

Menstrual blood as a diagnostic tool for endometriosis: systematic review

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Importance: To diagnose endometriosis remains challenging because confirmation still largely relies on invasive laparoscopy. Menstrual blood (MB) offers a promising, noninvasive source of diagnostic biomarkers that reflect the local uterine environment.

Objective: This systematic review aimed to critically evaluate the current landscape of molecular, cellular, and biochemical MB-derived potential biomarkers for endometriosis and to appraise their clinical usefulness and translational readiness by distinguishing promising biomarker candidates from exploratory biomarker candidates.

Evidence review: A systematic search of PubMed, Embase, and Web of Science identified 32 eligible studies investigating MB biomarkers in endometriosis. Data extraction followed PRISMA guidelines. Study quality was assessed using the Newcastle-Ottawa scale and a specifically designed “Early-stage biomarker quality assessment scale” (EBQAS) addressing validation strategy, methodological transparency, and reporting quality. Biomarker candidates were classified according to diagnostic accuracy, sample size, methodological quality, and clinical feasibility. Due to heterogeneity, a narrative synthesis was performed.

Findings: Most identified markers remained exploratory because of small sample sizes, limited diagnostic validation, and heterogeneous methodologies. A few markers were deemed promising biomarker candidates based on their performance and detection method. Specifically, these were P450 aromatase (sensitivity 94.6%, specificity 90.9%) measured by immunocytochemistry (ICC), transforming growth factor- β 1 (TGF- β 1) (AUC 97.3%), and insulin-like growth factor binding protein-1 (IGFBP1) (AUC 92%) measured by enzyme-linked immunosorbent assay (ELISA), and progesterone receptor B (PR-B) messenger ribonucleic acid (mRNA) expression (sensitivity 90.5%, specificity 85.7%), aromatase (AUC 97.7%), steroidogenic factor-1 (SF-1) (86.2%), and 17 β -hydroxysteroid dehydrogenase-2 (HSD17B2) (80.7%), all measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR). These markers were identified using clinically translatable analytical methods, including ELISA, RT-qPCR, and ICC.

Conclusion and Relevance: Menstrual blood is a clinically attractive substrate for noninvasive endometriosis diagnostics, but evidence remains limited to a few promising biomarker candidates within a largely exploratory field. Future research should prioritize multicenter validation and development of standardized, clinically feasible assays for routine diagnostics or point-of-care testing. (F S Rev® 2026;7:100116. ©2026 by American Society for Reproductive Medicine.)

Keywords: Menstrual blood, endometriosis, noninvasive diagnosis

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ESSENTIAL POINTS

- Promising menstrual blood (MB) biomarker candidates for endometriosis include P450 aromatase, TGF- β 1, PR-B mRNA expression, PR-B promoter methylation, aromatase/SF-1/HSD17B2 gene expression, and IGFBP1.
- These promising biomarker candidates were identified using clinically translatable methods, including ELISA, RT-qPCR, and immunocytochemistry, highlighting the feasibility of MB-based diagnostics for future routine clinical use.
- A broad range of exploratory biomarker candidates, particularly inflammatory proteins, extracellular vesicle cargo, stem cell-associated markers, and immune-cell signatures, showed differential expression between women with and without endometriosis but currently lack sufficient validation for clinical implementation.
- The current evidence base remains limited by small sample sizes, methodological heterogeneity, inconsistent reporting of diagnostic accuracy, and the absence of independent external validation cohorts, requiring cautious interpretation of most findings.
- Future research should prioritize multicenter validation of the most promising biomarker candidates and focus on simplified, standardized assays suitable for routine laboratory workflows or point-of-care testing to enable earlier noninvasive diagnosis of endometriosis.

Endometriosis is a chronic condition in which endometrial-like tissue grows outside the uterine lining, affecting approximately 10% (190 million) of women of reproductive age (1–4). It is commonly associated with a variety of symptoms, including pelvic pain, dyspareunia, heavy menstrual bleeding, back pain, bloating, nausea, and infertility (5). These symptoms can significantly affect quality of life, particularly sexual and emotional well-being. Individuals with endometriosis often experience reduced sexual satisfaction, strained relationships, and feelings of isolation, alongside an increased risk of depression, anxiety, and significant emotional burden (6).

The economic burden of endometriosis is also substantial. Direct annual medical costs range from \$1,109 per person in Canada to \$12,118 in the United States, whereas indirect costs, primarily related to lost productivity, range from \$3,314 in Austria to \$15,737 in the USA (7).

Diagnosis of endometriosis typically involves a combination of symptom-focused clinical history taking (focused on symptoms such as dysmenorrhea, dyspareunia, hypermenorrhea, and infertility), gynecological examination, and imaging techniques such as transvaginal sonography and, in certain cases, magnetic resonance imaging. However, laparoscopic surgery, a minimally invasive procedure, remains the gold standard for confirming the diagnosis and providing histological confirmation (8). Due to the lack of reliable noninvasive diagnostics, the invasive nature of laparoscopy, and the often vague or dismissed symptomatic presentation, diagnosis of endometriosis is often delayed by 7–10 years (9, 10).

Over the course of their lifetime, women experience approximately 400 menstrual cycles, losing an estimated 50 mL per cycle (11), amounting to approximately 20 L of menstrual blood (MB) in total. Despite its biological richness, MB remains largely underutilized for clinical diagnostics. It contains endometrial cells, immune cells, proteins, and microbial markers, making it a promising noninvasive sample for diagnosing various conditions. These include endometriosis, hormonal imbalances, cervical cancer, diabetes, and sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV) and human papillomavirus (HPV). A key advantage of using MB for diagnostic purposes is its ease

of collection, which can be performed at home without clinical supervision (12). This eliminates the discomfort associated with more invasive diagnostic procedures, such as needle anxiety, risks related to anesthesia, and the rare but serious risk of mortality associated with laparoscopy (13).

To date, only a limited number of original studies have explored the diagnostic potential of MB, and even fewer have focused specifically on endometriosis. Nevertheless, several biomarker candidates for endometriosis across various biological agents such as lipids, nucleic acids, cells or proteins have been identified. The menstrual blood has also shown diagnostic promise for STIs (e.g., hepatitis C virus [HCV], human papillomavirus [HPV], human immunodeficiency virus [HIV], chlamydia), metabolic conditions such as diabetes mellitus, and cervical cancer (12, 14, 15). In parallel, a growing femtech industry is actively exploring MB-based diagnostics, particularly through microbiome and biomarker analysis (16, 17–19).

Despite these promising developments, no systematic review has yet provided an overview of the diagnostic utility of MB specifically for endometriosis. Two narrative reviews have examined the broader applications of MB in diagnostics, including for endometriosis, but neither conducted a systematic assessment of identified biomarkers or study quality (12, 14).

OBJECTIVES

This systematic review aims to critically evaluate whether MB-derived molecular, cellular, and biochemical markers can serve as clinically applicable diagnostic tools for endometriosis. Specifically, the research questions were to systematically identify and synthesize reported markers; assess their diagnostic performance and level of validation; and differentiate between clinically translatable promising biomarker candidates and exploratory biomarker candidates, to determine the current state of evidence and highlight key gaps for future research.

MATERIAL AND METHODS

This systematic review was conducted according to the preferred reporting items for systematic reviews and meta-

analyses (PRISMA) (20). The review was prospectively registered in the PROSPERO international prospective register of systematic reviews (registration number: CRD420251019975).

Search strategy and selection process

A comprehensive literature search was conducted using PubMed (MEDLINE), Embase (Ovid), and Web of Science. Additional studies were identified manually through reference screening. The initial search was performed on December 3, 2024, and was updated on December 11, 2025, and April 18, 2026, to ensure continued relevance. All articles published until April 2026 were included. We included randomized control trials, nonrandomized control trials, and observational studies (e.g. cohort study [CS], case-control-study [CCS]). We excluded narrative reviews, systematic reviews, meta-analyses, and case reports. Although individual investigators have proficiency in additional languages, there was insufficient overlap to allow independent assessment of non-English studies by at least two reviewers. To maintain methodological rigor and avoid potential inaccuracies from machine translation, we limited inclusion to studies that could be evaluated directly in the original language by multiple reviewers. The detailed search process is described in the [Supplemental File 1](#) (available online): Search Strategy and Data Extraction Process. All studies assessed for eligibility and their exclusion criteria are listed in [Supplemental File 2](#): Reports Assessed for Eligibility.

For the PubMed search, the following search term was used: “endometriosis”[Title/Abstract] AND (“menstrual blood”[Title/Abstract] OR “menstrual effluent”[Title/Abstract]). Filters were applied to include all studies published from database inception until 2026, with abstracts available.

For the Embase search, the search term was: (endometriosis AND (menstrual blood OR menstrual effluent) AND (diagnosis OR diagnostics OR screening OR test)). Filters were set to include studies from database inception until 2026 and required the availability of abstracts. For the Web of Science search, the search term (((ALL = (menstrual blood)) OR ALL = (period blood)) OR ALL = (menstrual effluent)) AND ALL = (endometriosis) was used, and all studies published until 2026 were included.

Data extraction

Relevant information was extracted from each study and organized in Microsoft Excel (Version 16.109) using the following columns: search term, database, author, year, title, study design, objectives, results, study location, sample size (cases and controls), inclusion and exclusion criteria, methods for diagnosing endometriosis, methods for confirming the absence of endometriosis, endometriosis staging, sampling methods, sampling day, sample fluids, biochemical methods, identified biomarker candidates, differences in biomarker candidates between cases and controls, and their potential for diagnosing and understanding the pathogenesis of endometriosis. Information was extracted from the original publications, and original study investigators were not

contacted to obtain additional information not reported in the published manuscripts.

Risk of bias assessment

The quality of all included studies was evaluated using the Newcastle-Ottawa scale (NOS) alongside a scoring system specifically designed to address the prevalidation, exploratory stage of most of the MB biomarker research, with limited reporting of full diagnostic performance metrics, which would not be covered by standard biomarker scoring tools: “Early-stage biomarker quality assessment scale” (EBQAS) (see [Supplemental File 3](#): PICO, NOS, and EBQAS). The NOS rates studies across three domains, selection, comparability, and exposure assessment, allowing a maximum of 9 stars. Studies scoring 7–9 stars were classified as high quality, 4–6 stars as high risk of bias, and 0–3 stars as very high risk of bias. To complement this from a more laboratory perspective, the EBQAS awarded up to 15 additional stars based on three domains: validation strategy and cohort size, including the presence of independent validation cohorts and adequacy of sample size for diagnostic studies (5 stars); clarity and completeness of methodological reporting, covering sample collection, storage, analytical methods, and description of diagnostic accuracy assessment (5 stars); and transparency of results, including data presentation, statistical analysis, and reporting of diagnostic accuracy estimates with measures of precision (5 stars). In line with the NOS, we established thresholds for interpretation: studies scoring 11–15 stars were rated as high quality, 6–11 stars as high risk of bias, and 0–5 stars as very high risk of bias. With a total of 24 stars, the overall thresholds for assessing the risk of bias were set as follows: 18–25 stars were classified as high quality, 10–17 as high risk of bias, and 0–9 as very high risk of bias. Two investigators were involved in an independent assessment of the original articles, followed by consensus.

Biomarker assessment

To enable a structured interpretation of the identified targets, we differentiated between promising and exploratory biomarker candidates based on their level of diagnostic performance, validation, methodological robustness, and translational potential. Promising biomarker candidates were defined as targets that demonstrated evidence of high diagnostic performance, such as sensitivity ($\geq 75\%$), specificity ($\geq 75\%$), or area under the curve (AUC) ($\geq 75\%$), and were evaluated in study populations including at least 10 participants with and 10 participants without endometriosis in the overall cohort. In addition, promising biomarker candidates were required to show potential for clinical implementation, including the use of standardized, reproducible, and accessible analytical methods (e.g., enzyme-linked immunosorbent assay [ELISA], quantitative polymerase chain reaction [qPCR]), and feasibility for integration into routine clinical workflows or point-of-care testing. Preference was given to markers that could realistically be translated into noninvasive diagnostic tools. All promising biomarker candidates

needed to have a quality assessment score of ≥ 11 as defined by the NOS and EBQAS.

In contrast, exploratory biomarker candidates were defined as exploratory targets that showed differential expression between women with or without endometriosis but lacked formal evaluation of diagnostic accuracy, or if reported, showed less accurate performance (Sensitivity $<75\%$, Specificity $<75\%$, AUC $<75\%$). This category also included markers derived from small sample sizes (<10 participants per group), studies with high methodological heterogeneity, or those relying on complex, resource-intensive, or nonstandardized bioanalytical techniques (e.g., advanced omics platforms, cell culture-based functional assays, or extracellular vesicle isolation). All studies with a NOS plus EBQAS score of <11 were seen as exploratory biomarker candidates. Although all biomarker candidates identified in this review require further independent validation before clinical application can be considered, exploratory biomarker candidates remain substantially further from clinical implementation than promising biomarker candidates. Nevertheless, they provide important insights into the pathophysiology of endometriosis and represent a valuable source of hypotheses and candidate targets for future research. In addition to validation, these exploratory biomarkers will require further standardization and simplification of detection methods to support potential clinical translation.

RESULTS

Study selection

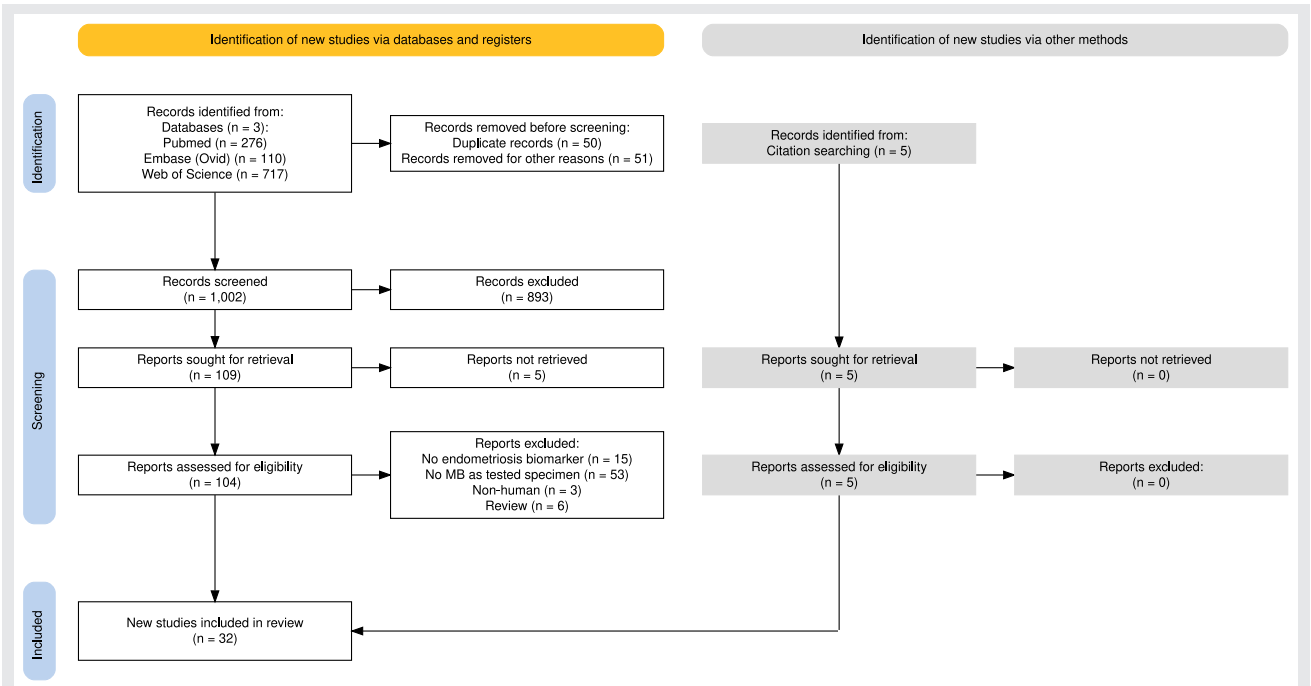
The search on PubMed yielded 276 articles (published between 1988 and 2026), Embase identified 110 articles (published between 1988 and 2026), and Web of Science identified 717 articles (published between 1951 and 2026) (Fig. 1). After removing 50 duplicates, 44 articles without abstracts, and 7 articles written in languages other than English or German, 997 articles were screened for relevance. This process excluded 871 articles. The remaining 107 articles were sought for retrieval and screened for full text. Of these, 53 articles were excluded because they did not analyze the specimen MB, 15 articles did not aim to diagnose endometriosis, 3 articles did not include human participants, 6 reviews could be additionally identified and excluded, and 5 articles were excluded because they were not accessible. This left 27 articles eligible for inclusion.

Additionally, 5 articles were identified through reference screening, resulting in a total of 32 articles included in the systematic review.

Study characteristics

A total of 32 studies met the inclusion criteria (Fig. 1). Most included studies followed a case-control design, comparing MB biomarker candidates between individuals with and without endometriosis. Two studies employed ex-

FIGURE 1



PRISMA flow chart of study selection. Flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies investigating menstrual blood biomarkers for the diagnosis of endometriosis.

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perimental in vitro approaches using MB-derived cells or extracellular vesicles to investigate mechanistic pathways (21, 22). No randomized controlled trials or longitudinal cohort studies were identified. Even though this review is focusing on the diagnostic potential of MB for endometriosis, all studies also contributed to the understanding of the pathological pathways of endometriosis. See [Supplementary Table 1: Study Characteristics](#).

The research was conducted across Australia (2 studies), Brazil (3 studies), Chile (1 study), China (3 studies), Germany (1 study), Indonesia (9 studies), Iran (2 studies), Japan (1 study), Republic of Korea (1 study), The Netherlands (1 study), UK United Kingdom (1 study), Ukraine (1 study), and USA (4 studies), Russia (1 study), and Canada (1 study). Most of the research was published between 2020 and 2026 (23/32 papers). A total of at least 632 cases, which are defined as women diagnosed with endometriosis and 578 controls, defined as women without confirmed or known endometriosis, were recruited. However, de Oliveira et al. (23) and Penariol et al. (24) recruited from the same cohort. Shih et al. (25) and Nayyar et al. (26) included an additional third group of symptomatic participants, defined as experiencing endometriosis typical symptoms (e.g. dysmenorrhea, abdominal bloating, or dyspareunia), but without a diagnosis (25, 26), and Wilson et al. (27) differentiated between an endometriosis case group without hormonal therapy (E1) and an endometriosis case group with hormonal therapy (E2) (27).

Across the included studies, definitions of endometriosis cases and controls varied considerably with respect to inclusion and exclusion criteria, as well as diagnostic verification methods. In most studies, cases were defined as women of reproductive age with confirmed endometriosis by surgery and histopathology, frequently requiring regular menstrual cycles and the absence of hormonal treatment for a defined period (commonly 3 months). Exclusion criteria often included pregnancy, malignancies, inflammatory or infectious diseases, and other gynecological conditions. Controls were typically women without endometriosis, either undergoing surgery for benign gynecological conditions (e.g., tubal ligation, infertility evaluation) or recruited as asymptomatic individuals, with similar restrictions regarding hormonal status and comorbidities.

Most researchers confirmed the diagnosis of endometriosis in the case group through laparoscopic surgery and/or pathology. Two researchers diagnosed endometriosis by visual inspection during surgery (28, 29). Four research groups further included endometriosis patients diagnosed through ultrasound or magnetic resonance imaging (21, 30–32). One study did not specify the diagnostic method used (33). The control group was primarily verified as not having endometriosis through laparoscopy. However, five studies did not provide specific definitions of their control group (27, 32–35). Five research groups recruited participants who self-reported no diagnosis of endometriosis as their control group (25, 26, 36–38). Of those, Miller et al. (37), Nayyar et al. (26), Shih et al. (25), and Warren et al. (38) all recruited participants with endometriosis through the ROSE study and

controls through the Gap registry. Four studies excluded endometriosis in their controls using ultrasound and other gynecological examinations (30, 31, 39, 40). Most studies staged the endometriosis group according to severity using the American Society of Reproductive Medicine (ASRM) classification (21–25, 28–30, 36, 39, 41–51). Van der Linden et al. (52) used the American Fertility Score (AFS), which was renamed to ASRM classification in 1996, to conduct endometriosis staging (53). Miller et al. (37) staged endometriosis using the World Endometriosis Research Foundation (WERF) assessment, but did not specify the types of endometriosis (37, 54). Eleven studies did not specify or conduct a staging procedure (26, 27, 31–35, 38, 40, 46, 55).

Most studies focused on the analysis of MB alone; however, five research groups also compared MB with peripheral blood (PB), Masuda et al. (28), Orlova et al. (49), Shimizu et al. (29), and Van der Linden et al. (52) compared MB with peritoneal fluid (PF) and Ji et al. (55) compared MB with both PF and fallopian tube fluid (FF). Few studies further sampled endometrial tissue during laparoscopy and compared it with MB (22, 29, 31, 32, 52, 55). The MB samples were collected between day 0 and 5, with some studies sampling on one day only and others on several days.

Sixteen studies sampled MB in a home setting using menstrual cups or menstrual sponges (22–27, 36–39, 42, 43, 46, 47, 49, 50). Other studies collected samples in clinical settings, employing methods such as syringe aspiration of the posterior fornix or the cervix (28, 29, 33, 40, 41, 44, 48, 52, 55), Cusco plastic speculums (30), Pipelle endometrial sampling catheters (21), suction catheters (35), Whatman filter paper-equipped pads (31, 32), or unspecified extractions (34, 45).

The biochemical analytical methods used in the publications were grouped into four categories:

1. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) analysis: Gene expression and epigenetic profiling were conducted using single-cell RNA sequencing, reverse transcription quantitative polymerase chain reaction (RT-qPCR), microRNA (miRNA) microarrays, DNA methylation analysis, and DNA/RNA sequencing (21–26, 29, 31–33, 37, 38, 43, 44, 46, 50, 55).
2. Enzyme activity assays: Studies used activity assays for enzymes such as N-acetyl- β -D-glycosaminidase (NAG), myeloperoxidase (MPO), indoleamine 2,3-dioxygenase 1 (IDO1), matrix metalloproteinases (MMP-2 and MMP-9), and employed the alkaline hematin technique to quantify MB components (37, 41, 43, 46).
3. Protein and lipid analysis: Protein-level investigations included ELISA, immunocytochemistry, immunohistochemistry, western blotting, two-dimensional electrophoresis (2-DE), and immunofluorescence. Mass spectrometry (MS)-based techniques such as high-performance liquid chromatography (HPLC)-MS, nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) using data-independent (DIA) and data-dependent acquisition (DDA), MS/MS and Ultra-high-performance

liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) were applied for proteomic and metabolomic profiling (22, 24–30, 34–36, 38–43, 45–50, 52, 55).

4. Cellular functional assays: Studies assessed cell behavior using proliferation, adhesion, migration, and invasion assays, as well as trans-well cultures, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), zymography, decidualization assays, flow cytometry, and in vitro colony-forming unit assays (21, 24, 26–28, 36–39, 43, 46, 50, 55).

Bioinformatic analysis reported in included studies: Selected omics-based studies included in this review performed downstream bioinformatic analyses of their generated datasets, including protein-protein interaction (PPI) network analyses using the STRING database, as well as gene ontology (GO) enrichment and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses to identify functional pathways and molecular interactions relevant to menstrual and reproductive health (24, 34).

Statistical analysis of biomarker performance: The diagnostic performance of the reported biomolecules was assessed using area under the receiver operating characteristic curve (AUC/ROC) analyses alongside sensitivity, specificity, and reported cut-off values (26, 30, 31, 33, 40, 42, 44, 47, 55), although most studies lacked formal diagnostic performance metrics analysis (21–25, 27–29, 32, 34–39, 41–43, 46, 48–50, 52).

Table 1 lists all biomarker candidates for endometriosis diagnosis assessed or discovered in the included studies.

Promising biomarker candidates

Among the 32 included studies, only six studies identified MB-derived markers demonstrating sufficient diagnostic performance, methodological quality, and translatability to be considered promising biomarker candidates for endometriosis. These biomolecules comprised proteins, gene expression markers, and menstrual stem cell-associated markers, although the level of validation, sample size, and translational readiness varied considerably

Protein markers. Promising protein biomarker candidates included transforming growth factor- β 1 (TGF- β 1), P450 aromatase, and insulin-like growth factor binding protein-1 (IGFBP1). The TGF- β 1, quantified by ELISA in MB collected via menstrual cup, showed good diagnostic accuracy in a cohort of 40 women with and 10 women without endometriosis, with an AUC of 97.3%, sensitivity of 90%, specificity of 80%, and a cut-off value of 515.0 ng/mL, while achieving one of the highest methodological quality scores (NOS+EBQAS:17) (47). The P450 aromatase, detected by immunocytochemistry in MB endometrial cells collected via menstrual cup ($n = 37$ with, $n = 33$ without endometriosis), demonstrated 94.6% sensitivity and 90.9% specificity at a cut-off >4 histo score, with a strong overall quality (NOS+EBQAS:14) (42). The IGFBP1, measured by ELISA in supernatants of functional decidualization assays of MB-derived stromal fibroblast cells ($n = 23$ with, $n = 9$ without

endometriosis), yielded an AUC of 92%, sensitivity of 87.5%, specificity of 91.7%, and a cut-off value of 14.21 pg/mL (NOS+EBQAS:13) (26).

Gene expression markers. Promising gene expression biomarker candidates showed the strongest overall diagnostic performance. Amanda et al. (31) identified P450 aromatase, steroidogenic factor-1 (SF-1), and 17 β -hydroxysteroid dehydrogenase-2 (HSD17B2) expression in MB using RT-qPCR on filter paper pad collected samples ($n = 20$ with, $n = 20$ without endometriosis), reporting AUCs of 97.7% (cut-off $>1.63 \cdot 2^{-\Delta\Delta C_t}$), 86.2% (cut-off $>1.71 \cdot 2^{-\Delta\Delta C_t}$), and 80.7% (cut-off $>1.83 \cdot 2^{-\Delta\Delta C_t}$), respectively, in a high-quality study (NOS+EBQAS:19, highest overall). Similarly, progesterone receptor B (PR-B) messenger RNA (mRNA), measured by RT-qPCR in laparoscopically collected MB ($n=21$ with, $n=21$ without endometriosis), showed 90.5% sensitivity and 85.7% specificity at a cut-off $\leq 1.1355 \mu\text{g/dL}$, with robust methodological quality (NOS+EBQAS: 14) (44). The PR-B promoter methylation (AUC 73.5%, sensitivity 77.8%, specificity 80%, cut-off $\leq 37.18 \%$) measured by pyrosequencing in a cohort of 52 cases and 30 controls (NOS+EBQAS:15) (33) showed promise. Although translation of sequencing-based methylation analyses into qPCR is still lacking herein, it is technically feasible.

Overall, the most promising clinically translatable biomarker candidates were *Aromatase*, TGF- β 1, PR-B expression, P450 aromatase, and IGFBP1 because they combined high diagnostic performance with relatively robust sample sizes, reproducible analytical methods (primarily ELISA, qPCR, and immunocytochemistry), feasible MB sampling approaches (predominantly menstrual cups or aspiration), and comparatively high methodological quality scores. However, all promising biomarker candidates still lack external validation.

Exploratory biomarker candidates

In contrast to the above-described promising biomarker candidates with potential for clinical translation, the majority (26 studies) of identified MB-derived markers remain exploratory, largely due to limited sample sizes, the absence of diagnostic validation, methodological heterogeneity, or reliance on complex analytical platforms that currently limit clinical implementation.

Protein markers. Protein biomarker candidates represented the largest exploratory group. Several proteins showed altered expression in women with endometriosis, although diagnostic accuracy was either not assessed or remained insufficient for biomarker classification. Vascular endothelial growth factor (VEGF), measured by immunocytochemistry in MB aspirated during laparoscopy, demonstrated only moderate diagnostic performance (AUC 67.2%, sensitivity 40%, specificity 93.3%, cut-off >6 points) despite a moderate methodological quality score (NOS+ EBQAS:14) (40). In addition, other studies found no significant differences in VEGF-A and sVEGF-R1 expression between women with and without endometriosis (41, 46). Further, MPO and

TABLE 1

Results—studies sorted into the categories promising biomarker candidates and exploratory biomarker candidates.

Biomarker status	Author	Sample size, mean age (y)		Category	Location	↑	Results ↓	—	Diagnostic accuracy as stated in the original studies for the candidates' molecules:
		Cases	Controls						
Promising biomarker candidates	Amanda et al. (31) 10/2024	20, 35.2	20, 33.1	Gene expression marker	MB	Genes: <i>P450 Aromatase, SF-1, HSD17B2</i>			Aromatase: AUC = 97.7% Sensitivity = 95% Specificity = 90% Cutoff value >1.63 SF-1: AUC = 86.2% Sensitivity = 90% Specificity = 80% Cutoff value >1.71 HSD17B2: AUC = 80.7% Sensitivity = 80% Specificity = 75% Cutoff value >1.83 AUC N/A Sensitivity = 90.5% Specificity = 85.7% Cutoff value ≤ 1.1355 µg/dL AUC = 97.3% Sensitivity = 90% Specificity = 80% Cutoff value = 515.0 ng/mL AUC N/A Sensitivity = 94.6% Specificity = 90.9% Cutoff value >4 histo score IGFBP1: AUC = 92% Sensitivity = 87.5% Specificity = 91.7% Cutoff value = 14.21 pg/mL PR-B methylation: AUC = 73.5% Sensitivity = 77.8% Specificity = 80% Cutoff value ≤ 37.18% (DNMT1: AUC = 66% Sensitivity = 74.1% Specificity = 68% Cutoff value ≤ 23.79%) AUC = 67.2% Sensitivity = 40% Specificity = 93.3% Cutoff value >6 points N/A
	Anwar et al. (a) (44) 06/2019	21, 35.6	21, 33.7	Gene expression marker	MB		Genes: <i>PR-B mRNA</i>		
	Effendi et al. (47) 12/2023	40, 31.6	10, 35.1	Protein marker	MB	Proteins: TGF-β1			
	Madjid et al. b) (42) 04/2020	37, 32.9	33, 31.8	Protein marker	Endometrial cells	Proteins: P450 aromatase			
	Nayyar et al. (26) 07/2020	30, 37.4 23 tested for diagnostic accuracy	Ctrl.: 34, 33.8 9 tested for diagnostic accuracy	Stem cell-specific marker	menstrual effluent-derived stromal fibroblast cells (ME-SFCs)	Proteins: PDPN	Proteins: IGFBP1 Genes: <i>ALDH1A1 mRNA</i>		
Exploratory biomarker candidates	Setiawan et al. (33) 06/2023	52, 34.3	30, 34.3	Gene expression marker	MB	Genes: <i>VEGF, DNMT1, PR-B gene promoter methylation</i>	Genes: <i>PR-B mRNA</i>		
	Anwar et al. (b) (40) 08/2020	30, 34.4	30, 31.1	Protein marker	MB	Proteins: VEGF			
	da Silva et al. (41) 09/2014	10, NA	7, N/A	Protein marker	MB			Proteins: NAG, MPO, TNF-α, VEGF	N/A
	De Oliveira et al. (23) 03/2022	10, 36.0	10, 35.0	Gene expression marker	MenSCs	Genes: <i>miR-200b-3p</i>		Genes: <i>miR-143-3p, miR-21-5p, miR-100-5p</i>	N/A
	Febriyeni et al. (32) 04/2024	18, 35.2	17, 33.0	Gene expression marker	MB	Genes: <i>CXCL16 mRNA</i>	Genes: <i>CXCL16 DNA methylation</i>		N/A
	Gurung et al. (36) 02/2025	8, 32.1	9, 30.2	Protein markers	Small extracellular vesicles (sEVs)	Proteins: A2M, SELL, FCN3, IgA, IgK, IgG, IgM, CD86	Proteins: DSC2, IL1RN, SDCBP2, SLC1A1, SLC15A1, LGALS7, IL18, IL1R1, S100P, LPB, PHB2, HRG		N/A
	Hwang et al. (35) 03/2013	9, N/A	9, N/A	Protein marker, gene expression marker	Eutopic endometrial cells	Genes: <i>Oct-04, CXCR4, SOX2, MET</i>	Proteins: CRMP, UCH-L1, MYL9		N/A
	Ji et al. (a) (34) 10/2023	8, 35.4 16, 33.3	8, 34.0 16, 31.7	Protein marker	MB	Proteins: CXCL5, IL-1RN		Proteins: CXCL3, CXCL1, CCL18	N/A
	Ji et al. (b) (55) 06/2024	5, 34.1	7, 36.2	Gene expression marker	EVs	Genes: <i>miR-4443, miR-8069</i>			<i>miR-4443</i> AUC = 74.1% <i>miR-4443</i> + dysmenorrhea AUC = 92.9% N/A
	Madjid et al. (a) (48) 10/2019	63, NA	86, NA	Protein marker	Endometrial cells	Proteins: Casp-3, Casp-3/Casp-9, MMP-9			N/A
	Madjid et al. (c) (45) 06/2020	30, 34.8	38, 31.6	Protein marker	Endometrial cells	Proteins: MMP-9	Proteins: TIMP-1		N/A

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TABLE 1

Continued.									
Biomarker status	Author	Sample size, mean age (y)		Category	Location	↑	Results		Diagnostic accuracy as stated in the original studies for the candidates' molecules:
		Cases	Controls				↓	—	
	Malik et al. (46) 01/2006	16, 34.9	16, 37.4	Protein marker, gene expression marker, menstrual blood loss	MB			Other: MBL Proteins: VEGF-A, sVEGF-R1, MMP9, MMP2 Genes: VEGF-A mRNA CFU, SUSD2 + eMSC, N-cadherin + eEPC	N/A
	Masuda et al. (28) 07/2021	32, 35.2	29, 33.7	Stem cell-specific marker	MenSCs				N/A
	Miller et al. (37) 08/2022	14, N/A	19, N/A	Protein marker, gene expression marker, cell type	MB	Genes: LCN2	Cells: T _H 17 cells, macrophages Proteins: TGF- α Genes: IL10, IL23A, IL6, CD74, CD83, CXCL16, CCL3	Cells: T _H -cells, Proteins: IL-1 α , IL-1 β , IL-4, IL-8, IL-17A, IL-18, IFN- α , IFN- γ , IP-10, EGF, TNF- α	N/A
	Nikoo et al. (43) 09/2014	6, 30.0	6, 29.0	Stem cell-specific marker	Menstrual blood stromal stem cells in women with endometriosis (E-MenSCs)	Proteins: IFN- γ , IL-10, MCP-1, CD9, CD10, CD29, IDO1, COX-2 Genes: IDO1, COX-2 Enzyme activity: IDO1 Proteins: CSF-1	Proteins: FOXP3 Genes: FOXP3	Proteins: CD105, CD44, Oct-04, CD73	N/A
	Orlova et al. (49) 08/2021	43, 31.0*	19, 30.8*	Protein marker	MB				N/A
	Penariol et al. (24) 09/2022	10, 36.0	10, 35.0	Stem cell-specific marker	Menstrual mesenchymal stem cells (MenSCs)	Genes: ATF3, ID1, ID3, FOSB, SNAI1, NR4A1, EGR1, LAMC3, ZFP36 Proteins: COL1A1, COL6A2, NID2	Proteins: MT2A, TYMP		N/A
	Peragallo-Papic et al. (50) 03/2026	10, 35.7	11, 34.0	Protein marker, gene expression marker	sEVs	1085 genes: IL18, CXCL8, TGF β I, FGF2, VEGFA, CCN1 (IGFBP10)	Proteins: CD63, CD9, CD81, Syntenin-1 1029 genes: MME (CD10), LIF, PGR Proteins: CD9, CD29 Genes: Oct-04, NANOG, BAX, BAX: BCL-2 ratio		N/A
	Sahraei et al. (17) 01/2022	3, N/A	3, N/A	Stem cell-specific marker	Menstrual blood-derived mesenchymal stem cells (MenSCs)	Proteins: CD10 Genes: SOX-2, BCL-2, MMP-2, MMP-9, IL-18, IL-6, IL-8, NF- κ B, COX-2, Cyclin D, VEGF, ER- α , β -catenin	Cells: CD8+ T cells, CD14/CD16 monocyte subset, Tregs	Cells: CD4/CD8 T cells, CD8+ T-cells-Perforin	Genes: SALL-4, HIF, TNF- α
	Schmitz et al. (39) 11/2021	12, 33.7	11, 32.5	Cell type, protein marker	MB			Cells: T-cells, monocytes, B-cells, CD69 on T cells, NK cell subset, NKp46+ in NK cells, Perforin + on NK cells, CD69+ on NK cells Proteins: ICAM-1, VCAM-1, IL-6, IL-8, TNF- α Cells: Stromal cells Genes: HSPA6+, SRGN+	N/A
	Shih et al. (25) 09/2022	11, 35.2	Ctrl.: 9, 33.4 Sympt.: 13, 32.3	Cell type, stem cell-specific marker, gene expression	Endometrial stromal cells	Cells: B-cells Genes: IL11+, MGP+	Cells: uNK-cells Genes: IFGBP1		N/A
	Shimizu et al. (29) 11/2017	13, 34.4	14, 32.0	Protein marker	MB			Protein: HMGB1	N/A
	Starodubtseva et al. (30) 07/2024	23, 28.0	16, 27.0	Lipid marker	MB	Lipids: Ceramides, cardiolipids, oxidized lipids, phosphatidylcholine 18:0_18:0, (plasmeyl-) phosphatidylethanolamines, plasmeyl-phosphatidylcholines, sphingomyelins, triacylglycerols, PE P-16:Q/18:1	(Plasmeyl-) phosphatidylcholines, SM, CerP	Plasmeylphosphatidylethanolamine PE P-16:Q/18:1 and cardiolipin CL 16:0_18:0_22:5_22:6 : AUC = 87%	
	Van der Linden et al. (52) 01/1994	8, N/A	8, N/A	Protein marker	MB			Proteins: IOG11 (α 281), J143 (α 3B1), HP2/1 (α 481), SAM1 (α 5B1), GOH3 (α 6B1), L-CAM (E-cadherin)	N/A
	Wang et al. (22) 05/2024	13, 37.2* mean age of all 20 cases	6, 39.4* Mean age of all 10 controls	Protein marker, gene expression marker	Menstrual blood endometrial cells (MESCs)	Proteins: OPN Genes: OPN mRNA			N/A
	Warren et al. (38) 03/2018	7, N/A	7, N/A	Cell type, protein marker, gene expression marker	Stromal fibroblast cells (SFCs)	Proteins: CD105	Cells: uNK-cells Genes: ALDH1A1	Proteins: IGFBP-1 Cells: Granulocytes, monocytes, T-cells, B-cells, SFCs, epithelial cells, endothelial cells Proteins: CD90, CD73 Proteins: CD62L Cells: B-cells	N/A
	Wilson et al. (27) 08/2025	E1:11, 33.8 E2: 3, 35.0	19, 33.5	Protein marker, Cell type	White blood cells (WBC), cDNA, acellular portion	Cells: T-cells, subpopulations of aged neutrophils, antiinflammatory macrophages Proteins: CD4, CD8	Cells: proinflammatory macrophages, cytotoxic T-cells Proteins: CD16, CXCR4, CD11b, CD11c, CX3CR1, MHC-II		N/A

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NAG showed significantly higher concentrations in MB compared with PB in women with endometriosis, although no significant group differences were observed for TNF- α , MPO, or NAG when comparing MB samples directly between women with and without endometriosis (41). Matrix metalloproteinases-9 was reported as elevated in endometriosis in two studies (45, 48), while its inhibitor TIMP-1 was decreased, although these findings were not replicated by Malik et al. (46), who found no significant differences in MMP-9 and MMP-2 between women with and without endometriosis. Caspase-3 and the Caspase-3 / Caspase-9 ratio also appeared elevated in endometriosis (48).

Ji et al. (34) identified 95 differentially expressed proteins (DEPs) and performed a PPI network analysis, revealing 48 nodes and 71 edges. Among the top 10 hub genes, CXCL1, CXCL3, CXCL5, CCL18, and interleukin 1 receptor antagonist (IL1RN) were included in the cluster. The ELISA validation confirmed significantly elevated expression of CXCL5 and IL1RN in endometriosis (34).

Hwang et al. (35) also investigated potential biomarker candidates for the diagnosis of endometriosis and detected three DEPs: CRMP2, UCH-L1, and MYL9. Among these, CRMP2 emerged as the most promising, showing significantly lower expression in women with endometriosis (35). These findings were supported by Gurung et al. (36), who identified 4,179 proteins downregulated and 22 proteins upregulated in MB-derived extracellular vesicles (EVs) in endometriosis. Among the downregulated proteins, they highlighted DSC2, IL1RN, SDCBP2, SLC1A1, SLC15A1, LGALS7, IL18, IL1R1 and S100P, among the upregulated A2M, SELL, and FCN3 immunoglobulins, such as Ig λ , IgK, Ig δ , IgG, IgM, and CD86 (36). Peragallo-Papic et al. (50) also investigated EVs and identified decreased CD63, CD9, CD81, and Syntenin-1 in MB-derived EVs.

No significant differences regarding HMGB1 expression between endometriosis cases and controls in MB were observed. However, levels of HMGB1 in MB compared with PB and PF were significantly higher (29). Furthermore, Wang et al. (22) investigated the role of osteopontin (OPN) in the pathogenesis of endometriosis and identified upregulated OPN expression in MB from endometriosis patients. Miller et al. (37) identified decreased TGF- α expression, and Orlova et al. (49) increased colony-stimulating factor (CSF-1).

Lipid markers. Only one study has examined the lipidome of MB in endometriosis. They used reversed-phase HPLC and MS on speculum-collected MB from women with ($n = 23$) and without ($n = 16$) endometriosis, and found 40 lipids significantly differently expressed (30). The endometriosis group showed increased ceramides, cardiolipins, oxidized lipids, PC 16:0_18:0, (plasmenyl)-phosphatidylethanolamines, plasmanylphosphatidylcholines, sphingomyelins, and triacylglycerols. Further, the researchers found decreased (plasmenyl)-phosphatidylcholines, sphingomyelin (SM) d18:0/18:0, and CerP d18:0/22:0 in MB from the endometriosis group (30). They furthermore identified plasmenyl-phosphatidylethanolamine (PE P)-16:0/18:1 and cardiolipin (CL) 16:0_18:0_22:5_22:6 as discriminatory lipid species,

with a combined AUC of 87%. Although sensitivity, specificity, and cut-off values were not reported, the study demonstrated good methodological quality (NOS+EBQAS:14) and introduced lipidomic profiling as a novel exploratory avenue for biomarker discovery. However, the requirement for specialized MS-based platforms and the current lack of simplified targeted assays may limit immediate clinical translatability.

Gene expression markers. Several studies examined gene expression markers in MB. Hwang et al. (35) identified increased mRNA expression levels of *Oct-4*, *CXCR4*, *SOX-2*, and *MET* in women with endometriosis. Ji et al. (55) identified 202 miRNAs upregulated and 221 miRNAs downregulated in EVs derived from MB of women with endometriosis. Among these, *miR-4443* and *miR-8069* were significantly elevated and tested for diagnostic accuracy with an AUC of 74.1% and an AUC of even 92.9% if in combination with dysmenorrhea. However, despite these promising findings, they were classified as exploratory rather than promising biomarker candidates due to the very small sample size ($n = 5$ with, $n = 7$ without endometriosis) (55). Additionally, *miR-200b-3p* was reported as increased in the endometriosis group (23). Epigenetic-related markers also demonstrated potential diagnostic relevance, with *DNMT1* expression measured by RT-qPCR showing moderate diagnostic performance (AUC 66%, sensitivity 74.1%, specificity 68%, cut-off $\leq 23.79\%$). However, the marker did not fulfil the predefined criteria for classification as a promising biomarker candidate due to its limited diagnostic accuracy (33). Additional angiogenesis- and inflammation-related genes emerged. In line with the protein measurements, *VEGF-A* expression showed inconsistent results, with two studies reporting increased levels in MB from women with endometriosis, whereas another found no differences between cases and controls (33, 46, 50). Additional dysregulated gene expression markers included *CXCL16* mRNA, *LCN2*, *IL10*, *IL23A*, *IL6*, *CD74*, *CD83*, *CXCL16*, *CCL3*, *IL1B*, *CXCL8*, *TGFBI*, *FGF2*, *CCN1* (*IGFBP10*), *MME* (*CD10*), *LIF*, and *PGR* (32, 37, 50). Lastly, *OPN* mRNA was elevated in women with endometriosis (22).

Cell types and EVs. Several studies assessed immune cell populations, with mixed findings. No significant differences were observed in major lymphatic (B cells and T cells) and myeloid (monocytes and granulocytes) cell types, as well as in epithelial, endothelial, and stromal fibroblast cells in MB across groups in two studies (27, 38, 39). However, one study reported a relative enrichment of B cells in the endometriosis group (25). Wilson et al. (27) analyzed white blood cells in MB and identified distinct morphological differences between women with and without endometriosis.

Findings on natural killer (NK) cells also varied. Although two studies reported a decrease in uterine NK (uNK) cells among endometriosis patients, Schmitz et al. (39) found no significant differences in the NK cell population between cases and controls (25, 38).

Further cellular subtyping revealed more nuanced changes. Regulatory T cells (Tregs) and CD8+ T cells were found to be elevated, whereas perforin-positive CD8+ T cells

and the ratio of CD4/CD8 T cells were reduced in MB from women with endometriosis. In contrast, T_H17 cells were reported as downregulated in the case group by Miller et al. (37). Additionally, CD14^{high}/CD16^{low} myeloid cells were nonsignificantly enriched in endometriosis patients (39). In contrast, Wilson et al. (27) reported increased T-cells and subpopulations of aged neutrophils, antiinflammatory macrophages and decreased proinflammatory macrophages and cytotoxic T-cells. They further, identified additional dysregulated proteins such as CD4, CD8, CD16, CXCR4, CD11b, CD11c, CXCR1, and MHC-II.

In summary, although broad immune cell populations showed limited discriminatory power, specific changes in T cell subsets and uNK cells may reflect underlying immunopathology in endometriosis but need to be further tested for diagnostic potential.

Stem cell-specific markers. The MB-derived stem cells (MenSCs) are increasingly recognized for their role in the pathogenesis of endometriosis and their potential as diagnostic and therapeutic targets. Several studies have examined the molecular, morphological, and functional characteristics of MenSCs in women with and without endometriosis, identifying specific differences that hold potential for diagnostic and therapeutic applications. Table 2 shows a detailed list of MB-derived stem cell markers for diagnosing endometriosis.

Endometrial stromal fibroblast cells. Gene expression analyses revealed elevated levels of *IDO1*, *COX-2*, *IL11+*, and *MGP+* and reduced expression of *IGFBP-1*, *ALDH1A1*, and *FOXP-3*. In addition to reduced IGFBP1 production, impaired decidualization is indicated (25, 26, 38, 43).

Last, protein expression analysis found coherent results with *IDO1*, *COX-2*, and *PDPN* as elevated and *FOXP3* and *IGFBP1* decreased (26, 43).

Endometrial mesenchymal stem cells. Studies focusing on endometrial stromal fibroblast cells (eSFCs) derived from MB have demonstrated altered cellular behavior and surface marker expression in patients with endometriosis. The MenSCs isolated from these individuals showed increased proliferation, invasion, and migration abilities, along with a higher shape index. Additionally, inflammatory cytokine concentration with higher levels of IFN- γ , IL-10, and MCP-1 was detected in these cells (26, 43). Experimental induction of inflammation with TNF and IL-1 β in control cells resulted in a phenotype closely resembling that of endometriosis cells (26). The enzymatic activity of *IDO1* was increased in MenSCs from women with endometriosis (43).

Alterations in surface markers were reported, with upregulation of CD9, CD10, and CD29, whereas CD90 and CD73 expression on E-MenSCs remained unchanged. The CD105 expression was also reported as elevated by Warren et al. (38), while being found unchanged by Nikoo et al. (43).

Research on endometrial mesenchymal stem cells (eMSCs) derived from MB further supports the presence of endometriosis-specific cellular signatures. Morphological differences were reported, along with distinct patterns in surface marker expression. The CD10 expression was found to

be elevated, whereas CD9 and CD29 expression were reduced in eMSCs from the case group (21).

Gene expression profiling highlighted upregulation of markers such as *ATF3*, *ID1*, *ID3*, *FOSB*, *SNA1L*, *NR4A1*, *EGR1*, *LAMC3*, *ZFP36*, *SOX-2*, *BCL-2*, *MMP-2*, and *MMP-9* in endometriosis. Additionally, gene expression of inflammatory and mitotic regulators such as *IL-6*, *IL-8*, *IL-1 β* , *NF- κ B*, *COX-2*, *Cyclin D*, *VEGF*, *ER- α* , and *β -catenin* were elevated in eMSCs from women with endometriosis, suggesting heightened inflammatory and angiogenic activity (21). Conversely, stemness and apoptotic genetic markers, including *Oct-4*, *NANOG*, and *BAX*, were downregulated in eMSCs (21, 24).

Proteomic analysis further revealed increased levels of COL1A1, COL6A2, and NID2, and reduced levels of MT2A and TYMP. Notably, multiple signaling pathways were differentially regulated, including upregulation of EMT and HIF1A targets, and downregulation of the PI3K/AKT/mTORC1 axis (24).

In contrast, no significant group differences were found in clonogenicity (colony-forming units [CFUs]) or sushi domain containing 2 (SUSD2+) cell concentrations in MB, although CFU levels were correlated with SUSD2+ eMSC and N-cadherin+ eEPC concentrations (28). These findings emphasize both the diagnostic complexity and the cellular heterogeneity of MenSCs in endometriosis.

Endometrial epithelial stem/progenitor cells. Only one study investigated epithelial progenitor cells (eEPCs) in MB, specifically targeting N-cadherin+ cells. Although these cells could be identified in the sample population, no significant differences were observed between women with and without endometriosis (28).

In conclusion, MenSCs, particularly eSFCs and eMSCs, demonstrate disease-specific molecular and functional characteristics that may serve as promising diagnostic targets. However, differences in epithelial progenitor cells remain inconclusive and require further investigation.

Menstrual blood loss. Malik et al. (46) were the only research team to investigate differences in menstrual blood loss (MBL) between women with and without endometriosis. Using a modified alkaline hematin method as described by Gannon et al. (56), they found no significant differences between the groups, with median MBL values of 28.4 mL in the endometriosis group and 32.1 mL in the control group (46).

Risk of bias in studies

The methodological quality of the 32 studies included was first evaluated using the NOS. All studies scored between 4 and 9 stars, with a mean score of 6.8. Based on NOS thresholds, 19 studies were considered high quality (≥ 7 stars), whereas 13 studies were classified as having a high risk of bias (4–6 stars). None of the studies fell into the very high risk of bias category (≤ 3 stars). The mean of the studies that identified promising biomarker candidates was 7, whereas the mean of all studies analyzing exploratory biomarker candidates was 7.1. See Table 3.

TABLE 2

Menstrual blood-derived stem cells for diagnosing endometriosis.

Stem cells Author		Endometrial stromal fibroblast cells (eSFCs)			Endometrial mesenchymal cells (eMSCs)			Endometrial epithelial progenitor (eEPCs)
		↑	↓	↔	↑	↓	↔	↔
Penariol et al. (24) 09/2022	Gene expression Protein expression Signaling pathways				<i>ATF3, ID1, ID3, FOSB, SNAI1, NR4A1, EGR1, LAMC3, ZFP36 COL1A1, COL6A2, NID2 EMT, HIF1A, TNF-α, TGF-β, IL6, -STAT3, mTORC1</i>	MTA2, TYMP PI3K		
Warren et al. (38) 03/2018	Gene expression CD marker		<i>IGFBP-1, ALDH1A1</i> CD105	CD90, CD73			Clonogenicity, SUSD2+	N-cadherin+
Masuda et al. (28) 07/2021	-							
Nayyar et al. (26) 07/2020	Characterization Gene expression Protein expression Gene expression	Cell migration, cell adhesion PDPN	<i>ALDH1A1</i> mRNA IGFBP1					
Sahraei et al. (17) 01/2022	CD marker Characterization				<i>SOX-2, BCL-2, IL-β, IL-8, NF- κB, COX-2, Cyclin D, VEGF, ER-α, β-Catenin</i> CD10	NANOG, BAX CD9, CD29	<i>SALL-4, TNF-α, HIF</i>	
Nikoo et al. (43) 09/2014	Characterization Gene expression Protein expression Protein activity CD marker Cytokines	Shape index, proliferation, invasion <i>IDO1, COX-2</i> <i>IDO1, COX-2</i> IDO1 CD9, CD10, CD29 IFN-γ, IL-10, MCP-1	<i>FOXP3</i> <i>FOXP3</i>					
Shih et al. (25) 09/2022	Gene expression	<i>IL11+</i> , <i>MGP+</i>	<i>IGFBP-1</i>					

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TABLE 3

Risk of bias assessment: Newcastle-Ottawa scale (NOS) and early-stage biomarker assessment scale (EBQAS).

Biomarker status	Author	Newcastle-Ottawa scale (NOS)								Total NOS	Early-stage biomarker assessment scale (EBQAS)			Total EBQAS	Total
		S1	S2	S3	S4	C1	E1	E2	E3		1	2	3		
Promising biomarker candidate	Amanda et al. (31) 10/2024	*	*	*	*	**	*	*	—	8	*	*****	*****	11	19
	Anwar et al. (a) (44) 06/2019	*	—	*	*	**	*	*	—	7	*	***	***	7	14
	Effendi et al. (47) 12/2023	*	*	*	*	**	*	*	—	8	*	***	*****	9	17
	Madjid et al. b) (42) 04/2020	*	—	*	*	**	*	*	—	7	—	**	**	4	11
	Nayyar et al. (26) 07/2020	*	—	*	—	*	*	*	—	5	—	****	****	8	13
	Setiawan et al. (33) 06/2023	—	*	*	—	**	*	*	—	7	*	***	****	8	15
	Anwar et al. (b) (40) 08/2020	*	*	*	*	**	*	*	—	8	*	**	****	6	14
Exploratory biomarker candidate	da Silva et al. (41) 09/2014	*	*	*	*	**	*	*	—	8	—	****	****	8	16
	De Oliveira et al. (23) 03/2022	*	—	*	*	**	*	*	—	7	—	****	***	7	14
	Febriyeni et al. (32) 04/2024	*	—	*	—	**	*	*	—	6	—	****	****	8	14
	Gurung et al. (36) 02/2025	*	*	*	—	**	*	*	—	7	—	****	****	8	15
	Hwang et al. (35) 03/2013	*	—	—	*	—	*	*	—	4	**	****	**	8	12
	Ji et al. (a) (34) 10/2023	*	—	—	—	**	*	*	*	6	**	****	****	8	14
	Ji et al. (b) (55) 06/2024	*	*	*	*	**	*	*	—	9	—	****	****	8	17
	Madjid et al. (a) (48) 10/2019	*	—	—	*	—	*	*	—	4	—	—	—	0	4
	Madjid et al. (c) (45) 06/2020	*	—	*	*	**	*	*	—	7	*	***	***	7	14
	Malik et al. (46) 01/2006	*	—	*	*	**	*	*	—	7	—	****	****	8	15
	Masuda et al. (28) 07/2021	*	*	*	*	**	*	*	—	8	*	****	***	8	16
	Miller et al. (37) 08/2022	—	—	*	—	**	*	*	—	5	—	****	****	8	13
	Nikoo et al. (43) 09/2014	*	—	*	*	*	*	*	—	6	*	***	**	6	12
	Orlova et al. (49) 08/2021	*	—	*	*	*	*	*	—	6	—	***	***	6	12

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TABLE 3

Continued.															
Biomarker status	Author	Newcastle-Ottawa scale (NOS)								Total NOS	Early-stage biomarker assessment scale (EBQAS)			Total EBQAS	Total
		S1	S2	S3	S4	C1	E1	E2	E3		1	2	3		
	Penariol et al. (24) 09/2022	*	*	*	*	**	*	*	—	8	—	****	**	6	14
	Peragallo-Papic et al. (50) 03/2026	*	—	*	*	**	*	*	—	7	—	****	****	8	15
	Sahraei et al. (17) 01/2022	*	*	*	*	—	*	*	—	6	—	***	***	6	12
	Schmitz et al. (39) 11/2021	*	*	*	—	**	*	*	—	7	—	****	****	8	15
	Shih et al. (25) 09/2022	*	*	—	—	**	*	*	—	6	—	***	***	6	12
	Shimizu et al. (29) 11/2017	*	—	*	*	**	*	*	—	7	—	****	***	7	14
	Starodubtseva et al. (30) 07/2024	*	—	—	*	**	*	*	—	6	*	****	****	8	14
	Van der Linden et al. (52) 01/1994	*	—	*	—	—	*	*	—	4	—	****	**	6	10
	Wang et al. (22) 05/2024	*	—	*	*	**	*	*	—	7	—	***	****	7	14
	Warren et al. (38) 03/2018	*	—	*	*	**	*	*	—	7	*	***	****	8	15
	Wilson et al. (27) 08/2025	*	—	*	—	**	*	*	—	6	—	****	***	7	13
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To complement this, we applied an EBQAS framework tailored to the mainly prevalidation and exploratory biomarker research in MB, focused on the technical aspects and reporting of the studies included. The EBQAS scores ranged from 0 to 11, with a mean of 7.3. A total of 17 studies reached high quality (≥ 8 stars), 14 had a high risk of bias (4–7 stars), and one showed a very high risk of bias (≤ 3 stars). The mean of the studies with promising biomarker candidates was 7.8, and the mean of the exploratory biomarker candidate studies was 7.5. See [Table 3](#).

When combining NOS and EBQAS scores, the overall quality ranged from 4 to 19 stars, reflecting substantial variation in methodological clarity, validation practices, and transparency of reporting across studies. All studies in the category of promising biomarker candidates had a score between 13 and 19.

DISCUSSION

Diagnosing endometriosis remains challenging, and MB represents a promising noninvasive alternative for biomarker discovery. However, current evidence is limited because most studies in this emerging field have employed diverse exploratory methodologies with small sample sizes and heterogeneous designs. Consequently, few biomarker candidates have been tested for diagnostic accuracy, and none have yet been validated in independent cohorts or by different research groups, precluding a formal meta-analysis or systematic quantitative synthesis.

This systematic review summarizes current research on the use of MB as a noninvasive diagnostic tool for endometriosis. Across 32 studies, a wide range of biomarker candidates spanning proteins, lipids, gene expression markers, and cell types were identified. We classified the studied biomolecules as promising or exploratory biomarker candidates based on diagnostic performance, methodological quality, reproducibility, and translational feasibility, recognizing that clinical applicability depends not only on accuracy but also on assay feasibility and scalability.

The most convincing biomarker candidates identified in this review were linked to dysregulated steroidogenesis, a central mechanism in endometriosis pathophysiology. Both protein ([42](#)) and gene expression-based ([31](#)) studies consistently demonstrated increased aromatase expression in MB-derived samples from women with endometriosis. The P450 aromatase detected in MB-derived endometrial cells by immunocytochemistry showed excellent diagnostic performance, with a sensitivity of 94.6% and specificity of 90.9% in a cohort of 37 women with and 33 women without endometriosis ([42](#)). Similarly, RT-qPCR-based analyses of *aromatase*, *SF-1*, and *HSD17B2* expression demonstrated some of the highest AUC values reported across all included studies, with *aromatase* reaching an AUC of 97.7%, followed by *SF-1* (AUC 86.2%) and *HSD17B2* (AUC 80.7%) in a cohort of 20 women with and 20 women without endometriosis ([31](#)). Importantly, the biological interconnection within steroidogenic pathways relevant to endometriosis mechanistically anchors these potential biomarkers. The *SF-1* promotes aromatase expression and local estrogen biosynthesis, whereas

reduced *HSD17B2* expression impairs estradiol inactivation, collectively contributing to the hyperestrogenic microenvironment characteristic of endometriosis ([57](#)).

From a translational perspective, these findings are particularly relevant because they were obtained using relatively accessible analytical methods, either immunocytochemistry of MB ([42](#)) or qPCR using patient-friendly and scalable pad-based MB collection ([31](#)). Immunocytochemical detection is already well established in pathology laboratories globally, facilitating broader clinical implementation, including in resource-variable healthcare settings. Although histological scoring systems may introduce some degree of observer subjectivity, ongoing advances in automated digital pathology and image analysis may improve reproducibility and standardization. Likewise, qPCR-based approaches may be adaptable to emerging cartridge-based and near point-of-care molecular diagnostic platforms, potentially supporting more accessible noninvasive testing strategies in the future. Furthermore, these studies generally demonstrated comparatively strong methodological reporting and quality assessment scores. Together, these characteristics make steroidogenic pathway markers among the most clinically promising biomarker candidates identified in this review, although external validation in larger multicenter cohorts remains essential.

Altered progesterone signaling represented another recurrent biological theme among biomarker candidates. Reduced *PR-B* expression and altered *PR-B promoter* methylation, together with changes in *DNMT1* expression ([33](#), [44](#)), support the concept of progesterone resistance in endometriosis. The *PR-B* mRNA demonstrated strong diagnostic performance using RT-qPCR-based approaches with a sensitivity of 90.5% and specificity of 85.7% in a cohort of 21 women with and 21 women without endometriosis ([44](#)). Supporting these findings, a larger study including 52 women with and 30 women without endometriosis (for whom the diagnostic criteria for case and control classification were not clearly reported) ([33](#)) also demonstrated significant downregulation of *PR-B* expression, although diagnostic accuracy measures were not reported. In the same study, *PR-B promoter* methylation analyses showed more moderate accuracy (AUC of 73.5%) ([33](#)) but relied on sequencing-based methodologies that may currently be less readily translatable into routine clinical diagnostics. Nevertheless, the consistent identification of *PR-B*-related alterations across two studies strengthens the biological plausibility of progesterone signaling pathways, which have long been implicated in endometriosis pathophysiology ([58](#)), as a source of noninvasive promising biomarker candidates.

Among protein-based promising biomarker candidates, TGF- β 1 ([47](#)) and IGFBP1 ([26](#)) demonstrated favorable diagnostic performance. The TGF- β 1 showed excellent discrimination, with an AUC of 97.3%, sensitivity of 90%, and specificity of 80% in a study including 40 women with and 10 women without endometriosis ([47](#)). It is particularly attractive for clinical translation because it can be measured directly from MB using inexpensive and widely available ELISA-based assays compatible with standard laboratory

workflows. The relative simplicity, scalability, and low cost of ELISA-based detection may also support broader implementation across diverse healthcare settings. In the future, simplified immunoassay formats such as quantitative lateral flow-based platforms may further enhance accessibility and support near point-of-care applications. However, the relatively small control group and lack of external validation warrant cautious interpretation. Although IGFBP1 demonstrated strong diagnostic accuracy, with an AUC of 92%, sensitivity of 87.5%, and specificity of 91.7% in 30 women with and 34 women without endometriosis (26), its assessment required decidualization assays and stromal cell culture of MB-derived cells, making large-scale routine implementation more challenging despite potential feasibility in specialized diagnostic laboratories.

In addition to these promising candidates, numerous exploratory biomarker candidates reflecting inflammatory, angiogenic, immune, stem cell-associated, and metabolic alterations were identified. The biomolecules studied included gene expression markers (21–25, 32, 34,35, 37, 38, 43, 46), matrix remodeling proteins (21, 22, 24, 27, 29, 34,35, 37–41, 43, 45, 46, 48–50, 52), immune cell populations (25, 27, 28, 37, 38), EV-associated miRNAs (36, 50, 55), and lipidomic signatures (30).

Angiogenic imbalance is a hallmark of endometriosis (59), and VEGF was one of the earliest and most widely studied MB biomarker candidates. Although several studies reported elevated VEGF protein and mRNA expression in women with endometriosis (21, 33, 40), findings remained inconsistent across studies (41, 46). Although VEGF can be measured using relatively accessible methods such as ELISA (41, 46), immunocytochemistry (40), and RT-qPCR (21, 33), inconsistent replication currently limits its diagnostic utility and highlights the need for harmonized validation strategies. Similarly, altered expression of stemness-associated genes (21, 24, 25, 28, 43), inflammatory mediators (41, 44–46, 49), and EV-associated miRNAs (36, 50, 55) suggests dysregulated angiogenic, inflammatory, and regenerative pathways in endometriosis and collectively, these exploratory biomarkers reflect multiple pathophysiological domains that may ultimately contribute to the understanding of endometriosis or even future biomarker candidates. However, most studies were limited by small sample sizes, lack of diagnostic validation, inconsistent replication, and highly heterogeneous methodologies.

Despite promising findings, this systematic review identified substantial methodological inconsistencies across studies that challenge the comparability and generalizability of results. Most studies included small cohorts, ranging from just 3 (21) to a maximum of 63 (48) participants in the endometriosis group. Further, heterogeneous sampling protocols and variable menstrual cycle timing may influence biomarker expression. The MB collection methods differed considerably between studies, ranging from clinically supervised aspiration techniques (28, 29, 33, 40, 41, 44, 48, 52, 55) to home-based menstrual cup or pad collection (22–27, 36–39, 42, 43, 46, 47, 49, 50). Although home-based sampling may improve accessibility and participant acceptability, the lack of standardization in sample handling and processing

remains a major challenge for reproducibility (56). Additional limitations relate to inconsistent diagnostic verification and control group definitions. Although most studies surgically confirmed endometriosis in affected participants (21–30, 34–38, 40–50, 52), control participants were often incompletely characterized, with some studies relying on self-reported absence of disease, applying different diagnostic procedures between groups (30, 31, 39, 40) or failing to specify any diagnostic method at all (27, 32–35). These inconsistencies may have introduced misclassification bias and obscured biomarker differences.

Menstrual blood presents a promising, noninvasive alternative to the current surgical gold standard of endometriosis diagnosis. However, to fully harness its diagnostic potential, further research is essential. Future studies should prioritize large multicenter validation cohorts, harmonized MB collection and processing protocols, and standardized reporting of diagnostic accuracy measures. Comparative studies investigating the relationship between biomolecules identified in MB and PB may further clarify their respective diagnostic value, whereas evaluation of biomarker fluctuations across the menstrual cycle could help define optimal sampling windows. In addition, future research may explore the potential of MB-based biomarkers as tools for longitudinal monitoring of disease activity and treatment response, facilitated by the noninvasive and easily repeatable nature of sample collection. Emphasis should be placed on biomarker candidates, which are measurable using reproducible, low-cost assays suitable for routine laboratory workflows and adaptable to diverse healthcare settings, including potential future point-of-care testing. In parallel, studies assessing the feasibility, acceptability, and implementation of MB sampling in clinical practice across different healthcare systems will be important to support equitable and scalable integration into endometriosis care pathways.

Overall, although MB represents a highly promising source for noninvasive endometriosis diagnostics, current evidence remains largely exploratory. Yet, several biomarker candidates demonstrate encouraging diagnostic and translational potential but require rigorous validation in well-designed multicenter studies before clinical implementation.

CONCLUSION

This systematic review highlights the potential of MB as a noninvasive diagnostic tool for endometriosis primarily due to its simple, repeatable, and patient-friendly sampling, which can be performed independently using menstrual cups, pads, or minimally invasive aspiration methods.

A broad range of molecular, cellular, and biochemical markers were shown to be differentially expressed between women with and without endometriosis; only a limited number of these candidates were evaluated for diagnostic accuracy, and even fewer fulfilled the criteria of promising biomarker candidates based on diagnostic performance, methodological robustness, and clinical feasibility. These included P450 aromatase, TGF- β 1, PR-B mRNA expression,

PR-B promoter methylation, aromatase, SF-1, HSD17B2, and IGFBP1.

Although these findings underscore the biological plausibility of MB-based diagnostics, methodological heterogeneity and small sample sizes limit current evidence. When selecting future candidates for validation, priority should be given to biomarker candidates that are easily detectable and feasible to measure in routine clinical laboratories or even suitable for near point-of-care testing. With further development, MB could provide a widely accessible, noninvasive screening for endometriosis and a diagnostic alternative to laparoscopy.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT and OpenAI to improve the clarity, structure, and language of the manuscript. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRedit Authorship Contribution Statement

Ruth Niedermeier: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Noemi Castelletti:** Writing – review & editing, Supervision, Methodology. **Andreas Bender:** Writing – review & editing, Supervision, Methodology. **Michael Hoelscher:** Writing – review & editing, Supervision. **Olena Ivanova:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kathrin Held:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Interests

R.N. has nothing to disclose. N.C. has nothing to disclose. A.B. has nothing to disclose. M.H. has nothing to disclose. O.I. has nothing to disclose. K.H. has nothing to disclose.

SUPPLEMENTAL MATERIAL

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