Ultrasound studies of the endometrial-myometrial junction for the diagnosis of adenomyosis and endometrial cancer

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STATEMENT OF ORIGINALITY AND PERSONAL CONTRIBUTION TO WORK

I, Joel Naftalin, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. I was personally involved in the design of all studies, applications for ethical approval and recruitment of all patients. I performed all ultrasound examinations except where indicated, and collected all the data. All statistical analyses were done, either by me or by UCL statistician Paul Bassett.

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Joel Naftalin

ABSTRACT

This thesis investigated the ultrasound assessment of the endometrial-myometrial junction (EMJ) and its clinical relevance to the uterine diseases, adenomyosis and endometrial cancer. The inter- and intraobserver variability in the classification of EMJ visualisation using three-dimensional ultrasound was assessed and a high level of agreement was found. Endometrial thickness and parity were found to be significantly associated with the quality of EMJ visualisation.

Seven recognised ultrasound features of adenomyosis, including an irregular endometrial-myometrial junction, were investigated for their role in the ultrasound diagnosis of adenomyosis. The other features were an asymmetrically thickened myometrium, parallel shadowing, linear striations, myometrial cysts, hyperechoic lesions and adenomyomas. The presence of any of these features was considered diagnostic of adenomyosis. The inter- and intraobserver variability of ultrasound diagnosis of adenomyosis was also investigated and a good level of agreement was found. This was the case when real-time ultrasound assessments were compared with assessments made from stored uterine volumes, as well as when both assessments were made from stored volumes.

Transvaginal ultrasound was used to assess the prevalence of adenomyosis in women attending a general gynaecology clinic in a large prospective observational study. Women were considered to have adenomyosis if one or more ultrasound feature of adenomyosis was found. Using this criterion, the prevalence was estimated to be 20.9% with 7.6% of women being excluded from the data analysis. It was also found that age, gravidity and pelvic endometriosis were all significantly associated with the presence of adenomyosis.

Menorrhagia was evaluated in order to assess if it was associated with adenomyosis. Multivariable analysis revealed that while adenomyosis was not significantly associated with menorrhagia when assessed as a binary outcome, when severity of disease was taken into account, there was a significant association. A similar analysis found that adenomyosis was significantly associated with dysmenorrhoea.

A second-stage ultrasound test that incorporated assessment of the EMJ was investigated for its use in the diagnosis of endometrial cancer in women presenting with post-menopausal bleeding. It was found to significantly increase the specificity of ultrasound in the diagnosis of endometrial cancer while having a minimal impact on sensitivity.

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HYPOTHESIS

This thesis will investigate the following hypotheses:

- Three-dimensional transvaginal ultrasound can be used as an accurate and reproducible method for assessment of the endometrial-myometrial junction.
- Transvaginal ultrasound is an accurate and reproducible imaging modality for the diagnosis of adenomyosis and for assessment of its severity
- Adenomyosis is a common condition
- Adenomyosis is associated with both menorrhagia and dysmenorrhoea
- Ultrasound assessment of the endometrial-myometrial junction can be used to improve the accuracy of transvaginal ultrasound in the diagnosis of endometrial cancer

AIMS

The aims of this thesis are:

- To examine the reproducibility of three-dimensional ultrasound assessment of the endometrial-myometrial junction
- To examine the reproducibility of ultrasound diagnosis of adenomyosis using three-dimensional stored uterine volumes
- To determine the prevalence of ultrasound features of adenomyosis in a population of women attending a general gynaecology clinic, using transvaginal ultrasound.
- To determine if adenomyosis is associated with menorrhagia and dysmenorrhoea
- To evaluate a second-stage test, incorporating assessment of the endometrial-myometrial junction, designed to improve the accuracy of transvaginal ultrasound in the diagnosis of endometrial cancer

PART I BACKGROUND

1.0 TECHNICAL ASPECTS OF ULTRASOUND

1.1 Introduction

Ultrasound has transformed many aspects of gynaecological practice. Prior to its use in medicine, diagnosis of gynaecological conditions was based almost entirely on clinical examination, surgical and histological findings. The first published clinical use of ultrasound was in the field of gynaecology when Professor Ian Donald and his registrar John McVicar used transabdominal ultrasound to differentiate between solid and cystic abdominal masses (Donald et al. 1958). Professor Donald combined his clinical experience and his knowledge of SONAR, gleaned during World War II, with the technical expertise of Tom Brown, an engineer with an interest in the use of ultrasound in metallurgy, to create and use the first clinically useful ultrasound machine. The ability to provide instant, clinically useful information using a safe, non-invasive modality was transformational and has revolutionised gynaecological practice. There have been many significant technical advances, most recently three-dimensional (3D) ultrasound, yet it is still B-mode ultrasound, first used by Donald and his team over 50 years ago, that is the primary imaging tool in gynaecology today.

1.2 Principles

Medical ultrasound is based on the principle of passing a current through a piezoelectric crystal to create ultrasound pulses, which can then be transmitted through the relevant body tissue. When these pulses encounter an interface between tissues of differing acoustic impedance or density, a proportion of the emitted soundwaves will be reflected back towards the piezolelectric element from which they were emitted (echoes). The echoes are then converted into an electric current, the amount of current produced being dependent on the number of echoes received. The conversion of this current into an image therefore gives a visual representation of the varying densities within the body tissue.

1.3 Image formation

1.3.1 Brightness mode image formation

A brightness-mode or B-mode image is a cross-sectional image representing tissues and organ-boundaries within the body. The image is constructed from echoes generated by the reflection of ultrasound waves at tissue boundaries. Each echo is displayed at a point in the image which corresponds to the position of its origin in the tissue being isonated. The brightness of the image at each point is related to the strength or amplitude of the echo, giving rise to the term brightness mode or B-mode (Martin 2003).

1.3.2 Transvaginal ultrasound

The frequency of ultrasound pulses used is a compromise between image resolution and the depth of penetration required. Higher ultrasound frequency results in better image resolution but there is greater attenuation of the beam within the tissues. Therefore, transabdominal ultrasound probes, which have to pass through multiple tissue layers before reaching the abdominal cavity, tend to use lower frequencies (3.5-5MHz) at the expense of image resolution. The ability of transvaginal ultrasound (TVUS) probes to get much closer to the organs of interest, enables the use of higher frequencies (8-15MHz) leading to improved image resolution.

1.4 Doppler

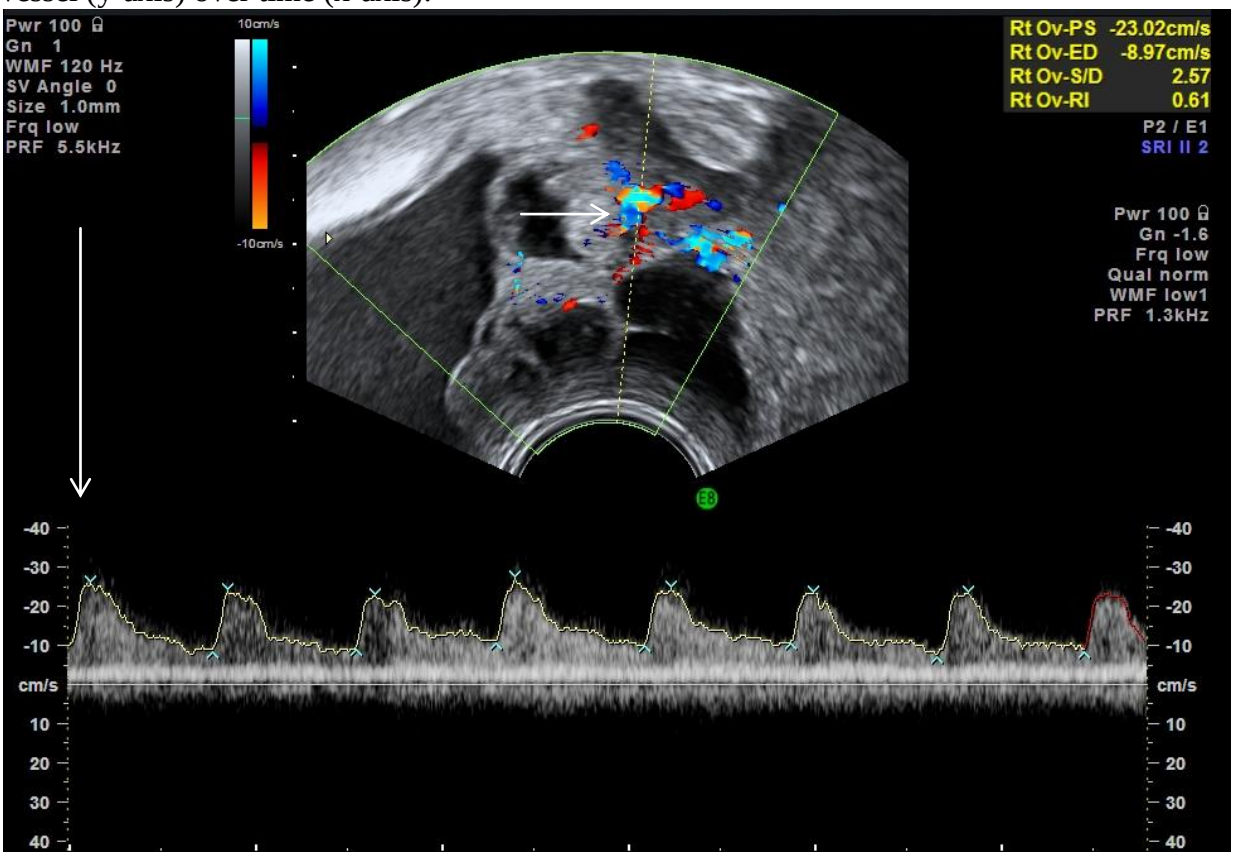
1.4.1 The Doppler effect

The Doppler effect is the apparent difference between the frequency at which waves (sound or light) leave a source and that at which they reach an observer, caused by the relative motion of the observer and the wave source (McNay and Fleming 1999). The effect takes its name from the Austrian physicist Christian Johann Doppler who presented the idea in 1842

(Roguin 2002).When the object emitting the waves is stationary the observed frequency is the same as the emitted frequency, however if the sound source is moving towards the observer, the experienced frequency is higher as the sound waves become more compressed and the opposite happens if the sound source is moving away from the observer. This change in frequency is called the Doppler shift and is proportional to the relative velocity of the source to the observer.

This effect can be applied clinically and is widely utilised in both obstetric and gynaecological ultrasound in order to assess the velocity of blood flow through blood vessels. When the ultrasound beam is reflected off moving blood, there are two Doppler shifts, one when the transmitted ultrasound strikes circulating blood cells and a second when circulating blood cells emit the reflected ultrasound. Evaluation of these Doppler shifts, alongside knowledge of the transmitted ultrasound frequency, the velocity of sound through the tissue and the angle of insonation, allow the calculation of the velocity of blood passing through the vessel (Hoskins et al. 2003). This estimated velocity of blood flow over time can be shown on the ultrasound machine as a tracing (Figure 1).

Figure 1 – Image capture showing B-mode ultrasound image with Doppler velocimetry of a blood vessel within a malignant ovary (short white arrow). The graph at the bottom (long white arrow) is the tracing showing the estimated velocity of blood flow through the blood vessel (y-axis) over time (x-axis).



1.4.2. Colour Doppler Imaging

In colour Doppler scanning, the same process is applied across an area of tissue rather than a specific blood vessel. The velocity signals are presented as a colour coded overlay, superimposed on the real-time B-mode image. This allows production of an angiogram-like map that provides information on the morphological arrangement of the vascular tree in the tissue of interest. While its sensitivity is good enough to enable visualisation of vessels smaller than one millimetre (De Souza and Cosgrove 2003), it is restricted by its reliance on frequency shifts.

Figure 2a –B-mode image of uterine corpus (long white arrow) and a pelvic mass (short white arrow) with appearances suggestive of a uterine fibroid.

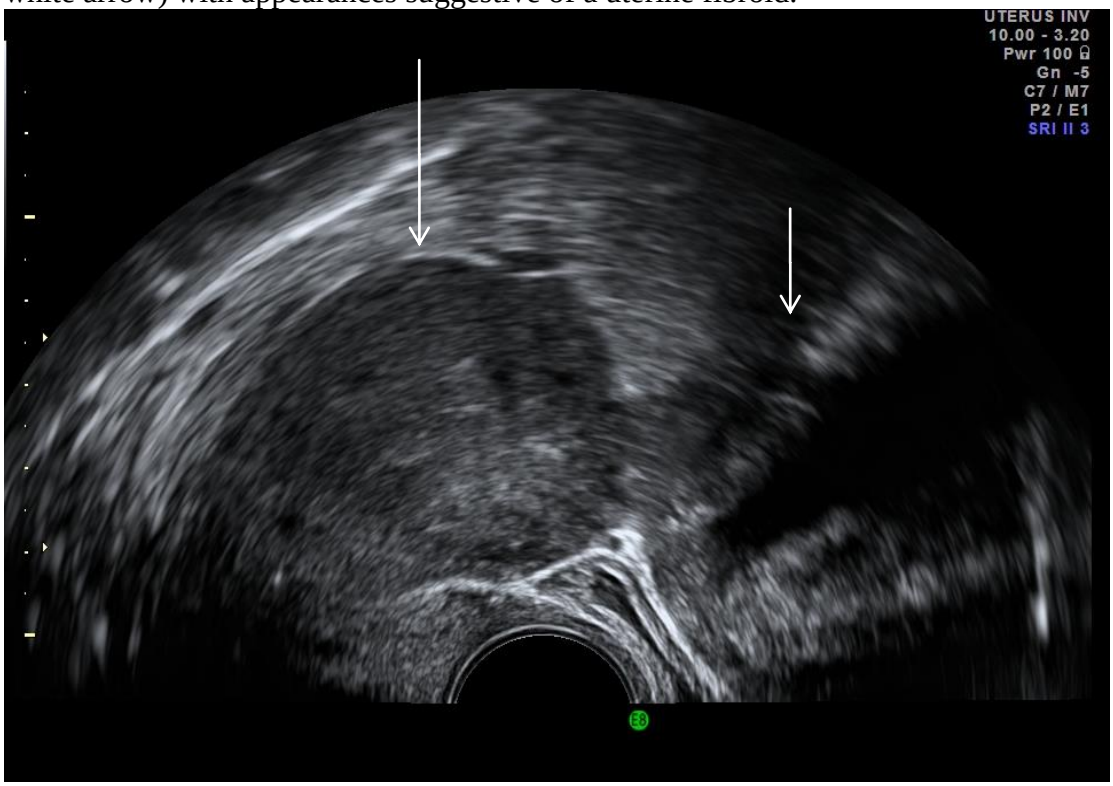
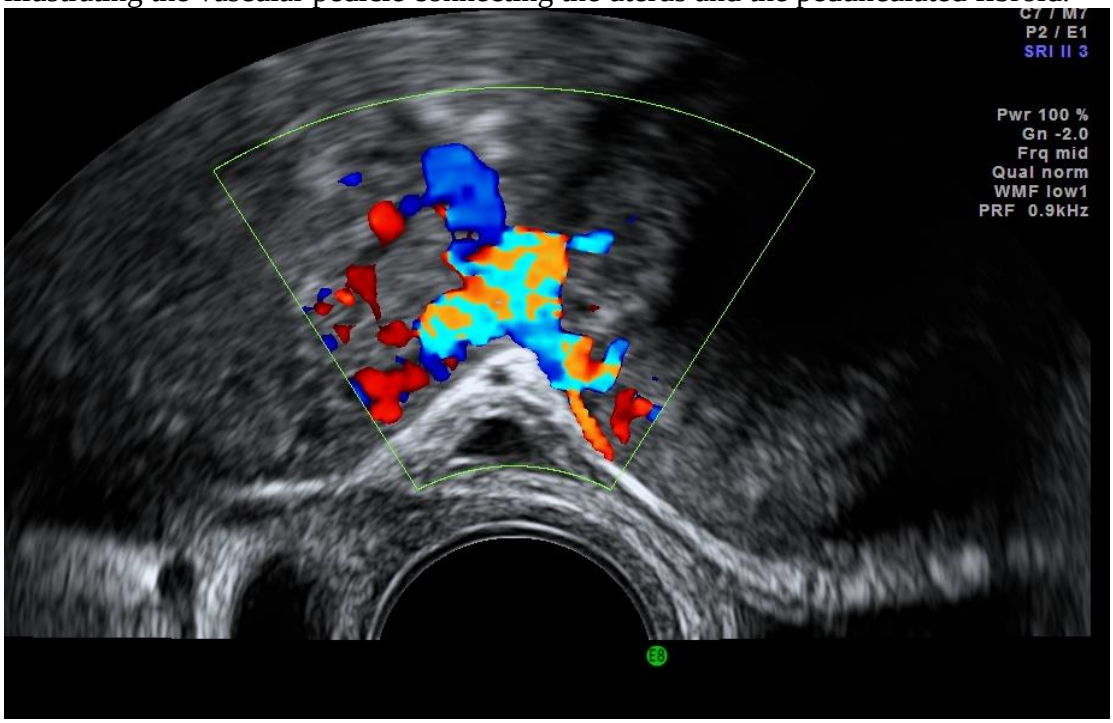


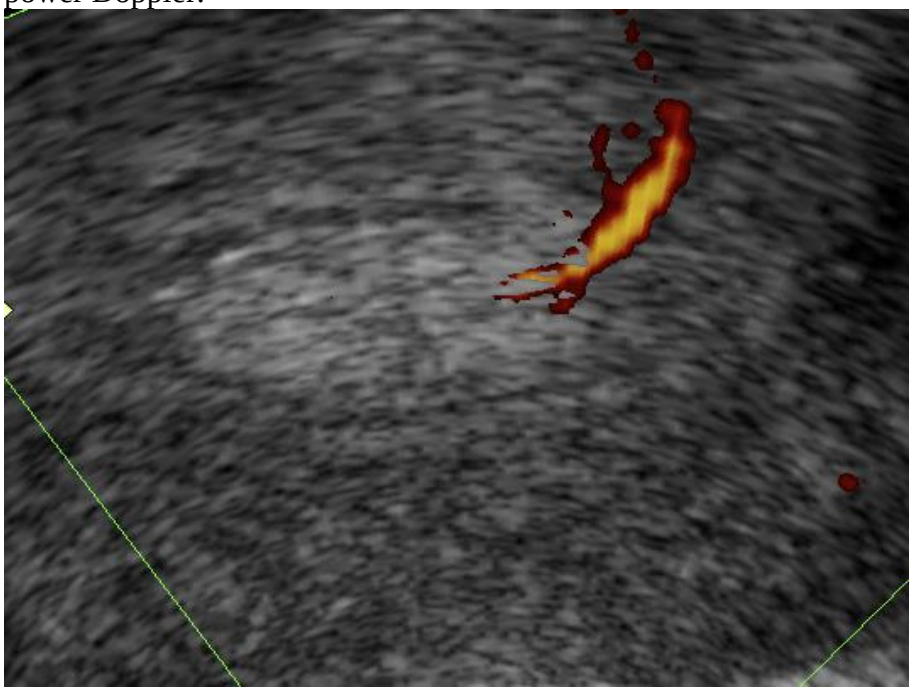
Figure 2b – B-mode image of same uterine corpus and pelvic mass, with colour Doppler illustrating the vascular pedicle connecting the uterus and the pedunculated fibroid.



1.4.3 Power Doppler Imaging

Power Doppler has greater sensitivity than colour Doppler imaging, because the display depends on the amplitude of Doppler signal rather than the frequency shift. Thus, power Doppler imaging provides information on the concentration of moving blood at the expense of knowing its velocity and direction. In addition to being more sensitive than colour Doppler imaging, it is relatively angle independent, more accurate in depicting luminal edges and better in visualising the continuity of flow (Rubin 1999).

Figure 3 – TVUS image showing an endometrial polyp with its 'feeder' vessel delineated by power Doppler.



1.5 Three-dimensional ultrasound

3D ultrasound is a relatively recent diagnostic modality, which allows detailed evaluation of pelvic organs by collecting a series of sequential ultrasound images and converting them into an ultrasound volume. This information is digitally stored as a dataset which is reconstructed in such a way as to allow visualisation of an organ from any chosen angle and in any arbitrary

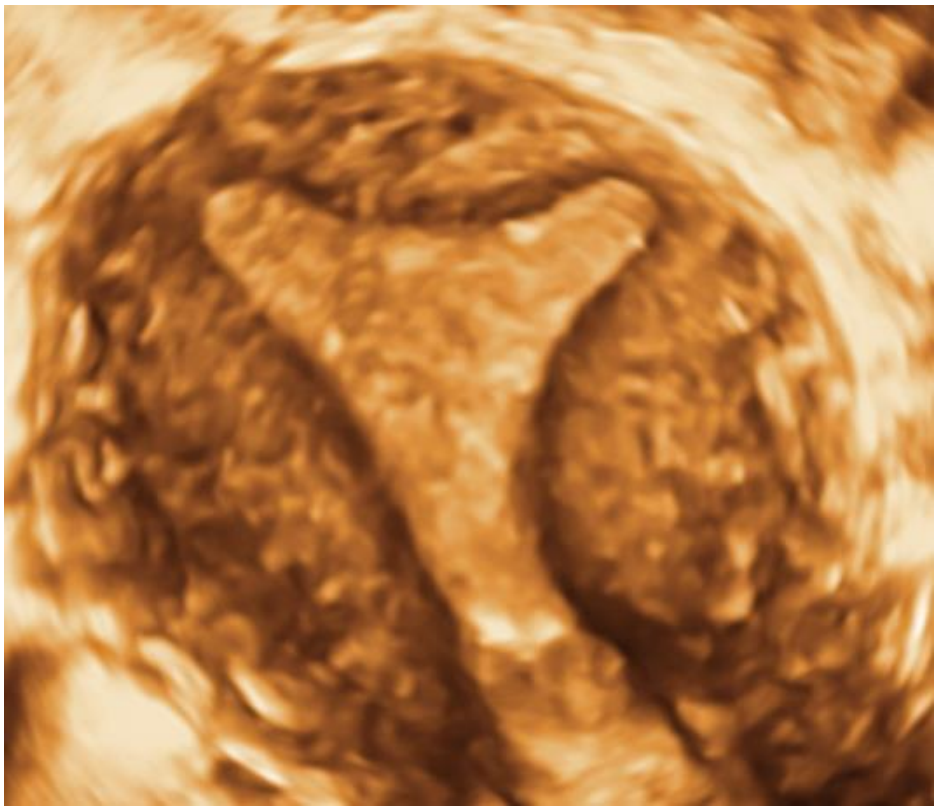
plane (Jurkovic 2002). In the field of obstetrics and gynaecology 3D ultrasound has so far had the greatest impact on the diagnosis of uterine abnormalities.

1.5.1 Three–dimensional ultrasound volume acquisition

3D ultrasound involves the acquisition of a series of consecutive, parallel B-mode images by sequential movement of either the transducer or the ultrasound beam. This series of images is then stored in the computer as a volume set which can be examined using three methods: section reconstruction, surface rendering and volume rendering. The 3D ultrasound equipment used in this thesis is designed by Kretz Technik, Zipf, Austria (Voluson E8). This commonly used system combines both short volume acquisition time and fast computing, enabling almost immediate online data analysis and near real-time surface rendering. Volume acquisition with this system is performed using mechanical movement of the ultrasound transducer within the transvaginal probe.

An important aspect of 3D ultrasound is the ability to display and manipulate any chosen section from within the dataset. This confers many advantages over B-mode imaging. Firstly, it allows the acquisition of views that are not possible with conventional two-dimensional sonography, largely as a result of restrictions on probe movement imposed by pelvic anatomy (Baba et al. 1997). The example of this most relevant to this thesis is the coronal view of the normal female uterus (Figure 4). Secondly, the ability to view the organ in three orthogonal planes enables a more detailed analysis of the organ to be performed. Finally, the storage of images for manipulation enables examination of the volume set by multiple operators at different times.

Figure 4 – A 3D coronal view of a normal uterus.

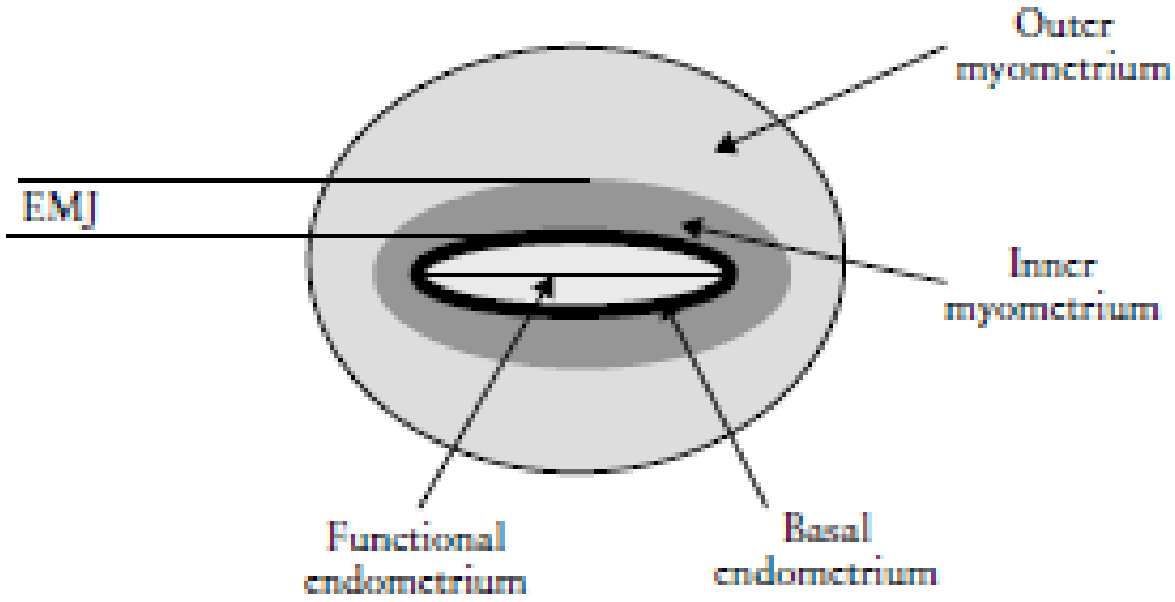


2.0 ENDOMETRIAL-MYOMETRIAL JUNCTION (EMJ)

2.1 Introduction

Assessment of the uterus has historically focussed on what were considered to be its two constituent parts: the endometrium and the myometrium. Magnetic resonance imaging (MRI) studies in the early 1980s revealed that the myometrium might be further divisible into two distinct compartments: the inner and outer myometrium (Hricak et al. 1983). Despite being macroscopically and microscopically indistinct, subsequent research has shown these two compartments of the myometrium to have quite different embryology, anatomy and physiology. The inner myometrium has been variously termed the uterine junctional zone (JZ) (Fusi et al. 2006), the endometrial-subendometrial unit or stratum sub-vasculare (Noe et al. 1999), the subendometrial myometrium (Lyons et al. 1991) the archimetra (Leyendecker et al. 1998) or archimyometrium (Leyendecker et al. 2006). While these terms are often used interchangeably it is not always clear whether they are referring to exactly the same entity. For the purposes of this thesis, this region will be referred to as the EMJ. It is the transitional zone between the mucous membrane that is the endometrium and the outer smooth muscle layer of the myometrium (Figure 5).

Figure 5 - Schematic diagram of a uterus in transverse section showing the endometrial and myometrial layers and the EMJ (Naftalin and Jurkovic 2009).



2.2 Embryology of the EMJ

The endometrium and the EMJ both arise from the paramesonephric ducts, whereas the outer myometrium is of mesenchymal origin (Daels 1974; Brosens et al. 1998) The paramesonephric ducts are formed at about six weeks' gestation from the coelomic invagination of mesodermal cells at the level of the third thoracic somite (Koff 1933; O'Rahilly 1977). The caudal portions of these tubes make contact with each other and fuse in the midline, giving rise to the uterus and vaginal canal. The septum that initially divides the uterine cavity is then reabsorbed leaving a single cavity. The endometrial glands originate at around 19 weeks' gestation from out-pouchings of the columnar epithelium that lines the primitive uterine cavity. The smooth muscle cells of the EMJ can be seen from around 21 weeks, while the outer layers of the myometrium develop in the third trimester or even postnatally (Noe et al. 1998).

2.3 Anatomy and histology of the EMJ

The myometrium consists of bundles of smooth muscle fibres, intermixed with areolar tissue, blood vessels, lymphatic vessels, and nerves. The muscle fibres of the EMJ have predominantly circular orientation, in contrast to the longitudinal smooth-muscle orientation of the outer layers of myometrium (Wetzstein and Renn 1970). The endometrium, the mucous membrane that lines the uterine cavity, is composed of a single layer of columnar epithelium.

The EMJ is structurally distinct from other mucosal-muscle interfaces within the human body in that it lacks a submucosal layer. Most tissues with a mucosa have a subjacent, histologically recognizable submucosa that protects the underlying tissue from mucosal invasion, e.g., stomach, intestine, trachea, and bronchi (Marcus 1961, Emge 1962). While the superficial and basal layers of the endometrium are clearly distinguishable on light microscopy, there is no histological distinction between the inner myometrium and the outer myometrium on light microscopy. Tetlow et al. demonstrated increased vascularity of the EMJ when compared with the rest of the myometrium and also found that the muscle fibres of this zone were more densely packed than in other zones of the myometrium. They concluded that these architectural findings would account for the hypoechoic appearance of the EMJ on both TVUS and MRI (Tetlow et al. 1999).

2.4 Physiology and hormonal regulation of the EMJ

The endometrium and EMJ do not just share an embryological origin. While they are physiologically distinct, they are both under the cyclical influence of ovarian sex steroids. The main function of the EMJ appears to be modulation of uterine peristalsis, an area thought to play an increasingly significant role in fertility. Uterine contractions emanating from the

EMJ were first visualised and subsequently described by Birnholz who visualised them on transabdominal ultrasound (Birnholz 1984). EMJ contractions vary in orientation, amplitude and frequency throughout the cycle under the influence of oestradiol and progesterone. In the menstrual phase these contractions are fundo-cervical in direction (de Ziegler et al. 2001) facilitating menstruation. The direction reverses during the follicular phase becoming predominantly cervico-fundal with the amplitude and frequency of contractions increasing significantly as ovulation approaches. There is evidence that this pattern of contractions in the late follicular phase facilitates sperm transport (Kunz et al.1996). After ovulation there is a decrease in overall EMJ contractility under the influence of progesterone. Ijland et al. suggested that this may help to facilitate implantation of the developing blastocyst, while improving its supply of oxygen and nutrients (Ijland et al.1996).

2.5 Imaging of the EMJ

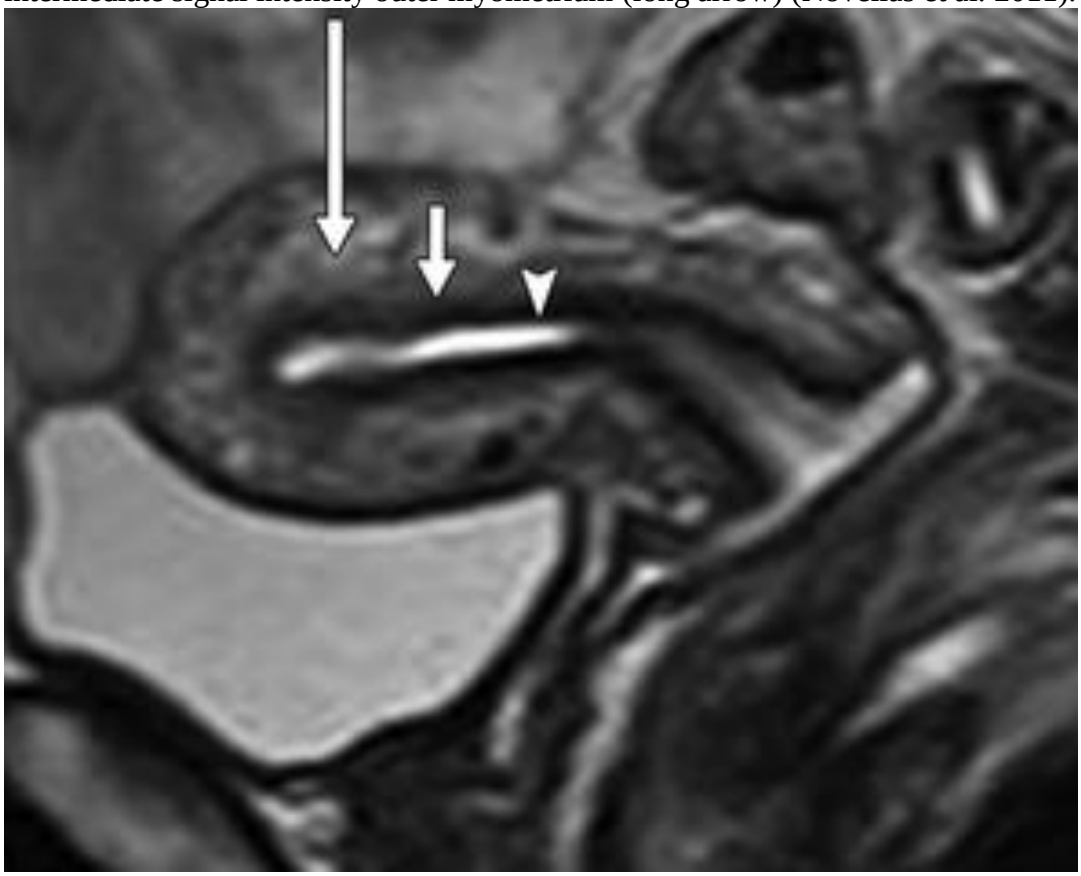
Assessment of the EMJ has traditionally been part of the MRI evaluation of the uterus, with changes in this area being a key component of the diagnosis of adenomyosis. With regard to ultrasound, although the EMJ is visible, it has not historically played a major part in the evaluation of uterine pathology. This may be partly due to the fact that in order to obtain clear images of the EMJ, high resolution ultrasound equipment with 3D imaging facility is often required, which has only been available in recent years. This may also explain why the majority of studies addressing visualisation and the appearance of the EMJ have used MRI.

2.5.1 Magnetic Resonance Imaging

Hricak et al. first described the uterus as comprising three distinct zones when visualised on MRI (Hricak et al 1983). MR imaging of uterine zonal anatomy is best demonstrated on T2-

weighted images. The endometrium is of generally high signal intensity and is therefore visualised as a thin white stripe. The inner myometrium or JZ, as most MRI studies refer to the inner myometrium, is of uniformly low signal intensity while the outer myometrium is of intermediate signal intensity (Lee et al. 1985) (Figure 6).

Figure 6 – Sagittal T2-weighted image of a uterus showing normal zonal anatomy with a high signal intensity endometrium (arrowhead), low-signal intensity JZ (short arrow) and intermediate signal intensity outer myometrium (long arrow) (Novellas et al. 2011).



Because of this observed contrast of the three uterine zones, MRI is very good at delineating and measuring the size of these zones. Many studies have attempted to derive a normal range for JZ thickness, with the upper limit now considered to be 8mm (Novellas et al. 2011). This measurement is crucial in the MRI diagnosis of adenomyosis, which is largely dependent on increased thickness of the JZ (Reinhold et al. 1996). It is worth noting that the thickness and appearance of the JZ is hormone-dependent and therefore cyclical with the maximal thickness

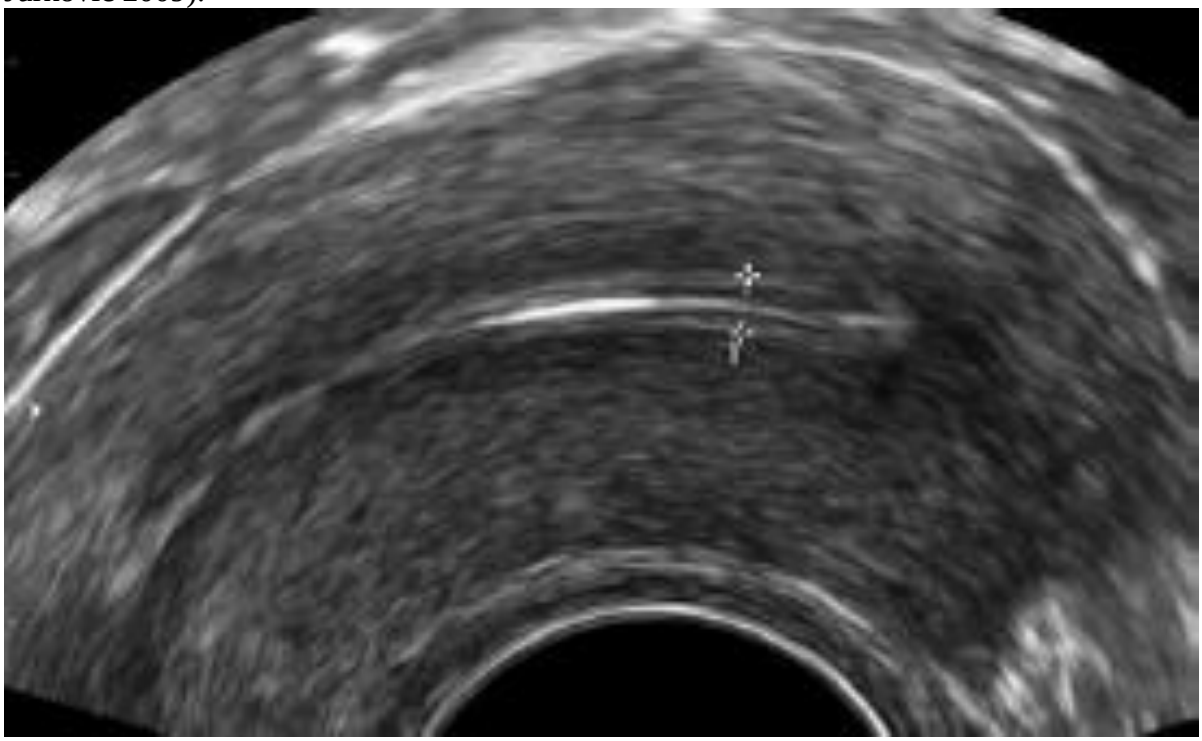
of the JZ being reached during the menstrual phase of the menstrual cycle (Fusi et al. 2006). Furthermore, prior to menarche, during pregnancy and after the menopause, uterine zonal anatomy is less distinct (Demas et al. 1986; Brosens et al. 1998; Willms et al. 1995) with the JZ and the outer myometrium being poorly distinguished. This finding is reversed in postmenopausal women by the taking of hormone replacement therapy (McCarthy et al. 1986). These effects mean that the JZ cannot be measured in a significant proportion of women; up to 30% of premenopausal women (Bazot et al. 2001) and 50% of postmenopausal women (Fusi et al. 2006).

2.5.3 Ultrasound

Formal ultrasound examination of the uterus includes a detailed assessment of the morphological characteristics of the myometrium and endometrium. These two main functional components of the uterus display different acoustic properties, which facilitates their differentiation on the ultrasound image. On ultrasound, the inner myometrium has been described as a hypoechoic band or 'halo' that regularly encircles the endometrium (Kunz et al. 2000). While this zone is distinct from both the endometrium and the outer myometrium, the ultrasonic delineation of uterine zonal anatomy is less clear than on MRI. Ultrasound, however, has the ability to visualise very clearly the basal endometrial layer, which forms the actual interface between the inner myometrium and the endometrium. On two-dimensional ultrasound scan, the EMJ is best seen in the longitudinal section, which enables the examination of its anterior and posterior aspects. In this view the EMJ is seen as consisting of two distinctive structures: basal endometrium and inner myometrium. In normal uteri the basal endometrium is seen as a continuous uninterrupted hyperechoic line and in practical

terms it represents the endometrial–myometrial interface (Naftalin and Jurkovic 2009) (Figure 7).

Figure 7 – A TVUS image showing a longitudinal view of a normal uterus in the proliferative phase of the cycle. The callipers are placed on the basal endometrial layer which appears as a regular continuous hyperechoic line. The surrounding inner myometrium appears hypoechoic in comparison to both the outer endometrial and outer myometrial layers (Naftalin and Jurkovic 2009).



On ultrasound, as with MRI, the differences in appearance of the inner and the outer myometrium are influenced by ovarian sex steroid hormone levels. For example, prior to menarche when the levels of the ovarian sex steroids are low, just as with MRI, the inner and outer myometrium are indistinct on ultrasound (Newren 1997). There are, however, no reported ultrasound studies of the cyclical changes seen within the EMJ (Naftalin and Jurkovic 2009).

With the development of 3D ultrasound has come the ability to create a 3D reconstruction of uterine anatomy in the coronal plane. This allows ultrasound assessment of both the lateral

and fundal aspects of the EMJ, which were otherwise impossible to see clearly on standard B-mode ultrasound imaging. The ability to see the entire lateral borders of the EMJ in a single view has, literally, added a new dimension to the ability to visualise the EMJ on ultrasound. Exacoustos et al. applied measurements taken routinely in the MRI assessment of adenomyosis to ultrasound assessment of the uterus (Figures 8a & 8b). They took various measurements of the JZ taken using a 3D coronal view of the uterus including the minimal JZ thickness (JZmin), the maximal JZ thickness (JZmax) and the maximal myometrial thickness, as a means of diagnosing adenomyosis (Exacoustos et al. 2011). They considered a JZmax >8mm and a difference between the JZmin and the JZmax of >4mm to have high diagnostic accuracy however these measurements have not been tested prospectively and furthermore, no studies have been performed that attempt to describe a normal range for JZ thickness, as measured by 3D ultrasound.

Figure 8a – A 3D coronal view of a normal uterus showing measurements of the maximal JZ thickness (callipers 1 and 4), minimal JZ thickness (calliper 3) and maximal myometrial thickness (calliper 2) (Exacoustos et al. 2011)

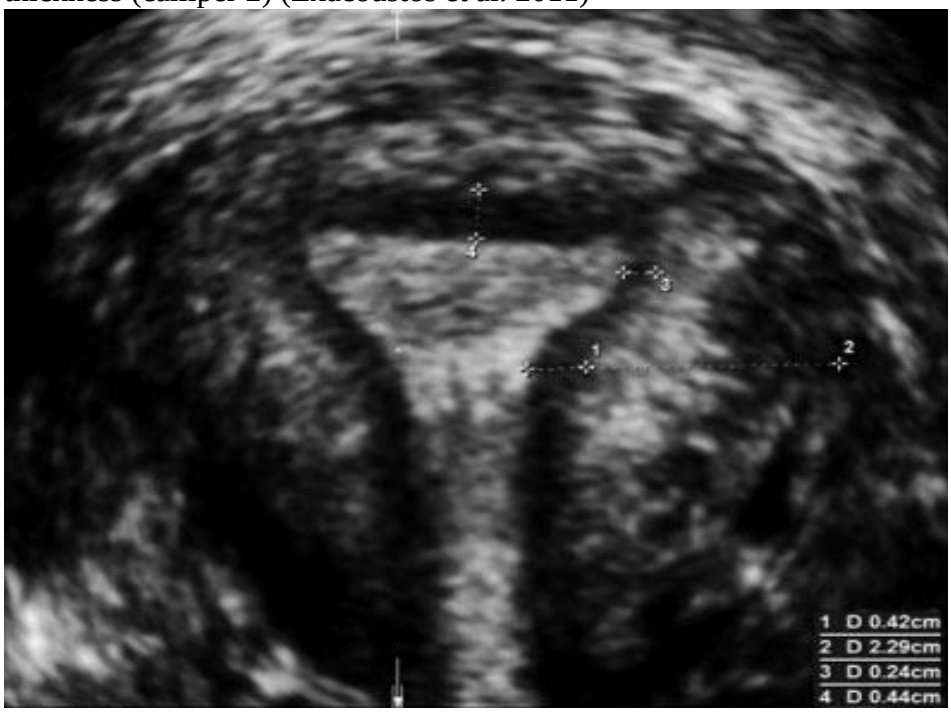
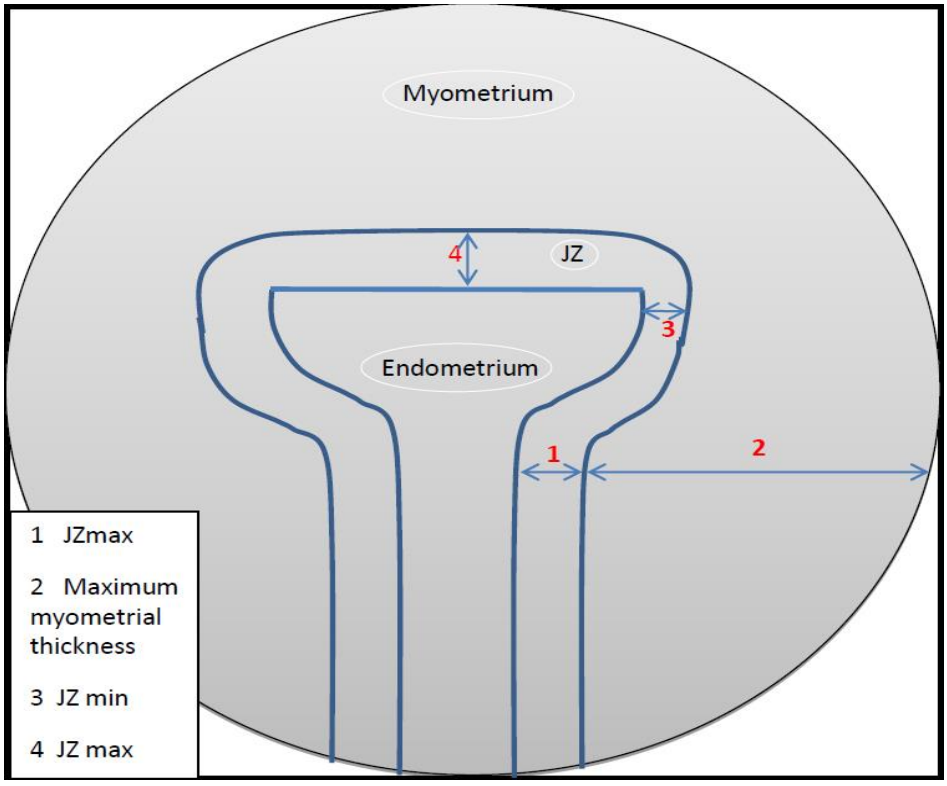


Figure 8b – A schematic representation of Figure 8a



2.6 Pathology of the EMJ

2.6.1 Adenomyosis

Adenomyosis is a condition defined by the presence of endometrial glands or stroma within the myometrium. While there is still much that is poorly understood about the condition, it is accepted that it often involves the EMJ, while some would consider it a disease of the EMJ.

2.6.1.1 History of adenomyosis

Determining the history of adenomyosis, as with many other aspects of the disease, is fraught with difficulties. It remains unclear as to when it was first described with Scroen (1690), Rokitansky (1860) and Cullen (1896) all being credited (Benagiano and Brosens 2006). Further difficulty stems from the fact that for some time, adenomyosis and endometriosis were considered to be the same condition. The first detailed descriptions of adenomyosis came from Cullen in 1896 (Cullen 1896). He subsequently dedicated an entire book to the condition, entitled 'Adenomyoma of the uterus' (Cullen 1908). It was Frankl who first used the term 'adenomyosis' explaining "I have chosen the name of adenomyosis, which does not suggest any inflammatory genesis as do terms like adenometritis, adenomyositis and adenomyometritis…..We were never able to find any trace of an inflammatory infiltration, either in the musculature or the mucosa of this region". He also noted continuity between adenomyosis and the endometrium, suggesting an endometrial origin to the disease (Frankl 1925). It was with Sampson's classical description of peritoneal endometrium and the first use of the term 'endometriosis' two years later, that the conditions started to be considered as two separate entities (Sampson 1927). The modern definition of adenomyosis – 'benign invasion of the endometrium into the myometrium'- was provided by Bird et al. in 1972 (Bird et al. 1972).

2.6.1.2 Pathogenesis of adenomyosis

The precise pathogenesis of adenomyosis remains unknown with many theories being proposed. The most widely held theory is that it results from invagination of endometrial tissue, across the EMJ, into the myometrium. There are numerous histological and ultrasound reports of endometrial tissue being seen in continuity with adenomyosis within the myometrium (Frankl 1925; Ferenczy 1998; Naftalin and Jurkovic 2009; Verma et al. 2009) that would appear to corroborate this theory. Other suggested mechanisms for the disease include *de novo* development of endometrial tissue from mullerian remnants, although this is thought to apply more to adenomyosis found in extra-uterine sites, such as the recto-vaginal septum (Nisolle and Donnez 1997).

Even if endometrial invagination is the cause, there is still significant uncertainty about what factors might trigger this invasion. As with most chronic conditions, the aetiology is likely to be multi-factorial involving some or all of the factors described below. The 'invasive potential' of endometrial tissue has been investigated as a possible cause. In-vitro studies have shown that endometrial cells have a similar invasive potential to metastatic bladder cell lines (Gaetje et al. 1995). Others have looked for differences between normal endometrial glands and 'adenomyotic' endometrial glands found in the myometrium. Lei et al. found increased expression of human chorionic gonadotrophin (hcg) / luteinizing hormone (LH) receptor mRNA in endometrial glands found in foci of adenomyosis, compared with normal endometrial glands (Lei et al. 1993). A similarly increased hcg/LH receptor expression has been found in endometrial cancer cells when compared to normal endometrium (Lei et al. 1992), and in invasive trophoblast when compared to non-invasive trophoblast in choriocarcinomas (Lin et al. 1994). It is possible therefore that this increased receptor expression may be related to the potential of endometrial tissue to invade into the myometrium and form adenomyotic foci (Ferenczy 1998).

Myometrial characteristics may have a role in this endometrial invasion. The EMJ is unusual in lacking a sub-mucosal layer. Most muscle-mucosa interfaces within the body, having a distinct subjacent histologically recognizable sub-mucosal layer that protects the underlying tissue from invasion (Marcus 1961, Emge 1962). The absence of this layer might explain why invasion can occur, but does not explain why it happens in particular areas of the myometrium but not others.

Endometrium may cross the EMJ at areas of myometrial 'weakness'. A few theories of how myometrial 'weakness' might occur have been proposed. Surgery provides a mechanical model for this. Many studies have looked for an association between adenomyosis and uterine surgeries, including caesarean section, uterine curettage and termination of pregnancy. All these operations involve operating across or close to the EMJ and could easily create defects in the EMJ or myometrium. Indeed, in the case of Caesarean section, it would be impossible to perform the operation without breaching the EMJ. Defects in the myometrium following Caesarean section are clearly visible on ultrasound and have been reported in the literature (Ofili-Yebovi et al. 2008). Studies, however, have not consistently found an association between Caesarean delivery and adenomyosis (Bergholt et al. 2001; Harris et al. 1985). Uterine curettage might create defects in the myometrium through which endometrial tissue might subsequently pass or could even push endometrial tissue through the EMJ and into the myometrium at the time of surgery. Adenomyosis has been elicited in pregnant rabbits by curetting one uterine horn and leaving the pregnancy in the other horn (Lewinski 1931). In human studies, several authors (Levgur et al. 2000; Vavilis et al. 1997; Curtis et al. 2002) have found an association between adenomyosis and previous surgical termination of pregnancy. Uterine softening in early pregnancy (Munsick 1985) might increase the risk of EMJ trauma, perhaps explaining the association reported between

adenomyosis and uterine instrumentation in pregnancy, but not outside of pregnancy (Levgur et al. 2000; Curtis et al. 2002).

Since adenomyosis is more common in parous women, mechanical events at parturition have been considered to be the main pathogenenic factors for the development of adenomyosis (Kitawaki 2006). However there are few data to support that it is parturition rather than pregnancy *per se* that is the cause. Certainly the physiological process of trophoblast invasion, common to all pregnancies, involves invasion up to the EMJ. MR images during a conception cycle have shown focal changes within the EMJ (Turnbull et al. 1995) and it is known that trophoblast invasion is able to progress beyond the EMJ, albeit pathologically, in cases of placenta accreta, increta and percreta. Perhaps the process of trophoblast invasion, in some women, alters the EMJ leaving it susceptible to adenomyosis in the future.

Uterine autotrauma, mediated by hyperperistalsis of the inner myometrium, has been suggested as a further mechanism by which both adenomyosis and endometriosis might spontaneously form. This theory holds that chronic peristalsis or periods of hyperperistalsis in the inner myometrium cause EMJ microtrauma and that a cycle of permanent hyperperistalsis, mediated by increased oestrogen production, leads to overt autotrauma. This combination of hyperperistalsis and overt trauma to the EMJ would physically force basal endometrial tissue into the myometrium (Leyendecker et al. 2009) causing adenomyosis. The increased presence of oestrogen plays a key role in this hypothesis. Adenomyosis has been found to be associated with oestrogen-dependent conditions including uterine fibroids (Azziz 1989), endometriosis (Kunz et al. 2000), and endometrial hyperplasia (Bergholt et al. 2001). It also appears to be most prevalent in women of reproductive age and regresses after the menopause (Kitawaki 2006). This has led some researchers to investigate the role of oestrogen in the pathogenesis of adenomyosis. It has been reported that oestrogen receptors are always found in adenomyotic tissue (Tamaya et al. 1979). Other investigators have found

that adenomyotic tissue contains aromatase, an enzyme that catalyses the conversion of androgens to oestrogens, suggesting that local oestrogen production within adenomyotic tissue may contribute to overall oestrogen concentrations (Urabe et al. 1989). Further evidence of this local production of oestrogen comes from a study that looked at oestrogen levels in both peripheral and menstrual blood in women with and without adenomyosis. While there was no difference in the oestrogen levels in peripheral blood, there were significantly increased oestrogen levels in the menstrual blood of women with adenomyosis (Takahashi et al. 1989).

A genetic predisposition to adenomyosis has been suggested from early reports suggesting heredity (Emge 1962; Arnold et al. 1995) however few studies have investigated the genetics of adenomyosis. Pandis et al. found a common chromosomal abnormality [7] [q21.q231.2] in three cases of adenomyosis (Pandis et al. 1995). Similarly Kitawaki found that the PP genotype is less frequently observed in women with endometriosis and adenomyosis when compared with women with neither condition (Kitawaki et al. 2001). Wang et al. 2002 found no chromosomal gain or loss in 25 cases of adenomyosis using comparative genomic hybridization (Wang et al. 2002).

2.6.1.3 Diagnosis of adenomyosis

2.6.1.3.1 Histology

The histological diagnosis of adenomyosis requires endometrial glands or stroma to be found beyond the EMJ and up until relatively recently, histopathological examination of the uterus was the only way to diagnose the condition. While recent advances in imaging make an accurate, non-invasive diagnosis possible, almost all the published studies of the diagnostic accuracy of these imaging techniques have used histology as the gold standard. There are,

however, substantial disadvantages to the continued use of histology for the diagnosis of adenomyosis.

There remains significant disagreement as to the depth of infiltration required for the diagnostic criteria to be met. It has been suggested that units of microscopic fields should be used for this depth, however reported depths vary and include half a low-power field of view (Kurman 1994), one low-power field of view (Rosai 1989), one medium-power field of view (Gompel and Silverberg 1985) and one high-power field of view (Entmann 1988). Furthermore, there is significant variation in the size of microscopic fields when different microscopes are used (Ellis and Whitehead 1981) leading to the suggestion that a different measure should be used. The depth of infiltration as a proportion of the total uterine wall thickness has been proposed with depths greater than 25% (Ferenczy 1998) and greater than one third of the total thickness being used in the literature (Hendrickson & Kempson 1990), however uterine wall thickness is not uniform throughout the uterus, particularly in the presence of adenomyosis. Other studies have looked at specific depths of infiltration. Bergholt et al. re-examined hysterectomy specimens and applied differing depths of infiltration of endometrial glands into the myometrium ($\geq$1mm, $\geq$3mm & $\geq$5mm) for the diagnosis of adenomyosis and showed that the reported prevalence varied from 11.5%–18.2% (Bergholt et al. 2001). Further variation was found depending on whether or not the presence of myometrial hyperplasia was used as a diagnostic criterion. This followed on from the study by Bird et al that showed that the reported prevalence of adenomyosis varied depending on the number of slices of histological tissue examined (Bird et al. 1972).

Histopathological grading systems for severity of adenomyosis have been proposed. Bird & Molitor both used a histological system to grade the severity of adenomyosis based on depth of infiltration, whereby infiltration of endometrial tissue to the inner myometrium only was graded ‘slight’, infiltration to the middle third of the myometrium was termed ‘moderate’ and

infiltration to the outer third was termed ‘extensive’ (Bird et al. 1972; Molitor 1971) . Bird et al. also used a histological system to grade the degree of myometrial involvement. Here, 1-3 endometrial glands per low-power field was graded ‘slight’, 4-9 endometrial glands per low-power field was graded ‘moderate’ and 10 or more endometrial glands per low-power field was termed ‘marked’ (Bird et al. 1972). While these grading systems add an extra level of subtlety to the histological diagnosis of adenomyosis, they have not been prospectively evaluated, nor have their inter- and intra-observer variability been evaluated.

This variation in diagnostic criteria in the histological diagnosis of adenomyosis poses problems not only in the diagnosis of the disease, but also in the investigation of the disease. Most studies looking at associations with the condition have compared the prevalence of the condition in different hysterectomized populations eg women with a history of menorrhagia and those without a history of menorrhagia, and then looked for statistical significance. Analysis and comparison of different studies is difficult when different diagnostic criteria are being used, making it difficult to draw firm conclusions about the condition when studied in this way. Even where standardised diagnostic criteria are used it has been suggested that awareness of the condition amongst individual pathologists could confer a further source of variance with the frequency of diagnosis of adenomyosis varying from 12-58% among 15 different hospitals, and from 10-88% among 25 different histopathologists (Seidman and Kjerulff 1996). One explanation for this might be that histopathologists may see but not report adenomyosis unless specifically requested to do so, particularly if it is not relevant to the primary condition. This phenomenon may be exacerbated by the fact that once diagnosed post-hysterectomy, the condition has already been cured and so a diagnosis of adenomyosis will not influence future clinical management. This links to another fundamental problem with the use of histological examination of hysterectomy specimens to diagnose adenomyosis, which is that it precludes assessment of the success of conservative treatments

for the condition. This drawback of histological diagnosis does not apply to non-invasive diagnostic techniques, which must therefore be used for assessments of the effectiveness of conservative treatments for the condition.

Finally, as histology remains the gold standard for diagnosis, much of our current knowledge of the condition comes from histological examination of the entire uterus. There is great national, international and temporal variation in the use of, and indications for, hysterectomy (Bergholt et al. 2001; Reid and Mukri 2005). Furthermore, populations of women undergoing hysterectomy in different regions are likely to be heterogenous and hence subject to significant demographic variation. All of these factors, directly linked to the use of histology as gold standard for diagnosis, explain both the significant variation in reported prevalences of adenomyosis, as well as the lack of consensus in the literature with regard to its clinical impact.

2.6.1.3.2 Clinical

There are no symptoms or symptom complexes that are pathognomic of adenomyosis and the symptoms classically ascribed to adenomyosis; abnormal uterine bleeding, painful periods and pelvic pain, are non-specific and associated with many other gynaecological pathologies. Furthermore, adenomyosis is often asymptomatic with 35% of women diagnosed with the condition in one study not having any symptoms explained by the disease (Benson and Sneedon 1958). Against this background, it is unsurprising that the accuracy of clinical diagnosis of adenomyosis is poor. In one study, a putative diagnosis of adenomyosis was confirmed in under half of women who subsequently underwent a hysterectomy (Lee et al. 1984).

2.6.1.3.3 Ultrasound

2.6.1.3.3.1 Diagnostic accuracy of ultrasound

Many investigators have assessed the accuracy of transvaginal ultrasound in the diagnosis of adenomyosis, invariably in women undergoing hysterectomy, by comparing pre-operative ultrasound diagnosis with subsequent histological diagnosis. The reported sensitivity varies from 53%-89% and the reported specificity varies from 50%-99% (Dueholm 2006). The prevalence of adenomyosis in these studies varies widely which may reflect the differing histological criteria used, the different populations studied or the varying degree of patient selection involved. Generally, the more highly selected the patients are, the higher the sensitivity of diagnosis (Dueholm 2006). Large studies with no selection will, almost invariably, include women with either large uterine fibroids, or large numbers of fibroids. Fibroids of this nature are likely to prevent examination of the entire myometrium, meaning that ultrasound features of adenomyosis may be missed (Bazot et al. 2001). The difficulty in differentiating adenomyomas and fibroids may also lead to false positive diagnosis of adenomyosis. The presence of other pathologies might also reduce the accuracy of ultrasound in the diagnosis of adenomyosis. Studies of post-menopausal women, and even studies of pre-menopausal women if large enough, are likely to include some women with endometrial cancer. The presence of endometrial cancer, particularly if it involves the myometrium, makes the accurate diagnosis of adenomyosis much more challenging.

2.6.1.3.3.2 Ultrasound features of adenomyosis

Uterine enlargement in the absence of uterine fibroids and asymmetrical myometrial thickening are indirect signs of adenomyosis originally described on transabdominal ultrasound (Siedler et al. 1987; Bohlman et al. 1987). Transabdominal ultrasound, however, does not have sufficient resolution to be able to directly visualise some of the subtle

sonographic features of adenomyosis that have been described on transvaginal ultrasound (Bazot et al. 2002). The characteristic gross appearance and individual ultrasound features of adenomyosis on transvaginal ultrasound derive not only from the presence of endometrial glands and stroma within the myometrium, but also from the associated muscular hypertrophy and hyperplasia (Ferenczy 1998). Many of these features of adenomyosis are subtle and may be missed by those not trained to look for or recognise them. Some authors have suggested that the learning curve for those already familiar with TVUS to be able to diagnose adenomyosis can be up to 6 months (Dueholm 2006). These difficulties are compounded by the fact that the features of adenomyosis cannot always be seen on hard-copy images leading some authors to suggest that it must be diagnosed during real-time examination, which has implications for teaching (Reinhold et al. 1996).

The asymmetrical myometrial thickening seen on transabdominal ultrasound can also be seen on TVUS. Another common feature described is heterogenous, poorly defined areas within the myometrium (Reinhold et al. 1995) or increased myometrial echotexture (Reinhold et al. 1998). These may include or be accompanied by hyperechoic islands or anechoic myometrial cysts and lacunae (Fedele et al. 1992). Parallel shadowing can sometimes be seen distal to these myometrial features. Adenomyomas can be seen on TVUS but can be mistaken for uterine fibroids, with which they will often co-exist. It is particularly important to distinguish between the two in pre-operative imaging as adenomyomas are less amenable to surgical resection than uterine fibroids. Adenomyomas tend to be more elliptical than uterine fibroids and will be less well-circumscribed than uterine fibroids. They will also tend to have less calcification and less edge-shadowing (Reinhold et al. 1998). The use of Doppler can assist in distinguishing between adenomyomas and uterine fibroids. Blood flow around fibroids will be circumferential with minimal or absent blood flow seen within the fibroid, whereas with

adenomyomas, blood vessels will be seen following their normal course through the myometrium (Table 1).

Table 1 – Ultrasound features of fibroids and adenomyomas that aid differentiation on TVUS.

	Fibroid	Adenomyomas
Border definition	Clear	Poor
Echogenicity	Mixed with calcifications	Hypoechoic with striate effect
Shape	Globular	Elliptical
Blood flow on Doppler imaging	Circumferential	Undisturbed

Features seen closer to the endometrium include linear striations and an irregular or indistinct EMJ. Linear striations are seen as fine hyperechoic lines radiating from the endometrium into the myometrium. An irregular EMJ has been reported as a feature of adenomyosis on both B-mode (Reinhold et al. 1998) and 3D imaging (Ahmed et al. 2007). Recently, Exacoustos et al. used the 3D coronal view of the uterus to visualise the EMJ and measure its thickness, to assess its usefulness in diagnosing adenomyosis (Exacoustos et al. 2011). They found that alterations in the EMJ had good diagnostic accuracy for diagnosing adenomyosis. While this is clearly an area of current research focus, there remain no published studies that describe a normal range for EMJ thickness, when measured in this way.

A few studies have looked at which specific ultrasound features are the most accurate in the diagnosis of adenomyosis. Bazot et al. found that myometrial cysts were the most specific and sensitive criterion for diagnosing adenomyosis (Bazot et al. 2001). Kepkep et al. found that myometrial cysts, along with sub-endometrial linear striations and a regularly enlarged

globular uterus were the ultrasound features with the highest accuracy (74.3%, 71.4% and 80% respectively). Sub-endometrial linear striations were the feature with the highest specificity (95.5%) (Kepkep et al. 2007). It should be noted that while there was considerable overlap in the ultrasound features used in these studies, the features used were not identical.

2.6.1.3.4 MRI

2.6.1.3.4.1 Diagnostic accuracy of MRI

There are a small number of studies assessing the performance of MRI in the diagnosis of adenomyosis, all of which included women undergoing hysterectomy. They compared pre-operative MRI diagnosis with subsequent histological diagnosis and showed a sensitivity of 70-86% and a specificity of 86-93% with a mean accuracy of 87.5% (Bazot et al. 2001, Reinhold et al. 1996, Dueholm et al. 2001).

2.6.1.3.4.2 MRI features of adenomyosis

The most widely used feature for diagnosing adenomyosis on MRI is increased JZ thickness. Focal or diffuse thickening (>12mm) of the JZ on T2-weighted images is considered highly suggestive of adenomyosis, whereas adenomyosis is unlikely to be present if the JZ is less than 8mm thick (Reinhold et al. 1998). Focal thickening is considered more specific than generalised thickening which must be differentiated from the appearance of thickening caused by physiological inner myometrial contractions. The timing of the MRI must also be considered before diagnosing adenomyosis by this criterion, as JZ thickness varies considerably during the menstrual cycle (Masui et al. 2001). The JZ is most clearly visible during the late secretory phase (Imaoka et al. 2003) and JZ thickness commonly appears to be >12mm during the menstrual phase, particularly on days 1 and 2. For this reason, some authors advocate avoiding MRI imaging during the menstrual phase of the menstrual cycle in

order to avoid false positive diagnosis of adenomyosis (Tamai et al. 2006). There are further limitations in using JZ thickness as the sole criterion to diagnose adenomyosis. One study found that the JZ was unmeasurable in almost a third of the women in their study, 22% of whom were subsequently diagnosed with adenomyosis on histology (Bazot et al. 2001). Less common MRI features of adenomyosis that have been reported include areas of low-signal intensity within the myometrium with ill-defined borders (Reinhold et al. 1996) and islands of endometrial tissue visualised as punctate foci of high-signal intensity on T2-weighted images. If cyclical bleeding occurs within these glands, they become cystic and will appear as areas of high-signal intensity on T-1 weighted imaging (Outwater et al. 1998). Rarely, endometrium can be seen to be invading the myometrium, which gives the appearance on MRI of 'pseudowidening' of the endometrium (Reinhold et al. 1999). While visualisation of these direct signs of adenomyosis has been reported, MRI has less-than-ideal sensitivity in detecting them, for example small myometrial cysts are only detected in 50% of cases. It has been suggested that this may be because MRI has insufficient spatial resolution to identify these smaller features of adenomyosis (Novellas et al. 2011).

2.6.1.3.4.2 Comparison of diagnostic accuracy of MRI and TVUS

Current opinion is divided as to which imaging modality should be the first-line diagnostic tool in adenomyosis. The diagnostic accuracies of TVUS and MRI have been compared in three studies of women undergoing hysterectomy for benign reasons (Table 2). While small advantages were found with MRI, pooled results show the two techniques to be equivalent and of intermediate accuracy (Dueholm 2006). A significant difference was found in the inter-observer variability of the two diagnostic modalities with MRI having good inter-observer agreement (kappa value = 0.73) and TVUS having only fair inter-observer agreement (kappa value 0.38) (Dueholm et al. 2002). It may be that due to improvements in

the resolution of TVUS machines over the last decade, alongside better training in the ultrasound diagnosis of adenomyosis, the inter-observer agreement in the ultrasound diagnosis of adenomyosis is now better, but no published studies have reassessed the inter-observer variability of TVUS in the diagnosis of adenomyosis.

While TVUS, in the hands of experienced operators had comparable accuracy to MRI, MRI seemed to be a superior, less observer-dependent diagnostic tool in the diagnosis of adenomyosis. Nevertheless, a review concluded that where adenomyosis was suspected, TVUS should be the first-line diagnostic tool, with MRI reserved for cases where TVUS was inconclusive (Dueholm 2006). This conclusion may stem from the fact that TVUS is well-tolerated (Bennett and Richards 2000) and is both cheaper and more easily accessible than MRI.

Table 2 – Studies comparing the diagnostic accuracy of TVUS and MRI for the diagnosis of adenomyosis (Dueholm 2006).

	Reinhold et al. 1996 % (95% CI)	Bazot et al. 2001 % (95% CI)	Dueholm et al. 2001 % (95% CI)
TVUS:			
Sensitivity	89 (71-97)	65 (48-79)	74 (63-82)
Specificity	89 (80-95)	98 (90-100)	87 (81-91)
PPV	71 (54-99)	93 (75-99)	68 (58-77)
NPV	96 (89-99)	85 (75-91)	89 (84-92)
MRI:			
Sensitivity	86 (66-95)	78 (61-89)	78 (68-86)
Specificity	86 (76-92)	93 (84-97)	88 (83-92)
PPV	65 (47-79)	84 (67-93)	70 (60-79)
NPV	95 (87-98)	89 (80-95)	92 (87-95)

2.6.1.3.5 CT

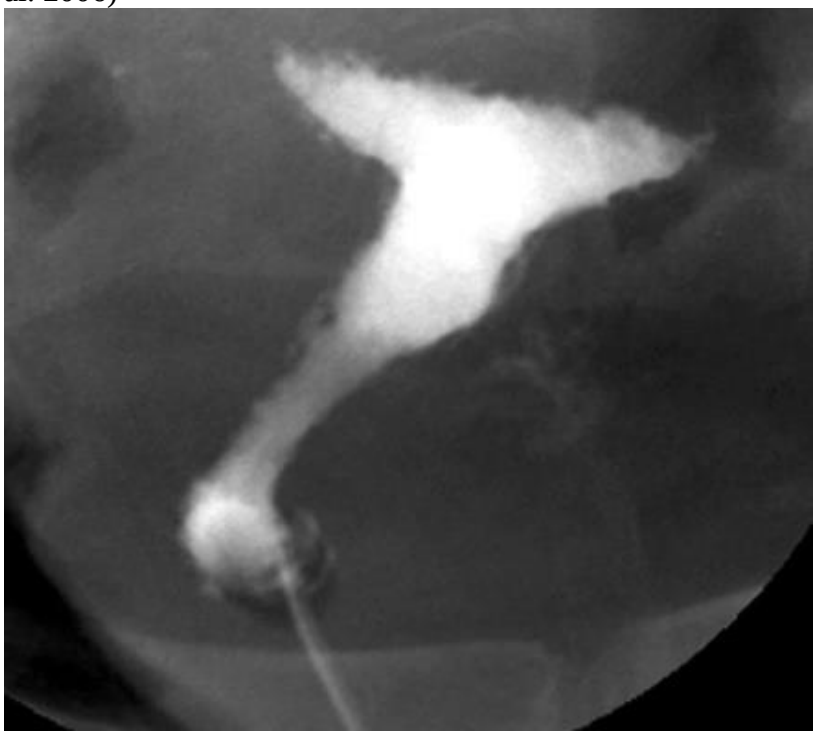
There are limited data on the use of computerised tomography (CT) for the diagnosis of adenomyosis. A retrospective study of 16 women with an MRI diagnosis of adenomyosis, 6 of whom had also had adenomyosis diagnosed on ultrasound scan, suggested that there were

features of adenomyosis that could be seen on CT scan, however the prospectively reported CT scans failed to diagnose adenomyosis in any of the women (Woodfield et al. 2009).

2.3.1.3.6 Hysterosalpingography

Adenomyosis has been diagnosed on hysterosalpingography, with features suggestive of adenomyosis including multiple small spicules with saccular endings (‘lollipop-like’ diverticula), extending from the endometrium into the myometrium (Wolf and Spataro 1988) and irregularity of the uterine contour with small outpouchings of contrast material (Simpson et al. 2006) (Figure 9). The diagnostic sensitivity is poor, however, and so hysterosalpingography is no longer used for the diagnosis of adenomyosis (Arnold et al. 1995).

Figure 9 - Hysterosalpingogram showing irregularity of the uterine contour with small outpouchings of contrast material. Findings consistent with diffuse adenomyosis (Simpson et al. 2006)



2.6.1.4 Clinical impact of adenomyosis

2.6.1.4.1. Prevalence of adenomyosis

The prevalence of adenomyosis in the general population remains unknown as, so far, it has only been possible to establish the diagnosis in pathological specimens, which confers a large selection bias. There is wide variation in the percentage of hysterectomy specimens found to have adenomyosis with reported prevalences of 5-70% (Azziz 1989). Explanations for this wide variation include the lack of consensus in the histological criteria used and variation in the number of tissue blocks examined.

2.6.1.4.2 Symptoms of adenomyosis

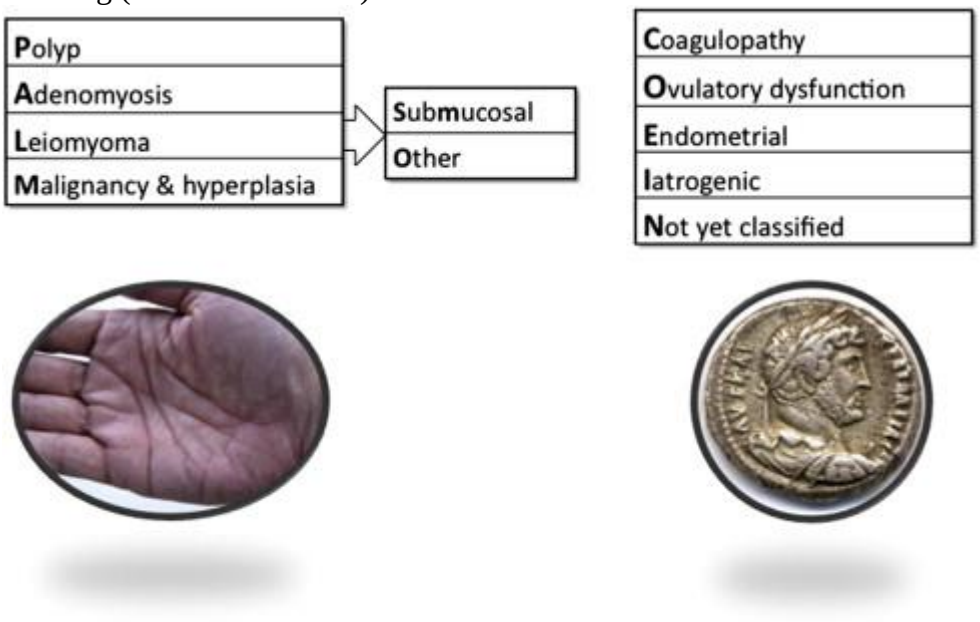
The exact clinical importance of adenomyosis remains uncertain (Dueholm 2006). The common symptoms said to be associated with adenomyosis are menorrhagia and dysmenorrhoea, with less commonly reported symptoms including chronic pelvic pain and dyspareunia. These symptoms are common to a number of other gynaecological conditions including uterine fibroids, endometrial polyps, endometrial hyperplasia and endometriosis. These conditions frequently co-existent, making assessment of causation of symptoms difficult. Furthermore, as is the case with endometrial polyps, fibroids and endometriosis, many women with adenomyosis are asymptomatic (Bird et al. 1972; Benson & Sneedon 1958; Molitor 1971).

2.6.1.4.2.1 Abnormal uterine bleeding

The first detailed description of adenomyosis by Cullen over a century ago described a condition associated with ‘lengthened menstrual periods’ (Cullen 1908). Many investigators since then have looked for an association between adenomyosis and abnormal uterine bleeding with conflicting results. While many studies have found an association between the

two (Emge 1962; Bird et al. 1972; Benson and Sneedon 1958), many others have not (Bergholt et al. 2001; Parrazzini et al. 1997; Weiss et al. 2009). Despite the uncertainty regarding an association, adenomyosis is still considered an important cause of abnormal uterine bleeding as evidenced by its appearance in PALM-COEIN, a mnemonic created by FIGO in 2011 as an aide-memoire for causes of abnormal uterine bleeding (Figure 10) (Munro et al. 2011).

Figure 10 – Illustration of PALM COEIN mnemonic for the causes of abnormal uterine bleeding (Munro et al. 2011).



It is not only the association between adenomyosis and abnormal uterine bleeding that is uncertain. The mechanism by which adenomyosis might cause bleeding symptoms also remains unclear. The finding that mefenamic acid can reduce menstrual loss suggests that prostaglandins may play a role (Azziz 1989). Impaired contractility of the adenomyotic uterus has been suggested as another causative mechanism (Bergeron et al. 2006). Uterine enlargement secondary to adenomyosis might result in a larger endometrial surface area and therefore a greater volume of menstrual loss although a study by Rees et al. found no

correlation between measured menstrual blood loss and endometrial volume-to-surface ratio (Rees et al. 1984).

2.6.1.4.2.2 Pain

As with abnormal uterine bleeding, pain symptoms featured in early descriptions of adenomyosis. Cullen described a condition associated with ‘a great deal of pain’ (Cullen 1908). Many authors have subsequently described an association between adenomyosis and pain symptoms, largely dysmenorrhoea (Emge 1962; Bird et al. 1972; Azziz 1989; Benson and Sneedon 1958), although once again, not all investigators have replicated these findings (Bergholt et al. 2001; Weiss et al. 2009). One prospective study found no difference in either frequency or severity of dysmenorrhoea or pelvic pain between 28 women with adenomyosis and 157 controls (Kilkku et al. 1984). Despite the lack of consensus as to the relationship between adenomyosis and pain, it is still considered a recognised cause of dysmenorrhoea (Peric and Fraser 2006). Dysmenorrhoea may be caused by uterine irritability and inflammation secondary to bleeding into foci of adenomyosis (Bergeron et al. 2006).

2.6.1.4.2.3 Reproductive function

Historically, adenomyosis has been considered a condition of parous women in their later reproductive years and so little work has focussed on its effect on reproductive function. Furthermore, an inability to diagnose the condition pre-hysterectomy has prevented investigation of the condition in a subfertile population. The advent of pre-operative diagnosis alongside an increase in maternal age has led to the condition being encountered more frequently in fertility clinics. Certainly, it is now accepted that the condition can be found in nulligravid and nulliparous women. Animal studies have suggested a deleterious effect on reproductive function with induced adenomyosis in baboons being associated with

lifelong infertility (Barrier et al. 2004). This has all contributed to an increasing focus on its role in reproductive function with some authors proposing that adenomyosis is a significant contributor to subfertility. As such, its diagnosis or exclusion should be mandatory in fertility investigations (Leyendecker et al. 2006). The prevalence of adenomyosis in a specific subset of fertility patients, women below the age of 36 with a confirmed diagnosis of endometriosis and fertile partners, was found to be as high as 90% (Kunz et al. 2005). It has been proposed that one mechanism through which adenomyosis might have a deleterious effect on fertility is through impairment of the inner myometrial contractions previously discussed as having a crucial role in sperm transport through the uterus (Leyendecker et al. 1996). The finding that adenomyosis has a negative impact on IVF/ICSI conception rates and live pregnancy rates (Salim et al. 2012; Tahlluri and Tremellen 2012) suggests that adenomyosis may negatively impact events after fertilisation and nidation as well.

2.6.2 Endometrial cancer

2.6.2.1 Introduction

Endometrial cancer was recently estimated to be the commonest gynaecological cancer in the developed world (Sankaranarayanan and Ferlay 2006). While the prognosis is good compared to other cancers, around 20% of women diagnosed with the condition will not survive beyond 5 years. All but the earliest endometrial cancers, or those developing in foci of adenomyosis, will involve the EMJ.

2.6.2.2 Prevalence

In 2007, 7536 new endometrial cancers were diagnosed in the UK, making it the fourth commonest cancer in women (ONS 2009). It is classically, although not exclusively, a disease of post-menopausal women and its incidence is rising in this population, whereas it seems to be stable or decreasing in premenopausal or perimenopausal women (Bray et al. 2005). This trend is likely to continue secondary to an ageing population, increasing obesity and a sharp fall in the number of hysterectomies being performed (Amant et al. 2005; Sorosky 2008; Reid and Mukri 2005).

2.6.2.3 Clinical presentation

Postmenopausal bleeding, defined as vaginal bleeding occurring at least a year after the last menstrual period, is the cardinal symptom of endometrial cancer. The probability of endometrial cancer in women presenting with postmenopausal bleeding is 5-10% (Amant et al. 2005). Prior to menopause, women with endometrial cancer may present with intermenstrual bleeding or irregular dysfunctional menstrual bleeding. Pain, vaginal discharge and pyometra are rarer symptoms and tend to be secondary to advanced cancer (Saso et al. 2011).

2.6.2.4 Risk factors for endometrial cancer

The development of endometrial cancer has been observed to follow two distinct pathways (Bohkman 1983). Type I cancers, which account for 80-90%, are usually oestrogen-dependent, endometrioid adenocarcinomas and generally have a good prognosis. Type II cancers are largely oestrogen-independent, have a variety of different histologies and tend to have a higher grade. Type II endometrial cancers therefore tend to have a worse prognosis as they tend to present later and behave more aggressively. As a significant majority of endometrial cancers are type I, most of the risk factors for the disease will link in some way to a relative increase in oestrogen exposure. A list of risk factors and protective factors can be seen in Table 3 (Saso et al. 2011).

Table 3 – Risk factors and protective factors for endometrial cancer (Saso et al. 2011).

Endogenous risk factors	Increasing age
	Obesity and physical inactivity
	Early menarche and late menopause
	Low parity or infertility
	Polycystic ovarian syndrome
	Family history
	Lynch syndrome (hereditary non-polyposis colorectal cancer)
	Oestrogen secreting tumours (granulosa or thecal cell tumours of the ovary)
	Diabetes mellitus
	Hypertension
	History of breast cancer
	Immunodeficiency
Exogenous risk factors	Unopposed oestrogen-only hormone replacement therapy
	Tamoxifen therapy
	Previous radiotherapy
	Dietary factors
Protective factors	Cigarette smoking
	Combined oral contraceptive use for at least one year
	Grand multiparity

2.6.2.5 Precursor lesions for endometrial cancer

Hyperoestrogenism is also a risk factor for endometrial hyperplasia, a premalignant condition that predisposes to endometrial cancer. Endometrial hyperplasia involves excessive cellular proliferation leading to an increased volume of endometrial tissue, with an increased ratio of endometrial glands to stroma (greater than 1:1). The WHO histological classification (Silverberg et al. 2003) is used in the UK. It classifies endometrial hyperplasia on the basis of the complexity of endometrial glands and the presence of any cytological atypia. Women with simple and complex endometrial hyperplasia have a risk of developing endometrial cancers of 1% and 3% respectively. Furthermore, 30-40% of women with complex atypical endometrial hyperplasia will have a concurrent adenocarcinoma, and those that do not, have a high risk of developing it (Mills and Longacre 2010)

2.6.2.6 Diagnosis of endometrial cancer

In the UK, if women report postmenopausal bleeding, they should be referred urgently to a gynaecological rapid-access clinic, where they should be seen within two weeks (NICE 2005). A variety of different diagnostic tools have been evaluated in the investigation of women with suspected endometrial cancer.

2.6.2.6.1 Blind endometrial biopsy

Dilatation and curettage (D&C) used to be the most common method to acquire a sample of the endometrium for histological analysis. It has fallen out of favour for a variety of reasons, including its need to be performed under general anaesthetic, its cost, its low sensitivity in diagnosing intrauterine pathology (Bettocchi et al. 2001) and its relatively high complication rate. Where a blind endometrial endometrial biopsy is required, D&C has largely been superceded by outpatient endometrial sampling devices, modelled on the pipelle de cornier

prototype. Endometrial sampling with a pipelle can be performed in an office setting, without general anaesthetic, and often without local anaesthetic. A meta-analysis has shown that pipelle biopsy has a detection rate for endometrial cancer of 99.6% in postmenopausal women and 91% in premenopausal women. It had a sensitivity of 81% for the detection of endometrial hyperplasia (Dijkhuizen et al. 2000). It appears to be more accurate in symptomatic and post-menopausal women (Clark et al. 2001). For it to be cost-effective as the first-line diagnostic tool, the population in which it is used should have a prevalence of endometrial cancer of at least 15% (Dijkhuizen et al. 2003).

2.6.2.6.2 Hysteroscopy

Hysteroscopy involves the passage of a thin scope through the cervix, allowing direct visualisation of the uterine cavity and endometrium. It can be performed as a daycase procedure under general anaesthetic, but is increasingly being performed under local anaesthetic in an outpatient setting where it has been shown to have equivalent rates of patient satisfaction but a shorter recovery time (Kremer et al. 2000). The advantage of hysteroscopy over blind endometrial biopsy is that it allows targeted biopsying of suspicious areas. Even if the endometrial cavity appears normal, it is recommended that an endometrial biopsy be performed with one study finding that 50% of the endometrial cancers diagnosed on endometrial sampling, were not identified at hysteroscopy (Lo et al. 2000). A further advantage of hysteroscopy is that where endometrial polyps are present, a polypectomy can accurately be performed, thus treating a potential cause of abnormal bleeding, even if it subsequently turns out to be benign.

Hysteroscopy under general anaesthetic has the same cost disadvantages as D&C, as well as being time-consuming for patients. While outpatient hysteroscopy has economic and clinical benefits it remains an invasive procedure associated with significant patient discomfort (Tahir

et al. 1999). Furthermore, it has a failure rate of up to 10% (Lo et al. 2000). In view of the invasive nature of outpatient hysteroscopy and its associated costs, transvaginal ultrasound has been recommended as the preferable first line investigation in women presenting with abnormal bleeding (Clark 2004).

2.6.2.6.3 Transvaginal ultrasound

In contrast to diagnostic hysteroscopy, TVUS is non-invasive, has no complications and is well-tolerated by women with postmenopausal bleeding (Nasri et al. 1991). It is therefore unsurprising that it has been extensively investigated as a tool for screening women with postmenopausal bleeding.

2.6.2.6.3.1 Endometrial thickness

In populations where the incidence of endometrial cancer is below 15%, using TVUS as the first line investigation, followed by endometrial biopsy if an abnormality is detected, has been shown to be more cost-effective than using endometrial biopsy as the first-line diagnostic tool (Dijkhuizen et al. 2003). In addition to saving money, using TVUS can potentially save women from undergoing invasive endometrial sampling. A meta-analysis of 35 studies involving 5892 women that evaluated different thresholds to define abnormal endometrial thickening showed that a cut-off of 5mm had a sensitivity of 96% for endometrial cancer. Thus a postmenopausal woman presenting with vaginal bleeding with a pre-test probability of endometrial cancer of 10%, would have a 1% chance of endometrial cancer with a normal TVUS. The authors concluded that TVUS assessment of endometrial thickness could reliably identify women with a very low likelihood of endometrial cancer who could thus avoid endometrial sampling. While the sensitivity of this cut-off for diagnosing endometrial cancer is very high, the specificity was just 61% (Smith-Bindman et

al. 1998). Therefore the positive predictive value of endometrial thickness measurement in diagnosing endometrial cancer is poor and many women therefore undergo ultimately unnecessary invasive procedures. A subsequent meta-analysis had similar results and concluded that while TVUS assessment of endometrial thickness was a good test for ruling out endometrial cancer, it was not very good at ruling it in (Gupta et al. 2002). TVUS remains the standard first-line test in the diagnosis of endometrial cancer, however because of the limitations of using endometrial thickness alone, many investigators have looked at ways to improve its specificity.

2.6.2.6.3.2 Endometrial thickness and morphology

Various endometrial ultrasound criteria have been assessed in attempts to improve the specificity of ultrasound in diagnosing endometrial cancer, with mixed results. While some have found assessments of endometrial and EMJ morphology useful in differentiating benign and malignant endometria (Randelzhofer et al. 2002; Epstein and Valentin 2006), others have not (Epstein et al. 2001; Epstein et al. 2002).

2.6.2.6.3.3 Transvaginal ultrasound with Doppler

There have been similarly contrasting results from studies assessing the use of Doppler to improve the accuracy of ultrasound in diagnosing endometrial cancer. An early study reported promising results in the use of colour Doppler to aid diagnosis of endometrial cancer (Bourne et al. 1991). Subsequent studies of colour Doppler flow in both the uterine and endometrial arteries were however, unable to show that it was useful in discriminating between benign and malignant endometrial lesions (Sladkevicius et al. 1994; Sheth et al. 1995). The evidence is no more consistent when Doppler has been used to assess the

morphology of endometrial vasculature. Alcazar et al. reported that a particular power Doppler pattern of vasculature could be observed in more than 80% of cases of endometrial cancer (Alcazar et al. 2003). Other investigators used an algorithm including power Doppler assessment of endometrial vascularity to estimate the risk of endometrial malignancy. While they concluded that their algorithm was better than subjective assessment, at differentiating benign and malignant endometria in women with an endometrial thickness of 5-15mm, this conclusion did not reach statistical significance (Epstein et al. 2002). Subsequent work by the same research group was unable to show that using colour Doppler to assess the number of blood vessels within the endometrium aided the diagnosis of endometrial cancer in women with postmenopausal bleeding (Epstein and Valentin 2006)

2.6.2.6.3.4 Three-dimensional ultrasound assessment of endometrial volume

Gruboeck et al. used compared endometrial thickness and endometrial volume measured by 3D ultrasound in the assessment of women with postmenopausal bleeding (Gruboeck et al. 1996). They found that endometrial volume assessment was a better screening tool than endometrial thickness measurement with an endometrial volume of 13mls having a sensitivity of 100% and a specificity of 99.8% for the diagnosis of endometrial cancer. A subsequent study again showed a higher sensitivity for endometrial volume than endometrial thickness (Mansour et al. 2007) although interestingly their cut-off value for endometrial volume was significantly different to that proposed in the study by Gruboeck et al. Furthermore, they used D&C as their gold standard which has been shown to be unreliable (Grimes 1982). Perhaps what is most noteworthy is that despite the early promise of 3D endometrial volume measurement in the screening of women with postmenopausal bleeding, a relatively small number of papers have subsequently been published on the subject. A recent review article (Alcazar and Jurado 2011) suggested that most of the published studies

showed that endometrial volume was superior to endometrial thickness. One study (Opolskiene and Valentin 2010) showed no advantage with 3D endometrial volume measurement.

2.6.2.7 Staging

Staging of endometrial cancer can be based on surgical, clinical, radiological of histopathological criteria, however surgical staging has been shown to have better prognostic value (Creasman et al. 2006). This led to FIGO updating the staging of endometrial cancer to more accurately reflect stage-for-stage prognosis (Table 4) (Creasman 2009). About 80% of women present with stage I disease and can be treated with hysterectomy and bilateral salpingo-oophorectomy.

Table 4 – Revised FIGO staging of endometrial cancer (Creasman et al. 2009).

Stage Ia	<50% myometrial invasion
Stage Ib	≥50% myometrial invasion
Stage II	Tumour confined to the uterine corpus but involving cervical stroma
Stage IIIa	Tumour invading the serosa of the uterine corpus and/or adnexa
Stage IIIb	Vaginal or parametrial involvement
Stage IIIc	Metastasis to pelvic or para-aortic lymph nodes
Stage IVa	Invasion of bladder and/or bowel mucosa
Stage IVb	Distant metastases including intra-abdominal and/or inguinal lymph nodes

3.0 Conclusion to Background

It is clear that there is an increasing focus on the EMJ and what its role in various pathologies might be. Gynaecologists and ultrasonographers are able to look at this area of the uterus in greater detail and depth and are even starting to make clinical decisions based on their assessments. Yet there is a danger that the clinical decision-making is out of step with what we know about the EMJ and its appearance on ultrasound. Obvious issues worth investigating are whether ultrasound assessment of the EMJ is reproducible and what clinical and demographic factors influence the appearance of the EMJ.

Adenomyosis, a disease of the EMJ, has been studied for considerably longer than the EMJ itself and yet still so many aspects of the disease remains unclear or uncertain. While histology has historically been the primary diagnostic tool for investigating adenomyosis, the significant reduction in the number of hysterectomies now being performed for benign disease has blunted its usefulness in investigating the disease. Almost all ultrasound studies of adenomyosis to date have assessed its diagnostic accuracy. While these studies have shown that transvaginal ultrasound has good accuracy in diagnosing adenomyosis, few if any ultrasound studies have attempted to investigate some of the many clinical uncertainties that remain about the disease. This has perhaps been due to concerns about the reproducibility of ultrasound diagnosis of adenomyosis. Significant uncertainties remain about the most basic aspects of the disease including its prevalence and even whether or not it has any clinical significance. Ultrasound should now be used to investigate these questions.

The role of ultrasound in the screening of women with post-menopausal bleeding is more established. Transvaginal ultrasound assessment of endometrial thickness remains the primary determinant of which women can be safely reassured and which women need endometrial sampling. Using ultrasound in this way has a high sensitivity for endometrial cancer but a low specificity meaning that many women undergo ultimately unnecessary

invasive investigations. Several investigators have looked at whether more sophisticated transvaginal ultrasound assessment of these women might be able to improve the diagnostic accuracy of ultrasound and assessment of the EMJ has been suggested as one aspect of the ultrasound examination that may aid this.

Given the above, I feel that the aims of this thesis should be to examine the reproducibility of ultrasound assessment of the EMJ and investigate what factors affect it visualisation, examine the reproducibility of ultrasound diagnosis of adenomyosis, determine the prevalence of ultrasound features of adenomyosis in women attending a general gynaecology clinic and investigate whether their presence is associated with clinical symptoms and lastly, determine whether assessment of the EMJ can increase the diagnostic accuracy of ultrasound in the investigation of women with post-menopausal bleeding

PART II MATERIALS AND METHODS

1.0 MATERIALS AND METHODS

1.1 Setting

1.1.1 The Gynaecological Diagnostic and Treatment Unit, University College Hospital

The work contained in this thesis was carried out in the Gynaecology Diagnostic and Outpatient Treatment Unit (GDOTU) of University College Hospital between October 2008 and March 2011. Situated in central London, University College Hospital is a teaching hospital and tertiary referral centre which primarily serves the needs of the people of Camden and Islington. The trust has an annual turnover of more than £769 million, employs over 6000 staff and has 665 inpatient beds. It sees over 789,000 outpatients a year and has around 125,000 inpatient admissions a year. The hospital was formed in 1994 and became an NHS foundation Trust in 2004. The Women's Health department at UCH offers specialist gynaecology services including gynaecological ultrasound scanning, early pregnancy care, ambulatory gynaecology, urogynaecology, colposcopy, menopause, gynaecological oncology, paediatric gynaecology, a specialist endometriosis centre, an assisted conception unit and a specialist clinic for African women. In the financial year 2009/10, there were 11,270 patient attendances at the GDOTU of which 6,487 were women with early pregnancy problems and 4,783 of which were non-pregnant women with gynaecological problems.

1.2 Ultrasound

1.2.1 Two-dimensional transvaginal ultrasound

In this thesis, all the ultrasound scans were performed by trained gynaecologists on a Voluson E8 ultrasound machine (GE Medical Systems, Milwaukee, WI, USA) using a 4-9 MHz probe with three dimensional facility. The scans were all perfomed in lithotomy position using a standardised protocol. First the uterus was examined in the transverse plane to identify the cervical canal and the uterine cavity. The probe was then rotated 90^{o} anti–clockwise and the

uterus and endometrium were visualised in the longitudinal plane. Care was taken to identify the point at which the endometrial thickness was greatest before measuring it in millimetres. If free fluid was present in the endometrial cavity, the fluid was measured separately and subtracted from the total thickness (Lee et al. 2005). Fibroids were diagnosed based on direct visualisation using previously described diagnostic criteria (Anderson 1999). Adenomyosis was diagnosed if any of the following ultrasound features of adenomyosis were present: asymmetrical myometrial thickening, myometrial cysts, linear striations, parallel shadowing, adenomyomas, hyperechoic islands and an irregular EMJ on either B-mode or 3-dimensional imaging (Table 5, Figures 11-17) (Naftalin et al. 2012b). Previously published ultrasound features of adenomyosis that were not used to diagnose adenomyosis in this thesis include a heterogenous myometrial echotexture and a globular uterine configuration. These were both excluded as they had been found to have a low positive predictive value (Bazot et al. 2001). The JZ measurements taken from 3D coronal views of the uterus proposed by Exacoustos et al. were not used as they were published after our study had started. Even so we would not have included them as their accuracy has yet to be prospectively assessed. Endometrial polyps were diagnosed based on direct visualisation using previously described criteria (Timmerman et al. 2003). The examination was then concluded by examining both adnexa and the pouch of Douglas, identifying both ovaries and any endometriotic nodules where possible.

Table 5 – Ultrasound features of adenomyosis used in this thesis (Naftalin et al. 2012b).

Asymmetrical myometrial thickening
Parallel shadowing
Linear striations
Myometrial cysts
Hyperechoic islands
Adenomyomas
Irregular EMJ

Figure 11 - Asymmetrical myometrial thickening. A longitudinal view of an anteverted uterus in which the distance from the endometrium to the anterior serosal surface (thick white line) is much greater than the distance from the endometrium to the posterior serosal surface (thin white line) (Naftalin et al. 2012b)

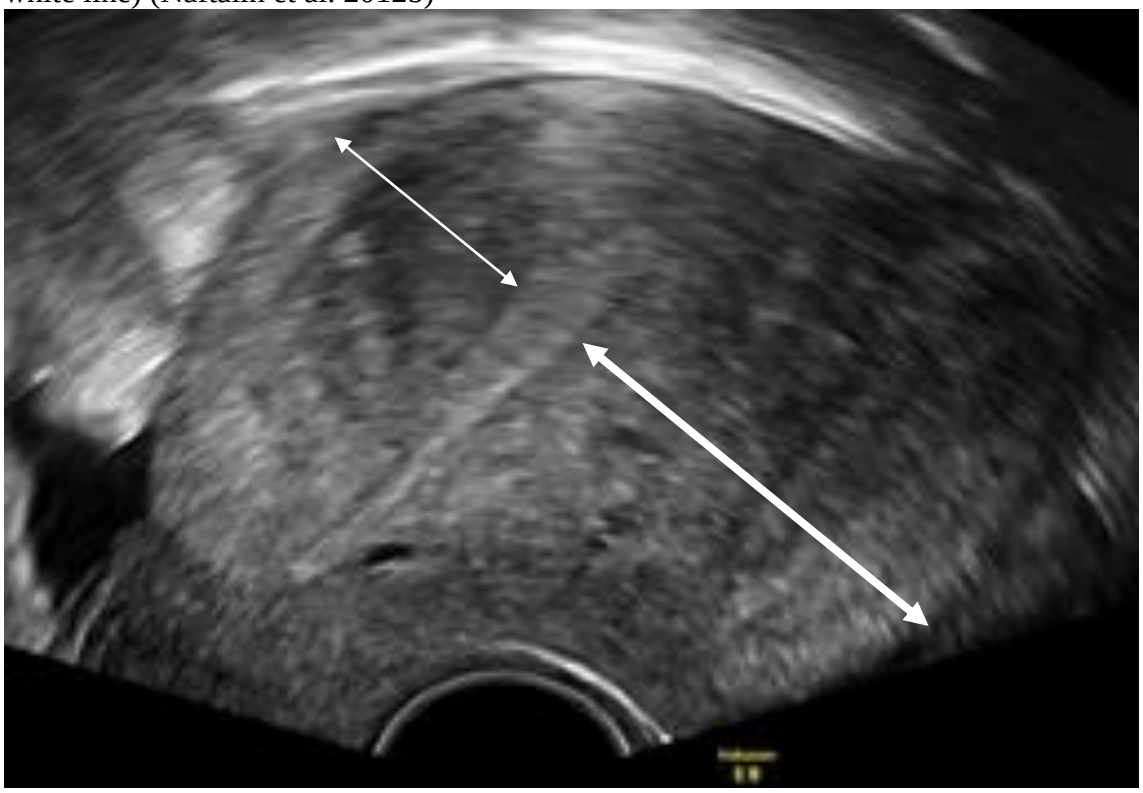


Figure 12 - Parallel shadowing. A longitudinal view of a retroverted uterus in which parallel hypoechoic lines can be seen running through the anterior myometrium (yellow s) (Naftalin et al. 2012b)

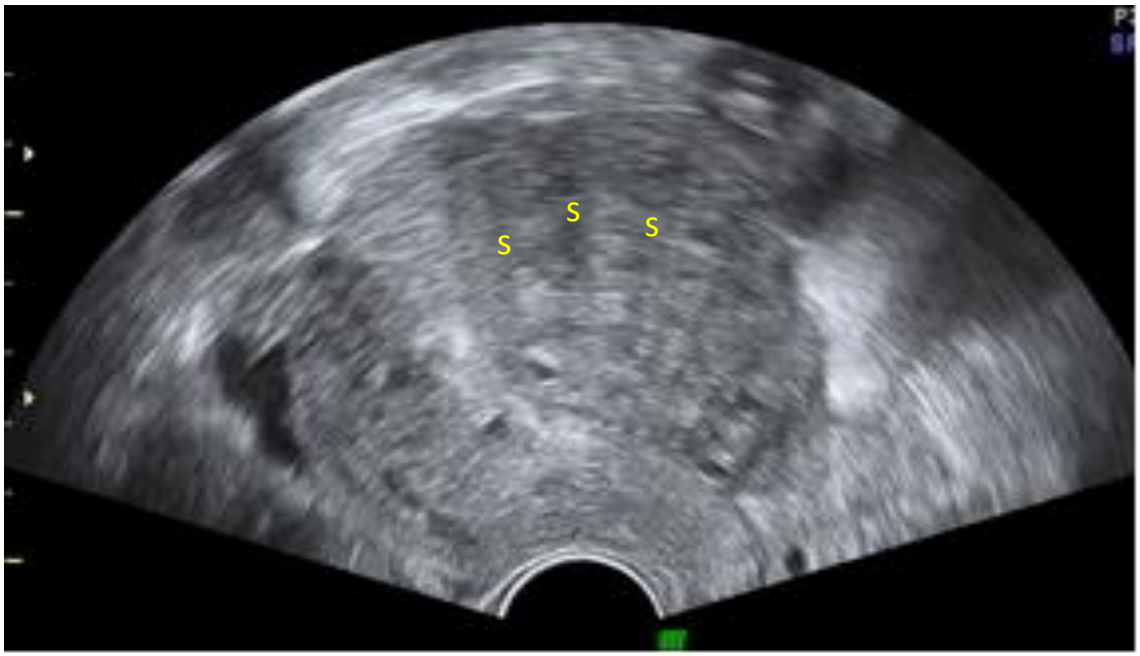


Figure 13 - Linear striations. A transverse view of the uterus showing a linear striation seen as thin hyperechoic lines (thin white arrow) extending from the endometrium into the myometrium (Naftalin et al. 2012b)

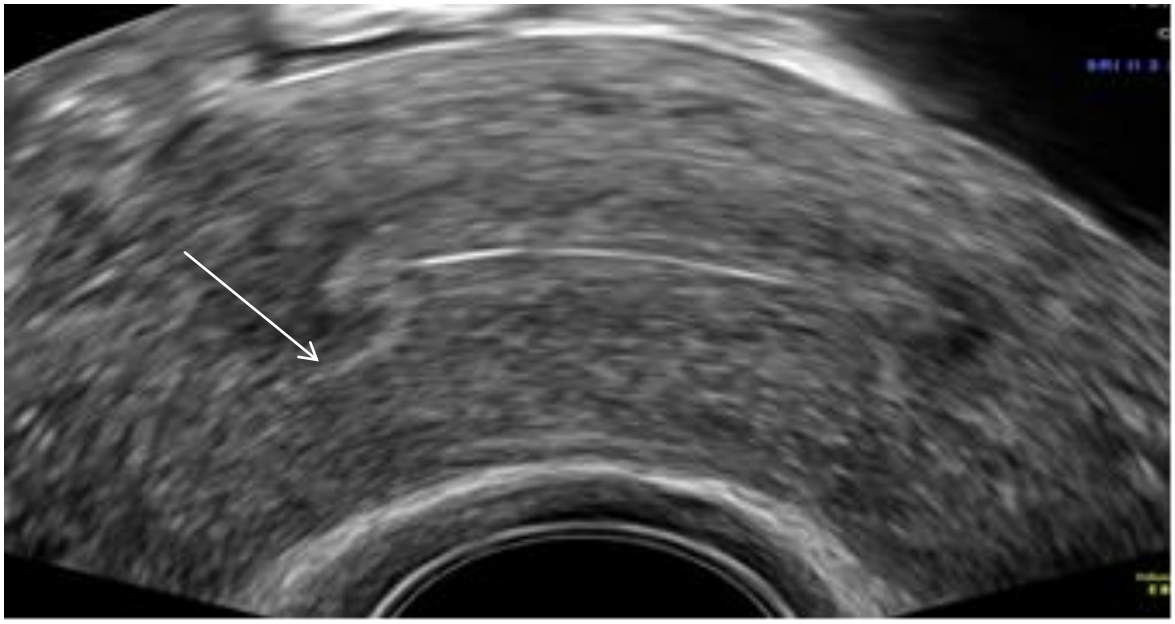


Figure 14 - Myometrial cysts. A transverse view of the uterus showing several myometrial cysts (thin white arrows) seen as anechoic lesions within the myometrium (Naftalin et al. 2012b)

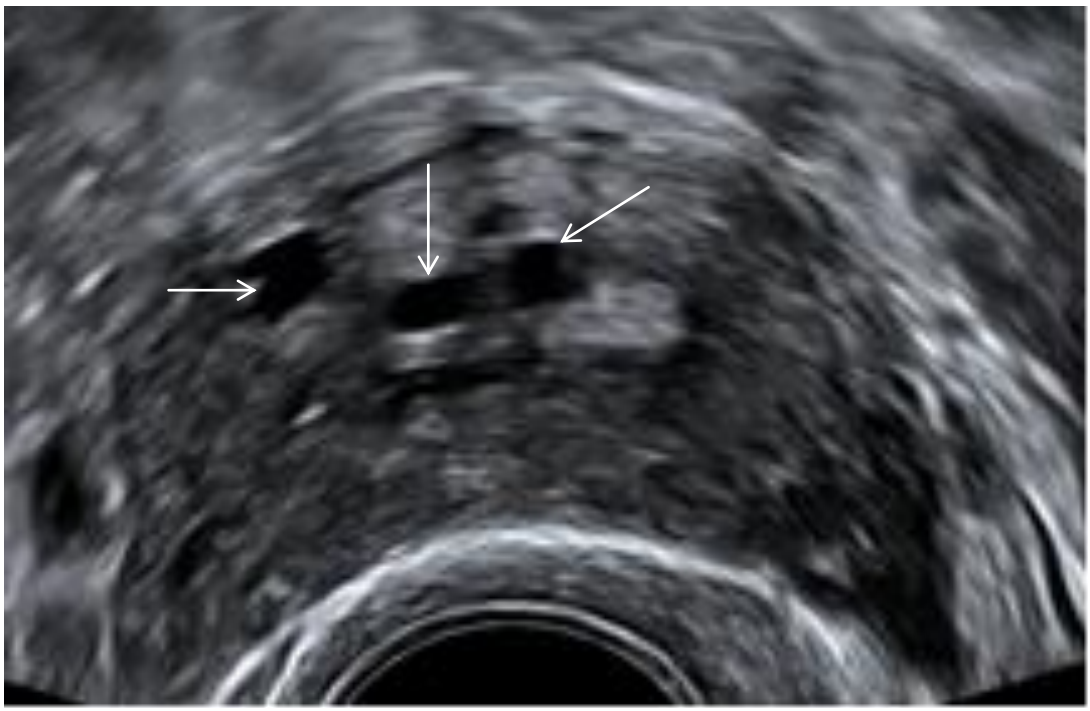


Figure 15 - Hyperechoic islands. A coronal view of a uterus where hyperechoic islands of tissue are seen in the fundal and lateral portions of the myometrium (thin white arrows). (Naftalin et al. 2012b)

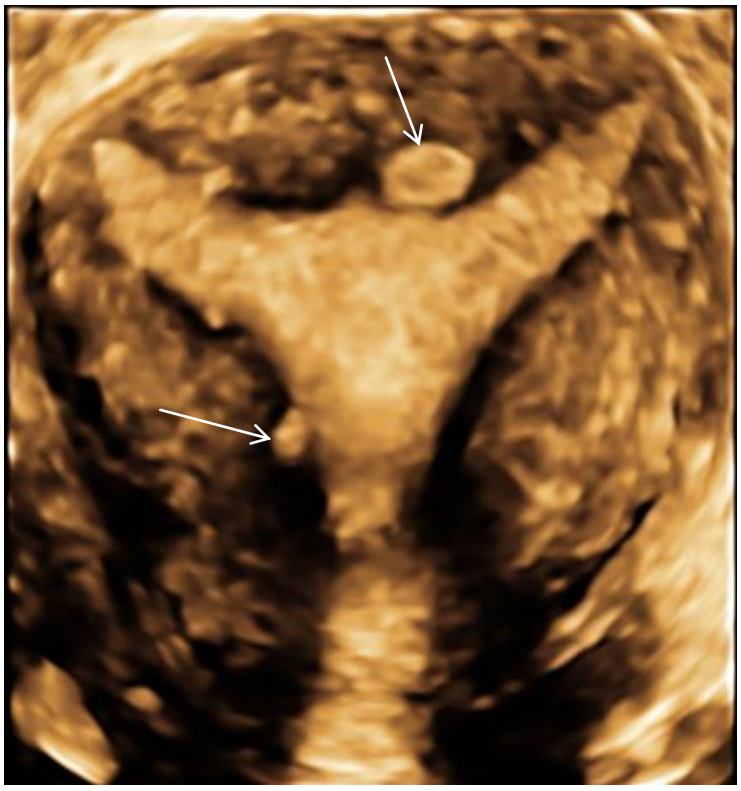


Figure 16 - Adenomyomas. A longitudinal view of a retroverted uterus showing an adenomyoma in the anterior myometrium (between white arrows). (Naftalin et al. 2012b)

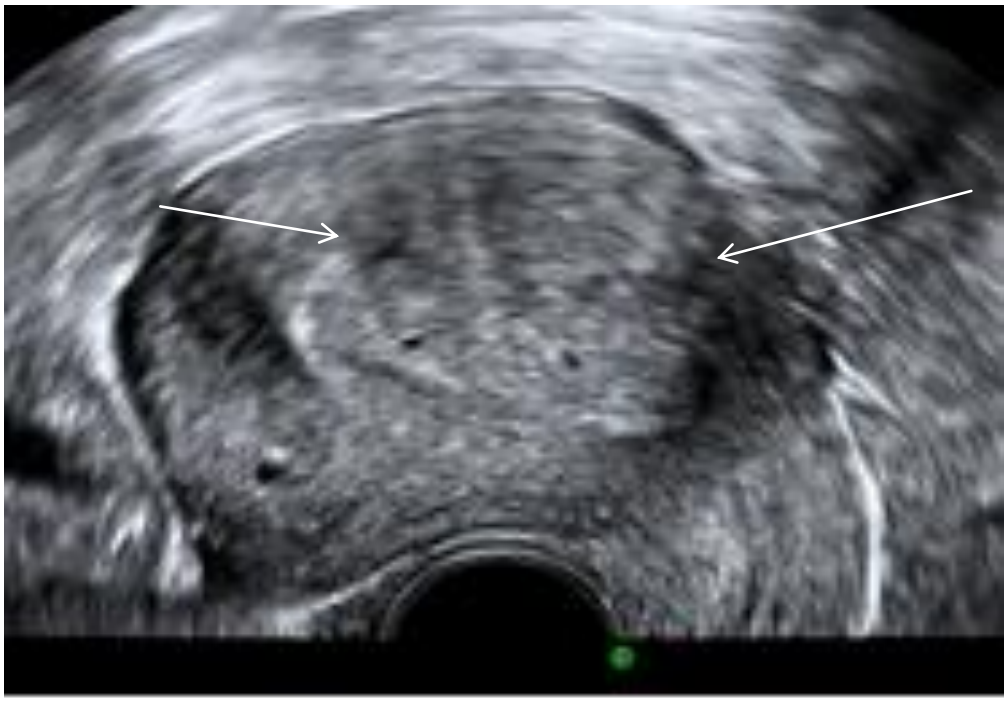
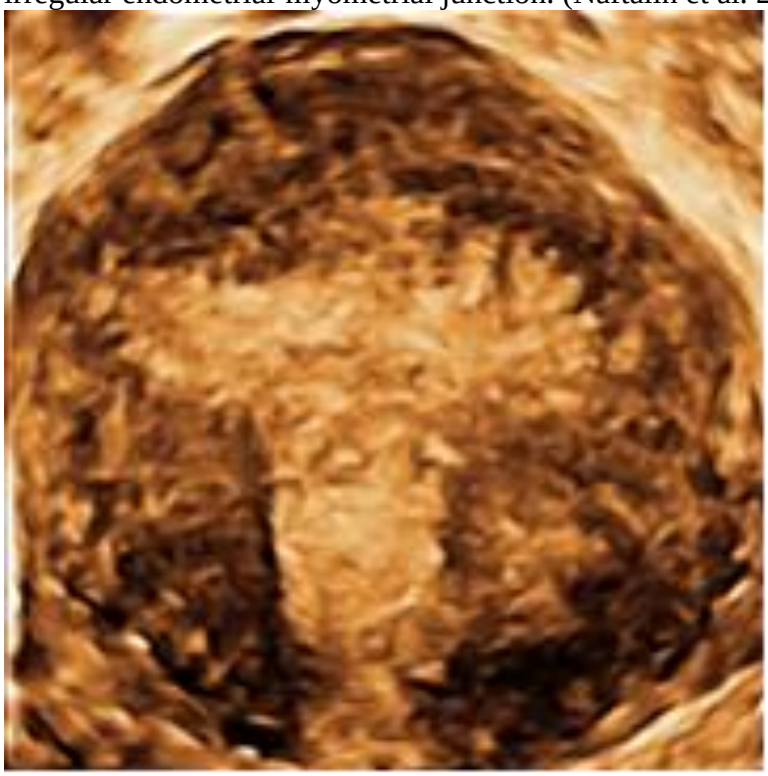


Figure 17 - Irregular endometrial-myometrial junction. A coronal view of a uterus with an irregular endometrial-myometrial junction. (Naftalin et al. 2012b)



An ultrasound diagnosis of endometriosis was made if ovarian endometriomas or endometriotic nodules were visualised on ultrasound scan. Ovarian cysts were classified as endometriomas when they appeared as well-circumscribed thick-walled cysts that contained homogeneous low-level internal echoes (‘ground glass’) (Tailor et al. 1999). Endometriotic nodules were typically visualised as stellate hypoechoic or isoechogenic solid masses with irregular outer margins (Bazot et al. 2007) which were tender on palpation and fixed to the surrounding pelvic structures.

1.2.2 Three-dimensional transvaginal ultrasound

All 3-D ultrasound volume acquisitions were performed immediately after the 2D TVUS by the same operator. The volume acquisition technique was performed in a standardised fashion using a maximum sweep angle of 120 degrees and the medium quality setting. The uterus was visualised in the longitudinal plane with the angle of the ultrasound beam and the axis of the endometrial cavity approaching 90 degrees. In order to minimise artefacts, the depth of the acquired volume was adjusted to cover the entire uterus with minimal inclusion of parametrial structures. The volumes were generated by automatic rotation of the mechanical transducer through 360^{o}. The probe was held steady and the patients were asked to hold their breath whilst volume acquisition was switched on. Following acquisition, the rendered volumes were saved to the hard drive of the machine for analysis.

1.2.2.1 Off-line assessment of uterine volumes

Assessment of 3D uterine volumes was performed offline. A coronal view was obtained using render mode, by placing a straight or curved line along the endometrial stripe on the sagittal and transverse views (Panel A and B of the multi-planar view). The multi-planar view

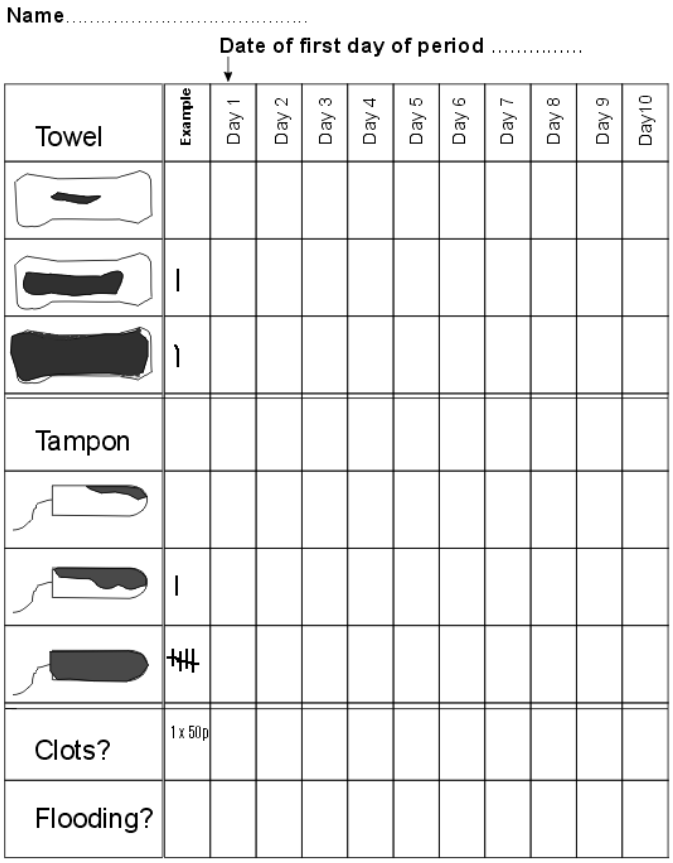
was then adjusted until a satisfactory coronal image was obtained of both the endometrial cavity and the external uterine contour, with visualisation of both interstitial portions of the fallopian tube. The gradient light and sepia settings were used to optimise the view of the EMJ, which was visualised as a hypoechoic area surrounding the endometrial cavity.

When using off-line evaluation of stored uterine volumes to diagnose adenomyosis, a detailed assessment of the entire myometrium was performed looking for the presence of each individual ultrasound feature of adenomyosis. Both operators were required to manipulate the rendered views, in whichever way they wanted, until they had visualised the entire myometrium and a diagnosis was reached.

1.3 Pictorial Blood Loss Assessment Charts

Pictorial Blood Loss Assessment charts (PBACs) were given to premenopausal women to complete in order to obtain semi—quantitative information about the amount of menstrual loss they had each cycle. PBACs use a scoring system to calculate total menstrual loss that accounts for both the number of sanitary towels or tampons a woman uses during her period and also the degree to which each item is soiled (Figure 18). A pictorial chart score of $\geq$100, when used as a diagnostic test for menorrhagia, has been shown to have a high specificity and sensitivity (Higham et al. 1990).

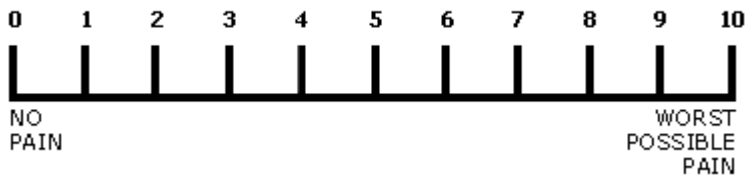
Figure 18 – A Pictorial blood loss analysis chart.



1.4 Numerical Rating Scale (NRS)

Premenopausal women were asked to complete an 11-point Numerical Rating Scale (NRS) (Figure 19) in order to obtain a subjective assessment of how painful they found their periods. The NRS is a scale where the extremes are no pain and pain as bad as it could be and patients are asked to put a mark on the scale to represent the intensity of the pain being measured.

Figure 19 – An 11-point numerical rating scale.



Women were given the scale and asked to put a mark on the scale to represent the intensity of the worst pain they experienced with their last period. NRSs have been shown to be sensitive (Jensen et al. 1986), simple to use with a low failure rate (Van Tubergen et al. 2002) and are considered to be preferable to traditional visual analog scales for assessment of pain intensity (Mannion 2007). They have been shown to have sufficient reliability and validity to be used in clinical research (Lara-Munoz et al. 2004).

1.5 Endometrial sampling (Pipelle & Hysteroscopy)

In women with postmenopausal bleeding found to have an endometrial thickness on TVUS of $\geq$5mm, endometrial sampling was performed. If the endometrium was globally thickened, and the woman was able to tolerate outpatient endometrial sampling, a pipelle de cornier was used (Prodimed, Neuillyen-Thelle, France). Women with a focally thickened endometrium were referred for either outpatient or day-case hysteroscopy.

1.6 Statistical analysis

Data were collected and stored in electronic databases (Microsoft Excel 2003). The Statistical Package for Social Sciences version 15.0 (Statistical Analysis Systems, Chicago, Illinois) was used to analyse data. Details of individual tests are documented in the relevant chapters. The level of statistical significance was chosen as $p<0.05$.

1.7 Ethical committee approval

The Local Research and Ethics Committee of University College Hospital granted approval for all studies undertaken. They advised that none of the studies required full formal ethical approval.

III Results

[1] INTER- AND INTRAOBSERVER VARIABILITY IN THREE-DIMENSIONAL ULTRASOUND ASSESSMENT OF THE ENDOMETRIAL-MYOMETRIAL JUNCTION

1.1 BACKGROUND

The endometrial-myometrial junction (EMJ) is thought to play an important role in both physiological and pathophysiological processes within the uterus. It has been suggested that a careful ultrasound examination of the EMJ junction can yield clinically useful information in a number of different pathologies and so should become part of the routine ultrasound examination of the uterus (Naftalin and Jurkovic 2009).

In order to obtain clear images of the EMJ, particularly at the lateral and fundal aspects of the uterine cavity, high resolution ultrasound equipment with 3D imaging facilities is often required, which has only become available in recent years. There are a small number of published studies involving assessment of 3D views of the EMJ (Exacoustos et al. 2011; Alcazar et al. 2009), however little is known about the reproducibility of 3D ultrasound assessment of the EMJ. The aim of this study was to examine inter- and intra-observer reproducibility of 3D ultrasound visualisation of the EMJ.

1.2 METHODS

Inter- and intraobserver variability in 3D ultrasound assessment of the EMJ was tested on a group of 30 women. Real time ultrasound examinations were performed and 3D volumes were obtained in the manner described in the materials and methods section. The investigator who performed the ultrasound examinations (Dr Will Hoo) was a specialist in gynaecological ultrasound with a special interest in benign gynaecological conditions. We included in the study only non-pregnant women with a normal uterus on ultrasound scan. After selecting the volumes for the study he did not participate in further reproducibility analysis. All saved

volumes were then examined independently by two other investigators (Mr Davor Jurkovic and Dr Joel Naftalin) who were blinded to each other's findings. The volumes were examined using planar reformatted sections and volume rendering. The quality of visualisation of the EMJ was graded as follows: 1.) Optimal – The EMJ was clearly visible and could be examined in its entirety, 2.) Satisfactory – Most, but not all parts of the EMJ could be clearly visualised, 3.) Unsatisfactory – Large parts of the EMJ could not be clearly visualised (Figs 20-22). It was considered that EMJ's graded as unsatisfactory were inadequate for clinical evaluation, whereas 1) & 2) could be used for clinical evaluation.

1.3 STATISTICAL ANALYSIS

Statistical analysis was performed, using SPSS software (SPSS Inc., Chicago IL). The inter-observer and intra-observer agreement for the classification of EMJ visualisation was assessed by calculating the kappa statistic.

1.4 RESULTS

There was complete agreement between the two operators in classifying the visualisation of the EMJ as being optimal or not. Both operators classified 16 EMJs as optimal, 8 as satisfactory and 2 as unsatisfactory. There was a discrepancy between the two observers in 4 cases. In two of these, operator 1 classified two cases as satisfactory which operator 2 had described as unsatisfactory. In the other two cases, the reverse occurred. The inter-observer agreement was good with a kappa value of 0.77 (Table 6). The intra-observer agreement was excellent for observer 1 with a kappa value of 0.83 (Table 7) and good for observer 2 with a kappa value of 0.70 (Table 8).

Figure 20 - Coronal view of uterus with EMJ visualisation classified as optimal (Naftalin et al. 2012a)

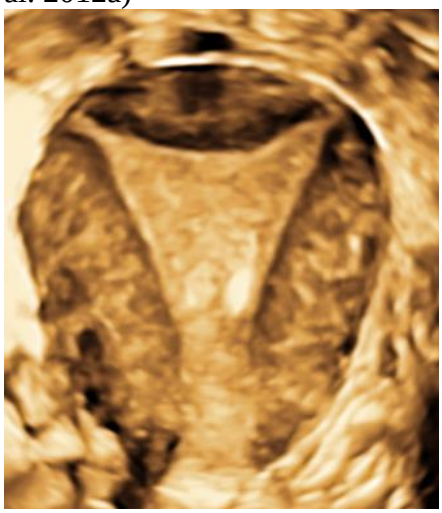


Figure 21 - Coronal view of uterus with EMJ visualisation classified as satisfactory (Naftalin et al. 2012a)

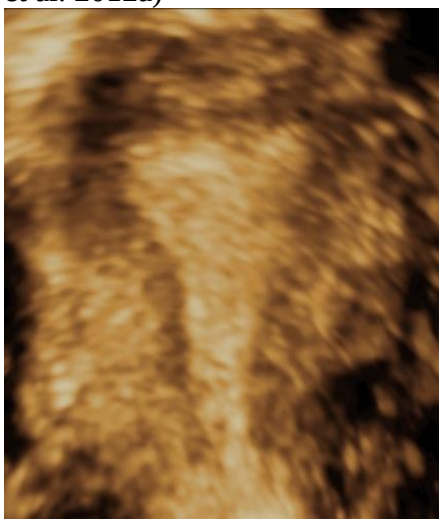


Figure 22 - Coronal view of uterus with EMJ visualisation classified as unsatisfactory (Naftalin et al. 2012a)

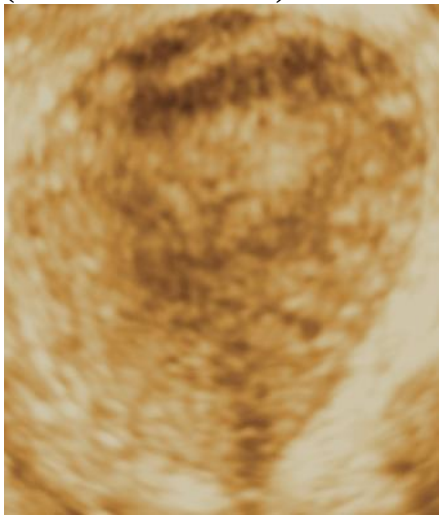


Table 6 - Inter-observer variability in the assessment of visualisation of the EMJ (Naftalin et al. 2012a)

	Operator 2				
Operator 1		Optimal	Satisfactory	Unsatisfactory	Total
	Optimal	16	0	0	16
	Satisfactory	0	8	2	10
	Unsatisfactory	0	2	2	4
	Total	16	10	4	30
k=0.77 (good agreement) **95%CI (0.58 – 0.96)** **Standard error of kappa = 0.097**					

Table 7 - Intra-observer variability (Operator 1) in the assessment of visualisation of the EMJ (Naftalin et al. 2012a)

	Operator 1 (2nd assessment)				
Operator 1 (1st assessment)		Optimal	Satisfactory	Unsatisfactory	Total
	Optimal	15	1	0	16
	Satisfactory	0	9	1	10
	Unsatisfactory	0	1	3	4
	Total	15	11	4	30
k=0.83 (excellent agreement) **95%CI (0.66 – 1)** **Standard error of kappa = 0.090**					

Table 8 - Intra-observer variability (Operator 2) in the assessment of visualisation of the EMJ (Naftalin et al. 2012a)

	Operator 2 (2nd assessment)				
Operator 2 (1st assessment)		Optimal	Satisfactory	Unsatisfactory	Total
	Optimal	16	0	0	16
	Satisfactory	3	7	0	10
	Unsatisfactory	0	2	2	4
	Total	19	9	2	30
k=0.70 (good agreement) **95%CI (0.47 – 0.93)** **Standard error of kappa = 0.117**					

[2] FACTORS AFFECTING VISUALISATION OF THE EMJ ON THREE-DIMENSIONAL ULTRASOUND

2.1 BACKGROUND

The EMJ is thought to play an important role in both physiological and pathophysiological processes within the uterus. Its appearance on ultrasound is known to be altered in the presence of certain pathologies, for example adenomyosis (Naftalin and Jurkovic 2009) and invasive endometrial cancer (Randelzhofer et al. 2002). It is not known, however, whether physiological factors affect its ultrasound appearance. The aim of this study was to identify demographic and physiological factors which might affect the quality of visualisation of the EMJ on 3D ultrasound.

2.2 METHODS

This was a prospective observational study of women attending our gynaecology clinic. In all women, indication for the scan, age, parity, date of the last menstrual period, menstrual pattern and the use of exogenous hormones was recorded and stored on a dedicated database (PIA Fetal Database, version 5.5.4.152, Viewpoint Bildverarbeitung GmbH, Munich, Germany). Postmenopausal status was defined as the absence of menstruation for ≥ 12 months in women ≥ 40 years old. Ultrasound examinations were performed in the manner described in the materials and methods section. The examination was then concluded by examining the adnexa and identifying both ovaries. The presence of a corpus luteum was determined on B-mode and Doppler examination. Premenopausal women with regular menstrual cycles, whose ovaries contained a corpus luteum were classified as being in the luteal phase of the cycle, and the remaining women with regular menstrual cycles were classified as being in the proliferative phase of the cycle.

We included in the study only women with a normal uterus on ultrasound scan. Those with evidence of any myometrial or endometrial pathology, pregnant women and those in whom the ovaries could not be seen clearly were excluded. Various demographic and physiological variables were recorded and we analysed their effect on the quality of EMJ visualisation on 3D volume rendering.

2.3 STATISTICAL ANALYSIS

Statistical analysis was performed, using SPSS software (SPSS Inc., Chicago IL). Spearman's rank correlation was used to examine the association of EMJ visualisation with age, parity and endometrial thickness. The Kruskal-Wallis test was used to examine the association between EMJ visualisation and the stage of menstrual cycle and the Mann-Whitney test was used to examine the association between EMJ visualisation and menopausal status. Subsequently, the joint effect of the various factors upon EMJ visualisation was examined in a multivariable analysis. As EMJ visualisation was measured using an ordinal scale, the analysis was performed using ordinal logistic regression.

2.4 RESULTS

413 consecutive, non-pregnant women were examined by a single operator (JN). 312 women (75.5%) were excluded due to the presence of uterine pathology and the remaining 101 (24.5%) were included in data analysis. The principal indications for ultrasound examination for the women included in data analysis are presented in Table 9. In 47/101 (46.5%) of the women, visualisation of the EMJ was classified as optimal, in 42/101 (41.6%) as satisfactory and in 12/101 (11.9%) as unsatisfactory. Spearman's rank correlation showed that the ability

to visualise the EMJ was significantly affected by a women's age, parity, and endometrial thickness as measured by ultrasound scan (Table 10).

The Kruskal-Wallis test did not show a significant effect of the stage of the menstrual cycle or menopausal status on the quality of EMJ visualisation (Table 11). A subsequent analysis assessing menopausal status only, using the Mann-Whitney test, showed a significant association between menopausal status and EMJ visualisation (Table 12).

The joint effect of the various factors on EMJ visualisation was examined in a multivariable analysis. A backwards selection procedure was used to retain only the statistically significant variables. The results indicated that only parity and endometrial thickness were significantly associated with EMJ visualisation. After adjusting for the effects of these two variables, there was no significant difference in EMJ visualisation between pre and post-menopausal women and the previously noted association between age and EMJ visualisation was no longer found to be significant.

The size of effect of each variable upon EMJ visualisation, in the form of odds ratios, can be seen in Table 13. These indicate the odds of being in the next best visualisation category (optimal visualisation rather than satisfactory visualisation, or satisfactory visualisation rather than unsatisfactory visualisation) for an increase in parity of one, or a 5mm increase in endometrial thickness. The results suggest that increasing parity is negatively associated with EMJ visualisation, whereby for each additional child delivered, the odds of being in the next best visualisation category were almost halved. The results for endometrial thickness suggest that increasing endometrial thickness is associated with better EMJ visualisation, a 5-mm

increase in endometrial thickness being associated with a three-fold increased risk of being in next best visualisation category.

Table 9 - Principal indications for examination (Naftalin et al. 2012a)

Principal indication for examination	*n (%)*
Pelvic pain/dyspareunia	31
Subfertility	22
Irregular/Intermenstrual bleeding	11
Heavy or painful menstrual bleeding	10
Amenorrhoea	7
Postmenopausal bleeding	7
Suspected ovarian cyst	5
Postcoital bleeding	2
Other	6

Table 10 – Result of Spearman's rank correlation in assessing the effect of age, parity and endometrial thickness on EMJ visualisation (Naftalin et al. 2012a)

Variable	EMJ Category	Median (IQR)	Correlation Coefficient	P-value
Age	Optimal Satisfactory Unsatisfactory	32 (27, 36) 32 (27, 42) 46 (39, 60)	0.30	**0.006**
Parity	Optimal Satisfactory Unsatisfactory	0 (0, 0) 0 (0, 1) 1.5 (0.5, 3.5)	0.33	<**0.001**
Endometrial Thickness	Optimal Satisfactory Unsatisfactory	6.3 (3.5, 9.5) 3.5 (2.5, 6.0) 3.1 (1.9, 4.9)	-0.36	<**0.001**

Table 11 – Result of Kruskal-Wallis test in assessing the effect of stage of menstrual cycle on EMJ visualisation (Naftalin et al. 2012a)

Stage of cycle	Optimal n (%)	Satisfactory n (%)	Unsatisfactory n (%)	P-value
Proliferative	19 (46%)	20 (49%)	2 (5%)	0.08
Secretory	11 (58%)	5 (26%)	3 (16%)	
Irregular / absent	10 (63%)	3 (19%)	3 (19%)	
Post-menopausal	2 (14%)	8 (57%)	4 (29%)	
Combined pill	5 (45%)	6 (55%)	0 (0%)	

Table 12 – Result of Mann-Whitney test in assessing the effect of menopausal status on EMJ visualisation (Naftalin et al. 2012a)

Menopausal status	Optimal n (%)	Satisfactory n (%)	Unsatisfactory n (%)	P-value
Pre-menopausal	45 (52%)	34 (39%)	8 (9%)	**0.004**
Post-menopausal	2 (14%)	8 (57%)	4 (29%)	

Table 13 – Results of multivariable analysis assessing the joint effect of the various factors on EMJ visualisation (Naftalin et al. 2012a)

Variable	Odds Ratio (95% CI)	P-value
Parity	0.53 (0.36, 0.78)	**0.001**
Endometrial Thickness (*)	3.00 (1.52, 5.88)	**0.001**

(*) Odds ratio given for a 5mm increase in endometrial thickness

[3] INTER- AND INTRAOBSERVER VARIABILITY IN THE ULTRASOUND DIAGNOSIS OF ADENOMYOSIS USING STORED 3D VOLUMES

3.1 BACKGROUND

The ability to diagnose adenomyosis without having to perform a hysterectomy has helped our understanding of the disease. There remains controversy, however, as to which imaging modality should be the first-line diagnostic tool. Ultrasound is relatively cheap, widely-accessible and well-tolerated and has been shown to have comparable accuracy to MRI in the diagnosis of adenomyosis (Dueholm 2006). However, it was found to have a significantly lower inter-observer variability (kappa=0.38) than MRI (kappa= 0.73) in the only published study to compare reproducibility of diagnosis in the two modalities (Dueholm et al. 2002). Furthermore, some authors have claimed that, unlike MRI, the ultrasound diagnosis of adenomyosis can only be made during a real-time scan, as the ultrasound features of adenomyosis cannot be reliably identified from static images (Reinhold 1999). The aim of this study was to assess the inter- and intra-observer variability in ultrasound assessment of adenomyosis using stored 3D uterine volumes. This assessment included diagnosis of adenomyosis, the number of ultrasound features of adenomyosis seen and a subjective assessment of severity of disease. The comparisons were made between:

a) real-time ultrasound scans and evaluation of the uterus from a stored 3D uterine volume (same operator performing scan and stored volume assessment but 3 years apart)
b) two different operators evaluating the same stored uterine volumes
c) the same operator evaluating the same stored uterine volumes on two separate occasions

3.2 METHODS

Out of a total of 985 archived 3D uterine volumes taken from women consecutively scanned women between January 2009 and January 2010 by the same operator, 36 were selected by one of the investigators (Dr Kate Pateman) for the examination of reproducibility of ultrasound diagnosis of adenomyosis. The volumes were selected to ensure a good case mix including normal uteri and those with adenomyosis. Those with adenomyosis were selected to ensure a wide range in both the number and type of ultrasound feature of adenomyosis. Volumes were not selected if it were not possible to adequately assess the entire myometrium. Reasons for this included the presence of an IUCD, multiple large fibroids and poor quality of stored volumes. The reproducibility assessments were performed more than 3 years after the original real-time scans in order to minimise bias in the assessment of intra-observer agreement between diagnosis on real-time ultrasound and on assessment of stored 3D uterine volumes. The first and second assessment of 3D stored uterine volumes (both assessments by Dr Joel Naftalin) were performed two weeks apart, and in a different order from the first assessment, (the order being selected by Dr Kate Pateman who played no further part in ultrasound assessment), again, in order to reduce bias. A third assessment of the stored volumes was then performed by a third investigator (Mr Davor Jurkovic) who was blinded to the results of all previous ultrasound assessments of the uteri. This was in order to assess inter-observer agreement in the ultrasound diagnosis of adenomyosis based on assessment of stored 3D ultrasound volumes of uteri.

3.3 STATISTICAL ANALYSIS

Statistical analysis was performed, using SPSS software (SPSS Inc., Chicago IL). Intra- and inter-observer agreement was assessed for the diagnosis of adenomyosis, assessment of the

number of ultrasound features of adenomyosis observed and subjectively assessed severity of adenomyosis and for the classification of each ultrasound feature of adenomyosis. Weighted and unweighted kappa analysis was used to assess the level of agreement in all cases.

3.4 RESULTS

The median age of the 36 women included in the data analysis was 41 (inter-quartile range: 35-47.75). 10/36 [27.7% (95% CI: 13.2% to 42.4%)] women were nulligravid and 12/36 [33.3% (95% CI: 17.9% to 48.7%)] were nulliparous. 6/36 [16.7% (95%CI; 4.5% to 28.8%)] women were menopausal. The indications for ultrasound scans are listed in Table 14. A total of 22/36 [60.1% (95% CI: 44.1% to 76.1%)] women had at least one ultrasound feature of adenomyosis at their original real-time ultrasound scan. The total number of ultrasound features seen on different scans can be seen in Table 15. 12/36 (33.3%) women had uterine fibroids and 2/36 (5.6%) women had endometrial polyps.

There was good intra-observer agreement, both when the observer was comparing real-time scan diagnosis of adenomyosis to diagnosis from stored uterine volumes, and when the observer was comparing two assessments of stored uterine volumes performed on two separate occasions, with kappa values of 0.67 and 0.83 respectively (Tables 16 and 21). There remained a good level of intra-observer agreement when comparison was made of the number of ultrasound features of adenomyosis recorded, with linear-weighted kappa values of 0.64 and 0.77 (Tables 17 and 22). There was excellent intra-observer agreement (kappa = 0.83) in the subjective assessment of severity of adenomyosis between the two evaluations of the stored uterine volumes (Table 23).

When two different operators assessed stored uterine volumes, there was good inter-observer agreement for the diagnosis of adenomyosis (kappa = 0.61) (Table 18), the number of

ultrasound features of adenomyosis seen (linear-weighted kappa = 0.63) (Table 19) and subjective assessment of severity of adenomyosis (linear-weighted kappa = 0.61) (Table 20).

Table 14 - Indications for ultrasound scan

Indication for ultrasound scan	n (%)
Pelvic pain	9
Menstrual symptoms	9
PCB/IMB/PMB	6
Ovarian surveillance	6
Subfertility	4
Other	3

Table 15 - Number of ultrasound features of adenomyosis seen on original real-time scan

Number of features seen	n (%)
0	14 (38.9)
1	4 (11.1)
2	3 (8.3)
3	5 (13.9)
4	4 (11.1)
5	4 (11.1)
6	2 (5.6)
7	0 (0)
Total	36 (100)

Table 16 - Kappa analysis of intra-observer agreement between diagnosis of adenomyosis on real-time ultrasound scan (operator 1) and on stored uterine volumes (operator 1)

	Diagnosis on stored volume (operator 1)			
Real time ultrasound diagnosis (operator 1)		Not adenomyosis	Adenomyosis	Total
	Not adenomyosis	13	1	14
	Adenomyosis	5	17	22
	Total	18	18	36
k=0.667 (good agreement) **95%CI (0.429-0.904)** **Standard error of kappa = 0.121**				

Table 17 - Linear-weighted kappa analysis of agreement on the number of ultrasound features seen on real-time ultrasound scan (operator 1) and on stored uterine volumes (operator 1)

	Number of ultrasound features of adenomyosis seen on stored uterine volumes (operator 1)								
Number of ultrasound features of adenomyosis seen on real-time ultrasound scan (operator 1)		0	1	2	3	4	5	6	7	Total
	0	13	0	1	0	0	0	0	0	14
	1	2	1	1	0	0	0	0	0	4
	2	2	0	1	0	0	0	0	0	3
	3	0	1	1	2	0	1	0	0	5
	4	1	0	2	0	1	0	0	0	4
	5	0	0	0	2	0	2	0	0	4
	6	0	0	0	0	1	0	1	0	2
	7	0	0	0	0	0	0	0	0	0
	Total	18	2	6	4	2	3	1	0	36
Linear-weighted kappa = 0.64 (good agreement) **95% CI (0.47-0.80)** **Standard error of kappa = 0.082**										

Table 18 - Kappa analysis of agreement of diagnosis of adenomyosis on stored uterine volumes (operator 1 and 2)

	Diagnosis on stored volume (operator 1)			
Real time ultrasound diagnosis (operator 1)		Not adenomyosis	Adenomyosis	Total
	Not adenomyosis	13	5	18
	Adenomyosis	2	16	18
	Total	15	21	36
k=0.61 (good agreement) **95%CI (0.36-0.87)** **Standard error of kappa = 0.130**				

Table 19a - Linear-weighted kappa analysis of inter-observer agreement on the number of ultrasound features seen on stored uterine volumes (operator 1 and 2)

	Number of ultrasound features of adenomyosis seen on stored uterine volumes (operator 2)								
Number of ultrasound features of adenomyosis seen on real-time ultrasound scan (operator 1)		0	1	2	3	4	5	6	7	Total
	0	13	1	3	1	0	0	0	0	18
	1	0	1	1	0	0	0	0	0	2
	2	2	0	2	2	0	0	0	0	6
	3	0	0	1	3	0	0	0	0	4
	4	0	0	0	1	1	0	0	0	2
	5	0	0	0	1	1	0	1	0	3
	6	0	0	0	0	1	0	0	0	1
	7	0	0	0	0	0	0	0	0	0
	Total	15	2	7	8	3	0	1	0	36
Linear-weighted kappa = 0.63 (good agreement) 95% CI (0.47 - 0.78) Standard error of kappa = 0.078										

Table 19b – Table showing the kappa values for inter-observer agreement between the presence of individual ultrasound features of adenomyosis (operator 1 and 2)

Ultrasound feature	**Kappa value (95% CI)**
Asymmetrical thickening	0.771 (0.53-1)
Parallel shadowing	1 (1-1)
Linear striations	0.571 (0.29-0.85)
Myometrial cysts	0.487 (0.22-0.753)
Hyperechoic islands	0.478 (0.135-0.822)
Adenomyomas	0 (0-0)
Irregular EMJ	0.650 (0.398-0.903)

Table 20 - Linear-weighted kappa analysis of inter-observer agreement on the severity of adenomyosis on stored uterine volumes (operator 1 and 2)

	Subjective assessment of severity of adenomyosis on stored uterine volumes (operator 2)					
Subjective assessment of severity of adenomyosis on uterine volumes (operator 1)		Absent	Mild	Moderate	Severe	Total
	Absent	13	5	0	0	18
	Mild	2	5	2	0	9
	Moderate	0	1	2	0	3
	Severe	0	1	3	2	6
	Total	15	12	7	2	36
Linear-weighted kappa = 0.61 (good agreement) **95%CI (0.44-0.78)** **Standard error of kappa = 0.087**						

Table 21 - Kappa analysis of intra-observer agreement of diagnosis of adenomyosis on stored uterine volumes (operator 1)

	Diagnosis on stored volume (operator 1)			
Real time ultrasound diagnosis (operator 1)		Not adenomyosis	Adenomyosis	Total
	Not adenomyosis	16	2	18
	Adenomyosis	1	17	18
	Total	17	19	36
k=0.83 (excellent agreement) **95%CI (0.65-1.0)** **Standard error of kappa = 0.092**				

Table 22 - Linear-weighted kappa analysis of intra-observer agreement on the number of ultrasound features seen on stored uterine volumes (operator 1)

	Number of ultrasound features of adenomyosis seen on stored uterine volumes (operator 2)								
Number of ultrasound features of adenomyosis seen on real-time ultrasound scan (operator 1)		0	1	2	3	4	5	6	7	Total
	0	16	1	1	0	0	0	0	0	18
	1	0	0	1	1	0	0	0	0	2
	2	1	1	3	0	1	0	0	0	6
	3	0	1	1	2	0	0	0	0	4
	4	0	0	0	0	1	1	0	0	2
	5	0	0	0	0	1	1	1	0	3
	6	0	0	0	0	0	0	1	0	1
	7	0	0	0	0	0	0	0	0	0
	Total	17	3	6	3	3	2	2	0	36
Linear-weighted kappa = 0.77 (good agreement) **95% CI (0.65 - 0.90)** **Standard error of kappa = 0.064**										

Table 23 - Linear-weighted kappa analysis of intra-observer agreement on the severity of adenomyosis on stored uterine volumes (operator 1)

	Subjective assessment of severity of adenomyosis on stored uterine volumes (operator 2)					
Subjective assessment of severity of adenomyosis on uterine volumes (operator 1)		Absent	Mild	Moderate	Severe	Total
	Absent	16	1	1	0	18
	Mild	1	6	2	0	9
	Moderate	0	0	3	0	3
	Severe	0	0	1	5	6
	Total	17	7	7	5	36
Linear-weighted kappa = 0.83 (excellent agreement) **95%CI (0.70-0.97)** **Standard error of kappa = 0.067**						

[3] HOW COMMON IS ADENOMYOSIS? A PROSPECTIVE STUDY OF PREVALENCE USING TRANSVAGINAL ULTRASOUND IN A GYNAECOLOGY CLINIC

3.1 BACKGROUND

The reliance on histological assessment of hysterectomy specimens for the diagnosis of adenomyosis has presented many difficulties in accurately assessing its prevalence. The lack of clear and uniform histological criteria raises concerns about the reproducibility of histological diagnosis of adenomyosis and partially explains the variation in the proportions of women diagnosed with adenomyosis in different studies (Bergholt et al, 2001). Furthermore, the reliance on histology for diagnosis introduces a heavy selection bias as only women who have undergone a hysterectomy can be diagnosed with the disease. This bias has been further amplified by the reduction in the number of hysterectomies being performed for benign disease (Reid and Mukri, 2005), resulting in the population of women from which conclusions are drawn becoming increasingly less representative of the general population. Continuous improvements in the resolution of TVUS have now enabled a more detailed assessment of uterine architecture. This has facilitated the detection of subtle features of adenomyosis, which could not have been seen with older equipment. TVUS, along with MRI, has recently been shown to have good levels of accuracy in the pre-operative diagnosis of adenomyosis (Dueholm 2006). TVUS is relatively inexpensive, widely available and well-tolerated by patients. The aim of this study was to assess the prevalence of adenomyosis in a population of women attending general gynaecological clinic using transvaginal ultrasound.

3.2 METHODS

This was a prospective observational study of women attending our general gynaecology clinic between January 2009 and January 2010. In all women, demographic data were recorded and a detailed clinical history was taken prior to undertaking the ultrasound scan. This included women's age, ethnicity, body mass index (kg/m^2), smoking history, age at menarche, gravidity and parity (number of all prior pregnancies: miscarriages, terminations of pregnancy and live births including mode of delivery), breastfeeding history (months of breastfeeding), contraceptive history (length of time taking exogenous hormones, IUCD usage) and menopausal status. Body mass index was calculated based on self-reported weight and height. Women who were not certain of these measurements had their height and weight measured using a calibrated scale and stadiometer.

Women were considered to have a history of endometriosis, if it had been diagnosed previously on laparoscopy or on ultrasound scan within our department using the criteria previously described in the methods section. In women who subsequently underwent hysterectomy, ultrasound findings were compared with the final histological diagnosis. Histopathological examinations were performed according to Molitor's method (Molitor, 1971).

3.3 STATISTICAL ANALYSIS

Statistical analysis was performed, to examine possible associations between various demographic and clinical variables and the presence of adenomyosis, using logistic regression. Initially the individual effect of each factor upon the outcome was assessed in a series of univariable analyses. Subsequently the joint effect of the variables was examined in a multivariable analysis. Before the multivariable analysis, variance inflation factors (VIFs)

were used to examine the colinearity between the variables. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago IL). Kappa analysis was used to determine the level of agreement between ultrasound and histological diagnosis of adenomyosis.

3.4 RESULTS

A total of 1066 consecutive women attended for clinical visits. None of them were pre-menarchal or pregnant. 45/1066 [4.2% (95%CI: 3.8%-5.6%)] women were excluded from the data analysis as they were unable to undergo a transvaginal scan. A further 36/1066 [3.4% (95%CI: 2.5%-4.6%)] women were excluded as they had previously undergone a hysterectomy. The median age of the 985 women included in the final data analysis was 40 (inter-quartile range: 32-48). 387/985 [39.3% (95% CI: 36.3%-42.4%)] women were nulligravid and 529/985 [53.7% (95% CI: 50.6%-56.8%)] were nulliparous. 174/985 [17.7% (95%CI: 15.4%-20.2%)] women were menopausal. The indications for clinical visits are listed in Table 24. The diagnoses following ultrasound assessment are listed in Table 25. A total of 206/985 [20.9% (95% CI: 18.5% - 23.6%)] women were diagnosed with adenomyosis. In 78/206 (37.9%) of them, adenomyosis was an isolated abnormality, whilst in 128/206 (62.1%) of women concomitant abnormalities were found (Table 26).

The results of the univariable analysis examining associations of demographic and clinical factors with adenomyosis are shown in Table 27. The multivariable analysis (Table 28) showed that age, gravidity and pelvic endometriosis were all significantly associated with the presence of adenomyosis. The prevalence of adenomyosis increased with age, reaching a peak between the ages of 40 and 59, after which the prevalence decreased. Women with a

previous or current diagnosis of endometriosis were significantly more likely to have adenomyosis with an odds ratio of 4.06 (95% CI: 2.25-7.33).

Of the 985 women, 45 [4.6% (95% CI: 3.4%-6.1%)] underwent hysterectomy within two years of their ultrasound scans. Of the 45 women who underwent hysterectomy, 19 did so for suspected gynaecological malignancies, 9 for menorrhagia, 6 for pelvic organ prolapse, 5 for urinary symptoms, 2 for pain symptoms and a further 2 had a hysterectomy prophylactically as they had a genetic predisposition to hereditary non-polyposis colorectal cancer. Women with uterine cancer (12 women with endometrial cancer and 2 with uterine sarcomas) or very large fibroids (4 women) were not used for comparison between TVUS and histology as their pathologies became the primary focus of histological examination. The invasive nature of the uterine cancers interfered with assessment of the EMJ and the overall uterine size of those women with very large numbers of fibroids made it difficult to obtain systematic representative sections from every part of the pathological specimens. The sensitivity and specificity of ultrasound in diagnosing adenomyosis in the remaining women was 81.8% (95% CI: 52.3%-94.9%) and 81.3% (95% CI: 57.0%-93.4%). The positive predictive value was 75% (95% CI: 42.8%-93.3%) and the negative predictaive value was 86.7% (95% CI: 58.4%-97.7%). A kappa analysis showed a good level of agreement between histology and transvaginal ultrasound in the diagnosis of adenomyosis [Kappa=0.62 (p=0.001), 95% CI (0.32-0.91)].

Table 24 – Primary indications for scan (Naftalin et al. 2012b)

Indication for TVUS	n (%)
Menorrhagia	121 (12.3)
Dysmenorrhoea	18 (1.8)
Menorrhagia / dysmenorrhoea	30 (3.0)
Pelvic pain	187 (18.9)
Infertility	129 (13.1)
Irregular bleeding / amenorrhoea	154 (15.6)
Postmenopausal bleeding	80 (8.1)
Other	266 (27.0)

Table 25 - Summary of ultrasound diagnoses recorded in the study population (n=985) (Naftalin et al. 2012b)

Ultrasound diagnosis	n (%)
No pathology	210 (48.7)
Adenomyosis	206 (20.9)
Fibroids	346 (35.1)
Endometriosis	63 (6.4)
Polycystic ovaries	164 (16.6)
Adnexal tumours	101 (10.3)
Thick endometrium in postmenopause	47 (4.8)
Endometrial polyps	45 (4.6)
Pelvic adhesions	26 (2.6)
Major congenital uterine anomalies	8 (0.8)
Other	9 (0.9)

Table 26 - Concomitant abnormalities found in women with adenomyosis (n=206) (Naftalin et al. 2012b)

Concomitant abnormalities	n (%)
Fibroids	47 (22.8)
Endometriosis	10 (4.9)
Polycystic ovaries	10 (4.9)
Adnexal tumours	17 (8.3)
Thick endometrium in postmenopause	1 (0.5)
Endometrial polyps	2 (1.0)
Pelvic adhesions	1 (0.5)
Major congenital uterine anomalies	0 (0)
Two or more pathologies	40 (19.4)
No additional pathology	78 (37.9)
Total	206 (100)

Table 27 – Results of univariable analysis looking at associations between demographic and clinical factors and adenomyosis (Naftalin et al. 2012b)

Variable	Category/term	Adenomyosis, n (%)	Odds ratio (95% CI)	p-value
Age[a]	Linear term Square term		65.3 (19.4, 220)	<**0.001**
Ethnicity	Caucasian Asian Afro-caribbean Oriental Middle eastern Mixed/other	119/640 (19) 25/80 (31) 35/149 (23) 8/38 (21) 10/40 (25) 7/38 (18)	1 1.99 (1.19, 3.32) 1.34 (0.88, 2.06) 1.17 (0.52, 2.61) 1.46 (0.69, 3.07) 0.99 (0.43, 2.30)	 0.14
Smoking (pack years)	0 1-10 >10	84/426 (20) 56/290 (19) 31/116 (27)	1 0.97 (0.67, 1.42) 1.48 (0.92, 2.39)	 0.21
BMI[b] (kg/m^2)			1.20 (1.03, 1.41)	**0.02**
Gravidity	0 ≥1	39/387 (10) 165/598 (28)	1 3.40 (2.33, 4.95)	 <**0.001**
Gravidity (detailed)	0 1 2 3-5 ≥6	39/387 (10) 38/187 (20) 35/131 (27) 65/220 (30) 27/60 (45)	1 2.28 (1.40, 3.70) 3.25 (1.96, 5.41) 3.74 (2.41, 5.81) 7.30 (3.98, 13.4)	 <**0.001**
Parity	0 1 2 ≥3	75/529 (14) 38/170 (22) 50/151 (33) 44/135 (33)	1 1.83 (1.18, 2.83) 3.14 (2.06, 4.78) 3.07 (1.98, 4.75)	 <**0.001**
Vaginal deliveries	0 1 2 ≥3	91/607 (15) 39/150 (26) 45/127 (35) 29/101 (29)	1 1.99 (1.30, 3.06) 3.11 (2.03, 4.77) 2.28 (1.41, 3.71)	 <**0.001**
Caesarean deliveries	0 ≥1	166/869 (19) 38/116 (33)	1 2.06 (1.35, 3.15)	 **0.001**
Miscarriages	0 ≥1	141/772 (18) 63/213 (29)	1 1.85 (1.30, 2.61)	 **0.001**
ERPC or STOP	0 ≥1	119/705 (17) 85/280 (30)	1 2.15 (1.56, 2.96)	 <**0.001**
Breastfeeding	≤6 months > 6 months	32/112 (29) 34/102 (33)	1 1.19 (0.76, 1.87)	 0.44
Age at menarche			1.02 (0.93, 1.12)	0.71
Use of intrauterine contraceptive devices	No Yes	100/519 (19) 44/151 (29)	1 1.72 (1.14, 2.60)	 **0.01**
Time on combined oral contraceptive pill (years)	0 <1 1-5 5-10 >10	77/328 (24) 22/162 (14) 46/215 (21) 38/174 (22) 21/106 (20)	1 0.51 (0.30, 0.86) 0.88 (0.58, 1.34) 0.91 (0.58, 1.41) 0.80 (0.47, 1.38)	 0.15
Time on progesterone-only contraception (years)	0 <1 1-5 >5	149/793 (19) 24/75 (32) 21/87 (24) 10/30 (33)	1 2.03 (1.21, 3.40) 1.37 (0.81, 2.31) 2.15 (0.99, 4.70)	 **0.01**
Menopausal status	No Yes	169/811 (21) 35/174 (20)	1 0.96 (0.64, 1.44)	 0.83
Endometriosis	No Yes	179/922 (19) 25/63 (40)	1 2.73 (1.61, 4.64)	 <**0.001**
Fibroids	No Yes	127/639 (20) 74/346 (21)	1 1.08 (0.78, 1.49)	 0.63

[a] – Odds ratio given for 10-unit increase in explanatory variable
[b] – Odds ratio given for 5-unit increase in explanatory variable

Table 28 - Results of multivariate analysis looking at associations between demographic and clinical variables and adenomyosis (Naftalin et al. 2012b)

Variable	Category / term	Odds Ratio (95% CI)	P-value
Age (**)	Linear term	34.3 (9.9, 118)	**<0.001**
	Squared term	0.70 (0.62, 0.80)	
Gravidity	0	1	
	1	1.83 (1.09, 3.06)	
	2	2.46 (1.44, 4.30)	
	3-5	2.66 (1.62, 4.28)	
	6+	4.90 (2.57, 9.35)	**<0.001**
Endometriosis	No	1	
	Yes	4.06 (2.25, 7.33)	**<0.001**

(**) Odds ratios given for a 10-unit increase in explanatory variable

[4] IS ADENOMYOSIS ASSOCIATED WITH MENORRHAGIA?

4.1 BACKGROUND

Original descriptions of adenomyosis reported an association with heavy and prolonged periods (Cullen 1908). Several later studies reported similar findings (Emge et al. 1962; Bird et al. 1972; Benson and Sneedon, 1958), but others have not shown significant differences in the prevalence of adenomyosis between women with and without a history of menorrhagia (Bergholt et al. 2001; Levgur et al. 2000; Parrazzini et al. 1997; Weiss et al. 2009). Most of these studies used retrospective histological examination of hysterectomy specimens to diagnose adenomyosis and compare it to clinical symptoms. The main problem with the use of histology for the diagnosis of adenomyosis in these studies is the heavy selection bias incurred (Mehasseb and Habiba 2009). Furthermore, in retrospective studies it is very difficult to account for confounding variables such as concomitant uterine fibroids which have an independent adverse effect on menstrual periods.

TVUS has recently been used for the non-invasive diagnosis of adenomyosis (Kepkep et al. 2007) and to study its prevalence (Naftalin et al. 2012b). There have been no large scale prospective studies using non-invasive techniques to assess the link between adenomyosis and clinical symptoms. The aim of this study was to investigate the possible association between adenomyosis and menorrhagia.

4.2 METHODS

This was a prospective observational study of premenopausal women attending our general gynaecology clinic. In all women, a detailed clinical history was taken prior to undertaking the ultrasound scan. Women were asked about the frequency and duration of menstrual periods and about any episodes of intermenstrual or postcoital bleeding. The amount of menstrual loss was assessed subjectively by asking whether they felt their periods were

excessively heavy or not. We also asked a subgroup of women to complete a PBAC in order to obtain semi-quantitative information about the amount of menstrual loss. Women were advised to complete the chart during their next period and then to return it back to the department by post (a stamped addressed envelope was provided).

Women who were unable to undergo a TVUS or had previously undergone a hysterectomy and those with a history of amenorrhoea or oligomenorrhoea with a cycle length greater than 60 days were excluded from the study. Women using contraceptives or taking medications that would affect menstrual flow, women who attended for ultrasound scan during their IVF cycle and those who underwent hysterectomy prior to their next period following the scan were not asked to complete a PBAC.

4.3 STATISTICAL ANALYSIS

Statistical analysis was performed in two stages, first for subjective assessment of menorrhagia and then for the semi-quantitative assessment of menstrual loss. Regression methods were used to examine possible associations between various demographic and clinical variables and menorrhagia. The subjective measure was a binary outcome, and so the analysis of this outcome was performed using logistic regression. The PBAC scores were measured on a continuous scale and so linear regression was used for the analysis. The scores were found to have a heavily positively skewed distribution and so were given a log transformation before analysis.

The analyses themselves were performed in two stages. Initially the separate association of each factor with the outcome was examined in a series of univariable analyses. Subsequently the joint effect of the variables upon each outcome was assessed in a multivariable analysis. To limit the number of variables in this analysis, only factors with a univariable p-value of

<0.2 were considered for this stage of the analysis. A backwards selection procedure was used to retain only the statistically significant variables in the final model. As menstrual loss was analysed on a log scale, the results are summarised in the form of ratios whereby the ratios represent the PBAC values when the symptoms was present relative to when it was absent. A Kappa analysis was used to analyse the agreement between the subjective assessment of menorrhagia and the PBACs, where a PBAC score ≥100 was considered to be consistent with menorrhagia.

4.4 RESULTS

A total of 892 consecutive premenopausal women attended for clinic visits between January 2009 and January 2010. 178 women were excluded (Figure 23) and 714 were entered into the data analysis. Their median age was 38 (IQR 30-43). 305 women [42.7% (95% CI. 39.1%-46.4%)] women were nulligravid and 424 [59.4% (95% CI. 55.7% - 63.0%)] were nulliparous. Principal indications for ultrasound scans are listed in Table 29. The diagnoses following ultrasound assessment are listed in Tables 30 & 31. All 714 women were entered into data analysis for subjective assessment of menorrhagia. 529 women were asked to complete PBACs and the analysis of the semi-quantitative assessment of menstrual loss was performed in the 304 women who returned their charts. There was moderately good agreement between the subjective binary assessment of menorrhagia and thePBAC assessment of menstrual loss, where a PBAC score of ≥100 was considered to be consistent with menorrhagia (k=0.529; 95% CI, 0.435 to 0.623) (Table 32).

The results of the univariable analysis examining the associations of demographic and clinical variables with subjective assessment of menorrhagia are shown in Table 33. The multivariable analysis (Table 34) showed that BMI, gravidity, fibroids, in particular sub-

mucous fibroids and endometrial polyps were all significantly associated with menorrhagia. Adenomyosis, when assessed as a binary outcome, was not significantly associated with subjective assessment of menorrhagia. There was, however a significant positive correlation between the number of features of adenomyosis on ultrasound scan and the volume of menstrual loss expressed as a PBAC score, with each additional feature of adenomyosis being associated with an average 22% [95% CI. 6% - 42% (p=0.005)] increase in the PBAC value (Figure 24).

Two further multivariable analyses were performed to compare the number of different morphological features of adenomyosis recorded on ultrasound in individual women and the presence of subjectively assessed menorrhagia. The first showed a significant positive relationship between the number of ultrasound features of adenomyosis and menstrual loss (Table 35 – Model 1). The second multivariable analysis (Table 35 - Model 2) compared women with no adenomyosis, women with <4 and those with ≥4 ultrasound features of adenomyosis. This analysis showed women with ≥4 ultrasound features of adenomyosis, but not women with <4 ultrasound features of adenomyosis, were significantly more likely to suffer with menorrhagia. A breakdown of the number of ultrasound features of adenomyosis recorded in the study population can be seen in Table 36. A final analysis looked at the association between menorrhagia and the specific ultrasound features seen. The outcome variable in the analysis was PBAC score which was measured on a log scale therefore the outcomes are expressed as ratios (Table 37). The results for the individual features suggested that only asymmetrical thickening and irregular EMJ were strongly associated with the PBAC scores. The presence of these features was associated with a higher PBAC score.

Figure 23 – Flowchart showing why women were excluded from data analysis (Naftalin et al. 2014)

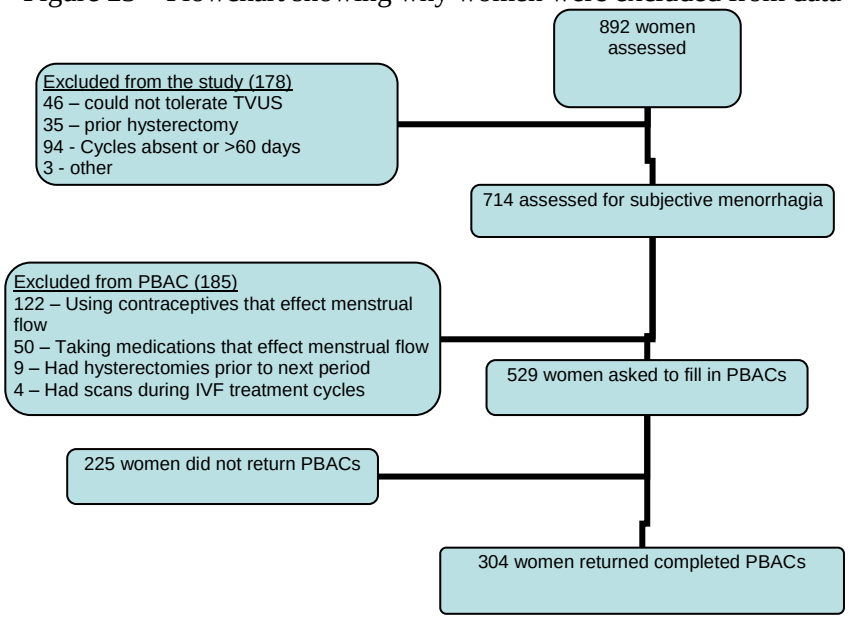


Table 29 – Principal indications for scan (n=714) (Naftalin et al. 2014)

Principal indication for scan	n (%)
Menorrhagia	119 (16.7)
Menorrhagia / dysmenorrhoea	31 (4.3)
Intermenstrual / postcoital bleeding	68 (9.5)
Mild Oligomenorrhoea	66 (9.2)
Pelvic pain	129 (18.1)
Dysmenorrhoea	18 (2.5)
Dyspareunia	12 (1.7)
Subfertility	115 (16.1)
Recurrent miscarriage	9 (1.3)
Other	147 (20.1)

Table 30 - Summary of ultrasound diagnoses recorded in the study population (n=714) (Naftalin et al. 2014)

Diagnosis	n (%)
No uterine pathology	336 (47.1)
Adenomyosis	100 (15.4)
Fibroids	176 (24.6)
Endometrial polyp	32 (4.5)
Two pathologies	69 (9.7)
Three pathologies	1 (0.14)
Total	714 (100)

Table 31 - Concomitant uterine abnormalities found in women with adenomyosis (n=157) (Naftalin et al. 2014)

	n (%)
Intramural/subserous fibroids	43 (27.4)
Submucous fibroids	10 (6.4)
Endometrial polyp	3 (1.9)
Two or more pathologies	1 (0.6)
No additional pathology	100 (63.7)
Total	157 (100)

Table 32 – Kappa analysis assessing agreement between subjective assessment of menorrhagia and PBAC charts (where PBAC score $\geq$100 considered to be consistent with menorrhagia) (Naftalin et al. 2014)

	Subjective menorrhagia	Subjectively normal	
PBAC score $\geq$100	119	48	167
PBAC score <100	24	113	137
	143	161	304
k=0.529 (moderate agreement) **95% CI (0.429-0.620)** **Standard error of kappa =0.048**			

Table 33 – Results of univariable analysis looking at associations between demographic and clinical factors and the subjective assessment of menorrhagia (n=714) (Naftalin et al. 2014)

Variable	Category	Menorrhagia n (%)	Odds Ratio (95% CI)	P-value
Age (*)	-		1.31 (0.19, 1.44)	**<0.001**
BMI (*)	-		1.50 (1.22, 1.84)	**<0.001**
Ethnicity	Caucasian	198/442 (45%)	1	**<0.001**
	Asian	37/66 (56%)	1.57 (0.94, 2.65)	
	Afro-Caribbean	79/122 (65%)	2.26 (1.49, 3.43)	
	Oriental	7/26 (27%)	0.45 (0.19, 1.10)	
	Middle Eastern	15/26 (58%)	1.68 (0.75, 3.74)	
	Mixed	16/32 (50%)	1.23 (0.60, 2.53)	
Gravidity	0	142/305 (47%)	1	**<0.001**
	1	44/133 (33%)	0.57 (0.37, 0.87)	
	2-3	86/161 (53%)	1.32 (0.90, 1.93)	
	4+	80/115 (70%)	2.62 (1.66, 4.14)	
Parity	0	180/418 (43%)	1	**<0.001**
	1	47/105 (45%)	1.07 (0.70, 1.65)	
	2	36/61 (59%)	1.90 (1.10, 3.28)	
	3+	89/130 (68%)	2.87 (1.89, 4.36)	
Adenomyosis	No	256/558 (46%)	1	**0.001**
	Yes	93/157 (59%)	1.89 (1.31, 2.71)	
Any fibroids	No	197/470 (42%)	1	**<0.001**
	Yes	155/244 (64%)	2.41 (1.75, 3.32)	
Submucous fibroids	No	280/624 (45%)	1	**<0.001**
	Yes	72/90 (80%)	4.91 (2.86, 8.43)	
Fibroids (combined)	None	197/470 (42%)	1	**<0.001**
	Any fibroids	83/154 (54%)	1.62 (1.12, 2.33)	
	Submucous fibroids	72/90 (80%)	5.54 (3.20, 9.59)	
Endometrial polyps	No	320/664 (48%)	1	**0.03**
	Yes	32/50 (64%)	1.91 (1.05, 3.47)	

* Odds ratios given for 5-unit increase in explanatory variable

Table 34 – Results of multivariable analysis looking at associations between demographic and clinical factors and the subjective assessment of menorrhagia (n=714) (Naftalin et al. 2014)

Variable	Category	Odds Ratio (95% CI)	P-value
BMI (*)	-	1.40 (1.13, 1.74)	**0.002**
Gravidity	0 1 2-3 4+	1 0.34 (0.18, 0.64) 1.01 (0.59, 1.74) 2.33 (1.26, 4.30)	**<0.001**
Fibroids (combined)	None Any fibroids Submucous fibroids	1 1.53 (0.91, 2.58) 5.60 (2.69, 11.6)	**<0.001**
Endometrial polyps	No Yes	1 2.81 (1.15, 11.7)	**0.02**

* Odds ratios given for 5-unit increase in explanatory variable

Table 35 – Results of multivariable analyses looking at the associations between demographic and clinical factors and the subjective assessment of menorrhagia, where the number of ultrasound features of adenomyosis was treated as a continuous variable (Model 1) and where the number of ultrasound features was treated as a categorical variable (Model 2) (n=714) (Naftalin et al. 2014)

Variable	Category	Odds Ratio (95% CI)	P-value
Model 1			
BMI (*)	-	1.38 (1.11, 1.71)	**0.004**
Gravidity	0 1 2-3 4+	1 0.31 (0.16, 0.60) 0.84 (0.48, 1.48) 1.77 (0.92, 3.39)	**<0.001**
Adenomyosis features	-	1.21 (1.04, 1.40)	**0.01**
Fibroids (combined)	None Any fibroids Submucous fibroids	1 1.53 (0.90, 2.58) 6.19 (2.96, 12.9)	**<0.001**
Endometrial polyps	No Yes	1 2.99 (1.21, 7.40)	**0.02**
Model 2			
BMI (*)	-	1.39 (1.11, 1.73)	**0.003**
Gravidity	0 1 2-3 4+	1 0.31 (0.16, 0.59) 0.91 (0.51, 1.61) 2.01 (1.04, 3.92)	**<0.001**
Adenomyosis features (categorical)	None 1 – 3 4+	1 0.73 (0.39, 1.36) 3.80 (1.62, 8.91)	**0.002**
Fibroids (combined)	None Any fibroids Submucous fibroids	1 1.49 (0.88, 2.53) 6.16 (2.93, 12.9)	**<0.001**
Endometrial polyps	No Yes	1 2.87 (1.16, 7.11)	**0.02**

* Odds ratios given for 5-unit increase in explanatory variable

Table 36 –The number of ultrasound features seen in the study population (n=157) (Naftalin et al. 2014)

Number of ultrasound features of adenomyosis seen	n (%)
1	18 (11.5)
2	37 (23.6)
3	41 (26.1)
4	32 (20.3)
5	16 (2.2)
6	10 (10.2)
7	3 (1.9)

Figure 24 – The relationship between increasing numbers of ultrasound features of adenomyosis and PBACassessed menstrual loss (n=304) (Naftalin et al. 2014)

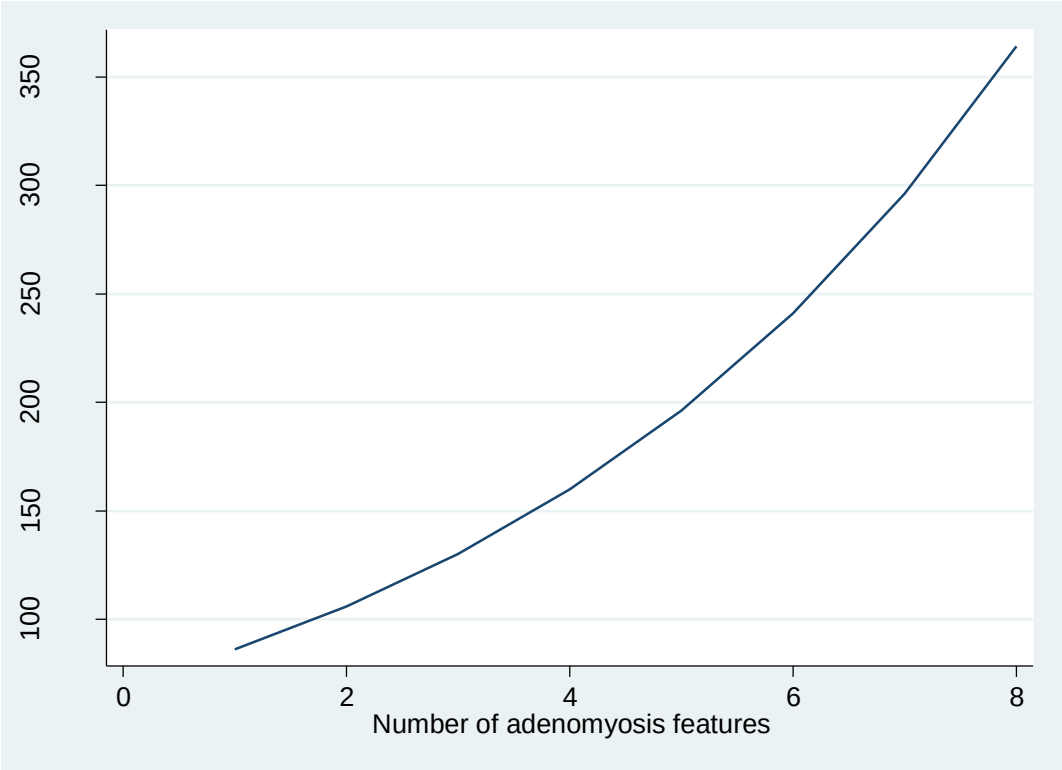


Table 37 – Results of the statistical analysis looking at the effect of the number and type of ultrasound features on the PBAC assessment of menorrhagia (n=304) (Naftalin et al. 2014)

Outcome	Ratio (95% CI)	P-value
Number of features	1.22 (1.06, 1.42)	**0.005**
Asymmetrical thickening	2.24 (1.36, 3.68)	**0.002**
Parallel shadowing	1.63 (0.80, 3.37)	0.18
Linear striations	0.96 (0.56, 1.63)	0.88
Myometrial Cysts	1.62 (0.99, 2.68)	0.06
Hyperechoic lesions	0.82 (0.45, 1.51)	0.53
Adenomyomas	1.07 (0.60, 1.89)	0.82
Irregular EMJ	1.83 (1.10, 3.05)	**0.02**

[5] IS ADENOMYOSIS ASSOCIATED WITH DYSMENORRHOEA?

5.1 BACKGROUND

Original descriptions of adenomyosis reported an association between the disease and 'a great deal of pain' (Cullen, 1908). Several later studies reported similar findings (Emge 1962; Bird et al. 1972; Benson and Sneedon 1958), but others have not shown significant differences in the prevalence of adenomyosis between women with and without a history of dysmenorrhoea (Bergholt et al. 2001; Parrazzini et al. 1997; Weiss et al. 2009). Most of these studies used retrospective histological examination of hysterectomy specimens to diagnose adenomyosis and compare it to clinical symptoms. The main problem with the use of histology for the diagnosis of adenomyosis in these studies is the heavy selection bias incurred (Mehasseb and Habiba, 2009). Furthermore, in retrospective studies it is very difficult to account for confounding variables such as endometriosis which are considered to have an independent adverse effect on menstrual pain.

TVUS has recently been used for the non-invasive diagnosis of adenomyosis (Kepkep et al. 2007) and to study its prevalence (Naftalin et al. 2012b). There have been no large scale prospective studies using non-invasive techniques to assess the link between adenomyosis and clinical symptoms. The aim of this study was to investigate the possible association between adenomyosis and dysmenorrhoea.

5.2 METHODS

This was a prospective observational study of premenopausal women attending our general gynaecology clinic. In all women, a detailed clinical and demographic history was taken prior to undertaking the ultrasound scan. In addition, an assessment was made as to how painful their periods were using an 11-point NRS. Women who were unable to undergo a

transvaginal scan or had previously undergone a hysterectomy and those with a history of amenorrhoea or oligomenorrhoea with a cycle length greater than 60 days were excluded from the study.

5.3 STATISTICAL ANALYSIS

Regression methods were used to examine factors associated with the subjective assessment of dysmenorrhoea. As the NRS scores were measured on a continuous scale, linear regression was used for the analysis. The scores were found to be approximately normally distributed and thus no transformation of the scores was given. The analysis was performed in two stages. Initially the separate association of each factor with the outcome was examined in a series of univariable analyses. Subsequently the joint effect of the variables upon each outcome was assessed in a multivariable analysis. To limit the number of variables in this analysis, only factors with a univariable p-value of <0.2 were considered for this stage of the analysis. A backwards selection procedure was used to retain only the statistically significant variables in the final model. Two variables, gravidity and parity, were found to have a small number of very high values. As a result these two variables were categorised for the purposes of analysis. Statistical analysis was performed, using SPSS software (SPSS Inc., Chicago IL).

5.4 RESULTS

A total of 892 consecutive premenopausal women attended for clinic visits between January 2009 and January 2010. 178 women were excluded (Figure 25) and 714 were entered into the data analysis. Their median age was 38 (IQR 30-43). 305/714 [42.7% (95% CI. 39.1%-46.4%)] women were nulligravid and 424/714 [59.4% (95% CI. 55.7% - 63.0%)] were

nulliparous. Primary indications for ultrasound scans are listed in Table 38. The diagnoses following ultrasound assessment are listed in Tables 39 & 40.

The results of the univariable analysis examining the associations of demographic and clinical variables with subjective assessment of dysmenorrhoea showed that there was a strong positive association between NRS score and the presence of both endometriosis and adenomyosis (Table 41). Women with adenomyosis had higher NRS scores, on average 1.1 units higher than those without adenomyosis. Women with endometriosis had NRS scores that were on average 1.6 units higher than. No other variables were significantly associated with NRS scores.

The multivariable analysis (Table 42) showed that, as in the univariable analyses, both adenomyosis and endometriosis had a significant association with dysmenorrhoea, as measured by NRS. The presence of either of these conditions was associated with higher pain scores. After adjusting for the effects of the other variable, the size of effects of each variable were slightly reduced from the size of effect observed in the univariable analyses. A linear regression analysis was performed assessing the association between both the number of ultrasound features of adenomyosis present and the specific ultrasound features seen. The outcome variable in the analysis was NRS score which was measured on a continuous scale. The NRS score was found to be approximately normally distributed. Linear regression was used to, in turn, to examine the effect of the total number of adenomyosis features, and the individual features, upon the outcome. The analysis of the NRS score included adjustments for both treatment and endometriosis, as these were thought to be potentially confounding factors. The shape of the relationship between the total number of features present and the outcome was examined. If the relationship was not found to be linear (a straight line), a curved relationship was assumed, which was achieved by adding a squared term into the

analysis. The results suggest that the number of ultrasound features of adenomyosis present was significantly associated with the NRS scores (Table 43). The relationship can be seen in Figure 26 which shows the fitted relationship between the number of ultrasound features of adenomyosis present and NRS score. The graph suggests that the number of features was not strongly associated with NRS score below around 4 features, however once the number of features was above this level, there was an increase in the pain score with increased number of features. The results for the individual features suggested that only asymmetrical thickening and irregular EMJ were strongly associated with the NRS scores. The presence of these features was associated with a higher NRS score. The largest effect was for irregular EMJ, where the presence of this symptom was associated with an increase of 1.6 units in the NRS score. A breakdown of the number of ultrasound features of adenomyosis seen in the study population can be seen in Table 44.

Figure 25 – Flowchart showing why women were excluded from data analysis.

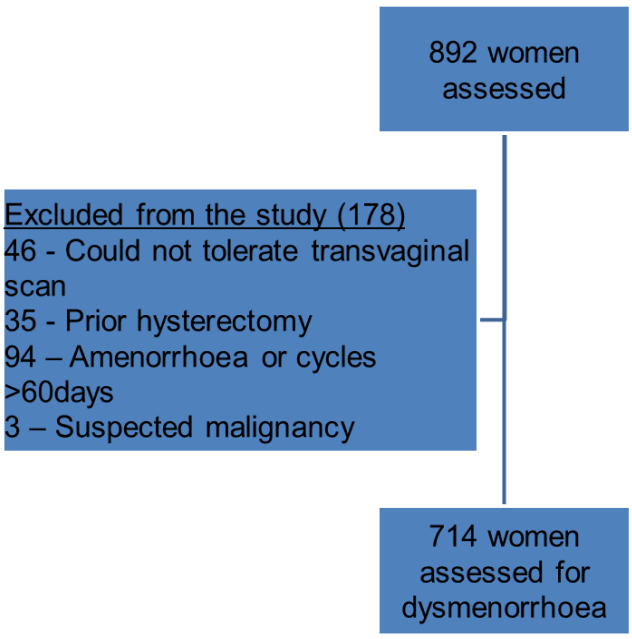


Table 38 – Primary indications for scan (n=714)

Principal indication for scan	n (%)
Menorrhagia	119 (16.7)
Menorrhagia / dysmenorrhoea	31 (4.3)
Intermenstrual / postcoital bleeding	68 (9.5)
Mild Oligomenorrhoea	66 (9.2)
Pelvic pain	129 (18.1)
Dysmenorrhoea	18 (2.5)
Dyspareunia	12 (1.7)
Subfertility	115 (16.1)
Recurrent miscarriage	9 (1.3)
Other	147 (20.1)

Table 39 Summary of ultrasound diagnoses recorded in the study population (n=714)

Ultrasound diagnoses	n (%)
No uterine pathology	336 (47.1)
Adenomyosis	100 (15.4)
Fibroids	176 (24.6)
Endometrial polyp	32 (4.5)
Two pathologies	69 (9.7)
Three pathologies	1 (0.14)
Total	714 (100)

Table 40 - Concomitant uterine abnormalities found in women with adenomyosis (n=157)

Concomitant uterine abnormalities	n (%)
Intramural/subserous fibroids	43 (27.4)
Submucous fibroids	10 (6.4)
Endometrial polyp	3 (1.9)
Two or more pathologies	1 (0.6)
No additional pathology	100 (63.7)
Total	157 (100)

Table 41 - Results of univariable analysis looking at associations between demographic and clinical factors and the subjective assessment of dysmenorrhoea (n=714)

Variable	Category	Coefficient (95% CI)	P-value
Age (*)	-	-0.04 (-0.17, 0.09)	0.53
BMI (*)	-	0.24 (-0.04, 0.51)	0.09
Ethnicity	Caucasian	0	0.10
	Asian	0.71 (-0.04, 1.46)	
	Afro-Caribbean	0.36 (-0.22, 0.93)	
	Oriental	-0.91 (-2.06, 0.24)	
	Middle Eastern	0.28 (-0.87, 1.43)	
	Mixed	0.77 (-0.27, 1.82)	
Gravidity	0	0	0.99
	1	-0.03 (-0.62, 0.57)	
	2-3	0.02 (-0.53, 0.58)	
	4+	0.02 (-0.61, 0.64)	
Parity	0	0	0.89
	1	-0.20 (-0.82, 0.42)	
	2	-0.11 (-0.89, 0.68)	
	3+	-0.17 (-0.75, 0.40)	
Adenomyosis	No	0	**<0.001**
	Yes	1.07 (0.56, 1.58)	
Any fibroids	No	0	0.38
	Yes	0.20 (-0.25, 0.65)	
SM fibroids	No	0	0.25
	Yes	-0.37 (-1.02, 0.27)	
Fibroids (combined)	None	0	0.11
	Non SM fibroids	0.47 (-0.06, 1.00)	
	SM fibroids	-0.26 (-0.92, 0.39)	
Endometriosis	No	0	**<0.001**
	Yes	1.57 (0.77, 2.37)	

(*) Regression coefficients given for a 5-unit increase in explanatory variable

Table 42 - Results of multivariable analysis looking at associations between demographic and clinical factors and the subjective assessment of dysmenorrhoea (n=714)

Variable	Category	Coefficient (95% CI)	P-value
Adenomyosis	No Yes	0 0.94 (0.43, 1.46)	**<0.001**
Endometriosis	No Yes	0 1.36 (0.56, 2.17)	**0.001**

Figure 26 – Relationship between the number of ultrasound features of adenomyosis present and NRS score.

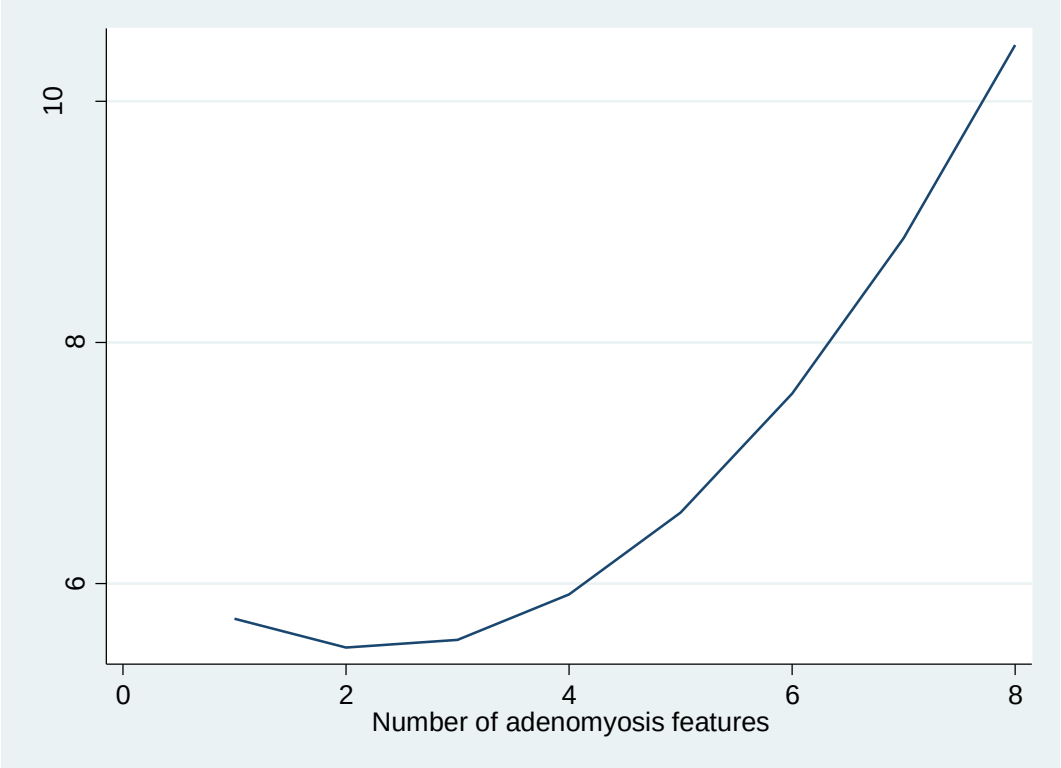


Table 43 - Results of the statistical analysis looking at the effect of the number and type of ultrasound features on the subjective assessment of dysmenorrhoea (n=304)

Outcome	Term	Coefficient (95% CI)	P-value
Number of features	Linear Squared	-0.7 (-1.8, 0.4) 0.2 (0.0, 0.3)	<**0.001**
Asymmetrical thickening	-	1.1 (0.2, 2.1)	**0.02**
Parallel shadowing	-	1.2 (-0.1, 2.5)	0.08
Linear striations	-	0.4 (-0.6, 1.3)	0.46
Myometrial Cysts	-	0.3 (-0.6, 1.3)	0.47
Hyperechoic lesions	-	0.3 (-0.8, 1.3)	0.62
Adenomyomas	-	0.1 (-1.0, 1.2)	0.86
Irregular EMJ	-	1.6 (0.7, 2.6)	**0.001**

Table 44 – The number of ultrasound features seen in the study population (n=157)

Number of ultrasound features of adenomyosis seen	n (%)
1	18 (11.5)
2	37 (23.6)
3	41 (26.1)
4	31 (19.7)
5	16 (2.2)
6	10 (10.2)
7	3 (1.9)

[6] EVALUATION OF A SECOND-STAGE TEST DESIGNED TO IMPROVE THE ACCURACY OF TRANSVAGINAL ULTRASOUND IN THE DIAGNOSIS OF ENDOMETRIAL CANCER

6.1 BACKGROUND

Endometrial cancer is the commonest cancer of the female genital tract in the developed world (Sankaranarayanan and Ferlay 2006). Ultrasound measurement of endometrial thickness is commonly used to triage women with post-menopausal bleeding for histological sampling. The cut-offs for endometrial sampling vary between different authors but $\geq$5mm is a commonly used cut-off. The sensitivity of this cut-off for diagnosing endometrial cancer is very high but the specificity is just 61% (CI: 59%-63%) (Smith-Bindman et al. 1998). Therefore the positive predictive value of endometrial thickness measurement in diagnosing endometrial cancer is poor and many women therefore undergo ultimately unnecessary invasive procedures.

A number of attempts have been made to improve the specificity of ultrasound in diagnosing endometrial cancer. Many studies have assessed whether different endometrial morphologies are more common in malignant lesions (Epstein et al. 2001; Epstein et al. 2002, Epstein and Valentin 2006, Randelzhofer et al. 2002). Other authors have looked at various Doppler parameters to assess whether they aided diagnosis of endometrial cancer (Bourne et al. 1991; Epstein and Valentin 2006).

The aim of our study was to assess whether a test that combined ultrasound examination of endometrial morphology and the integrity of the EMJ, with Doppler examination of the vasculature supplying endometrial lesions, could improve the specificity of ultrasound in

diagnosing endometrial cancer in women with postmenopausal bleeding and increased endometrial thickness on TVUS.

6.2 METHODS

This was a prospective observational study of women with a history of postmenopausal bleeding who attended our rapid-access clinic. In all women, a clinical history was taken and documented on a dedicated database (PIA Fetal Database, version 5.5.4.152, Viewpoint Bildverarbeitung GmbH, Munich, Germany). They all underwent clinical assessment which included speculum examination to exclude local vaginal and cervical abnormalities as a cause of bleeding prior to a TVUS. A smear test was performed only in women younger than 65 who were not up-to-date with their smears. Women who had not been sexually active and those with severe atrophic vaginal changes were offered a transrectal scan. Women in whom transvaginal or transrectal scans were not possible and those in whom ultrasound images of the uterine cavity were sub-optimal were excluded from the study and advised to undergo hysteroscopy. Women were also excluded from the study if they were taking hormone replacement therapy (HRT) or if they had had endometrial sampling performed within the six months prior to attending the clinic.

In women with suspected endometrial polyps or an endometrial thickness $\geq$5mm, a second-stage test was performed at the same time as the initial ultrasound examination. The second stage test involved a detailed subjective assessment of endometrial morphology, the integrity of the EMJ alongside a Doppler assessment of the endometrium. The endometrium was classified as being either homogeneous or heterogeneous in the absence of focal pathology. If focal pathology was present it was classified as being either regular or irregular. The EMJ was assessed and classified as being either regular or irregular.

For the purposes of the study, the assessment of endometrial morphology was classified as suspicious if two criteria were met. The first criterion was that either focal pathology was present and irregular or, if no focal pathology was present, that the endometrium was heterogenous in echotexture (Figure 27). The second criterion was that there must be evidence that any lesion breached the EMJ (Figure 28). With regard to the Doppler assessment, lesions with no detectable blood supply and those with a single feeding vessel were classified as benign (Figure 29) whilst those supplied by multiple vessels crossing the EMJ were considered suspicious (Figure 30).

Only women in whom both the endometrial morphology and the colour Doppler assessment were considered suspicious were classified, for the purposes of the study, as high risk for endometrial cancer. Patient management continued as per our clinical protocol and the ultrasound diagnosis was compared with the final histological diagnosis.

6.3 STATISTICAL ANALYSIS

A database file was set up using Microsoft Excel (Redmond, WA, US) for Windows to facilitate data entry and retrieval. Data were analysed using 2x2 tables to assess sensitivity and specificity.

6.4 RESULTS

125 women were assessed between September 2009 and November 2010. Eighteen women were excluded as they were using hormone replacement therapy. A further 7 women were excluded from statistical analysis; the endometrium could not be fully assessed on ultrasound in 4 of them, 2 of them declined surgery and 1 transferred her care to her local hospital following the ultrasound scan. Thus, 100 women were included in the study. Demographic

characteristics of the women included and excluded are shown in Table 45. The median age of women studied was 57.5 (inter-quartile range: 53-64). 20/100 [20% (95% CI: 12.2%-27.8%)] women were nulliparous. There was a high burden of co-morbidity with 32% (95% CI: 22.9%-41.1%) having hypertension, 12% (95% CI: 5.6%-18.4%) being hypothyroid, 12% (95% CI: 5.6%-18.4%) taking anti-coagulants and 12% (95% CI: 5.6%-18.4%) being diabetic.

Of the 100 women included, 49 had an endometrial thickness <5mm and no focal pathology. These women were reassured, discharged and advised to return should they experience any further vaginal bleeding. A histological diagnosis was obtained in all of the 51 women with either an endometrial thickness ≥5mm or focal pathology. The final diagnosis was obtained by pipelle in 15 cases and in a further 5 cases, pipelle sampling was inadequate and the women had to undergo an outpatient hysteroscopy to achieve a final diagnosis.

10/11 (90.9%) women eventually diagnosed with endometrial cancer were correctly identified as high risk for endometrial cancer using the second-stage test (Table 46). The one false negative was a woman with a carcinosarcomatous endometrial polyp. While this had an unusual appearance on ultrasound, it did not fit the criteria for ultrasound diagnosis of endometrial cancer according to the study protocol, as the EMJ was intact and only a single blood vessel could be seen crossing the basal endometrium on colour Doppler assessment. There were three false positives. All three of these women had fibroids and two of them had adenomyosis.

The sensitivity and specificity of the second-stage test in diagnosing endometrial cancer were 90.9% (95% CI: 62.3% - 98.4%) and 96.6% (95% CI: 90.1% - 98.9%) respectively. 38 women were classified as low risk for endometrial cancer of whom 37 had benign disease, giving a negative likelihood ratio of 0.094. Of the 13 women classified as high risk for

endometrial cancer, 10 were subsequently diagnosed with endometrial cancer, giving a positive likelihood ratio of 27.0. The effect of the second-stage test on the risk of endometrial cancer can be seen in Figure 31.

When endometrial morphology alone was assessed, alongside endometrial thickness, the sensitivity and specificity in diagnosing endometrial cancer were 90.9% (95% CI: 62.3% - 98.4%) and 95.5% (95% CI: 89% - 98.2%). The sensitivity was unchanged when Doppler assessment was analysed alone, alongside endometrial thickness and the specificity dropped slightly to 94.4% (95% CI: 87.5% - 97.6%).

Figure 27 – Longitudinal view of a uterus with a heterogenous endometrium. The patient was subsequently diagnosed with endometrial cancer.

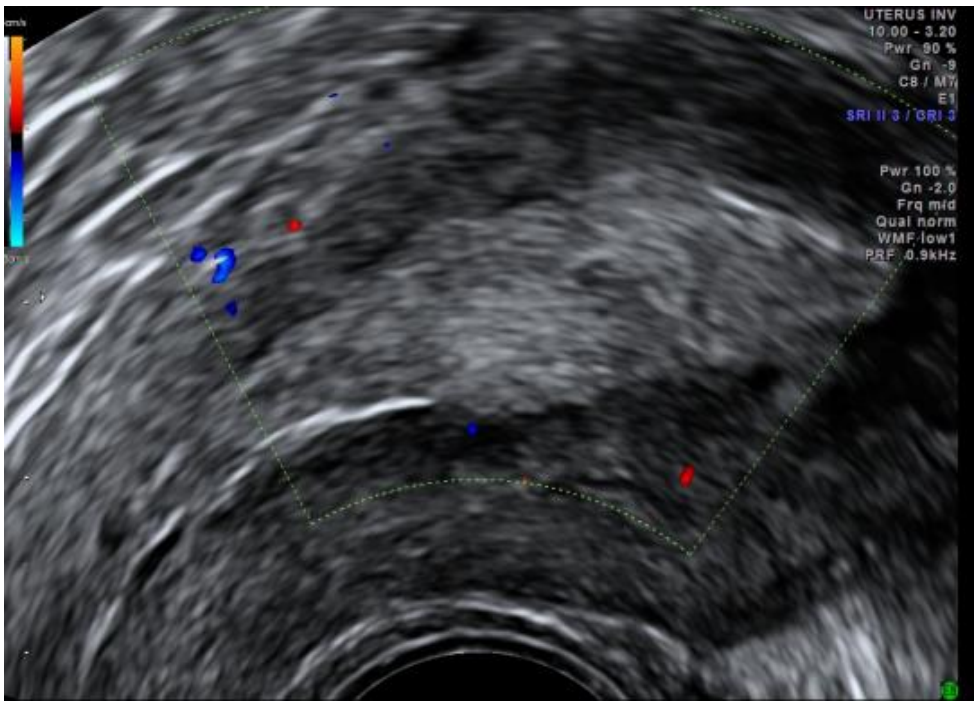


Figure 28 – Coronal view of a uterus where a hyperechoic tumour can be seen filling the right fundal portion of the endometrial cavity. A breach in the EMJ can be seen in the right cornuum (white arrow)

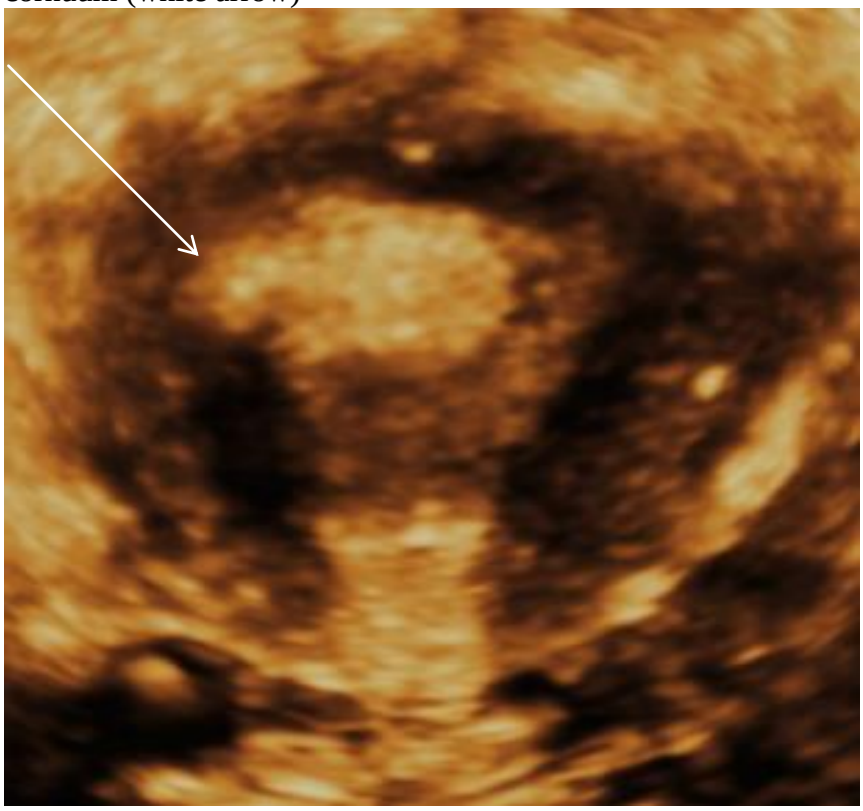


Figure 29 – Longitudinal view of a uterus using B-mode ultrasound with colour Doppler showing an endometrial polyp with single feeder vessel. The polyp was found to be benign.

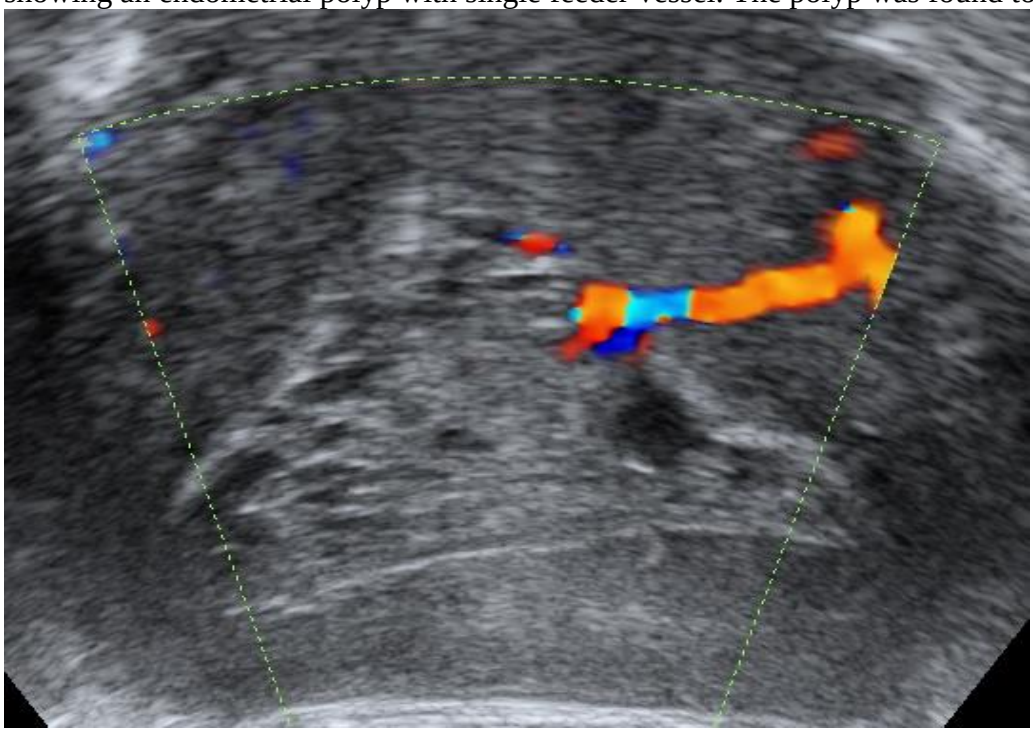


Figure 30 - Longitudinal view of a uterus using B-mode ultrasound with colour Doppler showing multiple vessels crossing the EMJ at the fundus. A pipelle biopsy subsequently revealed endometrial cancer.

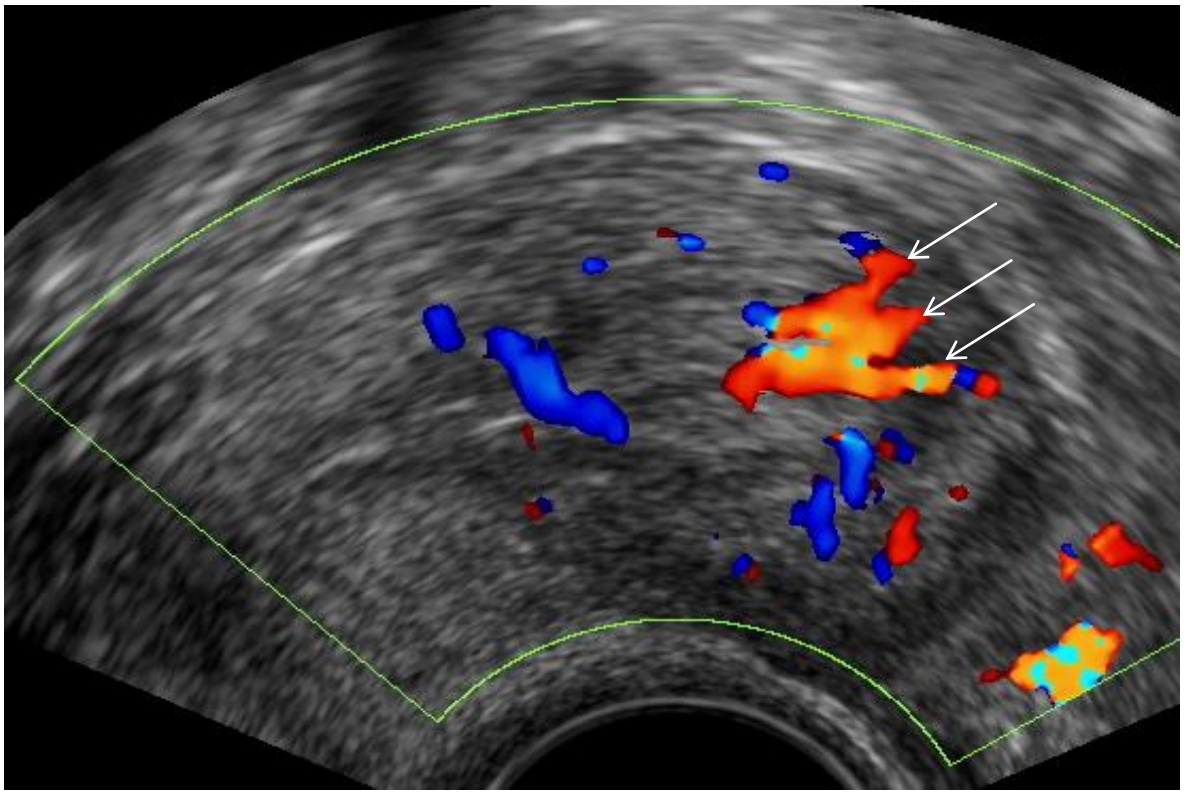


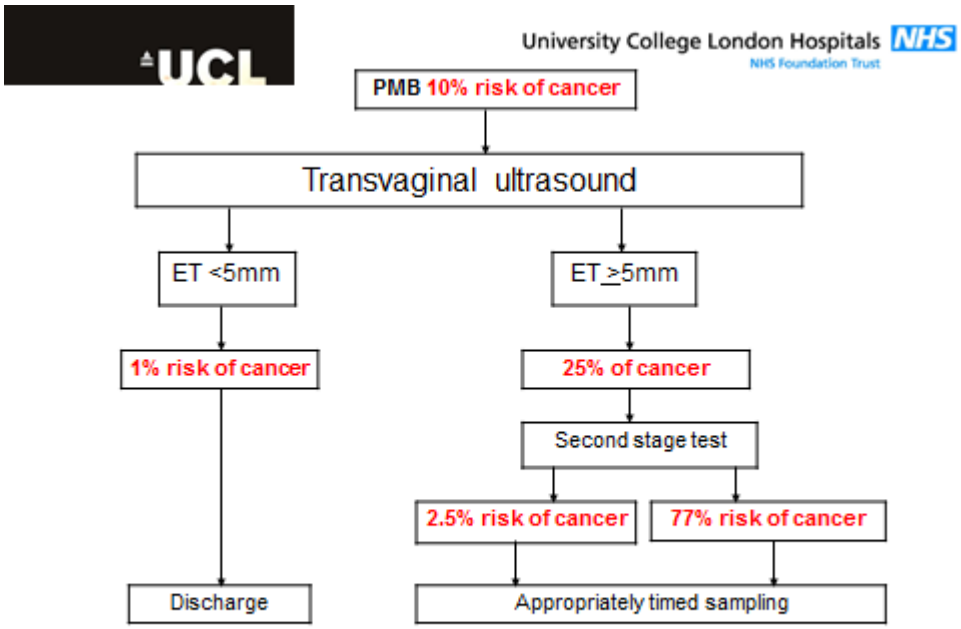
Table 45 – Demographics of women included and excluded from the study

Characteristic	Included (n=100)	Excluded (n=25)	Total (n =125)
Age in years (median; interquartile range)	57.5 (53-64)	57 (53-63)	57 (53-64)
Endometrial thickness mm (range) CI	6.3 (0.8-56.3) (4.8-7.8)	7.5 (1.4-10.5) (1.9-13.1)	7.4 (0.8-56.3) (5.9-8.9)
Nulliparous n (%)	20 (20.0)	7 (28.0)	27 (21.6)
Hypertension n (%)	32 (32.0)	7 (28.0)	39 (31.2)
Diabetes n (%)	12 (12.0)	2 (8.0)	14 (11.2)
Use of anticoagulants n (%)	12 (12.0)	1 (4.0)	13 (10.4)
Use of levothyroxine n (%)	12 (12.0)	1 (4.0)	13 (10.4)
Final diagnosis			
Malignant n (%)	11 (11.0)	1 (4)	12 (9.6)
Histological diagnosis obtained	51	19	70
Pipelle	15	3	18
Outpatient hysteroscopy	12	2	14
Inpatient hysteroscopy	23	8	31
Hysterectomy	1	2	3
Final histological diagnosis			
Atrophic/benign endometrium	16		
Benign endometrial polyp	21		
Simple hyperplasia	3		
Complex hyperplasia	0		
Cancer	11		
Histological subtype			
Endometrioid adenocarcinoma	10		
Carcinosarcoma	1		
Stage of Malignancies			
I	7		
II	1		
III	2		
IV	1		

Table 46 – Table showing how the endometria of women subsequently diagnosed with endometrial cancer were classified at the time of their original ultrasound scan.

Stage	Endometrial thickness (mm)	Endometrium	EMJ	Number of vessels	Ultrasound classification
Ia	8.3	Irregular	Intact	≥2	Malignant
Ia	12.6	Irregular	Disrupted	≥2	Malignant
Ia	12.9	Irregular	Disrupted	≥2	Malignant
Ia	14.5	Irregular	Intact	≥2	Malignant
Ia	17.7	Irregular	Intact	≥2	Malignant
Ib	13	Regular	Disrupted	≥2	Malignant
Ib	26.1	Regular	Disrupted	≥2	Malignant
IIb	13.6	Irregular	Disrupted	≥2	Malignant
IIIa	5	Irregular	Disrupted	≥2	Malignant
IIIb	31.4	Regular	Intact	1	Benign
IV	56.3	Irregular	Disrupted	≥2	Malignant

Figure 31 – Flowchart showing risks of endometrial cancer depending on results of TVUS



IV Discussions

[1] INTER- AND INTRAOBSERVER VARIABILITY IN THREE-DIMENSIONAL ULTRASOUND ASSESSMENT OF THE ENDOMETRIAL-MYOMETRIAL JUNCTION

1.0 DISCUSSION

In this study, there was complete agreement between operators in classifying how well the EMJ could be visualised as optimal or sub-optimal, where sub-optimal included both satisfactory and unsatisfactory views. The discrepancies between operators occurred where EMJs were classified as either satisfactory or unsatisfactory. This is perhaps unsurprising as judging whether most of the EMJ can be seen or not is more subjective and hence more likely to suffer from inter-observer variability, than judging whether all of the EMJ can be seen. Despite the observed variability, the overall reproducibility of examination was very good with kappa values between 0.70 and 0.83. This indicates that assessment of the EMJ on 3D scan is reproducible enough to be used in clinical practice. This is important because assessment of the regularity or integrity of the EMJ is increasingly being used to make clinical diagnoses, mostly for the ultrasound diagnosis of adenomyosis (Ahmed et al. 2007; Exacoustos et al. 2011) but also for the diagnosis of endometrial cancer (Randelzhofer et al. 2002). A reasonable reproducibility in the assessment of the EMJ should be considered a prerequisite if clinicians are to base clinical decisions on ultrasound findings. A criticism could be that the classification system used in this study was entirely arbitrary however there was no pre-existing classification system for assessment of the EMJ that could have been used. Furthermore, a similar approach has been used in the past when assessing reproducibility of ultrasound diagnosis of adnexal masses based on subjective evaluation (Yazbek 2010). It must be remembered that these results apply only to women with normal

uteri. Further work is needed to see if our findings are reproducible in different settings and different populations, more specifically, women with endometrial or myometrial pathology. In conclusion, this study shows that assessment of EMJ visualisation has both good inter- and intra-observer variability in women with normal uteri.

[2] FACTORS AFFECTING VISUALISATION OF THE EMJ ON THREE-DIMENSIONAL ULTRASOUND

2.0 DISCUSSION

This study showed that the ability to analyse the EMJ improves with increasing endometrial thickness. This finding is consistent with MRI studies that show that EMJ thickness increases in parallel with, although not to the same extent as, endometrial thickness (Novellas 2011). Ultrasound studies have shown that endometrial echogenicity also increases in parallel with endometrial thickness, particularly in the luteal phase of the cycle (Fleischer 1986). This would increase the visual contrast between the endometrium and the myometrium, which could further facilitate visualisation of the EMJ.

A more practical reason why increasing endometrial thickness enables better EMJ visualisation is that it allows greater flexibility in producing a clear rendered 3D coronal image. With a thin endometrium, curvature or asymmetry of the cavity can make it harder to find a plane through which the EMJ can be seen continuously. A thicker endometrium, and hence endometrial cavity, minimises this effect.

Other work, including MRI studies, has shown that the EMJ changes in both appearance and size at different stages of the menstrual cycle (McCarthy et al. 1986; Janus et al. 1988; Wiczyk et al. 1988). While our results were unable to show that stage of cycle had a significant effect on EMJ visualisation, this may be because the numbers were too small to determine a difference between the proliferative stage of the cycle and the luteal phase of the cycle, once post-menopausal women, women taking the pill and women with irregular cycles were excluded from the analysis.

We also found that the ability to see the EMJ decreases with increasing parity. The finding that parity has an effect on the EMJ is not unexpected. We know that the EMJ is involved in and changed by placentation (Brosens et al. 2010) and that there are differences in the appearance of the sub-endometria of nulliparous and multiparous women, as measured on 3D power Doppler angiography (Raine-Fenning et al. 2004). One might surmise that this effect was due to the presence of early or undiagnosed adenomyosis, a condition for which increased parity is thought to be a risk factor and which is known to affect the EMJ. This is purely speculative however, as none of the women included in the statistical analysis had any ultrasound features of adenomyosis, despite detailed assessment of the myometrium.

The study by Raine-Fenning et al also showed a statistically significant negative correlation between age and sub-endometrial vascularity (Raine-Fenning et al. 2004). MRI studies have reported a change in EMJ size as age increases (Novellas 2011). We found that age did not have a statistically significant effect on EMJ visualisation. Comparisons between these studies are difficult however, as they either used different imaging modalities or assessed subtly different aspects of the EMJ. These two difficulties endure throughout the literature and are compounded by a paucity of published material assessing EMJ visualisation.

Further work is needed to look at what factors, both physiological and pathological, affect the EMJ.

[3] INTER- AND INTRAOBSERVER VARIABILITY IN THE ULTRASOUND DIAGNOSIS OF ADENOMYOSIS

3.0 DISCUSSION

There was good intra-observer variability when comparing real-time B-mode ultrasound scan assessment and assessment of stored 3D uterine volumes for the diagnosis of adenomyosis, with a kappa value of 0.67. There was also good inter-observer variability when two different operators independently used assessment of stored 3D uterine volumes to diagnose adenomyosis, with a kappa value of 0.61. The intra- and inter-observer variability remained good or better when assessing agreement in the subjective classification of severity of adenomyosis on stored 3D uterine volumes, or in evaluating the number of ultrasound features of adenomyosis seen on stored 3D uterine volumes. When inter-observer variability was assessed there were seven disagreements in diagnosis. In all seven of these cases, the adenomyosis was subjectively classified as mild meaning that there was complete agreement in the diagnosis of adenomyosis, where adenomyosis was considered to be moderate or severe. Given that data from this thesis suggests that adenomyosis only becomes clinically significant, at least with regard to menorrhagia and dysmenorrhoea, until more than 2 ultrasound features are present, this means that there was complete agreement in the diagnosis of clinically significant adenomyosis.

The main strength of this study is that it was a prospective study with pre-defined objectives that assessed both inter- and intra-observer agreement. Furthermore, it not only assessed agreement of ultrasound diagnosis of adenomyosis, but also agreement in the number of ultrasound features seen as well as agreement in classification of severity of adenomyosis.

A potential weakness is that the numbers are relatively small. Another criticism might be that the prevalence of adenomyosis in the population studied was much higher (61.1%) than is

likely to be present in a general gynaecology clinic. This was done in order to ensure inclusion of stored 3D uterine volumes with a wide range in the number of ultrasound features seen in the original real-time scans so as to ensure that reproducibility of classification of severity of adenomyosis could be evaluated and to see whether subjective ultrasound classification of severity of adenomyosis influenced reproducibility of diagnosis of adenomyosis, which it did.

There has only been one other study assessing the inter-observer variability in diagnosis of adenomyosis on transvaginal ultrasound. This study found a considerably lower level of inter-observer agreement (kappa=0.38) than we found in our study (Dueholm et al. 2002). The study in question collected their data around ten years prior to data collection in our study and it may be that technical improvements in TVUS during that time period have led to increased resolution and therefore better visualisation of the ultrasound features of adenomyosis.

Consistent with this, is our finding that the intra-observer agreement was better when comparing two assessments using stored 3D volumes, then when comparing real-time ultrasound scan and an assessment using a stored uterine volume. The higher resolution of real-time ultrasound scan may have enabled visualisation of some of the more subtle ultrasound features that were not able to be visualised subsequently on 3D stored volumes. This may be one of the reasons why some authors have stated that real-time ultrasound scanning is mandatory for the accurate diagnosis of adenomyosis (Reinhold et al. 1996). Our finding that there was good intra-observer agreement between real-time ultrasound scan and stored 3D uterine volume assessment, performed over 3 years apart suggests that accurate diagnosis is possible without real-time ultrasound. This has clinical relevance as significant expertise in gynaecological ultrasound is required in order to make a diagnosis of adenomyosis. If the diagnosis can be made from assessment of stored 3D uterine volumes,

then less experienced sonographers who are uncertain of a diagnosis could store a 3D uterine volume for assessment by an experienced gynaecological sonographer at a later date. This has implications for service provision as it means that experienced sonographers do not have to be constantly available to supervise all scans in order for a diagnosis of adenomyosis to be made. Furthermore, patients would not need to undergo a second transvaginal ultrasound scan in order to confirm the presence of adenomyosis, if the diagnosis can be made on stored uterine volumes.

The inter-observer variability of visualisation of individual ultrasound features of adenomyosis was generally good with 3 of the seven features displaying moderate agreement, 2 displaying good agreement and one displaying perfect agreement. Two of the three features with the highest inter-observer variability were asymmetrical myometrial thickening (kappa value 0.77) and an irregular EMJ (kappa value 0.65). Data later on in this thesis shows that the presence of these two ultrasound features is independently associated with both dysmenorrhoea and increasing PBAC-assessed menstrual loss. Future studies should assess the real-time inter-observer agreement of ultrasound diagnosis of adenomyosis as well as the real-time inter-observer agreement of the individual ultrasound features of adenomyosis.

[4] HOW COMMON IS ADENOMYOSIS? A PROSPECTIVE STUDY OF PREVALENCE USING TRANSVAGINAL ULTRASOUND IN A GYNAECOLOGY CLINIC

4.0 DISCUSSION

The overall prevalence of adenomyosis in our study population was 20.9%. The prevalence increased with age reaching a peak of 32% in women aged 40-49. The prevalence was not significantly different in pre- and postmenopausal women. On multivariate analysis we found that the presence of adenomyosis was significantly associated with age, gravidity and endometriosis.

The main strength of this study is that it was a large prospective study with pre-defined objectives and clear diagnostic criteria. All examinations were performed by a single observer using high quality, top of the range equipment, which ensured a consistent approach to data collection and ultrasound examination, eliminating concerns regarding inter-observer variability. We have also recorded a large number of demographic and clinical variables which has enabled us to assess their possible effects on the prevalence of adenomyosis. A potential weakness is the lack of histological confirmation of diagnosis in the majority of women. In women who underwent a hysterectomy however, histological examination was also performed in a detailed, standardised fashion. The good agreement between pre-operative ultrasound diagnosis of adenomyosis and histological findings is reassuring and it confirms that targeted ultrasound examination can be used in clinical practice for non-invasive diagnosis of adenomyosis.

The majority of previous studies evaluating the prevalence of adenomyosis assessed populations of women who underwent hysterectomy. The sample sizes were typically very

small and they included mainly women with severe symptoms, who were more likely to have adenomyosis than asymptomatic women and those with mild symptoms. In view of this it is likely that the prevalence of adenomyosis in these studies was overestimated. Our estimate of prevalence of adenomyosis is likely to be closer to the true prevalence in the general population of women. As expected the prevalence of adenomyosis in our study was less than that in most recent studies (Weiss et al. 2008; Yeniel et al. 2007; Parazzini et al. 2009). In order to establish a true prevalence, a screening study involving a large number of unselected women would be required, but even then self-selection of women would provide a source of bias as those with past or present concerns about their gynaecological health would be more likely to volunteer for screening. Our estimate is therefore likely to be slightly higher than the genuine prevalence of the condition as we studied symptomatic women attending a gynaecology clinic.

Our finding of good agreement between ultrasound and histology has significant implications for clinical practice. Hysterectomy is rarely performed in modern practice for benign indications and it is neither practical nor realistic to use histology as a gold standard, in future clinical studies of adenomyosis. TVUS and MRI are the only non-invasive diagnostic techniques that could be used to investigate the prevalence of adenomyosis in a population of women attending outpatient clinics. MRI is much more expensive, time consuming and less accessible than ultrasound. In addition, previous studies have not demonstrated that MRI provides superior diagnostic accuracy compared to ultrasound (Dueholm 2006). Recent studies have shown that ultrasound diagnosis of adenomyosis is sensitive and specific enough to employ this technique for in-vivo studies of adenomyosis. The diagnostic criteria we used have been assessed previously and they were found to be satisfactory (Kepkep et al. 2007). It is therefore likely that in the future, TVUS will be the most common and often the only

method, which will be used to make the diagnosis of adenomyosis and monitor its response to conservative treatment.

We found a strong, statistically significant association between adenomyosis and endometriosis. Early descriptions of adenomyosis also described an association with endometriosis. Indeed, up until the 1920s they were considered to be part of the same entity. One recent study (Kunz et al. 2005) suggested that adenomyosis and endometriosis may be different expressions of the same pathological process; a process involving abnormal peristaltic activity within the inner myometrium. A number of studies have confirmed an association between adenomyosis and endometriosis (Bird et al. 1972; Emge 1972), but many other have not (Vavilis et al. 1997; Kilkku et al. 1984; Vercellini et al. 1995; Weiss et al. 2009). A possible explanation for these differences is a tendency to look for the association between adenomyosis and endometriosis at the time of hysterectomy. A number of women affected by both adenomyosis and endometriosis may have been successfully treated for endometriosis in the past. As a result they could be free of active endometriosis at the time of hysterectomy, which would typically take place sometime later. Furthermore, three of the studies that found no link between adenomyosis and endometriosis were retrospective. There are many potential methodological problems with retrospective studies, which could lead to under-reporting of endometriosis in study populations. These include the inability to detect endometriosis on histological examination of the uterus alone and the risk of under-reporting incidental endometriosis at the time of surgery and subsequent histological examination, even if the ovaries and fallopian tubes had been removed at the operation. We tried to overcome these difficulties by performing a prospective study and by taking into account both a past history of endometriosis confirmed at diagnostic laparoscopy and objective evidence of endometriosis on the TVUS.

Increasing age up to the menopause has long been considered a risk factor for adenomyosis. Molitor reported that adenomyosis becomes more common in later reproductive years with a decline in the frequency of diagnosis after menopause, however age has not consistently been shown to be associated with the disease and more recent studies evaluating age as an independent variable have not found an association (Bergholt et al. 2001; Vercellini et al. 1995; Weiss et al. 2009). These studies, however, are limited in their ability to assess age as an independent variable as they draw conclusions from women undergoing hysterectomy, who tend to present a relatively narrow age range. The advantage of using a non-invasive technique like ultrasound is that it can be applied to a much broader population of women. For example, in Bergholt's study just 31.2% of women were under the age of 45, whereas in our study 65.6% of women were under the age of 45. Our findings are therefore more likely to provide reliable information about the link between age and prevalence of adenomyosis.

Many pregnancy-related factors have been linked with adenomyosis including parity (Parazzini et al. 1997; Kilkku et al. 1984; Vavilis et al. 1997; Vercellini et al. 1995), caesarean delivery (Vavilis et al. 1997; Whitted et al. 2000) and terminations of pregnancy (Curtis et al. 2002; Levgur et al. 2000; Vavilis et al. 1997). Again, other studies have not replicated these findings (Bergholt et al. 2001; Parazzini et al. 1997; Panganamamula et al. 2004; Weiss et al. 2009). The univariate analysis in our study suggested that gravidity, parity, uterine instrumentation during pregnancy, spontaneous miscarriage, vaginal delivery and caesarean delivery were all associated with adenomyosis. After multivariate analysis only gravidity was found to be significantly associated with adenomyosis, although parity was not included in the multivariate analysis as analysis suggested it was co-linear with the other variables. This finding suggests that it may be the presence of a pregnancy rather than the

specifics of what occurs in any given pregnancy that may play a role in the development of adenomyosis.

Body mass index, intrauterine contraceptive device use, use of the progesterone-only contraceptive pill, vaginal or caesarean section delivery, miscarriage and either evacuation of retained products of conception or surgical termination of pregnancy all appeared to be significantly associated with adenomyosis prior to the multivariate analysis. It is likely that age accounted for most of these trends and that once the data were adjusted for age the significance was no longer seen. An important finding was the absence of a significant difference in the prevalence of adenomyosis between pre- and post-menopausal women. The prevalence of adenomyosis in postmenopausal women, however was less when compared to the prevalence in the group of women aged 40-49, when the condition was found to be most prevalent. This indicates that adenomyosis tends to regress after menopause, but still remains detectable in a significant number of women. Others have described a similar relationship (Kitawaki 2006). Kitawaki also noted that no studies have shown that use of the combined oral contraceptive pill causes regression of adenomyosis. Our data adds further weight to this and showed that there was no association between use of the combined oral contraceptive pill and the presence of adenomyosis.

Adenomyosis is a common condition and yet little is known about its aetiology, natural history and clinical significance. This was largely due to the inability to make a conclusive and accurate diagnosis of adenomyosis in the past using non-invasive diagnostic methods. Our study, and other recent studies, has shown that transvaginal ultrasound is an accurate method to diagnose adenomyosis. Due to its wide availability and acceptability, ultrasound could be used to facilitate large scale studies of adenomyosis in different populations of women. Future research should try to elucidate its natural history and to examine possible

associations between adenomyosis and different clinical symptoms including subfertility. This information should help to develop more effective preventative and treatment strategies in women affected by adenomyosis.

[5] IS ADENOMYOSIS ASSOCIATED WITH MENORRHAGIA?

5.0 DISCUSSION

Our results did not show a significant association between the presence of adenomyosis and menorrhagia on multivariable analysis. There was, however, a statistically significant positive correlation between amount of menstrual loss and the number of ultrasound features of adenomyosis seen. Other independent variables associated with menorrhagia included fibroids, in particular sub-mucous fibroids, endometrial polyps, gravidity and BMI. Sub-mucous fibroids were found to have the strongest association with menorrhagia.

The main strength of our study was that ultrasound rather than histology was used to diagnosis uterine pathology which helped to reduce selection bias. This also allowed the inclusion of a relatively large number of women with a greater range of symptoms and varying degrees of severity of adenomyosis who are likely, therefore, to be more representative of the population of women attending gynaecology clinics. In addition, this was a prospective study with clearly defined inclusion criteria and a standardised approach to the ultrasound examinations which were all performed by a single highly-trained operator using advanced, modern ultrasound equipment.

While two different assessments of menstrual loss were used, both methods had limitations. The subjective binary assessment of menorrhagia is simple to measure and easy to assess in a large population, however studies have shown that self-assessed menorrhagia may not be an accurate measure of excessive menstrual blood loss (Chimbira et al. 1980). Nevertheless, the use of a subjective assessment of menorrhagia is consistent with recent national guidance (NICE 2007) for clinicians. PBACs offer a relatively simple alternative to the more arduous and time-consuming laboratory methods of objectively measuring menstrual blood loss. It must be remembered however, that they are not as accurate and remain a subjective

assessment of menstrual blood loss. Furthermore, there are limitations to the way they were used in our study. It has been estimated that women may experience 20%-40% variation in menstrual loss between different periods (Hallberg & Nilsson 1964). The fact that PBACs were only used for one menstrual cycle in our study may therefore have limited the validity of the PBAC assessment. Another limitation in our use of PBACs is the fact that women filled in the charts based on usage with their own sanitary wear, which will be of variable absorbency across the study population. Studies have generally addressed this issue by providing standardised sanitary wear. We chose not do so for reasons of practicality and cost.

Despite these limitations the ease of use of PBACs enabled us to make a semi-quantitative assessment of menstrual loss in a large population. Furthermore, by making a semi-quantitative assessment of menstrual blood loss, their use allowed us to take account of the severity of menorrhagia in the study population.There was a fair level of agreement between both assessments of menorrhagia so we used both methods in data analysis.

There is a lack of consensus in the literature regarding the relationship between adenomyosis and menorrhagia. This is not surprising bearing in mind that the majority of studies were retrospective in nature and mainly included populations of women undergoing hysterectomy. These studies used differing criteria for the diagnosis of adenomyosis and few of them attempted to quantify severity of disease. In addition, none of the studies controlled for the presence of concomitant pathology and their potential effect on the volume of menstrual loss. Our study has clearly shown that severity of adenomyosis, as measured by the number of different ultrasound features of adenomyosis seen, correlates with the amount of menstrual loss. The severity of adenomyosis is difficult to express in quantitative terms as the lesions are often poorly defined and they may be disseminated throughout different parts of the myometrium. Levgur et al. used depth of foci of adenomyosis on histological examination to

assess severity of disease in their retrospective study (Levgur et al. 2000). While this study had relatively small numbers and used retrospective case note analysis to define menorrhagia, it was still able to uncover an association between severity of adenomyosis and menorrhagia.

Not only did our study show a positive association between the number of ultrasound features of adenomyosis seen and increasing PBAC-assessed menstrual loss, it also showed that some of the individual ultrasound features of adenomyosis were independently associated with increasing PBAC-assessed menstrual loss. Asymmetrical myometrial thickening and an irregular EMJ were both independently associated with PBAC assessed menstrual loss. It is not surprising that asymmetrical myometrial thickening has a greater effect on symptoms than other ultrasound features, as it is by definition, only seen when the disease is severe enough to have affected an entire wall of the uterus. This is in contrast to some of the other features, such as linear striations, that may affect only a small volume of the uterus. Irregularity of the EMJ may have a greater impact on menstrual loss than other features because any pathological effect is occurring at the interface where blood loss occurs, in contrast to myometrial cysts, for example, that by definition occur some distance from the endometrium. Despite the fact that some features appear to have a greater impact on menstrual loss than others, we think that it is better to express severity of adenomyosis semi-quantitatively using the number of different morphological features alone in an individual woman as an indirect measure of severity of disease. Any proposed system to formally grade severity of adenomyosis based on the number or type of features seen needs to be prospectively assessed before it can be brought into widespread clinical use.

The recognition that the severity of a condition is important when assessing clinical impact has already been adopted in clinical practice when studying the effect of fibroids on menstrual loss. It has been generally accepted that fibroids cause menorrhagia and that their

size and location determines their clinical significance (NICE 2007). Our study has also confirmed that the location of fibroids is a critical diagnostic feature as submucous fibroids were found to have a much more severe effect on menstrual loss than fibroids in other locations. It is also known however, that the majority of fibroids are asymptomatic (Divakar 2008). Furthermore, there is a surprisingly small amount of evidence showing an association between fibroids and menorrhagia (Parker 2007). While early reports (Miller et al. 1953) ascribed heavy periods to submucous fibroids only, others have found that neither the number, volume or location of fibroids were related to menstrual characteristics (Marino et al. 2004). This study was however, relatively small and did not have enough numbers to assess the effect of submucous fibroids. A larger study in which the population had a higher prevalence of fibroids found an association between both length of menses and "gushing bleeding" and the size of fibroids but not the number or their location (Wegienka et al. 2003). This study however, used telephone interviews as a means of assessing patient symptoms. Our study had a large sample size, used high quality ultrasound to assess the exact location of the fibroids and used both objective and semi-quantitative methods to measure menstrual loss. It is likely therefore, that our findings that fibroids are associated with heavy periods and that this association is stronger with sub-mucous fibroids, are accurate. A long-standing difficulty in assessing the association of fibroids and adenomyosis on menorrhagia is the potentially confounding effect that both conditions will have on each other, with both the prevalence and the burden of both conditions being positively associated with advancing age (Naftalin et al. 2012b; Mavrelos et al. 2010). By combining a thorough ultrasound assessment looking for all uterine pathologies with multiple demographic and clinical factors before using multivariate logistic regression analysis, our study was able to account for this potentially confounding effect.

Other variables found to be associated with menorrhagia in our study include endometrial polyps, increasing gravidity and increasing BMI. While endometrial polyps are frequently cited as a cause of menorrhagia (Munro et al. 2011), many studies have merely found a higher than expected prevalence of endometrial polyps in symptomatic populations of women and extrapolated causation. The only study that compared symptomatic women to asymptomatic women found a significantly higher prevalence of polyps in women with abnormal menstrual bleeding, however this study included women with prolonged periods and polymenorrhoea as well as women with menorrhagia. Furthermore, the authors concluded that small endometrial polyps are frequently asymptomatic (Clevenger-Hoeft et al. 1999). One study used PBACs to measure menstrual loss before and after performing hysteroscopic polypectomy and found that post-procedure, there was a significant reduction in menstrual loss (van Dongen et al. 2009). This suggests that endometrial polyps contribute significantly to menstrual loss and is in keeping with our finding that they are significantly associated with both PBAC-assessedand subjectively measured menorrhagia.

While there are little data assessing the effect of increasing BMI on menorrhagia, it is known that the peripheral conversion of adrenal and gonadal androgens to oestrogens in peripheral tissue is greater in obese women (Siiteri 1987). The increased endometrial proliferation caused by this oestrogen excess is known to progress to endometrial hyperplasia and endometrial cancer. Endometrial proliferation would also be expected to cause increased menstrual flow. Our study cannot explain the association between increasing gravidity and menorrhagia. It could be postulated that the increased blood loss may be due to the increase in uterine size seen with increasing gravidity (Verguts et al. 2013), however Rees et al. found no correlation between measured menstrual blood loss and endometrial volume-to-surface ratio (Rees et al. 1984).

[6] IS ADENOMYOSIS ASSOCIATED WITH DYSMENORRHOEA?

6.0 DISCUSSION

On multivariate analysis we found adenomyosis and endometriosis both had a significant independent positive correlation with dysmenorrhoea. There was also a positive correlation between the number of ultrasound features of adenomyosis seen and NRS score for painful periods.

The main strengths of our study were that ultrasound was used to diagnosis adenomyosis, minimising the heavy selection bias seen when histology is used to assess symptomatology in the past. This also allowed for the inclusion of a relatively large number of women. All the scans were performed using top of the range equipment by a single operator who was prospectively looking for the presence of adenomyosis. A further strength of the study was the use of a validated, reproducible pain scoring system to assess the extent of dysmenorrhoea. A potential weakness is the lack of histological confirmation of diagnosis in the majority of women studied however, in those women in the study population who underwent hysterectomy there was good agreement between ultrasound and histological diagnosis (Naftalin et al. 2012b).

Our finding that adenomyosis was significantly associated with pain symptoms, is neither new, nor surprising. The first detailed description of adenomyosis by Cullen over a century ago described one of the main symptoms as being a ‘great deal of pain’ (Cullen 1908). Many subsequent authors have described a similar clinical picture involving painful periods in women with adenomyosis (Emge 1962; Bird et al. 1972; Benson and Sneedon 1958). However, several authors have found no association between the presence of adenomyosis and pain-related symptoms (Bergholt et al. 2001; Parazzini et al. 1997; Weiss et al. 2009).

An important contributor to these conflicting reports is the use of histology as the means to diagnose adenomyosis. Pain symptoms have historically been a common indication for hysterectomy. Thus the hysterectomized population are significantly more likely to have pain symptoms than those women with an intact uterus. This makes it harder to show a significant difference in symptomatology between two groups undergoing hysterectomy. A recent study looking only at perimenopausal women undergoing hysterectomy (Weiss et al. 2009) having failed to show a significant difference in symptomatology between women found on histological examination to have adenomyosis and those who did not, concluded that adenomyosis was not a "disease per se, but rather a normal variant". This study, as with most other studies on the subject, used retrospective case note analysis to assess pain symptoms and assessed them as a binary outcome only. By using a quantitative assessment of pain our study was able to take account of severity of dysmenorrhoea in the data analysis and hence our findings are likely to be more accurate.

A further obstacle in assessing the association between adenomyosis and dysmenorrhoea is dealing with the confounding effect of endometriosis. Both conditions are a recognised cause of dysmenorrhoea and studies have revealed a strong association between the two conditions (Naftalin et al. 2012b). By performing a thorough pelvic ultrasound assessment looking for the presence of ultrasound features of adenomyosis as well as direct and indirect features of endometriosis, prior to using multivariable logistic regression analysis, our study was able to account for this potentially confounding effect.

[7] EVALUATION OF A SECOND-STAGE TEST DESIGNED TO IMPROVE THE ACCURACY OF TRANSVAGINAL ULTRASOUND IN THEDIAGNOSIS OF ENDOMETRIAL CANCER

7.0 DISCUSSION

We have shown that a second-stage ultrasound test applied to women presenting with postmenopausal bleeding and either an endometrial thickness ≥5mm or focal pathology, can significantly improve the specificity of ultrasound diagnosis of endometrial cancer. There are a number of advantages to being able to more accurately diagnose endometrial cancer in women with postmenopausal bleeding. It allows more efficient triaging of women with postmenopausal bleeding for invasive diagnostic testing. Without application of the second-stage test, women identified with focal endometrial lesions would normally have to wait for a hysteroscopy, which might take weeks, before reaching a tissue diagnosis. The identification of women with focal endometrial lesions with appearances suggesting a higher risk of endometrial cancer, facilitates same-day outpatient endometrial sampling, for example pipelle biopsy, which has been shown to have a sensitivity of 99.6% in detecting endometrial cancer (Dijkhuizen et al. 2000**)**. This would speed up the diagnostic process and reduce the time from presentation to treatment in those women, while reducing the total number of hysteroscopies required. This would both reduce the overall risk associated with diagnostic invasive procedures while also reducing service costs and waiting times.

The post-test odds of endometrial cancer in women with homogenous, avascular endometria and an intact EMJ are low (0.025). While this study is insufficient to conclude that an endometrial sample would not be required in this group, it could provide significant reassurance to patients and reduce the time-pressure for endometrial sampling in women

found to be low risk for endometrial cancer, despite having a thickened endometrium. Endometrial sampling could even be avoided in this group if, due to other co-morbidities, the invasive procedures required to get one represented too significant a risk.

Various endometrial ultrasound criteria have been assessed in attempts to improve the specificity of ultrasound in diagnosing endometrial cancer with mixed results. A study by Randelzhofer et al. agreed with our finding that endometrial morphology and regularity of the EMJ were useful criteria to differentiate benign and malignant endometria (Randelzhofer et al. 2002). In contrast, other investigators (Epstein et al. 2001; Epstein et al. 2002) concluded in two separate studies that there was significant overlap of ultrasound morphology between benign and malignant endometrial lesions. A later study by the same research group (Epstein and Valentin 2006) did find that heterogeneous echogenicity and an irregular surface of endometrial lesions on B-mode ultrasound scan were useful criteria for diagnosing endometrial malignancy in women with thickened endometria, but only when there was fluid within the uterine cavity. Differences in study design and population may explain why their conclusions differ from ours. Our population (mean age 59 years v 65 years) was younger and had a greater proportion of malignancies staged 2 or above (36.4% v 9.1%). Their study population also included women taking HRT whereas both our study and the study by Randelzhofer excluded patients taking HRT (Randelzhofer et al. 2002). Lastly, in their analysis, heterogeneous endometrium was close to statistical significance (P=0.08) and as the authors themselves concluded, it may just have been that their study was underpowered to detect a true difference as being statistically significant.

There have been similarly contrasting results from studies assessing the use of Doppler as a means to improve the accuracy of ultrasound in diagnosing endometrial cancer. Early studies using colour Doppler reported promising results in the diagnosis of endometrial cancer.

Bourne et al. used colour Doppler to measure and compare the pulsatility indices (PI) of the uterine arteries in 34 women with postmenopausal bleeding. They found that the PI was reduced in the 17 women subsequently found to have endometrial cancer and concluded that Doppler had great potential in the assessment of uterine pathology (Bourne et al. 1991). It is unclear however, from their methods, how women were selected for the study. Furthermore, the prevalence of endometrial cancer in the study population (50%) was much greater than in most other studies of women with postmenopausal bleeding. Subsequent studies of Doppler flow both in the uterine and endometrial arteries (Sladkevicius et al. 1994; Sheth et al. 1995) have been unable to show that Doppler is useful in discriminating between benign and malignant endometrial lesions.

Timmerman et al. showed that using colour Doppler to assess the distribution of blood vessels supplying focal endometrial lesions was helpful in the diagnosis of endometrial polyps (Timmerman et al. 2003), but they excluded women with a suspicion or confirmed endometrial cancer. Alcazar et al reported that in more than 80% of cases of endometrial cancer, a particular power Doppler pattern of vasculature could be observed (Alcazar et al. 2003). Epstein et al. used an algorithm including power Doppler assessment of endometrial vascularity to estimate the risk of endometrial malignancy. They concluded that their algorithm was better than subjective assessment at differentiating benign and malignant endometria in women with an endometrial thickness of 5-15mm, although this conclusion did not reach statistical significance (Epstein et al. 2002). Subsequent work by the same research group was unable to show that using colour Doppler to assess the number of blood vessels within the endometrium aided the diagnosis of malignancy in women with postmenopausal bleeding (Epstein and Valentin 2006). In contrast to our study however, they assessed the number of vessels seen within the endometrium as a whole. It may be that in order for colour

Doppler assessment of vascular morphology to be helpful, it needs to be limited to assessment of the number of vessels crossing the EMJ at the site of any endometrial irregularity or focal lesion.

Any test including assessment of endometrial morphology and/or Doppler examination will be limited by the fact that these methods are subjective. Furthermore, there were a relatively small number of women included our study. While, these results would need to be reproduced in larger numbers and in a different population prior to being introduced in a clinical setting, we have nevertheless shown that a large increase in the specificity of ultrasound in diagnosing endometrial cancer is possible without a large drop in sensitivity.

PART V Conclusions and further research

This thesis has explored aspects of the ultrasound assessment of the EMJ. It has shown that a classification of EMJ visualisation based on 3D ultrasound is reproducible and is therefore feasible to use in clinical practice. Endometrial thickness and parity have been shown to be significantly associated with quality of EMJ visualisation. These findings however, only relate to normal uteri and further work is required to assess what pathological variables affect EMJ visualisation.

In contrast to other work, it was found that there was a good level of intra- and interobserver agreement in the ultrasound diagnosis of adenomyosis. This was the case when real-time ultrasound assessments were compared with assessments made from stored uterine volumes, as well as when both assessments were made from stored volumes. Arguments against the use of TVUS for the pre-operative diagnosis of adenomyosis have centred on inadequate levels of inter-observer agreement and the reported necessity of real-time images to make the diagnosis. This finding strengthens the case for TVUS to be the pre-operative imaging modality of choice. It is also helpful to the clinician and to the patient, allowing a second opinion to be sought, in cases of uncertainty, without recourse to a second scan. Further work should focus on assessing the inter-observer agreement of TVUS in the diagnosis of adenomyosis when two real-time scans are performedon the same occasion.

TVUS was used to estimate the prevalence of adenomyosis in women attending a general gynaecology clinic in a large prospective observational study. The prevalence was estimated to be 20.9%. It was also found that age, gravidity and pelvic endometriosis were all significantly associated with the presence of adenomyosis. Better estimates of the prevalence of adenomyosis using TVUS could help improve understanding of the burden of the disease, in particular with regard to menstrual symptoms, pain symptoms and subfertility.

Furthermore, more accurate estimates of the prevalence of adenomyosis will lead to more

accurate assessment of the associations of the disease and will therefore aid studies in the future looking to identify women at increased risk of developing the condition.

TVUS was also used to investigate whether adenomyosis was associated with menstrual symptoms. Subjective assessments of dysmenorrhoea were used to look for an association between adenomyosis and dysmenorrhoea. Multivariable analysis revealed that both endometriosis and adenomyosis were independently associated with dysmenorrhoea. Both subjective and semi-quantitative assessments of menorrhagia were used in the same population to investigate if adenomyosis was associated with menorrhagia. Multivariable analysis showed that while adenomyosis was not significantly associated with menorrhagia when assessed as a binary outcome, when severity of disease was taken into account, there was a significant association. In both studies, severity of disease was based on the number of ultrasound features of adenomyosis seen. While this classification system has revealed insights into the relationship between adenomyosis and menstrual symptoms, its validity remains uncertain. A prospective evaluation is required.

Lastly, the role of a second-stage ultrasound test incorporating assessment of the EMJ in the diagnosis of endometrial cancer in women presenting with post-menopausal bleeding was investigated. Its use was found to significantly increase the specificity of ultrasound while having a minimal effect on sensitivity. It must however be noted that the sample size was small.

This thesis has, I hope, set a new benchmark for the ultrasound assessment of both the EMJ and the uterine diseases adenomyosis and endometrial cancer. By incorporating 3D ultrasound assessment with a more detailed analysis of the EMJ and the individual ultrasound features of adenomyosis, this thesis has increased our understanding of the EMJ, adenomyosis and paved the way for a new way of triaging women with post-menopausal

bleeding. It should also facilitate future work on adenomyosis and the EMJ. Key areas for further investigation are the prospective development of a clinically relevant ultrasound grading system for the severity of adenomyosis, the role of adenomyosis in subfertility and a comparison of the current model of assessing women with post-menopausal bleeding with the the second-stage test used in this thesis to assess women with post-menopausal bleeding.

Application of the ultrasound assessment used in this thesis to subfertile women could help determine how severity of adenomyosis affects fertility and also the impact of individual ultrasound features of adenomyosis on fertility. Continuation of this work in subfertile women who then conceive could provide novel information on the impact of adenomyosis on obstetric outcomes.

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Appendix

This thesis has resulted in the following publications:

- Naftalin J, Jurkovic D. The endometrial-myometrial junction: a fresh look at a busy crossing. *Ultrasound Obstet Gynecol* 2009;34(1):1-11

- Naftalin J, Hoo W, Nunes N, Mavrelos D, Nicks H, Jurkovic D. Inter- and intraobserver variability in three-dimensional ultrasound assessment of the endometrial-myometrial junction and factors affecting its visualization.*Ultrasound Obstet Gynecol* 2012;39(5):587-91

- Naftalin J, Hoo W, Pateman K, Mavrelos D, Holland T, Jurkovic D. How common is adenomyosis? A prospective study of prevalence using transvaginal ultrasound in a gynaecology clinic. *Hum Reprod* 2012;27(12);3432-9

- Naftalin J, Hoo W, Pateman K, Mavrelos D, Foo X, Jurkovic D. Is adenomyosis a cause of menorrhagia? *Hum Reprod 2014;29(3) 473-479*

Work stemming from, but not included in this thesis, resulted in the following publication:

- Naftalin J, Nunes N, Hoo W, Arora R, Jurkovic D. Endometrial cancer and ultrasound: why measuring endometrial thickness is sometimes not enough. *Ultrasound Obstet Gynecol* 2012 Jan;39(1):106-9