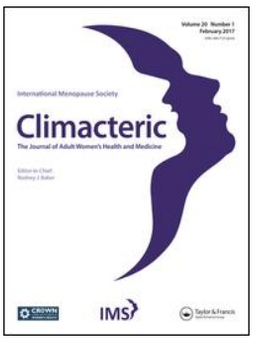

Climacteric

ISSN: 1369-7137 (Print) 1473-0804 (Online) Journal homepage: https://www.tandfonline.com/loi/icmt20

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To cite this article: L. Kiesel & M. Sourouni (2019): Diagnosis of endometriosis in the 21st century, Climacteric, DOI: 10.1080/13697137.2019.1578743

To link to this article: https://doi.org/10.1080/13697137.2019.1578743

Published online: 25 Mar 2019.

CLIMACTERIC
https://doi.org/10.1080/13697137.2019.1578743

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REVIEW

Diagnosis of endometriosis in the 21st century

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ABSTRACT

Endometriosis is a common disease but, due to the wide spectrum of symptoms, diagnosis can be delayed 8–12 years. Laparoscopy is nowadays the gold standard for diagnosis. A less invasive method could shorten the time to diagnosis. The aim of this review is to systematically summarize the literature regarding possible less invasive methods for the diagnosis of endometriosis. Electronic databases, including MEDLINE/PubMed, Cochrane, and Google Scholar, were searched to identify relevant studies; 53 publications contributed to this review. Low invasive tests including imaging, genetic tests, biomarkers, or miRNAs could be the key for establishing a less invasive diagnosis of endometriosis. The findings generally support that different methods can differently contribute to the diagnosis, also depending on the type of endometriosis. For example, transvaginal ultrasound has a sensitivity of 93% and a specificity of 96% in the diagnosis of endometrioma, while superficial/peritoneal endometriosis cannot be detected with imaging processes. Although several non-invasive tests including imaging, genetic tests, biomarkers, or miRNAs show promising diagnostic potential, further research is required before they can be recommended in routine clinical care. The combination of low invasive tests may be the solution to a reliable low invasive diagnosis of endometriosis.

ARTICLE HISTORY
Received 30 November 2018
Revised 24 January 2019
Accepted 27 January 2019
Published online 25 March 2019

KEYWORDS
Biomarkers in endometriosis; miRNAs in endometriosis; non-invasive diagnosis of endometriosis

Introduction

Endometriosis is defined by the World Endometriosis Society as an inflammatory condition characterized by endometrium-like tissue at sites outside the uterus[1]. Endometriosis afflicts 10% of women of reproductive age and 35–50% of women with pelvic pain or infertility. It is classified either as superficial or as deeply infiltrating endometriosis (DIE) when the endometriotic tissue penetrates the retroperitoneal space for a distance of 5 mm or more. Endometriosis may present in multiple locations in the pelvis including the uterus (adenomyosis), ovary (endometrioma), pelvic peritoneum, bladder/ureter, rectum, colon, uterosacral ligaments, rectovaginal septum, vaginal wall, pouch of Douglas, and so forth. More rare locations of endometriotic implants are distant sites such as the lungs, liver, pancreas, operative scars, and inguinal region[2], even the brain[3], with consequent variation in presenting symptoms.

There are different classification systems regarding the extent/severity of endometriosis. The revised American Society for Reproductive Medicine score is currently the most widely used throughout the world (stage I, minimal; stage II, mild; stage III, moderate; stage IV, severe). Although it is relatively easy to use, this score does not take into account the deeply infiltrating endometriotic lesions. For this reason, the ENZIAN classification was developed and provides a morphologically descriptive classification of DIE[4]. The ENZIAN classification does not, however, have a high level of international acceptance and is mainly used in German-speaking countries.

The clinical presentation of endometriosis may vary from asymptomatic and unexplained infertility to severe dysmenorrhea (painful periods), dyspareunia (painful intercourse), or chronic pelvic pain. Depending on the affected site, other more specific symptoms may be present (e.g. dysuria by bladder infection). The stage of the disease does not correlate with the presence or severity of symptoms and no symptom is specific to endometriosis; this is the main reason why endometriosis can remain undiagnosed for 8–12 years and the onset of the disease cannot be timed. However, it has been shown that an ultrasound-based endometriosis staging system correlates with and can predict the complexity of the surgery demanded in each case[5].

As already mentioned, endometriosis can be a cause of infertility. Therefore, an endometriosis fertility index has been developed as a clinical tool to predict pregnancy rates in women who have undergone surgery for endometriosis and attempt non-in vitro fertilization conception[6].

In addition, endometriosis has a profound effect on psychological and social well-being (depression, inability to work, sexual dysfunction) and imposes a substantial economic burden on society. Women with endometriosis incur significant direct medical costs (diagnosis, surgical therapy, hospital admission, fertility treatment). However, these costs are outweighed by indirect costs of endometriosis, including absenteeism and loss of productivity[7,8].

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Methods

Search strategy

To identify articles relevant to the topic, search terms such as 'imaging', 'miRNAs', 'blood biomarkers', 'urine biomarkers', 'endometrial biomarkers', 'genetic tests', 'low invasive tests', 'imaging' AND 'diagnosis of endometriosis' or 'role in endometriosis' or 'endometriosis' were used. Articles until August 2018 were considered.

Results

Diagnosis today

The only reliable diagnosis of endometriosis today is diagnostic laparoscopy with inspection of the abdominal cavity and histological confirmation of suspect lesions. The need for histological confirmation nevertheless remains debatable as macroscopically recognized endometriotic lesions are not always histologically confirmed. Conversely, occult microscopic endometriosis can be detected in biopsies of macroscopically normal peritoneum of women with and without visible endometriosis[9].

As surgery is risky and expensive, other tests including imaging, genetic tests, biomarkers, or miRNAs have been evaluated regarding their potential to detect endometriosis non-invasively. An accurate low invasive test could lead to diagnosis without the need for surgery, or at least reduce the need for it, so only women who were most likely to have endometriosis would undergo surgery.

Imaging as a low invasive method in the diagnosis of endometriosis

Different imaging processes have been evaluated regarding their diagnostic potential for endometriosis in a reliable systematic review of the Cochrane Database[10]; 49 studies (13 evaluated pelvic endometriosis, 10 evaluated endometriomas, 15 evaluated DIE, and 33 addressed endometriosis at specific anatomical sites) involving 4807 women were analyzed. The evaluated imaging processes included transvaginal ultrasound (TVUS) and magnetic resonance imaging (MRI). Most of the studies included are of low quality so the conclusions of this Cochrane Database review should be interpreted with caution.

Neither TVUS nor MRI qualified as a replacement or at least as a triage test for detecting pelvic endometriosis in general. As far as ovarian endometriosis is concerned, TVUS met the criteria for a SpPin triage test (specificity 96%) and almost for a SnNout test (sensitivity of 93%), while MRI qualified as a SnNout test (sensitivity 95%) and almost as a SpPin test (specificity 91%).

Regarding DIE in general, no imaging process qualified as a SpPin or SnNout test, although MRI closely approached the criteria for a SnNout test and TVUS closely approached the criteria for a SpPin test. For DIE in the rectovaginal septum, pouch of Douglas, uterosacral ligaments, and vaginal wall, TVUS qualified as a SpPin triage test. Ferrero et al.[11] showed that there is no difference in the potential of TVUS to detect endometriosis with or without bowel preparation. MRI qualified as a SpPin triage test for DIE in the pouch of Douglas, vaginal wall, and rectosigmoid. TVUS also qualified as a SpPin test for bladder endometriosis[12].

Another systematic review showed that MRI and TVUS have similar potential in the detection of DIE in the rectosigmoid, uterosacral ligaments, and rectovaginal septum[13].

These results are summarized in Table 1.

Data regarding more specific imaging processes such as transrectal ultrasonography (TRUS) or multidetector computerized tomography enema propose that the method can either only be used for affected rectosigmoid or that more studies are needed to provide meaningful results, respectively[10]. More specifically, transrectal sonography is a reliable method in the case of rectosigmoid endometriosis to measure the exact distance of the lesion from the anal margin as well as the degree of infiltration of the intestinal wall, but detection rates are better with TVUS. TRUS is, however, equally accurate at excluding endometriosis compared to TVUS. To sum up, TRUS is not necessarily part of the preoperative examination of the patient with rectosigmoid involvement but can be used in doubtful cases[14]. MRI, on the other hand, does not perform as well as TVUS in confirming or excluding rectal involvement and, given the fact that it is more expensive and unsuitable for claustrophobic patients, it is not included in the routine preoperative control of patients with rectal endometriosis[15].

Table 1. Sensitivity and specificity of different imaging methods in the diagnosis of endometriosis[10,12,13].

Location	*Author*	*Imaging method*	*Sensitivity (%)*	*Specificity (%)*	*Qualification*
Endometrioma	Nisenblat et al., 2016[10]	TVUS	93	96	SpPin test
		MRI	95	91	SnNout test
DIE	Nisenblat et al., 2016[10]	TVUS	79	94	SpPin test (only for rectovaginal septum, pouch of Douglas, uterosacral ligaments, and vaginal wall)
		MRI	94	77	SpPin test (for pouch of Douglas, vaginal wall, and rectosigmoid endometriosis)
DIE rectosigmoid	Guerriero et al., 2018[13]	TVUS	85	96	Undefined
		MRI	85	95	Undefined
DIE uterosacral ligaments	Guerriero et al., 2018[13]	TVUS	67	86	Undefined
		MRI	70	93	Undefined
DIE rectovaginal septum	Guerriero et al., 2018[13]	TVUS	59	97	Undefined
		MRI	66	97	Undefined
Bladder	Guerriero et al., 2015[12]	TVUS	62	100	SpPin test

DIE, deeply infiltrating endometriosis; MRI, magnetic resonance imaging; TVUS, transvaginal ultrasound.

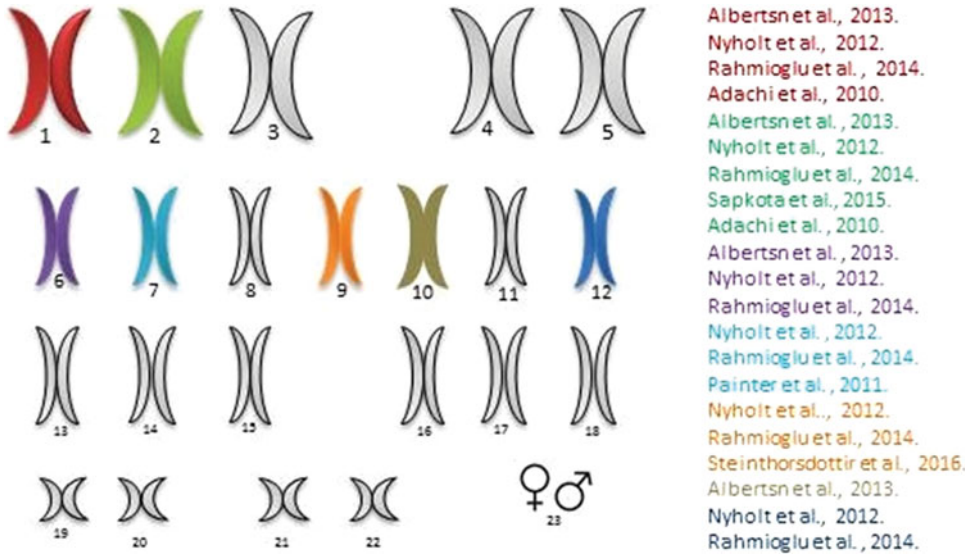

Figure 1. Single nucleotide polymorphisms associated with the risk of endometriosis found in different genome-wide significant loci on chromosomes 1, 2, 6, 7, 9, 10, and 12[23–30].

To conclude, none of the evaluated imaging modalities could accurately predict endometriosis so that they could replace surgery, but, depending on the location of the endometriotic lesion, different imaging methods seem to be more or less suitable for diagnosis. Nevertheless, it is difficult to compare results from published studies as authors use different terms when describing the sites affected by endometriosis. Therefore, the International Deep Endometriosis Analysis Group reached a consensus opinion regarding the sonographic evaluation of women with suspected endometriosis, the anatomic terminology for the compartmental evaluation and the measurement of lesions in the hope that a universal standardized terminology concerning endometriosis can be established. It that way, multicenter studies or comparison between different studies can be more reliable[16].

Genetic tests

The pathogenesis of endometriosis has not been fully elucidated. According to Sampson's theory[17], endometriosis occurs when endometrial tissue contained within menstrual fluid flows retrogradely through the fallopian tubes and implants at an ectopic site within the pelvic cavity. However, this theory explains neither the fact that although retrograde menstruation is seen in up to 90% of women[18], only 10% of women develop endometriosis, nor the fact that endometriotic lesions can also be detected in extra-abdominal locations. It is therefore suggested that other factors, for example environmental, immunological, and hormonal factors, play a role in the development of the disease. Among these are genetic factors as well.

Zondervan et al.[19] and Treloar et al.[20] in their linkage studies suggested that there may be one or more high-penetrance loci for endometriosis with (near-)Mendelian inheritance. The findings from twin studies[21,22] strengthen the hypothesis that genes influence a predisposition to endometriosis.

Genome-wide association studies have revealed 23 genome-wide significant loci that are associated with the risk of endometriosis[23–30] (Figure 1). In a preprint by Rahmioglu et al.[31], it seems that in total 27 genetic loci are associated with endometriosis. This result came from a meta-analysis of 15 genome-wide association studies and a replication analysis, including 58,115 cases and 733,480 controls, but has not yet been peer reviewed.

The loci were either intergenic or in/near genes with known functions of biological relevance to endometriosis, varying from roles in developmental pathways to cellular growth/carcinogenesis. It seems that some loci have a stronger correlation with stage III/IV cases[25], implying that they are likely to be implicated in the development of moderate to severe or ovarian disease.

Biomarkers in the diagnosis of endometriosis

Blood biomarkers, tissue/endometrial biomarkers, and urine biomarkers could serve as markers for diagnosis, stratification of patients, therapeutic efficacy, assessment of the best treatment option, drug design, or recurrence markers for endometriosis. Nevertheless, such biomarkers are not able to reveal the location of the endometriotic lesion. There are two approaches in the discovery of potential biomarkers: the hypothesis-driven approach and the hypothesis-generating approach. In the first, a hypothesis about the pathogenesis of endometriosis leads to the investigation of specified markers. In the second approach, a large number of biomarkers is investigated based on a system biology (e.g. 'Omics' approach, miRNAs).

Peripheral biomarkers

The systematic review by May et al.[32] includes over 100 biomarkers in 161 studies (Table 2). A popular biomarker among these is CA125. Elevated CA125 can be found under different circumstances including endometriosis but has no value as a

single test in the diagnosis. The authors concluded that peripheral biomarkers have potential as diagnostic markers. However, none of these have been clearly shown to be of clinical use and further research is necessary. Combination of markers may have greater sensitivity and specificity as diagnostic tests compared with single marker tests.

The Cochrane Library review regarding peripheral biomarkers examined 122 biomarkers in 144 studies[33] and also concluded that there are not enough data to accurately evaluate the use of peripheral biomarkers as diagnostic tools.

Tissue biomarkers

A systematic review by May et al.[34], including 200 biomarkers in endometrial tissue or menstrual fluid in 182 studies, identified differences between women with and without endometriosis, implying a diagnostic potential. However, larger well-designed studies are required to evaluate the true role of tissue biomarkers in the diagnosis of endometriosis. Among these biomarkers, PGP 9.5 was suggested to have a high diagnostic potential. Nevertheless, the literature on this is contradictory. Tokushige et al.[35], Al-Jefout et al.[36], and Meibody et al.[37] provide data supporting the high diagnostic potential of PGP 9.5 as a biomarker for endometriosis with a sensitivity and specificity of 98–100% and 85–100%, respectively. Contrarily, Cetin et al.[38] and Ellett et al.[39] brought to light data that PGP 9.5 has limited use as a diagnostic tool for endometriosis (sensitivity of 13–31% and specificity of 45–68%).

The Cochrane Library review examined 95 tissue biomarkers in 54 studies[40] and also came to the conclusion that more research is required to determine the true usefulness of such biomarkers in the diagnosis of endometriosis.

Urine biomarkers

The Cochrane Library review regarding biomarkers in urine[41] includes seven biomarkers in eight studies and concludes that more data are required in order to determine the role of such biomarkers in the diagnosis of endometriosis.

To sum up, further studies in well-defined populations are necessary to evaluate the diagnostic potential of endometrial, urinary, and blood biomarkers for the diagnosis of endometriosis[32–34,40,41]. The small number of studies, the small sample sizes, the various study designs, the heterogeneous laboratory methods as well as the risk of reporting bias – negative data are less likely to be published – are the reasons why the scientific community concludes that more research regarding the different biomarkers is needed and the reasons why the recommendation of the European Society of Human Reproduction and Embryology (ESHRE) is for clinicians not to use biomarkers in plasma, urine, or serum to diagnose endometriosis[59].

Table 2. Some of the biomarkers studied regarding their diagnostic potential for endometriosis (based on May et al.[32]).

Biological group	*Biomarkers*
Cytokines	IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-16, IL-18 IL-1α, IL-1β TGFβ, IFNγ, TNFα CCL 5 (Chemokine Ligand 5), MMIF (macrophage migration inhibitory factor), CCR (C-C chemokine receptor type 5)
Antibodies	Anti-endometrial antibodies Specific antibodies: anti-PEP, anti-carbonic anhydrase, ANA, anti-DNA, anti-RNA, anti-transferrin, IgG, anti-α2 HS-glycoprotein, anti-cardiolipin, anti-laminin-1; antibodies against lipid peroxide modified rabbit serum albumin, against copper oxidized low-density lipoprotein, against malondialdehyde-modified low-density lipoprotein
Glycoproteins	CA125, CA19-9, CA15-3, CA72, transferrin, α2-HS glycoprotein, AFP, haptoglobin-β, follistatin, gremlin-1, β2-microglobulin
Cell adhesion	ICAM-1, E-cadherin, osteopontin, VCAM-1, P-selectin, E-selectin
Growth factors	IGF-1, GM-CSF, EGF/EGFR, FGF-2, PDGF, HGF
Hormones	PR, TRH, E2, PR, testosterone, adiponectin, LH, leptin
Angiogenesis	VEGF, angiogenin, Flt-1
Apoptosis	Fas/Fast
Other	CRP, urocortin, PON-1, HSP70b', IMA, TRX, cfDANN, TATI, c-erbB-2, TIMP-1, MMP-2

miRNAs in the diagnosis of endometriosis

miRNAs, non-coding RNA with a size of 18–22 nucleotides, are modulators of gene expression and were discovered for the first time in *Caenorhabditis elegans* by Lee et al.[42] and Wightman et al.[43] in 1993. miRNAs contribute to the pathogenesis of endometriosis and infertility[44] as they have a role in angiogenesis and inflammation, in abnormal cell differentiation and invasion, and as modulators of gene expression[45]. They show stability in biological fluids and are resistant to RNAse degradation, so that they can act as cell-to-cell messengers.

Different miRNAs are found to be either upregulated or downregulated in patients with endometriosis compared to controls without endometriosis[46–52] (Figure 2). Nevertheless, miRNAs can be influenced by other diseases/conditions (e.g. cardiovascular disease, cancer, stress). An endogenous 'control' miRNA and standardized sample handling regarding RNA extraction and amplification are needed in the search for miRNA with diagnostic potential considering endometriosis[52].

Future targets in the diagnosis of endometriosis

A low invasive diagnosis of endometriosis is demanded. This could include imaging tests, genetic tests, biomarkers, or miRNAs as discussed. New approaches in the search for a low invasive diagnosis of endometriosis involve the 'Omics' approach (proteomics, metabolomics)[53,54] in combination with medical artificial intelligence such as text mining[55]. Text mining combined with bioinformatics can help us, for example, understand gene networks and pathways, candidate genes, and novel genes.

In some studies it has been suggested that the gut microbiota may be involved in the onset and progression of endometriosis[56] and that endometriosis may induce dysbiosis[57]. Besides, the intestinal microbial community regulates circulating estrogen levels via the enterohepatic circulation[58] and could therefore play a role in estrogen-related conditions like endometriosis. Targeting the fecal microbiota could open the door for a novel preventive, therapeutic as well as diagnostic approach for endometriosis.

Given the fact that endometriotic cells are similar to cancer cells in that they can build distant metastases (e.g. lungs)

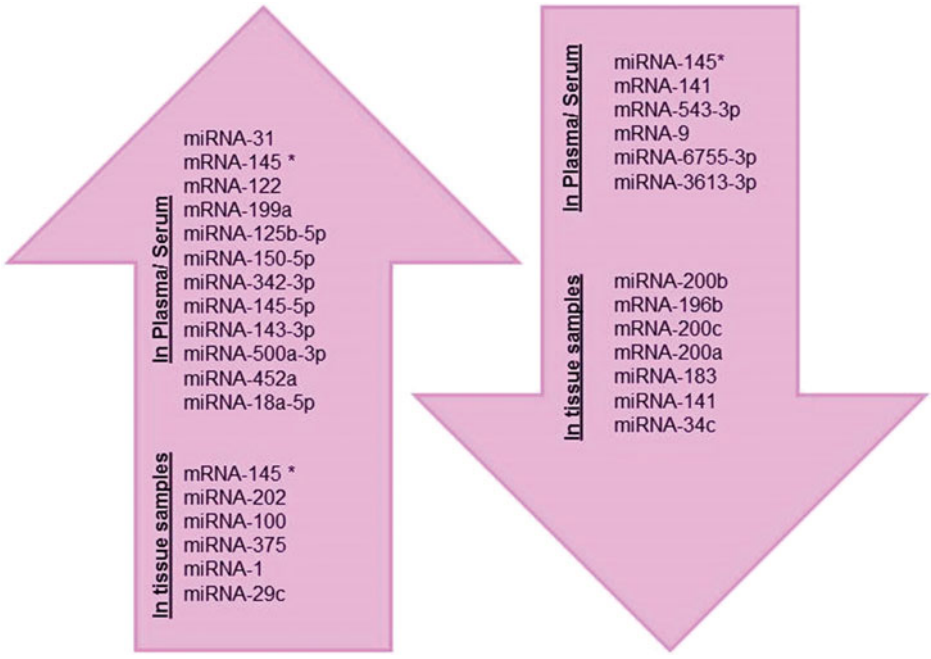

Figure 2. Upregulation and downregulation of miRNAs in plasma/serum and in tissue samples of patients with endometriosis[46–52]. *Concerning miRNA-145, controversial results have been reported: upregulated in Nothnick et al.[48] (plasma/serum) and Saare et al.[47] (tissue samples); downregulated in Wang et al.[49] (plasma/serum).

and attach to, invade, and damage adjacent tissues, seeing endometriosis as the mirror image of malignancy could be the key to unlock new diagnostic aspects. Looking, for example, into breast cancer, one sees that the behavior of the tumor and the effectiveness of treatment depend on the tumor biology and profile (e.g. hormone receptor and Her2 status, differentiation grade, etc.). Similarly, it could be that different molecular subtypes of endometriotic lesions (e.g. active vs. non-active lesions, progesterone resistant vs. progesterone sensitive) determine the characteristics of the disease regarding onset, progression, and therapeutic outcome. Different diagnostic steps may be essential depending on the biological profile of endometriotic lesions.

Conclusions

A low invasive test for endometriosis remains a challenge for the 21st century. But would a low invasive diagnostic test have only benefits? We must not forget the risk of a diagnostic test being used as a screening test in the context of the phenomenon called disease mongering. This is the strategy of raising public awareness about a treatable disease and widening its diagnostic boundaries in order to expand the markets of those who sell or provide treatment. To avoid this, the right indication for a test will always be needed and that includes unexplained subfertility and unexplained severe pelvic pain refractory to oral contraceptives or non-steroidal anti-inflammatory drugs. Many paths are already open in the search for a low invasive diagnosis of endometriosis and many more remain to be opened. At the present time, further research is necessary before any low invasive diagnosis of endometriosis can be recommended in routine clinical care. Combined tests are more likely to be efficient in the diagnosis than single ones.

Potential conflict of interest No potential conflict of interest was reported by the authors.

Source of funding Nil.

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