Reproductive Sciences (2022) 29:1667–1673
https://doi.org/10.1007/s43032-021-00668-2

REVIEW

The Search for Biomarkers in Endometriosis: a Long and Windy Road

Milena Králíčková[1,2,3] · Vaclav Vetvicka[4] · Luděk Fiala[5] · Antonio Simone Laganà[6] · Simone Garzon[6,7]

Received: 11 January 2021 / Accepted: 11 June 2021 / Published online: 22 June 2021

Abstract

Endometriosis is a complex and chronic estrogen-dependent disease, affecting a significant proportion of women of reproductive age. Despite the long interest and extensive research, the pathogenesis of the disease is still debated. Although available non-invasive diagnostic methods have adequate accuracy, an invasive approach by laparoscopy is often necessary to obtain histological confirmation. In this scenario, the search for an accurate, reliable, cost-effective, clinically applicable non-invasive biomarker plays a crucial role in a potentially early diagnosis and, in this way, shape the future management of the disease. Considering these elements, the current review aims to summarize the most significant and novel results about biomarkers for the diagnosis and follow-up of women affected by endometriosis.

Keywords Endometriosis · Biomarker · Immunology · MicroRNA · Human epididymis protein 4 · CA-125 · Stem cells

Introduction

Endometriosis is a common, chronic, hormone-dependent, inflammatory disease affecting 2–18% of women of reproductive age and over 40% of infertile patients [1]. The presence of endometrial-like tissue outside the uterine cavity characterizes the disease.

✉ Antonio Simone Laganà
antoniosimone.lagana@uninsubria.it

1 Department of Histology and Embryology, Faculty of Medicine, Charles University, Pilsen, Czech Republic

2 Department of Obstetrics and Gynecology, University Hospital, Faculty of Medicine, Charles University, Pilsen, Czech Republic

3 Biomedical Centre, Faculty of Medicine in Plzen, Charles University, Pilsen, Czech Republic

4 Department of Pathology, University of Louisville, Louisville, KY, USA

5 Institute of Sexology, First Faculty of Medicine, Charles University, Prague, Czech Republic

6 Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Piazza Biroldi 1, 21100 Varese, Italy

7 Department of Obstetrics and Gynecology, AOUI Verona, University of Verona, Verona, Italy

Retrograde menstruation with transplantation of shaded endometrium is considered the primary etiopathogenetic mechanism [2]. However, after more than a century of intensive research, we are still far from understanding endometriosis etiopathogenesis with multiple proposed molecular, genetic, and epigenetic mechanisms [3]. Indeed, the endometrium is a dynamic tissue with numerous molecular pathways activated and cyclically involved in cell proliferation, invasion, attachment, and possible migration [4]. All these mechanisms may participate in endometriosis development and represent targets for therapy and markers for diagnosis [4–6].

Understanding the endometriosis pathogenesis is paramount to develop further treatment options, given that available treatments have side effects, and not all patients benefit from them, particularly for non-surgical strategies [7–9]. However, developing new methods for an early diagnosis is of paramount importance as well. A median delay of 8 years has been reported from the onset of symptoms to a diagnosis of endometriosis [10]. Therefore, although clinician education and public awareness are essential to prevent delayed diagnosis and decrease the long-term morbidity resulting from untreated endometriosis [10], identifying possible disease markers may help reduce this issue.

Nevertheless, despite the significant efforts in establishing non-invasive methods to diagnose endometriosis, reliable tests are still missing. Most of the available studies are focused on finding a single marker in which expression would be significantly changed in patients with endometriosis. With this

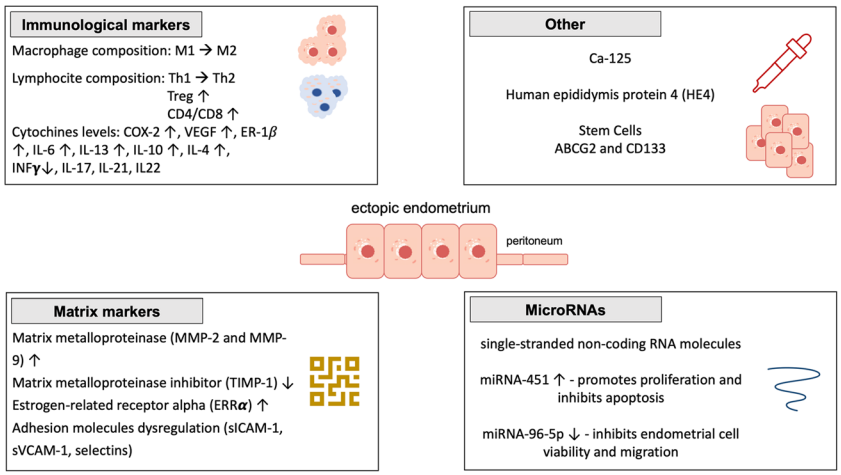

Fig. 1 Investigated biomarkers for endometriosis

approach, numerous non-invasive biomarkers have been suggested (Fig. 1) [11]. However, none of them proceeded into clinical practice yet.

Immunological Markers

The role of the immune system in endometriosis development has been well-established [12]. One of the immunological markers is a change in macrophage and lymphocyte populations' composition, particularly Th and Treg cells. A recent study found a high macrophage type 2 (M2) response in the peritoneal cavity of patients with endometriosis: increased $CD68^{low}/CD14^{low}$ subpopulation coupled with elevated levels of Tregs and Th cells. These results suggest that this subpopulation of macrophages might be responsible for an altered immune reaction in affected patients [13]. Moreover, a meta-analysis found a significant increase of neutrophil and decreased lymphocyte counts in patients with endometriosis, together with shortened activated partial thromboplastin time, which is probably connected with localized inflammatory responses [14].

Treg lymphocytes are well-established to play a critical role in controlling and modulating numerous immune responses. Therefore, the observed premenstrual rise of Tregs in endometriosis patients is a potentially important observation [15]. However, without additional confirmation, the possible role of these cells remains hypothetical. As Tregs cells can block the activation of the immune action, they might be involved in the tolerance to the endometriotic tissue, although more robust evidence is needed [16]. In this regard, the study followed surface markers on Tregs isolated from peripheral blood or peritoneal fluid of patients with endometriosis. The results showed that these patients had a higher number of $TNFRII^+$ and $CTLA\text{-}4^+$ Tregs in the blood and fewer $ICOS^+$ and $CD45RO^+$ Tregs in the peritoneal fluid than healthy controls. These data suggest different activation profiles and other memory markers between Tregs of healthy versus endometriotic women, but the real meanings of these findings are difficult to interpret [17]. Knowing whether the differential expression of these markers is the direct result of endometriosis or the cause of endometriosis is unclear. Therefore, the fundamental role of Tregs in the development or progression of endometriosis remains to be elucidated.

Some studies suggested TGF-β1, COX-2, VEGF, ER-1β, and aromatase levels elevated in endometriosis [18], but their expression is unfortunately not endometriosis-specific, which hampers their use as diagnostic factors. The role of TGF-β1 was further supported by in vitro findings: the activation of the TGF-β1 signaling lowered the invasive potential of endometrial cells; highly invasive phenotype expressed higher levels of SDC1, SDC4, and genes involved in TGF-β1 signaling [19]. However, these findings corresponded to only one subgroup of investigated patients and cannot be generalized.

The levels of IL-6 and IL-8 produced by endometriotic stromal cells were elevated in the peritoneal fluid of patients with endometriosis. In addition, in vitro experiments suggested that these levels can be suppressed by treatment with pyrvinium pamoate [20]. IL-8 is a pro-inflammatory cytokine, but the roles of IL-6 are both pro- and anti-inflammatory; therefore, the significance of increased production of these two particular cytokines is unclear. Pyrvinium pamoate is an inhibitor of several critical signaling pathways, including the Wnt/β-catenin pathway. Still, any suggestions of either using IL-6 or IL-8 levels as a marker or using this compound in endometriosis treatment are highly premature [21].

Other possible markers gaining attention recently are alarmins. In particular, the high-mobility group box 1 (HMGB1), which is involved in the mediation of inflammation and angiogenesis, appears interesting. The use of HMGB1 on endometriotic stromal cells resulted in the stimulation of VEGF production [22]. Another alarmin is IL-33, which regulates inflammation. Elevated levels in the peritoneal fluid and serum of women with endometriosis were

reported [23]. Moreover, IL-33 supplementation was observed to play a role in the growth of endometrial lesions [24].

Difficulties in searching for endometriosis biomarkers can be demonstrated in a study based on the antibody array approach. The study initially found 280 upregulated and 29 downregulated proteins using more than one hundred plasma samples, but a more detailed analysis confirmed only four of them being differentially expressed. In the end, only one of them, IL-31, demonstrated significant difference and potentiality to serve as a biomarker for endometriosis [25].

Similar conclusions can be reached from the novel use of mass cytometry, which revealed over forty distinct immune cell types in the peritoneal cavity. Still, only the level of $CD69^{+}$T lymphocytes was increased in patients with endometriosis [26]. Some connection with the inflammation level can be suggested, but what these findings mean is not clear. The different immune environments in the peritoneal fluid between healthy and patient's samples are established, but its clinical utility is not.

MicroRNAs

MicroRNAs (miRNAs) are short, single-stranded non-coding RNA molecules involved in various biological processes. Among their biological activities, they are considered both a marker and a regulating factor of endometriosis. A study identified 23 microRNAs differentially expressed in patients with endometriosis and healthy control. Subsequently, plasma miRNA expression patterns were suggested as a highly specific and reliable diagnostic biomarkers [27]. Some reports further hypothesized that more detailed miRNA studies would offer new treatments of endometriosis [28]. A PCR retrospective study found several miRNAs able to serve as a marker of endometriosis. The specificity of the panel was comparable to laparoscopy [29]. miRNA-451 was identified as a potential biomarker and is considered significantly contributing to endometriosis's pathogenesis [30]. With potential target genes, such as OSR1, CDKN2D, and TTN, and clear data on promoting proliferation and inhibiting apoptosis, miRNA-451 might be a potential target for the endometriosis treatment. Another study found that miR-96-5p is strongly downregulated in ectopic endometrial tissue. Experimental overexpression of this miRNA resulted in the inhibition of endometrial cell viability and migration. Besides, the direct target of miR-96-5p is TGFBR1. miR-96-5p regulates TGF-β/SMAD signaling pathway [31]. If confirmed, this might lead to potential reversion of endometriosis. As this miRNA is also a tumor suppressor, it might help inhibit ovarian cancer development in endometriotic patients [12].

However, many studies on miRNAs and endometriosis suffered from limited consistency and offered conflicting results despite the promising data. Another problem with miRNA in endometriosis originates from too high numbers of them. The careful combination of several candidates might yield better sensitivity. A genome-wide miRNA profiling suggested the presumption that the link between endometriosis and some miRNAs might exist. Still, it might be helpful in some groups only, as numerous factors such as infertility will significantly decrease its utility [32].

Other Markers

The remodeling of the extracellular matrix represents an essential condition for tissue implantation. In addition, matrix metalloproteinase (MMP) and its inhibitor TIMP-1 are present in shed endometrium cells and regulate the degradation of the extracellular matrix. The levels of MMP-9 were found higher, and the levels of TIMP-1 lower in patients with endometriosis [33]. Both levels, mainly when combined, might be helpful for the prediction of endometriosis. The possible role of MMP-9 was supported by findings showing the strong potential of MMP-9 as a biomarker of this disease [34]. A histochemical analysis investigated the expression of MMP-2 and MMP-9 in invasive colorectal endometriosis and superficial peritoneal endometriosis. This evaluation showed significant differences in expression patterns of these two markers, allowing them to be used to determine the invasiveness and aggressiveness of endometriosis [35].

An interesting possibility is the use of estrogen-related receptor alpha (ERRα). An RT-PCR study found that expression of ERRα correlated with the pathological stages of ovarian endometriosis [36], suggesting a potential use to determine the disease progression.

After some suggestions that cell adhesion molecules might be used as possible biomarkers of endometriosis, a detailed longitudinal study found no significant differences in the case of sICAM-1, sVCAM-1, and P- and E-selectin levels [37]. Similarly, a promoter of the neovascularization glycodelin A as a biomarker for endometriosis is questionable. Some analysts suggested high sensitivity [38]; other studies found no specificity at all [39].

Other potential markers are circulating non-coding RNAs. Individual studies did not get behind suggestions, but a meta-analysis of current literature allowed detailed subgroup analysis and meta-regression and found that these RNAs have a high potential to be a new non-invasive marker [40].

Another option is the genomic approach to the development of non-invasive markers. The expression of numerous genes has been found to differ between women with and without endometriosis, but this range was too broad, probably due to endometriosis's polygenic nature. Recently developed endometriosis knowledgebase allowed better comparison, narrowing genes' plethora to 13 candidates [41]. However, the use of gene expression for biomarkers of endometriosis

is still far away. Although these microRNAs look promising, their limitations result from the fact that no endometriotic lesion-specific microRNA has been identified yet.

Human Epididymis Protein 4

Human epididymis protein 4 (HE4) has been found elevated in endometriosis and hypothesized as a potential marker [42]. However, other studies showed no elevation of HE4 at all [43], making the diagnostic use of this biomarker questionable. Conversely, another study proposed the HE4 serum concentration as a marker for the differentiation of ovarian cancer from ovarian endometriotic cysts [44]. Consistently, a large study performed by four European centers showed that HE4 is a valuable biomarker for excluding malignant disease in patients with endometriosis [45]. From the current data, it is more likely that this marker is probably better suited for a diagnostic role in epithelial ovarian cancer [46], similar to other proposed markers such as CA-125, kallikrein 6, or osteopontin than as a marker of endometriosis. Despite numerous multicentric studies, the current lack of specificity suggests that the idea of HE4 as a biomarker of endometriosis should be abandoned.

CA-125

Tumor marker CA-125 is one of the most studied biomarkers [47]. CA-125 is a high molecular weight glycoprotein initially studied as a marker for ovarian tumors [48]. Its use in endometriosis is limited by a low specificity, as multiple conditions are associated with high levels of CA-125 [49].

The elevated levels of serum CA-125 in endometriosis correlate with the disease severity [50]. Studies comparing levels of CA135, CA19-9, and Ki-67 in late stages of endometriosis did not find strong correlation between serum levels and histochemical staining. However, the authors hypothesized that serum CA-125 levels might correlate with the proliferative activity of endometriotic epithelial cells [51]. The usefulness of the CA-125 marker was further supported by a study using 164 women, with CA-125 significantly increased levels. Further analysis revealed a good correlation with hsCRP and with the severity of endometriosis [52]. However, the problems with specificity, allowing us to distinguish between ovarian tumor and endometriosis, remain. They might be partially overcome by the simultaneous use of a panel of tumor markers, including CEA and CA19-9 [53], but CA-125 remains better suited for differentiation between ovarian clear cell carcinoma and endometrioid ovarian carcinoma [54].

Additional studies found that when used together with the neutrophil/lymphocyte ratio, CA-125 can be used to diagnose endometriosis [55]. Similar results were achieved when CA-125 was used simultaneously with fibrinogen level [56]. A recent study suggested that the CA-125 marker might be more important for predicting infertility associated with endometriosis than predicting endometriosis itself [21]. However, it is essential to note that this marker is currently not recommended as a biomarker for endometriosis. In addition, the possible use of this marker divides the community of researchers into two groups—one believing in clear correlation, and the second is convinced that the specificity and/or sensitivity is not enough.

Stem Cells

Stem cells are currently one of the proposed causes of endometriosis. It is not surprising that stem cells were also evaluated as a possible marker. One marker option is ABCG2 and CD133, with a known association with this disease's pathogenesis [57]. However, most of the markers associated with stem cells can be found in vitro only or differ based on stem cell origin, with extensive problems to valid stem cell–related markers [58]. Similar to the eutopic endometrium, epithelial and mesenchymal stem cells were isolated and proved to have high clonogenic and self-renewal capacities [59, 60]. The epithelial stem cells express epithelial cell adhesion molecule (EpCAM), cytokeratin, and alpha-6 integrin; conversely, stromal stem cells express CD133, Musashi RNA binding protein 1 (Msi-1), and spalt-like transcription factor 4 (SALL4) [60]. The endometriotic mesenchymal stem cells, similar to their eutopic endometrial counterparts, were found to retain the ability to differentiate in multiple mesodermal lineages, including adipocytes, chondrocytes, myocytes, and osteocytes [60]. The ability of the suspected epithelial progenitors to differentiate into mesodermal cellular lineages was not tested to the best of our knowledge. Even though the eutopic and ectopic mesenchymal stem cells are similar, many functional studies found essential differences that could explain much of the endometriosis characteristics. Indeed, ectopic mesenchymal stem cells have higher growth and proliferation rates than their eutopic counterparts [59]. Additionally, many investigators found increased migration and angiogenesis capacities of ectopic mesenchymal stem cells compared with eutopic stem cells [61]. These behavioral differences are attributed mainly to the altered expressions of genes related to growth, proliferation, migration, and self-renewal between these two cell groups: in particular, stage-specific embryonic antigen 3 (SSEA) and SRY-Box transcription factor 9 (SOX9)–expressing mesenchymal stem cells were detected; additionally, SSEA-expressing endometriotic cells were found to have increased telomerase activity when it was grown in cultures [62]. Stem cell–related markers look promising, and we can assume that they might be developed into clinically relevant markers.

Endometriosis was found to express the pluripotency gene triad highly: NANOG, SOX2, and octamer-binding transcription factor 4 (OCT-4) [63, 64]. The expression of NANOG and SOX2 was remarkably much higher than their expression

in the eutopic endometrium [63, 64]. Conversely, OCT-4 was found to be less expressed by endometriosis when compared with eutopic endometrium [63]. Chang et al. [65] also reported the upregulation of OCT-4 in ectopic lesions and suggested its possible involvement in lesion progression by stimulating the migration of endometrial cells. Moreover, two studies report the upregulatory influence of estrogen on SOX2 and NANOG [66, 67]. Based on these elements, overexpression of these two genes may maintain the self-renewal capacity and increase cell survival in endometriosis [63].

Conclusion

Despite decades of intensive research, endometriosis remains a significant health problem. The nature of this disease is highly complex, and despite decades of intensive research, the fast diagnosis, prediction, and most of all, treatment options remain elusive. Use or sensitivity of individual markers might also depend on the type and stage of endometriosis, further hindering the research.

The search for endometriosis biomarkers offered numerous promising markers, but none is used in clinical practice. Some markers are purely based on a known association between endometriosis and other biological reaction. In addition, many proposed markers are based more on the fact that some systems are involved in endometriosis and the subsequent assumption that its parts might serve as a marker. One example might be the hypotheses about the expression of CD200 and CD200R [68], which is based on the involvement in inflammatory diseases.

Most, if not all, biomarkers mentioned in this review have a common problem—unknown clinical cutoffs for sensitivity and specificity, which are usually low. Based on these setbacks, none of the possible biomarkers mentioned in this review has been recommended for clinical diagnosis. miRNAs might be an exception, but even they are not without problems. Severe discrepancies among expression levels and the vast number of different miRNAs make the usability questionable [69]. Therefore, our search for the diagnostic marker for the development and progression of endometriosis is still on.

Many, if not the majority, studies on markers in endometriosis suffer from numerous setbacks, including often being retrospective, often using a low-resolution technique, different diagnostic techniques, and low numbers of patients. In addition, the number of meta-analyses about non-invasive diagnosis and diagnostic markers is very low [70].

The current literature on potential biomarkers is already vast. However, no conclusion can be made. Some studies remind more of a fishing expedition testing one possible molecule after another, often without a clear explanation of the hypothesis behind the study. This hypothesis-free approach might rarely result in significant achievements. Just to expect the development of biomarker on the observation of differences between healthy women and patients with endometriosis is geared to fail.

However, the ever-improving palette of the newest technologies, including and combining metabolomics, genomics, and proteomics, offers promising tools for investigating a complete panel of molecules and genes. The use of a new generation of techniques and a better combination of possible markers might be a way to go [71]. A careful combination of individual biomarkers and evaluating a whole comprehensive panel instead of individual markers might open a new window [72].

In addition, most of the studies of biomarkers in endometriosis suffer from limitations caused by small numbers of patients. More extensive and long-term multicenter studies are necessary.

Author Contribution All the authors conform to the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, contributed to the intellectual content of the study, and approved the final version of the article.

Declarations

Ethics approval This article is a review and does not contain any research data collected and/or analyzed by the authors, so no formal ethical approval is required.

Conflict of interest The authors declare no competing interests.

References

1. Missmer SA, Cramer DW. The epidemiology of endometriosis. Obstet Gynecol Clin N Am. 2003;30:1–19 vii.
2. Baldi A, Campioni M, Signorile PG. Endometriosis: pathogenesis, diagnosis, therapy and association with cancer (review). Oncol Rep. 2008;19:843–6.
3. Laganà AS, Garzon S, Götte M, Viganò P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: molecular and cell biology insights. Int J Mol Sci. 2019;20(22):5615.
4. Sundqvist J, Andersson KL, Scarselli G, Gemzell-Danielsson K, Lalitkumar PG. Expression of adhesion, attachment and invasion markers in eutopic and ectopic endometrium: a link to the aetiology of endometriosis. Hum Reprod. 2012;27(9):2737–46.
5. Garzon S, Laganà AS, Barra F, Casarin J, Cromi A, Raffaelli R, et al. Aromatase inhibitors for the treatment of endometriosis: a systematic review about efficacy, safety and early clinical development. Expert Opin Investig Drugs. 2020;29:1377–88.
6. Riemma G, Laganà AS, Schiattarella A, Garzon S, Cobellis L, Autiero R, et al. Ion channels in the pathogenesis of endometriosis: a cutting-edge point of view. Int J Mol Sci. 2020;21(3):1114.
7. Della Corte L, Noventa M, Ciebiera M, Magliarditi M, Sleiman Z, Karaman E, et al. Phytotherapy in endometriosis: an up-to-date review. J Complement Integr Med. 2020;17(3):/j/jcim.2020.17.issue-3/jcim-2019-0084/jcim-2019-0084.xml.
8. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med. 2020;382:1244–56.

9. Garzon S, Laganà AS, Barra F, Casarin J, Cromi A, Raffaelli R, et al. Novel drug delivery methods for improving efficacy of endometriosis treatments. Expert Opin Drug Deliv. 2020;27:1–13. https://doi.org/10.1080/17425247.2021.1829589.
10. Ghai V, Jan H, Shakir F, Haines P, Kent A. Diagnostic delay for superficial and deep endometriosis in the United Kingdom. J Obstet Gynaecol. 2020;40(1):83–9.
11. Anastasiu CV, Moga MA, Elena Neculau A, Bălan A, Scârneciu I, Dragomir RM, et al. Biomarkers for the noninvasive diagnosis of endometriosis: state of the art and future perspectives. Int J Mol Sci. 2020;21(5):1750.
12. Králíčková M, Vetvicka V. Endometriosis and ovarian cancer. World J Clin Oncol. 2014;5:800–5.
13. Hudson QJ, Ashjaei K, Perricos A, Kuessel L, Husslein H, Wenzl R, et al. Endometriosis patients show an increased M2 response in the peritoneal CD14+low/CD68+low macrophage subpopulation coupled with an increase in the T-helper 2 and T-regulatory cells. Reprod Sci. 2020;27:1920–31.
14. Ottolina J, Bartiromo L, Dolci C, Salmeri N, Schimberni M, Villanacci R, et al. Assessment of coagulation parameters in women affected by endometriosis: validation study and systematic review of the literature. Diagnostics (Basel). 2020;10:567.
15. Berbic M, Fraser IS. Regulatory T cells and other leukocytes in the pathogenesis of endometriosis. J Reprod Immunol. 2011;88:149–55.
16. Osuga Y, Koga K, Hirota Y, Hirata T, Yoshino O, Taketani Y. Lymphocytes in endometriosis. Am J Reprod Immunol. 2011;65:1–10.
17. Gueuvoghlanian-Silva BY, Hernandes C, Correia RP, Podgaec S. Deep infiltrating endometriosis and activation and memory surface markers and cytokine expression in isolated Treg cells. Reprod Sci. 2020;27:599–610.
18. Meng Q, Sun W, Jiang J, Fletcher NM, Diamond MP, Saed GM. Identification of common mechanisms between endometriosis and ovarian cancer. J Assist Reprod Genet. 2011;28:917–23.
19. Ponandai-Srinivasan S, Saare M, Boggavarapu NR, Frisendahl C, Ehrström S, Riethmüller C, et al. Syndecan-1 modulates the invasive potential of endometrioma via TGF-β signalling in a subgroup of women with endometriosis. Hum Reprod. 2020;35:2280–93.
20. Karamian A, Paktinat S, Esfandyari S, Nazarian H, Ali Ziai S, Zarnani AH, et al. Pyrvinium pamoate induces in-vitro suppression of IL-6 and IL-8 produced by human endometriotic stromal cells. Hum Exp Toxicol. 2021;40:649–60.
21. Gica N, Panaitescu AM, Iancu G, Botezatu R, Peltecu G, Gica C. The role of biological markers in predicting infertility associated with non-obstructive endometriosis. Ginekol Pol. 2020;91:189–92.
22. Shimizu K, Kamada Y, Sakamoto A, Matsuda M, Nakatsuka M, Hiramatsu Y. High expression of high-mobility group box 1 in menstrual blood: implications for endometriosis. Reprod Sci. 2017;24:1532–7.
23. Santulli P, Borghese B, Chouzenoux S, Vaiman D, Borderie D, Streuli I, et al. Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis. Hum Reprod. 2012;27:2001–9.
24. Miller JE, Monsanto SP, Ahn SH, Khalaj K, Fazleabas AT, Young SL, et al. Interleukin-33 modulates inflammation in endometriosis. Sci Rep. 2017;7:17903.
25. Dorien O, Waelkens E, Vanhie A, Peterse D, Fassbender A, D'Hooghe T. The use of antibody arrays in the discovery of new plasma biomarkers for endometriosis. Reprod Sci. 2020;27:751–62.
26. Guo M, Bafligil C, Tapmeier T, Hubbard C, Manek S, Shang C, et al. Mass cytometry analysis reveals a distinct immune environment in peritoneal fluid in endometriosis: a characterisation study. BMC Med. 2020;18:3.
27. Suryawanshi S, Vlad AM, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, et al. Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. Clin Cancer Res. 2013;19:1213–24.
28. Siufi Neto J, Kho RM, Siufi DF, Baracat EC, Anderson KS, Abrão MS. Cellular, histologic, and molecular changes associated with endometriosis and ovarian cancer. J Minim Invasive Gynecol. 2014;21:55–63.
29. Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril. 2020;113:1232–41.
30. Gao S, Liu S, Gao ZM, Deng P, Wang DB. Reduced microRNA-451 expression in eutopic endometrium contributes to the pathogenesis of endometriosis. World J Clin Cases. 2019;7:2155–64.
31. Chen S, Luo Y, Cui L, Yang Q. miR-96-5p regulated TGF-β/SMAD signaling pathway and suppressed endometrial cell viability and migration via targeting TGFBR1. Cell Cycle. 2020;19:1740–53.
32. Vanhie A, O D, Peterse D, Beckers A, Cuéllar A, Fassbender A, et al. Plasma miRNAs as biomarkers for endometriosis. Hum Reprod. 2019;34:1650–60.
33. Madjid TH, Ardiansyah DF, Permadi W, Hernowo B. Expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in endometriosis menstrual blood. Diagnostics (Basel). 2020;10(6):364.
34. Bostanci Durmus A, Dincer Cengiz S, Yılmaz H, Candar T, Gursoy AY, Sinem CG. The levels of matrix metalloproteinase-9 and neutrophil gelatinase-associated lipocalin in different stages of endometriosis. J Obstet Gynaecol. 2019;39:991–5.
35. Weigel MT, Krämer J, Schem C, Wenners A, Alkatout I, Jonat W, et al. Differential expression of MMP-2, MMP-9 and PCNA in endometriosis and endometrial carcinoma. Eur J Obstet Gynecol Reprod Biol. 2012;160:74–8.
36. Liu C, Chen Y, Chen M, Mao X, Dong B, Sun P. A novel non-invasive molecular biomarker in ovarian endometriosis: estrogen-related receptor α. Arch Gynecol Obstet. 2020;302:405–14.
37. Proestling K, Wenzl R, Yotova I, Hauser C, Husslein H, Kuessel L. Investigating selected adhesion molecules as urinary biomarkers for diagnosing endometriosis. Reprod BioMed Online. 2020;40:555–8.
38. Kocbek V, Vouk K, Mueller MD, Rižner TL, Bersinger NA. Elevated glycodelin-A concentrations in serum and peritoneal fluid of women with ovarian endometriosis. Gynecol Endocrinol. 2013;29:455–9.
39. Drosdzol-Cop A, Skrzypulec-Plinta V. Selected cytokines and glycodelin A levels in serum and peritoneal fluid in girls with endometriosis. J Obstet Gynaecol Res. 2012;38:1245–53.
40. Li Y, Chen Q. Circulating non-coding RNAs as non-invasive diagnostic markers of endometriosis: a comprehensive meta-analysis. Arch Gynecol Obstet. 2019;300:1099–112.
41. Joseph S, Mahale SD. Endometriosis knowledgebase: a gene-based resource on endometriosis. Database (Oxford). 2019;2019:baz062.
42. Ortiz-Muñoz B, Aznar-Oroval E, García García A, Covisa Peris A, Perez Ballestero P, Sanchez Yepes M, et al. HE4, Ca125 and ROMA algorithm for differential diagnosis between benign gynaecological diseases and ovarian cancer. Tumour Biol. 2014;35:7249–58.
43. Jacob F, Meier M, Caduff R, Goldstein D, Pochechueva T, Hacker N, et al. No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting. Gynecol Oncol. 2011;121:487–91.
44. Huhtinen K, Suvitie P, Hiissa J, Junnila J, Huvila J, Kujari H, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. Br J Cancer. 2009;100:1315–9.

45. Zapardiel I, Gorostidi M, Ravaggi A, Allende MT, Silveira M, Abehsera D, et al. Utility serum marker HE4 for the differential diagnosis between endometriosis and adnexal malignancy. Int J Gynecol Cancer. 2016;26:52–5.
46. Bandiera E, Zanotti L, Fabricio AS, Bucca E, Squarcina E, Romani C, et al. Cancer antigen 125, human epididymis 4, kallikrein 6, osteopontin and soluble mesothelin-related peptide immunocomplexed with immunoglobulin M in epithelial ovarian cancer diagnosis. Clin Chem Lab Med. 2013;51:1815–24.
47. Bast RC Jr, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. Int J Biol Markers. 1998;13:179–87.
48. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981;68(5):1331–7.
49. Králíčková M, Losan P, Vetvicka V. Endometriosis and cancer. Womens Health (Lond). 2014;10:591–7.
50. Pittaway DE, Fayez JA. The use of CA-125 in the diagnosis and management of endometriosis. Fertil Steril. 1986;46:790–5.
51. Toki T, Kubota J, Lu X, Nakayama K. Immunohistochemical analysis of CA125, CA19-9, and Ki-67 in stage III or IV endometriosis: positive correlation between serum CA125 level and endometriotic epithelial cell proliferation. Acta Obstet Gynecol Scand. 2000;79:771–6.
52. Sütcü HK, Celik GIT, Akpak YK, Akar ME, Taşkın Ö, Ozdem S, et al. The value of CA-125, CA 19-9, Interleukin-6, Interleukin-8 and hsCRP in the diagnosis of endometriosis. Acta Medica Mediterranea. 2015;31:793–9.
53. Shinmura H, Yoneyama K, Harigane E, Tsunoda Y, Fukami T, Matsushima T, et al. Use of tumor markers to distinguish endometriosis-related ovarian neoplasms from ovarian endometrioma. Int J Gynecol Cancer. 2020;30:831–6.
54. Li M, Tan J, Zhang Y, Ai C, Wang H, Zhang H, et al. Assessing CT imaging features combined with CEA and CA125 levels to identify endometriosis-associated ovarian cancer. Abdom Radiol (NY). 2020;46:2367–75. https://doi.org/10.1007/s00261-020-02571-x.
55. Jing X, Li C, Sun J, Peng J, Dou Y, Xu X, et al. Systemic inflammatory response markers associated with infertility and endometrioma or uterine leiomyoma in endometriosis. Ther Clin Risk Manag. 2020;16:403–12.
56. Ding S, Lin Q, Zhu T, Li T, Zhu L, Wang J, et al. Is there a correlation between inflammatory markers and coagulation parameters in women with advanced ovarian endometriosis? BMC Womens Health. 2019;19:169.
57. Liu S, Zhou J, Wen J. Expression and significance of CD133 and ABCG2 in endometriosis. Clin Exp Obstet Gynecol. 2015;42:771–5.
58. Tempest N, Maclean A, Hapangama DK. Endometrial stem cell markers: current concepts and unresolved questions. Int J Mol Sci. 2018;19(10):3240.
59. Kao AP, Wang KH, Chang CC, Lee JN, Long CY, Chen HS, et al. Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells and the development of an in vivo endometriotic invasion model. Fertil Steril. 2011;95:1308–15.e1.
60. Chan RW, Ng EH, Yeung WS. Identification of cells with colony-forming activity, self-renewal capacity, and multipotency in ovarian endometriosis. Am J Pathol. 2011;178:2832–44.
61. Liu Y, Liang S, Yang F, Sun Y, Niu L, Ren Y, et al. Biological characteristics of endometriotic mesenchymal stem cells isolated from ectopic lesions of patients with endometriosis. Stem Cell Res Ther. 2020;11:346.
62. Valentijn AJ, Palial K, Al-Lamee H, Tempest N, Drury J, Von Zglinicki T, et al. SSEA-1 isolates human endometrial basal glandular epithelial cells: phenotypic and functional characterization and implications in the pathogenesis of endometriosis. Hum Reprod. 2013;28:2695–708.
63. Song Y, Xiao L, Fu J, Huang W, Wang Q, Zhang X, et al. Increased expression of the pluripotency markers sex-determining region Y-box 2 and Nanog homeobox in ovarian endometriosis. Reprod Biol Endocrinol. 2014;12:42.
64. Shariati F, Favaedi R, Ramazanali F, Ghoraeian P, Afsharian P, Aflatoonian B, et al. Increased expression of stemness genes REX-1, OCT-4, NANOG, and SOX-2 in women with ovarian endometriosis versus normal endometrium: a case-control study. Int J Reprod Biomed. 2019;16:ijrm.v16i12.3684.
65. Chang JH, Au HK, Lee WC, Chi CC, Ling TY, Wang LM, et al. Expression of the pluripotent transcription factor OCT4 promotes cell migration in endometriosis. Fertil Steril. 2013;99:1332–1339.e5.
66. Zhang Y, Eades G, Yao Y, Li Q, Zhou Q. Estrogen receptor α signaling regulates breast tumor-initiating cells by down-regulating miR-140 which targets the transcription factor SOX2. J Biol Chem. 2012;287(49):41514–22.
67. Yang L, Luo L, Ji W, Gong C, Wu D, Huang H, et al. Effect of low dose bisphenol A on the early differentiation of human embryonic stem cells into mammary epithelial cells. Toxicol Lett. 2013;218:187–93.
68. Abramiuk M, Grywalska E, Korona-Glowniak I, Niedzwiedzka-Rystwej P, Polak G, Kotarski J, et al. CD200 and CD200R expression on peripheral blood lymphocytes and serum CD200 concentration as a new marker of endometriosis. J Clin Med. 2020;9. https://doi.org/10.3390/jmc9093035.
69. Nothnick WB. MicroRNAs and endometriosis: distinguishing drivers from passengers in disease pathogenesis. Semin Reprod Med. 2017;35:173–80.
70. Li Y, Chen Q. Circulating non-coding RNA as non-invasive diagnostic markers of endometriosis: a comprehensive meta-analysis. Arch Gynecol Obstet. 2019;300:1099–112.
71. Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative re3al-time polymerase chain reaction. Fertil Steril. 2020;113:1232–41.
72. Liu C, Chen YJ, Chen M, Mao XD, Dong BH, Sun PM. A novel non-invasive molecular biomarker in ovarian endometriosis: estrogen-related receptor A. Arch Gynecol Obstet. 2020;302:405–14.