Biomarkers of endometriosis: How far have we come and where are we going?

Biološki označevalci endometrioze: Kje smo in kam smo namenjeni?

Vid Janša,1 Joško Osredkar,2 Eda Vrtačnik Bokal,3,4 Tea Lanišnik Rižner,5 Helena Ban Frangež3,4

Abstract

Endometriosis is a common gynaecological disease that is characterized by endometrium-like tissue outside the uterine cavity. Endometriosis significantly compromises the quality of life of women and is a major cause of infertility. The gold standard for diagnosis of endometriosis is visual inspection by laparoscopy, which significantly prolongs the time to final diagnosis. This lack of non-invasive diagnostic approaches is why the discovery of biomarkers for endometriosis has been defined as a research priority. In this report, we describe hypothesis-driven and hypothesis-generating approaches for biomarker discovery, along with some important potential biomarkers of endometriosis and their diagnostic characteristics, sensitivities, and specificities. Finally, we present our perspective on the discovery of biomarkers for endometriosis, and discuss some results from our previous and more recent studies. Future studies must focus on improving patient quality of life rather than on discovering significant differences, and therefore close collaboration between clinicians and pre-clinical researchers is essential.

Izvleček


DOI: https://doi.org/10.6016/ZdravVestn.3056

Copyright (c) 2021 Slovenian Medical Journal. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.
1 Introduction

Endometriosis is a common benign gynecological disease that affects up to 10% of women, with prevalence increasing to 50% for women with infertility or pelvic pain (1). It is characterized by endometrium-like tissue outside the uterine cavity. Despite multiple theories about the disease etiology (e.g., coelomic metaplasia theory, Mullerian rest theory, induction theory, stem-cell theory), none of these can as yet explain all types of endometriosis. The implantation (retrograde menstruation) theory is the most commonly accepted at present (2). Despite major efforts, endometriosis still remains a poorly understood disease with poorly known aetiology and complex pathogenesis. Degradation of the extracellular matrix, aberrant apoptosis, angiogenesis, enhanced cell adhesion, increased oxidative stress and inflammation processes, disturbed immune system, and other processes are involved according to a complex pathogenesis (3-5). As a result of these processes, endometrial cells survive and proliferate at ectopic sites, and evoke chronic pelvic inflammation (6).

Endometriosis significantly compromises the quality of life of women and is a major cause of infertility. As nonspecific symptoms and surgery represent the definitive diagnostic tool, it can take up to 11 years before women are correctly diagnosed and treated (7). The gold standard for diagnosis is visual surgical (laparoscopic) inspection of the pelvic organs, preferably coupled with histological confirmation (3). This procedure is invasive, requires general anaesthesia, is expensive, and can have complications. For these reasons, biomarker research was defined as a research priority in 2011 by the World Congress on Endometriosis, the World Endometriosis Society, and the World Endometriosis Research Foundation (1,7). To date, there are no reliable clinical markers for the diagnosis and prognosis of endometriosis.

2 Biomarker research

According to the National Institutes of Health Biomarkers Definitions Working Group, a biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention” (8). Properties of a perfect biomarker include high sensitivity, high specificity, simplicity, reproducibility, and minimal invasiveness. An ideal biomarker should enable diagnosis determination, especially in patients without specific symptoms. Correlation between biomarker levels and disease stage is also desirable (9). Unfortunately, many of these requirements are not attainable by many of the potential biomarkers of endometriosis.

Although the importance of reliable noninvasive biomarkers of endometriosis is recognized, the development of a clinically useful test is a long, expensive, and uncertain process (10). Developing diagnostic tests can be classified into four phases (11,12): phase I, the preclinical discovery phase that consists of exploratory studies aimed at the identification of potential biomarkers; phase II, the retrospective validation that includes preclinical development and validation of a potentially clinically useful diagnostic test; phase III, the prospective clinical validation and determination of...
clinical utility, which also defines the diagnostic accuracy and predictive value in the target population; and phase IV, the commercialization of the resulting diagnostic kits. Most of the endometriosis biomarker research has remained at phase I (13,14).

We must be aware of the importance of clinical endpoints during the process of biomarker discovery. A Cochrane systematic review by Nisenblat et al. explained that in the clinical setting, non-invasive biomarkers of endometriosis are needed as a replacement test for the diagnostic surgery, or as a triage test to select the patients who need this surgery (15).

The goal of clinical practice must be to improve the quality of life of the patient, reduce further morbidity, and provide rapid and accurate diagnosis and treatment.

3 Specific considerations in endometriosis biomarker research

Endometriosis is a heterogeneous disease that has been categorized into the endopelvic and extrapelvic forms (16). Endopelvic disease includes deep infiltrating endometriosis, ovarian endometrioma, and surface peritoneal endometriosis. Extrapelvic endometriosis includes relatively typical abdominal wall endometriosis as well as some rare locations, such as nasal, bladder, thorax, and even hepatic endometriosis (17). The widespread phenotype of endometriosis reminds us of the metastatic characteristic of cancer. Due to the heterogeneous nature of endometriosis, we cannot precisely explain the aetio-pathogenicity of the disease, and obviously there is the involvement of other mechanisms in addition to retrograde menstruation. Searching for biomarkers in aetiopathogenic heterogeneous diseases is challenging. This also increases the potential of false-negative laparoscopic surgery in symptomatic patients.

The major limitations in the discovery of biomarkers for endometriosis are a lack of correlation between the stage of the disease and the symptoms. Endometriosis can result in mild symptoms, or even be asymptomatic, which is also observed in daily clinical practice (18,19). The cyclic variation in the endometrial molecular characteristics also presents a significant challenge in biomarker research. An ideal biomarker for endometriosis would maintain its sensitivity and specificity regardless of the phase of the menstrual cycle (11).

When starting out on biomarker discovery for endometriosis, careful study design is a prerequisite. It is essential to enrol a group of patients from the target population who are stratified into cases and controls based on the gold standard of laparoscopy and histological evaluation. The patients have to be well characterized with regard to their clinical and life-style data. Standard operating procedures are needed to control and harmonize all of the pre-analytical steps (20).

4 Approaches in the search for biomarkers of endometriosis

Classical biomarker studies are ‘hypothesis-driven’ approaches that are based on assumptions regarding the pathophysiology of a disease. Using this approach, individual biomolecules or panels of biomolecules associated with the pathophysiological processes are investigated (e.g., cell proliferation, adhesion, invasion, angiogenesis, inflammation).
In contrast, “-omics” technologies aim to find general differences between patients without restricting the search to a specific panel of biomolecules. This is an approach that is 'hypothesis generating'. Hypothesis-generating research is especially appropriate in heterogeneous diseases such as endometriosis, as it is known that there is the need for a panel of biomarkers to reach sufficient sensitivity and specificity.

According to the opinion of the authors, one option in the research into biomarkers for endometriosis is to identify molecules with differential abundances in peritoneal fluid, and to determine the concentrations of these molecules in peripheral blood and other blood fluids (4). The peritoneal cavity represents a 'local endometriosis environment'. Ectopic endometrial cells within the peritoneal cavity can evoke local inflammation, which is mediated by immune cells and pro-inflammatory products in the peritoneal fluid. Additionally, the pathogenesis of endometriosis is poorly understood, and studies of the peritoneal fluid might provide the key to a better understanding of this disease. The surface of the peritoneal cavity is large, and it allows passive dialysis of substances between the blood plasma and the peritoneal fluid, where diffusion rates decrease as molecular weight increases (21-23). There is no doubt that peritoneal fluid has a complex role in the aetiopathogenesis of endometriosis, and to be able to reveal the underlying disease biology at the molecular level would be of great clinical importance.

5 How far have we come?

Over the last decade, there has been an upsurge in endometriosis biomarker research, although from the clinical point of view, patients and physicians have not seen any real benefits. Despite great research efforts, not a single potential biomarker has been validated for diagnosis or prognosis of endometriosis (15,24). Leading researchers have published several review articles with the aim being to identify all of the known potential biomarkers (4,13,15,24-27). These searches for biomarkers for non-invasive diagnostics have most commonly focused on peripheral blood, particularly on serum, but also on plasma, as well as urine, peritoneal fluid and saliva (4,13,26,27). Over 100 potential biomarkers of endometriosis have been identified, but neither a single biomarker nor a panel of biomarkers has been shown to be clinically useful to date. Here, we will review the most important recently investigated molecules.

Endometriotic lesions undergo cyclic bleeding, which results in inflammatory responses. Endometriosis is thus considered to be a chronic inflammatory disease. The most 'popular' biomarker is cancer antigen (CA)-125. It is a valuable tumour marker for ovarian malignancy and it is also known to be elevated in inflammatory events in the abdomen. CA-125 has been investigated at different cut-off values in patients with endometriosis, but none of those have met the criteria for triage or replacement tests (15). CA-125 lacks sensitivity and specificity. The cause of this sensitivity problem is that CA-125 is mainly elevated in advanced endometriosis stages, as opposed to early stages, while its specificity is poor because its levels also increase in other diseases (30,31). CA-125 thus shows better diagnostic characteristics for moderate-to-severe endometriosis, whereby a 14.7 U/mL cut-off allows diagnosis of moderate-to-severe endometriosis, with a sensitivity of 92% and a specificity of 87% (32,33).
Knific et al. recently proposed a model for diagnosis of all types of endometriosis, which included CA-125, body mass index, and information about the presence of ovarian cyst or dyspareunia and dysmenorrhea (34). The model had an area under the curve of 0.836, with a sensitivity of 74.0% and a specificity of 81.3% (34). By comparison, transvaginal ultrasound alone can detect endometrioma with 93% sensitivity and 96% specificity (35), although it has no diagnostic potential for peritoneal endometriosis, and a limited diagnostic potential for deep infiltrating endometriosis.

The cytokines represent another group of well-investigated molecules, although the data here are conflicting (36-38). The most studied cytokines in recent years have been interleukin (IL)-6 and tumour necrosis factor (TNF)-α. One study revealed that increased serum IL-6 and peritoneal fluid TNF-α differentiated between women with and without endometriosis (39); however, further studies did not confirm that result. In particular, IL-6 was significantly influenced by the stage of the disease and the phase of the menstrual cycle (36). Mihalyi et al. suggested a combination of cytokines as a potential biomarker: IL-6, IL-8, TNF-α, CA-125, CA 19-9, and C-reactive protein. These had 60% to 71% specificity and 87% to 92% sensitivity (40). Another study investigated 28 molecules from the plasma to identify a panel of biomarkers: vascular endothelial growth factor (VEGF), annexin V, CA-125, glycodelin, and soluble intracellular adhesion molecule 1. These had 63% to 81% specificity and 81% to 90% sensitivity (41). However, inconsistent with previous results, they also reported that the control group (i.e., patients without endometriosis at laparoscopy) showed increased levels of proinflammatory markers, including TNF-α, IL-6, and IL-1β. These results suggested a possible role for non-endometriotic pelvic pathology in the control group.

According to the retrograde menstrual flow theory, accumulation of iron from erythrocytes evokes oxidative stress (28). An imbalance between reactive oxygen species and the antioxidant response was thus proposed in the development of endometriotic lesions (29), with increased oxidative stress in patients with endometriosis. Studies of markers of oxidative stress and inflammation suggested myeloperoxidase, superoxide dismutase, and glutathione peroxidase. Myeloperoxidase activity distinguished between women with endometriosis versus controls with other benign gynecological disorders (e.g., myoma, non-endometriotic adhesions, non-endometriotic ovarian cysts, para-ovarian cysts, polycystic ovary syndrome, endometrial polyp, re-anastomosis after sterilization), but not versus controls with normal pelvis (42). Glutathione peroxidase showed no differences here, while superoxide dismutase was also significantly altered (28,43). Alternatively, significant reduction in serum levels of paraoxonase-1 were reported to distinguish between women without and with endometriosis with very promising accuracy, and with an area under the curve of 0.96, a sensitivity of 97%, and a specificity of 81% (44). However, further studies did not confirm this lower paraoxonase-1 activity in women with endometriosis (45).

The immunomodulatory protein galectin-9 might represent a marker for endometrial receptivity, and therefore it has been studied in the context of endometriosis (46). It was shown that women with other benign pelvic conditions, and even unexplained infertility, also have
significantly high serum levels of galectin-9, which thus means that this protein is not useful for the reality of clinical practice (46).

Endometrial cell survival after attachment to the peritoneum and neovascularization must be a key process in the development of endometriosis, and there has been a lot of effort put into identification of the relevant molecules in these fields. Disturbed apoptosis also has a role in these processes, and the soluble receptor that can protect cells against apoptosis by preventing Fas ligand from binding to cells, s-CD95/FAS, was shown to be elevated for endometriosis versus controls, and this difference was stage dependent (47). Matrix metalloproteinases are proteins that facilitate invasion of endometrial tissue fragments into the peritoneum, and these have been shown to be significantly increased in endometriosis versus controls (endometriosis-free women who had undergone laparoscopic surgery for infertility, or had nonmalignant conditions such as myoma, tubal ligation, ovarian biopsy) (48-51). VEGF promotes angiogenesis and vessel permeability, and it has been shown to be at higher concentrations in the peritoneal fluid of women with endometriosis, with greater differences seen for advanced stages of the disease (14,52). Analysis of VEGF after laparoscopic excision of endometriotic lesions showed reduced VEGF-A levels (53,54). Thus VEGF appears to have a role in the pathogenesis of endometriosis, although its potential as a single biomarker has not been shown. However, VEGF has been included in a biomarker panel (41), and it might also have potential as part of other biomarker combinations. Pigment epithelium-derived factor is an inhibitor of angiogenesis, and it was shown to be significantly decreased in women with endometriosis, where its levels were independent of the phase of the cycle and correlated with the pain symptoms (55,56). Urocortin-1 is a promoter of endometrial differentiation and decidualization, and influences endometrial adhesion and angiogenesis, and thus it might discriminate between patients with endometriosis and women with no other lesions; however, no cutoff plasma level accurately distinguished endometriosis from other pathological conditions (57).

The ‘-omic’ sciences (e.g., genomics, transcriptomics, proteomics, metabolomics) allow investigations of large numbers of molecules (e.g., the whole genome, transcriptome, proteome, metabolome) to generate new hypotheses. Endometriosis has been considered as an ideal target for these -omic sciences because of its heterogeneity, multiple phenotypes, obscure pathophysiology, association with other immune diseases, and lack of ideal diagnostic tools (58). To date, the number of -omics studies for the discovery of biomarkers for endometriosis have been relatively limited. The Rizner group at the Medical Faculty, University of Ljubljana, in collaboration with the Department of Gynaecology at the University Medical Centre Ljubljana and the Helmholtz Zentrum Munchen carried out the first successful exploration using the metabolomics approach for identification of biomarkers of endometriosis (59,60). Diagnostic algorithms with good diagnostic characteristics were identified for plasma and peritoneal fluid. Currently, we are awaiting the results of a multicenter validation study that included a cohort of 250 cases and controls from Ljubljana and Vienna.
6 Where are we going?

We have recently used proteomics approaches to analyze peritoneal fluid from women with endometriosis versus controls. To date, this is the first study to use high-content antibody protein microarrays, which allowed evaluation of more than 900 different proteins. The aim was to identify proteins that showed differential abundance and thus represented potential diagnostic and predictive biomarkers of endometriosis. We included 12 women with primary infertility, who were divided into a group of six women with laparoscopically and histologically confirmed endometriosis, and the control group of six women with unexplained primary infertility. Peritoneal fluid samples were collected during laparoscopy. Between endometriosis group and the controls, 18 antibodies defined differential abundances of 16 different proteins, all of which were up-regulated in the endometriosis group. Four of these proteins had not been associated with endometriosis before, and four of these proteins had never been investigated in peritoneal fluid. These 16 proteins are mainly related to fibrinogenesis, extracellular matrix remodelling, pathogenesis of inflammation, induction of dysfunctional immune system, and angiogenesis. We are currently validating these data with individual ELISA assays. We believe that our findings will bring new knowledge that will allow us to better understand the pathophysiology of endometriosis. If validated for plasma and/or serum samples, these newly discovered proteins have the potential to be used as individual biomarkers, or as a panel of biomarkers. However, there remains a long way to go before their application for diagnostic or prognostic purposes will be confirmed.

7 Conclusions

To date, the ‘-omic’ sciences have not identified any specific biomarkers for clinical use for patients with endometriosis. More studies should thus be undertaken using these -omics technologies, with standardization from sample collection to evaluation of the -omics data. For a complex and heterogeneous disease such as endometriosis, prediction will probably require diagnostic algorithms that include the concentrations of different molecules in combination with clinical data. Then these potential biomarker models and/or algorithms will need to be validated in independent groups of patients and in multicentre studies. Their transition from the discovery phase to their actual use in the clinical environment still remains uncertain, questionable, and subject to a long process. However, based on the more recent increased trend for high-quality -omics studies, the leading researchers in the field are optimistic that noninvasive biomarkers of endometriosis are not a myth, but have the potential to reach the clinic in the near future (4,7,11,13,14,27,37,58).

Acknowledgements

The author (T.L.R.) acknowledge the project (Biomarkers of endometriosis: transcriptomics and proteomics approach, J3-1755) was financially supported by the Slovenian Research Agency.
References


REVIEW ARTICLE

Biomarkers of endometriosis: How far have we come and where are we going?


