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REVIEW

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An integrated multi-tissue approach for endometriosis candidate biomarkers: a systematic review

Axelle Brulport^{1*}, Mathilde Bourdon^{2,3}, Daniel Vaiman², Christian Drouet^{2,4}, Khaled Pocate-Cheriet^{2,4}, Kheira Bouzid², Louis Marcellin^{2,3}, Pietro Santulli^{2,3}, Carole Abo^{2,3}, Maxime Jeljeli^{2,5}, Sandrine Chouzenoux², Charles Chapron^{2,3}, Frédéric Batteux^{2,5}, Camille Berthelot¹ and Ludivine Doridot²

Abstract

Biomarker identification could help in deciphering endometriosis pathophysiology in addition to their use in the development of non invasive diagnostic and prognostic approaches, that are essential to greatly improve patient care. Despite extensive efforts, no single potential biomarker or combination has been clinically validated for endometriosis.

Many studies have investigated endometriosis-associated biological markers in specific tissues, but an integrative approach across tissues is lacking. The aim of this review is to propose a comprehensive overview of identified biomarkers based on tissue or biological compartment, while taking into account endometriosis phenotypes (superficial, ovarian or deep, or rASRM stages), menstrual cycle phases, treatments and symptoms.

We searched PubMed and Embase databases for articles matching the following criteria: 'endometriosis' present in the title and the associated term 'biomarkers' found as Medical Subject Headings (MeSH) terms or in all fields. We restricted to publications in English and on human populations. Relevant articles published between 01 January 2005 (when endometriosis phenotypes start to be described in papers) and 01 September 2022 were critically analysed and discussed.

Four hundred forty seven articles on endometriosis biomarkers that included a control group without endometriosis and provided specific information on endometriosis phenotypes are included in this review. Presence of information or adjustment controlling for menstrual cycle phase, symptoms and treatments is highlighted, and the results are further summarized by biological compartment. The 9 biological compartments studied for endometriosis biomarker research are in order of frequency: peripheral blood, eutopic endometrium, peritoneal fluid, ovaries, urine, menstrual blood, saliva, feces and cervical mucus. Adjustments of results on disease phenotypes, cycle phases, treatments and symptoms are present in 70%, 29%, 3% and 6% of selected articles, respectively. A total of 1107 biomarkers were identified in these biological compartments. Of these, 74 were found in several biological compartments by at least two independent research teams and only 4 (TNF- α , MMP-9, TIMP-1 and miR-451) are detected in at least 3 tissues with cohorts of 30 women or more.

Integrative analysis is a crucial step to highlight potential pitfalls behind the lack of success in the search for clinically relevant endometriosis biomarkers, and to illuminate the physiopathology of this disease.

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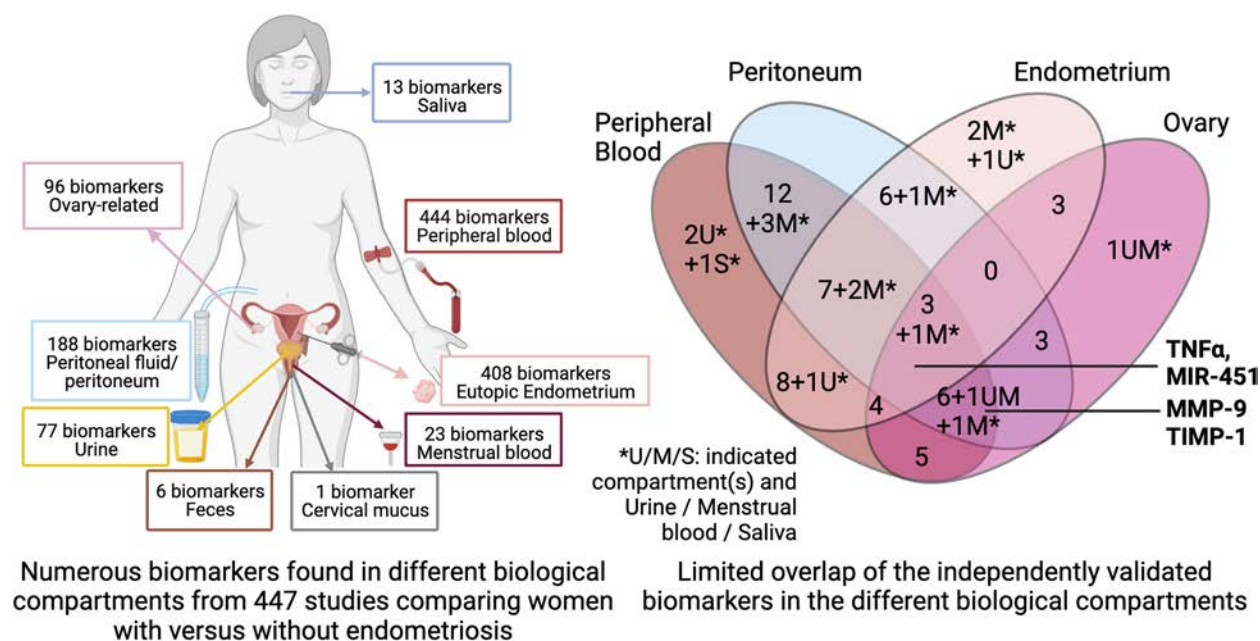
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Keywords Endometriosis, Candidate biomarkers, Biological compartments, Endometriosis phenotypes

Graphical Abstract



Introduction

Endometriosis is a chronic gynecological condition affecting 6%-10% of women of reproductive age [1]. Histologically, endometriosis corresponds to the dissemination of endometrial-like tissue, or lesions, outside the uterus. The reference method to diagnose endometriosis is surgery, through lesion visualisation and anatomical pathology evaluation. Endometriosis staging is currently based on surgeons' observations. The most widely used scoring is from the American Society for Reproductive Medicine (ASRM) that ranges endometriosis from stage I "minimal" to stage IV "severe", based on lesion localisation, size, appearance and presence of adhesions [2]. Currently, patient management practices promote non-invasive diagnostic methods such as transvaginal ultrasonography and magnetic resonance imaging (MRI) [3]. Not all staging features are accessible with non-invasive diagnostic approaches, which makes the ASRM classification difficult to use in this context. An alternative classification defines three phenotypes: superficial peritoneal lesions (located less than 5 mm below the peritoneum), ovarian endometriomas, and deep infiltrating endometriosis (located more than 5 mm below the peritoneum). The last two can usually be detected by imaging.

Clinically, endometriosis is associated with a wide range of symptoms and consequences: pelvic pain, severe pain during periods (dysmenorrhea), painful sex, painful urination (dysuria) and/or defecation (dyschesia), alternance of diarrhea/constipation, heavy menstrual bleeding, mood disorders, chronic fatigue and infertility [4]. Endometriosis is increasingly considered as a systemic disease rather than a pelvic pathology [5].

Because of its complex pathophysiology, symptoms heterogeneity, and diagnostic requirements, diagnosis delay for endometriosis ranges from 4 to 11 years [5]. Biomarker candidate research is therefore a key avenue to improve diagnosis. Despite extensive efforts, no single or combination of biomarkers has reached clinical validation for endometriosis, as extensively reviewed in Cochrane's reviews in 2016 [6–9]. While dozens of potential biomarkers have been investigated across varied biological compartments, few have been validated in independent studies, making their relevance unclear. To address this, we propose an original integrative review of the endometriosis biomarker literature across biological compartments. Our hypothesis is that endometriosis biomarkers recurrently identified across multiple tissues may be particularly relevant and play a more direct role in disease physiopathology, and an integrative multi-tissue

approach could highlight and prioritize these candidates, which may eventually lead to enhanced patient care. For all considered studies, we highlight if endometriosis subtypes, menstrual cycle phases, treatments and symptoms were accounted for. We particularly focus on biomarkers reproducibly detected by at least two independent research teams, and found in different biological compartments.

Materials and methods

Literature search

Pubmed and Embase (excluding Medline articles) were searched for English-language articles as follows: Endometriosis in the title AND 'biomarkers' as Medical

Subject Heading (MeSH) or All Fields terms. The strategy was designed in association with the referral Inter-University Library of Medicine of Université Paris Cité, France. All articles involving human subjects and published between 2005/01/01 and 2022/09/01 were selected for screening. The databases were last consulted on 30 September 2022. The review was conducted in accordance with The PRISMA 2020 statement for systematic review and not registered [10]. PRISMA 2020 Checklist is included in [Additional Material](#).

Eligibility criteria and study selection

The study selection strategy is summarized as a flowchart in Fig. 1. Both clinical and basic research studies

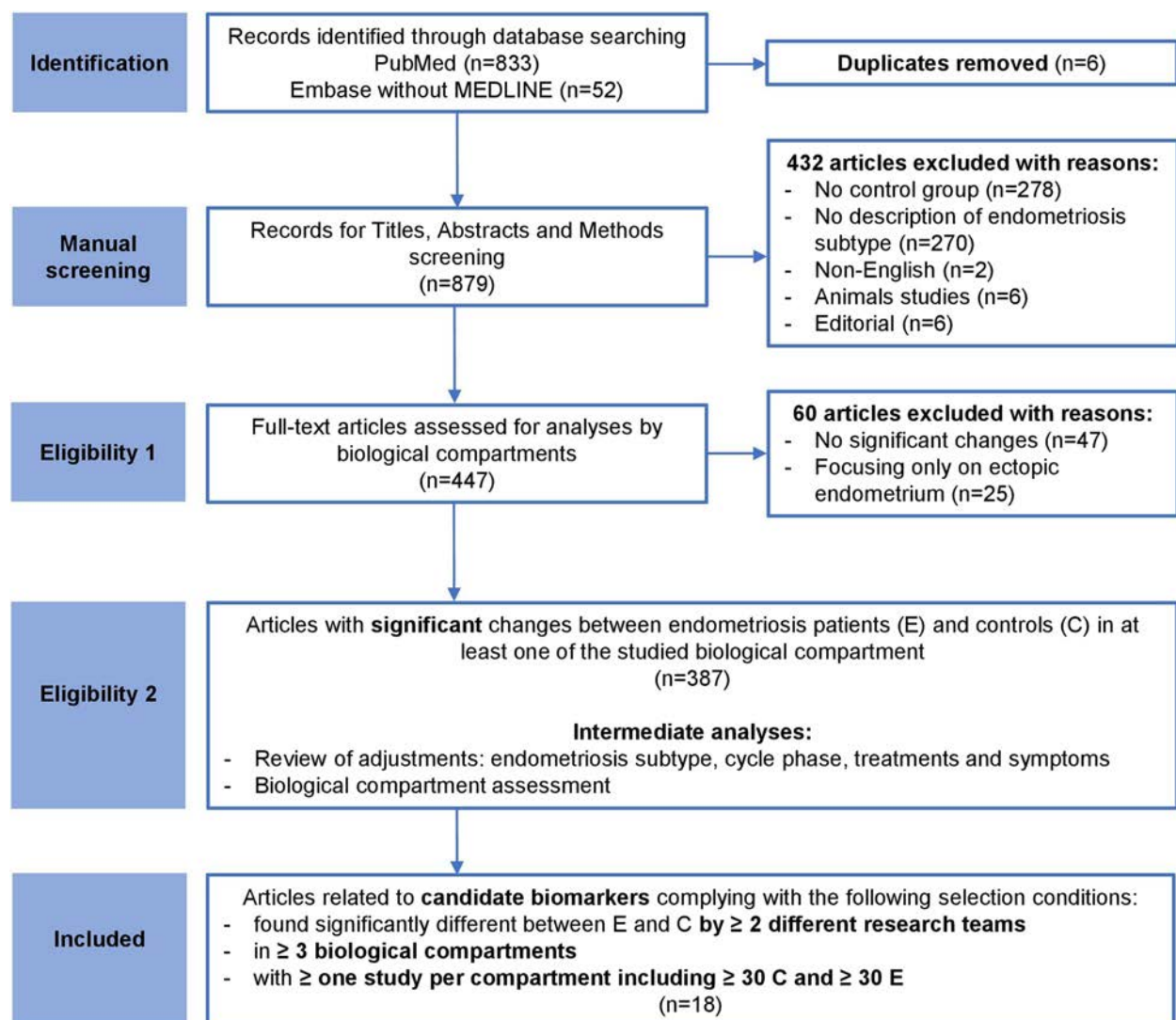


Fig. 1 PRISMA flowchart for the systematic review. Flowchart highlighting the different steps in the selection of articles included in the review, giving details of the inclusion and exclusion criteria and the analyses carried out

were considered. A total of 879 original publications were manually screened. At each step of the selection process, titles/abstracts/methods or full-text were screened by 2 independent reviewers (A.B. and one of the other co-authors) regarding eligibility criteria. Discrepancies concerning studies inclusion were resolved by A.B. and L.D. All review articles, editorial, animal subject studies and publications not written in English were excluded. Articles with the two following mandatory inclusion criteria were selected for further evaluation: i) presence of a control group without endometriosis and without malignant diseases, and ii) available information on endometriosis phenotype(s). For the remaining 447 articles, the full text was screened to extract the biomarkers studied, the significance and the direction of the variation observed between control and endometriosis groups and the biological compartment in which these changes were found. Only biomarkers significantly deregulated in endometriosis were considered. Biomarkers deregulated in ectopic endometrium only, and articles focusing exclusively on ectopic endometrium were excluded from this review because ectopic endometrium has no equivalent tissue in the control group. At this step, we obtained a list of 387 articles with 1107 significantly deregulated biomarkers in endometriosis and affected biological compartment(s). This list from the remaining 387 publications, validated independently by 2 reviewers, was reviewed a third time to extract information and/or adjustments for disease phenotypes, menstrual cycle phases, treatments and symptoms, which represent parameters of major importance in endometriosis. A list of candidate biomarkers per compartment was also created. Identified biomarkers and their instances in each compartment were tallied using a custom Perl script identifying unique character strings (case insensitive). To focus on sustained multi-tissue evidence, we selected articles related to the biomarkers identified by at least 2 different research teams (regardless of biological compartment) and across at least 3 biological compartments. For each candidate biomarker, at least one study per compartment including 30 or more controls and 30 or more patients with endometriosis was mandatory. All extracted data were analysed descriptively.

Data extraction and analysis

The data related to our final set of candidate biomarkers of interest in this review were extracted from 18 articles (see flowchart in Fig. 1). For each article, the following data were extracted by 2 independent reviewers: the quality of the control groups and the homogeneity of the confounding factors between the groups, the level of expression (mRNA, protein) and the direction and amplitude of variation of the candidate biomarker,

the adjustments of the results according to the subtypes of endometriosis, the phase of the menstrual cycle, the treatments and the symptoms and the ROC curve analysis to highlight diagnostic accuracy if available.

Assessment of risk of bias

Due to the great disparity in study designs (clinical and basic research) available for each candidate biomarkers of interest, we did not use ROC curve analysis as a selection criterion to address robustness, but instead included the presence of at least one study per biological compartment including at least 30 individuals per group as a mandatory criterion. For the creation of lists of candidate biomarkers by biological compartments, to avoid computing different aliases of the same gene/protein as different biomarkers, all identified candidate biomarkers were manually curated to unify writing styles and conventions before processing. Aliases were identified through the HUGO Gene Nomenclature Committee (HGNC) Multi symbol checker tool (<https://www.genenames.org/tools/multi-symbol-checker/>), replacing 76 aliases by their official gene symbols.

Results

Extensive but heterogeneous studies have explored potential endometriosis biomarkers

We systematically searched the PubMed and Embase databases for research articles on endometriosis biomarkers published between January 2005 and September 2022. Of the 879 publications retrieved after exclusion of duplicates, 278 focused on comparisons i) among endometriosis patients or between tissues in endometriosis patients or ii) between endometriosis and cancer patients (Fig. 1). We excluded these articles since their usability for endometriosis diagnosis is limited. 270 articles did not include information on the patient endometriosis phenotypes according to either the rARSM classification or lesions localization (Fig. 1). These articles were also excluded, as we chose to study how each candidate biomarker was potentially relevant to specific subtypes of patients. In total, we retained 447 publications for further analysis (Additional Table 1), of which 387 identified at least one biomarker with significantly modified levels in endometriosis patients compared to controls (Fig. 1).

Cycle phase, treatments and symptoms are rarely accounted or adjusted for

Information on endometriosis phenotypes was a mandatory inclusion criterion in this study, and 73% of selected publications adjusted the results accordingly, either intentionally or indirectly by including only a particular phenotype (Fig. 2). Biomarker levels can

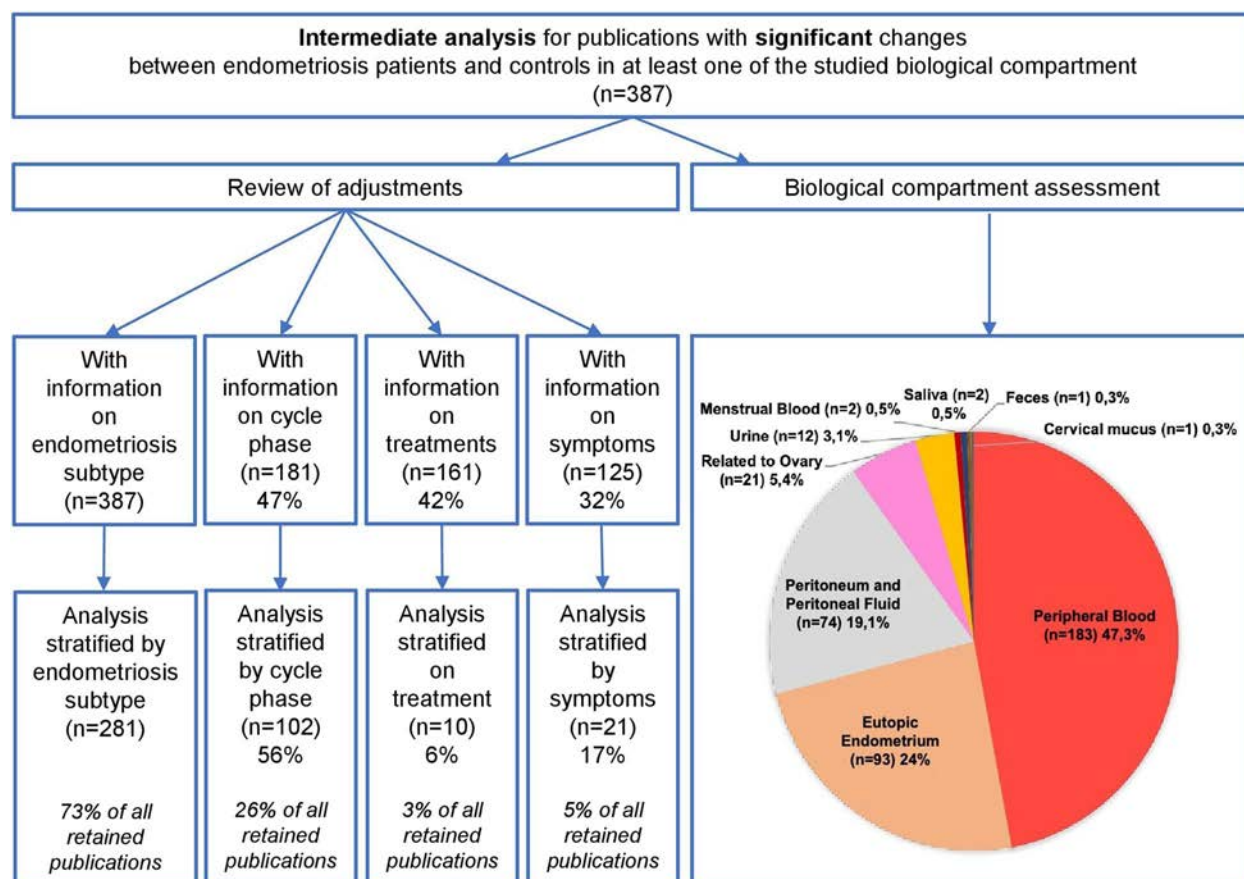


Fig. 2 Intermediate analyses carried out on 387 articles. Analyses performed i) on the adjustments of the results according to the subtype of endometriosis, the menstrual cycle phases, the treatments and the symptoms and ii) on the different biological compartments studied

vary with menstrual cycle phases [11], but only 47% of selected publications provided information about cycle phase. Just over half took this parameter into account when analysing the results (Fig. 2), and mainly because surgical teams operated on patients either in the follicular phase or in the luteal phase. Regarding treatments, 42% of articles provided some information (Fig. 2). Non-use of hormonal treatments in the 3 to 6 months prior to inclusion is often specified (and sometimes non-use of anti-inflammatory drugs in the days before inclusion), explaining why only 3% of the analysed publications adjust results for hormonal or symptomatic treatments (anti-inflammatory, painkillers, etc.; Fig. 2). Although endometriosis symptoms are very diverse, this aspect is the least documented, with only 32% of analysed articles taking symptoms into account (Fig. 2). Of these, 17% reported subgroup analyses depending on symptoms (Fig. 2). Infertility, a consequence of endometriosis often regarded as a symptom, was the most commonly considered.

One thousand one hundred seven biomarkers identified across nine unequally studied biological compartments

Among 447 retained publications, 387 identified a significant biomarker in at least one biological compartment (Fig. 1 and 2). The majority studied peripheral blood (183 articles, 47,3%) (Fig. 2, Additional Table 2). Other studied compartments were eutopic endometrium (93 articles, 24%), peritoneum and peritoneal fluid (74 articles, 19,1%), ovary (mainly follicular fluid or cumulus cells; 21 articles, 5,4%), urine (12 articles, 3,1%), menstrual blood (2 articles, 0,5%), saliva (2 articles, 0,5%), feces (1 article, 0,3%) and cervical mucus (1 article, 0,3%) (Fig. 2). 444 biomarkers were identified in peripheral blood, 408 in eutopic endometrium, 188 in peritoneum and peritoneal fluid, 96 in compartments related to ovary, 77 in urine, 23 in menstrual blood, 13 in saliva, 6 in feces and 1 in cervical mucus (Additional Table 2). In total, we listed 1107 candidate biomarkers of endometriosis, several of which were identified in different compartments. Interestingly, only a minority were reproducibly found in independent articles within the same compartment

(Additional Table 2), questioning the standardisation and reproducibility of endometriosis candidate biomarker studies. Moreover, only 74 were found in several biological compartments by at least two independent research teams (Table 1). These 74 biomarkers significantly modified in endometriosis were classified into molecular subtypes (Table 1) and used for the following selections. The names of the 74 candidate biomarkers identified, their direction of variation in the different biological compartments studied and the cohort sizes of the selected articles are detailed in Table 1.

Cohort sizes are relatively small except for peripheral blood

The majority of selected studies included between 10 and 50 women in each group with and without endometriosis (Fig. 3). Unsurprisingly, all studies including more than 500 women per group focused on peripheral blood. Biological compartments are also very unequally studied in cohorts with 100 to 500 women per group: 84.3% studied peripheral blood, 8.6% eutopic endometrium, 5.7% peritoneal fluid and 1.4% follicular fluid. Biological compartments that can be assessed non-invasively (urine, menstrual blood, saliva, feces, cervical mucus), have not been studied on a large scale.

Only 4 reproducible biomarkers have been consistently detected across tissues in large cohorts

We highlighted 4 candidate biomarkers identified by at least two different research teams in 3 biological compartments or more, with at least one well-powered study per compartment including 30 or more controls and 30 or more patients with endometriosis (Table 2). Here, we summarized the main lines of evidence supporting each of these 4 biomarkers as potential diagnostic elements for endometriosis.

TNF- α

Tumor necrosis factor alpha (TNF- α), a pro-inflammatory cytokine, was consistently reported as increased in larger cohorts of women with endometriosis in two biological compartments: peritoneal fluid [23, 135], and follicular fluid [34]. In the peritoneal fluid, this increase was further consolidated by consistent results from smaller cohorts [17, 31, 32], although two other studies found no significant differences between women with and without endometriosis [21, 51]. How TNF- α changes in peritoneal fluid tie in with endometriosis phenotypes and menstrual phases was unclear, with reported increases in both stages I/II and III/IV [32], only in stage III/IV with no difference between proliferative and secretory phases, or only in patients with endometrioma and in proliferative phase [135] – some of which may reflect inappropriate

statistical power to control for Type II errors when stratifying cohorts. Another increase was also detected in the endometrium at the mRNA level during menstrual phase [24]. While TNF- α was also reported as modified in blood, results were inconsistent, reporting increases [13, 29], decreases [12, 30] or no change [32, 39, 44, 60, 67], both within small or large cohorts. When focusing on large cohorts, increases were observed in serum while the decreases were in plasma, suggesting an importance on the blood collection method (Table 2). Unfortunately, diagnostic accuracy of TNF α was only assessed in blood and yielded low specificity and sensitivity, which is unsurprising in light of the discrepancies across studies.

MMP-9 or MMP-9/NGAL

Enzyme matrix metalloproteinase (MMPs), including MMP-9, are involved in extracellular matrix remodeling via proteolytic activity. They play a key role in physiological (like embryogenesis and wound healing) and pathophysiological (invasion and tissue destruction mechanisms) uterine processes [80]. In this review, we observed that MMP-9 levels appeared to be increased in endometriosis in all studies and regardless of the biological compartment studied. Interestingly, fertility status and menstrual cycle phases do not seem to affect the variations of this biomarker [79–82, 136]. Although still to be confirmed, the diagnostic value of this biomarker seems to be appropriate [79, 82]. We note that a therapeutic approach to reduce MMP-9 level through progesterone supplementation to improve IVF success rates in endometriosis patients showed promising success, suggesting that MMP-9 may have treatment as well as diagnosis value in endometriosis [81].

TIMP-1

TIMP-1, a metalloproteinase inhibitor, is involved in extracellular matrix remodeling which is particularly intense in ovary during follicular development and cyst formation and in endometrium during dynamic cyclic changes across the menstrual cycle [81]. TIMP-1 showed inconsistent regulation between different biological compartments in women with endometriosis, with reported decreases in blood and ovarian tissue and an increase in peritoneal fluid across well-powered cohorts [22, 81]. Although this remains to be confirmed, this candidate biomarker does not appear discriminative for disease stage and fertility status, but seems impacted by menstrual cycle phases [22].

miR451/miR451a

MiRNAs are small endogenous noncoding functional RNAs [122]. As they are released into the circulation, their interest as biomarkers has been the subject of

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	n = X&Y with X the number of samples from women without endometriosis, Y the number of samples from women with endometriosis
									Up/Down (in endometriosis patients vs controls) is indicated when studies are inconsistent
IFN γ		↑↓	↑		↓		↑		B: Up [39] n = 68&70 ; Up [13] n = 25&19; Down [30] n = 121&232 P: [40] n = 30&50 ; [17] n = 20&57 – O: [41] n = 29&20 (follicular fluid) M: [42] (in the supernatant of men- strual blood derived stem cells with an allogeneic stimulation) n = 6&6
	IL6	↑	↑	↑			↑*		B: [43] n = 72&38 ; [39] n = 68&70 ; [44] n = 31&38 ; [12] n = 93&201 ; [32] n = 17&33; [13] n = 25&19; [35] n = 20&48; [45] n = 22&47; [46] n = 35&45 ; [47] n = 60&80 ; [48] n = 32&40 ; [49] n = 35&43 ; [29] n = 103&190 ; [14] n = 12&75; P: [24] n = 11&24; [31] n = 22&30; [16] n = 16&67; [32] n = 17&33; [50] n = 28&70; [51] n = 42&36 ; [19] = 40&58 ; [35] n = 20&48; [45] n = 22&47 [23]; n = 35&45 ; [46] n = 35&45 ; [22] n = 45&126 ; [48] n = 32&40 ; E: [26] n = 5&5 – M: [28] n = 3&3
CCL5 (RANTES)		↑	↑	↑					B: [13] n = 25&19 – P: [17] n = 20&57; [52] n = 20&74 – E: [53] n = 5&15
	CXCL10 (IP-10)	↓	↑↓	↑*					B: [54] n = 70&77 – E: [55] n = 8&8 P: [56] Up n = 32&101 ; [33] Up n = 59&73 ; Up [38] n = 6&12; [54] Down n = 70&77 ; [52] n = 20&74

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
Immunity related markers / Cytokines	IL6R	↓*	↑	↓*					B and P: [45] <i>n</i> = 22&47 – E: [55] <i>n</i> = 8&8
	IL4	↑	↑		↑				B: [13] <i>n</i> = 25&19; [47] <i>n</i> = 60&80 P: [57] <i>n</i> = 31&38 ; [17] <i>n</i> = 20&57 – O: [41] <i>n</i> = 29&20 (follicular fluid)
	IL17A	↓	↑↓		↓				B: [58] <i>n</i> = 16&27 – P: [52] Up <i>n</i> = 20&74 [22], Down <i>n</i> = 45&126 O: [41] <i>n</i> = 29&20 (follicular fluid)
	IL2	↑↓	↑		↓				B: Up [13] <i>n</i> = 25&19, Down [44] <i>n</i> = 31&38 , Down [58] <i>n</i> = 16&27; P: [58] <i>n</i> = 15&27 – O: [59] <i>n</i> = 5&5 (follicular fluid)
	IL13	↑↓	↓		↑				B: Up [13] <i>n</i> = 25&19, Down [60] <i>n</i> = 46&57 P: [21] <i>n</i> = 38&56 – O: [41] <i>n</i> = 29&20
	IL10	↑	↑			↑			B: [13] <i>n</i> = 25&19; [58] <i>n</i> = 16&27; P: [51] <i>n</i> = 42&36 ; [23] 2018 <i>n</i> = 35&45 – M: [42] <i>n</i> = 6&6
	CCL2 (MCP-1)	↑	↑			↑			B: [61] <i>n</i> = 31&18; [62] <i>n</i> = 60&102 ; [39] <i>n</i> = 68&70 ; [29] <i>n</i> = 103&190 ; P: [56] <i>n</i> = 32&101 ; [18] <i>n</i> = 27&36; [21] <i>n</i> = 38&56 M: [42] <i>n</i> = 6&6
	IL9, IL37	↑	↑						B: IL37 [58] <i>n</i> = 36&27, IL9: [13] <i>n</i> = 25&19, B and P: [48] <i>n</i> = 32&40 P: IL9 [17] <i>n</i> = 20&57
	GM-CSF	↑	↑		↓*				B: [13] <i>n</i> = 25&19 – P: [17] <i>n</i> = 20&57 – O: [27] <i>n</i> = 9&9

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
IL1β		↑↓	↑				↑*		Up/Down (in endometriosis patients vs controls) is indicated when studies are inconsistent
IL12		↑↓	↑						B: Up [13] n = 25&19; [49] Up n = 35&43, Down [30] n = 121&232 P: [31] n = 22&30; [57] n = 31&38; [17] n = 20&57; [33] n = 59&73 M: [28] n = 3&3
FAS/CD95+		↑↓	↑						B: Up [13] n = 25&19, Down [60] n = 46&57 – P: [63] n = 33&72, [33] n = 59&73
IL1α		↑↓	↑*						B: [64] Up (soluble) n = 30&30, [40] Down (T cell surface) n = 30&50, P: [65] (in NK cells) n = 24&46; [66] n = 18&26 P: [52] n = 20&74 O: [41] Up n = 29&20 (follicular fluid), [27] Down n = 9&9 (cumulus cells)
IL3			↓		↑↓*				P [33]: n = 59&73 – O: [41] n = 29&20 (follicular fluid)
CCL22 (MDC)			↑		↓				P: [52] n = 20&74 – O: [41] n = 29&20 (follicular fluid)
IL32		↑		↑*					B: [67] n = 35&50 – E: [55] n = 8&8
CD8+ (cytotoxic T cells)		↓		↑					B: [40] n = 30&50, [68] n = 20&54 – E: [69] n = 15&15
CD3+ or CD4+ (T cells)		↑	↑ CD3	↑ CD4					B: [40] n = 30&50, [68] n = 20&54 P: [16] = 16&67 E: [69] n = 15&15
CD25HIGH/FOXP3+ /CD4+ (Treg cells)		↓	↑						B: [70] n = 15&17 P: [70] n = 15&17, [50] n = 28&70; [71] n = 25&25

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
Immunity related markers / Cytokines	CD68 (macrophages)		↑	↑					P [15]: n = 30&48 ; [31] <i>n</i> = 22&30; [72] <i>n</i> = 18&38; [45] <i>n</i> = 22&47 E: [73] n = 36&37
	GAL-3		↑	↓					P: [74] <i>n</i> = 8&15 – E: [75] n = 34&34
	PTGS2			↑	↓				E: [76] <i>n</i> = 21&26, O: [77] n = 40&38 (Cumulus cells)
	CXCL12 (SDF-1)	↑			↑*				B: [78] <i>n</i> = 10&11, O: [27] <i>n</i> = 9&9
ECM/Cell Markers/ Cell fate	MMP-9 (or MMP-9/NGAL)	↑	↑		↑	↑	↑*		B: [79] n = 31&60 ; [80] <i>n</i> = 26&50; [81] n = 140&200 – P [80]: <i>n</i> = 26&50
	MMP-2				↑			↑*	O: [81] n = 140&200 ; U: [82] n = 58&73 – M: [28] <i>n</i> = 3&3
	MMP-3	↑	↑	↑					O: [81] n = 140&200 – U: [83] <i>n</i> = 25&25; M: [28] <i>n</i> = 3&3
	MMP-7		↑	↑					B: [84] <i>n</i> = 20&40; [85] <i>n</i> = 20&40 P: [24] <i>n</i> = 11&24; E: [24] <i>n</i> = 11&24, [86] <i>n</i> = 20&23
	OSTEOPONTIN, PERIOSTIN	↑	↑	↑					P: [20] n = 34&124 ; E: [87] n = 110&109
	CA-125 aka MUC-16	↑							B: [25] n = 32&71 ; [91] n = 37&47 ; [92] <i>n</i> = 17&35; [93] n = 52&52 P: [94] n = 43&65 ; [92] <i>n</i> = 17&35; [93] n = 52&52 ; [25] n = 32&71 [91]; n = 37&47

Up/Down (in endometriosis patients vs controls) is indicated when studies are inconsistent

P [15]: **n = 30&48**; [31] **n = 22&30**; [72] **n = 18&38**; [45] **n = 22&47**
E: [73] **n = 36&37**
P: [74] **n = 8&15** – E: [75] **n = 34&34**
E: [76] **n = 21&26**, O: [77] **n = 40&38** (Cumulus cells)
B: [78] **n = 10&11**, O: [27] **n = 9&9**
B: [79] **n = 31&60**; [80] **n = 26&50**; [81] **n = 140&200** – P [80]: **n = 26&50**
O: [81] **n = 140&200**; U: [82] **n = 58&73** – M: [28] **n = 3&3**
O: [81] **n = 140&200** – U: [83] **n = 25&25**; M: [28] **n = 3&3**
B: [84] **n = 20&40**; [85] **n = 20&40**
P: [24] **n = 11&24**; E: [24] **n = 11&24**, [86] **n = 20&23**
P: [20] **n = 34&124**; E: [87] **n = 110&109**
Osteopontin: B and E: [88] **n = 41&40** – P: [38] **n = 6&12**
Periostin: B and P: [89] **n = 80&104** – E: [90] **n = 11&14**
B: [25] **n = 32&71**; [91] **n = 37&47**; [92] **n = 17&35**; [93] **n = 52&52**
P: [94] **n = 43&65**; [92] **n = 17&35**; [93] **n = 52&52**; [25] **n = 32&71** [91]; **n = 37&47**

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
Metabolites	CA-19-9	↑		↑					B: [95] <i>n</i> = 36&50; [96] <i>n</i> = 40&60; [91] <i>n</i> = 37&47 – P: [20] <i>n</i> = 34&124
	CYTOKERATIN-19	↑				↑			B: [97] <i>n</i> = 35&44 – U: [97] <i>n</i> = 35&44; [98] <i>n</i> = 6&11
	TIMP-1	↓	↑		↓				B: [81] <i>n</i> = 140&200 – P: [22] <i>n</i> = 45&126 – O: [81] <i>n</i> = 140&200
	FIBRONECTIN			↓	↑				E: [99] <i>n</i> = 18&40 – O: [100] <i>n</i> = 10&20
	TGF-β	↑	↑	↑	↑				B: [81] <i>n</i> = 140&200 – P: [50] <i>n</i> = 28&70 E: [101] <i>n</i> = 10&10 – O: [81] <i>n</i> = 140&200
	BCL2			↑			↑*		E: [102] <i>n</i> = 20&20 – M: [28] <i>n</i> = 3&3
	SOX2			↑			↑*		E: [103] <i>n</i> = 16&26 – M: [28] <i>n</i> = 3&3
	GLUTAMINE, ALANINE	↑		↓*	↓				B: Glutamine: [104] <i>n</i> = 15&22; Alanine: [105] <i>n</i> = 23&22 E: [106] <i>n</i> = 24&95 – O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)
	LYSINE	↑		↑↓		↓			B: [105] <i>n</i> = 23&22 – U: [108] <i>n</i> = 36&45 E: Up [109] <i>n</i> = 29&37; Down [106] <i>n</i> = 24&95
	LEUCINE	↑		↑↓					B: [105] <i>n</i> = 23&22 E Down: [106] <i>n</i> = 24&95; Up: [109] <i>n</i> = 29&37

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
									<i>n</i> = X&Y with X the number of samples from women without endometriosis, Y the number of samples from women with endometriosis
									Up/Down (in endometriosis patients vs controls) is indicated when studies are inconsistent
	ISOLEUCINE	↓			↑				B: [105] <i>n</i> = 23&22 – O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)
	THREONINE	↑			↓				B: [105] <i>n</i> = 23&22 – O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)
	VALINE	↑				↑			B: [105] <i>n</i> = 23&22; [110] <i>n</i> = 23&50 U: [108] <i>n</i> = 36&45

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
	B	P	E	O	U	M	Sa		
Metabolites	TYROSINE		↑	↓				E: [109] <i>n</i> = 29&37; [106] <i>n</i> = 24&95 O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)	
	TAURINE		↑		↑			E: [106] <i>n</i> = 24&95 – U: [108] <i>n</i> = 36&45	
	LACTATE, 3-HYDROXYBUTYRATE	↑			↑			B: [105] <i>n</i> = 23&22 O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)	
	GLUCOSE	↓		↑*	↓			B: [29] <i>n</i> = 103&190 ; [105] <i>n</i> = 23&22 – E [106]: <i>n</i> = 24&95 O: [107] <i>n</i> = 9&7 (samples <i>n</i> = 50&29)	
Hormones/ Growth Factors	ERα		↑	↑↓			↑*	P: [31] <i>n</i> = 22&30 – M: [28] <i>n</i> = 3&3 E: [111] Up <i>n</i> = 18&38, [112] Down <i>n</i> = 26&28	
	ERβ		↑	↓				P: [31] = 22&30, E [112]: <i>n</i> = 26&28	
	LEPTIN	↑	↓*					B: [113] <i>n</i> = 30&30 P: [19] <i>n</i> = 40&58	
	AMH	↓		↑*				B: [114] <i>n</i> = 93&57 , [115] <i>n</i> = 17&17 – E: [116] <i>n</i> = 20&23	
	FGF-2	↑*	↑	↑*				P: [52] <i>n</i> = 20&74, [117] <i>n</i> = 18&38	
	VEGF/VEGF-A	↑↓	↑	↑*			↑*	B: Up [30] <i>n</i> = 121&232 ; Up [29] <i>n</i> = 103&190 ; Up [118] <i>n</i> = 822&1109 ; Up [119] <i>n</i> = 30&30 ; Down [25] <i>n</i> = 32&71 ; Down [60] <i>n</i> = 46&57 ; P [22]: <i>n</i> = 45&126 – E: [26] <i>n</i> = 5&5 – M: [28] <i>n</i> = 3&3	
	PDGF-A		↑*	↓			P: [52] <i>n</i> = 20&74 – E: [25] <i>n</i> = 8&15		
	ACTIVIN-A	↑*		↑*			B: [120] <i>n</i> = 75&139 – P: [121] <i>n</i> = 3&6		

Table 1 (Continued)

Molecular subtypes	Biomarkers found in several tissues	Biological compartments							References by compartment: B for Blood, P for Peritoneum/ Peritoneal fluid, E for Endometrium, O for ovary, U for Urine, M for menstrual blood, and Sa for Saliva
		B	P	E	O	U	M	Sa	
miRNAs	MIR-451/MIR-451A	↑↓	↑	↑	↓				B: Up [122] n = 24&24; Up [123] n = 99&89; Down [124] n = 66&80 P: [22] n = 45&126 – E: [125] n = 20&40 – O: [126] n = 30&30 (follicular fluid)
	MIR-122, MIR-199A	↑	↑						B: [127] n = 25&60, [46] n = 35&45 – P: [46] n = 35&45
	MIR-15A-5P	↓		↓					B: [128] n = 30&60 – E: n = 3&3
	MIR-17/MIR-17-5P	↓		↓					B: [47] n = 60&80; [128] n = 30&60 – E: [129] n = 51&51
	MIR-135A	↑↓						↑	B: Down [130] n = 24&24; Up [131] n = 17&17 – Sa: [131] n = 17&17
Other	GLYCODELIN-A	↑	↑						B: [37] n = 86&170; [30] n = 121&232 [19]; n = 42&57; [35] n = 20&48 P: [37] n = 86&170; [19] n = 42&57; [32] n = 17&33, [35] n = 20&48
	HAPTOGLOBIN	↓	↑						B: [132] n = 15&15 – P: [133] n = 8&16
	PGP9.5		↑	↑*					P: [72] n = 18&38 – E: [134] n = 20&20

Arrows indicate the direction of change in endometriosis patients. *: change detected only in a subgroup of patients (either because of patient inclusion criteria restricting to a subgroup of patients, or detected only in a specific sub group of all included patients). ILX Interleukin X, CA-X Cancer Antigen X, MMP-X Matrix Metalloproteinase-X, CDX Cluster of Differentiation X, CXCLX Chemokine (C-X-C motif) ligand X, TNFα Tumor Necrosis Factor alpha, (s)ICAM-1 (soluble) Intercellular adhesion molecule-1, IFNγ Interferon gamma, CCL5 Chemokine (C-C motif) ligand 5, IP-10 Interferon gamma induced protein 10, IL6R Interleukin 6 receptor, MCP-1 Monocyte Chemoattractant protein-1, GM-CSF Granulocyte/macrophage-colony stimulating factor, MDC Macrophage-derived chemokine, GAL-3 Galectin-3, VEGF Vascular Endothelial Growth Factor, NGAL Neutrophil Gelatinase-Associated Lipocalin, TIMP-1 TIMP metalloproteinase inhibitor-1, MUC-16 Mucin-16, TGF-β Transforming growth factor beta, BCL2 B-cell lymphoma 2, ERα Estrogen receptor alpha, ERβ Estrogen receptor beta, AMH Anti-Müllerian hormone, FGF-2 Fibroblast growth factor 2, PDGFR Patelet-derived growth factor, PGP9.5 Protein gene product 9.5, PTGS2 Prostaglandin-endoperoxide synthase 2, SDF-1 Stromal cell-derived facotr-1.

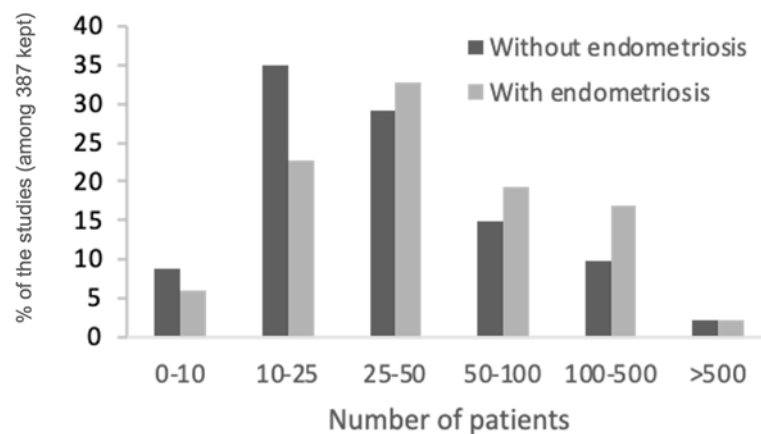


Fig. 3 Cohort size distribution. Analyses performed among the 387 articles kept and illustrating the distribution of studies according to cohort size

numerous studies, specific miRNA expression patterns are hallmarks for numerous diseases [122, 123]. These associations between miRNA expression profiles and diseases are often obtained by non-targeted screening (microarray or miRNome sequencing), and their mechanistic roles in physiology or pathophysiology are poorly studied. miR-451 seemed to perform well as a diagnostic marker of endometriosis across different biological fluids and study settings, particularly in combination with other miRNAs [123, 124]. This biomarker does not seem to be correlated with endometriosis severity [22, 123]. Understanding the observed discrepancies in the direction of variation will require studies with systematic adjustments for disease severity, menstrual cycle phases, treatments, symptoms, clinical characteristics of the cohorts and associated comorbidities.

Discussion

Endometriosis biomarker research investigated a large variety of biological compartments so far, some of which are relevant to the local mechanisms of endometriosis pathophysiology (eutopic endometrium, peritoneal fluid, ovary, menstrual blood, cervical mucus), while others approached endometriosis as a systemic disorder resulting in body-wide dysregulations (peripheral blood, urine, feces, saliva). A first valuable outcome of our review was that non-invasively accessible biological compartments (urine, menstrual blood, feces, saliva, cervical mucus) remained drastically understudied despite their potential to transform endometriosis diagnosis. These compartments can address disease modifications both at the systematic and local levels, and deserve more focused attention in the future.

We identified a total of 1107 candidate biomarkers across all nine studied biological compartments, suggesting that endometriosis is potentially associated with

widespread molecular modifications. However, agreement between studies, protocols and laboratories was strikingly low, with few candidate markers consistently modified within the same compartment and exhibiting similar directions of change. This suggests that many reported candidate biomarkers were either highly dependent on technical considerations, or represent false positives due to unaccounted confounders. Only 4 of these candidates were reproducibly detected across several compartments by different research teams and with appropriately powered cohorts, and we argued that these markers with widespread modifications should be first-line candidates for investigation in more accessible biological compartments.

As highlighted in this review, the relationships between marker variations and biological compartments were often obscured by uneven consideration of fundamental variables such as disease stage, symptoms, treatments, and menstrual cycle phase. Symptoms and treatments were the major missing elements in many study designs. These variables were rarely analyzed and often absent altogether. Regarding symptomatic treatments (painkillers, anti-inflammatory drugs), neither their effectiveness nor their frequency of use was reported. Menstrual cycle phases were also key variables as many metabolic and regulatory pathways vary throughout the cycle, including one-carbon metabolism [137] and miRNAs [128, 138], but were frequently overlooked. Most articles used the revised ASRM classification to rank phenotypes from endometriosis stage I (minimal) to stage IV (severe) [2]. Deep infiltrating endometriosis is then classified as stage III or IV regardless of the presence of endometrioma. However, presence of endometriomas seems decisive for some biomarkers regulation, especially metabolites. A more accurate classification like ERZIAN scoring may allow for better discrimination between different disease

Table 2 Candidate biomarkers found by independent teams in at least 3 biological compartments in large cohort

Biomarker	Patients	Potential confounding factors		Type of measured molecule related to the indicated biomarker	Main results		Missing information	Adjustments		Statistics as regards diagnostic accuracy				References					
		Endometriosis group (n)	Age		BMI (endometriosis patients vs controls)	Protein		Serum	Biological sample type	Global variation (endometriosis patients vs control)	Concerning endometriosis phenotype, cycle phase, hormonal treatments or symptoms	Endometriosis subtype	Cycle phase		Symptoms	Hormonal treatments	AUC	Sensitivity	Specificity
TNF- α	Healthy volunteers without detected endometriosis by ultrasound examination (n=103)	Surgically and histologically proven ovarian endometriosis (n=190)	Stage I/II (n=77) and III/IV (n=113)	NS	ND	Protein	Serum	Increased 1.4 times	Cycle phase, treatment for controls, symptoms	Higher in stage III/IV vs I/II	NA	ND or NA	ND or NA	0.776 (alone)	ND (alone)	ND	[29]		
	Without endometriosis (laparoscopic exam) (n=93)	Endometriosis patients (n=201)	Stage I/II (n=132) and III/IV (n=69)	ND	ND or NA	Protein	Plasma	Reduced 14.6 times	Pain symptoms, lesions localisations	Significant reduction for I/II and III/IV vs NS I/II vs III/IV	Change detected in all controls and secretory phase	ND or NA	NP	0.758 (all phase)	79.5% (alone)	73.7%	[12]		
	Without endometriosis (laparoscopic exam) (n=121)	Endometriosis patients (n=232)	Training subset (n=155), test subset (n=67)	NS	ND or NA	Protein	Plasma	Reduced 1.2 times (only in training set)	Lesions localisations	ND (for stages)	Change detected in all and proliferative phase	ND	NP	0.65 (for training set)	78%	57%	[30]		
	Without endometriosis (laparoscopic exam) (n=35)	Surgically and histologically proven endometriosis (n=45)	Stage I/II/III/IV (n=10/8/18/9)	NS	Lower	Protein	Plasma	NS	Pain symptoms other than dysmenorrhea	Higher in patients with vs without DIE	NS (between phase)	NS	ND	ND	ND	ND	[23]		
	Without endometriosis (laparoscopic exam) (n=59)	Endometriosis (n=73)	Stage I/II (n=31) and III/IV (n=42)	NS	NS or NA	Protein	Peritoneal fluid	Increased ~2 times	None	Higher in OE vs DIE (all phases)	Main result only found in proliferative phase	ND	NP	ND	ND	ND	[33]		
		PE (n=39), OE (n=18), DIE (n=18)																	

Table 2 (continued)

Biomarker	Patients	Potential confounding factors		Type of measured molecule related to the indicated biomarker	Main results	Missing information	Adjustments	Statistics as regards diagnostic accuracy					References		
		Endometriosis group (n)	Age					BMI (endometriosis patients vs controls)	Biological sample type	Global variation (endometriosis patients vs control)	Concerning endometriosis phenotype, cycle phase, hormonal treatments or symptoms	Endometriosis subtype		Cycle phase	Symptoms
	Control group (n)	Endometriosis group (n)	Age	BMI (endometriosis patients vs controls)	Biological sample type	Global variation (endometriosis patients vs control)	Concerning endometriosis phenotype, cycle phase, hormonal treatments or symptoms	Endometriosis subtype	Cycle phase	Symptoms	Hormonal treatments	AUC	Sensitivity	Specificity	
	Infertile patients undergoing ICSI without endometriosis as assessed by laparoscopy (n = 279)	Surgically and histologically proven endometriosis infertile patients undergoing ICSI (n = 47)	NS	NS	Protein	Follicular fluid	Pain symptoms, lesions localisations	ND	NP	NA	NP	ND	ND	ND	[34]

Table 2 (continued)

Biomarker	Patients	Potential confounding factors		Type of measured molecule related to the indicated biomarker	Main results		Missing information	Adjustments		Statistics as regards diagnostic accuracy				References	
		Endometriosis group (n)	Age		BMI (endometriosis patients vs controls)	Biological sample type		Global variation (endometriosis patients vs control)	Concerning endometriosis phenotype, cycle phase, hormonal treatments or symptoms	Endometriosis subtype	Cycle phase	Symptoms	Hormonal treatments		AUC
MMP-9 or MMP-9/NGAL	Healthy volunteers without endometriosis (laparoscopic exam) (n=31)	Surgically proven endometriosis (n=60) OE (n=31)	NS	NS	Protein	Serum	Increased 1.25 times	Cycle phase and symptoms	Higher in stage III/IV vs I/II	NA	NP	Predictive accuracy for severe endometriosis (threshold: 14.13 pg/ml) 0.878	80%	73.3%	[79]
	Infertile women without endometriosis but with tubal factor infertility undergoing IVF (n=200)	Infertile women with endometriosis confirmed by laparoscopy and histological analysis, undergoing IVF Stage III/IV (n=140)	NS	NS	Protein	Serum	Increased 1.5 times	Pain symptoms, lesions localisations	NP	NP	NA/NP for infertility	Progesterone supplementation decrease (1.4 times) MMP-9 serum level in pregnant women	ND	ND	[81]
TIMP-1	Surgically and histologically or clinically verified no evidence of endometriosis (n=58)	Surgically and histologically verified endometriosis (n=73)	NA	NA	Protein	Urine	Increased 1.5 times	Cycle phase, hormonal treatments, symptoms and lesions localisations	NP	NA	NA	The odds ratio of having MMP-9 or MMP-9/NGAL in the urine and having endometriosis was 7.8 (95% CI: 2.5–25.1; p<0.001) and 6.3 (95% CI: 1.7–22.8; p<0.001), respectively	ND	ND	[82]
	Infertile women without endometriosis but with tubal factor infertility undergoing IVF (n=200)	Infertile women with endometriosis confirmed by laparoscopy and histological analysis, undergoing IVF Stage III/IV (n=140)	NS	NS	Protein	Serum	Reduced 1.2 times	Pain symptoms, lesions localisations	NP	NP	NA/NP for infertility	NP	ND	ND	[81]
	Healthy volunteers without endometriosis (laparoscopic exam) (n=45)	Surgically proven endometriosis (n=126)	NA	NA	Protein	Peritoneal fluid	Reduced 1.2 times	Pain symptoms, lesions localisations	No statistical difference between stage I/II and III/IV	Increase only in secretory phase	NA/Same range of increase regardless of fertility status	NP	ND	ND	[22]

Table 2 (continued)

Biomarker	Patients	Potential confounding factors		Type of measured molecule related to the indicated biomarker	Main results		Missing information	Adjustements		Statistics as regards diagnostic accuracy				References		
		Control group (n)	Endometriosis group (n)		Age	BMI (endometriosis patients vs controls)		Biological sample type	Global variation (endometriosis patients vs control)	Endometriosis subtype	Cycle phase	Symptoms	Hormonal treatments		AUC	Sensitivity
miR-451/ miR-451a	Healthy volunteers without endometriosis (laparoscopic exam) (n=99)	Surgically proven endometriosis (n=89) Stage I/II/III/IV (n=27/17/36/19) (laparoscopic exam)	NS	NS	miRNA	Serum	Increased 4.5 times (qRT-PCR)	Concerning endometriosis phenotype, cycle phase, hormonal treatments or symptoms	Significant increase for I/II and III/IV vs controls NS I/II vs III/IV	NS	NA	NS	0.84 (alone)	90%	72.9%	[123]
														0.939 (in combination with miR-125b, miR-150, miR-342, miR-3613 and let-7b)	83%	
	Healthy volunteers without endometriosis (n=66)	Infertile women with endometriosis confirmed by laparoscopy and histological analysis, undergoing IVF/ICSI (n=80) Stage I/II (60) and III/IV (20)	NS	NS	miRNA	Serum	Decreased 1.7 times (qRT-PCR)	Other symptoms than infertility for patients, symptoms and treatment for control, cycle phase, lesions localisations	NP	NA	NA	NA	0.978	ND	ND	[124]
	Without endometriosis (laparoscopic exam) (n=45)	Surgically proven endometriosis (n=126)	NA	NA	miRNA	Peritoneal fluid	Increased 2.5 times (qRT-PCR)	Pain symptoms, lesions localisations	No statistical difference between stage I/II and III/IV	Significantly up-regulated during menstrual phase compared to proliferative and secretory phase in endometriosis women but not in control	NA/No impact of the fertility status	NP	ND	ND	ND	[22]
	Infertile women without endometriosis (laparoscopic exam), undergoing IVF All with OE (n=30)	Infertile women with endometriosis confirmed by laparoscopy, undergoing IVF Stage II/IV (n=30)	NS	NS	miRNA	Follicular fluid	Decreased 2 times (qRT-PCR)	Pain symptoms	NP	NP	NA/NP for infertility	NP	ND	ND	ND	[126]

ND: Not disclosed/done (despite available information), NA Not available, NS No significant difference/correlation, NP: Not possible (same for all samples), PE Peritoneal Endometriosis, OE Ovarian Endometriosis, DIE Deep Infiltrating Endometriosis, CSI IntraCytoplasmic Sperm Injection, IVF In Vitro Fertilization, US Ultrasound, VEGF Vascular Endothelial Growth Factor, sFLT-1 Soluble Fms-like tyrosine kinase 1, MCP1 Monocyte Chemoattractant protein 1, IL6 Interleukin 6, TNF-alpha Tumor Necrosis Factor-alpha, MMP-9 Matrix Metalloproteinase-9, NGAL Neutrophil Gelatinase-Associated Lipocalin, TIMP-1 TIMP metalloproteinase inhibitor 1

phenotypes [2]. All these parameters may contribute to explain the lack of reproducibility between studies, and standardizing data records may help alleviate this issue in future studies.

The top 4 candidate biomarkers of interest identified here belong to different molecular categories (miRNAs, extra-cellular matrix, and cytokines), and are involved in pathophysiological processes common to many diseases, especially extra-cellular matrix remodeling and inflammation. Previous studies have generally combined elements of the same molecular category together [122, 123], but combinations involving different molecular families are more rarely studied [30]. The 4 candidate biomarkers identified in this work were present in blood, an accessible and relevant biological compartment for diagnostic test development. They have never been combined together to test their diagnostic performance in endometriosis, but their association should be evaluated. Comparing sensitivity and specificity across studies to identify potential combinations of markers of interest remains difficult, as designs and cut-offs varied between studies and between biological compartments. We noted that formal meta-analyses of endometriosis biomarkers were largely absent, and will likely remain challenging due to the heterogeneity in study designs and data collection records that we highlighted above, limiting the reusability of available information.

Finally, this systematic study also came with some limitations. First, and despite our best efforts, we may have missed biomarkers that meet our selection criteria but are listed under different aliases during manual literature curation. We however expect that these instances were rare and did not affect the overarching conclusions of this study. Another important limiting factor was the design of the selected studies, which typically excluded rather than accounted for stratifying parameters of interest. Most of the highlighted adjustments were by exclusion of other categories of patients, for example by including only a single endometriosis phenotype in the cohort, or enrolling women in the same phase of the cycle. In this context, rigorously assessing the impact of adjustment and the differential effects of endometriosis subtypes, cycle phases, symptoms and treatments on biomarker levels remained challenging. Another potential source of bias was the heterogeneity of the control groups, a problem widely recognized for endometriosis research. Indeed, supposedly healthy donors may contain asymptomatic endometriosis patients, while most laparoscopically examined controls with confirmed absence of endometriosis had other gynecological or fertility issues. In most cases, these women presented benign comorbidities (e.g. leiomyomas, ovarian cysts) which were not matched with the case group and may impact the levels

of certain markers. While these markers were also of interest to eliminate other diseases during endometriosis diagnosis, they addressed a separate question compared to diagnosing endometriosis at large in the population. A final issue that may interfere with reproducibility concerned the methodology of the studies. At this time, few untargeted studies with large discovery and validation cohorts used omics technologies for high-throughput biomarker discovery. The majority of studies focused on a limited panel of predefined targets and many potential biomarkers were therefore not evaluated. We chose to focus on biomarkers reported by independent research teams and in multiple tissues to improve the relevance and the strength of evidence, but numerous biomarkers were probably unconfirmed because they have not been evaluated so far by independent team and in several compartments.

Conclusion

It appears necessary to rethink endometriosis candidate biomarkers research by designing studies that can be integrated at different levels: i) local and systemic biological compartments; ii) different disease phenotypes with improved characterisation; iii) treatments and their impacts; iv) symptoms; and v) menstrual cycle phases. Access to these parameters will require harmonisation of data collection methods following recommendations of the EPHeCT project [139]. Such harmonisation would enable meta-analyses, yield a considerable increase in cohort sizes, and facilitate investigations into the effects of these stratifying variables. As endometriosis biomarker discovery remains challenging, sensitivity may be improved by combining biomarkers from different molecular pathways. However, combining biomarkers across biological compartments seems unsustainable in clinical practice, and identifying the most relevant biological compartment remains an important challenge. To this regard, our study pinpoints numerous discrepancies in the results obtained in peripheral blood. Local approaches may lead to more consistent results, as is the case in peritoneal fluid, which can unfortunately not be assessed non-invasively. We therefore highlight the need to further investigate non-invasively accessible biological fluids, especially locally accessible such as menstrual fluid or cervical mucus.

Abbreviations

(r)ASRM	(The revised) American Society for Reproductive Medicine
MeSH	Medical Subject Headings
TNF- α	Tumor Necrosis Factor alpha
MMP-9	Enzyme Matrix MetalloProteinase 9
NGAL	Neutrophil Gelatinase-associated lipocalin
TIMP-1	TIMP metalloproteinase inhibitor
miR	Micro RNA
MRI	Magnetic Resonance Imaging
HGNC	HUGO Gene Nomenclature Committee

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12958-023-01181-8>.

Additional file 1: Additional material. PRISMA checklist

Additional file 2: Additional Table 1. References of the 447 selected articles

Additional file 3: Additional Table 2. Quantitative summary of significantly modified biomarkers in endometriosis identified by biological compartment

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Authors' contributions

All authors were actively involved in the preparation of the manuscript (conceptualization: A.B., L.D.; systematic literature search: A.B., M.B., D.V., C.D. K.P.C., K.B., L.M., P.S., C.A. M.J., S.C., C.C., F.B., C.B., L.D.; selection and data review: A.B., L.D.; manuscript and figure preparation: A.B., L.D.; manuscript review: A.B., M.B., D.V., C.D., C.B., L.D.).

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. The code will be made available on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Zondervan KT, Becker CM, Missmer SA. Endometriosis. *N Engl J Med*. 2020;382(13):1244–56.
- Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, et al. World Endometriosis Society consensus on the classification of endometriosis. *Hum Reprod*. 2017;32(2):315–24.
- Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat Rev Endocrinol*. 2019;15(11):666–82.
- Saunders PTK, Horne AW. Endometriosis: Etiology, pathobiology, and therapeutic prospects. *Cell*. 2021;184(11):2807–24.
- Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. *Lancet*. 2021;397(10276):839–52.
- Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, et al. Endometrial biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Gynaecology and Fertility Group, éditeur. Cochrane Database of Systematic Reviews*. <https://doi.org/10.1002/14651858.CD012165>
- Nisenblat V, Prentice L, Bossuyt PM, Farquhar C, Hull ML, Johnson N. Combination of the non-invasive tests for the diagnosis of endometriosis. *Cochrane Gynaecology and Fertility Group, éditeur. Cochrane Database of Systematic Reviews*. 2016;2016(7). Disponible sur: <https://doi.org/10.1002/14651858.CD012281>
- Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Gynaecology and Fertility Group, éditeur. Cochrane Database of Systematic Reviews*. 2016;2016(5). Disponible sur: <https://doi.org/10.1002/14651858.CD012179>
- Liu E, Nisenblat V, Farquhar C, Fraser I, Bossuyt PM, Johnson N, et al. Urinary biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Gynaecology and Fertility Group, éditeur. Cochrane Database of Systematic Reviews*. 2015;2015(12). Disponible sur: <https://doi.org/10.1002/14651858.CD012019>
- Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;n71. Disponible sur: <https://doi.org/10.1136/bmj.n71>
- Schisterman EF, Mumford SL, Sjaarda LA. Failure to consider the menstrual cycle phase may cause misinterpretation of clinical and research findings of cardiometabolic biomarkers in premenopausal women. *Epidemiol Rev*. 2014;36:71–82.
- 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.
- Monsanto SP, Edwards AK, Zhou J, Nagarkatti P, Nagarkatti M, Young SL, et al. Surgical removal of endometriotic lesions alters local and systemic proinflammatory cytokines in endometriosis patients. *Fertil Steril*. 2016;105(4):968–977.e5.
- Matta K, Lefebvre T, Vigneau E, Cariou V, Marchand P, Guittion Y, et al. Associations between persistent organic pollutants and endometriosis: A multiblock approach integrating metabolic and cytokine profiling. *Environment International*. 2022;158:106926. Disponible sur: <https://linkinghub.elsevier.com/retrieve/pii/S0160412021005511>
- Skrzypczak J, Szczepańska M, Puk E, Kamieniczna M, Kurpisz M. Peritoneal fluid cytokines and sICAM-1 in minimal endometriosis: search for discriminating factors between infertility and/or endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2005;122(1):95–103.
- Milewski Ł, Dziunycz P, Barcz E, Radomski D, Roszkowski PI, Korczak-Kowalska G, et al. Increased levels of human neutrophil peptides 1, 2, and 3 in peritoneal fluid of patients with endometriosis: association with neutrophils, T cells and IL-8. *J Reprod Immunol*. 2011;91(1–2):64–70.
- Beste MT, Pfäffle-Doyle N, Prentice EA, Morris SN, Lauffenburger DA, Isaacson KB, et al. Molecular network analysis of endometriosis reveals a role for c-Jun-regulated macrophage activation. *Sci Transl Med*. 2014;6(222):222ra16.
- Borrelli GM, Kaufmann AM, Abrão MS, Mechsner S. Addition of MCP-1 and MIP-3β to the IL-8 appraisal in peritoneal fluid enhances the probability of identifying women with endometriosis. *J Reprod Immunol*. 2015;109:66–73.
- Kocbek V, Vouk K, Bersinger NA, Mueller MD, Lanišnik RT. Panels of cytokines and other secretory proteins as potential biomarkers of ovarian endometriosis. *J Mol Diagn*. 2015;17(3):325–34.
- Henze D, Doecke WD, Hornung D, Agueusop I, von Ahsen O, Machens K, et al. Endometriosis Leads to an Increased Trefoil Factor 3 Concentration in the Peritoneal Cavity but Does Not Alter Systemic Levels. *Reprod Sci*. 2017;24(2):258–67.

21. Jørgensen H, Hill AS, Beste MT, Kumar MP, Chiswick E, Fedorcsak P, et al. Peritoneal fluid cytokines related to endometriosis in patients evaluated for infertility. *Fertil Steril*. 2017;107(5):1191–1199.e2.
22. Mari-Alexandre J, Barceló-Molina M, Belmonte-López E, García-Oms J, Estellés A, Braza-Boils A, et al. Micro-RNA profile and proteins in peritoneal fluid from women with endometriosis: their relationship with sterility. *Fertil Steril*. 2018;109(4):675–684.e2.
23. Jaeger-Lansky A, Schmidthalen K, Kuessel L, Gstöttner M, Waidhofer-Söllner P, Zlabinger GJ, et al. Local and systemic levels of cytokines and danger signals in endometriosis-affected women. *J Reprod Immunol*. 2018;130:7–10.
24. Kyama CM, Overbergh L, Debrock S, Valckx D, Vander Perre S, Meuleman C, et al. Increased peritoneal and endometrial gene expression of biologically relevant cytokines and growth factors during the menstrual phase in women with endometriosis. *Fertil Steril*. 2006;85(6):1667–75.
25. Szubert M, Suzin J, Duechler M, Szulawska A, Czyż M, Kowalczyk-Amico K. Evaluation of selected angiogenic and inflammatory markers in endometriosis before and after danazol treatment. *Reprod Fertil Dev*. 2014;26(3):414–20.
26. Heydari S, Kashani L, Noruzinia M. Dysregulation of Angiogenesis and Inflammatory Genes in Endometrial Mesenchymal Stem Cells and Their Contribution to Endometriosis. *IJAAI*. 2021; Disponible sur: <http://publi.sh.kne-publishing.com/index.php/IJAAI/article/view/8025>
27. Da Luz RM, Da Broi MG, Koopman L de O, Praça JR, da Silva-Jr WA, Ferriani RA, et al. Transcriptomic analysis of cumulus cells shows altered pathways in patients with minimal and mild endometriosis. *Sci Rep*. 2022;12(1):5775. Disponible sur: <https://www.nature.com/articles/s41598-022-09386-4>
28. Sahraei SS, Davoodi Asl F, Kalhor N, Sheykhasan M, Fazaeli H, Moud SS, et al. A Comparative Study of Gene Expression in Menstrual Blood-Derived Stromal Cells between Endometriosis and Healthy Women. Xu H, éditeur. *BioMed Research International*. 2022;2022:1–11. Disponible sur: <https://www.hindawi.com/journals/bmri/2022/7053521/>
29. Tang T, Lai H, Huang X, Gu L, Shi H. Application of serum markers in diagnosis and staging of ovarian endometriosis. *J Obstet Gynaecol Res*. 2021;47(4):1441–50.
30. 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.
31. Montagna P, Capellino S, Villaggio B, Remorgida V, Ragni N, Cutolo M, et al. Peritoneal fluid macrophages in endometriosis: correlation between the expression of estrogen receptors and inflammation. *Fertil Steril*. 2008;90(1):156–64.
32. 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(10):1245–53.
33. Zhou J, Chern BSM, Barton-Smith P, Phoon JW, Tan TY, Viardot-Foucault V, et al. Peritoneal Fluid Cytokines Reveal New Insights of Endometriosis Subphenotypes. *IJMS*. 2020;21(10):3515. Disponible sur: <https://www.mdpi.com/1422-0067/21/10/3515>
34. Wunder DM, Mueller MD, Birkhäuser MH, Bersinger NA. Increased ENA-78 in the follicular fluid of patients with endometriosis. *Acta Obstet Gynecol Scand*. 2006;85(3):336–42.
35. Mosbah A, Nabel Y, Khashaba E. Interleukin-6, intracellular adhesion molecule-1, and glycodelin A levels in serum and peritoneal fluid as biomarkers for endometriosis. *Int J Gynaecol Obstet*. 2016;134(3):247–51.
36. Kuessel L, Wenzl R, Proestling K, Balendran S, Pateisky P, Yotova 1st, et al. Soluble VCAM-1/soluble ICAM-1 ratio is a promising biomarker for diagnosing endometriosis. *Hum Reprod*. 2017;32(4):770–9.
37. O DF, Fassbender A, Van Bree R, Laenen A, Peterse DP, Vanhie A, et al. Technical Verification and Assessment of Independent Validation of Biomarker Models for Endometriosis. *Biomed Res Int*. 2019;2019:3673060.
38. Lee JC, Kim SH, Oh YS, Kim JH, Lee SR, Chae HD. Increased Expression of Retinol-Binding Protein 4 in Ovarian Endometrioma and Its Possible Role in the Pathogenesis of Endometriosis. *Int J Mol Sci*. 2021;22(11):5827.
39. Othman EEDR, Hornung D, Salem HT, Khalifa EA, El-Metwally TH, Al-Hendy A. Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2008;137(2):240–6.
40. Nasyrova RF, Sotnikova LS, Baystrukova NV, Krivoschchekova GV, Novitsky VV, Kupriyana IE, et al. Psychoimmune interactions in women of reproductive age with endometriosis. *Bull Exp Biol Med*. 2011;152(1):93–7.
41. Mao XD, Hu CY, Zhu MC, Ou HL, Qian YL. Immunological microenvironment alterations in follicles of women with proven severe endometriosis undergoing in vitro fertilization. *Mol Biol Rep*. 2019;46(5):4675–84.
42. Nikoo S, Ebtekar M, Jeddi-Tehrani M, Shervin A, Bozorgmehr M, Vafaei S, et al. Menstrual blood-derived stromal stem cells from women with and without endometriosis reveal different phenotypic and functional characteristics. *Mol Hum Reprod*. 2014;20(9):905–18.
43. Martínez S, Garrido N, Coperias JL, Pardo F, Desco J, García-Velasco JA, et al. Serum interleukin-6 levels are elevated in women with minimal-mild endometriosis. *Hum Reprod*. 2007;22(3):836–42.
44. Siedentopf F, Tariverdian N, Rütke M, Kantenich H, Arck PC. Immune status, psychosocial distress and reduced quality of life in infertile patients with endometriosis. *Am J Reprod Immunol*. 2008;60(5):449–61.
45. Li S, Fu X, Wu T, Yang L, Hu C, Wu R. Role of Interleukin-6 and Its Receptor in Endometriosis. *Med Sci Monit*. 2017;23:3801–7.
46. Maged AM, Deeb WS, El Amir A, Zaki SS, El Sawah H, Al Mohamady M, et al. Diagnostic accuracy of serum miR-122 and miR-199a in women with endometriosis. *Int J Gynaecol Obstet*. 2018;141(1):14–9.
47. Wang F, Wang H, Jin D, Zhang Y. Serum miR-17, IL-4, and IL-6 levels for diagnosis of endometriosis. *Medicine (Baltimore)*. 2018;97(24):e10853.
48. Jiang J, Jiang Z, Xue M. Serum and peritoneal fluid levels of interleukin-6 and interleukin-37 as biomarkers for endometriosis. *Gynecol Endocrinol*. 2019;35(7):571–5.
49. Kokot I, Piwowar A, Jędryka M, Solkiewicz K, Kratz EM. Diagnostic Significance of Selected Serum Inflammatory Markers in Women with Advanced Endometriosis. *IJMS*. 2021;22(5):2295.
50. Podgaec S, Rizzo LV, Fernandes LFC, Baracat EC, Abrao MS. CD4(+) CD25(high) Foxp3(+) cells increased in the peritoneal fluid of patients with endometriosis. *Am J Reprod Immunol*. 2012;68(4):301–8.
51. Wickiewicz D, Chrobak A, Gmyrek GB, Halberszadt A, Gabrys MS, Goluda M, et al. Diagnostic accuracy of interleukin-6 levels in peritoneal fluid for detection of endometriosis. *Arch Gynecol Obstet*. 2013;288(4):805–14.
52. Rakhila H, Al-Akoum M, Bergeron ME, Leboeuf M, Lemyre M, Akoum A, et al. Promotion of angiogenesis and proliferation cytokines patterns in peritoneal fluid from women with endometriosis. *J Reprod Immunol*. 2016;116:1–6.
53. Wang XQ, Yu J, Luo XZ, Shi YL, Wang Y, Wang L, et al. The high level of RANTES in the ectopic milieu recruits macrophages and induces their tolerance in progression of endometriosis. *J Mol Endocrinol*. 2010;45(5):291–9.
54. Galleri L, Luisi S, Rotondi M, Romagnani P, Cobellis L, Serio M, et al. Low serum and peritoneal fluid concentration of interferon-gamma-induced protein-10 (CXCL10) in women with endometriosis. *Fertil Steril*. 2009;91(2):331–4.
55. Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. *Fertil Steril*. 2016;106(6):1420–1431.e7.
56. Bersinger NA, Dechaud H, McKinnon B, Mueller MD. Analysis of cytokines in the peritoneal fluid of endometriosis patients as a function of the menstrual cycle stage using the Bio-Plex® platform. *Arch Physiol Biochem*. 2012;118(4):210–8.
57. Tariverdian N, Siedentopf F, Rütke M, Blois SM, Klapp BF, Kantenich H, et al. Intraperitoneal immune cell status in infertile women with and without endometriosis. *J Reprod Immunol*. 2009;80(1–2):80–90.
58. Fan YY, Chen HY, Chen W, Liu YN, Fu Y, Wang LN. Expression of inflammatory cytokines in serum and peritoneal fluid from patients with different stages of endometriosis. *Gynecol Endocrinol*. 2018;34(6):507–12.
59. Lo Turco EG, Souza GHMF, Garcia JS, Ferreira CR, Eberlin MN, Bertolla RP. Effect of endometriosis on the protein expression pattern of follicular fluid from patients submitted to controlled ovarian hyperstimulation for in vitro fertilization. *Hum Reprod*. 2010;25(7):1755–66.
60. Lee YH, Cui L, Fang J, Chern BSM, Tan HH, Chan JKY. Limited value of pro-inflammatory oxylipins and cytokines as circulating biomarkers in endometriosis - a targeted 'omics study. *Sci Rep*. 2016;6:26117.
61. Gmyrek GB, Sozanski R, Jerzak M, Chrobak A, Wickiewicz D, Skupnik A, et al. Evaluation of monocyte chemotactic protein-1 levels in peripheral

- blood of infertile women with endometriosis. *Eur J Obstet Gynecol Reprod Biol.* 2005;122(2):199–205.
62. Agic A, Djalali S, Wolfer MM, Halis G, Diedrich K, Hornung D. Combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood as a diagnostic test for endometriosis. *Reprod Sci.* 2008;15(9):906–11.
 63. Fairbanks F, Abrão MS, Podgaec S, Dias JAJ, de Oliveira RM, Rizzo LV. Interleukin-12 but not interleukin-18 is associated with severe endometriosis. *Fertil Steril.* 2009;91(2):320–4.
 64. Karakus S, Sancakdar E, Akkar O, Yildiz C, Demirpence O, Cetin A. Elevated Serum CD95/FAS and HIF-1 α 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.
 65. Eidukaite A, Siaurys A, Tamosiunas V. Aberrant expression of CD95 and CD69 molecules among CD56 cells in women with endometriosis. *Am J Reprod Immunol.* 2006;55(4):276–81.
 66. Gogacz M, Gałczyński K, Wojtaś M, Winkler I, Adamiak A, Romanek-Piva K, et al. Fas-Related Apoptosis of Peritoneal Fluid Macrophages in Endometriosis Patients: Understanding the Disease. *J Immunol Res.* 2017;2017:3175394.
 67. Choi YS, Kim S, Oh YS, Cho S, Hoon KS. Elevated serum interleukin-32 levels in patients with endometriosis: A cross-sectional study. *Am J Reprod Immunol.* 2019;82(2):e13149.
 68. Abramuk M, Grywalska E, Korona-Główniak I, Niedźwiedzka-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(9):1–24. Disponible sur: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7440448/>
 69. Wu L, Lv C, Su Y, Li C, Zhang H, Zhao X, et al. Expression of programmed death-1 (PD-1) and its ligand PD-L1 is upregulated in endometriosis and promoted by 17 β -estradiol. *Gynecol Endocrinol.* 2019;35(3):251–6.
 70. Olkowska-Truchanowicz J, Bocian K, Maksym RB, Białoszewska A, Włodarczyk D, Baranowski W, et al. CD4⁺ CD25⁺ FOXP3⁺ regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. *Hum Reprod.* 2013;28(1):119–24.
 71. Chen H, Qin S, Lei A, Li X, Gao Q, Dong J, et al. Expansion of monocytic myeloid-derived suppressor cells in endometriosis patients: A pilot study. *Int Immunopharmacol.* 2017;47:150–8.
 72. Tran LVP, Tokushige N, Berbic M, Markham R, Fraser IS. Macrophages and nerve fibres in peritoneal endometriosis. *Hum Reprod.* 2009;24(4):835–41.
 73. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. *Am J Reprod Immunol.* 2015;73(3):221–31.
 74. Caserta D, Di Benedetto L, Bordini G, D'Ambrosio A, Moscarini M. Levels of Galectin-3 and Stimulation Expressed Gene 2 in the peritoneal fluid of women with endometriosis: a pilot study. *Gynecol Endocrinol.* 2014;30(12):877–80.
 75. Yang H, Yin J, Ficarotta K, Hsu SH, Zhang W, Cheng C. Aberrant expression and hormonal regulation of Galectin-3 in endometriosis women with infertility. *J Endocrinol Invest.* 2016;39(7):785–91.
 76. Cho S, Park SH, Choi YS, Seo SK, Kim HY, Park KH, et al. Expression of cyclooxygenase-2 in eutopic endometrium and ovarian endometriotic tissue in women with severe endometriosis. *Gynecol Obstet Invest.* 2010;69(2):93–100.
 77. da Luz CM, da Broi MG, Donabela FC, Paro de Paz CC, Meola J, Navarro PA. PTGS2 down-regulation in cumulus cells of infertile women with endometriosis. *Reprod Biomed Online.* 2017;35(4):379–86.
 78. Li F, Alderman MH 3rd, Tal A, Mamillapalli R, Coolidge A, Hufnagel D, et al. Hematogenous Dissemination of Mesenchymal Stem Cells from Endometriosis. *Stem Cells.* 2018;36(6):881–90.
 79. Bostanci Durmus A, Dincer Cengiz S, Yilmaz H, Candar T, Gursay AY, Sinem CG. The levels of matrix metalloproteinase-9 and neutrophil gelatinase-associated lipocalin in different stages of endometriosis. *J Obstet Gynaecol.* 2019;39(7):991–5.
 80. Liu H, Wang J, Wang H, Tang N, Li Y, Zhang Y, et al. The plasma and peritoneal fluid concentrations of matrix metalloproteinase-9 are elevated in patients with endometriosis. *Ann Clin Biochem.* 2016;53(Pt 5):599–605.
 81. Singh AK, Chattopadhyay R, Chakravarty B, Chaudhury K. Altered circulating levels of matrix metalloproteinases 2 and 9 and their inhibitors and effect of progesterone supplementation in women with endometriosis undergoing in vitro fertilization. *Fertility and Sterility.* 2013;100(1):127–134.e1. Disponible sur: <https://linkinghub.elsevier.com/retrieve/pii/S001502821300407X>
 82. Becker CM, Louis G, Exarhopoulos A, Mechsner S, Ebert AD, Zurakowski D, et al. Matrix metalloproteinases are elevated in the urine of patients with endometriosis. *Fertil Steril.* 2010;94(6):2343–6.
 83. Chen X, Liu H, Sun W, Guo Z, Lang J. Elevated urine histone 4 levels in women with ovarian endometriosis revealed by discovery and parallel reaction monitoring proteomics. *J Proteomics.* 2019;204:103398.
 84. 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.
 85. Mabrouk M, Elmakky A, Caramelli E, Farina A, Mignemi G, Venturoli S, et al. Performance of peripheral (serum and molecular) blood markers for diagnosis of endometriosis. *Arch Gynecol Obstet.* 2012;285(5):1307–12.
 86. Zhao L, Gu C, Ye M, Zhang Z, Han W, Fan W, et al. Identification of global transcriptome abnormalities and potential biomarkers in eutopic endometria of women with endometriosis: A preliminary study. *Biomed Rep.* 2017;6(6):654–62. Disponible sur: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5440448/>
 87. Vallvé-Juanico J, López-Gil C, Ponomarenko J, Melnychuk T, Castellví J, Ballesteros A, et al. External validation of putative biomarkers in eutopic endometrium of women with endometriosis using NanoString technology. *J Assist Reprod Genet.* 2020;37(12):2981–7. Disponible sur: <https://doi.org/10.1007/s10815-020-01965-6>
 88. Cho S, Ahn YS, Choi YS, Seo SK, Nam A, Kim HY, et al. Endometrial osteopontin mRNA expression and plasma osteopontin levels are increased in patients with endometriosis. *Am J Reprod Immunol.* 2009;61(4):286–93.
 89. Zheng Q, Lu J, Li R, Hu C, Liu P. Elevated periostin in serum and peritoneal washing fluids as potential biomarkers for endometriosis. *Gynecol Endocrinol.* 2016;32(11):900–3.
 90. Ganieva U, Nakamura T, Osuka S, Bayasula, Nakanishi N, Kasahara Y, et al. Involvement of Transcription Factor 21 in the Pathogenesis of Fibrosis in Endometriosis. *Am J Pathol.* 2020;190(1):145–57.
 91. Rokhgireh S, Mehdizadeh Kashi A, Chaichian S, Delbandi AA, Allahqoli L, Ahmadi-Pishkuhi M, et al. The Diagnostic Accuracy of Combined Enolase/Cr, CA125, and CA19–9 in the Detection of Endometriosis. *BioMed Research International.* 2020;2020:1–9. Disponible sur: <https://www.hindawi.com/journals/bmri/2020/5208279/>
 92. Amaral VF do, Ferriani RA, Silva de S, Nogueira AA, Rosa e Silva JC, Rosa e Silva ACJ de S, et al. Positive correlation between serum and peritoneal fluid CA-125 levels in women with pelvic endometriosis. *Sao Paulo Med J.* 2006;124(4):223–7.
 93. Florio P, Reis FM, Torres PB, Calonaci F, Abrão MS, Nascimento LL, et al. High serum follistatin levels in women with ovarian endometriosis. *Hum Reprod.* 2009;24(10):2600–6.
 94. Matalliotakis IM, Goumenou AG, Mulyim N, Karkavitsas N, Koumantakis EE. High concentrations of the CA-125, CA 19–9 and CA 15–3 in the peritoneal fluid between patients with and without endometriosis. *Arch Gynecol Obstet.* 2005;271(1):40–5.
 95. Tuten A, Kucur M, Imamoglu M, Kaya B, Acikgoz AS, Yilmaz N, et al. Copeptin is associated with the severity of endometriosis. *Arch Gynecol Obstet.* 2014;290(1):75–82.
 96. OG Onur Güralp SA Serdar Acikgöz NT Nevin Tüten 2020 Evaluation of Serum Endocan Levels in Endometriosis: A case-control study *Clin Ter.* (6):517 22<https://doi.org/10.7417/CT.2020.2266>
 97. Draji HA, Abbas AAH, Abdullah TH. Serum and Urine Levels of Cytokeratin-19 in Endometriosis. *Ann Trop Med Public Health.* 2020;23(14). Disponible sur: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7440448/>
 98. Tokushige N, Markham R, Crossett B, Ahn SB, Nelaturi VL, Khan A, et al. Discovery of a novel biomarker in the urine in women with endometriosis. *Fertil Steril.* 2011;95(1):46–9.
 99. Holzer I, Machado Weber A, Marshall A, Freis A, Jauckus J, Strowitzki T, et al. GRN, NOTCH3, FN1, and PINK1 expression in eutopic

- endometrium - potential biomarkers in the detection of endometriosis - a pilot study. *J Assist Reprod Genet.* Nov2020;37(11):2723–32.
100. Regiani T, Cordeiro FB, da Costa L do VT, Salgueiro J, Cardozo K, Carvalho VM, et al. Follicular fluid alterations in endometriosis: label-free proteomics by MS(E) as a functional tool for endometriosis. *Syst Biol Reprod Med.* 2015;61(5):263–76.
 101. Goteri G, Altobelli E, Tossetta G, Zizzi A, Avellini C, Licini C, et al. High temperature requirement A1, transforming growth factor beta1, phosphoSmad2 and Ki67 in eutopic and ectopic endometrium of women with endometriosis. *Eur J Histochem.* 2015;59(4):2570.
 102. Park JS, Lee JH, Kim M, Chang HJ, Hwang KJ, Chang KH. Endometrium from women with endometriosis shows increased proliferation activity. *Fertil Steril.* 2009;92(4):1246–9.
 103. 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.
 104. Murgia F, Angioni S, D'Alterio MN, Pirarba S, Noto A, Santoru ML, et al. Metabolic Profile of Patients with Severe Endometriosis: a Prospective Experimental Study. *Reprod Sci.* 2021;28(3):728–35.
 105. Dutta M, Joshi M, Srivastava S, Lodh I, Chakravarty B, Chaudhury K. A metabonomics approach as a means for identification of potential biomarkers for early diagnosis of endometriosis. *Mol Biosyst.* 2012;8(12):3281–7.
 106. Dutta M, Singh B, Joshi M, Das D, Subramani E, Maan M, et al. Metabonomics reveals perturbations in endometrium and serum of minimal and mild endometriosis. *Sci Rep.* 2018;8(1):6466.
 107. Pocate-Cheriet K, Santulli P, Kateb F, Bourdon M, Maignien C, Batteux F, et al. The follicular fluid metabolome differs according to the endometriosis phenotype. *Reprod Biomed Online.* 2020;41(6):1023–37.
 108. Vicente-Muñoz S, Morcillo I, Puchades-Carrasco L, Payá V, Pellicer A, Pineda-Lucena A. Nuclear magnetic resonance metabolomic profiling of urine provides a noninvasive alternative to the identification of biomarkers associated with endometriosis. *Fertil Steril.* 2015;104(5):1202–9.
 109. Li J, Guan L, Zhang H, Gao Y, Sun J, Gong X, et al. Endometrium metabolomic profiling reveals potential biomarkers for diagnosis of endometriosis at minimal-mild stages. *Reprod Biol Endocrinol.* 2018;16(1):42.
 110. Vicente-Muñoz S, Morcillo I, Puchades-Carrasco L, Payá V, Pellicer A, Pineda-Lucena A. Pathophysiologic processes have an impact on the plasma metabolomic signature of endometriosis patients. *Fertil Steril.* 2016;106(7):1733–1741.e1.
 111. Moberg C, Bourlev V, Ilyasova N, Olovsson M. Levels of oestrogen receptor, progesterone receptor and α B-crystallin in eutopic endometrium in relation to pregnancy in women with endometriosis. *Hum Fertil (Camb).* 2015;18(1):30–7.
 112. Colón-Caraballo M, García M, Mendoza A, Flores I. Human Endometriosis Tissue Microarray Reveals Site-specific Expression of Estrogen Receptors, Progesterone Receptor, and Ki67. *Appl Immunohistochem Mol Morphol.* 2019;27(7):491–500.
 113. Seeber B, Sammel MD, Fan X, Gerton GL, Shaunik A, Chittams J, et al. Panel of markers can accurately predict endometriosis in a subset of patients. *Fertil Steril.* 2008;89(5):1073–81.
 114. Sánchez-Ferrer ML, Jiménez-Velázquez R, Mendiola J, Prieto-Sánchez MT, Cánovas-López L, Carmona-Barnosi A, et al. Accuracy of anogenital distance and anti-Müllerian hormone in the diagnosis of endometriosis without surgery. *Int J Gynaecol Obstet.* 2019;144(1):90–6.
 115. Lemos NA, Arbo E, Scalco R, Weiler E, Rosa V, Cunha-Filho JS. Decreased anti-Müllerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis. *Fertil Steril.* 2008;89(5):1064–8.
 116. Carrarelli P, Rocha ALL, Belmonte G, Zupi E, Abrão MS, Arcuri F, et al. Increased expression of antimüllerian hormone and its receptor in endometriosis. *Fertil Steril.* 2014;101(5):1353–8.
 117. Bourlev V, Larsson A, Olovsson M. Elevated levels of fibroblast growth factor-2 in serum from women with endometriosis. *Am J Obstet Gynecol.* 2006;194(3):755–9.
 118. Vodolazkaia A, Yesilyurt BT, Kyama CM, Bokor A, Schols D, Huskens D, et al. Vascular endothelial growth factor pathway in endometriosis: genetic variants and plasma biomarkers. *Fertil Steril.* 2016;105(4):988–96.
 119. Mohamed ML, El Behery MM, Mansour SAEA. 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.
 120. Reis FM, Luisi S, Abrão MS, Rocha ALL, Viganò P, Rezende CP, et al. Diagnostic value of serum activin A and follistatin levels in women with peritoneal, ovarian and deep infiltrating endometriosis. *Hum Reprod.* 2012;27(5):1445–50.
 121. Hou Z, Sun L, Gao L, Liao L, Mao Y, Liu J. Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosis. *Biomarkers.* 2009;14(8):604–18.
 122. Cosar E, Mamillapalli R, Ersoy GS, Cho S, Seifer B, Taylor HS. Serum microRNAs as diagnostic markers of endometriosis: a comprehensive array-based analysis. *Fertil Steril.* 2016;106(2):402–9.
 123. Moustafa S, Burn M, Mamillapalli R, Nematian S, Flores V, Taylor HS. Accurate diagnosis of endometriosis using serum microRNAs. *Am J Obstet Gynecol.* Oct2020;223(4):557.e1–557.e11.
 124. Wang L, Zhang J, Sun H, Ji X, Zhang S. Effect of miR-451 on IVF/ICSI-ET outcome in patient with endometriosis and infertility. *Am J Transl Res.* 2021;13(11):13051–8.
 125. Gao S, Liu S, Gao ZM, Deng P, Wang DB. Reduced microRNA-451 expression in eutopic endometrium contributes to the pathogenesis of endometriosis. *WJCC.* 2019;7(16):2155–64. Disponible sur: <https://www.wjnet.com/2307-8960/full/v7/i16/2155.htm>
 126. Li X, Zhang W, Fu J, Xu Y, Gu R, Qu R, et al. MicroRNA-451 is downregulated in the follicular fluid of women with endometriosis and influences mouse and human embryonic potential. *Reprod Biol Endocrinol.* 2019;17(1):96.
 127. Wang WT, Zhao YN, Han BW, Hong SJ, Chen YQ. Circulating microRNAs identified in a genome-wide serum microRNA expression analysis as noninvasive biomarkers for endometriosis. *J Clin Endocrinol Metab.* 2013;98(1):281–9.
 128. Vanhie A, O D, Peterse D, Beckers A, Cuéllar A, Fassbender A, et al. Plasma miRNAs as biomarkers for endometriosis. *Hum Reprod.* 2019;34(9):1650–60.
 129. Nabel Y, Elshahawy H, Mosbah A. Intrauterine Bacterial Colonization and Endometrial MicroRNA-17–5p Levels in Association to Endometriosis: A Study in an Egyptian Population. *Immunol Invest.* 2020;49(6):611–21.
 130. Cho S, Mutlu L, Grechukhina O, Taylor HS. Circulating microRNAs as potential biomarkers for endometriosis. *Fertil Steril.* 2015;103(5):1252–1260.e1.
 131. Perricos A, Proestling K, Husslein H, Kuessel L, Hudson QJ, Wenzl R, et al. Hsa-mir-135a Shows Potential as A Putative Diagnostic Biomarker in Saliva and Plasma for Endometriosis. *Biomolecules.* 2022;12(8):1144. Disponible sur: <https://www.mdpi.com/2218-273X/12/8/1144>
 132. Hwang JH, Oh JJ, Wang T, Jin YC, Lee JS, Choi JR, et al. Identification of biomarkers for endometriosis in eutopic endometrial cells from patients with endometriosis using a proteomics approach. *Mol Med Rep.* 2013;8(1):183–8.
 133. Wölfler MM, Meinhold-Heerlein IM, Söhngen L, Rath W, Knüchel R, Neulen J, et al. Two-dimensional gel electrophoresis in peritoneal fluid samples identifies differential protein regulation in patients suffering from peritoneal or ovarian endometriosis. *Fertil Steril.* 2011;95(8):2764–8.
 134. Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, et al. Density of small diameter sensory nerve fibres in endometrium: a semi-invasive diagnostic test for minimal to mild endometriosis. *Hum Reprod.* 2009;24(12):3025–32.
 135. Zhou L, Chen Y, Gao J, Shankar S, Zhang G. Diagnostic Value of Circulating MicroRNAs for Endometriosis: a Meta-analysis. *Reprod Sci.* 2020;27(3):793–805.
 136. Sahraei SS, Davoodi Asl F, Kalhor N, Sheykhasan M, Fazaeli H, Moud SS, et al. A Comparative Study of Gene Expression in Menstrual Blood-Derived Stromal Cells between Endometriosis and Healthy Women. Xu H, éditeur. *BioMed Research International.* 2022;2022:1–11. Disponible sur: <https://www.hindawi.com/journals/bmri/2022/7053521/>
 137. Kim R, Nijhout HF, Reed MC. One-carbon metabolism during the menstrual cycle and pregnancy. *PLoS Comput Biol.* 2021;17(12):e1009708.
 138. MacGregor KA, Rodriguez-Sanchez N, Di Virgilio TG, Barwell ND, Gallagher IJ, Moran CN. Changes in adipose tissue microRNA expression

across the menstrual cycle in regularly menstruating females: a pilot study. *Physiol Genomics*. 2022;54(1):1–10.

139. Vitonis AF, Vincent K, Rahmioglu N, Fassbender A, Buck Louis GM, Hummelshoj L, et al. World Endometriosis Research Foundation Endometriosis Phenome and biobanking harmonization project: II. Clinical and covariate phenotype data collection in endometriosis research. *Fertility and Sterility*. 2014;102(5):1223–32. Disponible sur: <https://linkinghub.elsevier.com/retrieve/pii/S0015028214018858>

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