Current biomarkers for the detection of endometriosis

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Abstract

A clinically reliable non-invasive test for endometriosis is expected to reduce the diagnostic delay. Although varieties of biomarkers have been investigated for decades, and cancer antigen-125, cancer antigen-199, interleukin-6, and urocortin were the most studied ones among hundreds of biomarkers, no clinically reliable biomarkers have been confirmed so far. Some emerging technologies including "omics" technologies, molecular imaging techniques, and microRNAs are promising in solving these challenges, but their utility to detect endometriosis has yet to be verified. New combinations of researched indicators or other non-invasive methods and further exploration of the emerging technologies may be new targets and future research hotspots for non-invasive diagnosis of endometriosis. In conclusion, researches of biomarkers for the detection of endometriosis are still ongoing and may benefit from novel molecular biology, bioinformatics methods and a combination of more diverse monitoring methods. Though it will be a daunting task, the identification of a specific set of diagnostic biomarkers will undoubtedly improve the status of endometriosis.

Keywords: Biomarkers; Endometriosis; Non-invasive diagnosis

Introduction

Endometriosis is defined as the presence of endometrial-like tissue outside the uterine cavity. Symptoms of endometriosis often affect patients' psychologic and social well-being and impose a substantial economic burden on society. For this reason, endometriosis is considered a disabling condition that may significantly compromise social relationships, sexuality, and mental health.[1-3] Approximately 10% of women of reproductive ages were affected by endometriosis and 30% to 50% of them suffer from infertility. Despite its negative impact on the quality of patients' life, many issues related to endometriosis remain unclear. The mechanisms underlying the development of the disease, early and better diagnostic methods, as well as treatment options, are still worth discussing in this field.

As the age of menarche getting earlier, the incidence of endometriosis in young girls is also increasing. Although the World Endometriosis Association has reached a consensus that the development of reliable non-invasive tests is one of the primary research priorities of endometriosis,[4] studies regarding endometriosis biomarkers are still in the earliest stage. Hence, a clinically reliable test for the detection of endometriosis is expected to show profound impacts on improving patients' life quality and reducing healthcare and individual costs. Generally, the predetermined criteria for blood tests that can be used clinically to replace the surgical diagnosis of endometriosis is a sensitivity of 0.94 and a specificity of 0.79 (replacement test).[5] Nisenblat *et al*[5] suggested in their study that indicators with sensitivity ≥0.95 and specificity ≥0.50 can be used to accurately exclude negative results (SnOUT test), while indicators with specificity ≥0.95 and sensitivity ≥0.50 can be used for the accurately diagnosis of positive results (SpIN test). As shown in Table 1, we summarized the specificity and sensitivity required for the criteria of replacement test, SnOUT test, and SpIN test.

The main purpose of this review is to outline current studies in the verification of potential non-invasive diagnostic biomarkers for endometriosis through retrospective analysis of related articles and put forward our prospects for future research directions.

Classical Blood Biomarkers

The etiology of endometriosis is complex, which is still poorly understood so far. Currently, various hypotheses have been proposed, such as menstrual blood regurgitation, chronic inflammatory condition, coelomic metaplasia, and so on.[6] The typical chronic inflammatory process of endometriosis involves many factors, such as hormones, cytokines, glycoproteins, and angiogenic factors, which

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Table 1: The criteria for replacement test and triage tests.

Items	Replacement test	SnOUT triage test	SpIN triage test
Sensitivity	≥0.94	≥0.95	≥0.50
Specificity	≥0.79	≥0.50	≥0.95

SnOUT: High sensitivity tests have few false negative results and act to rule conditions out; SpIN: High specificity tests have few false positive results and act to rule conditions in.

are related to the pathogenesis of the disease and some of these factors may be expected to perform as endometriosis biomarkers.[7] As illustrated in Figure 1, a variety of other blood markers have also been investigated during the past decades, including markers of apoptosis; cell adhesion molecules and other matrix-related proteins; cytoskeleton molecules; nerve growth markers; oxidative stress markers; tumor markers; and other peptides/proteins shown to influence key events in endometriosis.[8,9] Among the above-mentioned factors, cancer antigen-125 (CA-125), cancer antigen-199 (CA-199), urocortin (UCN), and interleukin-6 (IL-6) have received much attention as the promising biomarkers for endometriosis. However, it is a pity that all of these emerging indicators are far from meeting the criteria for diagnostic biomarkers. In our own studies, we also found that circulating endometrium cells (CECs) have great potential for the development of an early, non-invasive diagnostic assay.[10] In addition, the improvement of emerging molecular diagnostic technologies and the combination of these promising biomarkers would be new targets and focus for future research.

CA-125

CA-125, a common blood biomarker for endometriosis, has been extensively studied.[11] More than 20 years ago, a meta-analysis had demonstrated elevated levels of CA-125 in patients with endometriosis especially with the most advanced stages.[12] Further studies have shown that levels of CA-125 also vary with the clinical type and American Society for Reproductive Medicine stage of endometriosis and fluctuate during the menstrual cycle.[13,14] The reported diagnostic estimates for CA-125 with a cutoff of >43.0 IU/mL in one study demonstrated a sensitivity of 1.00 (95% confidence interval [CI] 0.92–1.00) and a specificity of 0.80 (95% CI 0.56–0.94) which met the criteria for a replacement test,[15] but this cutoff value came from only one individual study and limited to moderate-severe forms of endometriosis. Nisenblat *et al* recently reviewed the accuracy of serum CA-125 in the diagnosis of endometriosis[5] and found that all the other cutoff thresholds for CA-125, which ranged from >10.0 to 43.0 IU/mL, had not meet the replacement or triage test criteria and that only CA-125 with cutoffs >16.0 to 17.6 IU/mL approached the criteria for the SpIN classification test. Overall, CA-125 seems to be hampered as a single clinically reliable diagnosis biomarker of endometriosis.[16] However, CA-125 still plays a major role as a benchmark molecule in the study of other biomarkers,[10,17] and further large-scale diagnostic studies are needed to assess the role of CA-125 with a cutoff value >43.0 IU/mL in a wide range of endometriosis population.

CA-199

Another common glycoprotein, CA-199, has been found to be elevated in endometriosis.[18] The cutoff thresholds of CA-199 used to detect endometriosis in various studies are diverse, ranging from >7.5 to >37.0 IU/mL. Due to the inconsistency in the methods, a meta-analysis[5] included only three of these studies and assessed the cutoff value of CA-199 >37.0 IU/mL. The total sensitivity was 0.36 (95% CI 0.26–0.45) and the specificity was 0.87 (95% CI 0.75–0.99). The results of other studies with the cutoff value of CA-199 >7.5, >9.5, and >10.67 IU/mL were reported separately,[5] but none of them were clinically meaningful diagnostic estimates. Therefore, CA-199 fails to meet the ideal criteria for a single adequate diagnostic test in endometriosis according to current research.

IL-6

In endometriosis, cytokines[19] seem to have a profound effect on the implantation of endometriotic foci by reducing immunologic surveillance and identifying and destroying endometrial cells. Among them, IL-6 has been the most studied ones in the past few decades. Foda and Aal found a fair sensitivity of 0.70 (95% CI 0.57–0.80) and a high specificity of 1.00 (95% CI 0.88–1.00) with a cutoff value of IL-6 >12.20 pg/mL and tumor necrosis factor alpha >12.45 pg/mL simultaneously, which meet the criteria for SpIN triage test.[20] However, May *et al*[21] and Nisenblat *et al*[5] found inconsistent results that the association between endometriosis and elevated serum levels of IL-6 and tumor necrosis factor alpha is not really noticeable. Jee *et al*[22] also reported that there was no significant difference in peripheral blood IL-6 and sCD163 levels between women with or without ovarian endometriosis, which supports the negative findings presented for IL-6. Therefore, it is better that future researchers focus on the diagnostic efficacy of IL-6 combined with other cytokines instead of IL-6 alone.

Urocortin

UCN is mainly expressed by eutopic and ectopic human endometria to promote the process of decasualization.[23] Though UCN is generally considered to be involved in the pathogenesis of endometriosis,[24] it remains unclear whether UCN may be used as a reliable biomarker. Most recently, Pergialiotis *et al*[25] accumulated current results associated with the expression of UCN in their systematic review and found that the specificity of UCN even reach 90% with the cutoff value >33 pg/mL. However, the wide variation of the included studies precludes meta-analysis of available data on UCN. Therefore, further validation in larger studies is still required to reach firm conclusions with respect to its predictive accuracy.

Circulating endometrium cells

The identification of peripheral blood circulating cells has been used for clinical diagnosis of cancer for many years,

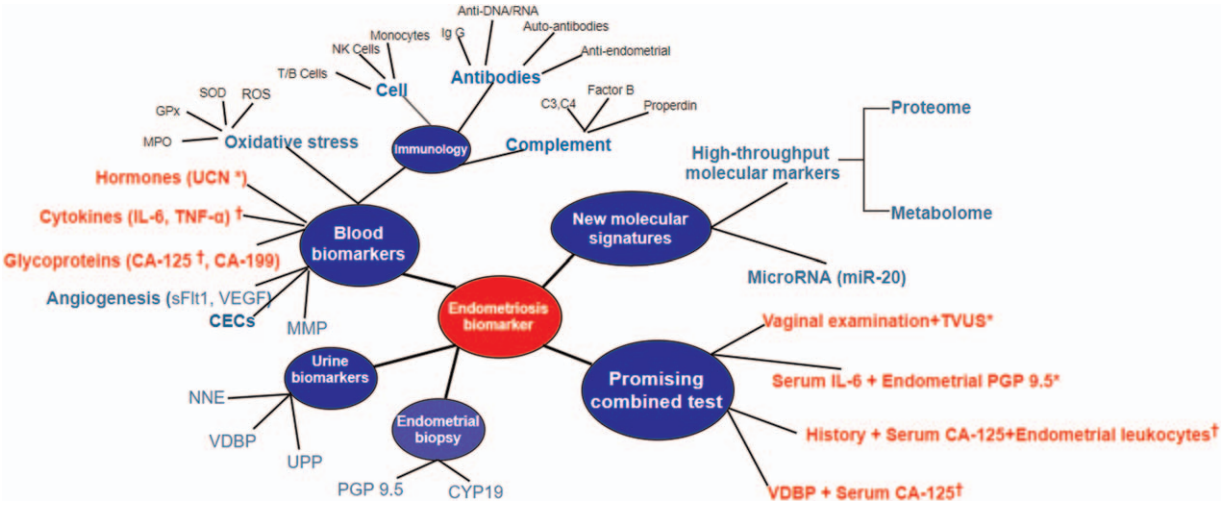

Figure 1: Potential biomarkers of endometriosis in peripheral blood, uterine materials, or urine. *Biomarkers reported that might meet the criteria for a replacement test. †Biomarkers reported that might meet the criteria for a SpIN triage test. UCN: Urocortin; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor alpha; CA-125: Cancer antigen-125; CA-199: Cancer antigen-199; VEGF: Vascular endothelial growth factor; sFlt1: Soluble FMS-like tyrosine kinase-1; CECs: Circulating endometrial cells; MMP: Matrix metalloproteinase; NNE: Non-neuronal enolase; VDBP: Vitamin D binding protein; UPP: Urinary peptide profiling; MPO: Myeloperoxidase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; ROS: Reactive oxygen species; NK cell: Natural killer cell; IgG: Immunoglobulin G; PGP 9.5: Protein gene product 9.5; CYP 19: Aromatase cytochrome P450 isoform; C3: Complement C3; C4: Complement C3; TVUS: Transvaginal ultrasound.

such as colorectal carcinoma, pancreatic cancer, prostatic cancer, and lung cancer.[26,27] Although endometriosis is a benign disease, it has many malignant features such as dissemination, implantation, and metastasis. Bobek *et al*[28] firstly reported the presence of endometrial cells in peripheral blood of patients with endometriosis, referred as circulating endometrial cells in 2014. In our own research,[10] peripheral blood was collected from patients for CEC analysis 1 day before surgery and then the size-based microfluidic chip and immunofluorescence staining were applied to capture and identify CECs. Our results showed that the CEC assay had 89.5% sensitivity and 87.5% specificity in distinguishing endometriosis from other benign ovarian masses and had 89.5% sensitivity and 80.0% specificity in distinguishing endometriosis from healthy controls, which showed a great superiority in diagnosing endometriosis compared with CA-125.[10] However, the use of CECs as a biomarker for endometriosis is a comparatively new concept, and many aspects still require to be investigated. Firstly, it is unachievable to determine the absolute quantity of the CECs captured by the current microfluidic chip techniques. Besides, malignant tumors and the shedding of vascular endothelial cells that also express cytokeratin and estrogen receptor/progesterone receptor may inevitably interfere with the results. Further explorations of single-cell sorting and sequencing of captured cells are in process in our team and may help define more specific characteristics of CECs for its clinical use as endometriosis biomarker.

New Molecular Signatures

Recently, molecular biology technologies related to bioinformatics analysis have been rapidly developed. The so-called omic sciences, an emerging technology that integrates genomics, transcriptomic, proteomics, metabolomics, etc, have been widely used by researchers in the study of complex diseases in the past few years.[29,30] The wide scanning provided by omics technology makes possible a generic approach for countless molecules and can be considered as a promising tool for discovering endometriosis biomarkers. Besides, considering the close relationship between endometriosis and genetic factors, it is worth mentioning that microRNA (miRNA) as an emerging technology in this area.

High-throughput molecular markers

Proteome

Recently, protein "fingerprint" technology has become a hot topic in the diagnosis of endometriosis. Studies on proteomics both in peripheral blood and endometrium have shown promising results. Nisenblat *et al*[5] reviewed four studies on assessing the accuracy of the proteome in detecting endometriosis in their recent Cochrane systematic review and only one study detecting six protein peaks (1629.00, 3047.00, 3526.00, 3774.00, 5046.00, and 5068.00 Da) met the criteria for a SpIN triage test with the sensitivity of 0.66 (95% CI 0.52–0.77) and a specificity of 0.99 (95% CI 0.93–1.00).[31] Besides, proteomics techniques are not only expensive but also time consuming. Currently, some new mass spectrometry-based methods have been developed which may bring a new change in the near future.[32] Further evaluations of this diagnostic approach through using a standardized analysis process with similar sets of markers and defined cutoff thresholds is required to fully evaluate the diagnostic tool.

Metabolome

Metabolomics analysis is promising in the diagnosis of endometriosis in view of the fact that ectopic endometrial

tissue has specific pathologic metabolic pathways. Metabolomic tests revealed significant differences in endometrium between patients with endometriosis and control women.[33-35] Plasma levels of fucose, proline, lysine/arginine, choline-containing metabolites, and lipoproteins are elevated in women with endometriosis, and these changes may be related to the spread and severity of the disease.[36] Elevated levels of lactate, carnitine, β-glucose, phosphatidylcholine, pyruvate, valine, and sphingomyelin were also found in the follicular fluid of endometriosis.[37,38] One system review[5] included four studies assessed the accuracy of the metabolism in detecting endometriosis and found that only one study met the criteria for a SpIN triage test with a sensitivity of 0.66 (95% CI 0.52–0.77) and a specificity of 0.99 (95% CI 0.93–1.00). And recently, a panel of plasma acylcarnitines was reported to represent a potential diagnostic approach,[39] promising to be a practical diagnostic tool.

MicroRNA

Studies have found that the miRNAs obtainable from diseased tissues and other body fluids were able to detect various diseases.[40] Today, advances in sequencing and microarray technology have made it possible to investigate systemic levels of miRNAs[41] and long non-coding RNA.[42] Deregulation of miRNAs is involved in the pathophysiology of endometriosis and they have been investigated as potential biomarkers.[43] Agrawal *et al* analyzed studies of circulating miRNAs in endometriosis in a recent systematic review.[44] They found that only miR-20 was differentially expressed in multiple studies among 42 different dysregulated miRNAs. Hence, there has been no miRNA, single or in a panel, that can be utilized as an endometriosis biomarker so far. Further validations in a large population using a standardized reproducible methodology are required to further clarify the diagnostic potential of miRNAs.

Combined Test

Nisenblat *et al*[45] evaluated the combined tests as replacement tests or triage tests for the diagnosis of endometriosis in one Cochrane review. Eleven eligible studies were included and fifteen different diagnostic combinations were assessed in this review. There are two combinations that meet the criteria for a replacement test (serum IL-6 >15.4 pg/mL and endometrial Protein Gene Product 9.5 (PGP 9.5) for pelvic endometriosis; vaginal examination and transvaginal ultrasound for rectal endometriosis) and two met the criteria for SpIN triage tests (urine vitamin D binding protein and serum CA-125 >2755 IU/mL; history, serum CA-125 >35 IU/mL and endometrial leukocytes) for pelvic endometriosis and a combination of vaginal examination and transvaginal ultrasound reached the threshold for a SpIN test for obliterated pouch of Douglas, vaginal wall endometriosis and rectovaginal septum endometriosis. However, the clinical utility of the combined endometriosis diagnostic test is still unclear due to the limitations and heterogeneity of the included studies. Recently, Pateisky *et al*[46] found in a prospective cohort study that specific plasma miRNA characteristics were associated with endometriosis and that hsa-miR-154-5p alone or combined with other types may be potentially applicable for non-invasive diagnosis of the disease. In summary, it is greatly worthwhile and essential to further evaluate the diagnostic potential of any type of combined test that has been identified in a few studies as potentially valuable for the detection of endometriosis.

Others

Urine biomarkers

In the development of biomarkers for endometriosis, urine is significantly less targeted relative to blood. And only 11% of endometriosis biomarkers have been reported based on urine since 2010.[47] Wang *et al*[41] evaluated the accuracy of biomarkers obtained from urine in a Cochrane review. Their study included eight studies, five of which evaluated the diagnostic performance of four urine biomarkers for endometriosis. Results showed that three biomarkers (non-neuronal enolase,[48] vitamin D binding protein,[49] and urinary peptide profiling[50,51]) can better distinguish women with or without endometriosis while cytokeratin 19[52] showed no significant difference. Overall, none of the urinary biomarkers mentioned above met the criteria for a replacement test or a triage test though several urine biomarkers may have diagnostic potential and further evaluation is still required before the introduction of routine clinical practice.

Endometrial biomarkers

There is evidence that gene expression, intrinsic regulatory mechanisms, and hormonal responses play roles in both eutopic and ectopic endometrium in women with endometriosis.[53] Therefore, symptomatic endometriosis may be deduced or diagnosed by endometrial biopsy or intrauterine fluid component estimation.[54] One Cochrane review evaluated the diagnostic accuracy of biomarkers obtained from endometrial tissue.[55] Only studies of the neurofibrillary marker PGP 9.5 and the hormone marker aromatase cytochrome P450 isoform (CYP19) have sufficient numbers to obtain meaningful results and the accuracy of PGP 9.5 appears to be sufficient to replace surgical diagnosis. However, this test does not currently appear to be suitable for diagnostic purposes due to the rigorous methods of sample collection.[56] Other biomarkers such as 17-β hydroxysteroid dehydrogenase type 2, endometrial proteome, caldesmon, interleukin-1 receptor type II, and some other neuromarkers also show good prospects in detecting endometriosis but further high-quality studies are still needed to accurately evaluate the diagnostic potential of these endometrial biomarkers.[55]

Perspective and Conclusion

For such a complex disease, a biomarker panel that combines several different markers will most likely be more accurate than any single biomarker in the diagnosis of endometriosis.[45] Studies have confirmed that some biomarker panels such as peptide peaks,[57] proteins,[58] and metabolites[39] have a promising prospect. And, with

the development of new omics technology and multiple immunoassay techniques, more valuable biomarker panels may be discovered in the near future.[59]

Given that endometriosis is a disease caused by genetic and environmental factors, several emergent technologies brought genetic risk factors into the focus of research. The emergence of genome-wide association studies makes it possible to detect single-nucleotide polymorphisms which are closely related to the high risk of a particular disease or condition.[60,61] Single-nucleotide polymorphisms in six genomic regions have been identified to be possibly involved in endometriosis pathophysiology according to Pagliardini *et al*'s meta-analysis.[62] Another technique worth mentioning is miRNAs, which means small non-coding RNAs that repress translation thereby regulating the degree of gene expression.[44] Panir *et al*'s study[43] suggested that miRNA dysregulation may be involved in the pathophysiology of endometriosis and Xu *et al*[63] founded that circular RNAs are differentially expressed between eutopic and normal endometrium, but nowadays there is no non-coding RNA that can be used as reliable biomarkers for endometriosis no matter single or in panel.[44] However, with the continuous progress of experimental technologies, there will be more promising emerging technologies for us to try and explore in the future.

Despite decades of research, there are still major challenges in the diagnosis and treatment of endometriosis. A wide range of factors including hormones, cytokines, glycoproteins, angiogenic factors, cytoskeleton molecules, nerve growth markers, oxidative stress markers, tumor markers, etc, have been extensively studied, but none of them can singly or accurately identify the disease successfully. A biomarker panel or a combination of different non-invasive diagnostic methods is likely to be a promising target for the diagnosis of endometriosis. Research on biomarkers is still open and valuable, and future new molecular biology and bioinformatics methods may bring the dawn of solving this problem.

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Conflicts of interest

None.

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