Non-invasive methods of diagnosis of endometriosis
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Purpose of review
Laparoscopy is the gold standard for the diagnosis of endometriosis but the need for visual evidence of the disease is a major stumbling-block for both effective clinical management of affected patients as well as for research into this common and debilitating reproductive disease. Laparoscopy is invasive and often causes a delay in diagnosis and treatment, especially in symptomatic teenagers and young women. Moreover, the visual inspection of the pelvis has major limitations, particularly for the diagnosis of retroperitoneal lesions. It is therefore not surprising that considerable efforts are being made to improve imaging techniques and to evaluate the diagnostic value of potential molecular markers of disease.

Recent findings
High-resolution transvaginal ultrasonography and, in selected cases, magnetic resonance imaging improve the diagnosis of retroperitoneal pelvic endometriosis as well as the identification of lesions that involve pelvic organs. A variety of serum and endometrial markers are being evaluated for their diagnostic potential, particularly in endometriosis associated infertility. The first gene profiling studies are showing positive results and proteomic technology is being applied to identify novel diagnostic protein expression patterns.

Summary
Current imaging techniques, such as transvaginal ultrasonography, are useful to screen the pelvis for the presence of retroperitoneal endometriosis but fail to diagnose peritoneal lesions, small ovarian endometriomas and adhesions. Postgenomic technologies and identification of novel serum and endometrial markers are likely to revolutionize future diagnosis of endometriosis.

Keywords
endometriosis, ultrasound, magnetic resonance imaging, serum, endometrial markers

Introduction
Accurate non-invasive diagnostic techniques are urgently needed if the clinical management of women with endometriosis is to be more effective. It has been shown that the younger the patient is at the onset of symptoms, the longer it takes before the diagnosis of endometriosis is made [1*]. Transvaginal ultrasonography (TVS), magnetic resonance imaging (MRI) and endometrial and serum markers have the potential to improve the diagnosis and can be useful in the follow-up of patients. Endometriosis research has also entered the post-genomic era and powerful genomic and proteomic technology is being applied in the search for novel diagnostic approaches. This review focuses on recent studies evaluating diagnostic imaging techniques and describes the development of molecular markers for the diagnosis of endometriosis.

Diagnostic imaging of endometriotic lesions
With the current practice of combining diagnostic and operative procedures in laparoscopy, the risk exists that unexpected preoperative findings may lead to undertreatment or unnecessary surgery. Imaging techniques are therefore becoming increasingly important to determine preoperatively the presence and extent of the surgical pathology. Whenever possible with the use of non-invasive techniques, the decision to operate or not should be based on the accurate preoperative diagnosis and proper assessment of the extent of the disease. Several publications during the last year have contributed to an improved preoperative diagnosis of the extent of deep, adenomyotic endometriosis.

Superficial pelvic endometriosis and ovarian endometriomas
Superficial pelvic endometriosis and ovarian endometriomas are predominantly hemorrhagic lesions. During laparoscopy, these lesions are readily identified on the basis of the presence of old or recent bleeding. Both TVS and MRI have a low sensitivity for the diagnosis of peritoneal endometriotic implants and adhesions. In a systematic review, Moore et al. [2*] concluded that TVS is useful in the diagnosis of ovarian endometriomas, if the diameter is 20 mm or more. As TVS costs less than MRI, the ultrasonographic technique may be the preferred method of confirming a sizeable endometrioma [3].

Recto-vaginal and recto-sigmoid endometriosis
Retroperitoneal endometriosis represents a dominantly nodular, myoproliferative lesion interspersed with a
sparse amount of glandular and stromal tissue and microendometriomas. Similar to uterine adenomyosis, these lesions have no capsule and are in continuity with the surrounding fibromuscular or muscular structures. Recto-vaginal septum endometriosis is a misnomer as involvement of this septum rarely occurs. The nodule can eventually reach the upper extremity of the rectovaginal septum, but on MRI the septum invariably appears distinct and regular \[4\]. The lesion frequently extends laterally into the parametrium and, if larger than 3 cm, may involve the ureters \[5\]. The physical examination to evaluate the extent of the involvement of the posterior pelvic organs remains inadequate \[6,7\] and further investigations are required.

Sonovaginography is described as a new technique using the instillation of saline in the vagina in combination with TVS to improve the localization, extension and infiltration of rectovaginal endometriosis, which are important elements for the choice of appropriate surgery (Table 1) \[8\]. Chapron and collaborators \[4\] recently described the magnetic resonance appearance of rectovaginal endometriotic nodules in eight affected patients. On \(T_1\)-weighted images the signal intensity of rectovaginal nodule is isointense to the myometrium with hyperintensive spots remaining visible in the fat suppressed sequences, indicating the presence of microendometriomas. On the \(T_2\)-weighed images the signal intensity of the nodules is isointense or hypointense to the myometrium with hyperintensive spots. The nodules have an irregular contour and are indistinguishable from the uterovaginal structures. In some cases a hyperintensity transition zone can be identified between the rectum and the nodule which has been termed the ‘safety margin’. In other cases, this safety margin is not seen and thickening of the rectum wall is noticed. The safety margin is likely to represent interposing fat tissue. The retraction between the torus uterinum, the endometriotic nodule and the rectum results in obliteration of the pouch of Douglas.

In a small study, Koga et al. \[9\] detected infiltrating rectosigmoid endometriosis through a combination of transvaginal and transrectal ultrasonography. The hypoechoic irregular-shaped area corresponded to a layer of hypertrophic muscular propria of the lesion, while the hyperechoic rim represented the layer including the mucosa, submucosa and serosa. These lesions may be constricting or may produce an eccentric intraluminal filling defect, resembling colon carcinoma. It is important to note that, unlike colon carcinoma, endometriosis does not breach the bowel mucosa or cause mucosal ulcerations. Bazot et al. \[7\] compared in a series of 30 symptomatic patients the accuracy of TVS and rectal endoscopic sonography (RES) for the diagnosis of posterior pelvic endometriosis involvement and found TVS as efficient as RES (Table 1). However, the main limitation of TVS is its inability to determine the exact distance of the rectal lesions from the anal margin or to evaluate the depth of rectal wall involvement. Therefore, TVS can be recommended as a screening tool if recto-vaginal endometriosis is suspected, while RES is reserved for cases in which colorectal involvement is suspected, and prior to surgery.

**Bladder endometriosis**

Nodular bladder endometriosis is not easily palpable at vaginal examination. Typically it is found in patients with dysmenorrhea associated with urinary symptoms such as micturition frequency. TVS may reveal a solid nodule within the posterior bladder wall if the bladder is slightly filled. Colour Doppler studies may detect low to moderate vascularity and mild pressure with the vaginal probe often elicits focal pain \[10\].

In a series of 12 patients with nodular bladder endometriosis, varying between 10 and 31 mm in diameter, TVS was normal in four patients, but MRI, using a body coil, enabled visualization of the lesions in all patients \[11\]. Furthermore, the use of an endocavitary coil was found superior to a body coil in determining the extent of infiltration of the bladder wall. In contrast, Vercellini \[12\] reported that MRI did not yield additional information when compared with ultrasonography.

<table>
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<th>Reference</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
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<td>RES [7]</td>
<td>82</td>
<td>88</td>
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PPV, positive predictive value; NPV, negative predictive value; TVS, transvaginal ultrasonography; SVG, sonovaginography; RES, rectal endoscopic sonography.
Obstructive uropathy secondary to endometriosis

Ureteral obstruction is an infrequent but serious complication of deep pelvic endometriosis. According to some textbooks the proportion of lesions located on the left side is higher than on the right side, but this was not confirmed by a recent study [5*].

Diaphragmatic endometriosis

Although there are some case reports on the diagnosis of diaphragmatic endometriosis using computed tomography and MRI, Redwine [13] found, in a series of eight patients, that imaging scans were of little clinical use because a negative scan did not exclude the presence of diaphragmatic lesions.

Endometrial and serum markers

Endometrial and serum markers for the diagnosis of endometriosis are increasingly taking advantage of novel strategies in the search for markers of diseases. This is reflected by the innovative techniques described in recent publications.

Endometrial markers

Kitawaki and co-workers [14] reported that detection of aromatase P450 protein in endometrial biopsy samples strongly correlates with the presence of endometriosis or adenomyosis. The authors suggested that this approach could be used as an outpatient screening test for endometriosis, with a sensitivity and a specificity of 91% and 100%, respectively. However, in a prospective study, Dheenadayalu et al. [15*] reported that endometrial aromatase P450 messenger RNA expression, detected by reverse transcriptase–polymerase chain reaction and Southern blot analysis, is not confined to women with endometriosis but is also associated with most hormone-dependent proliferative disorders of the uterus, including leiomyomata, adenomyosis, and proximal tubal disease. As a diagnostic marker for endometriosis, P450arom messenger RNA expression yielded a sensitivity of 82%, a specificity of 59%, a positive predictive value of 76%, and a negative predictive value of 67%. If additional uterine pathology was taken into account, the sensitivity increased to 84%, the specificity to 72%, the positive predictive value to 87%, but the negative predictive value remained unchanged (67%). The authors concluded that although endometrial aromatase P450 messenger RNA expression is predictive of the presence of pelvic disease, the relative high incidence of false negatives and lack of specificity are likely to impair clinical application.

Serum markers

There continues to be considerable interest in the serum markers for endometriosis. Harada et al. [16*] investigated the clinical value of the serum levels of CA-19-9 versus CA-125 and suggested that CA-19-9 is a useful marker for the severity of the disease.

A recent report suggested that serum levels of interleukin-6, with a cut-off level of 2 pg/mL, could discriminate between patients with and without endometriosis [17]. Similarly, Matarese et al. [18] reported that increased leptin levels in serum and peritoneal fluid of patients with pelvic endometriosis. The authors suggested that the proinflammatory and neoangiogenic actions of this adipocyte-derived helical cytokine might contribute to the pathogenesis of endometriosis. However, follow-up studies have failed to confirm that the presence of pelvic endometriosis is associated with elevated serum leptin concentrations [19]. Clearly, larger prospective studies are required to determine the diagnostic potential of measuring circulating inflammatory cytokine levels in endometriosis.

Functional genomics and proteomics

There is increasing evidence to suggest that endometriosis is a polygenic and multifactorial disease, indicating that multiple distinct pathways could be involved in its pathogenesis. Furthermore, many cardinal features of endometriosis, such as inflammation and neoangiogenesis, are shared with a plethora of other diseases, rendering it unlikely that a single biochemical marker will yield sufficient sensitivity and specificity to be used in clinical practice. In recent years, the human genome-sequencing project has been the driving force in the development of functional genomics. Global gene profiling studies are poised to revolutionize the diagnosis and treatment of endometriosis and other human diseases [20].

Microarray technology allows simultaneous analysis of the expression of large numbers of genes and has been used to characterize the expression of genes, gene families, and signal transduction pathways during the implantation window in human endometrium [21]. A similar approach has been used to characterize gene expression upon decidualization of human endometrial stromal cells in culture [22,23]. Recently, Eyster et al. [24*] used complementary DNA microarrays to identify differentially expressed genes between eutopic and ectopic endometrium and reported that the expression of eight genes from a total of 4133 genes on the microarray was increased in endometriotic implants.

The potential of proteomic pattern technology as a diagnostic tool has recently been demonstrated for ovarian cancer. Petricoin and coworkers [25*] first identified an optimum discriminatory proteomic pattern from analysis of serum from 50 unaffected women and 50 women with ovarian cancer. Subsequently, the discovered pattern was used to classify an independent
The reader will learn how accurate ultrasound is for the diagnosis of the ovarian endometrioma.

Conclusions

Current imaging techniques do not allow accurate staging of endometriosis as they lack the resolution necessary to visualize superficial implants and small ovarian endometriomas and cannot detect the presence, type or extent of endometriotic adhesions. However, TVS and MRI are increasingly used as non-invasive techniques to explore the retroperitoneal space, and specifically the presence and extent of deep pelvic endometriosis and bowel involvement.

An accurate test for endometriosis, based on biochemical analysis of eutopic endometrium or peripheral blood samples, could potentially reduce the number of uninformative laparoscopies and provide a rational basis for initiating medical treatment. However, any attempt at evaluating the diagnostic accuracy of a biochemical marker of endometriosis will have to overcome two intrinsic biases: first, the inevitable selection of patients requiring laparoscopy which inevitably results in a high disease prevalence and, secondly, the intrinsic limitations of the visual diagnosis of endometriosis [10]. There will be inevitable discrepancies between biochemical markers of the disease and the visual assessment of pelvic lesions. Therefore, biochemical markers should focus on predicting clinical correlates of endometriosis, such as infertility and pain, rather than on the ectopic lesions per se.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


This interesting paper demonstrates the need for non-invasive diagnosis of endometriosis in symptomatic teenagers and young women.


The reader will learn how accurate ultrasound is for the diagnosis of the ovarian endometrioma.


This provides clear proof that rectovaginal septum endometriosis is frequently a misnomer.


The paper describes when ureter endometriosis should be suspected and investigated.


These authors describe an interesting simple improvement of TVS for the investigation of rectovaginal endometriosis.


This is a comprehensive review of non-invasive techniques for the orientation of diagnosis and research in the field of endometriosis.


The reader will appreciate the importance of magnetic resonance imaging for the diagnosis of bladder endometriosis.


The paper reveals that proliferative uterine diseases including endometriosis are characterized by elevated aromatase P450 RNA expression.


The paper suggests that a combination of serum markers may be useful to evaluate the severity of endometriosis.


Further study of gene expression may expand our understanding of the nature of endometriosis.


The first paper to show the use of proteomics pattern for the diagnosis of ovarian cancer justifies the hope that a disease like endometriosis may be diagnosed using a serum sample.