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

Biološki označevalci endometrioze: Kje smo in kam smo namenjeni?

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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

Endometrioza je ena najpogostejših benignih ginekoloških bolezni, ki jo opredelimo kot prisotnost endometriju podobnega tkiva zunaj maternične votline. Bolezen poslabša kakovost življenja bolnic in je eden od vodilnih vzrokov za neplodnost. Zlati diagnostični standard za dokaz endometrioze je še vedno neposredna vizualizacija sprememb ob laparoskopiji, kar podaljša čas do postavitve končne diagnoze. Pomanjkanje neinvazivnih diagnostičnih možnosti je razlog, da raziskovanje bioloških označevalcev endometrioze pomeni prednostno raziskovalno področje. Članek prikazuje različne pristope k odkrivanju bioloških označevalcev endometrioze (na hipotezo usmerjene raziskave in raziskave, pri katerih rezultati generirajo nadaljnje hipoteze, (angl. hypothesis-driven and hypothesis-generating approaches)) ter pomembnejše možne biološke označevalce endometrioze in njihove lastnosti, občutljivost in specifičnost. Predstavljamo tudi svoj pogled na raziskovanje možnih bioloških označevalcev endometrioze in rezultate naših raziskav. Nadaljnje raziskovanje mora v ospredje postaviti klinični cilj – izboljšati kakovost življenja bolnic, zato je nujno dobro sodelovanje med raziskovalci v kliničnem in predkliničnem okolju.

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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 aetiopathogenicity 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 gynaecological disorders (e.g., myoma, non-endometriotic adhesions, nonendometriotic 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 promotor 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 cut-off 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 heterogenous 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).

References

1. Rogers PA, D'Hooghe TM, Fazleabas A, Giudice LC, Montgomery GW, Petraglia G, et al. Defining future directions for endometriosis research: workshop report from the 2011 World Congress of Endometriosis In Montpellier, France. Reprod Sci. 2013;20(5):483-9. DOI: 10.1177/1933719113477495

2. Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissues into the peritoneal cavity. Am J Obstet Gynecol. 1927;14(4):422-69. DOI: 10.1016/S0002-9378(15)30003-X

3. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al.; European Society of Human Reproduction and Embryology. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29(3):400-12. DOI: 10.1093/humrep/det457 PMID: 24435778

4. Rižner TL. Diagnostic potential of peritoneal fluid biomarkers of endometriosis. Expert Rev Mol Diagn. 2015;15(4):557-80. DOI: 10.1586/14737159.2015.1015994 PMID: 25719220

5. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril. 2012;98(3):511-9. DOI: 10.1016/j.fertnstert.2012.06.029 PMID: 22819144

6. Agic A, Xu H, Finas D, Banz C, Diedrich K, Hornung D. Is endometriosis associated with systemic subclinical inflammation? Gynecol Obstet Invest. 2006;62(3):139-47. DOI: 10.1159/000093121 PMID: 16679772

7. Rogers PA, Adamson GD, Al-Jefout M, Becker CM, D'Hooghe TM, Dunselman GA, et al.; WES/WERF Consortium for Research Priorities in Endometriosis. Research priorities for endometriosis. Reprod Sci. 2017;24(2):202-26. DOI: 10.1177/1933719116654991 PMID: 27368878

8. Biomarkers Definitions Working Group.Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95. DOI: 10.1067/mcp.2001.113989 PMID: 11240971

9. Guo Y, Fu Z, Van Eyk JE. A proteomic primer for the clinician. Proc Am Thorac Soc. 2007;4(1):9-17. DOI: 10.1513/pats.200608-156JG PMID: 17202286

10. Surinova S, Schiess R, Hüttenhain R, Cerciello F, Wollscheid B, Aebersold R. On the development of plasma protein biomarkers. J Proteome Res. 2011;10(1):5-16. DOI: 10.1021/pr1008515 PMID: 21142170

11. Fassbender A, Vodolazkaia A, Saunders P, Lebovic D, Waelkens E, De Moor B, et al. Biomarkers of endometriosis. Fertil Steril. 2013;99(4):1135-45. DOI: 10.1016/j.fertnstert.2013.01.097 PMID: 23414923

12. Fassbender A. Biomarkers of endometriosis. In: Harada TE, editor. Endometriosis: Pathogenesis and Treatment. Berlin: Springer; 2014. pp. 321-39.

13. Rižner TL. Noninvasive biomarkers of endometriosis: myth or reality? Expert Rev Mol Diagn. 2014;14(3):365-85. DOI: 10.1586/14737159.2014.899905 PMID: 24649822

14. Fassbender A, Burney RO, Dorien FO, D'Hooghe T, Giudice L. Update on biomarkers for the detection of endometriosis. BioMed Res Int. 2015;2015:130854. DOI: 10.1155/2015/130854 PMID: 26240814

15. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. 2016;5(5):CD012179. DOI: 10.1002/14651858.CD012179 PMID: 27132058

16. Machairiotis N, Stylianaki A, Dryllis G, Zarogoulidis P, Kouroutou P, Tsiamis N, et al. Extrapelvic endometriosis: a rare entity or an under diagnosed condition? Diagn Pathol. 2013;8(1):194. DOI: 10.1186/1746-1596-8-194 PMID: 24294950

17. Ahn SH, Singh V, Tayade C. Biomarkers in endometriosis: challenges and opportunities. Fertil Steril. 2017;107(3):523-32. DOI: 10.1016/j.fertnstert.2017.01.009 PMID: 28189296

18. Rawson JM. Prevalence of endometriosis in asymptomatic women. J Reprod Med. 1991;36(7):513-5. PMID: 1834839

19. Thomas EJ. The relevance of asymptomatic endometriosis. Hum Reprod. 1996;11(3):103-9. DOI: 10.1093/humrep/11.suppl_3.103 PMID: 9147105

20. Rizner TL, Adamski J. Paramount importance of sample quality in pre-clinical and clinical research-Need for standard operating procedures (SOPs). J Steroid Biochem Mol Biol. 2019;186:1-3. DOI: 10.1016/j.jsbmb.2018.09.017 PMID: 30261262

21. Koninckx PR, Kennedy SH, Barlow DH. Endometriotic disease: the role of peritoneal fluid. Hum Reprod Update. 1998;4(5):741-51. DOI: 10.1093/humupd/4.5.741 PMID: 10027629

22. Young VJ, Brown JK, Saunders PT, Horne AW. The role of the peritoneum in the pathogenesis of endometriosis. Hum Reprod Update. 2013;19(5):558-69. DOI: 10.1093/humupd/dmt024 PMID: 23720497

23. Bedaiwy MA, Falcone T. Peritoneal fluid environment in endometriosis. Clinicopathological implications. Minerva Ginecol. 2003;55(4):333-45. PMID: 14581858

24. Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, et al. Endometrial biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. 2016;4:CD012165. DOI: 10.1002/14651858.CD012165 PMID: 27094925

25. May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. Hum Reprod Update. 2010;16(6):651-74. DOI: 10.1093/humupd/dmq009 PMID: 20462942

26. Burney RO. Biomarker development in endometriosis. Scand J Clin Lab Invest Suppl. 2014;244:75-81. DOI: 10.3109/00365513.2014.936692 PMID: 25083898

27. Lanišnik Rižner T. Molecular biomarkers of endometriosis – options for non-invasive diagnostics? Zdrav Vestn. 2014;83:782-91.

28. Ekarattanawong S, Tanprasertkul C, Somprasit C, Chamod P, Tiengtip R, Bhamarapravatana K, et al. Possibility of using superoxide dismutase and glutathione peroxidase as endometriosis biomarkers. Int J Womens Health. 2017;9:711-6. DOI: 10.2147/IJWH.S141021 PMID: 29026339

29. Scutiero G, Iannone P, Bernardi G, Bonaccorsi G, Spadaro S, Volta CA, et al. Oxidative stress and endometriosis: a systematic review of the literature. Oxid Med Cell Longev. 2017;2017:7265238. DOI: 10.1155/2017/7265238 PMID: 29057034

30. Mol BW, Bayram N, Lijmer JG, Wiegerinck MA, Bongers MY, van der Veen F, et al. The performance of CA-125 measurement in the detection of endometriosis: a meta-analysis. Fertil Steril. 1998;70(6):1101-8. DOI: 10.1016/S0015-0282(98)00355-0 PMID: 9848302

31. Hirsch M, Duffy J, Davis CJ, Nieves Plana M, Khan KS; International Collaboration to Harmonise Outcomes and Measures for Endometriosis. Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis. BJOG. 2016;123(11):1761-8. DOI: 10.1111/1471-0528.14055 PMID: 27173590

32. 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(8):1315-9. DOI: 10.1038/sj.bjc.6605011 PMID: 19337252

33. Szubert M, Suzin J, Wierzbowski T, Kowalczyk-Amico K. CA-125 concentration in serum and peritoneal fluid in patients with endometriosis - preliminary results. Arch Med Sci. 2012;8(3):504-8. DOI: 10.5114/aoms.2012.29529 PMID: 22852007

34. Knific T, Vouk K, Vogler A, Osredkar J, Gstöttner M, Wenzl R, et al. Models including serum CA-125, BMI, cyst pathology, dysmenorrhea or dyspareunia for diagnosis of endometriosis. Biomarkers Med. 2018;12(7):737-47. DOI: 10.2217/bmm-2017-0426 PMID: 29865858

35. Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. 2016;2:CD009591. DOI: 10.1002/14651858.CD009591.pub2 PMID: 26919512

36. May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. Hum Reprod Update. 2010;16(6):651-74. DOI: 10.1093/humupd/dmq009 PMID: 20462942

37. Kocbek V, Vouk K, Bersinger NA, Mueller MD, Lanišnik Rižner T. Panels of cytokines and other secretory proteins as potential biomarkers of ovarian endometriosis. J Mol Diagn. 2015;17(3):325-34. DOI: 10.1016/j.jmoldx.2015.01.006 PMID: 25797583

38. Knific T, Fishman D, Vogler A, Gstöttner M, Wenzl R, Peterson H, et al. Multiplex analysis of 40 cytokines do not allow separation between endometriosis patients and controls. Sci Rep. 2019;9(1):16738. DOI: 10.1038/s41598-019-52899-8 PMID: 31723213

39. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod. 2002;17(2):426-31. DOI: 10.1093/humrep/17.2.426 PMID: 11821289

40. Mihalyi A, Gevaert O, Kyama CM, Simsa P, Pochet N, De Smet F, et al. Non-invasive diagnosis of endometriosis based on a combined analysis of six plasma biomarkers. Hum Reprod. 2010;25(3):654-64. DOI: 10.1093/humrep/dep425 PMID: 20007161

41. Vodolazkaia A, El-Aalamat Y, Popovic D, Mihalyi A, Bossuyt X, Kyama CM, et al. Evaluation of a panel of 28 biomarkers for the non-invasive diagnosis of endometriosis. Hum Reprod. 2012;27(9):2698-711. DOI: 10.1093/humrep/des234 PMID: 22736326

42. O DF, Waelkens E, Peterse DP, Lebovic D, Meuleman C, Tomassetti C, et al. Evaluation of total, active, and specific myeloperoxidase levels in women with and without endometriosis. Gynecol Obstet Invest. 2018;83(2):133-9. DOI: 10.1159/000475664 PMID: 28511185

43. Prieto L, Quesada JF, Cambero O, Pacheco A, Pellicer A, Codoceo R, et al. Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. Fertil Steril. 2012;98(1):126-30. DOI: 10.1016/j.fertnstert.2012.03.052 PMID: 22578534

44. Verit FF, Erel O, Celik N. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. Hum Reprod. 2008;23(1):100-4. DOI: 10.1093/humrep/dem340 PMID: 18000171

45. Bragatto FB, Barbosa CP, Christofolini DM, Peluso C, dos Santos AA, Mafra FA, et al. There is no relationship between Paraoxonase serum level activity in women with endometriosis and the stage of the disease: an observational study. Reprod Health. 2013;10(1):32. DOI: 10.1186/1742-4755-10-32 PMID: 23799909

46. Brubel R, Bokor A, Pohl A, Schilli GK, Szereday L, Bacher-Szamuel R, et al. Serum galectin-9 as a noninvasive biomarker for the detection of endometriosis and pelvic pain or infertility-related gynecologic disorders. Fertil Steril. 2017;108(6):1016-1025.e2. DOI: 10.1016/j.fertnstert.2017.09.008 PMID: 29202955

47. Karakus S, Sancakdar E, Akkar O, Yildiz C, Demirpence O, Cetin A. Elevated serum CD95/FAS and HIF-1α levels, but not Tie-2 levels, may be biomarkers in patients with severe endometriosis: a preliminary report. J Minim Invasive Gynecol. 2016;23(4):573-7. DOI: 10.1016/j.jmig.2016.01.025 PMID: 26851415

48. Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med. 2003;9(5):223-8. DOI: 10.1016/S1471-4914(03)00051-0 PMID: 12763528

49. Huang HF, Hong LH, Tan Y, Sheng JZ. Matrix metalloproteinase 2 is associated with changes in steroid hormones in the sera and peritoneal fluid of patients with endometriosis. Fertil Steril. 2004;81(5):1235-9. DOI: 10.1016/j.fertnstert.2003.10.027 PMID: 15136083

50. Malvezzi H, Aguiar VG, Paz CC, Tanus-Santos JE, Penna IA, Navarro PA. Increased circulating MMP-2 levels in infertile patients with moderate and severe pelvic endometriosis. Reprod Sci. 2013;20(5):557-62. DOI: 10.1177/1933719112459234 PMID: 23171686

51. De Sanctis P, Elmakky A, Farina A, Caramelli E, Seracchioli R, Mabrouk M, et al. Matrix metalloproteinase-3 mRNA: a promising peripheral blood marker for diagnosis of endometriosis. Gynecol Obstet Invest. 2011;71(2):118-23. DOI: 10.1159/000320752 PMID: 21150162

52. Zubrzycka A, Zubrzycki M, Janecka A, Zubrzycka M. New horizons in the etiopathogenesis and noninvasive diagnosis of endometriosis. Curr Mol Med. 2015;15(8):697-713. DOI: 10.2174/1566524015666150921105218 PMID: 26391550

53. Mohamed ML, El Behery MM, Mansour SA. Comparative study between VEGF-A and CA-125 in diagnosis and follow-up of advanced endometriosis after conservative laparoscopic surgery. Arch Gynecol Obstet. 2013;287(1):77-82. DOI: 10.1007/s00404-012-2539-4 PMID: 22930151

54. Bourlev V, Iljasova N, Adamyan L, Larsson A, Olovsson M. Signs of reduced angiogenic activity after surgical removal of deeply infiltrating endometriosis. Fertil Steril. 2010;94(1):52-7. DOI: 10.1016/j.fertnstert.2009.02.019 PMID: 19324337

55. Chen L, Fan R, Huang X, Xu H, Zhang X. Reduced levels of serum pigment epithelium-derived factor in women with endometriosis. Reprod Sci. 2012;19(1):64-9. DOI: 10.1177/1933719111413300 PMID: 22051848

56. Fu G, Che X, Sun Y, Huang X, Xu H, Zhou C, et al. Pigment epithelial-derived factor expression in endometriotic lesions in a rat model of endometriosis. Acta Histochem. 2013;115(4):301-7. DOI: 10.1016/j.acthis.2012.08.006 PMID: 22975116

57. Maia LM, Rocha AL, Del Puerto HL, Petraglia F, Reis FM. Plasma urocortin-1 as a preoperative marker of endometriosis in symptomatic women. Gynecol Endocrinol. 2018;34(3):202-5. DOI: 10.1080/09513590.2017.1380188 PMID: 28925754

58. Coutinho LM, Ferreira MC, Rocha AL, Carneiro MM, Reis FM. New biomarkers in endometriosis. Adv Clin Chem. 2019;89:59-77. DOI: 10.1016/bs.acc.2018.12.002 PMID: 30797471

59. Vouk K, Hevir N, Ribić-Pucelj M, Haarpaintner G, Scherb H, Osredkar J, et al. Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis. Hum Reprod. 2012;27(10):2955-65. DOI: 10.1093/humrep/des152 PMID: 22859507

60. Vouk K, Ribič-Pucelj M, Adamski J, Rižner TL. Altered levels of acylcarnitines, phosphatidylcholines, and sphingomyelins in peritoneal fluid from ovarian endometriosis patients. J Steroid Biochem Mol Biol. 2016;159:60-9. DOI: 10.1016/j.jsbmb.2016.02.023 PMID: 26921767