1 Library preparation

Total RNA was extracted from tissue stored in RNA later using RNeasy total RNA Kit (Qiagen, Hilden, Germany). The quantity and quality of purified RNA was measured using the Qubit high sensitivity RNA assay kit with Qubit 4 Fluorometer (Invitrogen, Singapore) and RNA high sensitivity 6000 Pico Kit on bioanalyzer respectively (Agilent Technologies, Santa Clara, US). cDNA library preparation for next generation mRNA sequencing was performed using the SMART-seq2 protocol (127). Tagmentation of DNA and addition of sample index barcodes were performed. The final amplified libraries were cleaned and the samples were pooled and sequenced. cDNA libraries for small RNA sequencing were constructed according to a highly sensitive small RNA sequencing protocol (128). The small RNA libraries were indexed and the samples were pooled and sequenced. Both mRNA and small RNA sequencing were performed on an Illumina Nextseq 550 sequencer at the Bioinformatics and Expression Analysis core facility at Karolinska University Hospital, Sweden.

3.7.1.2 Bioinformatic analysis

mRNA sequencing data were analyzed for DE genes between the paired samples using Partek flow genomic analysis software (Partek, St. Louis, USA). Filtered DE genes were explored for treatment effect using hierarchical clustering and pathway enrichment tools. Small RNA sequencing data were analyzed for DE genes between the paired samples using Bioconductor software DESeq (version 1.24.0). Raw and processed data files from both mRNA and small RNA sequencing were deposited in NCBI's Gene Expression Omnibus.

3.7.1.3 Gene expression analysis

Extracted RNA from the paired samples were converted to cDNA using SuperScript® VILO™ kit (Invitrogen®, Thermo Fisher Scientific, Waltham, USA). The gene expression patterns of the top three downregulated glycolytic genes were validated using the following set of Taqman® gene probes: PGK1, GAPDH, ENO1, ki67, BAX, and PRL. cDNA was used in the real-time PCR and analyzed on a Step One Plus Real-time PCR system (Applied Biosystems, USA).

3.7.1.4 Immunohistochemistry to detect Ki67

Immunohistochemistry was performed on paraffin-embedded endometrial tissues using a standardized protocol (129). Primary antibody against Ki67 was diluted using diluent DaVinci Green (Biocare Medical, Concord, CA) and incubated overnight at 4 °C. Rabbit

MACH 3^{TM} Probe and its respective HRP polymer (Biocare Medical, Concord, CA) and Betazoid DAB Chromogen (Biocare Medical, Concord, CA) were used to detect the antibody. Tissue sections were counterstained using haematoxylin. Immunopositivity of Ki67 stained areas and images were analyzed at 20X magnification using a built-in tool in the Leica analysis software.

3.7.1.5 Enzyme-linked immunosorbent assay (Elisa) for detection of PRL

Protein lysates were extracted from endometrial tissues. Total protein was quantified using a Qubit protein assay kit. PRL levels were measured using Prolactin ELISA Kit, wherein the plate is pre-coated with capture antibody. Tissue lysate from paired samples and standards provided in the kit were dispensed as duplicates to each well along with Prolactin-HRP conjugates. Later, they were detected by measuring the absorbance at 450nm.

3.8 STATISTICAL ANALYSES

3.8.1 Study I

Data were manually entered into the REDCap data entry and management program (112, 113) by statisticians at the Mayo Clinic, USA. Continuous data with normal distribution is reported as mean ±SD; the median and interquartile range is used for data that is not normally distributed. Changes in scores from baseline to 3, 6, and 9 months, respectively, were compared using Wilcoxon signed rank test. All statistical analyses were 2 sided, and $P< 0.05$ was considered statistically significant. Analyses were performed using SAS 9.4 (SAS Institute, Inc, Cary, NC) by statisticians at the Mayo Clinic, USA.

3.8.2 Study II

The Wilcoxon signed-rank test was used for continuous variables and McNemar's test was used for categorical variables. All p-values were two-sided and a $p<0.05$ was considered statistically significant.

3.8.3 Study III

Each rater entered the assessments into the REDCap data entry and management program (112, 113). Statistical data analysis was performed using the software Statistical Package for the Social Science (SPSS) (version 26, IBM Corporation, Armonk, NY, USA).
Fleiss kappa (κ) was used as a measure of inter-rater agreement for categorical data. Kappa values were categorized as 'Poor' ($\kappa \leq 0.20$), 'Fair' (0.21-0.40), 'Moderate' (0.41-0.60), 'Good' (0.61-0.80), and 'Very good' (0.81-1.00) (130).

The intraclass correlation coefficient (ICC) was used as a measure of reliability for quantitative data, categorized as 'Poor' (≤0.5), 'Moderate' (0.5-0.75), 'Good' (0.75-0.9)

and ‘Excellent’ (>0.9) and their 95% confident intervals were calculated based on individual-rating, absolute-agreement, 2-way random-effects model (131, 132).

3.8.4 Study IV

GraphPad Prism 8 (GraphPad Software Inc., USA) was used for statistical analysis and graphical illustrations. Statistical analysis was performed between the baseline and treatment groups using a paired T-test.

4 ETHICAL CONSIDERATIONS

The following approvals were obtained prior to recruitment of study subjects:

Studies I and II and IV were approved by the regional ethical committee at Karolinska Institutet, Sweden (2013/2060-31/1) and registered at clinicaltrials.gov (NCT01821001). ***Study I*** was also approved by the Institutional Review Board at Mayo Clinic, Rochester, MN, USA.

Study I was approved by the Swedish Medical Product Agency (EudraCT 2013-004409-14).

Study III was approved by the regional ethical committee at Karolinska Institutet, Sweden (2016-1751-32).

All women included in ***study I*** were informed about the study orally and written and signed a consent form. The women could discontinue their participation whenever they wanted to, without giving any reason for doing so and their choice would not impact their care in the future. The women remained anonymous via a study ID number. The key to the code is kept locked in at the WHO center together with the questionnaires.

The radiological images for ***study III*** were mostly collected during recruitment for study I. Women that underwent MRI and expert TVS for investigation of adenomyosis were asked if their images could be used for the study. There are restrictions on sending MRIs between hospitals, and it is difficult to keep them anonymous. We therefore used raters from Karolinska University Hospital in Sweden to assess the MRIs. The MRIs were pseudonymized at the PACS before being assessed by the radiologists. The TVS images were pseudonymized at the Voluson E10 or E8, before being transferred to a memory stick and sent by snail mail to the raters.

For ***study IV***, endometrial biopsies and blood samples were coded with a study number, before transportation to the research laboratory. The samples were given the same study number corresponding to the women in ***study I***. The collection of blood samples and endometrial biopsies are procedures that cause discomfort. The women were therefore encouraged to take paracetamol and Ibuprofen 30-45 minutes before the appointment. However, the samples were collected as per clinical routine for other conditions. The biopsies are kept in a biobank for 10 years, before they are destroyed.

5 RESULTS AND DISCUSSIONS

5.1 STUDY I

Since this was a pilot study there was no data on which to base a power calculation. However, when planning the study, it was decided to include in total 30 women from the two sites. This was based on the minimum number of women necessary to assess the UFS-QOL. An endpoint in improved symptom in the SSS of 10 points was set, since this endpoint have been used at the Mayo Clinic in previous studies of leiomyomas (117, 118). A subanalyses of the data was made in April 2016. The primary endpoint was reached with improvement in SSS of more than 10 points. At that time, 1 woman from the USA was enrolled, and 22 women from Sweden. 1 woman from the USA and 11 women from Sweden had completed the protocol. A decision was made at the Mayo Clinic to halt enrollment due to funding limitations. The limited sample size may have affected the results. However, the trial is a hypothesis generating study based on observational data and to some extend animal studies for the first time also confirmed in humans .

19 women completed 6 months of treatment with a daily dose of 5mg bromocriptine vaginally.

Table 2. PBLAC score and VAS from the study subjects at baseline and after 6 months of treatment with bromocriptine.

Study subject	Baseline PBLAC	6 months PBLAC	Baseline VAS	6 months VAS
1	222	34	6,3	7
2	367	260	3,2	4,3
3	294	242	4,9	2
4	358	348	2,4	0
5	645	91	8	2,2
6	824	783	8,5	0,9
7	339	217	10	3
8	missing	69	4,1	2,6
9	67	106	4	2,2
10	384	32	8,3	0
11	543	423	1,5	1,5
12	292	65	4,3	0
13	208	384	4	0,4
14	646	288	6,7	8,8
15	327	279	10	9,3
16	325	147	8,6	6,3
17	1967	1620	5,1	8,4
18	122	76	1,7	0,3
19	3690	1170	5	6

5.1.1 Menstrual bleeding

There were significant changes in PBLAC and AMCOQ indicating an improvement in menstrual bleeding severity after 6 months of treatment with bromocriptine.

PBLAC at baseline 349 [292-645] decreased to 242 [76-384] at 6 months ($p < 0.001$) (Table 2). AMCOQ at baseline 51 [40-61] decreased to 35 [21-48] at 6 months ($p < 0.001$). Although menstrual bleeding improved significantly, the majority of women scored PBLAC >100 at 6 months, indicating continuation of HMB. However, there are known limitations in PBLAC as women assess their use of menstrual protection differently, with low correlation with true blood loss, especially with increasing scores (133, 134). Also, higher limit than 100 to define HMB is suggested (133-135). There are many different kinds of tampons and pads on the market, which makes comparison difficult. In our study, we handed out tampons and pads to the study subjects, for standardization. For trials, it may be more reliable to use a menstrual cup when assessing the amount of bleeding. However, menstrual cups were not available at the time of planning the study. The AMCOQ, which also includes questions regarding the impact of bleeding on daily life, may be more reliable than PBLAC in assessing HMB.

5.1.2 Pelvic pain

A significant improvement of pelvic pain measured by MPQ and VAS was shown.

VAS at baseline 5 [4-8.3] decreased to 2.2 [0.4-6.3] at 6 months ($p = 0.001$). MPQ at baseline 10 [5-22] decreased to 6 [3-16] at 6 months ($p = 0.025$). Although pain was not an inclusion criterion, pain was a common symptom. 13/19 women reported moderate to severe pain at baseline. After 6 months of treatment, 12 women reported VAS < 3 whereas 3 women reported no pain at all (Table 2). This shows that bromocriptine has a remarkable effect on pelvic pain. VAS is easy to measure and reliable in assessing pain.

5.1.3 Quality of life

Quality of life assessed by UFS-QOL improved. SSS at baseline 60 [44-72] was reduced to 44 [28-59] at 6 months ($p < 0.001$).

EHP30 consists of 5 core measurements and a modular questionnaire. Core measures “Control and powerlessness” and “emotional well-being” showed a significant improvement after treatment, whereas “pain”, “social support” and “self-image” did not show any significant changes. The majority of women indicated the modular questions to be not applicable and thus not presented in the study. EHP30 is validated for endometriosis and is a commonly used tool to evaluate treatment effects. Endometriosis and adenomyosis have many symptoms in common, but there are also differences, which is why the EHP30 may not be a suitable instrument for adenomyosis. In this study, the women only had symptoms during the bleeding period, whereas it is common that women with

endometriosis have symptoms throughout the entire month. Therefore, the symptoms associated with endometriosis may have more impact on the social and work life than symptoms associated with adenomyosis. It is also common that women with endometriosis have pain during intercourse and have impaired fertility that interfere with their family life. Thus, a lot of questions in EHP30 are not applicable for women with adenomyosis.

The FSFI questionnaire consists of questions about sexual function. Very few of the women in this study reported impaired sexual function at baseline, thus no improvement could be assessed after treatment with bromocriptine.

5.2 STUDY II

5.2.1 MRI

No significant reduction in JZmax was found at 6 months although 9/18 women showed a decrease in JZmax. Two women showed a considerable reduction of JZmax, from 19.2 to 9.5 mm and 21.0 to 15.6 mm.

JZmax was found to be in the range 12.1-27.7 at baseline. Notable, the range in JZmax decreased to 9.5-25.7 at 6 months. A similar shift in range was also found for JZdiff and JZmax/myo.

Cysts in the JZ were found in 9/18 (50 %) at baseline, which was reduced to 5/18 (27 %) at 6 months. However, no statistically significant difference was found.

5.2.2 TVS

Asymmetric wall thickness at baseline was assessed in 13/18 women at baseline and 6/18 women at 6 months ($p = 0.02$).

The JZmax 8,5mm [5.2-14.0] at baseline showed a significant decrease to 7.9mm [5-11.2] at 6 months ($p = 0.02$).

Cysts in the myometrium were found in 6/18 (33 %) at baseline and 5/18 (28 %) at 6 months.

The subtle changes seen in MRI and TVS may indicate that vaginal bromocriptine has an impact on adenomyosis, although only significant in asymmetric wall thickness and JZmax measured with 3D TVS. Absence of significant changes in JZmax measured with MRI might be explained by the wide range and the small sample size. Moreover, 6 months observation time might be too short to be able to see significant changes. A few women showed a substantial difference, while other women did not show any differences at all. As far as we know, this is the first human study where radiological images are assessed before and after treatment in women with adenomyosis.

5.3 STUDY III

The inter-rater agreement for diagnosing adenomyosis and for the individual imaging features associated with the disease are listed in Tables 3 and 4.

Table 3. Inter-rater agreement for categorical data, Fleiss kappa. The table is reused from paper III.

Variable	**MRI**			**TVS**		
	Kappa		95% CI	Kappa		95% CI
Globular uterus shape	.199	poor	.195-.202	.482	moderate	.48-.485
Wall asymmetry	.552	moderate	.548-.556	.414	moderate	.411-.417
Irregular JZ	.527	moderate	.522-.531	.309	fair	.307-.312
Cysts in the JZ/Myometrium	.327	fair	.324-.331	.192	poor	.190-.195
Fan shaped shadowing	-		-	.357	fair	.354-.359
Buds or Striations	-		-	.209	fair	.206-.211
Hyperechogenic Islands	-		-	.289	fair	.286-.292
Diagnosis	.283	fair	.280-.287	.420	moderate	.417-.422

Table 4. Inter-rater reliability for continuous data, Intraclass correlation, ICC. The table is reused from paper III

Variable	**MRI**			**TVS**		
	ICC		95% CI	ICC		95% CI
JZmax	.821**	good	.709-.898	.082[#]	poor	-.11- .252
JZmin	.572**	moderate	.408-.724	-.31[##]	poor	-.67- -.104
Myometrial thickness at the same level as JZmax	.830**	good	.739-.900	-		-
JZmax/myom	.570**	moderate	.382-.731	-		-
JZdiff	.713**	good	.583-.823	-		-

**Missing value: 15 cases are excluded since at least one rater has evaluated JZ as ”not assessable”.
[#] Missing value: 29 cases are excluded since at least one rater has evaluated the JZ as “not assessable”.
[##] Missing value: 42 cases are excluded since at least one rater has evaluated the JZ as “not assessable”

5.3.1 MRI

For continuous variables, inter-rater reliability for JZ max was ‘good’ and the reliability for other JZ measurements was ‘moderate to good’. This was expected since the diagnosis of adenomyosis traditionally is made by assessment of different features in the JZ (11, 64, 68, 69, 136).

The diagnosis of adenomyosis by MRI is highly reproducible (136, 137) with a high inter-rater agreement for various JZ measurements (136).
For categorical variables, interrater agreement for "wall asymmetry" and "irregular JZ" was 'moderate' and the reliability for various JZ measurements were 'good to moderate'. Therefore, it is remarkable that the agreement for diagnosis was 'fair'.
The lack of standardized criteria for which features to include when making the diagnosis is the most likely reason. Further, if the diagnosis was made on JZ measurements alone, the agreement for diagnosis may have been higher.

5.3.2 TVS

An ultrasonographer uses pattern recognition when assessing adenomyosis. In this study, the features proposed by the MUSA group (70) were assessed. The overall agreement for the diagnosis was 'moderate', while most of the different features showed less agreement.

Different raters may value features differently, but still get the same overall impression if the subject has adenomyosis or not. "Globular uterus shape" and "asymmetric wall thickness" had the highest agreement in the assessed features. The inter-rater agreement for cysts in the myometrium was 'poor'. Small cysts in the myometrium may be difficult to detect with TVS, especially when other features, such as shadowing, are present.

With 3D TVS, it is possible to visualize the JZ. However, JZ measurements with 3D TVS have been shown to have limited reproducibility (138-140). In our study, one or more rater classified the JZ as "not assessable" in 29/51 cases. Those cases get excluded in the ICC analyzes. In the remaining 22/51 cases, the ICC was 0.08 [-.11 to 0.252], thus unreliable.

Our findings show that pattern recognition is the best method to make the diagnosis by TVS. More studies are needed to learn which features are more reliable and to make standardized definition of the disease. Measurement of the JZ with TVS is challenging and is unlikely to be useful for diagnosing adenomyosis in clinical practice.

5.3.3 Comparing MRI and TVS

The agreement for cysts was 'fair' by MRI and 'poor' by TVS. Traditionally, "cysts" in the myometrium have a high impact when diagnosing adenomyosis with both MRI and TVS. It is worth having in mind that cysts are only present in one-third to half of the affected women (11, 64, 136). The low agreement in our study emphasizes that the assessment of cysts shall be done with caution.

Both modalities have shown unsatisfactory inter-rater agreement for the diagnosis, indicating that adenomyosis is challenging even for experts.

5.4 STUDY IV

5.4.1 PRL expression

A significant reduction in serum PRL levels was observed at 6 months (mean difference (MD): -4.82 ± 3.88, p<0.001) (Figure 9A). This is in line with previous studies that have shown vaginal administration of bromocriptine to be efficient in decreasing serum levels of PRL (108, 109). However, we did not observe any differences in expression of PRL or the PRL-R in the eutopic endometrium after treatment (Figure 9 B-D). Therefore, this study did not prove our hypothesis that decreased levels of PRL in the eutopic endometrium following bromocriptine treatment is associated with improvement in bleeding and pain. However, previous animal studies compared levels of PRL in the endometrium compared with healthy tissue (45, 50, 141), and did not investigate the mechanism of action of bromocriptine. Thus, the role of PRL in adenomyosis needs to be further studied.

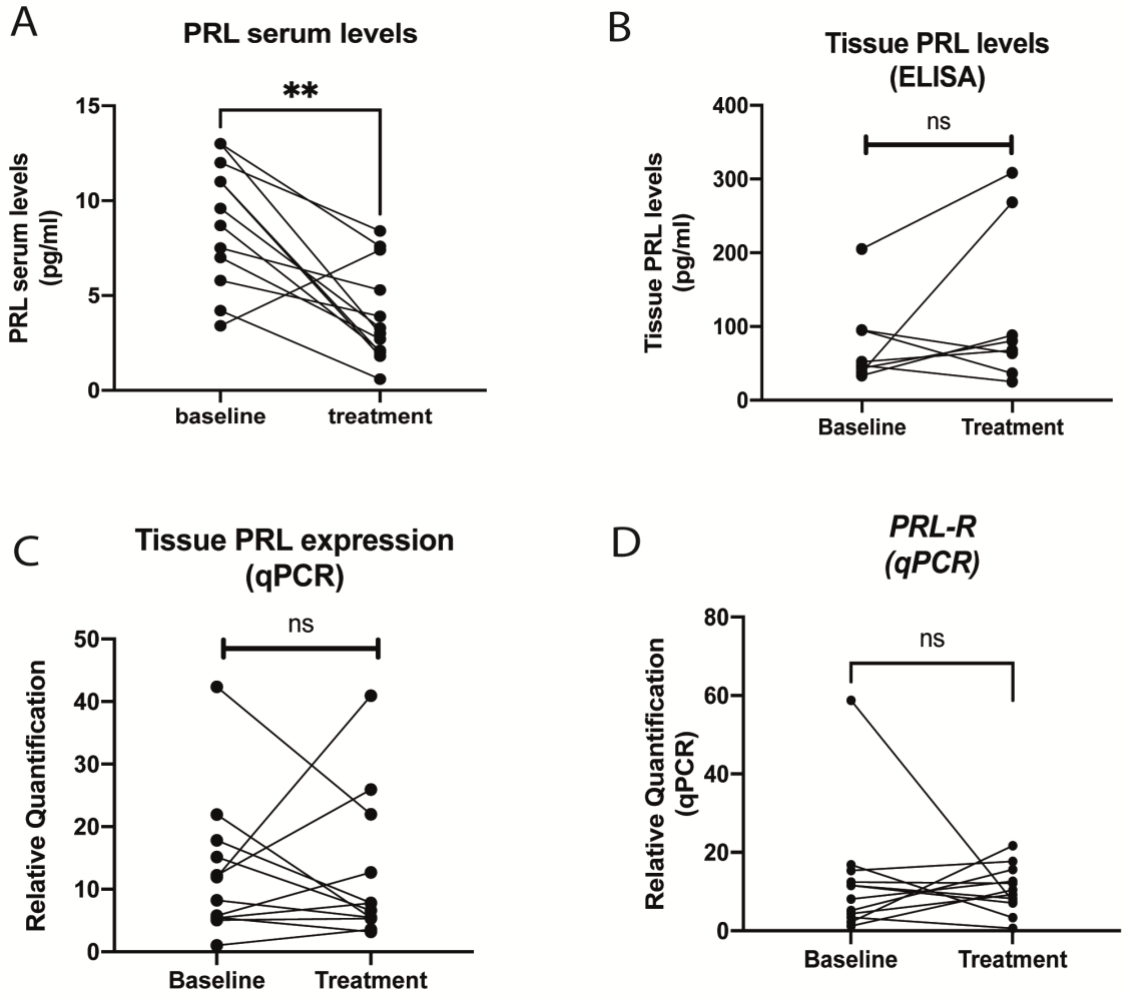

Figure 9: Levels before and after treatment with bromocriptine A) PRL in serum. B) PRL in tissue (ELISA). C) PRL in tissue (PCR). D) the PRL receptor in tissue. The figure is reused from paper IV.

5.4.2 Differentially expressed genes

5.4.2.1 Ki67 and BAX

Gene expression analysis of Ki67, a marker of proliferation, showed a significant downregulation between baseline and bromocriptine treatment (MD: -13.95 ± 9.59; $p<0.001$) (Figure 10A). Correspondingly, BAX, a marker of apoptosis, showed a significant upregulation (MD: 37.70 ± 60.68; $p<0.01$) (Figure 10B). Immunohistochemical analysis of Ki67 showed no significant change when analyzing all samples. However, two out of three "good responders" showed a dramatic reduction in Ki67 staining intensity (Figure 11). Previous studies have shown higher concentrations of Ki67 (16) and lower concentrations of BAX (142) in eutopic endometrium from women with adenomyosis than in healthy controls, suggesting that women with adenomyosis have altered proliferation and apoptosis.

Our results suggest that bromocriptine might stimulate apoptosis and suppress proliferation in the endometrium in women with adenomyosis.

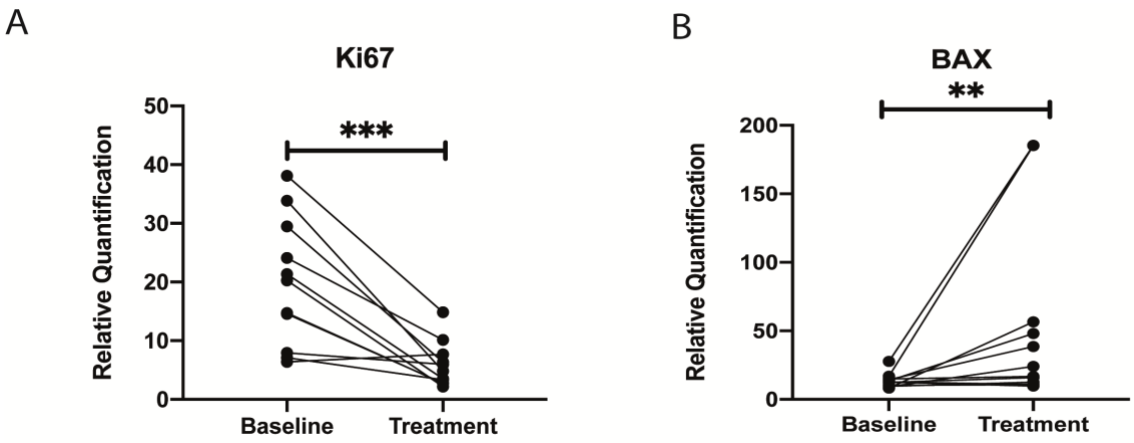

Figure 10: Real-time PCR for target genes before and after treatment A) The ki67 gene, involved in proliferation B) The BAX gene, involved in apoptosis. The figure is reused from paper IV.

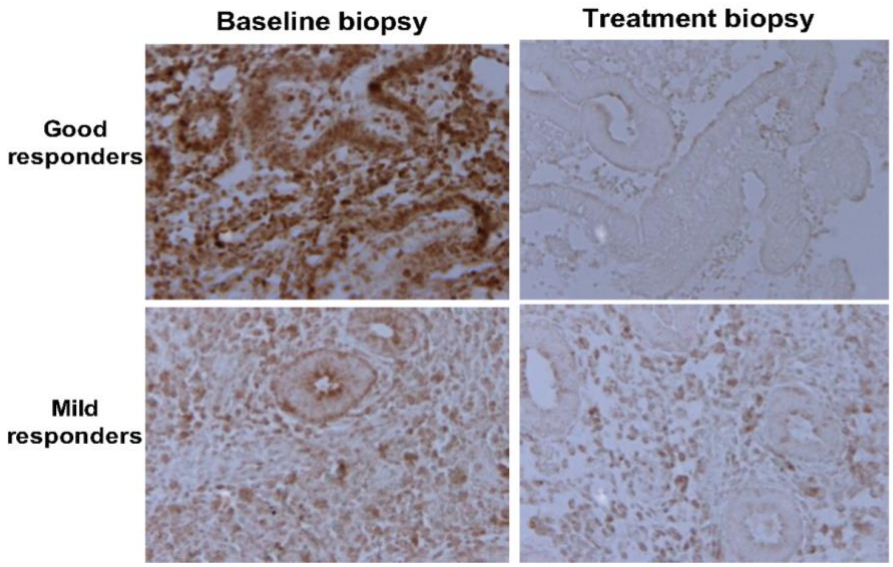

Figure 11: Immunohistochemical analysis (40X magnification) for Ki67 staining at baseline and after 6 months of treatment with bromocriptine. Ki67 positive staining in brown from Good and Mild responders shows a remarkable reduction in staining intensity in biopsies from Good responders before and after treatment. The reduction in Ki67 corresponds to less proliferation. The figure is reused from paper IV.

5.4.2.2 Glucose metabolism

mRNA sequencing showed 1821 DE genes out of 37131 annotated genes. Nine out of 12 paired samples showed a dominance of decreased gene expression. Over all 22 out of 31 downregulated genes within carbon metabolic pathways were associated with glucose metabolism.

Small RNA sequencing data showed 47 miRNAs that were differentially regulated (31 downregulated and 16 upregulated). A literature search indicated a strong association with the downregulated target genes involved in carbon metabolism. Specifically, glycolytic enzymes were downregulated. All the validated genes, PGK1 (MD: -2.36 ± 2.17, $p < 0.01$), GAPDH (MD: -1.13 ± 1.60, $p < 0.05$), and ENO1 (MD: -1.75 ± 2.60, $p < 0.05$) showed a significant reduction in gene expression after bromocriptine treatment (Figure 12).

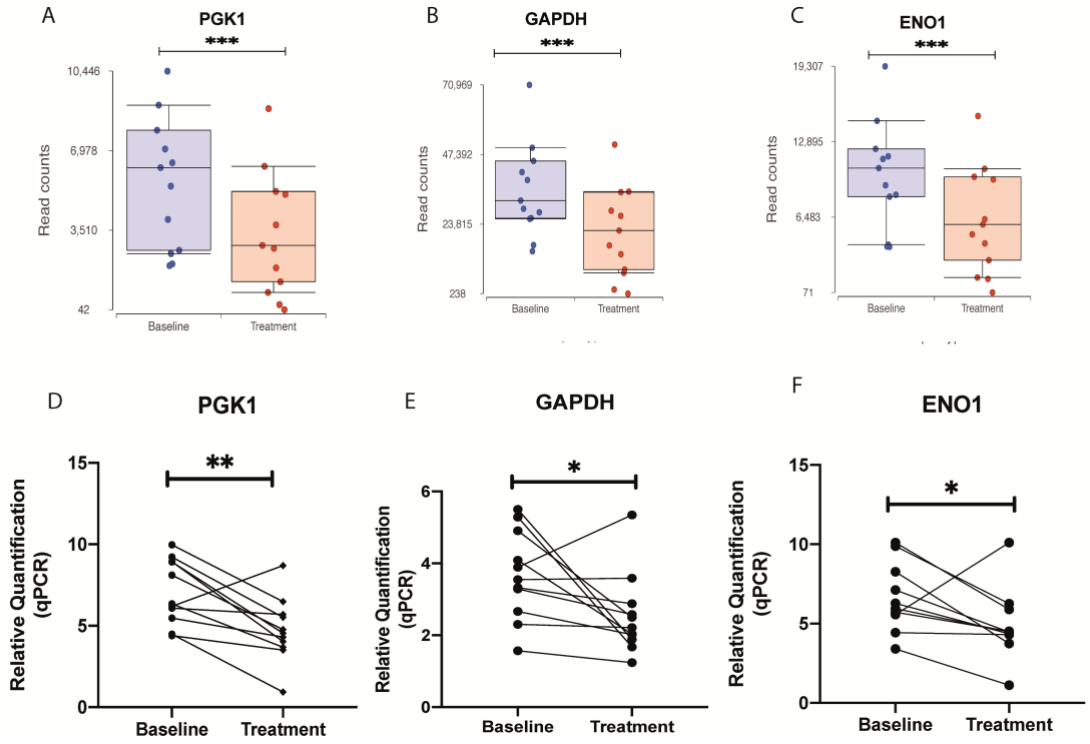

Figure 12: Gene expression in selected genes involved in the glycolytic pathway. The figure is reused from paper IV.

The results of our mRNA sequencing clearly indicate that the expression of genes regulating glucose metabolism was affected following bromocriptine treatment. This is in line with previous studies that have shown decreased hepatic glucose production after bromocriptine treatment as a result of increased dopamine levels (143, 144). Thus, bromocriptine leads to impaired glucose metabolism and can be used as a medication for diabetic patients. This mechanism may be a result of bromocriptine itself, not an act of PRL.

5.5 METHODOLOGICAL CONSIDERATIONS

5.5.1 Study I

The study is a prospective single-arm pilot study including 19 women who were treated with vaginal bromocriptine for 6 months. A properly powered double-blinded randomized controlled trial would have had advantages.

The changes in symptoms and quality of life are assessed through questionnaires. There are no questionnaires validated for adenomyosis. Questionnaires commonly used for diseases with similar symptoms, namely endometriosis, and leiomyomas, were used. Nevertheless, some of the questions in the questionnaires are not applicable for adenomyosis, since the symptoms are slightly different. Therefore, we used several different questionnaires to

demonstrate the changes in symptoms. When we started the study, the knowledge of the disease was limited, and we did not know which questionnaires were more appropriate to describe the disease. The questionnaires were not available in the Swedish language. The main supervisor and the doctoral student translated the questions from English to Swedish. It would have been an advantage to have a translated questionnaire validated for adenomyosis.

The women that participated in the study were mainly recruited by the doctoral student while working at a private gynecologic clinic, Aleris Specialistvård Sabbatsberg. A few women were referred from other centers. The doctoral student was the study coordinator and had all contact with participating women. We considered it to be more suitable for the women to have all appointments at Aleris Specialistvård Sabbatsberg, instead of at the WHO clinical trial center at the Karolinska University Hospital. The reason for this, was concerns that the women would discontinue their participation in the study, if they had appointments in different geographical places.

Bromocriptine is a well-known drug. Vaginal administration is well tolerated and effective in reducing circulating PRL levels in women with hyperprolactinemia(108). The dose 5mg is a common dose for inhibition of lactation and for treatment of Parkinson´s disease. We do not know which dosage is adequate for the treatment of adenomyosis. The uptake through the vagina may be higher than the uptake through the gastro intestinal canal, as has been shown for other drugs (145). Therefore, a lower dose than 5 mg may be sufficient. More studies are needed to investigate what dose is sufficient in the treatment of adenomyosis. Vaginal administration has fewer gastrointestinal side effects than is seen with oral administration (109). Some women developed tiredness, nausea, headache, and dizziness while increasing the dosage. Three women dropped out because of side effects at the beginning of the study. After changing the protocol to a slower increase in dosage, no women dropped out and only a few women complained of side effects.

5.5.2 Study II

In this study, radiological features with MRI and TVS were compared before and after treatment with bromocriptine. The images were assessed by one dedicated gynecologic ultrasonographer and one dedicated radiologist. When evaluating the images at 6 months, the raters were not blinded to assessments made by themselves at baseline. However, they were encouraged not to look at the baseline assessments. The predetermined features for the images were entered in a datasheet, not in a dedicated data program, as we used in other studies.

5.5.3 Study III

A strength of the study was the use of the REDCap data entry and management program. All predetermined features had to be filled in before moving to next case. This minimizes

the risk of missing data. The raters were able to stop and continue later, to minimize the risk of fatigue.

For MRI, the quality of the images is dependent on which protocol is used when examining the women. At the time of the study, no specific protocol for adenomyosis was available. Consensus guidelines suggesting technical protocols for MR imaging of endometriosis were published after the enrollment of the study subjects (146, 147). The women were asked to fast for 4 hours prior to the examination to reduce motion artifacts caused by small bowel peristalsis, but no antispasmodic drug or abdominal belt was used. Furthermore, no oblique axial T2-weighted sequence perpendicular to the long uterine axis was included in the MRI protocol (11, 136). Thus, several MRIs were affected by artifacts, thus hampering the quality of the images and making the images more difficult to assess. We did not exclude any images although the quality in some images was poor. The quality of the images may have had an impact on the assessment. If perfect quality, the agreement between raters may have increased.

A limitation of the study is the use of stored offline TVS videos and volumes instead of real-time examinations. However, it would be impossible to do reproducibility studies with multiple raters in TVS without using recorded material. The quality of the stored video clips was good, but the length is only a few seconds per clip. Some details can then be challenging to assess. During a real-time examination, the ultrasonographer can focus on details of interest. Also, some women did not have stored video clips or 3D volumes. Those cases were excluded.

5.5.4 Study IV

The endometrial biopsies were planned to be collected during the proliferation phase of the menstrual cycle. Women with adenomyosis have HMB and some of them have a prolonged bleeding period. At baseline, 5 women reported menstrual bleeding lasting 10 days or longer. Therefore, a few biopsies were taken in late proliferation phase and 2 biopsies in the beginning of the secretory phase. It would be preferable to take the biopsies in the secretory phase. Then the biopsies could be scheduled to be taken in a stringent interval controlled by the LH peak. The amount of tissue will increase if taken in the secretory phase, and the risk for blood content will be decreased. Also, some of the biopsies had to be excluded due to low RNA content.

The study sample was small in this pilot study and some tissue was lost during storage, thus including 12/18 paired samples. Unfortunately, 3/6 excluded paired samples belonged to “good responders”.

To get reliable results, the analyses need to be conducted in a trial with a larger samples size and samples taken in a stringent interval during the menstrual cycle. However, whether proliferation phase or the secretory phase is the best phase to study alterations in the eutopic endometrium in women with adenomyosis, needs to be further explored.

6 CONCLUSIONS

6.1 STUDY I

Significant improvement in menstrual bleeding, pain, and quality of life in women with adenomyosis after bromocriptine treatment suggests a novel therapeutic agent for this common disease with limited alternative therapies.

6.2 STUDY II

TVS showed a significant decrease in JZmax and asymmetric myometrial wall thickness after treatment with bromocriptine. The MRI features did not change. The changes seen by TVS in this small pilot study may indicate that vaginal bromocriptine has an impact on adenomyosis that is reflected in the radiological appearance.

6.3 STUDY III

The inter-rater agreement for diagnosing adenomyosis was higher for TVS than for MRI despite MRI manifesting higher inter-rater agreement in most features assessed by both modalities. Measurement of JZ in the coronal plane with 3D TVS is unreliable and is unlikely to be useful for diagnosing adenomyosis.

6.4 STUDY IV

Vaginal administration of bromocriptine in women with adenomyosis has an anti-proliferative effect on endometrial cells by downregulating genes associated with glucose metabolism. The bromocriptine induces growth inhibition via a non-PRL mediated mechanism.

7 FUTURE PERSPECTIVES

Our knowledge of adenomyosis is scarce. Studies are needed to elucidate the prevalence and symptomatology. Uniform diagnosis criteria by histology and radiology are lacking and the underlying pathophysiology needs to be explored.

Until recently, the only available treatment for HMB, regardless of underlying disease, has been a hysterectomy. The progress in research and the development of drugs for various gynecological diseases has increased tremendously during the past years. Women's desire to preserve their fertility has brought the research in the field forward. However, when validating studies, we need to keep in mind that it may be different diseases or different stages that correspond to the different features. We need to elucidate the progress of the disease. Most studies use histology as the golden standard and compare the findings with radiology. To diagnose adenomyosis with histology, hypertrophied myometrium surrounding the ectopic endometrium some distance from the basal layer, needs to be present. When the myometrium becomes hypertrophied is not known. It may be present at the same time as the ectopic endometrium develops in the myometrium or later. It may be different stages of the disease and may not correspond to the thickened JZ assessed by MRI or features seen with TVS. Therefore, to compare different radiological or clinical findings with histology as the gold standard may not be correct. Further, it is challenging to distinguish between diffuse, focal and cystic adenomyosis, and the difference between them needs to be explored.

It is important that women with symptoms get a correct diagnosis. Therefore, it is necessary to establish uniform imaging diagnosis criteria for adenomyosis. Whether MRI or TVS is the better modality for diagnosing adenomyosis is debated. However, it will save money for society and time for the woman if gynecologists are able to make the diagnosis using TVS. Further reproducibility studies in TVS with multiple raters are needed to find out which features are more reliable and robust in diagnosing adenomyosis.

The treatment potential of bromocriptine needs to be further explored. Randomized controlled trials (RCT) with bromocriptine and placebo are crucial to investigate treatment effects for adenomyosis. In the clinical trial included in this thesis, a daily dose of 5mg bromocriptine was used. However, which dose is sufficient to bring symptom relief needs to be determined. There is an ongoing multicenter RCT comparing placebo with a vaginal ring releasing 13.5µg Quinagolide a day. Quinagolide is a Selective dopamine D2 agonist, thus the same drug as bromocriptine. Women with endometriomas, deep endometriosis, and adenomyosis are included. It will be interesting to follow the results. However, other medical treatments such as progestins, COCs and progesterone receptor modulators need to be studied in women with adenomyosis and without comorbidity with endometriosis or leiomyomas.

In this thesis, we investigated changes in PRL and DE genes in women with adenomyosis before and after treatment with bromocriptine. However, the sample size was small in this pilot study and the results have to be validated in larger studies. With new techniques, it is possible to investigate the action of large number of genes in tissue samples. To learn the mechanism underlying the development of adenomyosis, the action of genes in the eutopic endometrium from women with adenomyosis compared with eutopic endometrium from healthy women needs to be explored. Further, whether the eutopic and ectopic endometrium in women with adenomyosis differs, remains unknown and need to be explored.

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