The Use of Transvaginal Ultrasound and Biochemical Markers in the Diagnosis of Endometriosis.

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Abstract

Endometriosis is the presence of endometrial-like tissue outside the uterus, which induces a chronic, inflammatory reaction. It is a common and debilitating gynaecological condition, which may cause severe pain, significant impairment of quality of life and infertility.

Non-invasive techniques to establish the presence and severity of pelvic endometriosis would be valuable to patients in a number of ways: to guide patient choice regarding treatment; to plan fertility or medical treatment; to enable referral to the most appropriate centre and surgeon if surgery is chosen; to enable pre-operative counselling; and better plan the operation including the involvement of other specialties as indicated.

This thesis aims to assess: the ability of ultrasound to pre-operatively predict the presence and severity of pelvic endometriosis; the reproducibility of these findings; the benefit of tenderness mapping, symptomatology and serum CA125 measurement both on their own and in addition to ultrasound.

The introduction to this thesis discusses: the pathogenesis and impact of endometriosis; the literature regarding the diagnostic tests available including MRI and ultrasound; and the usefulness of serum markers.

Study one assesses the reproducibility of the assessment of severity of pelvic endometriosis by transvaginal ultrasound. Study two assesses the accuracy of the

ultrasound diagnosis of the specific features of pelvic endometriosis and assesses the impact on the diagnostic accuracy of lesion location and total number of lesions. Study three assesses the ability of ultrasound to accurately assess the overall severity of pelvic endometriosis and therefore to enable preoperative triaging of patients. Study four assesses if symptoms alone or in combination with the ultrasound findings are diagnostically useful. In addition, tenderness mapping is assessed as an addition to the ultrasound findings. Lastly, in study five CA125 is assessed as a test for endometriosis on its own and as an addition to the ultrasound findings.

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Statement of originality and personal contribution to work.

I was personally involved in the design of all studies, application for ethical approval and recruitment of all patients. I performed the majority of the ultrasound examinations, collected all data, performed all statistical analysis and interpretation of the results, with the advice and guidance of those mentioned in the acknowledgements.

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I would like thank Dr Susan Bewley for giving some much needed perspective and showing me the light at the end of the tunnel. Finally I would like to thank my wife and family for putting up with me through these many years.

Literature search method

I performed a search of Pubmed using the search term Endometrio*. This brought up all of the papers with endometriosis and endometrioma. Secondly these were filtered using the terms ultrasound, diagnosis, TVS, transvaginal, transrectal.

I also performed a search of google scholar which has a wider catchment for similar terms to the above. The benefit of google is that it shows the most cited papers first.

I also searched within specific journals such as Ultrasound in Obstetrics and Gynaecology, Fertility and Sterility and Human Reproduction for the search term endometriosis.

Chapter 1: Introduction

1.1 Introduction

Endometriosis is a disease defined as the abnormal location of tissue similar to the lining of the uterus (endometrium)[1]. This is usually confined to the pelvis and is associated with painful periods, pelvic pain, reduced fertility and pain during sexual intercourse.

Endometriosis is a common condition but the prevalence of this condition in the general population is uncertain, as to date surgery is required as the gold standard for diagnosis. Amongst women undergoing surgery the prevalence of endometriosis varies widely depending on the indication for the operation. The reported prevalence varies from 40 to 60% in women with dysmenorrhea, and 20 to 30% in women being investigated for subfertility[2]. Endometriosis is detected in 2–50% of laparoscopies done in women with no symptoms (e.g. in a context of tubal ligation), whilst among those undergoing abdominal hysterectomy, it can be as high as 25%[3].

1.2 Clinical presentation

Patients with endometriosis present with a range of clinical symptoms. The classical presentation is with pain before or during menstruation and pain during sexual intercourse. Many women however do not present with these symptoms. Some may have abdominal distension or bloating and pain not related to the menstrual cycle. This creates diagnostic challenges for the gynaecologist, as there are many other conditions such as irritable bowel and pelvic inflammatory disease that can present in

very similar ways. Also, as described above, some patients can have endometriosis found at laparoscopy for either laparoscopic sterilisation or as part of the investigation for infertility when they are completely pain free.

1.3 Pain pathways

The simplest way to describe pain conduction pathways from the viscera to the brain is that nociceptors transmit signals to the spinal cord via primary afferent nerve fibres and the painful stimulus is then transmitted to the brain and the sensation of pain is experienced by the person. However, painful sensations are subject to a series of modulatory mechanisms which can occur in the spinal cord via descending fibres or via pharmacological agents. At each ascending neurological level pain sensation is influenced by modulatory activity including mental state such as anxiety, prior sensitisation, stress, life events, the individuals understanding of the causes of pain and social conditioning. All of these factors create highly complex interaction between the pathological process and the person who perceives the pain[4].

1.4 Types of disease

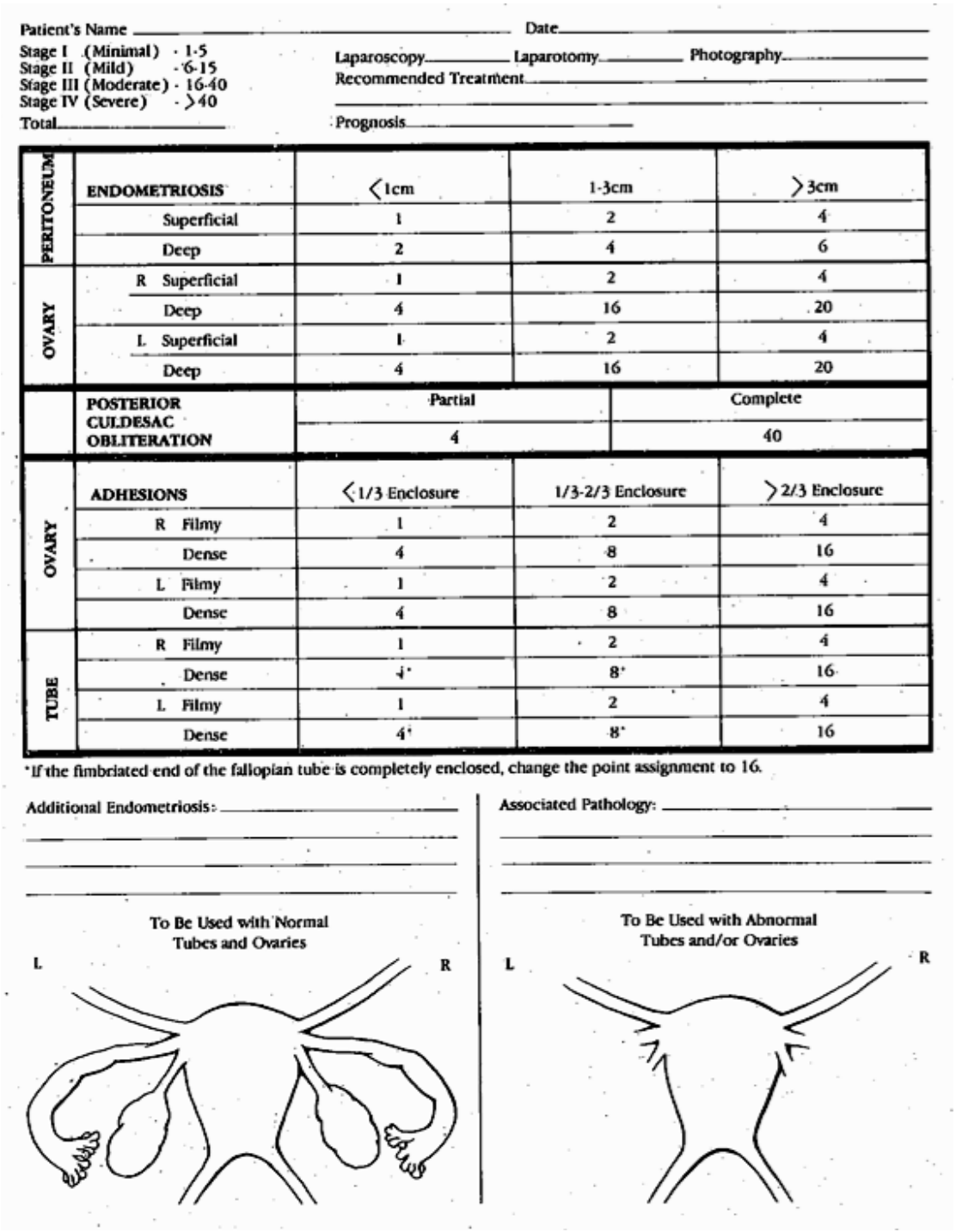
It has been proposed that pelvic endometrosis is three separate, but related disease entities[5]: superficial peritoneal, ovarian endometriomas and deeply infiltrating disease (DIE). There are likely to be different underlying mechanisms involved in the development of these different disease states. These mechanisms will be discussed in more detail in chapter 2.

1.5 Diagnosis and staging of endometriosis at laparoscopy

Currently, laparoscopy is the gold standard for the diagnosis and staging of endometriosis. Diagnostic laparoscopy is associated with 0.06% risk of major complications (e.g. bowel perforation) whilst this risk is increased to 1.3% in operative laparoscopy[6]. Repeated laparoscopies increase the operative risks due to scarring and adhesions.

The revised American Society of Reproductive Medicine[7] (rASRM) staging system for the operative diagnosis of endometriosis is a weighted scoring system which takes into account all the features of pelvic endometriosis. Features include superficial and deep ovarian and peritoneal endometriosis, extent of pouch of Douglas obliteration, density and degree of enclosure of the ovaries and tubes by adhesions and any other evidence of bowel or urinary tract endometriosis. The benefit of this system is that it is a systematic objective standardised approach to the findings at surgery and the staging of the disease. Below is the scoring system as published in 1985[7].

Figure 1. Revised American Society of Reproductive Medicine staging of pelvic endometriosis at laparoscopy guidelines



Patient's Name _____ Date _____

Stage I (Minimal) - 1-5
Stage II (Mild) - 6-15
Stage III (Moderate) - 16-40
Stage IV (Severe) - >40
Total _____

Laparoscopy _____ Laparotomy _____ Photography _____
Recommended Treatment _____
Prognosis _____

PERITONEUM	ENDOMETRIOSIS	<1cm	1-3cm	>3cm
	Superficial	1	2	4
	Deep	2	4	6
OVARY	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20

POSTERIOR CULDESAC OBLITERATION	Partial	Complete
	4	40

OVARY	ADHESIONS	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
TUBE	R Filmy	1	2	4
	Dense	4*	8*	16
	L Filmy	1	2	4
	Dense	4*	8*	16

*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Additional Endometriosis: _____

Associated Pathology: _____

To Be Used with Normal Tubes and Ovaries

L R

To Be Used with Abnormal Tubes and/or Ovaries

L R

As can be seen from the above guidelines deeply invasive endometriosis (DIE), in isolation, can score a maximum of 6 points even if invading into bowel. Thus it is possible for DIE to be scored as mild disease when a bowel resection would be required to excise this disease. This type of operation would carry a significant operative risk. The deficiency of this system is a reflection of the fact that the scoring relates to how the disease is likely to affect fertility. Any updated scoring system for endometriosis should include the operative risk together with the impact on future fertility.

1.6 Alternative imaging modalities to ultrasound for the diagnosis of pelvic endometriosis.

Computerised Tomography (CT) gives reasonable quality images of the bowel but is poor at identifying other soft tissue structures and there is a considerable amount of radiation involved, especially when the pelvis is being assessed. For this reason magnetic resonance imaging (MRI) is now the preferred alternative to CT. When the patient is starved for 6 hours prior to examination and a smooth muscle relaxant is administered reasonable quality images of bowel can be obtained on MRI. In addition high quality images of the uterus, adnexa, vagina, bladder and upper urinary tract can be obtained. This enables visualisation of the whole pelvis in one examination. Although static images can not give any information regarding pelvic organ mobility or tenderness, because of the intense desmoplastic reaction and fibrosis that is often seen around areas of DIE, such as to the uterus and adnexa, these distortions in pelvic anatomy can be seen on MRI. Other signs of adhesions on MRI include triangular tenting and fluid collections. Retraction of the ovaries from the ovarian fossa towards the midline is usually seen in deep pelvic endometriosis[8].

On MRI, colonic endometriotic implants appear as areas of irregular wall thickening or low signal intensity plaques on the T2 images, and are sometimes associated with hemorrhagic foci that are hyperintense on fat suppressed T1 images[8] (T1 and T2 are settings on the MRI machine which give different levels of contrast and signal intensity between various tissue types). Other areas appear as hypo or hyper intense foci with morphological abnormalities such as stellate margins[9].

When MRI was a relatively new imaging modality the quality of the images and the knowledge of the morphological changes resulted in a relatively low accuracy for detecting extra-ovarian disease. In 1989 Arrive[10] used MRI to assess 30 women with clinically suspected endometriosis and compared this with the surgical findings. Three of the five normal cases and 16 of the 25 cases of endometriosis were correctly identified with MRI (sensitivity, 64%; specificity, 60%; accuracy, 63%). MRI demonstrated seven of eight endometriomas but only 14 of 29 adhesions and only six of 45 endometrial implants. They concluded that MR imaging could not be used as the primary modality in the detection, characterization, and staging of endometriosis. In the same year Zawin[11] found a slightly better sensitivity of 71% and specificity of 82% for the MRI detection of pelvic endometriosis but also concluded that it could not be used as a first line investigation.

Kinkel in 1999[12] assessed with MRI 30 women who had proven endometriosis to establish the morphological features of the various locations of DIE. They found that proximal nodularity was different for uterosacral ligaments with deep endometriosis but there was no difference in signal intensity between normal and abnormal

uterosacral ligaments. They also found that rectal endometriosis was missed in two of three patients and showed non-specific rectal wall thickening in one patient. It was concluded that MR imaging can diagnose deep endometriosis of uterosacral ligaments, the bladder and the pouch of Douglas, but lacks sensitivity in detecting rectal endometriosis without rectal distension.

Bazot[9] in 2004 compared MRI and endometriosis found at surgery and found that the sensitivity, specificity and accuracy of MR imaging for deep pelvic endometriosis were 90.3%, 91%, and 90.8% respectively. The sensitivity, specificity, and accuracy, respectively, of MR imaging for the diagnosis of endometriosis in specific sites were as follows: USL, 76%, 83.3% and 80.5%; vagina, 76%, 95.4%, and 93.3%; rectovaginal septum, 80%, 97.8%, and 96.9%; rectosigmoid, 88%, 97.8%, and 94.9%; and bladder, 88%, 98.9%, and 97.9%. They conclude that MRI demonstrates high accuracy in prediction of deep pelvic endometriosis in specific locations. However, this study was performed in a major Parisian teaching hospital with the images being read by a radiologist with a specialist interest in endometriosis diagnosis and the patient population was of women being referred for specialist endometriosis surgery with an ultrasound diagnosis of endometriosis already. For this reason the results may be superior to those seen in everyday practice.

There have been various studies related to differing modes of ultrasound compared with MRI for the assessment of the specific locations of DIE. Bazot[13] in 2007 compared the accuracy of MRI and rectal endoscopic sonography (RES) for the evaluation of DIE in 88 patients. Their results showed a sensitivity and specificity of MRI and RES, respectively, of 84.8% and 45.6%, 88.8% and 40%, for uterosacral

endometriosis; 77.7% and 7.4%, 70% and 100%, for vaginal endometriosis and 88.3 and 90%, 92.8 and 89.3 for colorectal endometriosis. Their conclusion was that MRI is more accurate than RES for the diagnosis of uterosacral and vaginal endometriosis, whereas the two methods are similarly accurate for colorectal endometriosis.

Bazot[14] in 2008 went on to compare physical examination, transvaginal sonography, RES and MRI for the diagnosis of DIE in 92 patients with clinically suspected endometriosis. They found that MRI performs similarly to TVS and RES for the diagnosis of intestinal endometriosis but has higher sensitivity and likelihood ratios for uterosacral ligament and vaginal endometriosis. They also found that clinical examination can detect deep endometriosis in 81.5% of cases but that the localisation of disease was poor with much greater accuracy on the other imaging modalities.

Grasso[15] in 2009 compared three dimensional transvaginal ultrasound with MRI and found that the sensitivity and specificity of MRI for the diagnosis of deep infiltrating endometriosis in specific sites were: USL 69.2% and 94.3%; vagina 83.3% and 88.8%; rectovaginal septum 76.4% and 100%; rectosigmoid 75% and 100%; bladder 83.3% and 100%. This compares values for 3D TVS of : USL 50% and 94.7%; vagina 84% and 80%; rectovaginal septum 76.9% and 100%; rectosigmoid 33.3% and 100%; bladder 25% and 100%. This shows that the 3D TVS has lower sensitivities than MRI but similarly high specificities. However this study was performed in only 33 patients of whom only 66% had DIE and therefore further investigation with larger studies are needed to validate these findings.

The main criticism of MRI is that it is less readily available and significantly more expensive than ultrasound examination. For this reason MRI is usually reserved as a second line imaging modality.

1.7 Ultrasound diagnosis of endometriosis

Transvaginal ultrasound has been reported to be a safe, non invasive investigation and has been shown to have a high degree of accuracy in the detection and exclusion of ovarian endometriomas (cysts on the ovary containing endometrium)[16]. However, when this thesis was conceived there were no prospective studies confirming the accuracy of transvaginal ultrasound for the diagnosis of non-ovarian endometriosis. Since then there have been a number of prospective studies detailing the accuracy of the diagnosis of deeply infiltrating disease. The transrectal and transvaginal ultrasound assessment of endometriosis will be considered in sections 1.8 and 1.9 respectively.

1.8 Transrectal ultrasound diagnosis of endometriosis.

In contrast there have been various studies assessing the use of endorectal ultrasound in the assessment of rectovaginal septum and uterosacral ligament endometriosis[17-20]. Ohba[20] in 1996 performed transrectal ultrasound examination on patients after a laparoscopic diagnosis of uterosacral ligament endometriosis and found that this showed thick and irregularly shaped uterosacral ligaments. Normal uterosacral ligaments appeared as low echoic homogeneous arcs on each side of the uterine cervix. They measured the diameter of the uterosacral ligaments and found that the patients who had endometriosis affecting the ligaments had diameters that were significantly thicker than the normal ligaments ($P<0.05$). They also found that the

patients with endometriosis were more likely to have tenderness on palpation of the uterosacral ligaments if the diameter was >14mm. This possibly suggests that more advanced infiltration gives rise to more symptoms. However, as the ligaments were not biopsied, it is not confirmed that the thickening was due to endometriosis infiltration. Also this study was attempting to show that there are differences that it is possible to detect on transrectal ultrasound. No attempt was made to assess the accuracy of the diagnosis by this method.

In a retrospective study by Chapron[18] in 1998 rectal endoscopic ultrasonography showed deep invasion of the bowel mucosa in all 16 patients who underwent laparotomy with bowel resection. The histological results confirmed in each of these 16 patients (100%) that there was deep infiltration of the intestinal wall by endometriotic lesions. This study can be criticised as being retrospective and therefore open to bias. However, the findings open the possibility of assessing preoperatively which patients could have their endometriosis treated without opening the bowel and which would need a full bowel resection.

In a study by Griffiths[17] in 2008 transrectal ultrasound for the diagnosis of deeply invasive endometriosis the positive likelihood ratio was 10.89 and the negative likelihood ratio was 0.24 suggesting a high degree of accuracy. However this study does not state whether it was pro or retrospective and it was not clear whether the patients had previously had a diagnosis of endometriosis made at laparoscopy. These findings suggest that transrectal ultrasonography may have a high degree of accuracy for the diagnosis of bowel endometriosis. However prospective observational studies are necessary to validate these findings.

Although these studies suggest a high degree of accuracy for transrectal ultrasonography for all these studies the patients had enemas as preparation. This is given to empty the bowel and induces diarrhoea, which is quite unpleasant for the patient. Some patients also find the procedure so unpleasant that sedation or even general anaesthetic is sometimes required. In addition a circumferential rectal probe was used which does not allow for accurate visualisation of the other pelvic organs such as the bladder, uterus and ovaries. In order to study the other pelvic organs an alternative imaging modality would have to be used such as tranvaginal ultrasound or MRI.

In 2003 Bazot[21] compared rectal endoscopic sonography (RES) with transvaginal ultrasound (TVS). They took 30 patients and preoperatively assessed them for signs of deeply infiltrating endometriosis. They found that the sensitivity and specificity of TVS and RES for the diagnosis of uterosacral ligament involvement was 75% and 75%, and 83% and 67% respectively. For the diagnosis of rectosigmoid endometriosis the results were 95% and 82%, and 100% and 88% respectively. They conclude that the results of TVS are similar to those possible with RES and therefore TVS should be used at first line assessment. They state that one of the advantages of RES is that the frequencies used are often higher than the TVS transducers and therefore give good detail analysis of the bowel mucosa but poor penetration of surrounding structures. This explains why it is difficult to assess the bladder or anterior structures of the pelvis. For this reason TVS gives a broader assessment of the pelvis. They state that this is the first study to assess the use of TVS for rectal lesions. TVS will be discussed in more detail in the next section

1.9 Studies assessing the role of transvaginal ultrasound assessment of extra-ovarian endometriosis.

As seen in section 1.8 non- ovarian endometriosis features such as uterosacral ligament involvement were first assessed on transrectal sonography (TRS)[20] in 1996. In 1998 another study by Chapron[18] suggested that rectal endoscopic sonography (RES) was useful for the diagnosis of intestinal endometriosis.

In 2002 Ballyguier[22] compared MRI and TVS for the diagnosis of DIE. They found that TVS was good at locating bladder lesions but was less good than MRI at detecting rectal and posterior lesions. However, this was a retrospective study and only included 12 patients, all with histologically proven endometriosis of the bladder. Their conclusions were likely to be biased because of these drawbacks.

In 2003, Bazot et al[21] demonstrated that TVS was as accurate as RES for the diagnosis of intestinal endometriosis and may provide useful information for assessing uterosacral ligament endometriosis. This study is discussed in relation to RES in section 1.7. However, it was the first study to assess TVS in the diagnosis of rectal endometriosis involvement. In 2004 the same group went on to prospectively assess the role of TVS in 142 women when compared with laparoscopy and histology. They found that the sensitivity and specificity of TVS for the diagnosis of DIE of the: bladder 71.4% and 100%; uterosacral ligaments (USL) was 70.6% and 95.9%; vagina was 29.4% and 100%; rectovaginal septum was 28.6 and 99.3%; and for interstinal disease 87.2% and 96.8% respectively. They concluded that the assessment of bladder and intestinal disease was accurate but the assessment of uterosacral, vaginal

and rectovaginal septum involvement was less accurate. They noted that retroflexion of the uterus, subserous fibroids and endometriotic ovarian cysts which were adherent to the uterosacral ligaments can obscure the ligaments and therefore make them difficult to visualise on TVS. They also note that in the assessment of rectal and intestinal disease TVS can not give a distance from the rectal lesion to the anal margin. TVS is also restricted in the ability to assess the sigmoid colon due to distance and the presence of faecal matter.

Bazot[23] repeated the comparison between TVS and RES in 2007 and found that the accuracy of TVS had improved and the sensitivity for the diagnosis of USL disease was 80.8%. TVS was now better than RES for all areas except intestinal disease. This improvement may have been due to variations in the populations between these studies or to improved technique with greater experience. However, the authors reaffirm that TVS should be the first line imaging modality of choice.

Abrao[24] in 2007 compared the use of bimanual examination, TVS and MRI for detection of DIE of the rectosigmoid and/or 'retrocervical sites' as defined by the author in 104 patients, demonstrating higher sensitivity, specificity, NPV and PPV for TVS in cases of rectal DIE when compared with MRI and clinical examination. However, it is not clear whether radical resection of diseased tissue, including rectal resection and/or dissection of POD obliteration by adhesions, was performed in all patients affected with DIE. This information is important for the full evaluation of the test.

Hudelist[25] in 2009 studied the additional benefit of TVS with clinical examination in 200 women. They found that the sensitivity of per vaginum (PV) examination was 23% for left ovary, 38% for right ovary, 52% for right USL, 74% for left USL, 25% for bladder, 46% for rectum, 64% for vagina, 70% for pouch of Douglas, and 88% for rectovaginal septum. The specificities were all above 98% except for left USL which was 89%. With the addition of TVS to the PV exam the sensitivities improved to over 80% except for bladder of which was 75% and right USL of 67%. The specificities were maintained at their high level. These patients were all examined by senior gynaecologists who all work in a tertiary endometriosis centre and therefore their PV examination skills would be at a very high level. Even in this context the accuracy was improved by the addition of ultrasound examination and therefore it is likely that the benefit of TVS would be greater for less experienced clinicians.

Hudelist[26] then went on to compare TVS and PV exam directly and found that TVS was much more accurate than PV exam especially for ovarian endometriomas and rectosigmoid disease. In 27 women with proven endometriomas, vaginal examination yielded a sensitivity of only 41%, in contrast to the finding obtained by TVS with a sensitivity of 96%. These differences were less obvious for the rectovaginal space or vaginal deep infiltrating endometriosis with lower sensitivities and NPVs for both modalities when compared with values for ovarian or rectosigmoidal endometriosis. The largest difference between TVS and PV exam was for the detection of rectosigmoidal endometriosis. In this study, 31 women (24.0%) had rectosigmoidal deep infiltrating endometriosis, of which 39% were preoperatively diagnosed by PV exam and 90% of them by TVS. The authors explain the obviously lower sensitivity and accuracy of vaginal examination by two factors. First, rectosigmoidal

endometriotic nodules situated at or above the level of the uterine fundus cannot be palpated digitally but can be visualized by TVS. Second, it is difficult to assign palpable nodules situated posterior to the cervix to specific anatomical structures such as the uterosacral ligaments, rectovaginal septum, vagina, rectum or sigmoid. This causes false-negative findings with regard to rectal or sigmoid deep infiltrating endometriosis. By contrast, TVS clearly facilitates the differential visualization and possible endometriotic involvement of these locations.

There are few studies on the accuracy of TVS for the diagnosis of ovarian adhesions either due to endometriosis or to other causes. No study has previously assessed mobility as either minimal, moderate or severe adhesions for the ovary in accordance with the ASRM classification[7]. Guerriero et al[27] used the combination of 3 features as suggestive of ovarian adhesions: blurring of the ovarian margin, the inability to mobilise the ovary on palpation (fixation) and an increased distance from the probe. They found that these tests either combined or individually gave a kappa value of between 0.25 and 0.51. Okaro[28] examined women with chronic pelvic pain prior to laparoscopy for the presence of ovarian adhesions and classified them as either mobile or fixed. They found a high degree of agreement between TVS and laparoscopy at identifying ovarian adhesions (0.81 kappa). Yazbek[29] examined the role of ultrasound for the preoperative assessment of adnexal masses. They found a sensitivity of 44% and a specificity of 98% in the diagnosis of severe pelvic adhesions. Guerriero[30] used a technique of applying pressure between the uterus and ovary. If they remained linked then this was suggestive of adhesions. This gave a sensitivity and specificity of 89% and 90% respectively for fixation of the ovaries to the uterus.

The preoperative diagnosis of partial or complete obliteration of the pouch of Douglas has not been reported on directly before. Hudelist[25] gave a high accuracy for the diagnosis of pouch of Douglas endometriosis but did not report obliteration separately. Yazbek[29] described the technique for diagnosing POD obliteration but did not report this finding separately from severe pelvic adhesions.

The assessment of adhesions is important preoperatively as surgery for severe adhesions carries a higher risk of complication and therefore needs to be performed by more experienced surgeons. The patients may also decide that their symptoms are not severe enough to warrant surgery. Therefore the accurate preoperative assessment will help in counselling patients regarding the best treatment option for them.

No studies have yet assessed the role of transvaginal ultrasound for the severity of endometriosis compared with the ASRM staging system. In addition, no studies have assessed the interobserver variability of transvaginal ultrasound for the diagnosis of the severity of pelvic endometriosis.

Summary table of the important imaging studies with comparison of methods (reproduced from Nightingale, Ballard and Wright 2012 Gynaecological Surgery with permission)

Author	Study period	Study population	Age	TVS	TRS	MRI	Blinding
Abrao	2004-2006	104 Consecutive women with clinically suspected endometriosis	Mean 33.8 years (SD 6.1)	HDI 5000 ultrasound scanner with 5-9MHz transducer within 3months before surgery. Rectal enema used	N/A	1.5T scanner with a Torso phase array coil. Contrast agent gadolinium 0.2mmol/kg. No bowel preparation used	TVS carried out blinded to clinical data. MRI radiologist blinded to clinical data and TVS results
Bazot	2000–2004	81 Consecutive women referred for surgical management of DIE	Median 31.9 years	Ultramark HDI 5000 or Siemens Elegra ultrasound machine. 5-9MHz transducer. No bowel preparation used	Olympus GF UM 20 Echo endoscope, 7.5 and 12MHz.		Sonographers informed of women's clinical history and symptoms but blinded to physical examination and previous imaging. Different physicians performed TVS and TRS
Bazot	2000–2005	Retrospective study of 92 consecutive women with clinically suspected pelvic endometriosis	Median 31.8 years	Ultramark HDI 5000 or Siements Elegra ultrasound machine, 5-9MHz transducer. No bowel preparation used	Olympus GF UM 20 Echo endoscope, 7.5 and 12MHz probe	1.5T scanner. Bowel preparation given. Contrast agent gadolinium	All examinations conducted by different physicians with knowledge of clinical history and symptoms but blind to results of physical exam and other imaging
Chamié	2005-2007	92 Women with a history and clinical examination consistent with endometriosis	Mean 33 years	N/A	N/A	GE Signa 1.5T scanner. Contrast agent gadolinium. No bowel preparation used	MRI images interpreted independently by 2 radiologists blinded to patient history
Chapron		Retrospective study of 81 consecutive patients with histologically proven DIE. MRI and transrectal ultrasound given prior to planned surgery	Mean 31.0 (SD6.7)	N/A	Olympus GF-UM20 scope ultrasound machine with 7.5 and 12MHz probes	1.5T Tesla Unit with a phased-array coil. No contrast aged used	Patients already had a diagnosis of DIE but the ultrasonographer and radiologist were blind to clinical information when they interpreted the results of the tests
Delpy	1998–2003	31 Women with suspected rectovaginal endometriosis based on clinical symptoms and/or abnormal clinical examination	Mean 31.5 years	N/A (for rectal infiltration)	7.5MHz radial-scanning miniprobe (Fujinon) fitted with a distal balloon	N/A	Blind to precise clinical findings but with the knowledge of suspected endometriosis. Surgery conducted with full knowledge of imaging results
Guerriero	2005-2007	88 Consecutive women with clinically suspected endometriosis	Mean 33 years (SD 5)	1Week before surgery using Technos MPX with 6.5-7.0MHz transducer. Paid special attention to tender areas. No rectal enema used.	N/A	N/A	Not reported
Hudelist	2007–2008	200 Women with clinically suspected	Median 33 years	Logic 9 or Accuvix XQ ultrasound machine 5-9MHz			PV examination performed first followed by TVS by

		endometriosis		transducer combined with bimanual PV examination within 2months of surgery. No rectal enema used			the same examiner
Menada	2006–2007	90 Women with clinically suspected rectovaginal endometriosis	Median 32 years	Siemens Sonoline Antares ultrasound machine. 3.6-8.0MHz multifrequency transducer			TVS carried out independently by 2 ultrasonographers with the knowledge of clinically suspected disease by blind to any other clinical information
Piketty	2005–2007	134 Women with clinically suspected DIE	Mean 32.1years (SD 5)	Toshiba ultrasound machine. 5-9MHz transducer. No rectal enema used	Olympus UM 160 following rectal enema. 5, 7.5 and 12MHz frequencies used	N/A	Examiners told DIE was suspected but were not given information on clinical findings or other imaging findings

Summary table of study results: imaging of rectal or recto-sigmoid involvement in deep infiltrating endometriosis (reproduced from Nightingale, Ballard and Wright 2012 Gynaecological Surgery with permission)

Study	Pre-test probability* (*n*)	Sensitivity% (95% CI)	Specificity% (95% CI)	LR+ (95% CI)	LR– (95% CI)	Post-test probability for study population: positive test (%)	Post-test probability for study population: negative test (%)
Transvaginal Ultrasound							
Abrao	52% (54/104)	98 (95,100)	100 (100,100)	∞	0.02 (0.00, 0.1)	>99	2
Menada TVS	83% (75/90)	56.5	92.5	7.57	0.47	97	60
Menada RWC-TVS**,***	83% (75/90)	95.7	100.0	∞	0.04	>99	15
Guerriero	44% (39/88)	67 (55,73)	92 (84, 100)	8.2 (3.1,21.4)	0.4 (0.2,0.6)	87	22
Bazot	67% (54/81)	93 (86,100)	100 (100,100)	∞	0.07 (0.03,0.2)	>99	12
Hudelist	24% (48/200)	96 (90,100)	98 (96, 100)	48.6 (15.8, 149.1)	0.04 (0.01,0.2)	94	1
Piketty	56% (75/134)	91 (84,97)	97 (92,100)	26.3 (6.7, 102.8)	0.1 (0.05, 0.2)	97	11
Bazot	68% (63/92)	94 (88,100)	100 (100,100)	∞	0.06 (0.02, 0.2)	>99	11
Trans-rectal ultrasound							
Chapron	42% (34/81)	97 (91,100)	89 (81,98)	9.1 (4.0,20.9)	0.03 (0.00, 0.2)	87	2
Delpy	40% (12/40)	92 (76,100)	67 (45,88)	2.8 (1.4,5.0)	0.1 (0.02,0.8)	65	8
Bazot	67% (54/81)	89 (81,97)	93 (83,100)	12.0 (3.2,45.7)	0.1 (0.06,0.3)	96	20
Piketty	56% (75/134)	96 (92,100)	100 (100,100)	∞	0.04 (0.01,0.1)	>99	5
Bazot	68% (63.92)	89 (81,97)	93 (84,100)	12.9 (3.4,49.2)	0.1 (0.06,0.2)	96	20
Magnetic Resonance Imaging (MRI)							
Chapron	42% (34/81)	76 (62,91)	98 (94,100)	35.9 (5.1,252.1)	0.2 (0.1,0.4)	98	15

Abrao	52% (54/104)	83 (73,93)	98 (94,100)	41.7 (6.0,291.1)	0.2 (0.09, 0.3)	98	16
Chamie	54% (50/92)	86 (76,96)	93 (85,100)	12.0 (4.0,36.0)	0.2 (0.08, 0.3)	93	15
Bazot	68% (63.92)	87 (79,96)	93 (84,100)	12.7 (3.3,48.4)	0,1 (0.07,0.3)	96	23

*Pre-test probability is the prevalence of deep infiltrating endometriosis involving the rectum or rectosigmoid colon.
**Figures published in the paper given, no data were available to construct a 2 × 2 table in order to calculate 95% confidence intervals
***RWC-TVS, water contrast in the rectum combined with TVS

1.10 Surgical treatment of endometriosis.

The success of surgery for pelvic endometriosis is highly dependent on the expertise and training of the operating surgeon[31]. In an attempt to optimise the treatment of women suffering from severe endometriosis, tertiary referral endometriosis centres have been established[32] (RCOG green top guideline). These centres provide comprehensive care to endometriosis patients including high quality surgical care. The capacity of tertiary centres, however, is limited and the critical issue in routine clinical practice is the ability to assess the severity of endometriosis in order to facilitate the triaging of women for treatment. In cases of mild and moderate endometriosis, treatment can be provided immediately, even when the operation is performed by non-expert laparoscopic surgeons. In cases of severe disease, however, treatment cannot be provided unless the surgeon has significant laparoscopic skills in the surgical excision of endometriosis[32-34]. This is especially important if the disease involves the rectovaginal septum as this can extend anteriorly into the vagina, posteriorly into the rectum and laterally to effect the ureters. Laparoscopy is associated with surgical and anaesthetic risks, and it is very costly compared to non-invasive diagnostic methods. A non-invasive method, which would enable a reliable diagnosis of severe endometriosis, could facilitate early referral to tertiary endometriosis centres or to local surgeons with special interest and skills in laparoscopic surgery for endometriosis. In chapter 8 we examine the ability of preoperative transvaginal ultrasound to assess the severity of pelvic endometriosis when compared with the findings at laparoscopy. The aim of the study was to establish whether pre-operative ultrasound examination is an accurate method to diagnose severe pelvic endometriosis.

Chapter 2: Pathophysiology of endometriosis.

2.1 Pathophysiology of endometriosis.

The natural history of endometriosis is not well understood and there are no non-invasive studies regarding the natural history of this disease. Risk factors for endometriosis include early menarche and late menopause. In contrast the use of the oral contraceptive pill, which suppresses menstruation, is protective. These findings suggest that the development of disease seems to be related to exposure to menstruation[35,36]. The exact aetiology is not well understood and several theories have been proposed.

2.2 Retrograde menstruation

This remains the foremost theory and was first proposed by Sampson in 1927[36]. He postulated that endometrial tissue is deposited in the peritoneal cavity via retrograde flow through the fallopian tubes during menstruation. These cells then adhere, invade and proliferate within the structures of the peritoneal cavity.

Animal studies have shown that adhesion occurs when endometrial tissue from baboons, mice and humans is transplanted into the peritoneal cavity[37]. Stromal endometrial cells have a high level of VEGF (vascular endothelial growth factor), which suggests an active vascular network is necessary for graft survival and implicates them in graft attachment. Glandular cells have a high proliferation rate in freshly-implanted endometriotic lesions, suggesting a more prominent role for these cell types in graft growth[38]. Stromal cells may contribute to adhesion via secretion of extracellular matrix components and the endocrine environment is likely to play a role

in this activity. Additionally, IL-6 and TNF-alpha have been show to increase endometrial cell adhesion to the peritoneum in mice[37].

The theory of transplantation via retrograde menstruation is supported by evidence that endometriosis is more common in adolescents with obstructive outflow reproductive tract anomalies[39]. It is also supported by the fact that dependent areas of the pelvis, where pooling of menstrual effluent occurs, are more likely to be affected[40]. However it is unclear whether retrograde menstuation occurs more frequently in women with endometriosis than in those without. The presence of retrograde menstruation has been described as being between 76 and 90% of women investigate with laparoscopy[38,41]. In one of these studies[41] the presence of retrograde menstruation was higher (97%) in women with endometriosis than in those without. However in the other study[38] no difference was found. The transplantation theory plausibly explains peritoneal endometriosis but does not adequately explain deeply-infiltrating, ovarian or distant site disease.

2.3 Epithelial transformation

Meyer proposed the metaplasia theory in 1919[42] which was further refined by Gruenwald[43] into the coelomic metaplasia theory. This suggests that cells in the lining of the coelomic cavity are able to differentiate into endometrial tissue under the influence of certain factors. Cases of endometriosis in which retrograde menstruation cannot occur, such as Rokitanshky-Kuster-Hauser syndrome support this theory[44-46]. Additionally it has been suggested that some types of deeply infiltrating disease are due to local metaplasia[47 48].

2.4 Embryonic cells

Distant site endometriosis cannot be explained by either of the above theories, and the theory of embryonic cell transformation has been proposed to explain this. Pluripotent embryonic stem cells may undergo differentiation into functioning endometrium at any site in the body. This may explain case reports of catamenial haemo- and pneumothorax[49], catamenial nasal haemorrhage, brain and other distant site deposits. The alternative theory for these sites is the lymphatic / blood borne metastasis theory.

2.5 Familial / genetic characteristics

There appears to be a genetic basis for the development of endometriosis. In a study by Kennedy[50] 230 women with surgically confirmed endometriosis in 100 families were identified. The families consisted of 19 mother-daughter pairs, 1 set of cousins and 56 sister pairs. There were 5 families with 3 affected sisters, 1 family with 5 affected sisters, and 18 families with ≥3 affected members in more than one generation. The study confirmed a familial tendency for endometriosis and supports the hypothesis that endometriosis has a genetic basis. However the pattern of inheritance is not Mendelian and is more likely to represent a polygenic and /or multifactorial etiology.

The 5–8% risk observed for first-degree relatives is more consistent with polygenic/multifactorial tendencies than with a single mutant gene (25 or 50%). Either one assumes that more than a single gene is involved or that multiple alleles exist at a single locus. One or more Mendelian forms could coexist but polygenic inheritance is obligatory if endometriosis is assumed to be a single entity. Additional support for polygenic inheritance is the increased severity in familial cases[51,52]. As

predicted, the greater the severity of a polygenic disorder, the greater the underlying genetic liability[53]. Thus, the higher the proportion of affected relatives, the greater the likelihood that the affected individual has severe endometriosis.

The number of genes necessary to explain observed heritability is surprisingly small. This is illustrated by the following reasoning: If three genes exist, each with two alleles, there are 27 different classes (3n). If three alleles exist per locus and two loci exist, 36 genotypes (6n) would be possible. As the number of genotypic classes increases, a histograph showing the distribution of genotypes (phenotypes) in the population will more closely approximate a normal distribution. Thus, continuous variation can be approximated in the population by only a few genes.

Various specific genes have been suggested as playing a role in the development of the disease including the 1031T/C polymorphism of the TNF-alpha gene[54] and various polymorphisms of the N-acetyl transferase 2 (NAT 2) gene[55].

Chapter 3. Biochemical markers for endometriosis

3.1 CA125

Many biochemical markers have been investigated for the evaluation of endometriosis. The most widely researched marker is Cancer Antigen 125 (CA125), which is known to be raised in moderate to severe endometriosis. This was first discovered by Bast[56] in 1981and is a mucin-type glycoprotein with a molecular weight of over 200,000 daltons[57]. It has two major binding domains A and B: these bind the monoclonal antibodies OC125 and M11 respectively[58]. Apart from endometriosis, there are many other benign and malignant diseases, which can raise CA125 including uterine leiomyomas[59,60], adenomyosis[61] and pelvic inflammatory disease[62] all of which can be associated with pelvic pain.

A meta-analysis[63] by Mol showed that CA125 is a better discriminator for stage 3 and 4 disease than stage 1 and 2 disease. In this study the test's performance in diagnosing all disease stages was limited: the estimated sensitivity was only 28% for a specificity of 90% (corresponding likelihood ratio of a raised level is 2.8). The test's performance for moderate–severe endometriosis was better: for a specificity of 89%, the sensitivity was 47% (corresponding likelihood ratio of a raised level is 4.3). However, most of the studies included in this meta-analysis used a CA125 cut off level of 35IU/ml.

An interesting study by Kafali[64] looked at 28 patients who were having laparoscopies for infertility. CA125 levels in the menstrual phase of the cycle were compared with CA125 levels in the rest of the cycle. They found that there was no difference in the

patients who did not have endometriosis but there was a large difference in patients who did have endometriosis. Using a cut-off point of an 83% increase in CA125 levels between the menstrual and the non-menstrual phase of the cycle gave a sensitivity of 93% and a specificity of 92% specificity. All of the endometriosis found was either stage 1 or 2.

Since then there have been studies to further investigate the role of CA125 in the diagnosis of deeply infiltrating endometriosis as separate from ovarian disease. In a cohort study by Patrelli[65] serum CA125 values were significantly elevated in patients with ovarian and mixed endometriosis lesions (median levels 48 U/mL), compared with those who had exclusively extraovarian foci (median levels 27 U/mL), however the location did not affect the subsequent fertility rate after two years of follow up.

3.2 VEGF, TNF-alpha and CRP

Xavier[66] showed that serum VEGF is raised in the late secretory phase of the cycle in patients with endometriosis. Serum TNF-alpha concentrations were also raised throughout the cycle in women with endometriosis compared with controls[66]. A positive correlation between CRP and VEGF was also found throughout the menstrual cycle except for the early secretory phase. However there was no statistical difference in CRP between women with endometriosis compared with controls. Fasciani[67] found that serum VEGF concentrations were significantly raised in patients with endometriomas and malignant ovarian disease when compared with other benign ovarian cysts. Conversely Gagne[68] found no increase in serum VEGF level in endometriosis patient when compared with controls.

3.3 IL-6

IL-6 is a regulator of inflammation and immunity, which may play a role in linking the endocrine and the immune systems. It also modulates secretion of other cytokines, promotes T-cell activation and B-cell differentiation and inhibits growth of various human cell lines[69]. IL-6 is produced by monocytes, macrophages, fibroblasts, endothelial cells, vascular smooth-muscle cells and endometrial epithelial stromal cells and by several endocrine glands, including the pituitary and the pancreas[70]. In one study, it was found that IL-6 was significantly elevated in the serum of endometriosis patients but not in their peritoneal fluid as compared with patients with unexplained infertility and tubal ligation/reanastomosis[70]

Darai[71] found that IL-6 was raised in the serum of patients with endometriosis when compare with other benign ovarian cysts but was reduced when compared with malignant ovarian tumours. Serum TNF-alpha levels were equally raised with endometriomas and malignant disease when compared with other benign tumours. Pellicer . found that IL- is raised in the serum and follicular fluid of patients undergoing IVF treatment compared with controls.

No studies have tested these five serum markers (CA125, CRP, TNF-alpha, VEGF and IL-6) together in patients undergoing laparoscopy for chronic pelvic pain to evaluate if they are helpful at distinguishing those with and without endometriosis. In addition no studies have attempted to integrate these markers together with history and ultrasound characteristics into a model for the diagnosis of endometriosis.

3.4 Combination of serum markers.

A study by Mihalyi[72] has shown that it is possible to exclude endometriosis on a

serum test. In this cohort study they tested for IL6 and 8, tumour necrosis factor-alpha, high-sensitivity C-reactive protein (hsCRP), and cancer antigens CA125 and CA-19-9. Using stepwise logistic regression, moderate–severe endometriosis was diagnosed with a sensitivity of 100% (specificity 84%) and minimal–mild endometriosis was detected with a sensitivity of 87% (specificity 71%) during the secretory phase. Using LSSVM analysis, minimal–mild endometriosis was diagnosed with a sensitivity of 94% (specificity 61%) during the secretory phase and with a sensitivity of 92% (specificity 63%) during the menstrual phase. Their aim was to provide a test that would have a high sensitivity and moderate specificity therefore enabling women without endometriosis to avoid having an invasive laparoscopy unnecessarily. However, before these tests can be used in a clinical setting they would have to be validated by larger prospective studies.

4.0 Chapter 4: Thesis hypotheses

- The hypothesis is that transvaginal ultrasound performed as part of a routine work up for patients with chronic pelvic pain is an accurate and reproducible diagnostic technique for the exclusion and staging of pelvic endometriosis. This should facilitate the correct referral to a general gynaecologist for assessment if mild to moderate endometriosis is expected and to a specialist tertiary referral centre if severe disease is expected.
- The inclusion of subjective features such as symptomatology and tenderness during the TVS examination can help improve the accuracy of the diagnosis of the presence and severity of endometriosis.
- Combining serum CA125 levels with TVS characteristics will provide better diagnostic accuracy, in terms of the presence and severity of endometriosis, than either test alone.

4.1 Thesis aims

The aim of this thesis is to assess:

- the sensitivity of ultrasound for the detection of endometriosis in a population of women with chronic pelvic pain when compared with laparoscopy.
- the inter-observer variability in the diagnosis of endometriosis between two ultrasound operators.
- the ability of transvaginal ultrasound performed by experienced operators to predict the severity of pelvic endometriosis.
- if it is possible to predict the presence and severity of pelvic endometriosis from symptomatology and tenderness at TVS.
- if the addition of symptomatology and tenderness at TVS can help in the diagnosis of pelvic endometriosis in patients who have false negative findings for pelvic endometriosis at transvaginal ultrasound examination, and in doing so establish which patients would benefit from laparoscopy in spite of a normal TVS result.
- if combining serum CA125 levels with TVS characteristics will provide better diagnostic accuracy, in terms of the presence and severity of endometriosis, than either test alone.

Chapter 5: General methods

The methods which follow are applicable to all studies with specific details for each study being explained in those chapters.

These were prospective, observational, multicentre studies, which were conducted at King's College Hospital and at University College Hospital in London. These are major teaching hospitals and the latter included a specialist tertiary endometriosis centre. Consecutive women with clinically suspected or proven pelvic endometriosis were invited to join the study. The inclusion criteria were: pre-menopausal women with a clinical suspicion of endometriosis awaiting diagnostic laparoscopy; women diagnosed with pelvic endometriosis at diagnostic laparoscopy awaiting operative treatment, age 16 or over, ability to provide informed consent. Women who could not undergo a transvaginal ultrasound scan and those who became pregnant whilst awaiting surgery were excluded from the study.

The studies were ethically approved and an information leaflet was given to all eligible women before assessment. Informed consent was obtained from all patients who agreed to take part in the studies.

Procedures

All women were assessed by the attending clinicians who obtained a detailed history, which was recorded on a dedicated clinical database (ViewPoint, GE Healthcare, Fairfield, Connecticut, USA). Women were specifically asked about symptoms

associated with endometriosis such as dysmenorrhoea, chronic pelvic pain, dyspareunia, subfertility, dyschezia and cyclic rectal bleeding.

Transvaginal ultrasound examination was performed by four ultrasound operators who were all gynaecologists with a high level of expertise in gynaecological ultrasonography. The ultrasound operators were blinded to any previous surgical findings. All patients were operated on by four different laparoscopic surgeons with a high level of expertise in laparoscopic surgery. When moderate, severe or deeply invasive disease (DIE) was present a complete surgical exploration of the pelvis was performed, involving dissection of the pouch of Douglas when obliterated and resection of any DIE, especially of bowel or RVS, so as not to miss any disease. The operating surgeons were blinded to the detailed transvaginal ultrasound findings.

Transvaginal ultrasound assessment of pelvic endometriosis

All women were examined in the dorsal lithotomy position using a high resolution transvaginal ultrasound probe. The examinations were performed in a standardised and systematic way. Firstly the uterus was assessed in the transverse and sagittal planes. Next the ovaries were found and their size was measured in three orthogonal planes.

Ovarian cysts were diagnosed as endometriomas when they appeared as well circumscribed thick walled cysts which contained homogenous low level internal echos ("ground glass")[73] (Figure 2). Measurements were recorded from the inside of the cyst wall in three orthogonal planes. The adnexa were also systematically examined for the presence of tubal dilatation.

Ovarian mobility was assessed by a combination of gentle pressure with the vaginal probe and abdominal pressure with the examiner's free hand as in a bimanual examination. The ovary was deemed to be completely free when all of its borders could be seen sliding across the surrounding structures. Minimal adhesions were considered to be present when some of the surrounding structures could not be separated from the ovary with gentle pressure but the ovary could be mobilised from the majority (approximately >2/3) of the surrounding structures. Moderate adhesions were thought to be present when the ovarian mobility was reduced due to adhesions with the surrounding structures but the structures on 2/3-1/3 of the surface of the ovary were sliding across it on gentle pressure. Fixed ovaries could not be mobilised at all with gentle pressure nor separated from the surrounding structures (Figures 3 and 4). If the tubes were dilated the mobility of the dilated tubes was documented in a similar fashion. Normal fallopian tubes are difficult to identify in the absence of background fluid in the pelvis and therefore it was not possible to score non dilated tubes for adhesions. It is difficult to see filmy adhesions on TVS unless there is fluid entrapped within the adhesions, giving rise to the "flapping sail sign"[74], or unless the mobility of the affected organs is reduced and therefore these features were not scored separately at TVS.

The presence of adhesions in the pouch of Douglas was assessed next. The uterus was gently mobilised by a combination of pressure on the cervix with the ultrasound probe alternating with pressure on the fundus from the examiners free hand on the abdominal wall. The aim was to watch the interface of the posterior uterine serosa and the bowel behind to ensure that the two structures were sliding easily across one

another. If these two surfaces were completely free of one another this was assessed as no adhesions present. Complete obliteration was assessed as the absence of any sliding between the serosa on the posterior surface of the cervix or uterus and the bowel behind. Partial obliteration of the pouch of Douglas was present if there were some adhesions between the bowel and the uterus but some free sliding was seen. Partial obliteration was also present when adnexal structures were firmly adherent to the posterior aspect of the uterus but the bowel appeared to be free.

Endometriotic nodules or deeply invasive endometriosis (DIE) were typically visualised as stellate hypoechoic or isoechogenic solid masses with irregular outer margins[75] which were tender on palpation and fixed to the surrounding pelvic structures. They were usually located in the uterosacral ligaments, adnexa, rectovaginal septum, and urinary bladder (Figure 7). Endometriotic nodules located in the wall of the rectosigmoid colon tend to appear as hypoechoic thickenings of bowel muscularis propria (Figures 5 and 6), which sometimes protrude into the lumen of the bowel[76]. The presence and largest diameter of any deep lesions were documented.

All these findings were recorded on a database file using a Microsoft Excel for Windows spreadsheet to facilitate data entry and retrieval. The severity of endometriosis as assessed by TVS was compared with laparoscopic findings using the same rAFS classification.

Statistical Analysis

All statistical analyses were carried out using Medcalc version 9.2.0.2 (Medcalc Software, Mariakerke, Belgium). The diagnostic accuracy of the tests was assessed using sensitivity, specificity, positive (PPV) and negative (NPV) predictive value, and positive (LR+) and negative (LR−) likelihood ratio measures. Overall levels of agreement for non binary data was calculated using Cohen's quadratic weighted Kappa coefficient. Kappa values of 0.81-1.0 indicated very good agreement, Kappa values of 0.61-0.80 good agreement, Kappa values of 0.41-0.60 moderate agreement, Kappa values of 0.21-0.40 fair agreement and Kappa values <0.20 poor agreement[77-79]. The Kruskal-Wallis one-way analysis of variance was used to assess for statistical difference between rank sum of the groups as the data was not normally distributed.

Figure 2. Transvaginal ultrasound image of an ovarian endometrioma with the typical ground glass appearance.

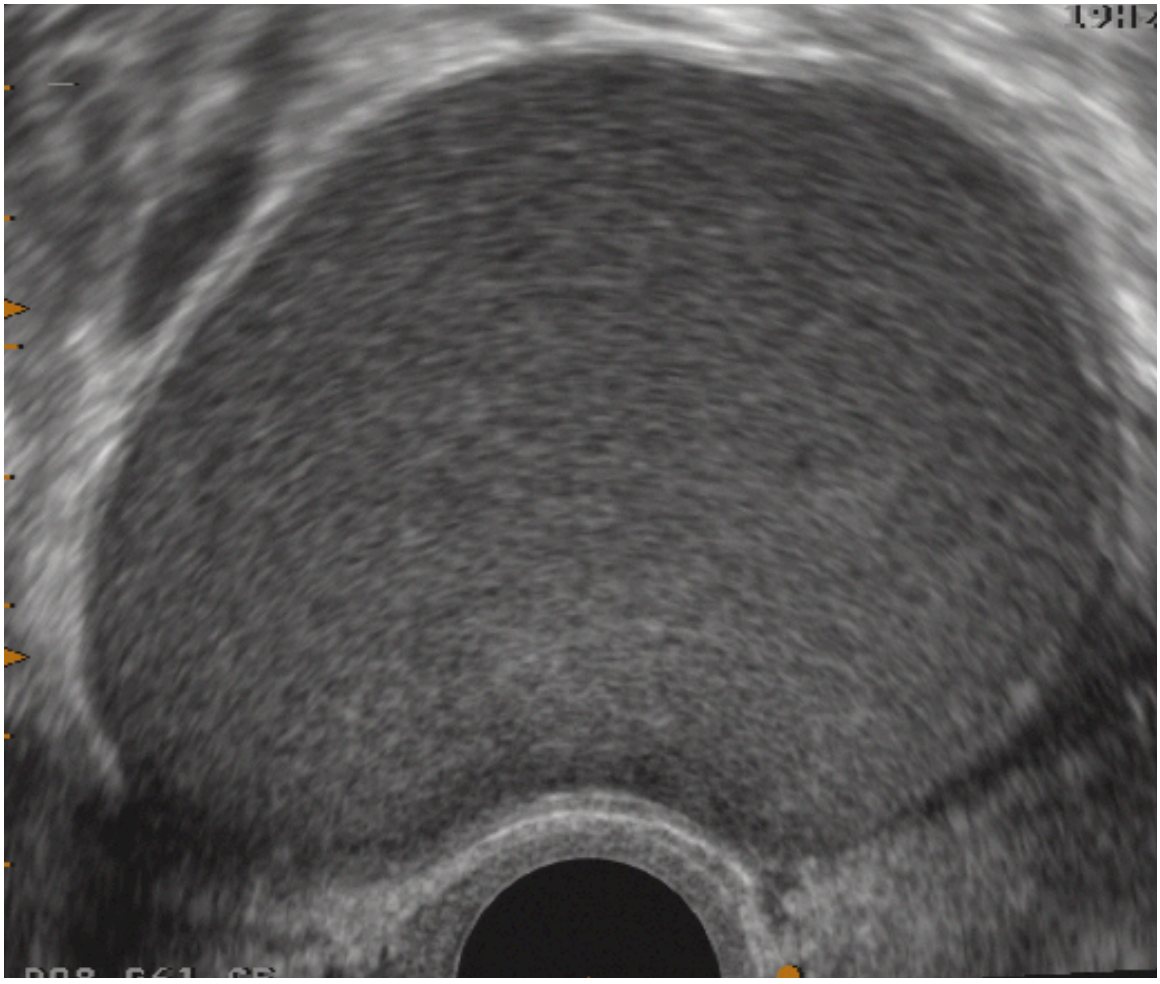


Figure 3. Transvaginal ultrasound image of an ovarian endometrioma which is firmly adherent to the fundus of the uterus

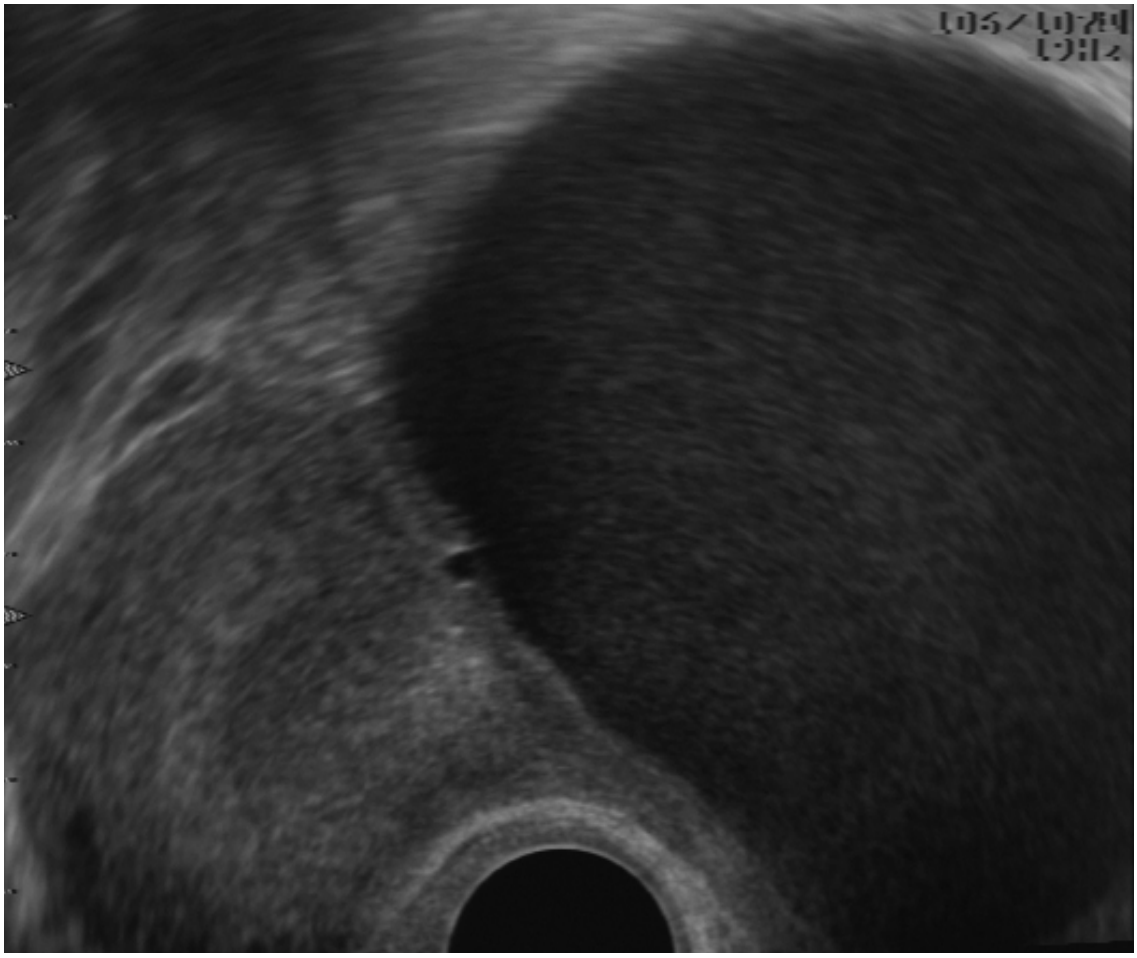
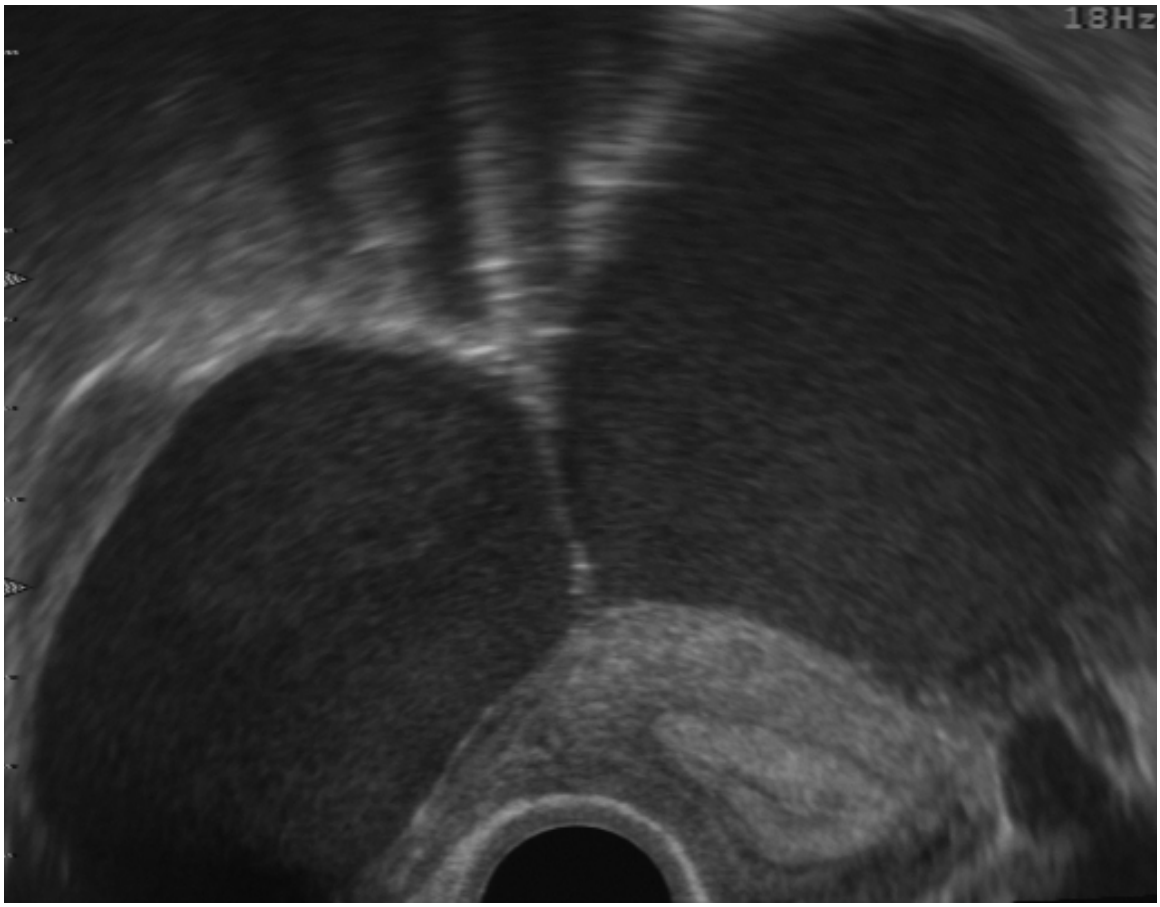


Figure 4. Transvaginal ultrasound image of ovarian endometriomas within each ovary causing adhesions between the ovaries and the posterior aspect of the uterus: "The kissing ovaries" sign.



Figures 5 and 6. Two transvaginal ultrasound images of hypoechoic thickening of the muscularis layer of the rectum due to deeply infiltrating endometriosis.

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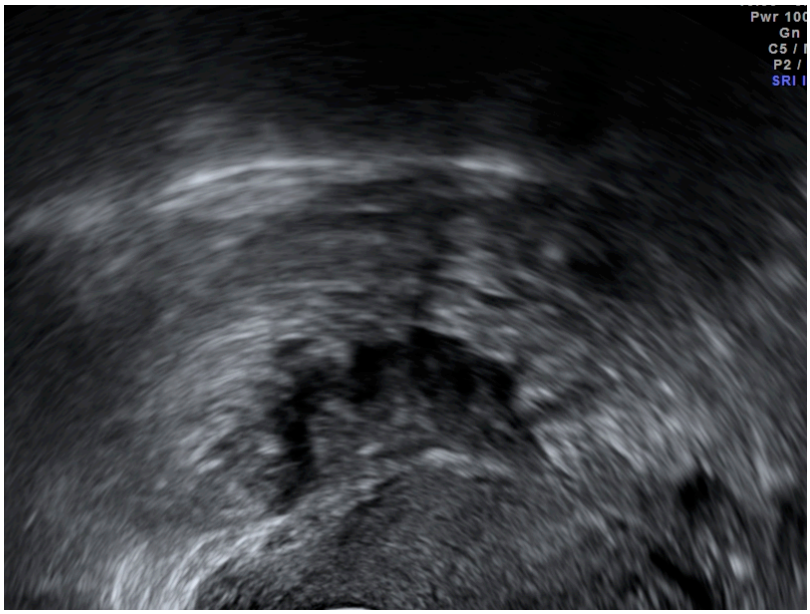


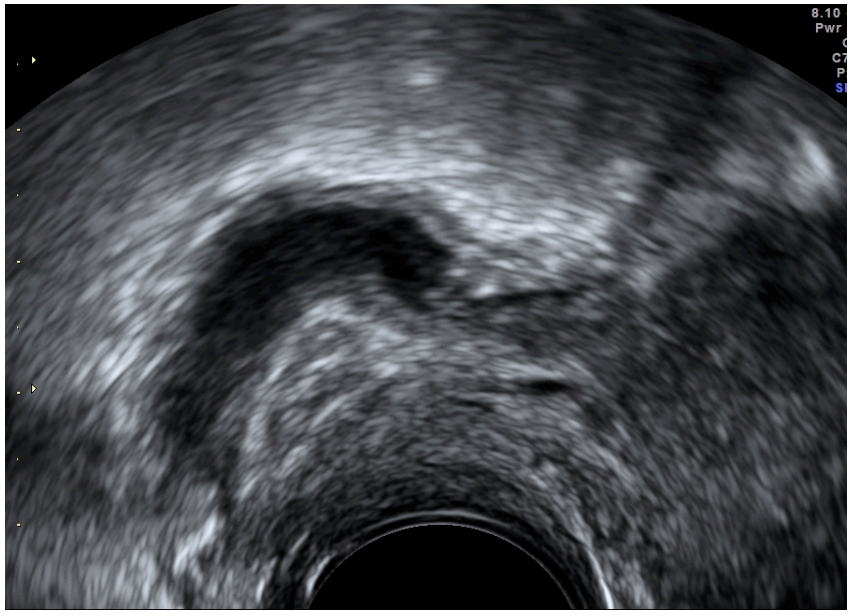
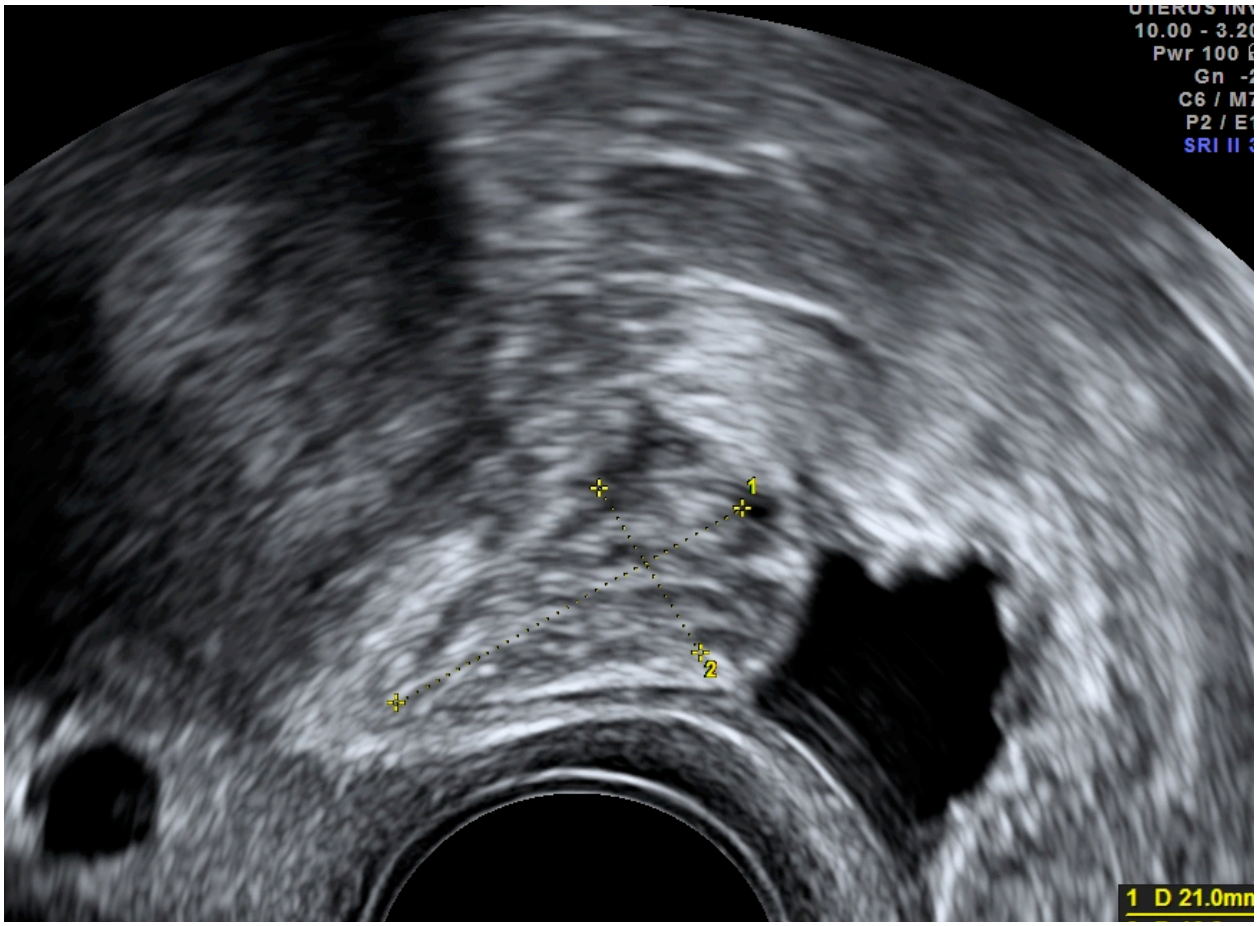


Figure 7. Transvaginal ultrasound image of deeply infiltrating endometriosis of the bladder.



Chapter 6: Study 1

The reproducibility of the assessment of severity of pelvic endometriosis by transvaginal ultrasound.

Introduction

As stated in the introduction (chapter 1.5) there is no clear consensus regarding the definition of severe endometriosis[80,81] and the most commonly used classification, the American Society of Reproductive Medicine (ASRM) classification[7] , has advantages and disadvantages. The advantage of this classification is that it is widely used in clinical practice and provides a formalized systematic approach to documenting the impact of the disease on the patient's fertility. However many authors recognize that the features of deeply infiltrating endometriosis (DIE) are often the most symptomatic[2,82] and difficult to treat[83]. These features are poorly represented in the ASRM classification and therefore need to be documented separately.

In keeping with the recommendations of Khan[84] any diagnostic test has to have the reliability (the test has to give a similar measurement when repeated by different observers) tested prior to the validity (the measurement must be accurate when compared with the "true" state of the attribute estimated by a suitable reference standard. Therefore in this study we tested the inter-observer reproducibility of the diagnosis of both the individual features of endometriosis and the overall severity. No previous study has tested this before.

The aim of the study was to establish whether TVS is a reproducible technique for the preoperative assessment of the severity of pelvic endometriosis.

Materials and Methods

This was a prospective, observational, multicentre study, which was conducted at King's College Hospital and at University College Hospital in London. These are major teaching hospitals and the latter included a specialist tertiary endometriosis centre. Women with clinically suspected or proven pelvic endometriosis were invited to join the study. The inclusion criteria were: pre-menopausal women with a clinical suspicion of endometriosis awaiting diagnostic laparoscopy; women diagnosed with pelvic endometriosis at diagnostic laparoscopy awaiting operative treatment; age 16 or over; ability to provide informed consent. Women who could not undergo a transvaginal ultrasound scan and those who became pregnant whilst awaiting surgery were excluded from the study.

The study was ethically approved and an information leaflet was given to all eligible women before assessment. Informed consent was obtained from all patients who agreed to take part in the study.

Methods.

All women were assessed separately by both attending clinicians who obtained a detailed history, which was recorded on a dedicated clinical database (ViewPoint, GE Healthcare, Fairfield, Connecticut, USA). Women were specifically asked about symptoms associated with endometriosis such as dysmenorrhoea, chronic pelvic pain, dyspareunia, subfertility, dyschezia and cyclic rectal bleeding.

Transvaginal ultrasound examination was performed by two ultrasound observers who were both gynecologists with a high level of expertise in gynecological ultrasonography. All patients were examined by both ultrasound observers and the observers were blinded to each other's findings. The ultrasound observers were also blinded to any previous surgical findings. All patients were operated on by two different laparoscopic surgeons with a high level of expertise. When moderate, severe or deeply invasive disease (DIE) was present a complete surgical exploration of the pelvis was performed, involving dissection of the pouch of Douglas (when obliterated) and resection of any DIE, especially of bowel or RVS, so as not to miss any disease. The operating surgeons were blinded to the detailed transvaginal ultrasound findings.

Transvaginal ultrasound assessment of pelvic endometriosis was performed as described in the general method section (chapter 5).

Statistical Analysis

All statistical analyses were carried out using Medcalc version 9.2.0.2 (Medcalc Software, Mariakerke, Belgium). In order to determine any systematic bias between the two diagnostic methods and to assess the relationship between any differences and the magnitude of the scores, the differences in score were plotted against the mean of the two scores on a scatter diagram. Systematic bias between the two observers was determined by calculating the 95% confidence interval of the mean (mean ± 2 standard errors) as described by Bland and Altman[77]. If 0 lay between this interval then no bias was assumed to exist. Cohen's quadratic weighted Kappa coefficient was calculated to determine agreement between the two observers in classifying the stage

of the disease and also the presence of the individual features of the disease. Kappa values of 0.81-1.0 indicated very good agreement, Kappa values of 0.61-0.80 good agreement, Kappa values of 0.41-0.60 moderate agreement, Kappa values of 0.21-0.40 fair agreement and Kappa values <0.20 poor agreement.

Results

In the three year period from August 2006 to July 2009 thirty four patients were recruited to the study and all had TVS examination performed by both ultrasound observers (A and B). Of these patients one cancelled her laparoscopy and was therefore excluded from the final analysis. The mean age of the patients at recruitment was 33.8 years (range 18-51 years). The presenting symptoms were: dysmenorrhoea (29/33, 87.9%), dysparunia (20/33, 60.6%), chronic pelvic pain (19/33, 57.5%), infertility (11/33) 33.3%), Dyschezia (11/33, 33.3%), cyclic rectal bleeding (1/33, 3%). Of these patients 12 (36.4%) had no endometriosis found, 1(3%) had minimal disease, 1 (3%) had mild disease, 5 (15.2%) had moderate disease and 14 (42.4%) had severe disease. The mean interval between scan and laparoscopy was 35.2 days (95% CI 32.0–38.6; SD, 21.1).

There was a good overall level of agreement between the ultrasound observers at identifying absent, minimal, mild, moderate and severe disease (quadratic weighted kappa = 0.931, standard error (Kw_ = 0) = 0.172, standard error (Kw_#0) = 0.034). The agreement between the TVS and laparoscopy findings for stage were also very good (kappa = 0.955, standard error (Kw=0) =0.174, (Kw_#0)=0.021 for observer A and Kappa = 0.966, standard error (Kw=0) =0.174, (Kw#0)=0.019 for observer B). The correlation coefficient for the ASRM scores was 0.987 (95 % CI 0.973-0.993) for

observers A and B, 0.963 (95 % CI 0.926-0.982) for observer A and laparoscopy and 0.966 (95 % CI 0.932-0.983) for observer B and laparoscopy. When the ASRM score was compared using a scatter diagram of the differences in score between the two observers plotted against the mean score, (Figure 8) (after Bland and Altman) this showed the limits of agreement were -16.1 and 12.4. The mean difference was -1.82 (95% confidence interval for the mean = -4.35 to 0.71). As the confidence interval for the mean difference contains zero no bias was assumed to exist. Visual inspection of scatter also revealed that the magnitude of differences between the scores recorded by each observer did not change with increasing score magnitude (Figures 9 and 10).

Table 1 shows the inter-rater agreement between observers A and B, and between each of the observers and laparoscopy, for assessing the individual features of severe endometriosis.

Figure 8. Bland Altman plot of difference between ASRM scores for ultrasound observers A and B plotted against the average score for both observers.

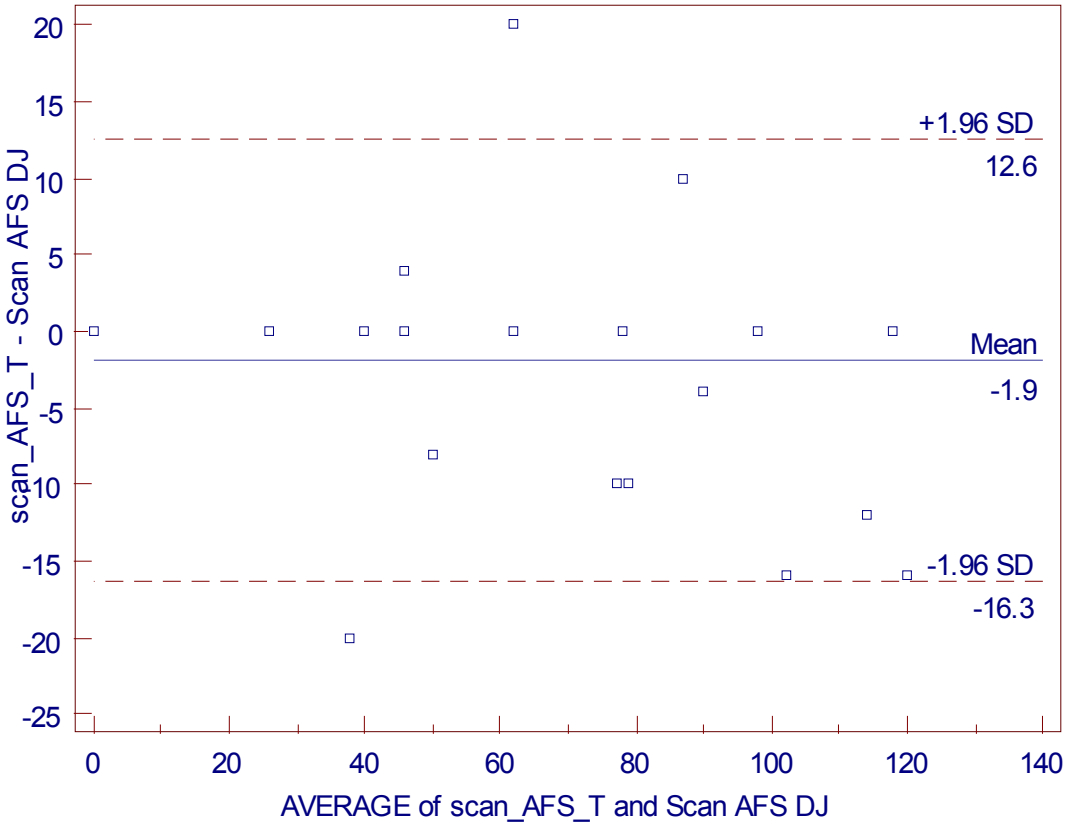


Figure 9. Bland Altman plot of difference between ASRM scores for ultrasound observer A and score at laparoscopy plotted against the average score for both.

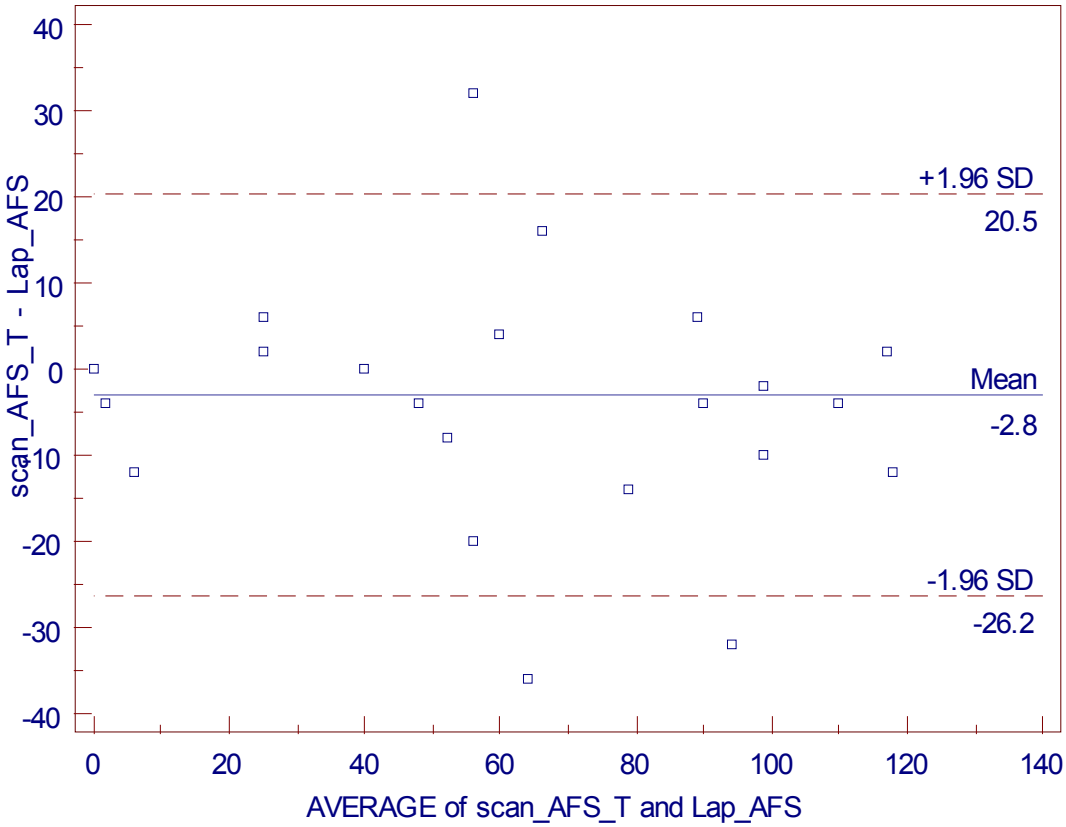


Figure 10. Bland Altman plot of difference between ASRM scores for ultrasound observer B and score at laparoscopy plotted against the average score for both.

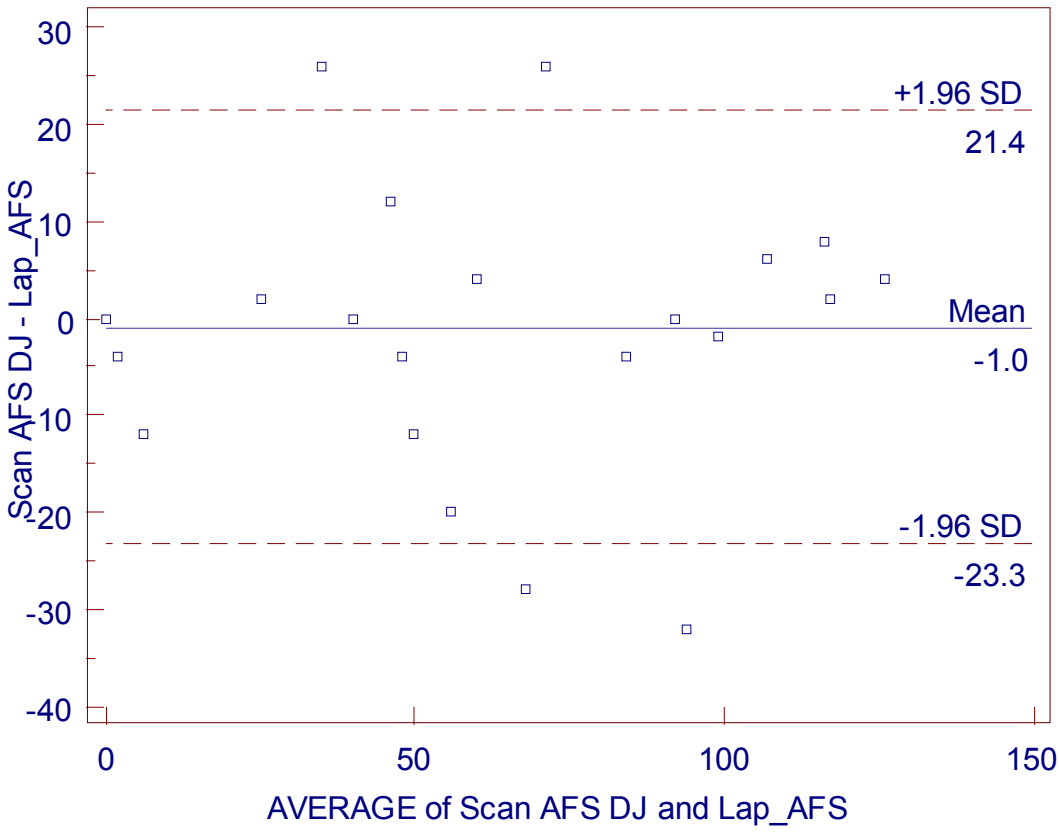


Table 1 Inter-rater agreement between observers A and B, and between each of the observers and laparoscopy, for assessing the individual features of severe endometriosis.

Feature	Prevalence n and %	Inter-rater agreement between observers A and B Kappa (Standard Error Kw'=0, and Kw'#0)	Inter-rater agreement between observer A and Laparoscopy (Standard Error Kw'=0, and Kw'#0)	Inter-rater agreement between observer B and Laparoscopy (Standard Error Kw'=0, and Kw'#0)
Bladder DIE	1/33 (3.0%)	1 (0.171, 0.000)	1 (0.171, 0.000)	1 (0.171, 0.000)
POD obliteration (partial or complete)	19/33 (57.6%)	0.947 (0.171, 0.031)	0.963 (0.174, 0.026)	0.982 (0.174, 0.018)
Ovarian adhesions overall	34/66 (51.5%)	0.927 (0.123, 0.039)	0.751 (0.123, 0.073)	0.837 (0.122, 0.056)
Bowel DIE	12/33 (36.4%)	0.555 (0.164, 0.155)	0.644 (0.160, 0.137)	0.934 (0.171, 0.654)
RVS DIE	11/33 (33.3%)	0.530 (0.165, 0.180)	0.530 (0.151, 0.154)	0.783 (0.167, 0.177)
USL DIE	9/66 (13.6%)	0.645 (0.115, 0.187)	0.463 (0.104, 0.176)	0.327 (0.120, 0.171)
PSW DIE	1/66 (1.5%)	0.548 (0.122, 0.232)	-0.023 (0.106, 0.018)	0.385 (0.097, 0.274)

Legend for table 1. DIE = deeply infiltrating endometriosis, POD = pouch of Douglas, RVS = rectovaginal septum, USL = uterosacral ligament, PSW = pelvic side wall.

Discussion

Assessment of the reproducibility of a diagnostic test is an essential part of evaluating its accuracy. This is the first study to prospectively examine inter-observer variability of TVS for the assessment of pelvic endometriosis.

Overall TVS performed well at assessing the stage of disease with very good levels of agreement between observers A and B, and between each of these observers and the stage found at laparoscopy. There were also very high correlation coefficients for the ASRM score between observers A and B, and between these observers' scores and the scores given to the findings at laparoscopy.

When the detection rates of individual features of endometriosis were compared between the observers, there were very good levels of agreement regarding diagnosis of endometriosis of the bladder, ovarian adhesions and pouch of Douglas obliteration. The high level of agreement for the diagnosis of bladder endometriosis is concordant with previous studies, which showed a high level of accuracy in the TVS diagnosis of bladder endometriosis[85,86]. There was also a good level of agreement for the diagnosis of ovarian adhesions. The preoperative diagnosis of ovarian adhesions has not been extensively investigated; however, Okara[28] found a high level of accuracy with kappa = 0.80. Prior to this in a study by Guerriero[27] in 1997 a moderate level of accuracy was found (Kappa = 0.50). The preoperative diagnosis of partial or complete obliteration of the pouch of Douglas has not been reported on directly before. Hudelist[26] gave a high accuracy for the diagnosis of pouch of Douglas endometriosis but did not report obliteration separately.

There were moderate levels of agreement between observers A and B regarding endometriosis of the bowel and rectovaginal septum. There were high levels of agreement between observer B and laparoscopy for both these features. However, observer A did not perform as well for these features and this is likely to be the cause of reduced level of agreement between A and B. Previous studies have also shown lower sensitivity for the diagnosis of endometriosis in these area[26,86].

There were fair to poor levels of agreement for endometriosis affecting the uterosacral ligaments and pelvic side walls. This could be explained by the low prevalence of endometriosis in these areas in the study population. Low accuracy of TVS for diagnosing endometriosis of the uterosacral ligaments and pelvic side walls has also been previously reported[83,86] . The diagnosis of endometriosis in these locations is not critical for the management as surgical excision can usually be achieved without involvement of other surgical specialists.

Although there are no studies assessing the reproducibility of TVS in the diagnosis of endometriosis, previous studies have examined the inter-observer variability of laparoscopy for the diagnosis of endometriosis. Interestingly, although laparoscopy is currently the gold standard test, previous studies have shown significant intra- and inter-observer variability.

Hornstein[87] asked 5 specialists to view video recordings of laparoscopies of patients with endometriosis and score according to the ASRM classification. Each video was viewed twice in order to assess intra- observer variability. The greatest variability occurred in endometriosis of the ovary and cul-de-sac obliteration, with less

variability observed for peritoneum endometriosis and for ovarian and tubal adhesions. Buchweitz[88] in 2005 asked one hundred and eight gynecologic surgeons to view three videos of laparoscopies. The patients had stage one disease, stage two disease and no disease. The surgeons were asked to indicate the endometriotic lesions on a prepared surgical sketch and to classify the site according to the ASRM classification. They found a correct classification of the endometriotic disease stages I and II in only 22% and 13% of the cases, respectively and concluded that the visual assessment of a videoed laparoscopy with minimal and mild endometriosis is subject to considerable intra and inter-observer variability.

Weijenborg[89] in 2007 asked two observers to review 90 videos of laparoscopies and found a high level of agreement in the stage of the disease but fair to moderate level of agreement of regarding the presence or absence of adhesions in the intra- and inter-observer setting, respectively.

In our study, we were not able to assess intra-observer reproducibility as all the patients were examined real time and it was not felt to be appropriate to subject patients to additional repeated examinations solely for the purpose of this study. Further research in to the intra-observer variability of TVS in endometriosis diagnosis would be valuable.

We have included data regarding agreement between observers A and B and laparoscopy findings for stage, ARSM score and individual features in order to explain some of the variation in inter-observer agreement. The data shows high levels of agreement for A and B with respect to laparoscopy findings for the stage of disease

and the ASRM score. However, when there was poorer diagnostic accuracy for a specific feature of disease, the inter-observer agreement also worsened.

Conclusion

Although laparoscopy remains the gold standard for the diagnosis of endometriosis, our results confirm that TVS is a suitable and reproducible method for the initial assessment of patients with suspected endometriosis and it may be used to appropriately triage patients with severe disease to local specialists or tertiary endometriosis centres.

Chapter 7. Study 2

Ultrasound mapping of pelvic endometriosis: does the location and number of lesions affect the diagnostic accuracy?

Aim

The aim of this study is to assess the accuracy of pre-operative transvaginal ultrasound scanning (TVS) in identifying the specific features of pelvic endometriosis and pelvic adhesions in a population of women with chronic pelvic pain when compared with laparoscopy.

Methods

The methods for this study were the same as detailed in the general methods section above.

Statistical Analysis

All statistical analyses were carried out using Medcalc version 9.2.0.2 (Medcalc Software, Mariakerke, Belgium). The diagnostic accuracy of the tests was assessed using sensitivity, specificity, positive (PPV) and negative (NPV) predictive value, and positive (LR+) and negative (LR−) likelihood ratio measures. Area under the receiver operator curves were calculated for the overall accuracy.

Results.

From July 2006 to September 2009 we recruited 237 women into this study. 39 women were excluded from the final analysis: twenty nine because they were not assessed by one of the two designated ultrasound operators, five became pregnant whilst awaiting surgery, one cancelled her operation, one laparoscopy was unsuccessful and three women were lost to follow up.

198 women were included in the final analysis. The mean age was 35.0 (95% CI 33.98 – 35.97, SD 7.10) (range 19-50) years. The presenting symptoms were dysmenorrhoea for 143/198 (72.2%), chronic pelvic pain for 98/198 (49.5%), dyspareunia for 91/198 (45.9%), infertility for 42/198 (21.2%), dyschezia for 19/198 (9.6%) and cyclic rectal bleeding for 3/198 (1.5%) women. A single presenting symptom was present in 72/198 (36.4%) women, two presenting symptoms in 66/198 (33.3%), three presenting symptoms in 39/198 (19.7%), four or more symptoms in 19/198 (9.6%) women.

At laparoscopy 126 /198 (63.6%) women had endometriosis. Of these women 30 /126 (23.8%) had stage 1 endometriosis by the rASRM classification, 24/126 (19.0%) had stage 2, 21/126 (16.7%) had stage 3 and 51/126 (40%) had stage 4 disease. Of the 104 women with focal lesions (excluding women with only diffuse superficial peritoneal disease) 28/104 (26.9%) women had endometriosis in a single location whilst the remaining 73.1% had endometriosis in two or more locations.

The ultrasound examinations were performed by two examiners: examiner A performed 104 (52.5%), examiner B 94 (47.5%). All patients were operated on by

one of four laparoscopic surgeons: surgeon A operated on 79 (39.9%), surgeon B on 54 (27.3%), surgeon C on 35 (17.7%) and surgeon D on 30 (15.2%) women. The mean interval between TVS and operation was 36.8 days (95% CI 33.4 – 41.1, SD 22.9) (range 0-87 days).

Table 2 shows the prevalence of the individual features of pelvic endometriosis at laparoscopy. Table 3 gives the details of the individual locations of endometriosis in relation to whether they were isolated lesions or multifocal lesions. Of the 104 women with focal lesions (excluding women with only diffuse superficial peritoneal disease) 28/104 (26.9%) of these women had endometriosis in a single location whilst the remaining 73.1% had endometriosis in two or more locations.

Ovarian endometriomas were rarely isolated lesions as ovarian adhesions were also present in 48/51 (94%) of cases. 27/51 (52.9%) women with endometriomas had unilateral and 24/51 (47.1%) had bilateral lesions. There was no significant difference in the frequency of endometriomas located in the right or left ovary (Chi-square =0.327 p=0.51). Women with bilateral endometriomas were no more likely to have associated DIE 16/24 (66.6%) compared to women with unilateral endometriomas 14/27 (51.8%) (Chi-square =0.621 p=0.431 stat).

Table 2. The prevalence of endometriotic lesions at different anatomical locations at laparoscopy

Site of disease	N (%)
Endometrioma on either ovary Unilateral Bilateral	51/198 (25.7%) 27/198 (13.6%) 24/198 (12.1%)
Moderate/ severe adhesions on either ovary Unilateral Bilateral	78/198 (39.4%) 30/198 (15.2%) 48/198 (24.2%)
DIE of USL unilateral DIE of USL bilateral	8/198 (4.0%) 12/198 (6.1%)
Complete obilteration of POD Partial obilteration of POD	30/198 (15.2%) 24/198 (12.1%)
DIE of Rectum / Sigmoid	11/198 (5.6 %)
DIE of RVS	32/198 (16.2%)
DIE of bladder	5/198 (2.5%)
DIE of utero vesical fold (separate from bladder)	6/198 (3.0%)
DIE of PSW unilateral DIE of PSW bilateral	7/198 (3.5%) 3/198 (1.5%)

DIE is deeply infiltrating endometriosis, USL is uterosacral ligaments, POD is pouch of Douglas, RVS is rectovaginal septum, PSW is pelvic side wall.

Table 3 Isolated and multiple endometriotic lesions in respect to their locations.

Site of disease	Endometriosis of a single location N (%)	Endometriosis multiple locations N(%)
Ovarian endometrioma n=51	2/51 (3.9%)	49/51 (96.1%)
Ovarian adhesions n= 85	16/85 (18.8%)	69/85 (81.2%)
Adhesions in POD n=54	1/54 (1.9%)	53/54 (98.1%)
USL DIE n= 23	5/23 (21.7%)	18/23 (88.3%)
RV or POD DIE n= 32	1/32 (3.1%)	31/32 (96.9%)
DIE of rectum or sigmoid n=9	0/9 (0%)	9/9 (100%)
DIE of bladder n=5	1/5 (20%)	4/5 (80%)
DIE of UVF n=6	1/6 (16.7%)	5/6 (83.3%)
DIE of PSW n=9	1/9 (11.1%)	8/9 (88.9%)
Total	28/104 (26.9%)	76/104 (73.1%)

DIE is deeply infiltrating endometriosis, USL is uterosacral ligaments, POD is pouch of Douglas, RVS is rectovaginal septum, UVF is utero vesical fold, PSW is pelvic side wall. All bilateral structures were considered as one location (ovaries, USL, PSW).

Diagnostic accuracy of pre-operative TVS for each of the specific anatomical locations of endometriosis is shown in Table 4. There was a significant difference between the sensitivities for the different locations (Chi squared= 74.97, P<0.0001) while the specificities were similar (p>0.05). The positive likelihood ratio (LR+) was very useful (>10) for the TVS diagnosis of endometriosis of the following anatomical locations: ovarian endometriomas; moderate or severe ovarian adhesions; pouch of Douglas adhesions; and deeply infiltrating endometriosis (DIE) of the bladder; rectum or sigmoid; rectovaginum; uterovesical fold; and the uterosacral ligaments. Only for pelvic side wall DIE and mild ovarian adhesions was the LR+ moderately useful (5-10). The negative likelihood ratio (LR-) was very useful (<0.1) for bladder DIE and moderately useful (0.1-0.2) for ovarian endometriomas, moderate or severe ovarian adhesions, and pouch of Douglas adhesions. The sensitivity was highest for bladder and ovarian endometriomas and lowest for DIE of the uterovasical fold, pelvic side wall and uterosacral ligaments.

Table 4 Accuracy of pre-operative ultrasound diagnosis of endometriotic lesions affecting different pelvic organs.

Site of disease	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Area under ROC Curve
Ovarian endometrioma N= 75	84.0 (95% CI 73.7 – 91.4)	95.6(95% CI 92.8 – 97.6)	81.8 (95% CI 71.8-90.6)	96.2 (95% CI 93.5-97.8)	19.26 (95% CI 11.431-32.451)	0.167 (95% CI 0.10-0.281)	0.898 (95% CI 0.864 – 0.926) P = 0.0001
DIE of bladder N=5	100 (95% CI 48.0-100)	100 (95% CI 98.1 - 100)	100 (95% CI 48.0-100)	100 (95% CI 98.1 - 100)	∞ (95% CI 0- ∞)	0.00 (95% CI 0- ∞)	1.00 (95% CI 0.981 – 1.00) P = 0.000
DIE Rectum / Sigmoid N=9	33.3 (95% CI 12.1-64.6)	98.9 (95% CI 96.2-99.7)	60 (95% CI 23.1-88.2)	96.9 (95% CI 93.4 - 98.6)	31.5 (95% CI 5.992-165.6)	0.674 (95% CI 0.424-1.07)	0.661 (95% CI 0.591 – 0.727) P= 0.111
RV DIE N= 32	50.0 (95% CI 33.6 – 66.4)	100 (95% CI 97.7 – 100)	100 (95% CI 80.6-100)	96.9 (95% CI 93.4-98.6)	∞ (95% CI 0- ∞)	0.50 (95% CI 0.354-0.707)	0.758 (95% CI 0.692– 0.816) P = 0.0001
DIE of UVF N=6	16.7 (95% CI 2.8 – 63.9)	99.0 (95% CI 96.3 – 99.8)	33.3 (95% CI 6.1-79.2)	97.4 (95% CI 94.2-98.9)	16.0 (95% CI 1.68-153.94)	0.84 (95% CI 0.589-1.205)	0.578 (95% CI 0.506 – 0.648) P = 0.528
DIE of PSW N=13	15.4 (95% CI 2.4 – 45.5)	98.17 (95% CI 96.3 – 99.3)	22.2 (95% CI 0.063-0.547)	97.2 (95% CI 95.0-98.4)	8.421 (95% CI 1.933-36.65)	0.862 (95% CI 0.683-1.087)	0.568 (95% CI 0.517 – 0.617) P = 0.419
DIE of USL N=40	10.0 (95% CI 2.9 – 23.7)	99.16(95% CI 97.6 – 99.8)	57.1 (95% CI 25.0-84.2)	90.7 (95% CI 87.5-93.2)	11.867 (95% CI 2.754-51.14)	0.908 (95% CI 0.818-1.007)	0.546 (95% CI 0.495 – 0.596) P = 0.351

PPV is positive predictive value, NPV is negative predictive value, +ve LH is positive likelihood ratio, -ve LH is negative likelihood ratio, ROC is receiver operating characteristics, DIE is deeply infiltrating endometriosis, RV is rectovaginal, UVF is utero vesical fold (separate from bladder), PSW is pelvic side wall, USL is uterosacral ligaments. (All bilateral anatomical locations were treated separately).

The LR+ and –LR for all adhesions on the ovaries were moderately and somewhat useful respectively. However for the assessment of moderate or severe adhesions on the ovary the LR+ and –LR was very and moderately useful respectively as detailed in table 4. When the diagnosis of ovarian adhesions was stratified according to the ASRM classification into mild, moderate and severe the overall level of agreement between scan and laparoscopy was very good (Table 6). The LR+ and –LR for adhesions in the pouch of Douglas were very and moderately useful respectively as detailed in table 5. When pouch of Douglas obliteration was assessed according to the ASRM classification into partial and complete obliteration the overall level of agreement between scan and laparoscopy was very good (Table 7). Table 8 shows that the accuracy of the diagnosis of DIE increases significantly with the total number of endometriotic lesions present. This data is represented graphically in figure 11. Table 8 shows that although the number of endometriotic lesions seen on scan significantly increases with the number of lesions present (fig 12) the proportion of the total lesions correctly diagnosed increases to a maximum at three lesions present at laparoscopy then declines (fig 13).

Table 5 Accuracy of pre-operative ultrasound diagnosis of pelvic adhesions in women with suspected endometriosis.

Site of disease	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Area under ROC Curve
Any adhesions on ovary N=130	79.6 (95% CI 72.0 – 85.5)	91.9 (95% CI 87.9 – 94.6)	83.8 (95% CI 76.6-89.2)	89.5 (95% CI 85.2-92.6)	9.81 (95% CI 6.456– 14.92)	0.222 (95% CI 0.160-0.310)	0.865 (95% CI 0.827 – 0.897) P = 0.0001
Mod/ Severe adhesions on ovary N=123	83.7 (95% CI 76.2 – 89.2)	94.1 (95% CI 90.7 – 96.4)	86.6 (95% CI 79.3-91.6)	92.8 (95% CI 89.1-95.3)	14.288 (95% CI 8.826-23.131)	0.173 (95% CI 0.116-0.258)	0.889 (95% CI 0.854 – 0.919) P = 0.0001
Severe adhesions on ovary N=103	83.5 (95% CI 75.1-89.4)	93.5 (95% CI 90.1-95.8)	81.9 (95% CI 73.5)	94.2 (95% CI 90.8-96.3)	12.876 (95% CI 8.266-20.057)	0.176 (95% CI 0.114-0.273)	0.867 (95% CI 0.830- 0.899) P = 0.0001
Any adhesions in POD N= 54	83.3 (95% CI 71.3-91.0)	95.1 (95% CI 90.3-97.6)	86.5 (95% CI 74.7-93.3)	93.8 (95% CI 88.7-96.7)	17.143 (95% CI 8.242-35.656)	0.175 (95% CI 0.096-0.318)	0.892 (95% CI 0.841-0.932) P = 0.0001
Complete obliteration of POD N= 30	83.3 (95% CI 66.4-0.927)	97.0 (95% CI 93.2-98.7)	83.3 (95% CI 66.4-92.7)	97.0 (95% CI 93.2-98.7)	28.0 (95% CI 11.636-67.376)	0.172 (95% CI 0.077-0.383)	0.902 (95% CI 0.852-0.939) P = 0.0001

PPV is positive predictive value, NPV is negative predictive value, +ve LH is positive likelihood ratio, -ve LH is negative likelihood ratio, ROC is receiver operating characteristics, POD is pouch of Douglas.

Table 6 Comparison of ultrasound and laparoscopy for the assessment of severity of ovarian adhesions.

	TVS assessment of ovarian adhesions				
Laparoscopic assessment of ovarian adhesions	Absent	Minimal	Moderate	Severe	Total
Absent	238	6	5	10	259 (65.4%)
Minimal	10	3	0	1	14 (3.5%)
Moderate	7	1	4	8	20 (5.1%)
Severe	11	1	5	86	103 (26.0%)
Total	266 (67.2%)	11 (2.8%)	14 (3.5%)	105 (26.5%)	396

Weighted Kappa = 0.801 (standard error (Kw'=0) 0.050 and (Kw'#0) 0.031).

Table 7 Comparison of ultrasound and laparoscopy for the assessment of severity of adhesions in the pouch of Douglas.

	Pouch of Douglas obliteration at TVS			
Pouch of Douglas obliteration at laparoscopy	No adhesions	Partial obliteration	Complete obliteration	Total
No adhesions	137	4	3	144 (72.7%)
Partial obliteration	9	13	2	24 (12.1%)
Complete obliteration	0	5	25	30 (15.2%)
Total	146 (73.7%)	22 (11.1%)	30 (15.2%)	198

Weighted Kappa kappa = 0.852 (standard error (Kw'=0) 0.071 and (Kw'#0) 0.038)

Table 8. Women with DIE separated into groups by total number of endometriotic lesions compared with the accuracy of diagnosis of DIE in each group.

Total number of endometriotic lesions	Number of women (n=61)	Number correctly diagnosed with DIE (n, %)
Single lesions	10	1 (10.0%)
2 lesions	8	3 (37.5%)
3 lesions	16	9 (56.3%)
4 lesions	16	11 (68.8%)
5 lesions or more	11	8 (72.7%)

Kruskal-Wallis test of correlation between total number of lesions and % of women correctly identified with DIE (P = 0.0228).

Figure 11. Bar chart of total number of endometriotic lesions at laparoscopy against percentage of women correctly diagnosed with DIE in each group.

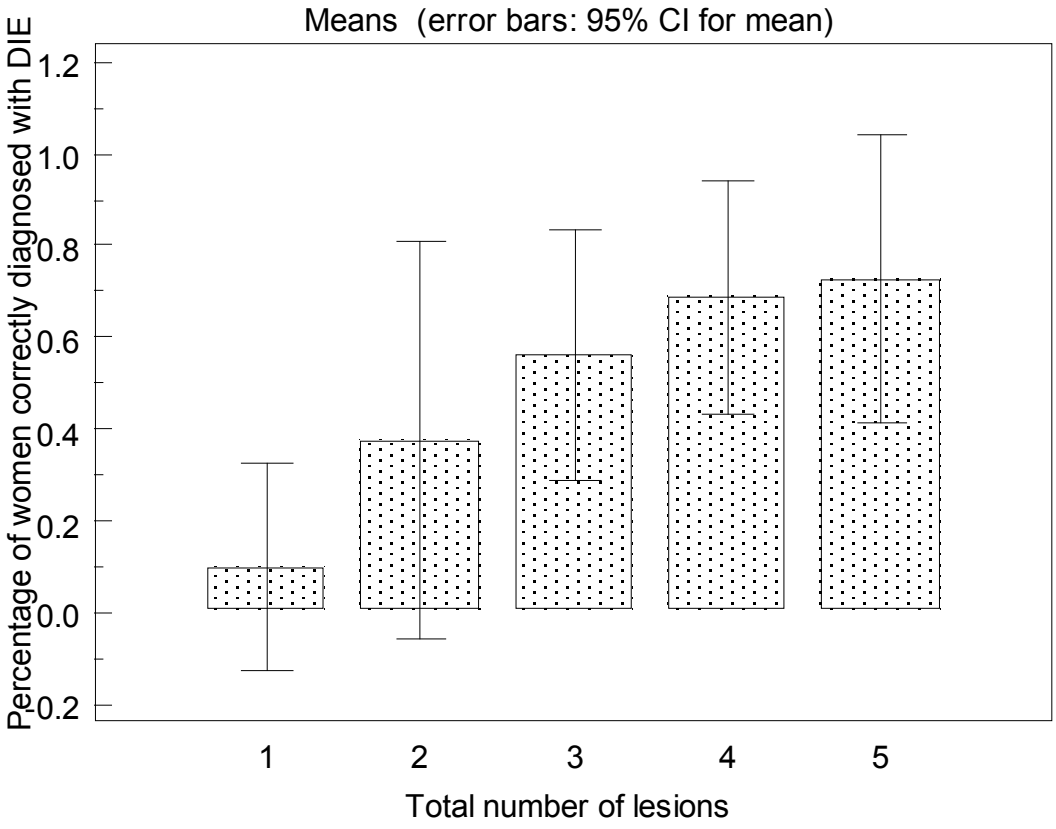


Table 9 shows the mean number and mean proportion of lesions diagnosed on scan for all women with endometriotic lesions grouped by total number of lesions.

Total number of lesions	Number of women N=104	Mean number of lesions diagnosed on scan	Mean proportion of total lesions diagnosed on scan
Single lesions	28	0.429 (95% CI 0.207 to 0.651)	0.3929 (95% CI 0.2000 to 0.5857)
2 lesions	25	1.800 (95% CI 1.4232 to 2.1768)	0.8000 (95% CI 0.6541 to 0.9459)
3 lesions	24	2.8750 (95% CI 2.4562 to 3.2938)	0.8750 (95% CI 0.7749 to 0.9751)
4 lesions	16	3.5625 (95% CI 3.0871 to 4.0379)	0.8594 (95% CI 0.7756 to 0.9432)
5 lesions or more	11	3.5455 (95% CI 2.4471 to 4.6438)	0.6450 (95% CI 0.4584 to 0.8316)
		P< 0.0001*	P= 0.0008*

*Kruskal-Wallis test of correlation between total number of lesions at laparoscopy and mean number of lesions diagnosed on scan and mean proportion of total lesions diagnosed on scan respectively.

Figure 12 Bar chart of total number of endometriotic lesions at laparoscopy against the mean number of lesions seen on scan in each group.

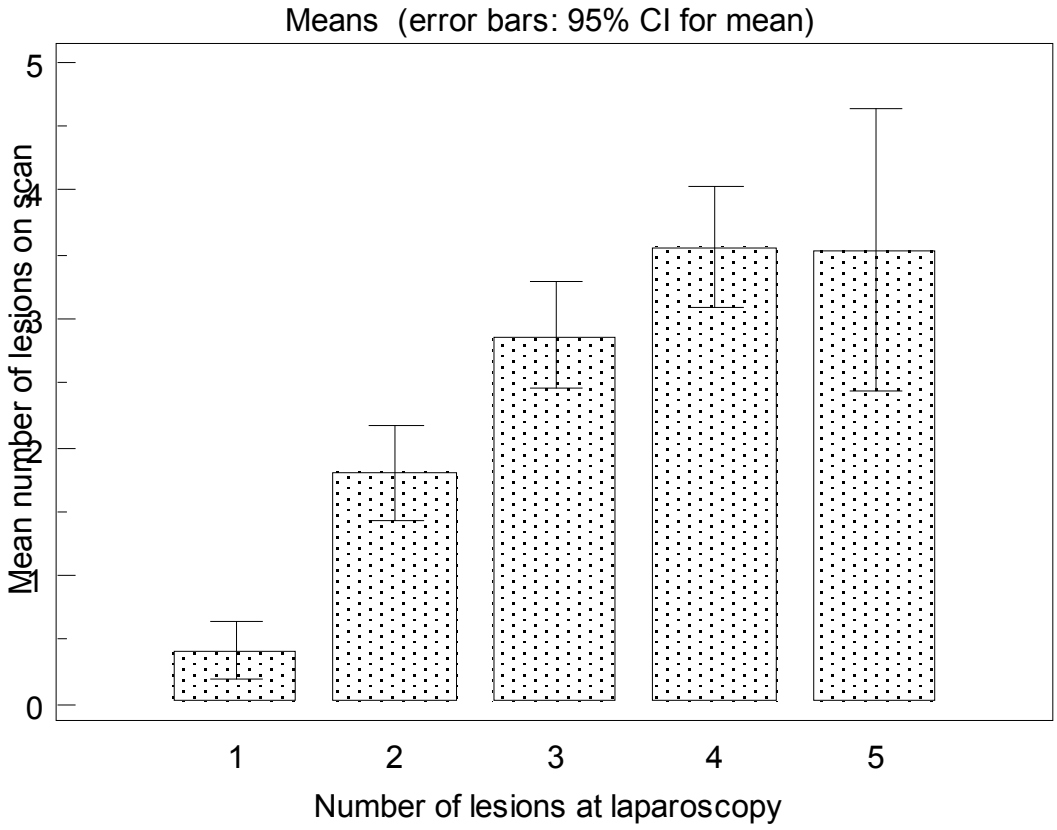
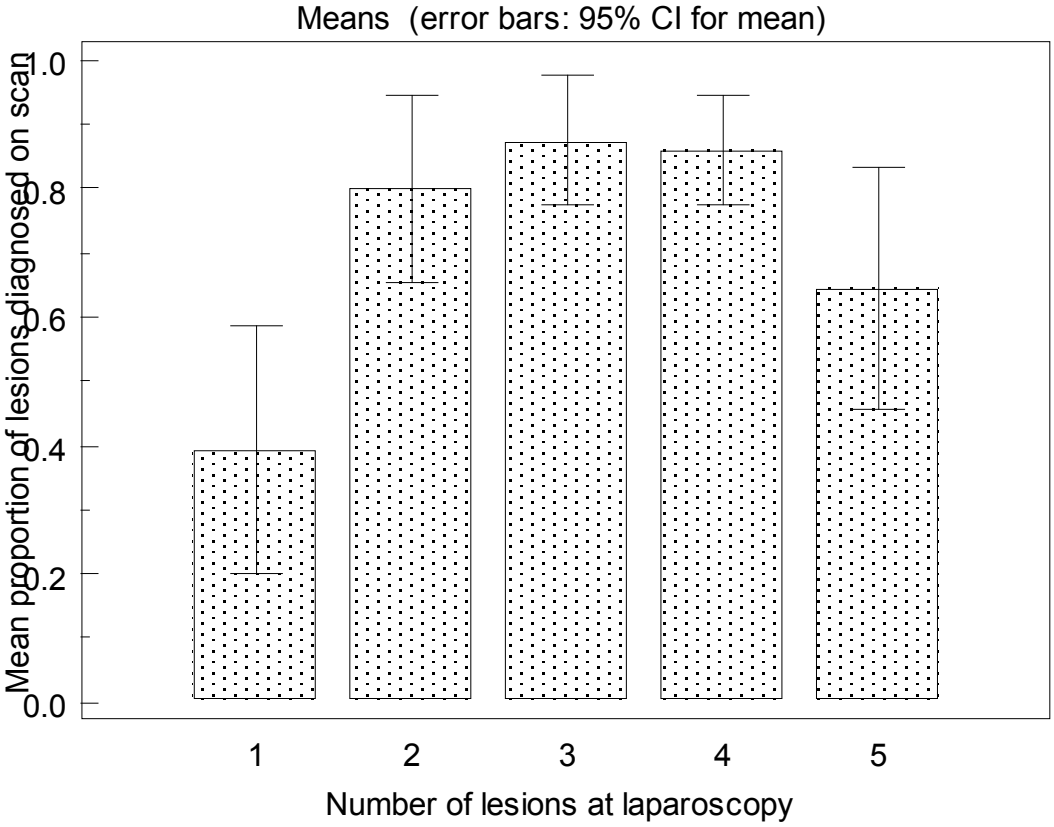


Figure 13 Bar chart of total number of endometriotic lesions seen at laparoscopy against mean proportion of lesions diagnosed on scan in each group.



Discussion

Our study has shown that it is possible, using transvaginal ultrasound scanning, to predict features of pelvic endometriosis with a consistently high specificity and a high level of sensitivity for the most difficult to treat features eg RVS, bowel and bladder disease. The total number of endometriotic lesions found at laparoscopy has statistically significant positive effect on the accuracy of ultrasound diagnosis of deeply infiltrating lesions. The sensitivity of the ultrasound diagnosis was significantly affected by the location of the endometriotic lesions but the specificity remained high throughout. We have shown for the first time that ultrasound enables detection and assessment of severity of adhesions affecting the ovaries and pouch of Douglas.

A high accuracy for the diagnosis of the features of pelvic endometriosis is helpful for the management of these patients as they can be counselled regarding the most appropriate treatment modality. If surgery is decided upon these patients can be directed to the most appropriate surgeon for their needs, which may mean being referred to a tertiary centre in patients who have bladder/ bowel DIE or an obliterated pouch of Douglas. When these patients and their clinicians have decided that they would like to proceed with operative treatment an accurate diagnosis of the extent of the disease will enable a full discussion of the risks and benefits and therefore enable informed consent. It will also aid the surgeon in planning the operation and ensuring that the necessary staff are present such as colorectal surgeons for bowel disease. Preoperative underestimation of the severity of DIE lesions is the reason why complete surgical exision may not be achieved resulting in progression of the

remaining disease[90]. For patients suffering from infertility it may aid in counselling them on the likely cause of infertility and consequences of pursuing a surgical approach to their management.

Our study has shown a high accuracy in the diagnosis of ovarian adhesions, pouch of Douglas obliteration and bladder lesions. The accuracy for these features was similar to the accuracy for ovarian endometriomas which were previously thought of as the only feature of pelvic endometriosis which it is possible to diagnose on TVS[16]. Previous studies have stated that left sided endometriomas are more common than right[817] but there was no statistically significant difference in our data set. Our study has also shown that only 26.9% of patients with focal endometriosis will have disease in only one location and therefore in all cases the examiner should perform a detailed search for lesions in other typical locations.

There are few studies on the accuracy of TVS for the diagnosis of ovarian adhesions. Our study has shown a high level of accuracy for this diagnosis with a kappa value of 0.798. No study has previously assessed mobility as either minimal, moderate or severe adhesions for the ovary in accordance with the ASRM classification[7]. Guerriero[27] used the combination of 3 features as suggestive of ovarian adhesions: blurring of the ovarian margin, the inability to mobilise the ovary on palpation (fixation) and an increased distance from the probe. They found that these tests either combined or individually gave a kappa value of between 0.25 and 0.51. Okaro[28] examined women with chronic pelvic pain prior to laparoscopy for the presence of ovarian adhesions and classified them as either mobile or fixed. They found a high degree of agreement between TVS and laparoscopy at identifying ovarian adhesions

(0.81 kappa). This compares with the results of our study of an area under the ROC of 0.883 for the presence of either moderate or severe adhesions and a kappa of 0.798 for the three stages of severity. Yazbek[29] examined the role of ultrasound for the preoperative assessment of adnexal masses. They found a sensitivity of 44% and a specificity of 98% in the diagnosis of severe pelvic adhesions. The technique for examination of adhesions was similar to that used in this paper but they do not state ovarian adhesions separately. Guerriero[30] used a technique of applying pressure between the uterus and ovary. If they remained linked then this was suggestive of adhesions. This gave a sensitivity and specificity of 89% and 90% respectively for fixation of the ovaries to the uterus.

The preoperative diagnosis of partial or complete obliteration of the pouch of Douglas has not been reported on directly before. Our study shows a high accuracy of this diagnosis. Hudelist[91] gave a high accuracy for the diagnosis of pouch of Douglas endometriosis but did not report obliteration separately. Yazbek[29] described the technique for diagnosing POD obliteration but did not report this finding separately from severe pelvic adhesions.

The high level of accuracy for the diagnosis of bladder endometriosis is concordant with previous studies, which showed a high level of accuracy in the TVS diagnosis of bladder endometriosis[23,85,92].

There were poor levels of sensitivity for the diagnosis of endometriosis affecting the uterosacral ligaments and pelvic side walls. The low accuracy of TVS for diagnosing endometriosis of the uterosacral ligaments and pelvic side walls has also been

previously reported[83,86]. Hudelist[26] reports higher levels of sensitivity for the diagnosis of uterosacral disease however these levels were lower than for almost all of the other locations of DIE. The preoperative diagnosis of endometriosis in these locations is not critical for the management as these are rarely missed at laparoscopy and surgical excision can usually be achieved without involvement of other surgical specialists.

Our study showed a high specificity of the diagnosis of rectovaginal septum disease and a lower sensitivity. This agrees with the results of a recent review by Hudelist[93] encompassing 10 studies on the diagnostic accuracy of TVS for intestinal endometriosis. He found sensitivities ranging from 67- 98% and specificities of 92-100%.

Hudelist[26] compared pelvic examination alone with pelvic examination combined with TVS for the preoperative assessment of patients with suspected pelvic endometriosis. They found that by combining these two procedures it is possible to get sensitivities of 67- 100% for the diagnosis of features similar to our own. They maintained high specificities with these findings of 86-100%. It is likely that our pick up rate would be improved by the inclusion of a thorough pelvic examination prior to TVS.

The effect of the number of lesions on the sensitivity of ultrasound diagnosis of specific endometriotic lesions in different locations has not been assessed before. Our data shows that the accuracy of the diagnosis of individual specific lesions increases with their absolute number up to a maximum of three lesions. With increasing number

of lesions above that level the sensitivity declines. A possible reason for this could be that in more severe disease the adhesions tend to obscure other small lesions further away from the ultrasound probe. There is also a possibility of operator bias as in women with evidence of severe disease documentation of the presence of small lesions such as those located at utero-sacral ligaments becomes less clinically relevant.

Our study could be criticised for not more accurately differentiating between DIE of the rectum, sigmoid, RVS and vagina. We could also be criticised for including subjective assessments such as ovarian and pouch of Douglas mobility which can not be recorded with ease. However our diagnostic accuracy of diagnosing ovarian and pouch of Douglas disease was higher than for other features and therefore is unlikely to be biased due to its subject nature. It remains to be seen in further studies whether these subjective features are as reproducible as the more objective features. Our study can also be criticised as both examiners were gynaecologists with a special interest in TVS. As most of the TVS performed in the UK is by sonographers who have less experience of pelvic anatomy and pathology the findings may not be applicable to most units.

Conclusions

Our study has shown that the specificity of the ultrasound diagnosis of pelvic endometriotic lesions is high with low false positive rates. The negative diagnostic rate was less high especially in the diagnosis of bowel, rectovaginal, uterosacral ligament, pelvic side wall and uterosacral ligament lesions. Therefore women with significant symptoms and a negative diagnosis still require further investigation.

Chapter 8 Study 3

The prediction of the severity of endometriosis by transvaginal ultrasound examination compared with findings at laparoscopy.

Aim

The aim of this study was to assess the ability of transvaginal ultrasound performed by experienced operators to predict the severity of pelvic endometriosis.

Methods

The methods for this study were the same as detailed in the general methods section above.

Statistical analysis and sample size power calculation

As no previous studies have been conducted to assess the accuracy of expert ultrasound scanning in the assessment of severity of endometriosis there are no figures on which to base a power calculation. In clinical practice it would be ideal to identify all cases of endometriosis. Our hypothesis was that it would be clinically acceptable if transvaginal ultrasound had a sensitivity of 90% in identifying severe pelvic endometriosis. This study was designed to have 90% power to detect a 10 % difference between the sensitivity of diagnostic laparoscopy and transvaginal ultrasonography in detecting severe pelvic endometriosis with a two-sided α of 0.05.

The study needed a minimum of 190 patients, but we recruited 211 patients to allow for loss of power due to cancellations or pregnancy.

All statistical analyses were carried out using Medcalc version 9.2.0.2 (Medcalc Software, Mariakerke, Belgium). The diagnostic accuracy of the tests was assessed using sensitivity, specificity, and positive (LR+) and negative (LR−) likelihood ratio measures. Correlation was calculated using the coefficient of correlation R. In order to determine any systematic bias between the two diagnostic methods and to assess the relationship between any differences and the magnitude of the scores, the differences in score were plotted against the mean of the two scores on a scatter diagram (Figure 15). Systematic bias between the two observers was determined by calculating the 95% confidence interval of the mean (mean ± 1.96 standard deviations) as described by Bland and Altman[77,78] Overall levels of agreement was calculated using Cohen's quadratic weighted Kappa coefficient. Kappa values of 0.81-1.0 indicated very good agreement, Kappa values of 0.61-0.80 good agreement, Kappa values of 0.41-0.60 moderate agreement, Kappa values of 0.21-0.40 fair agreement and Kappa values <0.20 poor agreement[79] .

Results

In the period of 30 months from July 2006 to December 2008 we recruited 211 women into this study. 10 women were excluded from the final analysis: five became pregnant whilst awaiting surgery, one cancelled her operation, one laparoscopy was unsuccessful and three women were lost to follow up.

201 women were included in the final analysis. The mean age was 34.9 (95% CI 33.98 – 35.86, SD 6.79) (range 19-51) years. The presenting symptoms were dysmenorrhoea for 142/201 (70.6%), chronic pelvic pain for 104/201 (51.7%), dyspareunia for 78/201 (38.8%), infertility for 38/201 (18.9%), dyschezia for 7/201 (3.5%) and cyclic rectal bleeding for 2 (1%) women. A single presenting symptom was present in 72 (35.6%) women, two presenting symptoms in 78 (38.8%) and three or more symptoms in 51 (25.4%) women.

The ultrasound examinations were performed by four examiners: examiner A performed 104 (51.7%), examiner B 68 (33.8%), examiner C 18 (9%) and D 11 (5.5%) examinations. All patients were operated on by one of four laparoscopic surgeons: surgeon A operated on 70 (34.8%), surgeon B on 52 (25.9%), surgeon C on 45 (22.3%) and surgeon D on 34 (16.9%) women. The mean interval between TVS and operation was 37.5 days (95% CI 34.3 – 40.8, SD 23.2) (range 0-87 days).

Table 10 shows the findings on ultrasound compared with laparoscopy. There was a good level of agreement between ultrasound and laparoscopy in identifying absent, minimal, mild, moderate and severe disease (Quadratic Weighted Kappa = 0.786, Standard error (Kw'=0) = 0.068, Standard error (Kw'#0) = 0.033).

Table 10. A comparison of ultrasound and laparoscopic assessment of the severity of pelvic endometrisois using rAFS classification.

	Laparoscopy					
Ultrasound	Absent	Minimal	Mild	Moderate	Severe	Total (%)
Absent	59	29	27	3	2	120 (59.7)
Minimal	0	1	0	0	0	1 (0.5)
Mild	1	1	4	2	1	9 (4.5)
Moderate	2	1	0	20	4	27 (13.4)
Severe	0	1	0	2	41	44 (21.9)
Total (%)	62 (30.9)	33 (16.4)	31 (15.4)	27 (13.4)	48 (23.9)	201 (100)

Table 11. Diagnostic accuracy of ultrasound in diagnosing different stages of pelvic endometriosis using laparoscopy as the gold standard.

	Sensitivity	Specificity	LR +ve	LR -ve
Absent vs Present	81/142 (57.04%, 95% CI 0.485 – 0.652)	59/62 (95.2%, 95% CI 0.856- 0.987)	11.78 (95% CI 3.87 – 35.88)	0.45 (95% CI 0.373 – 0.546)
Absent to mild vs moderate to severe	67/ 75 (89.0 %, 95% CI 0.795 – 0.949)	122/ 126 (96.8%, 95% CI 0.916- 0.989)	28.14 (95% CI 10.69 – 74.0)	0.11 (95% CI 0.057 – 0.212)
Absent to moderate vs Severe	41/48 (85.4%, 95% CI 0.716– 0.934)	150/ 153 (98.0%, 95% CI 0.939- 0.994)	43.5 (95%CI 14.12 – 134.38)	0.149 (95%CI 0.0749 – 0.295)

LR +ve = positive likelihood ratio, LR –ve = negative likelihood ratio

Table 12. A comparison of inter-observer reproducibility of ultrasound diagnosis of severe pelvic endometriosis using laparoscopy as the gold standard.

Examiner	A	B
Sensitivity (%)	9/11 (81.8, 95% CI 47.7-96.8)	28/30 (93.0, 95% CI 783.71-987.25)
Specificity (%)	92/93 (98.9, 95% CI 93.3-99.9)	37/38 (97.4, 95% CI 864.52-99.59)
False positive rate (%)	0.96 (1/104) (95% CI 0.17-5.3)	1.5 (1/68) (95% CI 0.26-7.9)
False negative rate (%)	1.9 (2/104) (95% CI 0.53-6.7)	0.96 (2/68) (95% CI 0.81-10.1)
LR +ve	76.1 (95% CI 10.6- 545)	33.4 (95% CI 4.82 – 231)
LR –ve	0.184 (95% CI 0.0524 – 0.644)	0.099 (95% CI 0.0339- 0.292)
PPV (%)	89.8	96.6
NPV (%)	97.8	94.9
Accuracy (Area under ROC curve)*	0.904	0.938

*comparison of area under ROC curves P=0.627
LR +ve = positive likelihood ratio, LR –ve = negative likelihood ratio, PPV = positive predictive value, NPV = negative predictive value, ROC = receiver operator curve.

Sensitivity, specificity, positive and negative likelihood ratios of transvaginal ultrasonography in diagnosing pelvic endometriosis are shown in Table 11. Table 12 shows the accuracy of examiners A and B for detecting severe pelvic endometriosis. There was no significant difference found in overall accuracy between these two examiners when the area under the ROC curve was compared. The numbers examined by C and D were not sufficient to make individual comparisons of accuracy meaningful and therefore the results of these examiners were not presented in Table 12.

Deeply invasive endometriosis (DIE) is given a maximum score of six on the rAFS classification and therefore it is helpful to record the presence of these lesions separately. Table 13 shows the presence of DIE in relation to severity as classified by rAFS. The 17 cases of mild disease where DIE was present included the uterosacral ligaments in 12 (70.6 % 95% CI 46.8- 86.7), pelvic side wall in 4 (23.5% 95% CI 9.6- 47.3), uterovesical fold/ bladder in 2 (11.8% 95% CI 3.3-34.3), pararectal space in 1 (5.9% 95% CI 1.1-27.0), rectovaginal septum in 1 (5.9% 95% CI 1.1-27.0) and rectum in 1 (5.9% 95% CI 1.1-27.0). 13 (76.5% 95% CI 52.7- 90.4) cases had one site of DIE and the other 4 (23.5% 95% CI 9.6- 47.3) had two sites. Only one case, involving the bladder, was correctly diagnosed as having DIE on TVS. Table 4 shows the prevalence of DIE, and TVS sensitivity for DIE, in relation to severity classified by rAFS.

Table 13. The prevalence of DIE and TVS sensitivity for DIE in relation to severity classified by rAFS

Severity as classified by rAFS score	None	Minimal	Mild	Moderate	Severe
Total cases	62	33	31	27	48
DIE present prevalence (%, 95% CI)	0 (0, 0- 5.8)	0 (0, 0- 10.4)	17 (54.8, 35.2- 67.5)	17 (63.0, 44.2- 78.5)	37 (77.1, 63.5- 86.7)
DIE correctly diagnosed on TVS Sensitivity (%, 95% CI)	N/A	N/A	1/17 (5.9, 1.1- 27.0)	6/17 (35.3, 17.3- 58.7)	18/37 (48.7, 33.5 - 64.1)

Table 14 shows the sensitivity, specificity, positive and negative likelihood ratios and areas under the ROC curves for the diagnosis of: DIE involving the bladder and uterovesical fold; DIE of the rectovaginal septum and bowel; and complete obliteration of the pouch of Douglas.

Table 14. Diagnostic accuracy of TVS in the assessment of features of severe endometriosis not clearly scored by rAFS. These features are: deeply invasive endometriosis of the bladder or utero-vesical fold; deeply invasive endometriosis of the rectovaginal septum, rectum or sigmoid colon; obliterated pouch of Douglas.

	DIE of bladder or utero-vesical fold	DIE of Rectovaginal septum or rectum/sigmoid	Obliterated pouch of Douglas	Any of these featuresCombined features
Sensitivity %	5/9 (55.56 95% CI 21.4-86.0)	14/31 (45.16 95% CI 27.3-64.0)	18/25 (72.00 95% CI 50.6 – 87.9)	23/38 (60.53 95% CI 43.4 – 75.9)
Specificity %	192/192 (100 95% CI 98.1 – 100)	170/170 (100 95% CI 97.8 – 100)	171/176 (97.16 95% CI 93.4 – 99.0)	156/163 (95.71 95% CI 91.3 – 98.2)
+LR	N/A	N/A	25.06 (95% CI 10.32 – 62.2)	14.09 (95% CI 6.533 – 30.41)
-LR	0.44 (95% CI 0.214 – 0.923)	0.55 (95% CI 0.398 – 0.755)	0.29 (95% CI 0.154 – 0.541)	0.412 (95% CI 0.278 – 0.612)
Area under ROC curve	0.778 (95% CI 0.714 – 0.833) (P=0.0027)	0.726 (95% CI 0.659 – 0.786) (P= 0.0001)	0.846 (95% CI 0.788 – 0.893) (P= 0.0001)	0.781 (95% CI 0.718 – 0.836) (P= 0.0001)

LR +ve = positive likelihood ratio, LR –ve = negative likelihood ratio, ROC = receiver operator curve.

Histological confirmation of endometriosis was not possible in all cases as the study design did not state that histology was necessary. However, where available the histology results are shown in table 15. Table 16 shows the distribution of the individual features of endometriosis according to the overall stage of disease.

Table 15. Histological confirmation of endometriosis in relation to severity.

Severity	None	Minimal	Mild	Moderate	Severe
Total cases	62	33	31	27	48
Histology available (%, 95% CI)	9 (14.5, 7.8- 25.3)	3 (9.1, 3.1-23.6)	9 (29.0, 16.1-46.6)	25 (92.6, 76.6- 97.9))	44 (91.7, 80.5- 96.7)
Endometriosis confirmed (%, 95% CI)	0 (0, 0-29.9)	3 (43.9- 100 %)	7 (77.8, 45-93.7)	25 (100, 86.7- 100)	44 (100, 92.0- 100)

Table 16. Distribution of features of endometriosis in relation to the overall severity of disease by rAFS at laparoscopy.

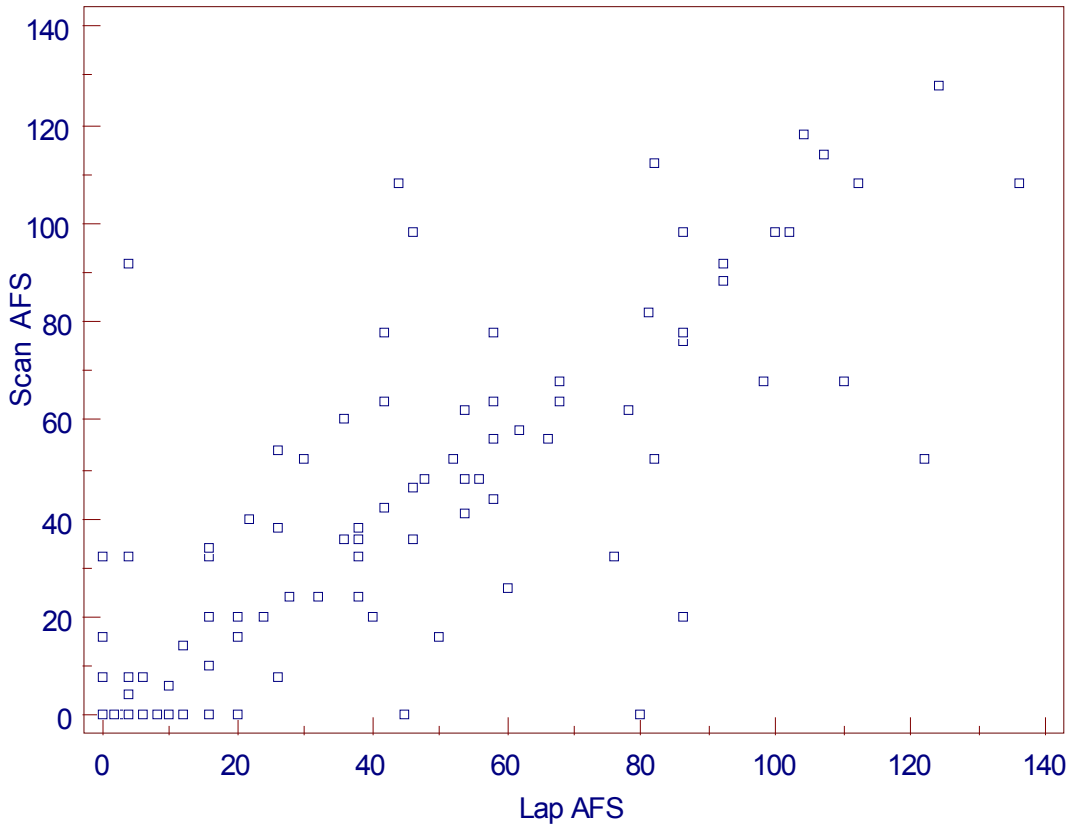
Features found at operation	Minimal (n=33)	Mild (n=31)	Moderate (n=27)	Severe (n=48)
Superficial peritoneal	33	22	16	28
Deep peritoneal	0	17	17	37
No endometriotic cysts	33	31	11	11
cysts* <1cm	0	0	0	0
cysts* 1-3cm	0	0	11	16
cysts* >3cm	0	0	5	21
Partial POD obliteration	0	0	7	12
Complete POD obliteration	0	0	0	24
Dense ovarian adhesions** <1/3	0	4	1	0
Dense ovarian adhesions** 1/3-2/3	0	1	5	4
Dense ovarian adhesions** >2/3	0	1	14	41
Tubal adhesions (either side)	0	0	0	7
Tubal dilatation (either side) dilatation	0	2	0	2

Features present which would upstage the disease under the newer system.

* equals largest cyst on either ovary.
** on either ovary which ever is worse affected

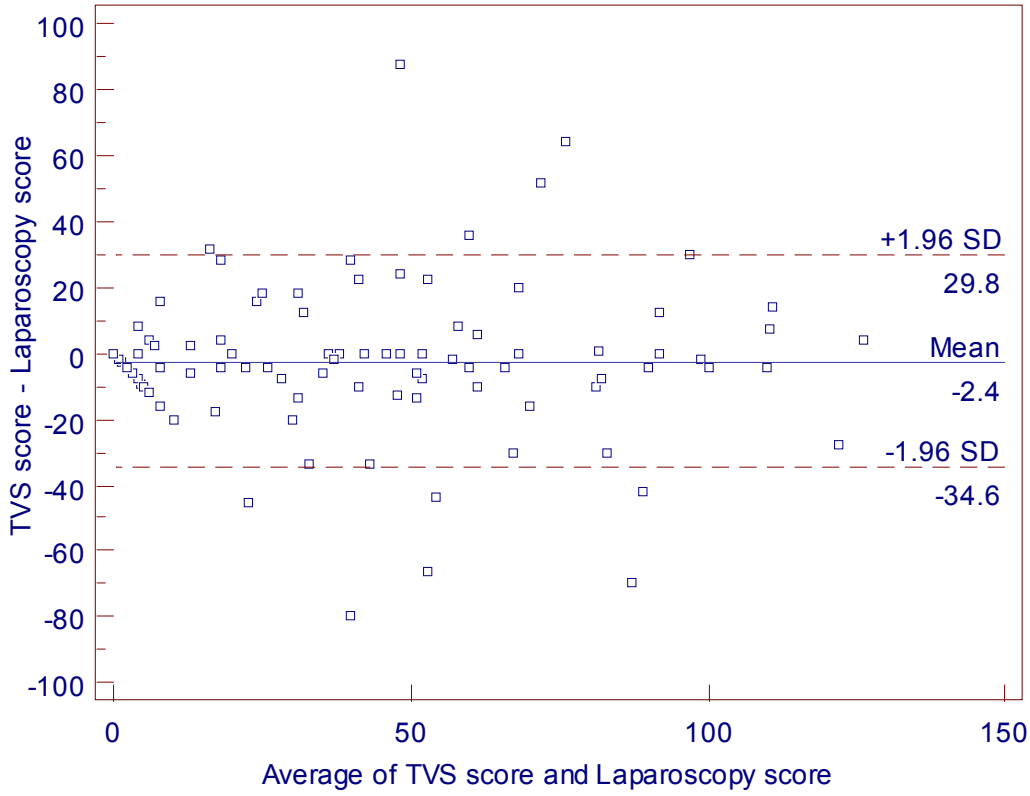
Fig. 14 demonstrates correlation of ultrasound and laparoscopic assessment of the severity of pelvic endometriosis as classified by rAFS. The inter-method correlation coefficient was 0.867 (95% CI 0.829-0.898). The mean difference between TVS and laparoscopy in assessing severity of endometriosis was -2.398 (95% CI -4.685 to -0.1112) and the limits of agreement were -34.62 (95% CI -38.54 to -30.709) to 29.83 (95% CI 25.91 to 33.74). The difference is normally distributed as 95% of the values lie within 1.96 standard deviations of the mean. Visual inspection of the scatterplot revealed that the magnitude of the difference did not change with increasing severity of endometriosis. (Fig. 15).

Figure 14: A scatterplot of ultrasound and laparoscopic findings in individual women with and without evidence of pelvic endometriosis. The severity of the disease was determined using the rAFS classification.



Sample size	201
Correlation coefficient r	0.8677
Significance level	P<0.0001
95% Confidence interval for r	0.8289 to 0.8982

Figure. 15. Scatterplot of the difference in rASF score of the severity of endometriosis between TVS and laparoscopy versus mean score.



There were seven false negative cases for severe endometriosis: two were diagnosed as absent for endometriosis, one as minimal and four as moderate disease. The two cases of severe endometriosis which were classified as not having endometriosis both had the pouch of Douglas correctly classified as partially or completely obliterated by adhesions but the endometriotic nodules were not seen and there were no ovarian endometriomas present. These cases were correctly classified as having severe adhesions but not as a consequence of endometriosis. The one case of severe disease which was classified as having minimal endometriosis had a rectovaginal septum nodule with an obliterated pouch of Douglas which was not seen on TVS. Of the four cases of severe disease which were diagnosed as moderate disease three of them had the pouch of Douglas incorrectly classified as partially obliterated when it was completely obliterated and the other had ovaries which were fixed when they were classified as mobile. There were three cases of false positive for severe endometriosis: one had mild disease and the other two had moderate disease. The mild case had the pouch of Douglas misclassified as obliterated. One of the moderate cases had smaller ovarian endometriomas at laparoscopy than on scan and the other had a unilateral endometrioma on laparoscopy when there were bilateral cysts on scan. There were 29 false negative cases for minimal disease and 27 false negative cases for mild disease. The majority of these false negatives had superficial peritoneal disease only.

Discussion

Our study confirms that transvaginal ultrasound is an accurate diagnostic method for the assessment of women with suspected pelvic endometriosis. We used the rASRM classification to assess the severity of endometriosis on both ultrasound and laparoscopy[94]. We chose this particular scoring system as it is a well-established standard and it is widely used in clinical practice for the assessment of pelvic endometriosis. There was a high level of agreement between transvaginal ultrasound and laparoscopy in assessing the severity of disease. The accuracy of ultrasound was 94% in cases of moderate and severe pelvic endometriosis, however, the sensitivity of diagnosis in minimal and mild pelvic endometriosis was relatively low. Minimal endometriosis is characterised by the presence of small and superficial endometriotic lesions, which are difficult to detect on ultrasound or indeed on any other imaging modality. Mild disease, as defined by rASRM, can either be due to superficial disease or to isolated deeply infiltrating lesions. These isolated lesions are especially difficult to diagnose as there are no other signs to raise the suspicion of their presence. Inspection of scatter plots of differences between ultrasound and laparoscopy showed that ultrasound is significantly biased towards underestimating the severity of endometriosis. The lower sensitivity of ultrasound in the diagnosis of superficial disease and isolated deeper nodules is likely to be the main factor contributing to this finding.

The rASRM system has been criticised for scoring a maximum of six points for DIE of the peritoneum and no points being allocated for disease of the bowel[80]. This can raise the possibility of isolated DIE being given a low score although the symptoms and surgical difficulty would suggest a higher stage of disease. Other systems have been developed to complement the rASRM system but these are not widely known

and they are rarely used in routine clinical practice[95,96] . For these reasons we recommend stating the exact site and extent of any DIE found on either TVS or at surgery in addition to using the rAFS scoring system. Table 13 shows that with increasing severity of disease, DIE becomes more common and the sensitivity of TVS at diagnosing DIE also increases.

An easier way of diagnosing severe disease would be to use the presence of DIE on the bladder, bowel or RVS or obliteration of the pouch of Douglas could be used as an alternative way of diagnosing severe endometriosis. It could be argued that the detection of DIE in any of these locations amounts to severe disease and warrants surgery by an expert laparoscopic surgeon. The sensitivity of ultrasound in the diagnosis of DIE in these locations varies between 45 and 72% but the specificity is very high, between 97 and 100% (Table 14). By adopting this approach the diagnosis of severe endometriosis on ultrasound becomes simpler and quicker. Table 5 shows accuracy of diagnosis of these features individually and the overall diagnosis of severe disease when any of these features are present. These results show that TVS is highly specific but less sensitive at diagnosing these features. The moderate sensitivity is likely to be due to the difficulty in diagnosing isolated DIE without the presence of large endometriomas or severe adhesions to raise the suspicion of DIE. Histological diagnosis was not a condition of inclusion into this study and biopsies were not sent in all cases. However when biopsies were sent in moderate or severe cases endometriosis was confirmed. Endometriosis was confirmed in 77.8% of mild cases when histology was available.

Although, the diagnosis of moderate and severe disease was made with a high level of accuracy, there were occasional false positive and false negative findings. The false negatives in general were due to difficulty in identifying DIE and in classifying pouch

of Douglas obliteration. The high specificity of ultrasound diagnosis of endometriosis is an important finding. This indicates that women with evidence of severe disease could be referred for expert surgical treatment based on ultrasound findings alone without the need for confirmatory diagnostic laparoscopy.

Ultrasound examinations in this study were performed by operators with a high level of expertise in gynaecological ultrasonography. There was no significant difference in diagnostic accuracy between the two operators who performed the majority of ultrasound examinations in this study. This is in keeping with the findings of chapter 7 (study 3). It remains to be seen whether the accuracy of ultrasound diagnosis of endometriosis will remain high when the examinations are performed by less experienced operators.

Previous studies have shown that ultrasound is an accurate test to diagnose ovarian endometriomas[16] . However, the results were less promising in cases of non-ovarian pelvic endometriosis. Some authors have advocated transrectal scans in order to improve ultrasound diagnosis of DIE. This technique was particularly helpful for the diagnosis of utero-sacral and intestinal endometriosis[18-21,97] . Bazot[86] however, achieved better diagnosis of uterosacral and rectosigmoid endometriosis using transvaginal ultrasonography compared to the transrectal approach. They also showed that TVS is very accurate in the diagnosis of intestinal and bladder endometriosis, but less so in detecting uterosacral, vaginal and rectovaginal septum involvement. Our results are concordant with their findings as we also had difficulties in identifying the disease in the rectovaginal septum.

Okaro[28] assessed ovarian mobility in terms of being either mobile or fixed. They described their method as mobilisation of the ovary with gentle pressure from the probe. They found a good level of agreement (kappa=0.80) between the scan

diagnosis and the laparoscopy findings. We agree with their findings as we also found a high level of accuracy in assessing ovarian mobility. Our study methods, however, differed in two ways to this study. When assessing ovarian mobility we used a bimanual technique with gentle pressure from the probe and palpation across the abdominal wall with the examiners free hand. Also we assessed for mild and moderate levels of adhesions in addition to fixed or completely mobile ovaries. This is important, as a small amount of adhesions is easier to divide and therefore require less surgical skill. However, our study could be criticised for using a subjective distinction between the levels of ovarian adhesions. This subjective classification may be a source of bias towards under or over estimating the level of adhesions and consequently the overall severity. It is yet to be established whether these subjective criteria are reproducible in further inter observer studies.

In our study some of the false positives and false negatives for severe disease came from the misdiagnosis of pouch of Douglas obliteration. Bazot[86] also found this to be a difficult diagnosis to make. They gave a TVS diagnosis rate of complete and partial obliteration of 23 and 11 patients respectively out of the study population of 142 cases. At surgery they found 44 cases of complete obliteration and 13 cases of partial obliteration. They defined pouch of Douglas obliteration as complete when "uterus, adnexa and rectosigmoid colon were stuck together with loss of peritoneal structure and incomplete when peritoneal limits were partially identified with presence or absence of suspended or lateralized fluid collection". Our definition of obliteration is easier to use, however it is sometimes not an easy feature to assess accurately. Hudelist[25] compared bimanual digital examination to TVS combined with bimanual examination. They found that the combined technique gave very high levels of

accuracy and these were much higher than bimanual examination alone. We did not perform digital examinations in our study and it may be that our sensitivities would be increased using a combined technique. However this study does not compare TVS alone with the combined technique and therefore it may be that using their technique TVS may perform just as well as the combined technique. Abrao[24] compared TVS with digital vaginal examination and MRI and found that TVS had better sensitivity, specificity and accuracy in cases of deep "retro-cervical" and rectosigmoid endometriosis when compared with the other two techniques.

Guerriero[98] studied a novel technique for diagnosing "deep endometriosis" which included vaginal and rectovaginal septum disease. This involved using 12mls of gel within the probe cover to create a stand-off to visualise the near field area. Their sensitivity and specificity were 90% and 95% respectively and they concluded that this was an accurate and inexpensive technique for evaluating patients for deep endometriosis. This technique may have benefits over the standard TVS routines but it is more complex and would require a greater degree of operator skill and experience to be reliable. Further more this study suffers from selection bias as all the patients included were suspected of having deeply infiltrating rectovaginal endometriosis on clinical history or examination. Therefore it is difficult to apply these findings to a population of women with chronic pelvic pain. It is thus unlikely that the accuracy of the technique described by Guerriero would be reproducible in our study due to the mix of patients with various grades of severity of endometriosis. Also a direct comparison with the standard routine would be required in order to conclude that this technique is superior. Our study, however, differs from previously published research in that we were attempting to establish the ability of TVS to give an overall

assessment of the severity of pelvic endometriosis, rather than trying to examine the accuracy in diagnosing individual morphological features of the disease.

In a previous study[99] examined the value of MRI for staging of pelvic endometriosis. This study used a scoring system based on the rAFS with modifications to allow for MRI interpretation. They found a high degree of agreement between the MRI findings and operative findings (κ =0.916) with concordant staging in 42 of 44 patients. The authors recognised, however, that MRI is not a good test to diagnose adhesions or complete obliteration of the pouch of Douglas. In some cases endometriotic nodules were not seen on MRI and superficial disease was almost impossible to assess, which is similar to the results of our study using TVS. Although it is clear that both MRI and TVS are to some extent limited in the assessment of pelvic endometriosis, the ability of TVS to examine for the presence of adhesions directly using dynamic manipulation of the pelvic organs may be an important advantage over MRI.

Our study is limited by a lack of agreement regarding the classification of severe endometriosis. The rAFS classification, which we used in our study, does not provide a very accurate description of deep infiltrating endometriosis. Although this is clearly an important limitation, none of the other systems to assess the severity of endometriosis is widely used in clinical practice and for this reason we have used the most widely accepted standard, the rAFS classification. As has been described previously this is useful for fertility assessment but is less good at assessing the difficulty involved in complete resection. Our study is also limited by the fact that gynaecologists with significant experience of in diagnostic ultrasound and endometriosis performed all of the TVS. This makes the conclusions difficult to extrapolate to other centres where the level of experience may not be as high.

In conclusion, our study has shown that a targeted transvaginal ultrasound scan is an accurate test to diagnose severe pelvic endometriosis. This implies that in women with evidence of severe disease on ultrasound, a confirmatory diagnostic laparoscopy may not be required and these women could be referred directly to an expert minimally invasive endometriosis surgeon locally or a regional tertiary referral endometriosis centre. This approach could facilitate more effective triaging women of women with severe endometriosis resulting in shorter, safer, more rational, and cost effective management.

Chapter 9 Study 4

The value of symptomatology and tenderness in addition to TVS in the diagnosis of severity of pelvic endometriosis.

Introduction.

The clinical presentation of endometriosis is variable: some women experience several severe symptoms whilst others experience no symptoms at all and are diagnosed incidentally during a procedure such as laparoscopic sterilisation [100-102] . In addition, many of the symptoms of endometriosis such as dysmenorrhoea and deep dyspareunia are common in women of reproductive age and could be caused by a number of pathologies such as adenomyosis, adhesions, infections, or where no organic cause can be found.

A variety of studies have attempted to address the apparent lack of correlation between symptomatology and presence, location and severity of pelvic endometriosis on laparoscopy. Epidemiological data[2] suggests that dysmenorrhoea, deep dysparunia and non-cyclic pelvic pain are all more likely to occur in patients with endometriosis than in those without. In a large primary care based study[103] which included 5540 cases, with over 10000 controls, it was found that dysmenorrhoea, pelvic pain, dysparunia and menorrhagia were strongly correlated with a diagnosis of endometriosis and had odds ratios of 9.8, 13.5, 9.4 and 5.0 respectively.

However, the direct relationship between types of pain and types of lesion found is less clear [104-108]. No relationship was found between the ASRM stage of disease and the severity of pain[109] . Fauconnnier[82] found that severe dysmenorrhoea was associated with an increase in pouch of Douglas adhesions, dyspareunia with uterosacral ligament lesions, non cyclic pain with bowel lesions and dyschezia with deeply infiltrating endometriosis lesions (DIE) of the vagina. Koninckz[110] found that the depth of invasion of DIE related to severity of pain. Porpora [109] found that pain was only associated with ovarian endometriomas when adnexal adhesions were present.

No previous studies have examined if symptomatology can be used in conjunction with the findings at transvaginal ultrasound examination to improve the diagnostic pickup rate for pelvic endometriosis and reduce the rate of unnecessary laparoscopic procedures.

Aims

The aims of this study were:

- to examine if it is possible to predict the presence and severity of pelvic endometriosis from the symptomatology and tenderness at TVS
- to examine if the addition of symptomatology and tenderness at TVS can help in the diagnosis of pelvic endometriosis in patients who have false negative findings for pelvic endometriosis at transvaginal ultrasound examination, and in doing so establish which patients would benefit from laparoscopy in spite of a normal TVS result.

Methods and statistical analysis

The methodology for this study was similar to the previous chapters. The symptoms which are associated with pelvic endometriosis of dysmenorrhoea, deep dysparunia, non-cyclic pelvic pain, menorrhagia, infertility, painful defaecation (dyschezia) and cyclic rectal bleeding were recorded prospectively. Tenderness was assessed on TVS by the examiner asking the patient to inform them of any tenderness throughout the scan. These symptoms were then used to build logistic and multivariate regression models for the presence and stage of pelvic endometriosis respectively. Kruskal Wallis rank correlation was used to assess the correlation that each feature had with the stage of endometriosis at laparoscopy. Logistic regression analysis was used to assess the predictive value of each subjective feature for the presence of endometriosis. Multiple regression analysis was used to assess the predictive value of each subjective feature on the stage of endometriosis.

Results

In the period of 38 months from July 2006 to September 2009 we recruited 237 women into this study. 39 women were excluded from the final analysis: twenty nine because they were not assessed by one of the two designated ultrasound operators, five became pregnant whilst awaiting surgery, one cancelled her operation, one laparoscopy was unsuccessful and three women were lost to follow up.

198 women were included in the final analysis. The mean age was 35.0 (95% CI 33.98 – 35.97, SD 7.10) (range 19-50) years. The presenting symptoms were dysmenorrhoea for 143/198 (72.2%), chronic pelvic pain for 98/198 (49.5%),

dyspareunia for 91/198 (45.9%), infertility for 42/198 (21.2%) and dyschezia for 18/198 (9.6%) women. Tenderness was found on ultrasound examination in 57/198 (28.8%) of women. A single presenting symptom was present in 72 (36.4%) women, two presenting symptoms in 66 (33.3%), three presenting symptoms in 39 (19.7%), four or more symptoms in 19 (9.6%) women.

The ultrasound examinations were performed by two examiners: examiner A performed 104 (52.5%), examiner B 94 (47.5%). All patients were operated on by one of four laparoscopic surgeons: surgeon A operated on 79 (39.9%), surgeon B on 54 (27.3%), surgeon C on 35 (17.7%) and surgeon D on 30 (15.2%) women. The mean interval between TVS and operation was 36.8 days (95% CI 33.4 – 41.1, SD 22.9) (range 0-87 days).

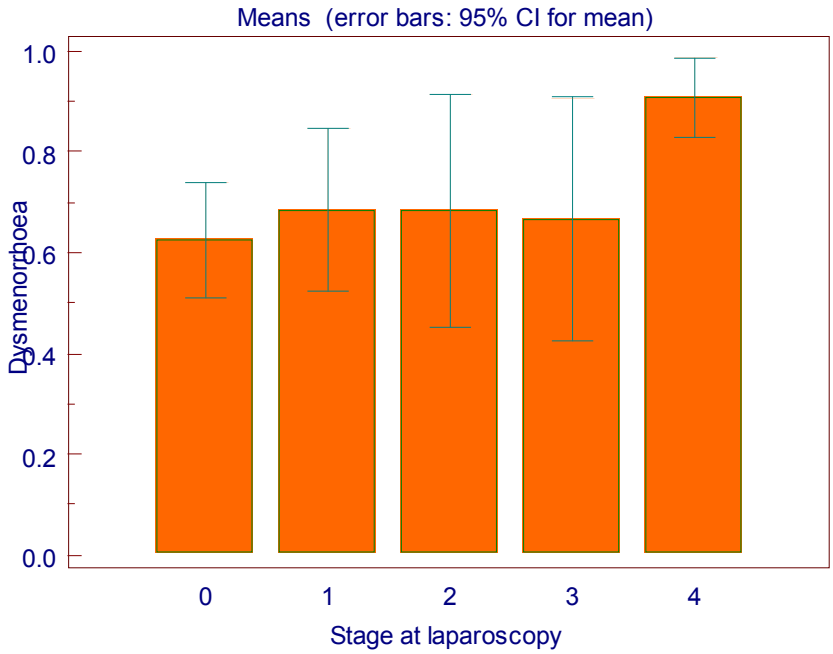
Table 17 shows the frequency of the symptoms and tenderness on TVS in relation to the ASRM stage of endometriosis. When the significance level (P) was calculated using the Kruskal- Wallis test for correlation only dyschezia and dysmenorrhoea were significantly associated. The association with infertility neared significance (P=0.053). Figure 16 shows the data from table 17 as bar graphs for each symptom and tenderness in relation to the stage of endometriosis.

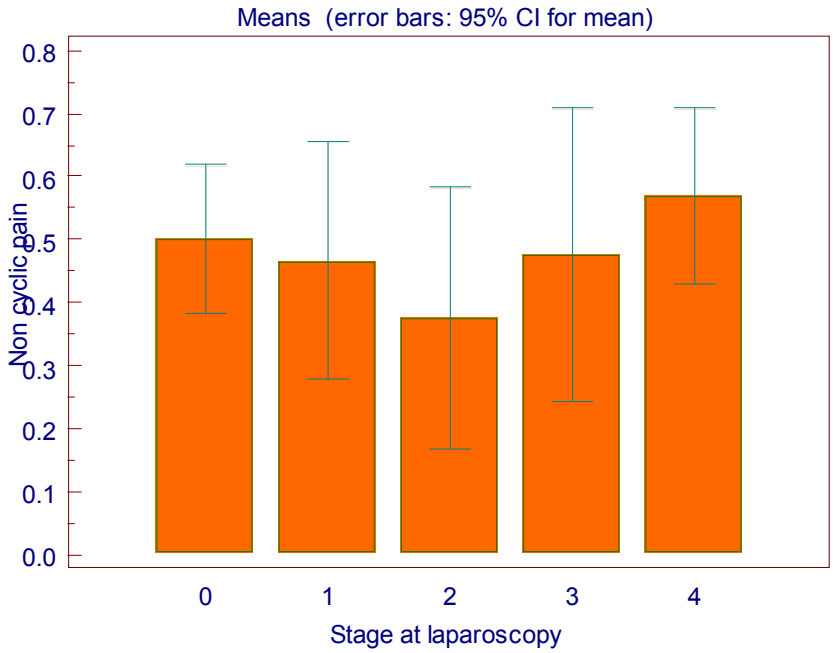
Table 17. The frequency of symptoms of endometriosis and tenderness on TVS in relation to ASRM stage of endometriosis. (Absent, minimal, mild, moderate or severe). P calculated using Kruskal- Wallis test for correlation.

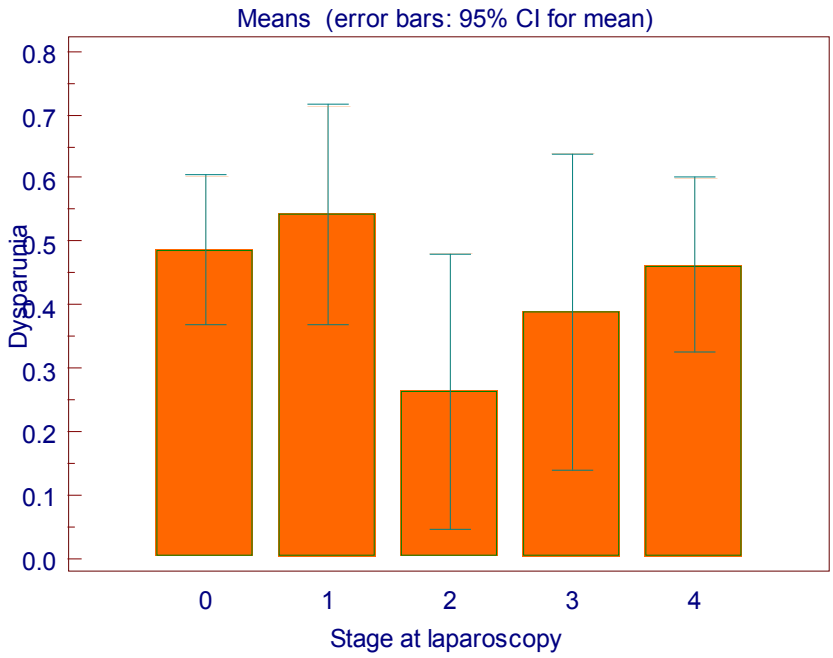
Stage	Dysmenorrhoea (n=143)	Non cyclic pain (n=98)	Dyspareunia (n=91)	Infertility (n=42)	Menorrhagia (n=29)	Dyschezia (n=18)	Tenderness (n=57)
0 (n=72)	45 /72 62.5%	36/72 50.0%	35/72 48.6%	11/72 15.3%	9/72 12.5%	1/72 1.4%	20/72 27.8%
1 (n=35)	24 /35 68.6%	16/35 45.7%	19/35 54.3%	7/35 20.0%	6/35 17.1%	5/35 14.3%	5/35 14.3%
2 (n=19)	13 /19 68.4%	7/19 36.8%	5/19 26.3%	2/19 10.5%	3/19 15.8%	0/19 0%	7/19 36.8%
3 (n=18)	13/18 72.2%	9/18 50.0%	8/18 44.4%	3/18 16.7%	3/18 16.7%	3/18 16.7%	7/18 38.9
4 (n=54)	48 /54 88.9%	30/54 55.6%	24/54 44.4%	19/54 35.2%	8/54 14.8%	9/54 16.7%	18/54 33.3%
P	0.0103*	0.6253	0.3456	0.0534	0.9388	0.0072*	0.2452

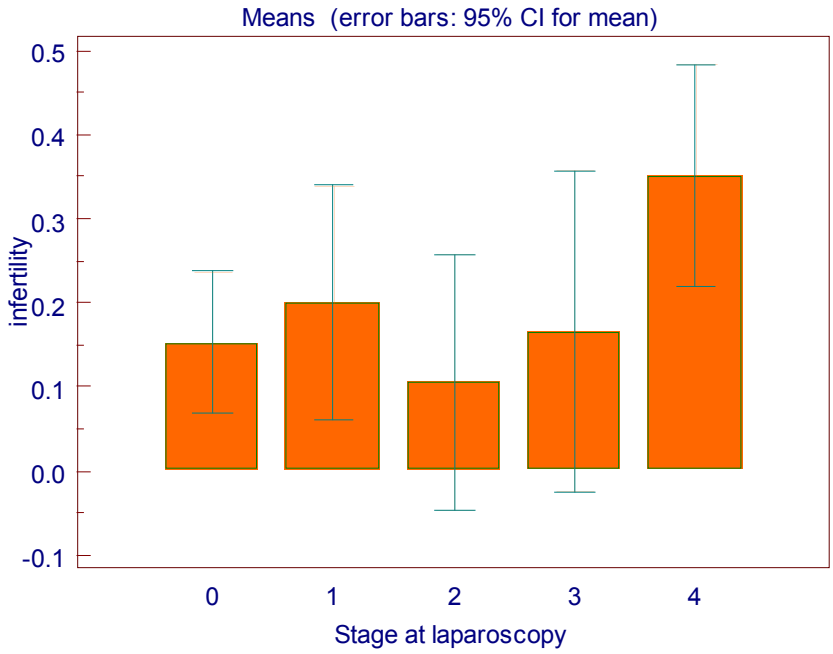
* = $P < 0.05$

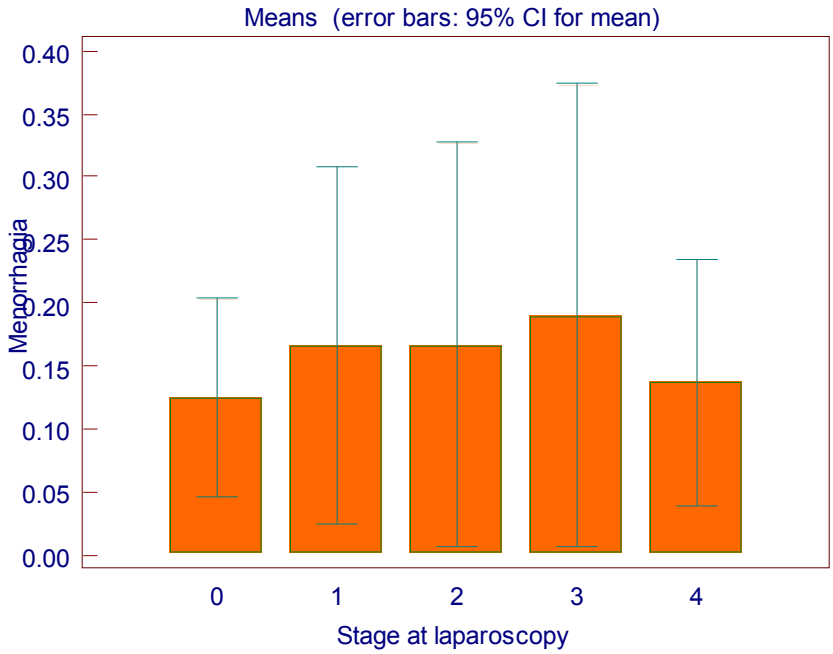
Figures 16-22 Bar graphs of mean number of patients who have each symptom and tenderness on TVS by stage at laparoscopy.

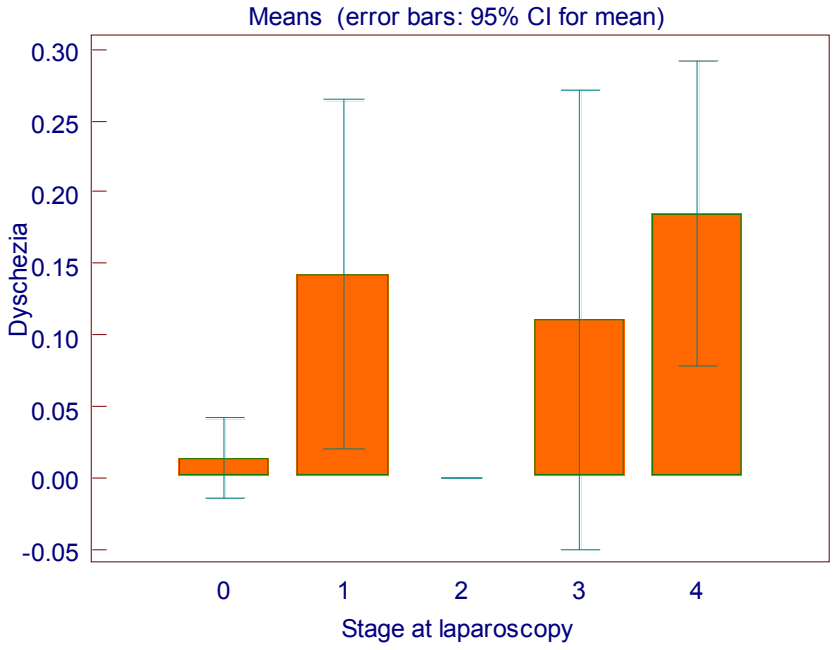

Means (error bars: 95% CI for mean)
Dysmenorrhoea
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Stage at laparoscopy


Means (error bars: 95% CI for mean)
Non cyclic pain
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
Stage at laparoscopy


Means (error bars: 95% CI for mean)
Dyspareunia
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
Stage at laparoscopy


Means (error bars: 95% CI for mean)
infertility
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
1
2
3
4
Stage at laparoscopy


Means (error bars: 95% CI for mean)
Menorrhagia
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
Stage at laparoscopy


Means (error bars: 95% CI for mean)
Dyschezia
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
1
2
3
4
Stage at laparoscopy

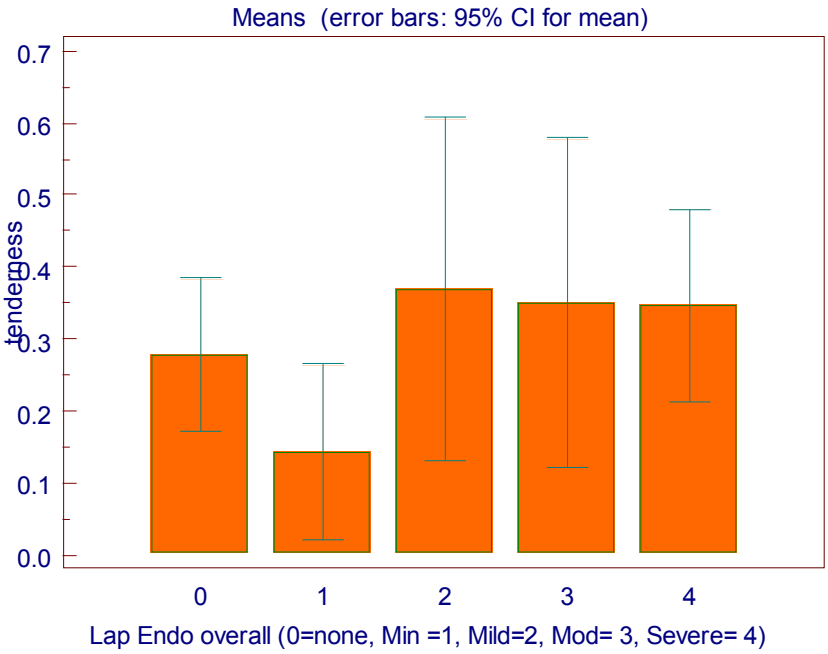

Means (error bars: 95% CI for mean)
tenderness
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
Lap Endo overall (0=none, Min =1, Mild=2, Mod= 3, Severe= 4)

Table 18 shows the multivariate regression for how the symptoms and tenderness on TVS correlate with the ASRM stage at laparoscopy. The presence in the history of dysmenorrhoea, dyschezia and infertility were all significantly associated with the stage of endometriosis. Dysparunia was negatively associated with the stage of endometriosis. Menorrhagia, pain when not menstruating and tenderness on TVS were not significantly associated with the stage of endometriosis.

Table 18 Results of multivariate analysis of the prediction of ASRM stage according to symptoms and tenderness on TVS.

	Coefficient	Std.Error	T	P
Constant	0.7463			
Dyschezia	0.9804	0.3994	2.454	0.0150*
Dysmenorrhoea	0.8681	0.2670	3.252	0.0014*
Dysparunia	-0.4697	0.2292	-2.049	0.0418*
Infertility	0.7876	0.2799	2.814	0.0054*
Menorrhagia	0.07125	0.3211	0.222	0.8246
Non cyclic pain	0.3607	0.2315	1.558	0.1208
Tenderness	0.4155	0.2556	1.626	0.1057

Dependent variable: ASRM stage $R2 = 0.1493$ ($P < 0.001$).
* = P< 0.05

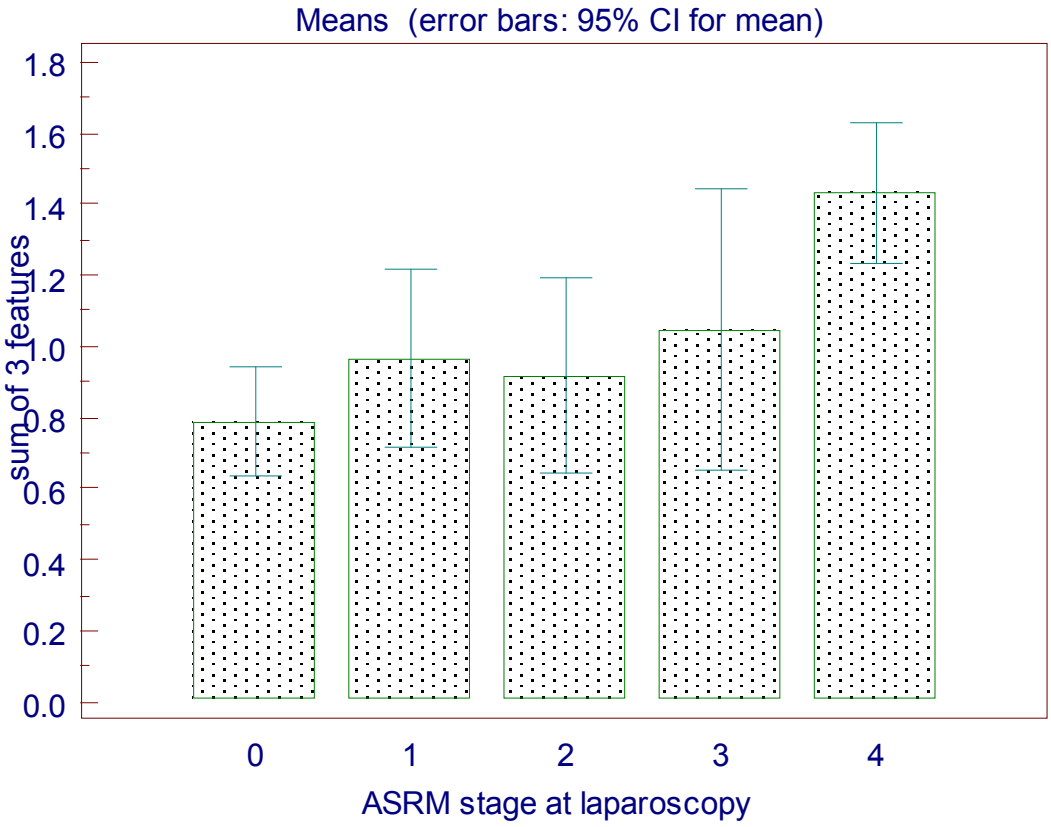
Table 19 shows the frequency of the sum of the presence of dysmenorrhoea, infertility and dyschezia in relation to the stage of endometriosis at laparoscopy.

Table 19. Table of the stage at laparoscopy and the numerical sum of the presence of dysmenorrhoea, infertility, and dyschezia (sum of 3 symptoms).

Sum of 3 symptoms	Stage at laparoscopy				
	0 (n= 72)	1 (n= 35)	2 (n= 19)	3 (n= 18)	4 (n= 54)
0 (n= 46)	24	9	5	5	3
1 (n= 101)	39	18	12	8	24
2 (n= 48)	9	8	2	4	25
3 (n= 3)	0	0	0	1	2

Figure 2 is a bar graph of the mean of the sum of the presence of dysmenorrhoea, infertility, and dyschezia (sum of 3 features). The coefficient of correlation was 0.3279 (95% CI 0.1974 to 0.4469) P<0.0001. The addition of non cyclic pelvic pain (ie the sum of 4 features) gave a coefficient of correlation = 0.3253 (95% CI 0.1947 to 0.4446) P<0.0001. As the coefficient of correlation was less with the addition of cyclic pelvic pain this has not been included in the table. Although there is a strong correlation between the sum of these symptoms and the stage this is only suggestive clinically as there was considerable overlap between the stages. However, all three symptoms were only present in stage 3 and 4 disease. Also with none of these symptoms present there was a 24/46 (52%) chance of having no disease found. With 2 of these symptoms there was a 25/48 (52.1%) chance of having stage 4 disease.

Figure 23. Bar graph of the correlation between the stage at laparoscopy and the mean of the numerical sum of the presence of dysmenorrhoea, infertility, and dyschezia (sum of 3 features).



Coefficient of correlation = 0.3279 (95% CI 0.1974 to 0.4469) P<0.0001
The addition of non cyclic pelvic pain (ie the sum of 4 features) gave a coefficient of correlation = 0.3253 (95% CI 0.1947 to 0.4446) P<0.0001.

Table 20 shows the results of logistic regression analysis for the presence or absence of endometriosis on ultrasound. This shows that only dyschezia was a significant predictor for the presence of endometriosis on laparoscopy.

Table 20 Results of logistic regression analysis for the symptoms and tenderness on TVS with respect to presence of endometriosis at laparoscopy.

Symptoms	Coefficient	Std.Error	P	Odds Ratio	95% CI
Constant	-0.06166				
Dyschezia	2.4312	1.0569	0.02143*	11.3721	1.4328 to 90.2587
Dysmenorrhoea	0.6506	0.3625	0.07267	1.9167	0.9419 to 3.9003
Dysparunia	-0.4851	0.3209	0.1306	0.6156	0.3282 to 1.1546
Infertility	0.6395	0.4103	0.1190	1.8956	0.8482 to 4.2362
Menorrhagia	0.2377	0.4585	0.6041	1.2684	0.5164 to 3.1153
Non cyclic pain	0.1566	0.3302	0.6353	1.1695	0.6122 to 2.2341
Tenderness on TVS	0.05935	0.3569	0.8679	1.0611	0.5272 to 2.1358

As TVS has a low sensitivity especially when assessing minimal and mild endometriosis we attempted to establish if the symptomatology and tenderness could improve upon the sensitivity of TVS for endometriosis. We took all of the patients who did not have endometriosis found at TVS and assessed to establish if the addition of the symptomatology and tenderness on TVS would improve the diagnosis of both the presence or the stage of disease. We found that neither the symptoms nor tenderness on TVS were predictive of the presence or stage of endometriosis in patients who had a negative TVS. In addition only the presence of tenderness on TVS showed a significant increase in frequency with stage of disease in these patients.

When the predictive value of the symptoms and tenderness were assessed using logistic regression for the presence of individual locations for deeply infiltrating endometriosis (DIE) we found significant associations between: rectovaginal disease and dysmenorrhoea and tenderness; pouch of Douglas obliteration and infertility and dysmenorrhoea; pelvic side wall disease and menorrhagia; uterosacral ligament DIE and tenderness. Dysparunia was negatively associated with DIE of any location. There were no significant predictors for utero vesical fold, bladder or bowel DIE. These findings are detailed in table 21 and summarised in table 22.

Table 21. Results of logistic regression analysis for significant predictors of deeply infiltrating endometriosis (DIE) in various locations.

Location of DIE	Predictor	Coefficient	Std.Error	P	Odds Ratio	95% CI for OR
DIE of any location	Dysparunia	-0.7290	0.3714	0.04965	0.4824	0.2330 to 0.9989
Rectovaginal septum	Dysmenorrhoea	1.3799	0.6729	0.0403	3.9744	1.0629 to 14.8610
	Tenderness	1.1197	0.5047	0.0265	3.0640	1.1395 to 8.2387
Pouch of Douglas partial or complete obliteration	Dysmenorrhoea	1.2795	0.5183	0.01357	3.5950	1.3016 to 9.9293
	Infertility	1.7487	0.4623	0.0001552	5.7469	2.3224 to 14.2212
	Tenderness	0.8815	0.4563	0.05336	2.4146	0.9873 to 5.9054
Pouch of Douglas complete obliteration	Dysmenorrhoea	1.6271	0.7939	0.04043	5.0889	1.0735 to 24.1231
	Infertility	1.9247	0.5569	0.0005476	6.8530	2.3008 to 20.4123
Pelvic Side Wall	Menorrhagia	1.7907	0.8943	0.04523	5.9938	1.0387 to 34.5866
Uterosacral ligament	Tenderness	1.0475	0.5217	0.04464	2.8506	1.0254 to 7.9251

There were no significant predictors for utero vesical fold, bladder or bowel DIE.

Table 22. Results showing positive and negative predictors of deeply infiltrating endometriosis (DIE) in various locations.

Location Of DIE	Dyschezia	Dysmenorrhoea	Dysparunia	Infertility	Menorrhagia	Non cyclic pain	Tenderness
Any location			-ve				+
Bladder							
Bowel							
RVS		+					+
POD partial or complete		+		+			+/-
POD complete		+		+			
PSW					+		
USL							+
UVF							

Discussion

This study has shown that the frequency of dysmenorrhoea, infertility and dyschezia are associated with the stage of endometriosis at laparoscopy according to the ASRM classification. In addition the sum of these three symptoms is strongly associated with the stage of disease. However, our findings show that deep dysparunia, non cyclic pelvic pain, menorrhagia and tenderness on TVS were not associated with the stage of endometriosis at laparoscopy nor with the presence or absence of endometriosis at laparoscopy. In addition none of these features helps improve the TVS pickup rate of minimal and mild disease in women who have a negative TVS.

These findings are in contrast with Porpora[109] who showed that the severity of symptoms did not correlate with ARSM stage of disease. However, they were using visual analogue scores to assess the severity of the symptoms rather than the presence of individual symptoms.

However, although the frequency of certain symptoms does increase with the stage of disease there is still a moderate frequency of these symptoms (apart from dyschezia) in women without endometriosis. In our data set only a history of dysmenorrhoea with infertility and dyschezia is strongly predictive of moderate to severe disease. A combination of two of these symptoms makes a diagnosis likely but by no means certain. This means that a detailed history of symptomatology alone will only increase the likelihood of disease if these symptoms are present but will not allow for an accurate diagnosis of the presence or stage of endometriosis based on history alone. Also the small numbers of patients in our dataset means that firm conclusions are difficult to be certain of without validating the findings with a larger dataset.

When the symptoms were correlated with individual locations of deeply infiltrating endometriosis (DIE) there were some strong associations. We did not find that dyschezia was a predictor of any location of DIE. This is in contrast to Fauconnier[82] who found that dyschezia was related to DIE of the vagina. In this study they found a correlation between deep dysparunia and uterosacral ligament disease (USL). In contrast our data showed a negative correlation between deep dyparunia and USL involvement but this was not statistically significant. Deep dysparunia was common in the women found not to have any endometriosis at surgery. This may be explained by the complex nature of sexual symptoms. The only finding which was significantly associated with USL involvement was tenderness during the TVS examination. Dysmenorrhoea and infertility were strong predictors of partial and complete pouch of Douglas obliteration. This agrees with the findings of Fauconnier[82] who found an association with dysmenorrhoea and pouch of Douglas obliteration.

Unfortunately when all of the patients who were correctly diagnosed with endometriosis on TVS were removed from the analysis in order to assess the benefit of the addition of symptomatology to TVS we did not find any strong associations. This means that there is minimal benefit offered by assessing the symptoms over and above a detailed TVS by a gynaecologist with expertise in assessing pelvic endometrioisis. However, a detailed history is always worth assessing as for sonographers who are less familiar with assessing severe endometriosis a history of dysmenorrhoea, infertility and dyschezia would suggest that there is likely to be significant endometriosis and will make the diagnosis easier. Experienced ultrasound

operators will also benefit from taking a full symptom history in that they will have a good idea of what they will find before starting the ultrasound assessment.

One of the major limitations with this study is that the symptoms and assessment of tenderness were assessed by the Gynaecologist who was performing the ultrasound. This is a potential source of inaccuracy and it would have been more reliable if the patients had filled out detailed validated quality of life questionnaires including a detailed assessment of symptoms. It would also have been better if patients were asked to give a visual analogue score for tenderness in various locations during the TVS examination. Both of these measures would have reduced potential sources of bias.

Conclusions

The frequency of dysmenorrhoea, dyschezia and infertility increased with the ASRM stage of endometriosis. The presence of two or more of these symptoms is suggestive of advanced endometriosis. The overall number of symptoms also increases with stage. Dyspareunia was negatively associated with the stage of endometriosis. Menorrhagia, pain when not menstruating and tenderness on TVS were not significantly associated with the stage of endometriosis. Certain symptoms are associated with certain locations of DIE. The symptomatology does not help in diagnosing minimal and mild endometriosis in patients who have no signs of endometriosis found on TVS.

Chapter 10 Study 5

The use of serum CA125 in addition to TVS for the diagnosis of presence and severity of endometriosis when compared with laparoscopy.

Introduction

It has been shown in the previous chapters that transvaginal ultrasound is valuable for the preoperative diagnosis of more advanced endometriosis. The addition of subjective aspects of assessment such as patient symptomatology and tenderness on examination with the transvaginal probe do not add significantly to the accuracy of the ultrasound diagnosis. Serum markers have been investigated for their role in the evaluation of endometriosis with limited results [63,64,66,67,71,111-129]. No single serum test or combination of tests has as yet given a highly sensitive and specific test to stage or exclude pelvic endometriosis. The elevation of cancer antigen 125 (CA125) in association with endometriosis has been know about since the mid 1980s[63,130] Cancer antigen 125 is a high molecular weight mucin glycoprotein which is encoded by the MUC16 gene. It was first discovered by Bast in 1981[56]. This protein plays a role as a lubricating barrier against foreign particles and infectious agents on the surface of many epithelial cells. This protein is expressed by a few normal tissues such as the endometrium, fallopian tube epithelium, lung parenchyma and cornea[131]. When there is rapid production or turnover of these cells, for example due to inflammation or malignancy, this surface antigen in released into the blood. This has given rise to its use in the detection of ovarian cancer. However, any condition which

causes irritation of the peritoneum, pericardium or pleura can elevate the levels. Benign conditions which increase the production of CA125 include pregnancy, fibroids, pelvic inflammatory disease, congestive cardiac failure with pleural effusions and endometriosis.

The association between raised CA125 levels and pelvic endometriosis has been know since 1986 and since then many studies have attempted to elucidate the exact relationship and clinical usefulness of this test. A meta-analysis performed by Mol[63] found 23 studies which investigated the use of CA125 as a non invasive test for the presence and stage of endometriosis. The sensitivies of these studies ranged between 0.16 and 1.00 and the specificities ranged between 0.38 and 1.00. The meta analysis found that measurement of CA125 was more accurate at diagnosing stage 3-4 disease than stage 1-2. In addition there was difficulty in the meta – analysis in finding the CA125 level at which the accuracy is greatest over all 23 studies. The higher the level then the higher the specificity will be but sensitivity will be sacrificed. However most of the quoted studies used a CA125 level of 35 IU/ml as a cutoff level. No studies have used CA125 in conjunction with TVS to diagnose the presence and severity of pelvic endometriosis.

Aim

To assess the value of serum CA125 for the diagnosis of presence and severity of endometriosis and to assess its role in improving the diagnosis of endometriosis at TVS.

Methods

This prospective observational study was conducted at King's College Hospital Early Pregnancy and Gynaecology Assessment Unit. Subjects were recruited consecutively from April 2007 to September 2008. The study was approved by the Ethics Committee and the Research & Development Committee for this hospital.

Patients

Consecutive women with chronic pelvic pain, defined as pelvic pain for more than six months duration, who were due to be admitted for a diagnostic laparoscopy were asked to donate a blood sample. Patients were recruited from the Early Pregnancy and Gynaecology Assessment Unit at King's College Hospital. The inclusion criteria were: all women referred with a clinical suspicion of endometriosis and booked for a diagnostic laparoscopy; written informed consent obtained; aged 16 or over. Exclusion criteria were: previous proven endometriosis at laparoscopy or other pelvic surgery; unable to comprehend study and provide informed consent.

An information leaflet about the study was given to all eligible patients before assessment. Written informed consent was obtained from all patients who agreed to take part in the study.

Procedure

Data, including age, parity and gravidity and the name of the examining doctor were recorded. A detailed clinical history was recorded including symptomatology associated with endometriosis such as dysmenorrhoea (painful periods), dysparunia

(painful sexual intercourse), menorrhagia (heavy or prolonged periods), dyschezia (painful defecation), infertility (for greater than one year), and cyclic rectal bleeding.

Women were given a blood form and asked to return to have the blood taken from a peripheral vein on day 1 of the menstrual cycle (the first day of bleeding). If day one of the menstrual cycle was on a Saturday or a Sunday they were asked to come on the Monday. Blood samples were taken by the phlebotomy service of King's College Hospital. Blood was Centrifuged at was centrifuged at 300 rpm for 15 mins and separated into six aliquots. Within two hours of collection samples were stored at -30^{o}C and transferred to –70^{o}C within 24 hours. The samples were analysed for CA125 with the Immuno-1 analyser (Bayer diagnostics, Tarrytown, NY, USA). The exact method used in the analysis of these samples is in the appendix.

A database file was set up using Microsoft Excel for Windows spreadsheet to facilitate data entry and retrieval. Univariate analyses were carried out using the MedCalc software package using the Mann–Whitney test and Kruskal–Wallis test with Dunn's multiple comparison test. Additionally, receiver operating characteristic (ROC) curves were constructed.

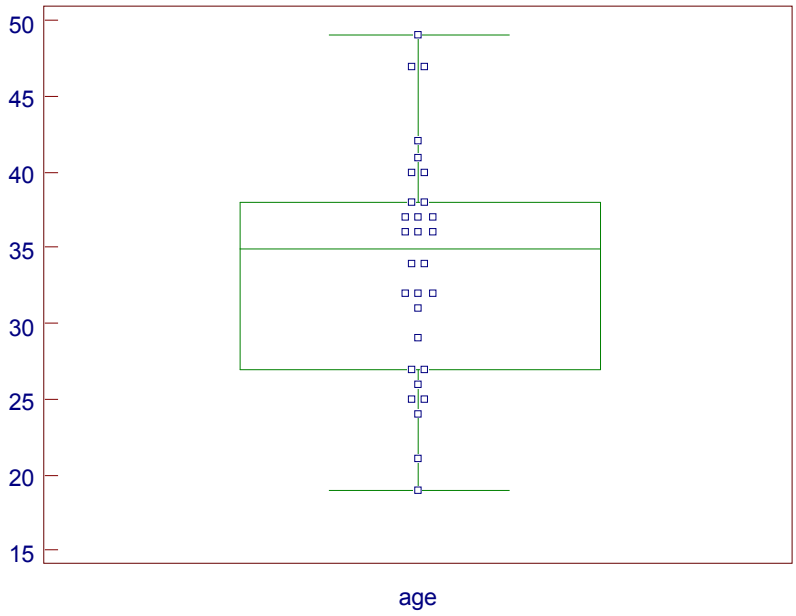
All patients had their laparoscopies performed at Kings College Hospital. The severity of endometriosis was recorded in accordance with the revised American Fertility Society guidelines1985 [7]. This includes a detailed description of the presence and extent of endometriosis affecting the peritoneum, ovaries, tubes, and pouch of Douglas. Each element is given a score depending on severity. The operating surgeons were blinded to the serum biochemical marker results.

Results

67 patients were recruited. Of these 30 patients returned for their serum to be sampled on day 1-3 of the menstrual cycle. 37 patients did not return to have serum sampled and are excluded from the analysis. These were analysed on the day of collection but the results were sent to the research team only.

The mean age of the patients was 34.0 years (95% CI 29.8 to 37.6) with a range of 19 to 49 years and standard deviation of 7.62 years.

Figure 24. Box and whisker plot of age.



The correlation coefficient for the CA125 values vs the ASRM stage of endometriosis, the ASRM score and the presence or absence of endometriosis, is shown in table 23. This shows that there was a statistically significant correlation

between the CA125 level and the stage of endometriosis but not with the presence or absence of disease. However the Mann-Whitney test (table 24) for difference between the mean of two groups shows a significant difference in the values between the two groups.

Table 23. Results of correlation coefficient coefficient data for CA125 compared with the ASRM stage and score and the presence or absence of endometriosis.

CA125 correlated with:	Correlation coefficient r	95% CI for r	P value
ARSM stage at laparoscopy (0-4)	0.5651	0.257 to 0.768	0.0011
ASRM score at laparoscopy	0.4336	0.087 to 0.687	0.0167
Presence of endometriosis (N/Y)	0.2744	-0.095 to 0.578	0.1423

Figure 25 box plot of Log CA125 results separated into two groups: patients with endometriosis present and those without endometriosis at laparoscopy.

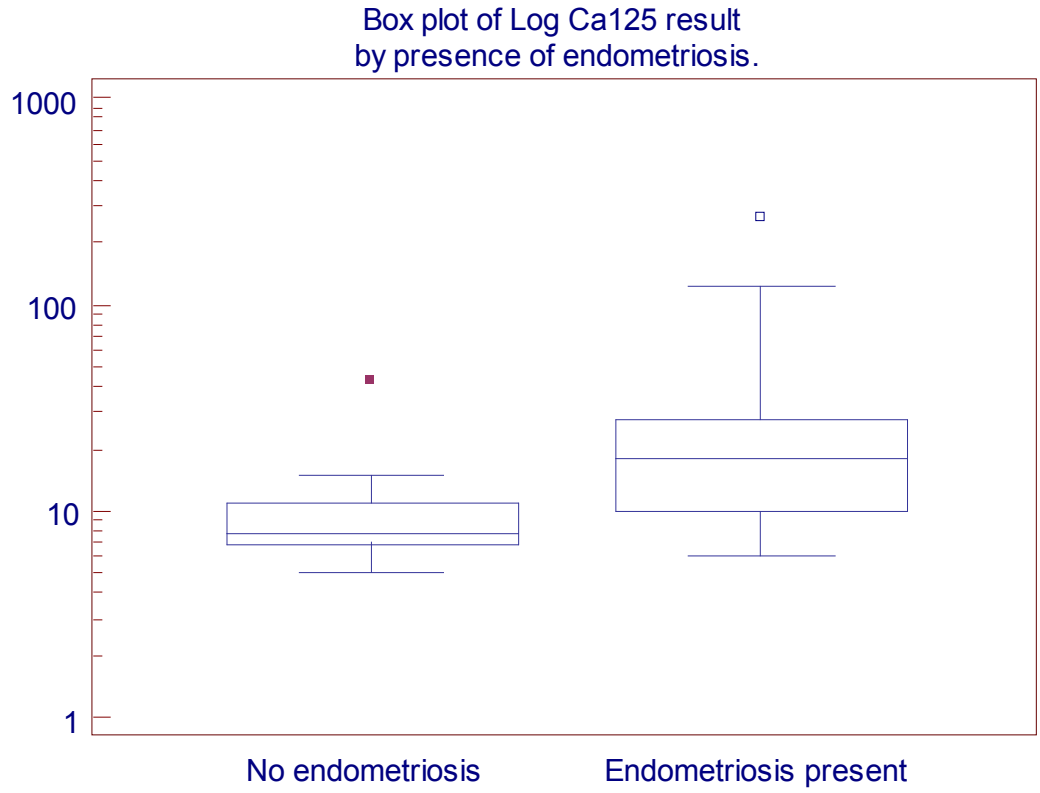


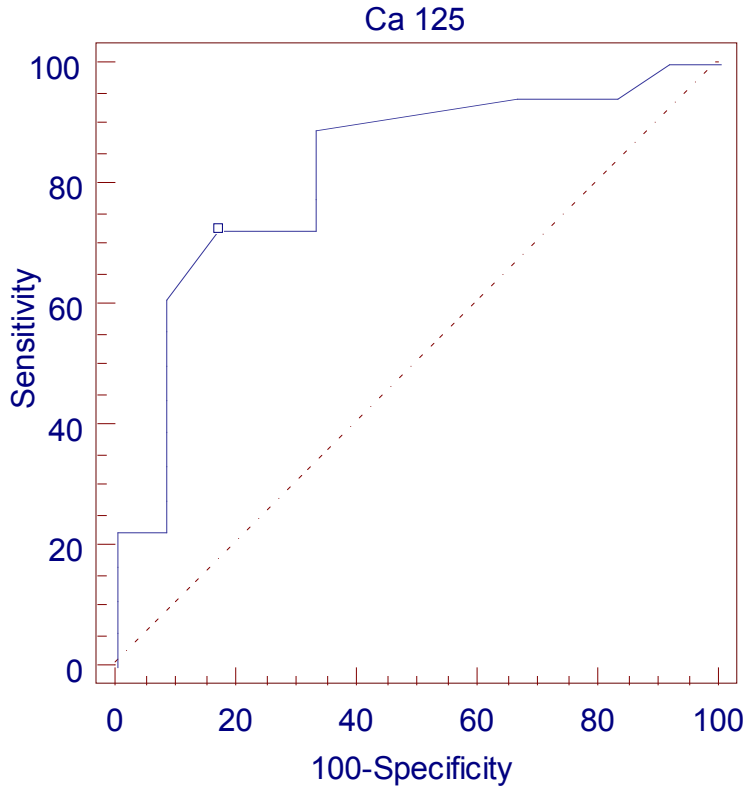
Table 24. Results of Mann-Whitney test for difference between those with endometriosis and those without.

	Patients without endometriosis	Patients with endometriosis
Sample size	12	18
Lowest value	5	6
Highest value	43	266
Median	8	18
95% CI for the median	6.55 to 12.82	9.69 to 33.25
Interquartile range	7 to 11	10 to 28
Two-tailed probability		P=0.0033

Table 25. Results of CA125 values and coordinates on the ROC curve.

CA125 value	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=5	100.00	81.3 - 100.0	0.00	0.0 - 26.6	1.00	
>5	100.00	81.3 - 100.0	8.33	1.4 - 38.5	1.09	0.00
>6	94.44	72.6 - 99.1	16.67	2.6 - 48.4	1.13	0.33
>7	94.44	72.6 - 99.1	33.33	10.1 - 65.1	1.42	0.17
>8	88.89	65.2 - 98.3	66.67	34.9 - 89.9	2.67	0.17
>9	77.78	52.4 - 93.5	66.67	34.9 - 89.9	2.33	0.33
>10	72.22	46.5 - 90.2	66.67	34.9 - 89.9	2.17	0.42
>11 *	72.22	46.5 - 90.2	83.33	51.6 - 97.4	4.33	0.33
>15	61.11	35.8 - 82.6	91.67	61.5 - 98.6	7.33	0.42
>16	55.56	30.8 - 78.4	91.67	61.5 - 98.6	6.67	0.48
>17	50.00	26.1 - 73.9	91.67	61.5 - 98.6	6.00	0.55
>19	44.44	21.6 - 69.2	91.67	61.5 - 98.6	5.33	0.61
>21	38.89	17.4 - 64.2	91.67	61.5 - 98.6	4.67	0.67
>22	33.33	13.4 - 59.0	91.67	61.5 - 98.6	4.00	0.73
>23	27.78	9.8 - 53.5	91.67	61.5 - 98.6	3.33	0.79
>28	22.22	6.5 - 47.6	91.67	61.5 - 98.6	2.67	0.85
>43	22.22	6.5 - 47.6	100.00	73.4 - 100.0		0.78
>45	16.67	3.8 - 41.4	100.00	73.4 - 100.0		0.83
>54	11.11	1.7 - 34.8	100.00	73.4 - 100.0		0.89
>122	5.56	0.9 - 27.4	100.00	73.4 - 100.0		0.94
>266	0.00	0.0 - 18.7	100.00	73.4 - 100.0		1.00

Figure 26. ROC curve for the CA125 value in relation to the diagnosis of the presence of endometriosis.



The highest point of accuracy is marked by the point which corresponds to a CA125 level of >11 U/ml. Area under ROC curve = 0.822 (95% CI 0.639 to 0.936) P=0.0001.

From table 25 it can be seen that for a level of CA125 above 8 U/ml this gives a sensitivity of 88.9 % and a specificity of 66.7 %. This would mean that at this cut off level approximately one patient would be missed in every ten with endometriosis and out of three patients who were offered a laparoscopy two patients would have endometriosis.

When the stage of endometriosis was considered there was a significant increase in CA125 levels with the stage, Kruskal-Wallis rank correlation P=0.0099.

When the CA125 data was used to assess for stage 3 or 4 endometriosis (Figure 28) the results were more accurate than assessing for just the presence of disease. The results (table 26) show that for a cut off level of CA125 >11 U/ml the sensitivity of detecting stage 3 to 4 disease would be 100% and the specificity would be 65.2%. Therefore at this cut off level no patients with moderate to severe disease would be missed and approximately 3 laparoscopies would be performed to pick up two cases of moderate to severe disease.

Figure 27. Scatter plot of log CA125 result in relation to the ASRM stage at laparoscopy.

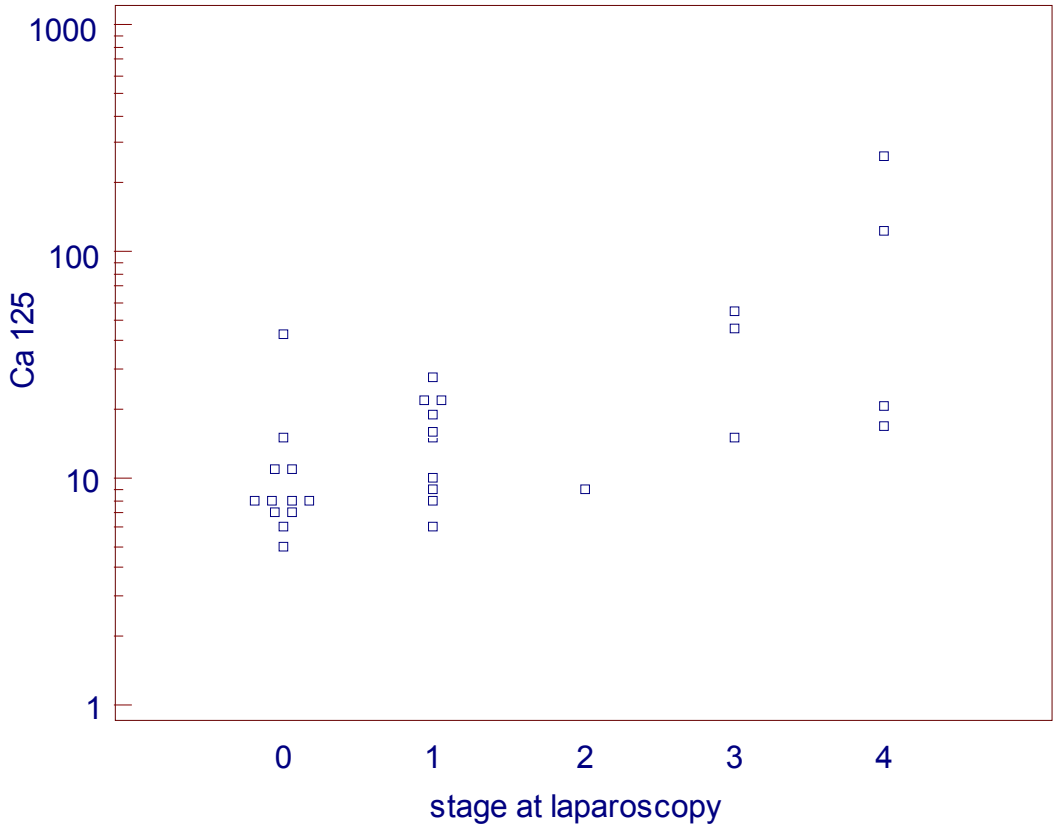
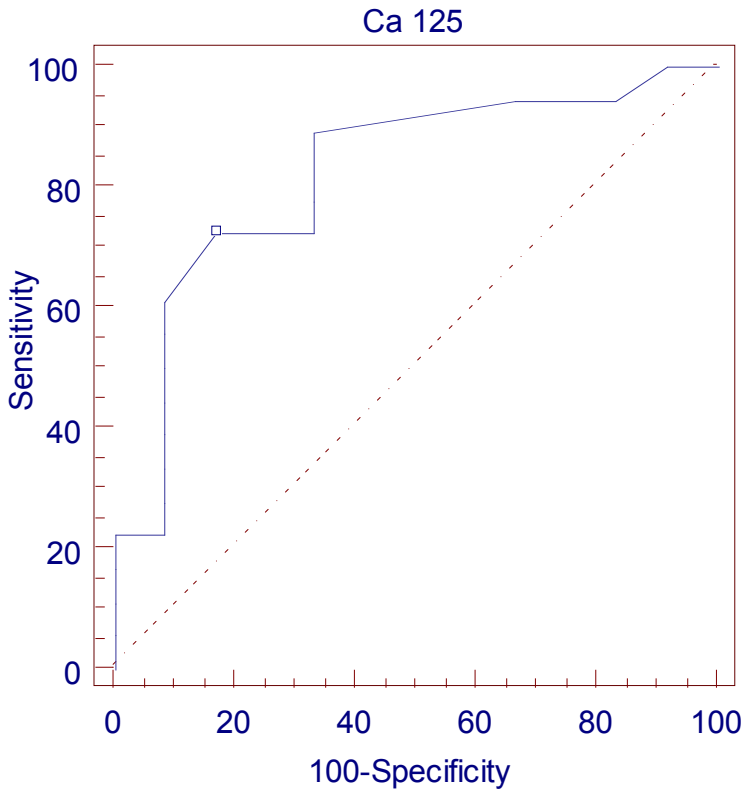


Figure 28. ROC curve for the CA125 value in relation to the diagnosis of the presence of stage 3 or 4 endometriosis.



The highest point of accuracy is marked by the point which corresponds to a CA125 level of >11 U/ml. Area under ROC curve = 0.901 (95% CI 0.735 to 0.978) P=0.0001.

Table 26. Results of CA125 values and coordinates on the ROC curve.

CA125 value	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=5	100.00	58.9 - 100.0	0.00	0.0 - 15.0	1.00	
>5	100.00	58.9 - 100.0	4.35	0.7 - 22.0	1.05	0.00
>6	100.00	58.9 - 100.0	13.04	2.9 - 33.6	1.15	0.00
>7	100.00	58.9 - 100.0	21.74	7.5 - 43.7	1.28	0.00
>8	100.00	58.9 - 100.0	43.48	23.2 - 65.5	1.77	0.00
>9	100.00	58.9 - 100.0	52.17	30.6 - 73.2	2.09	0.00
>10	100.00	58.9 - 100.0	56.52	34.5 - 76.8	2.30	0.00
>11 *	100.00	58.9 - 100.0	65.22	42.7 - 83.6	2.87	0.00
>15	85.71	42.2 - 97.6	73.91	51.6 - 89.7	3.29	0.19
>16	85.71	42.2 - 97.6	78.26	56.3 - 92.5	3.94	0.18
>17	71.43	29.3 - 95.5	78.26	56.3 - 92.5	3.29	0.37
>19	71.43	29.3 - 95.5	82.61	61.2 - 94.9	4.11	0.35
>21	57.14	18.8 - 89.6	82.61	61.2 - 94.9	3.29	0.52
>22	57.14	18.8 - 89.6	86.96	66.4 - 97.1	4.38	0.49
>23	57.14	18.8 - 89.6	91.30	71.9 - 98.7	6.57	0.47
>28	57.14	18.8 - 89.6	95.65	78.0 - 99.3	13.14	0.45
>43	57.14	18.8 - 89.6	100.00	85.0 - 100.0		0.43
>45	42.86	10.4 - 81.2	100.00	85.0 - 100.0		0.57
>54	28.57	4.5 - 70.7	100.00	85.0 - 100.0		0.71
>122	14.29	2.4 - 57.8	100.00	85.0 - 100.0		0.86
>266	0.00	0.0 - 41.1	100.00	85.0 - 100.0		1.00

However all of the cases of stage 3 and 4 disease in this study were correctly diagnosed on TV ultrasound scan and therefore the added benefit for these patients of an additional test is minimal. We wanted to establish if patients who have no abnormality found at TVS would benefit from a serum test to improve the pick up rate of endometriosis. The patients who had disease positively identified on scan were excluded from the data set and then the data was analysed as follows.

Positive diagnosis of endometriosis on scan excluded.

Three patients had stage 3 disease and 4 patients had stage 4 disease. These were excluded from the results and this left 23 patients. 10 of these patients had stage 1-2 disease found at laparoscopy and 13 had no disease found. The results in figure 6 and table 4 show that when the correctly diagnosed stage 3 and 4 patients were removed from the analysis CA125 does not perform as well at distinguishing between those who have endometriosis and those without. However, a CA125 result cut off of > 8 U/ml would give a sensitivity of 70% and a specificity of 53 % for detecting stage 1 and 2 disease and therefore this would give more confidence to recommend laparoscopy for these patients.

Figure 29. Scatter plot of log CA125 result with stage of endometriosis at laparoscopy.

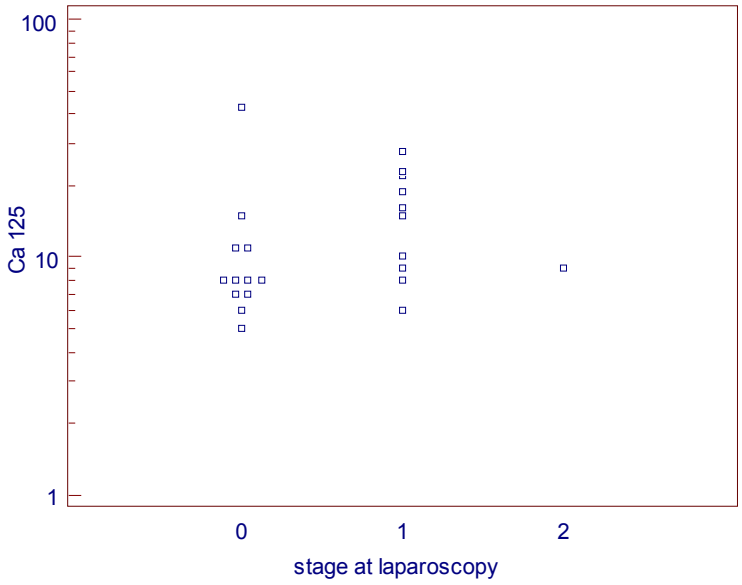


Figure 30. Box and whisker plot of all patients with a negative scan. Plot of log CA125 result with stage at laparoscopy.

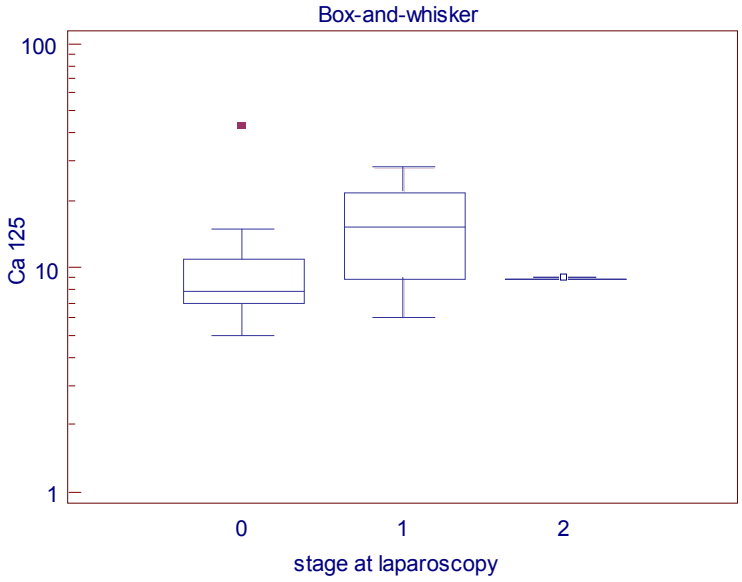
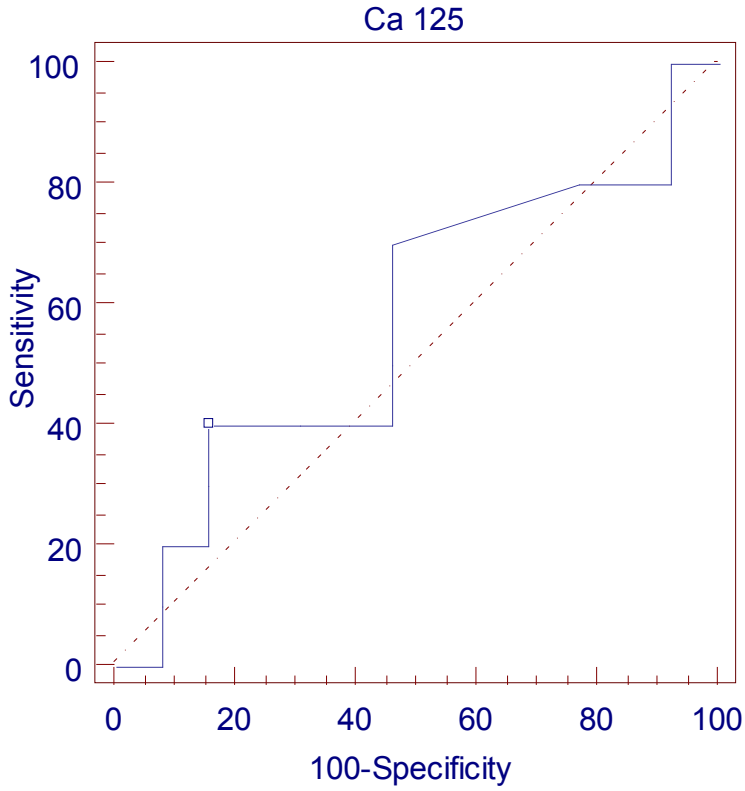


Figure 31. ROC curve for the CA125 value in relation to the diagnosis of the presence of Stage 1 and 2 endometriosis (Patients correctly diagnosed at TVS excluded).



The highest point of accuracy is marked by the point which corresponds to a CA125 level of >15 U/ml. Area under ROC curve = 0.569 (95% CI 0.349 to 0.771) P=0.5745.

Table 27. Results of CA125 values and coordinates on the ROC curve.

CA125 value	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=5	100.00	69.0 - 100.0	0.00	0.0 - 24.9	1.00	
>5	100.00	69.0 - 100.0	7.69	1.3 - 36.1	1.08	0.00
>6	80.00	44.4 - 96.9	7.69	1.3 - 36.1	0.87	2.60
>7	80.00	44.4 - 96.9	23.08	5.3 - 53.8	1.04	0.87
>8	70.00	34.8 - 93.0	53.85	25.2 - 80.7	1.52	0.56
>10	40.00	12.4 - 73.6	53.85	25.2 - 80.7	0.87	1.11
>15 *	40.00	12.4 - 73.6	84.62	54.5 - 97.6	2.60	0.71
>19	20.00	3.1 - 55.6	84.62	54.5 - 97.6	1.30	0.95
>22	20.00	3.1 - 55.6	92.31	63.9 - 98.7	2.60	0.87
>28	0.00	0.0 - 31.0	92.31	63.9 - 98.7	0.00	1.08
>43	0.00	0.0 - 31.0	100.00	75.1 - 100.0		1.00

Discussion

Our results have shown a significant association (P=0.0011) between the ASRM stage of endometriosis and the CA125 level. There was also a significant difference between the mean CA125 levels in those patients with endometriosis and those without, however there was considerable overlap in the results between the two groups therefore making the clinical usefulness uncertain. Our results also show a reasonable accuracy of diagnosing stage 3 and 4 endometriosis with a sensitivity of 100% and specificity of 65.2% at a CA125 cut off level of >11 (level of greatest accuracy) and a sensitivity of 85.7% and specificity of 78.3% at a CA125 cut off level of >16. At a CA125 cut off level of 35 the sensitivity would be 57.1% with and specificity of 95.7%. For the presence of disease at any stage at a cut off level of CA125 >11 the sensitivity is 72.2% and the specificity is 83.3 and at a cut off level of >35 the sensitivity is 22.2% and the specificity is 91.7%.

These results are in agreement with the Meta-analysis performed by Mol[63] (Ref Mol) which showed that the CA125 is a better discriminator for stage 3 and 4 disease than stage 1 and 2 disease. In this study the test's performance in diagnosing all disease stages was limited: the estimated sensitivity was only 28% for a specificity of 90% (corresponding likelihood ratio of a raised level is 2.8). The test's performance for moderate–severe endometriosis was better: for a specificity of 89%, the sensitivity was 47% (corresponding likelihood ratio of a raised level is 4.3). However, most of the studies included in this meta-analysis used a CA125 cut off level of 35IU/ml.

An interesting study by Kafali[64] looked at 28 patients who were having laparoscopies for infertility. CA125 levels in the menstrual phase of the cycle were compared with

CA125 levels in the rest of the cycle. They found that there was no difference in the patients who did not have endometriosis but there was a large difference in patients who did have endometriosis. Using a cutoff point of an 83% increase in CA125 levels between the menstrual and the non menstrual phase of the cycle gave sensitivity of 93% and a specificity of 92% specificity. All of the endometriosis found was either stage 1 or 2. This study however has small numbers and the results would need to be repeated in larger studies before the clinical usefulness could be established.

Since then there have been studies to further investigate the role of CA125 in the diagnosis of deeply infiltrating endometriosis as separate from ovarian disease. In a cohort study by Patrelli[65] serum CA-125 values were significantly elevated in patients with ovarian and mixed endometriosis lesions (median levels 48 U/mL), compared with those who had exclusively extraovarian foci (median levels 27 U/mL), however the location did not affect the subsequent fertility rate after 2 years of follow up. However, all of the included patients had proven endometriosis and 94% of their patients had stage three or four disease which suggests that this study population was more typical of a tertiary referral centre and the results are not applicable to the general population with suspected endometriosis due to the symptoms.

However, when assessing the additional benefit that CA125 gives to the ultrasound diagnosis our results did not show any benefit. This was partly because all of the stage 3 and 4 disease was correctly diagnosed by TVS and therefore the CA125 level could not improve on the false negative rate for stage 3 and 4 disease. It was also due to the fact that the CA125 levels in patients with stage 1 and 2 disease was not

statistically higher than CA125 levels for patients with no endometriosis (P= 0.056). There was also significant overlap in the ranges of the CA125 results for these two groups. This agrees with the results of the Mol meta-analysis which showed a poor ability to exclude the absence of endometriosis with CA125.

One study which has shown promise in excluding endometriosis on a serum test is by Mihalyi[72] . In this cohort study they tested for IL6 and 8, tumour necrosis factor-alpha, high-sensitivity C-reactive protein (hsCRP), and cancer antigens CA-125 and CA-19-9. Using stepwise logistic regression, moderate–severe endometriosis was diagnosed with a sensitivity of 100% (specificity 84%) and minimal–mild endometriosis was detected with a sensitivity of 87% (specificity 71%) during the secretory phase. Using LSSVM analysis, minimal–mild endometriosis was diagnosed with a sensitivity of 94% (specificity 61%) during the secretory phase and with a sensitivity of 92% (specificity 63%) during the menstrual phase. Their aim was to provide a test which would have a high sensitivity and moderate specificity therefore enabling women without endometriosis to avoid having an invasive laparoscopy unnecessarily. However, these tests were performed on stored samples and it remains to be seen whether these complicated multi-marker models can improve patient management in a clinical setting.

Conclusions

This study has shown that CA125 levels are associated with the presence and stage of endometriosis at laparoscopy. However the diagnostic accuracy of TVS is not improved by the addition of CA125 to the model. The numbers in this study were small and further investigation is needed to establish if the difference between CA125

levels in the mid proliferative phase and menstrual phase of the cycle, or a multi marker test with a sophisticated statistical model, could improve on the differentiation between patients who have no disease and those who have early or advanced disease.

Chapter 11: Conclusions and further research

The main aim of this thesis was to show that targeted transvaginal ultrasound (TVS) has the potential to correctly distinguish moderate and severe endometriosis from minimal or mild. This would enable accurate triaging of patients with severe disease to specialist centres where advanced level surgery enabling complete excision would be possible, without the need for unnecessary diagnostic surgery. This may also reduce the long delay that many women experience between the onset of symptoms and receiving effective treatment. This thesis was successful in showing in studies 1-3 that TVS, when performed by ultrasound operators with the appropriate experience, is an accurate and reproducible method for diagnosing moderate and severe disease and therefore enabling the successful triaging of patients. The research methods were in agreement with the STARD criteria for diagnostic tests[132].

The other benefits to the patient of this ability are: to guide patient choice regarding medical or surgical treatment; to plan fertility or medical treatment if surgery is not chosen; to enable referral to the most appropriate centre and surgeon if surgery is chosen; to enable the surgeon to counsel the patient about the likely extent of surgery and potential risks; and to allow the surgeon to prepare sufficiently for surgery including the involvement of other specialties as indicated.

Further research would be helpful to validate these findings in different clinical settings with larger numbers of patients and different patient populations. Future research would also be helpful to show that patient outcomes are improved by the application of ultrasound based triaging in terms of time interval to definitive treatment, reduction in diagnostic laparoscopy, patient satisfaction with the treatments offered and cost effectiveness.

However although it is possible to accurately diagnose moderate and severe endometriosis using TVS the accuracy for diagnosing minimal and mild endometriosis is poor. This is due to the fact that superficial peritoneal deposits of endometriosis are too small to see on ultrasound. Currently laparoscopy would be the only was to assess these patients which is expensive, has risks and causes inconvenience and pain. Therefore the secondary aim of this thesis was to assess if it is possible to add any tests or aspects from the history in order to improve the diagnosis of minimal or mild endometriosis either on their own or in conjunction with TVS. In this respect the thesis was less successful than in the primary aim.

In study 4 symptomatology was assessed and this showed that although the frequency of some symptoms increases with increasing severity there is significant overlap in symptomatology between patients with no endometriosis and patients with severe endoemtriosis. This means that symptomatology alone can only give a suggestion of the presence of disease but in not reliable. When symptoms were added to the assessment of patients who did not have endometriosis found on TVS there was no additional benefit found.

Further research into the severity of the symptoms with larger numbers of patients and a more accurate description of their symptoms including visual analogue scores for severity may further our understanding of the benefit of the symptomatology on its own and as an addition to ultrasound.

Study 5 assess if the inclusion in the model of serum CA125 levels with TVS would be helpful. I found that although serum CA125 levels as associated with the presence and stage of endometriosis at laparoscopy the diagnostic accuracy of TVS is not improved by the addition of CA125 to the model. The numbers in this study were

small and further investigation is needed to establish if the difference between CA125 levels in the mid proliferative phase and menstrual phase of the cycle, or a multi marker test with a sophisticated statistical model, could improve on the differentiation between patients who have no disease and those who have early or advanced disease.

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Appendix

Patient information sheet Version 3 dated Dec 2006

Please take some time to read this information sheet. You might need to discuss its content during your consultation.

1. **Study title**

The accuracy of gynaecological ultrasound examination for the diagnosis of severe pelvic endometriosis.

2. **Invitation paragraph**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, LONDON, N16 0BW.

3. **What is the purpose of the study?**

To assess the accuracy of diagnosing severe endometriosis on pelvic ultrasound scan. Women who have been referred to hospital with features of endometriosis such as heavy periods, pelvic pain, reduced fertility and pain with sexual intercourse will be investigated for pelvic endometriosis. This often involves an ultrasound scan and a diagnostic laparoscopy. If from your history your symptoms are suggestive of endometriosis you will be offered the opportunity to take part in a study investigating the accuracy of ultrasound in the diagnosis of severe endometriosis. Those women who join the study will be offered a specialist ultrasound scan performed by an expert in this area who will record detailed scan findings prior to the laparoscopy. A blood test will also be performed just after your period starts. By comparing the detailed ultrasound findings and blood test results with the findings at the operations the accuracy of the ultrasound scans can be calculated. More accurate tests help patients and their Doctors plan their care better.

4. **Why have I been chosen?**

You have been referred to this hospital with some of the features of endometriosis such as heavy periods, pelvic pain, reduced fertility and pain

with sexual intercourse. Based on your symptoms and an examination you have been offered an operation to help in the diagnosis and treatment of your symptoms. An expert ultrasound scan and blood tests will be performed to look for any signs of endometriosis in the pelvis. The operative findings can be compared to the ultrasound scan findings to assess the accuracy of the ultrasound scan findings.

5. Do I have to take part?

It is up to you to decide whether or not to take part in the study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

6. What will happen to me if I take part?

The research will take part from January 2006 until February 2008. You will be taking part in an observational study. This means that your care will not change whether you chose to take part in the study or not. All patients taking part will be offered an ultrasound examination at King's College Hospital prior to their operation. In addition a blood sample will be taken during early menstruation. Thereafter, your care will be followed up during your hospital stay or at another clinic appointment.

7. What do I have to do?

By participating in this study, you will not have any additional responsibilities. However, you are required to follow any relevant information regarding your treatment, which will be given to you at the assessment clinic. Your recovery period, following surgical intervention, can vary from 1 week to 2 weeks.

8. What is the procedure that is being tested?

We are testing the accuracy of ultrasound scanning and a blood test at detecting the severity of endometriosis.

9. What are the alternatives for diagnosis or treatment?

An ultrasound scan is usually the first method of investigation when there is suspicion of endometriosis. There are many alternatives for treatment including contraceptives (such as the pill, certain types of coil and other contraceptions which help with period pain) and non contraceptive medicines including tablets and injections. This will be discussed with you in more detail by your gynaecologist.

10. What are the side effects of taking part?

You should not expect to have side effects by taking part in this study. Ultrasound scanning is safe and radiation-free. Taking part in the study will not change your care in any way. We will simply be recording your care in slightly more detail than normal in order to help improve the care we can offer.

11. What are the possible disadvantages and risks of taking part?

There are no disadvantages to taking part in this study.

12. What are the possible benefits of taking part?

There are no direct advantages to taking part in the study but you will be making a contribution to medical science. This should lead to improvements in care in the future.

13. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the method that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to discontinue. If you decide to discontinue in the study, you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw from the study. These reasons will be explained and arrange for your care to discontinue. An analysis of the data collected will be carried out after one year of starting, to monitor the efficiency of the study.

14. What happens when the research study stops?

The study is planned to terminate in February 2008. The findings and recommendations will be discussed and published in a medical journal. If the research stops earlier than planned, for reasons out of our control, your future care will not be affected.

15. What if something goes wrong?

It is not anticipated that this would directly result in harm being caused to any patients. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to make a complaint about any aspect of the way you have been

approached or treated during the course of this study, the National Health Service complaints procedure will be made available to you.

16. Will my taking part in this study be kept confidential?

We will inform your General Practitioner of your participation in the study unless instructed otherwise by you. All information collected about you during the course of the study will be kept strictly confidential. The data will be stored on password-protected computers in a locked room. Any information, which leaves the hospital, will have your personal details removed, so your identity cannot be recognised.

17. What will happen to the results of the research study?

The results of the study will be discussed and analysed to make recommendations. We are hoping to publish the outcome of this research by December 2008 in a medical journal. Your identity will not be disclosed in any publication.

18. Who is organising and funding the research?

The study is funded by the Departments of Obstetrics and Gynaecology at King's College Hospital.

19. Who has reviewed the study?

Research & Development and Research Ethics Committee at Kings College Hospital.

20. Contact for further information

Should you need further information about the study, please do not hesitate to contact me at the following address:

Dr Tom Holland,
Suite 8, 3rd floor,
Golden Jubilee Wing,
King's College Hospital,
Denmark Hill,

London

SE5 9RS

Thank you for taking part in this study.

A copy of the information sheet and a signed consent for participating in the study will be given for your record.

Consent form version 2 Dec 2006

King's College Hospital
Consent Form for Diagnosis of severity of endometriosis study

Patient agreement to study participation

Patient details

Patient's surname/family name……

Patient's first names ……

Date of birth ……

Responsible health professional

Job title …… ……

NHS number (or other identifier)…….

Female

Special requirements……
(e.g. other language/other communication method)

One copy to be retained in patient's notes

and one copy to be given to the patient

CONSENT FORM

Centre number: ……

Study number: ……

Patient Identifier number

For this trial: ……

NB Three copies should be made, for (1) patient, (2) researcher, (3) hospital notes

Title of Project:

The accuracy of gynaecological ultrasound examination for the diagnosis of severe pelvic endometriosis.

Name of Researcher: *Tom Holland*

Please initial box

1. *I confirm that I have read and understand the information* sheet dated June 2006 (version 2.) for the study "the accuracy of gynaecological ultrasound examination for the diagnosis of severe pelvic endometriosis" and have had the opportunity to ask questions. ☐

2. *I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.* ☐

3. *I understand that sections of any of my medical notes may be looked at by responsible individuals from Kings College Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.* ☐

4. *I understand that a blood sample will be kept to be used for research into the diagnosis of pelvic pain and endometriosis.* ☐

5. *I agree to take part in the above study and for my GP to be informed.* ☐

Name of Patient	*Date*	*Signature*
Name of Person taking consent	*Date*	*Signature*
Researcher	*Date*	*Signature*

Serum markers blood form

KING'S CLINICAL BIOCHEMISTRY - TRIAL FORM				
SURNAME	**FORENAME**		**TRIAL** **Endometriosis Study**	
UNIT NUMBER **(55442)_____**	**CONSULTANT** **Mr Jurkovic (JURD)**	**WARD/CLINIC** **To be Collected (TBC)**	**D.O.B.**	**SEX**
SPECIMEN REQUIREMENTS **2 clotted samples**		**INVESTIGATIONS REQUIRED** **CA125** **(Save 5 serum aliquots in freezer)**		
DATE	**TIME**	**NAME** **Tom Holland**	**BLEEP No.**	

Ethical committee approval

King's College Hospital Research Ethics Committee
Camberwell Building
King's College Hospital
94 Denmark Hill
London SE5 9RS
Email: Janet.Browning@kingsch.nhs.uk
Tel: 020 7346 3923 Fax 020 7346 5085

20 July 2006

Mr Davor Jurkovic
Consultant
King's College Hospital
Denmark Hill
London SE5 9RS

Dear Mr Jurkovic

Full title of study: **The accuracy of gynaecological ultrasound examination for the diagnosis of severe pelvic endometriosis.**
REC reference number: **06/Q0703/119**

The King's Research Ethics Committee considered your project at its meeting on 19th July 2006. We are happy to grant it ethical approval. We do, however, think the Patient Information Sheet should indicate somewhere that the ultrasound is to be performed transvaginally. We wish you well with your study.

The favourable opinion applies to the research sites listed on the attached form. However, all researchers and local research collaborators who intend to participate in this study at NHS sites should notify the R&D Department for the relevant care organisation and seek research governance approval.

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully. Please note that a condition of approval is that a progress report is submitted annually.

The documents reviewed and approved at the meeting were:

Document	*Version*
Application	form signed 22/06/2006
Investigator CV	
Protocol	Version 1 dated June 2006
GP/Consultant Information Sheets	Version 1 dated June 2006
Participant Information Sheet	Version 1 dated June 2006
Participant Consent Form	Version 1 dated June 2006

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/Q0703/119 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr David Jewitt
Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
Standard approval conditions
Site approval form (SF1)

Copy to: R&D Department

King's College Hospital Research Ethics Committee

Attendance at Committee meeting on 19 July 2006

Committee Members:

Name	Profession	Present?	Notes
Dr David Jewitt	Consultant Cardiologist	Yes	
Dr Brian O'Connor	Consultant Chest Physician	No	
Dr Colin Ball	Consultant Paediatrician	No	
Mr Chris Barrass	Director of Pharmacy	No	
Ms Barbara Beckles	Senior Performance and Policy Officer	No	
Dr Nora Donaldson	Head of Clinical Research Statistics	No	
Professor John Garrett	Professor Emeritus, Oral Pathology	Yes	
Professor David Scott	Honorary Consultant Rheumatologist	Yes	
Mr Evan Stone QC	Barrister	Yes	
Dr Julia Wendon	Consultant Physician	No	
Mr Kai Lok Chan	Assistant Director of Pharmacy	Yes	
Mr Juan Gonzalez	Assistant Head of Research Statistics	No	
Rev David Rushton	Hospital Chaplain	No	
Professor A J Strong	Professor of Neurosurgery & Consultant Neurosurgeon	Yes	
Dr Luke Zander	Retired GP	No	
Ms Mandy Skinner	Observer	Yes	
Mrs Cathy Walton	Consultant Midwife	Yes	
Dr Will Bernal	Consultant Liver Intensivist	Yes	
Ms Janet Browning	Research Ethics Committee Coordinator	Yes	
Mr Edward Martin	Statistician/Observer	Yes	

Also in attendance:

Name	Position (or reason for attending)

King's College Hospital Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	06/Q0703/119	**Issue number:**	2	**Date of issue:**	20 July 2006
Chief Investigator:	Mr Davor Jurkovic				
Full title of study:	The accuracy of gynaecological ultrasound examination for the diagnosis of severe pelvic endometriosis.				

This study was given a favourable ethical opinion by King's College Hospital Research Ethics Committee on 19 July 2006. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* [1]
Mr Davor Jurkovic		Kings College Hospital, London	King's College Hospital Research Ethics Committee	20/07/2006	

Approved by the Chair on behalf of the REC:

..... (Signature of ~~Chair~~/Administrator)
(delete as applicable)

J BROWNING (Name)