Check for updates

Accuracy and utility of blood and urine biomarkers for the noninvasive diagnosis of endometriosis: a systematic literature review and meta-analysis

Wioletta Dolińska, B.Sc.,[a] Hannah Draper, M.B.Ch.B. M.Phil.,[a,b] Lara Othman, M.B.Ch.B.,[c] Chloe Thompson, B.Sc.,[a] Samantha Girvan, M.Phil,[a] Keith Cunningham, M.D.,[b] Jane Allen, M.B.Ch.B.,[b] Alan Rigby, M.Sc.,[c] Kevin Phillips, M.B.Ch.B.,[b] and Barbara-ann Guinn, Ph.D.[a]

[a] Centre for Biomedicine, Hull York Medical School, University of Hull, Hull, United Kingdom; [b] British Society for Gynaecological Endoscopy-accredited Endometriosis Centre, Hull University Teaching Hospitals NHS Trust, Hull, United Kingdom; and [c] Hull York Medical School, University of Hull, Hull, United Kingdom

Objective: Endometriosis is a chronic, incurable condition associated with debilitating pain and subfertility affecting over 190 million women worldwide, which has no reliable noninvasive diagnostic tool. We aimed to determine the state-of-the-art in noninvasive liquid biopsy biomarker detection and predict the most promising biomarkers for endometriosis detection.

Evidence Review: A systematic review of the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines was conducted using the PubMed, MEDLINE, Scopus, and Cochrane Library databases. Primary research studies examining blood or urine biomarkers in humans published in English up until August 2022 were included. Studies with more than 10 patients with clear methodology and surgical staging of endometriosis were included, whereas studies that included gynecological malignancies or who did not perform laparoscopy in the control group were excluded. The articles were assessed for the risk of bias using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. One investigator extracted the data, and 2 investigators checked the accuracy. Extracted data were analyzed descriptively, the box plots of pooled data were calculated using RStudio, and the likelihood ratios were determined.

Results: A total of 8,244 and 3,619 manuscripts for blood and urine biomarkers were identified. After screening on the basis of the title, abstract, full text, and quality assurance, 18 of these studies assessing blood biomarkers and 15 examining urine biomarkers were eligible for data extraction. However, there were inconsistencies in the results indicating that standardized techniques would be essential for direct comparisons to be made in the future. In 4 of the eligible studies, the urine biomarkers were juxtaposed with blood markers; however, in most cases, the combination of blood and urine biomarkers resulted in an increase in the area under the curve value, sensitivity, and specificity. One study presented biomarkers with a likelihood ratio of >10. However, currently, none of the biomarkers have been shown to be clinically useful, and further research is necessary to determine their utility in clinical practice.

Conclusion: Multiple biomarkers described here provide exciting avenues for further study particularly as part of diagnostic panels, including the endometrial antigens tropomyosin 3, stomatin-like protein 2, and tropomodulin 3, microribonucleic acids, and interleukins. There is a need for standardized protocols to be used to achieve consistent, reproducible results that will facilitate the development of a clinically applicable noninvasive test for endometriosis. (Fertil Steril Rev® 2023;4:116–30. ©2023 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, urine, blood biomarker, plasma, serum

Received August 22, 2022; revised December 20, 2022; accepted December 21, 2022.

W.D. has nothing to disclose. H.D. has nothing to disclose. L.O. has nothing to disclose. C.T. has nothing to disclose. S.G. has nothing to disclose. K.C. has nothing to disclose. J.A. has nothing to disclose. A.R. has nothing to disclose. K.P. has nothing to disclose. B.-a.G. has nothing to disclose.

W.D. and H.D. should be considered similar in author order.

Supported by the University of Hull graduate internship scheme (to W.D.) and an INSPIRE grant awarded to the Hull York Medical School by the Academy of Medical Sciences through the Wellcome Trust (Ref: IR5\1018).

Supported by the Hull University Teaching Hospitals NHS Trust as a Clinical Research Fellow (to H.D.).

Reprint requests: Barbara-ann Guinn, Ph.D., Centre for Biomedicine, Hull York Medical School, University of Hull, Cottingham Rd, Hull 6 7RX, United Kingdom (E-mail: Barbara.Guinn@hyms.ac.uk).

Fertil Steril Rev® Vol. 4, No. 2, April 2023 2666-5719

© 2023 The Authors. Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.1016/j.xfnr.2022.12.001

ESSENTIAL POINTS

- There are currently no noninvasive biomarkers for the detection of endometriosis with adequate sensitivity and specificity to be used in clinical practice.
- A number of blood and urine biomarkers provide exciting avenues for further study, such as micro–ribonucleic acids, tropomyosin 3, stomatin-like protein 2, tropomodulin 3, and interleukins, that could be useful alone or in combination.
- There is a need for collaborative research using standardized protocols in endometriosis research to achieve consistent, reproducible results that are clinically applicable.

Endometriosis is a chronic, incurable condition associated with debilitating pain and subfertility affecting over 190 million women worldwide (1). Endometriosis is estimated to affect up to 1 in 10 women (2), increasing to 50% of women seeking treatment for fertility (3) and 75% of adolescents with pelvic pain (4). It is an inflammatory condition (recently reviewed in the study by Machairiotis et al. (5)) characterized by the growth of endometrial tissue, glands, and stroma outside of the endometrial cavity. Endometriotic lesions are most commonly found within the pelvis but can be found in distant locations (6), such as the pleural cavity and nervous system. Symptoms are typically dependent on the extent, location, degree of invasion, and associated adhesions. These typically include pelvic pain, dysmenorrhea, dysuria, dyspareunia, infertility, dyschezia, cyclical hematuria, and gastrointestinal disturbance (7) as well as depression and anxiety (8). However, there is no reliable correlation between the extent of disease and severity of symptoms (9). Despite extensive research, the precise mechanisms of the development of endometriosis remain controversial (10).

Endometriosis can be broadly divided into 3 categories: superficial (peritoneal); ovarian; and deep endometrioses (11), with ongoing debates as to how to classify, stage, and report the disease (12). Recently, recommendations on best practice diagnosis and management have been published (11). However, laparoscopy and biopsy for histology remain the gold standard for diagnosing endometriosis. Although these are routinely performed procedures, these are not without risk with 2 per 1,000 women experiencing a serious complication, such as visceral injury (13). Notwithstanding the psychological benefits of providing photographic and histologic proof of diagnosis, surgery remains expensive with a burden of morbidity and even mortality (14). There is also no proven superiority of surgically treating superficial endometriosis, suggesting that several women undergo diagnostic laparoscopy with no clear therapeutic benefit (11). Although there are little robust data, it is conceivable that receiving a timely diagnosis of endometriosis, and appropriate treatment, may help to avoid the far-reaching consequences that delayed diagnosis has on women and their families (15). Considering the knowledge that we have already acquired, an increased understanding of the pathophysiology of endometriosis (16, 17) and the difficulty in its diagnosis (reviewed in the study by Chapron et al. (18), the detection of noninvasive biomarkers has been of increasing interest to a number of stakeholders. These include gynecologists, the US Food and Drug Administration, the World Endometriosis Research Foundation, biomedical scientists, family physicians, insurance providers, and patients and their families.

Urine and blood are excellent sources for noninvasive and minimally invasive biomarkers. Both types of liquid biopsy show a high abundance of endogenous metabolites, genetic products, peptides/proteins, and secreted organelles (19). Additionally, the urine proteome is less complex and more stable, allowing the detection of changes in low-abundance proteins (20). It is sensitive to internal changes, giving a chance to identify a biomarker at an early stage of disease (21). Blood and urine samples are safe, easily accessible, acceptable, and less expensive than laparoscopy, and sample processing and storage are also relatively simple (19). Previous systematic literature reviews (SLRs) identified noninvasive biomarkers of endometriosis in urine (22), menstrual fluid (23), blood (24), and serum, urine, and blood (25). Each stated that although biomarkers were identified, none consistently met the criteria needed to replace or triage a diagnostic test. Laparoscopy remains the gold standard but not the only indication for a diagnosis of endometriosis (18). This SLR focuses on the evaluation of studies that have identified urine and blood biomarkers in literature data sets from their inception to August 2022 to determine the clinical potential of the biomarkers identified to date to be used for the noninvasive detection of endometriosis. By performing this review, we hoped to determine the state-of-the-art in noninvasive liquid biopsy biomarker detection and predict the future of biomarkers for endometriosis detection.

MATERIALS AND METHODS

Literature Search and Study Selection

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for systematic reviews were adhered to (26, 27). The search strategy was developed on the basis of index terms found in 3–6 sentinel articles that were identified in an initial screen of the literature using PubMed. We started with the key terms (endometriosis) AND (blood biomarkers) OR (urine biomarkers) and then searched the Medical Subject Headings (MeSH) database to further develop a targeted string of terms.

Four databases, PubMed, MEDLINE, Scopus, and Cochrane Library, were used to screen for blood biomarkers with the following MeSH terms: (endometriosis*) AND (detection*) (by C.T.) and (endometriosis*) AND (blood*) AND (detection*) AND (biomarker*) (by W.D.), identifying articles published up to August 17, 2022.

We screened 6 databases for urine biomarkers—PubMed, MEDLINE, Embase, Scopus, Cochrane Library, and Web of Science—with the following MeSH terms: (endometriosis*) AND (urine*), identifying articles published up until August 17, 2022.

Articles were exported into Excel files, 1 for blood and 1 for urine (Supplemental Tables 1 and 2, available online). Subsequently, duplicates were removed, and articles were screened on the basis of the title and abstract, and if chosen for further assessment, the article was read fully. For the purpose of these literature searches, we used prespecified inclusion/exclusion criteria (Table 1). At the stage of abstract reading, our search excluded books, systematic reviews, meta-analyses, and conference articles. The assessment of whether an article met the inclusion/exclusion criteria was performed by 2 independent researchers for urine (W.D., L.O.) and blood (W.D., C.T). Any disparities in chosen articles were discussed between the reviewers, and a consensus was achieved. However, when disagreements remained, they were resolved by a third reviewer (B.G.). A fourth researcher (H.D.) provided the quality assurance of the articles.

Review articles were only removed once the cited articles in all selected manuscripts had been screened against the inclusion/exclusion criteria as detailed earlier. This "reverse snowballing" step helps ensure that relevant literature is successfully found as part of a systematic review (28).

Data Extraction and Quality Assessment

Quality assurance was performed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool (29) (Supplemental Table 3), giving the reviewers a chance to determine whether there are any applicability concerns and risks of bias across the selected articles.

All studies that were used for data extraction (Supplemental Table 4) had a case-control design and focused on the measurement and comparison of the level of a specific blood or urine biomarker, with comparisons between patients with endometriosis and a control group. Across all of the studies, the participants, in both the control and case groups, were of reproductive age, and the number of women included in the studies ranged from 39 (30) to 1,931 (31). We used the QUADAS-2 tool to assess whether studies considered the role of the menstrual cycle on biomarker expression in either blood or urine through noting that sample collection occurred at a particular phase of the menstrual cycle, collecting multiple samples at different phases, or performing subgroup analyses.

Statistical Analysis and Meta-Analysis

Collated data were imported into RStudio using readr. The "NA" values were removed, and the remaining data were visualized using the geom_boxplot function of ggplot2.

The likelihood ratios (LRs) were calculated for all studies that presented their sensitivity and specificity. Values above 10 were considered to have strong evidence to rule in endometriosis (32, 33).

TABLE 1

Inclusion and exclusion criteria used for screening articles that focused on blood and/or urine biomarkers.

Inclusion criteria	Exclusion criteria
English-language articles	Studies of less than 10 participants
Noninvasive biomarkers of any kind	Studies not directly associated with blood biomarkers for endometriosis
Primary research articles	Studies that involved animal models/cell lines
Adult females	Pediatrics, pubescent females and males
Human studies with more than 10 participants	Unrelated gynecological diseases
Patients with endometriosis	Biomarkers retrieved invasively
Studies with a description of the methodology used for the measurement of changes in blood/urine composition	Gynecological malignancies included in the control group
	Only 1 type of endometriosis considered
	Different grading system than that of the ASRM or rAFS
	Lack of laparoscopy in the control group
	Blood biomarkers only: most cases (>80%) were diagnosed with stage I-II OR stage III–IV endometriosis in accordance with the ASRM or rAFS grading system OR lack of stage specification

Note: ASRM = American Society for Reproductive Medicine; rAFS = revised American Fertility Society.

Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

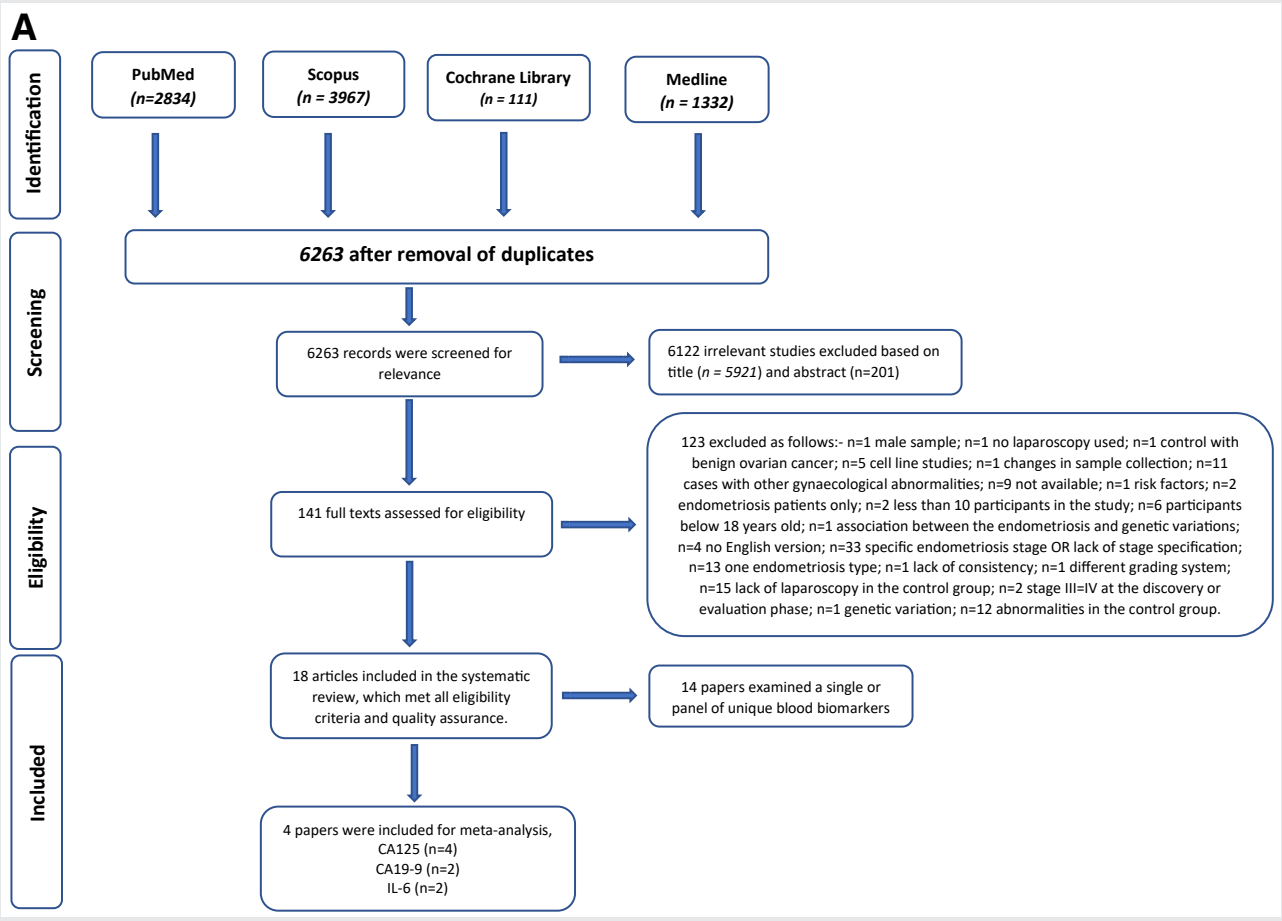
RESULTS

Study Selection, Quality Assurance, and Study Characteristics

The searches amassed 8,244 and 3,619 manuscripts for blood and urine biomarkers, respectively, of which 1,981 and 641 were duplicates (Fig. 1). Manuscripts were then screened on the basis of the title and abstract. The full-text versions of 141 studies on blood biomarkers were assessed for eligibility, and 18 articles were included in this review (Supplemental Table 1). Quality assurance was performed using the QUADAS-2 tool (Table 2 and Supplemental Table 3), and none of them were excluded considering the risk of bias and applicability. Of the 38 full-text studies on urine biomarkers considered, 19 were suitable for quality assurance after which 4 were excluded (Table 3).

We excluded studies that had not performed laparoscopy on their control groups. This was because the most clinically relevant control group would be those patients who are symptomatic of endometriosis because this is the population a test to exclude "true negatives" would be applied to. However, performing laparoscopy on asymptomatic women is not possible in the absence of any indication for surgery, and

FIGURE 1



Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram showing the process of study selection at each stage of the systematic literature review. (A) Blood and (B) urine biomarkers. CA = cancer antigen; IL = interleukin.

Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

therefore, these individuals would not be a truly healthy group. The studies included in this review approached this dilemma in different ways: 24 used symptomatic controls; 7 used asymptomatic women undergoing laparoscopy for other indications, such as sterilization; and 2 used symptomatic patients who had undergone a laparoscopic evaluation and self-reported healthy volunteers (Supplemental Table 5).

Meta-Analysis

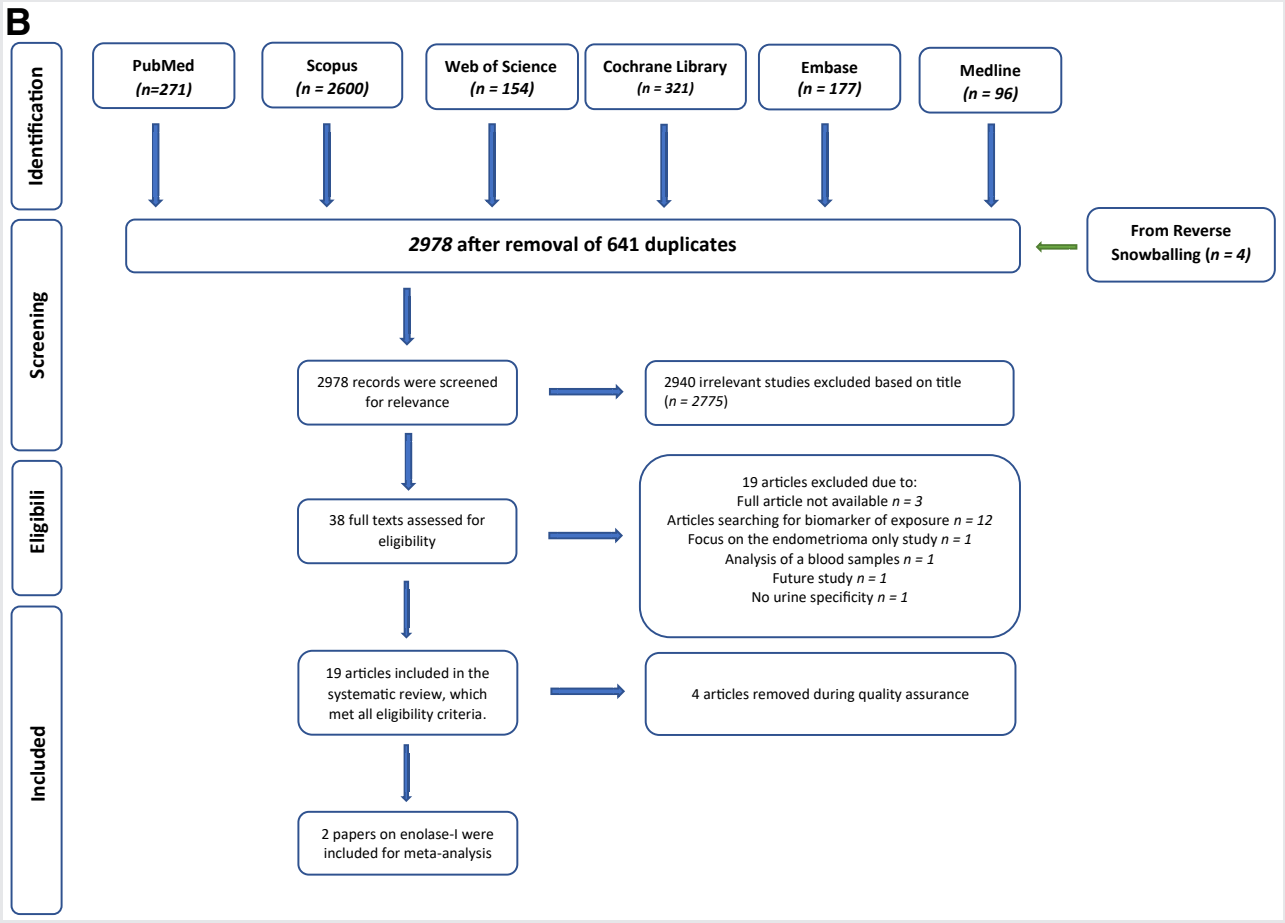
Fifty-eight potential biomarkers were investigated across 33 studies; however, only 7 biomarkers were assessed in more than 1 study. Cancer antigen (CA)125 was the most widely investigated biomarker and was analyzed in 6 studies (34, 35, 36, 37, 38, 39); however, the sensitivity and specificity were only presented in 5 of these studies. The blood biomarkers CA19-9 (36, 39) and Interleukin (IL)-6 (36, 40) were assessed in 2 studies each. The urine biomarkers cytokeratin-19 (CK19) (41, 42), E-selectin (43, 44), and P-selectin (43, 44) were presented in 2 studies each; however, there were nonsignificant differences in 1 (CK19) or both (E- and P-selectin) markers, resulting in enolase-1 (ENO1) being the only urine biomarker where meta-analysis was possible. The box plots demonstrating the pooled data are presented in Figure 2A, which showed the lack of reproducibility between cohorts with differing patient selection criteria.

The LRs were calculated for 35 single or combined biomarkers retrieved from 19 studies that presented the sensitivity and specificity with a range of 1–19.5 (Fig. 2B). Only 1 study presented biomarkers with an LR of >10, suggesting strong evidence of the presence of endometriosis (34): stomatin-like protein (SLP)2a; tropomodulin (TMOD)3b; TOMD3c; and TMOD3d, with positive LRs of 12.5 15.5, 11.1, and 19.4, respectively. Among the least useful were cytokeratin (LR, 1.1) and ENO1 (LR, 1.1).

Blood Biomarkers

Cancer antigen 125 is a well-established marker of epithelial cell ovarian cancer; however, its level is also increased in serum samples from patients with endometriosis due to the stimulation of the coelomic epithelia (35). The predictive

FIGURE 1 Continued



Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

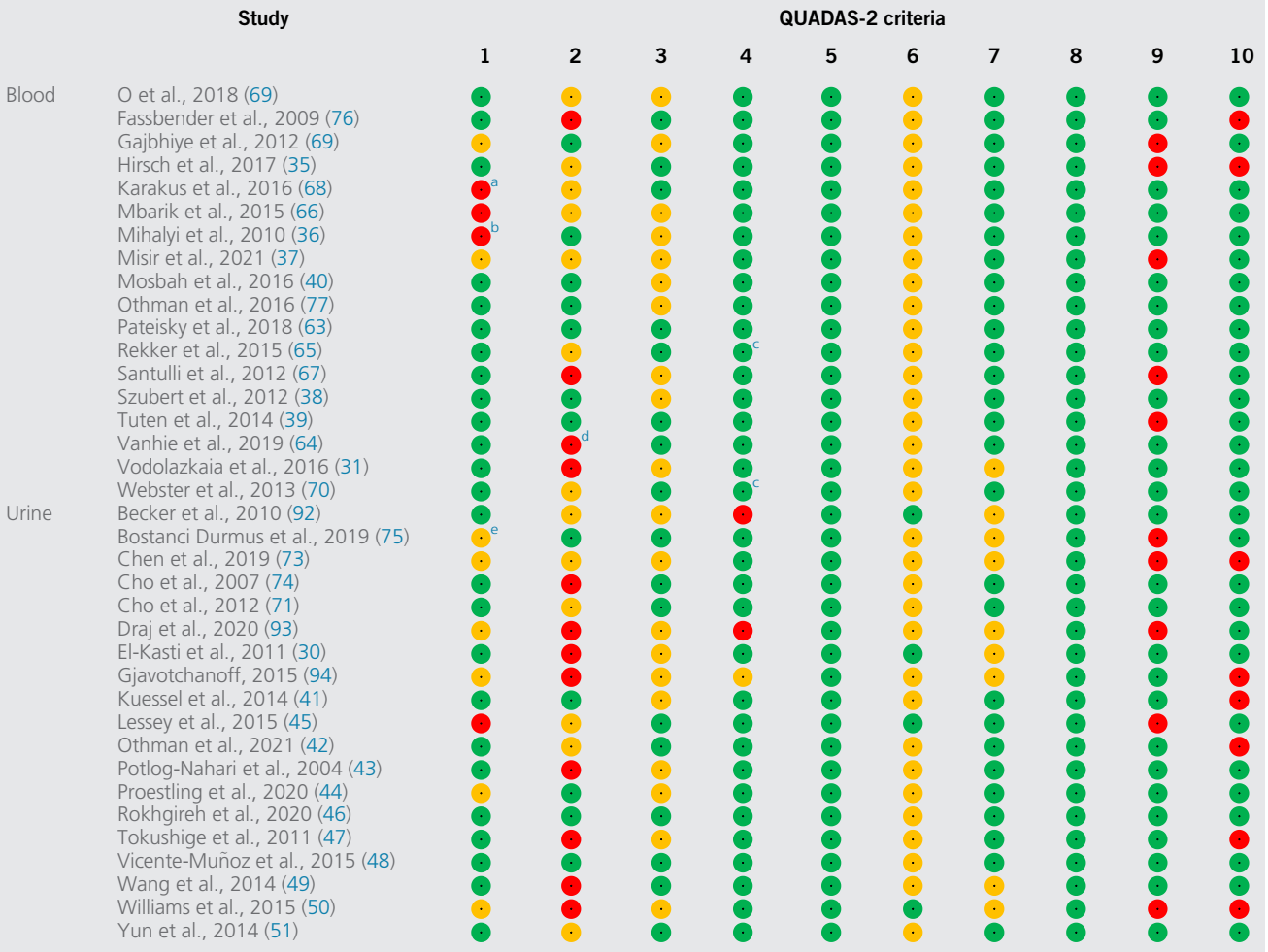
potential of CA125 was assessed in 5 studies alone and in conjunction with other markers (34, 35, 36, 38, 39) (Fig. 2). Four studies had comparable numbers of participants (30, 45,42–44,46–62), with the study by Mihalyi et al. (36) having 294. There were a broad range of results with an overall sensitivity reported as 32% (34) to 87.5% (39). It should be noted that different cutoffs were used during area under the curve (AUC) calculations because these are determined by the data generated and the statistical approach used. The differences in the cutoffs used may account, at least in part, for the differences in diagnostic accuracy observed in the study by Hirsch et al. (35) improving the specificity by using a higher cutoff of 30 U/mL than that in the study by Szubert et al. (38) who used 11 U/mL with improved sensitivity, although neither identified a biomarker suitable for clinical use currently.

One source of variation between these studies was the use of exogenous hormones and the stage in the menstrual cycle when samples were taken. Although 2 studies did exclude participants who had used hormonal treatments in the past 3 months (34, 39), only Mihalyi et al. (36) investigated the effect of the menstrual cycle. Mihalyi et al. (36) noted significant differences across the menstrual cycle and with stages of endometriosis with sensitivity varying from 100% for stage III/IV during the menstrual phase to as low as 65.1% for all stages during the proliferative phase. A less extreme variation was noted for specificity (71.1%–73.7%). Studies that did not account for the phase of the menstrual cycle through either taking samples in the same phase or performing subgroup analysis may underestimate the clinical utility.

Micro–ribonucleic acids (miRNAs) control gene expression posttranscriptionally by inhibiting miRNA translation or promoting mRNA degradation in the cytoplasm. Most are localized in the cells but have been detected in extracellular body fluids, such as serum, plasma, spinal fluid, follicular fluid, saliva, and urine, making them attractive potential biomarkers. Micro–ribonucleic acids have been shown to be involved in endometriosis with differential expression demonstrated between eutopic and ectopic endometrial tissues (52). They are also known to regulate pathways involved in proliferation, inflammation, and angiogenesis (63), all relevant to endometriosis pathogenesis. Four of the SLR selected studies

TABLE 2

Outcome of the quality assurance performed using the QUADAS-2 tool for blood and urine biomarkers.



Note: Quality Assessment of Diagnostic Accuracy Studies-2.
[a] The control group did not have symptoms associated with endometriosis.
[b] Infertility with or without pain.
[c] Two control groups.
[d] Validation cohort well described.
[e] The control group underwent laparoscopy for a variety of indications.

Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

assessed miRNAs. Vanhie et al. (64) used a multivariate logistic regression with stepwise feature selection to build 3 diagnostic models for endometriosis. The minimal-mild endometriosis model had a sensitivity, specificity, and AUC of 78%, 37%, and 60% (LR, 1.2), respectively. The other models did not reach significance, although this may, in part, be due to the homogeneous nature of the patient group being almost entirely superficial peritoneal endometriosis, whereas moderate-severe included extensive adhesions, endometriomas, and endometriotic nodules. Pateisky et al. (63) assessed miRNA-154-5p and demonstrated an AUC, sensitivity, and specificity of 0.72, 67%, and 69% (LR, 2), respectively, in their validation cohort of 64 cases and controls.

Misir et al. (37) assessed miR-34a, a tumor suppressor miRNA that has roles in cell cycle, proliferation, apoptosis, and metastasis and is transcriptionally regulated by p53. They also evaluated miR-200c, which is part of the family that regulates cellular transformation and expression of several genes, including epithelial-mesenchymal transition. These molecules play an important role in angiogenesis, tumor development, and metastasis. miR-34a-5p was significantly down-regulated in all stages of endometriosis with an AUC of 0.686–0.826, whereas miR-200c was up-regulated in endometriosis compared with the control group with an AUC of 1.00. This is a promising preliminary study in a carefully selected population with predominantly superficial disease although no power calculation was performed.

Rekker et al. (65) evaluated miRNAs 200a, 200b, and 141 in 61 participants with endometriosis. Of note, they assessed whether the time of day affected miRNA expression and found that the levels were lower in the morning than in the evening; therefore, for their final analysis of a combined

signature of 3 miRNAs, they used evening samples only (n = 32) and achieved a sensitivity, specificity, and AUC of 84.4%, 66.7%, and 0.76, respectively. The LRs were improved by combining the panel but remained insufficient to gain a clinical diagnosis at 2.5. However, the study demonstrated the potential of combined markers to improve precision. All 3 miRNAs evaluated within this study demonstrated variation with sampling time and emphasize the need to take blood sampling time into account when studying circulating miRNAs as biomarkers.

Pateisky et al. (63) evaluated miRNA expression in plasma samples and found that 2 miRNAs, miRNA-326 and miRNA-485-3p, showed menstrual cycle–specific regulation in healthy individuals, with lower levels observed during the secretory phase. However, this regulation was lost in patients with endometriosis. Other miRNAs, such as miRNA-154-5p, were independent of menstrual cycle and hormonal intake. Vanhie et al. (64) considered the effect of the menstrual cycle on 41 miRNAs in the luteal, menstrual, and follicular phases as assessed by endometrial biopsy histology. They demonstrated that 16 miRNAs had significant differences in levels during at least 1 phase in the menstrual cycle in the discovery cohort and 4 in the validation cohort. However, no miRNA was significantly affected by cycle in both the discovery and validation cohorts, leading to the conclusion that this is likely due to biologic variability rather than a true menstrual effect. Rekker et al. (65) also noted no difference in miRNA expression with menstrual phase although they did note a difference between morning and evening samples. They also noted that not all healthy volunteer samples were taken at the same time of day, which may impact the interpretation of their results.

These studies support a possible biologic link between miRNAs and endometriosis. There are several factors that may limit the clinical utility of miRNAs, including high natural variation in circulating levels and significant differences in levels depending on the time of day and menstrual phase. Future studies will need to carefully consider their design with rigorous methodology required, sufficient statistical power, and independent validation steps to establish whether miRNAs are clinically useful markers.

Immunologic factors and angiogenesis are likely to play key roles in the pathogenesis of endometriosis (66). The immunologic aberrations observed in endometriosis are both local and systemic with chronic inflammation activating host immune responses leading to humoral and cell-mediated inflammation. Mbarik et al. (66) and Santulli et al. (67) examined the IL-33 expression, a member of the IL-1 family that induces the synthesis of cytokines and can trigger the fibrotic process. Santulli et al. (67) found no difference between patients with endometriosis (n = 510) and symptomatic controls (n = 132). A post hoc analysis showed significant differences in the IL-33 levels between patients with deep endometriosis and the control group ($P = .022$) and patients with deep endometriosis and those with superficial endometriosis ($P < .001$). This is in contrast to Mbarik et al. (66) who found high levels of IL-33 expression in patients (2.48 ng/mL) compared with those in controls ($P = .0068$), with a significant stepwise increase between stage I–II and III–IV endometrioses albeit in a much smaller sample size. Furthermore, they also investigated ST2, which mediates the effects of IL-33 on the inflammatory process by suppressing IL-33 activity; however, there was no difference in serum ST2 expression between the endometriosis and control groups. Both used commercial enzyme-linked immunosorbent assay (ELISA) kits but found a factor difference in the mean IL-33 levels (2.48 ng/nL (36) and 7.5 pg/mL (67)). Interleukin-33 may act as a profibrotic mediator involved in the pathogenesis of the disease because it is associated with deep disease (38), and it could be hypothesized that IL-33 activates endometrium cells in vivo to recruit inflammatory cells (66) and, therefore, may be implicated in the etiology of endometriosis but is unlikely to be a diagnostic marker.

Mosbah et al. (40) investigated IL-7 and glycodelin A, which is a secretory phase protein produced by endometrial epithelial cells as a response to progesterone exposure and is thought to have both an immunosuppressive effect and a role in glandular morphogenesis. The IL-7 levels were significantly higher in patients with endometriosis (n = 48) than in the control group (n = 20) of patients undergoing laparoscopic tubal ligation ($P < .001$; AUC, 0.88; specificity, 80%; and sensitivity, 93%). Similarly, glycodelin A had an AUC, specificity, and sensitivity of 0.96, 75%, and 91%, respectively. Interleukin-7 and glycodelin A were both correlated with disease stage although the LRs were 4.6 and 3.6, respectively, which remained below the threshold of clinical utility. However, further investigation with a broader, more clinically relevant cohort would be justified.

Interleukin-6, IL-8, CA125, CA19-9, high-sensitivity C-reactive protein, and tumor necrosis factor-alpha (TNF-α) have been assessed in patients with endometriosis (n = 201) and controls (n = 93) (36). Using stepwise logistic regression, moderate-severe endometriosis could be diagnosed with a sensitivity and specificity of 100% and 74%, respectively, whereas minimal-mild endometriosis could be diagnosed with a sensitivity and specificity of 87% and 71%, respectively. The least-squares support vector machine model gained slightly superior results compared with logistic regression for minimal-mild endometriosis. They noted that the greatest sensitivity was observed during the secretory phase and the least sensitivity was observed during the proliferative phase regardless of disease stage, and when considered in the secretory phase, the LR increased to 6.11. The sensitivity and specificity increased in all disease stages when the discrete menstrual cycle phases were considered separately compared with those when pooled.

Tumor necrosis factor-alpha is a proinflammatory cytokine that is implicated in endometriosis pathogenesis and works through 2 types of transmembrane receptors that have soluble forms (soluble tumor necrosis factor receptor [sTNFR]-I and sTNFR-II). Othman et al. (42) assessed sTNFR-I and sTNFR-II in 62 patients with endometriosis and 55 controls. Soluble tumor necrosis factor receptor-I achieved a moderate predictive value (AUC, 0.62) in all stages of endometriosis, whereas sTNFR-II did not. When early-stage disease was considered alone, this marginally improved (AUC, 0.68). There was no difference between patients with advanced endometriosis and the control group, which could

limit its clinical utility as a diagnostic biomarker. Contrary to Mihalyi et al. (36), Othman et al. (42) found no difference between the expression levels in the proliferative and secretory phases. C3a induces a broad variety of inflammatory reactions, such as IL-1 and TNF release. Fassbender et al. (53) assessed the C3a levels in 109 patients with endometriosis and 51 controls during the follicular and luteal menstrual phases but found no difference. The investigators suggest considering complement activation in the cervix and endometrium; however, this would represent a more invasive screening tool.

Other biomarkers that have been assessed for their suitability include copeptin, the more stable C terminal portion of the antidiuretic hormone. It is produced in the hypothalamus, secreted by the pituitary, and elevated in critical acute and chronic inflammatory conditions. Tuten et al. (64) found that the copeptin, CA125, CA15-3, and CA19-9 levels were higher in patients with endometriosis (n = 50) than in the control group (n = 36) ($P = .002$, $P = .001$, $P = .017$, and $P = .015$, respectively), whereas the copeptin and CA19-9 levels were significantly higher in stage III–IV patients than in the stage I–II group ($P = .004$ and $P = .036$, respectively). Although the serum copeptin levels were associated with disease severity, the LR was only 1.5, attributing little clinical utility even in a study with a relatively homogeneous population. A study of soluble Fas ligand, CD95/FAS, and hypoxia-inducible factor 1-alpha serum levels showed that the CD95/FAS and hypoxia-inducible factor 1-alpha levels ($P < .05$) were higher in patients with endometriosis (n = 30) than in healthy controls (n = 30) in the follicular phase of the menstrual cycle (68). In addition, the serum levels of these proteins were higher in stage III/IV endometriosis than in I/II endometriosis ($P < .05$) (68).

Gajbhiye et al. (34) presented the only biomarkers with LRs greater than 10 suggesting strong evidence to rule in endometriosis. They evaluated whether tropomyosin 3, SLP2, and TMOD3 could be biomarkers for the early detection of minimal-mild endometriosis (comparing 50 patients with endometriosis with 27 secretory-phase controls). Stomatin-like protein 2a, TMOD3b, TOMD3c, and TMOD3d had positive LRs of 12.5, 15.5, 11.1, and 19.4, respectively (Supplemental Table 4). This was a well-designed trial consisting of 2 independently recruited cohorts for biomarker identification and validation. Women in the control group were excluded if they had received hormonal medication or had any relevant pathology or autoimmune diseases. They were also screened for immunologic factors, such as antithyroid, antinuclear, antiphospholipid, antihistone, or antilupus antibodies. This resulted in a homogeneous control group that could lose significance when applied to a wider population but would warrant further investigation.

Vodolazkaia et al. (31) assessed single nucleotide polymorphisms involved in the angiogenesis (vascular endothelial growth factor [VEGF] pathway) in serum samples from a large cohort of patients with endometriosis, symptomatic controls, and healthy volunteers. They found the placental growth factor (PLGF) levels to be elevated in endometriosis (mild-moderate disease in the menstrual phase) with moderate diagnostic performance (AUC, 0.73; sensitivity, 74%; and specificity, 80%; LR, 3). They note cyclical variation in expression during the menstrual cycle and a possible link between genetic variants in the PLGF rs2268613 gene influencing the PLGF plasma levels in Caucasian women. The myeloperoxidase enzyme, a proinflammatory enzyme and marker for neutrophil activation and oxidative stress, was investigated in 212 patients with endometriosis and 121 controls, patients undergoing laparoscopy for infertility and/or pain but with no evidence of endometriosis (69). A significant difference was only observed between women with endometriosis and benign disorders; therefore, myeloperoxidase is not suitable as a single biomarker, and it is likely to be involved in many other pathological processes, making it unsuitable as an endometriosis biomarker. Circulating angiogenic cells (CACs) are a diverse group of cells that have a role in enhancing neovascularization and have been implicated in a murine model of endometriosis. Webster et al. (70) measured the CAC levels in 47 patients with endometriosis and 30 controls in the 4 phases of the menstrual cycle. The CAC levels varied considerably between individuals; however, there were no consistent fluctuations during the menstrual cycle, suggesting that CACs were unaffected by it. There were no statistically significant differences in the levels between controls and patients with endometriosis at any stage, limiting their potential as a biomarker of endometriosis, although it remains plausible that they participate in angiogenesis within endometriotic lesions and, therefore, the pathogenesis of endometriosis.

Urine Biomarkers

Of all eligible studies that examined urine biomarkers, 5 investigated multiple protein targets. El-Kasti et al. (30) determined 13 statistically significant urinary peptides between the control group and patients with moderate/severe endometriosis using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry. The peaks with a molecular mass of 1,767.1 Da ($P = .019$) differentiated between the control group and patients with severe endometriosis at the periovulatory phase of the menstrual cycle, resulting in a sensitivity and specificity of 75% and 84.6%, respectively, whereas during the luteal phase, a peptide of 1,824.3 Da ($P = .045$) distinguished between both groups with a sensitivity and specificity of 75% and 71.4%, respectively. The comparison of the urinary peptide profiles between the control group and patients with endometriosis with stage I–II disease did not indicate any significant difference; however, a difference was observed when comparing the patients with endometriosis at stages I–II and III–IV.

In the study by Cho et al. (71), the number of significantly different urinary protein spots between patients with endometriosis and controls was higher than that in the study by El-Kasti et al. (30). They defined the protein with the greatest difference in the level as vitamin D binding protein (VDBP), and the ELISA analysis revealed that the VDBP level was significantly higher in urine from patients with endometriosis than in the control group ($P = .001$ and $P = .001$ after the creatinine [Cr] correction). Nevertheless, the median VDBP-Cr level between the patients with and without endometriosis was statistically significant only in the secretory phase of the

TABLE 3

Reasons that articles on urine biomarkers were excluded after quality assurance assessment.

Reference	Title	Reason for exclusion
Becker et al., 2010 (92)	Matrix metalloproteinases are elevated in the urine of patients with endometriosis	Lack of an appropriate diagnosis—had a group where clinical diagnosis of endometriosis without surgery was included
Draj et al., 2020 (93)	Serum and urine levels of cytokeratin-19 in endometriosis	Lack of an appropriate diagnosis—the control group was based on being asymptomatic with a regular menstrual cycle and being fertile
Gjavotchanoff, 2015 (94)	CYFRA 21-1 in urine: a diagnostic marker for endometriosis?	Lack of a reference standard being used, lack information about how the diagnosis had been obtained in case of patients with endometriosis
Tokushige et al., 2011 (47)	Discovery of a novel biomarker in the urine in women with endometriosis	No statistical analysis was performed to show the significance of the difference in the cytokeratin-19 levels between patients with endometriosis and controls

Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

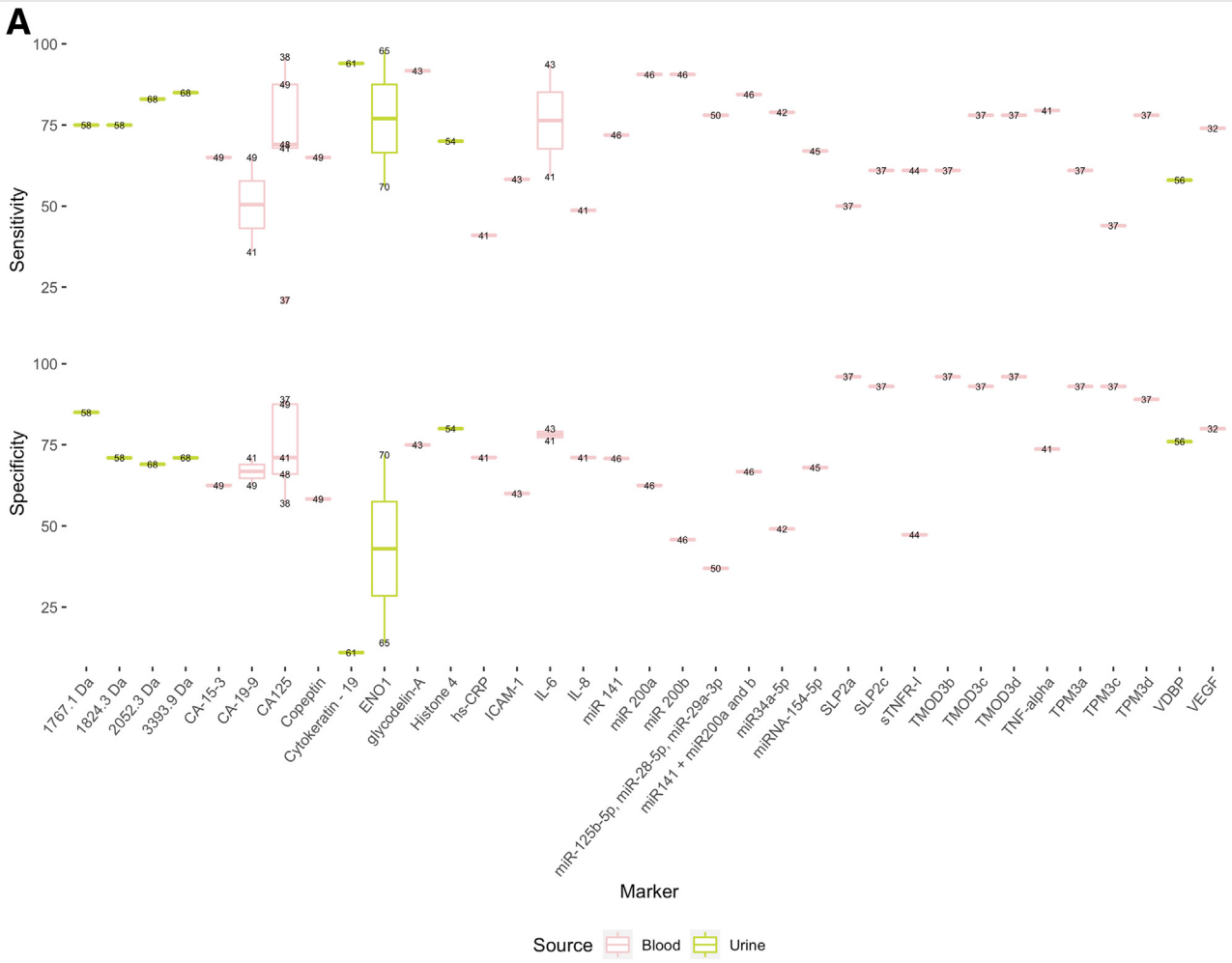
menstrual cycle. The sensitivity and specificity for VDBP-Cr were not higher than those for the peptides detected by El-Kasti et al. (30), which were 57.9% and 76.3%, respectively. Furthermore, the comparison of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry sample spectra generated by Wang et al. (49) identified 36 peptides that were significantly different between patients with endometriosis and the control group. Two of them, 2,052.3 and 3,393.9 Da, were down-regulated, showing the greatest difference in the peptide peaks with $P < 10^{-5}$ and sensitivity levels of 83.2% and 84.6%, whereas the specificity levels were 68.9% and 71.3%, respectively. In contrast to Cho et al. (71) and El-Kasti et al. (30), Wang et al. (72) found that the menstrual cycle phases did not alter the peptide patterns. In an independent blinded investigation, Wang et al. (72) also found 2 other peptides that differed in patients with endometriosis when compared with healthy donor controls. The peak with a molecular mass of 1,579.2 Da decreased in patients with endometriosis and was defined as a fragment of the collagen alpha-6 (IV) chain precursor, whereas the peak with a mass of 891.6 Da increased and was identified as a fragment of the type VIII, IX, and XV collagen $\alpha 1$ chain precursor. Williams et al. (50) did not find any association between urinary peptide/protein and endometriosis. Chen et al. (73) showed that there were 127 differentially expressed proteins between patients with endometriosis and the unexplained infertility control group, of which 99 were up-regulated and 28 were down-regulated. The first study by Chen et al. (73) used tandem mass tag–parallel reaction monitoring for endometriosis biomarker detection and identified histone 4 with equally high levels of sensitivity and specificity of 70% and 80%, respectively. This was marginally improved through the use of combined multiple markers giving a combined AUC of 0.863.

Two of the studies chosen for further analysis focused on CK19. Kuessel et al. (41) found no difference in the urinary CK19 levels between patients diagnosed with endometriosis and controls (ELISA, $P = .51$). In their study, the classification of samples to the proliferative or secretory menstrual cycle phase did not decrease the P values, ($P = .42$ and $P = .92$, respectively). After the exclusion of patients who received hormonal therapy up to 3 months before laparoscopy, there was still no significant difference between the case and control groups ($P = .51$). Additionally, no significant difference was observed after the classification of the remaining participants to the proliferative phase ($P = .57$) or the secretory phase ($P = .77$). Similarly, Lessey et al. (45) revealed the absence of any significant difference in the CK19 levels between patients with endometriosis and controls using chemiluminescent microparticle immunoassays ($P = .59$). Kuessel et al. (41) did not give a power calculation and used fewer patients than Lessey et al. (45) who found no clinical difference in an adequately powered study. Although the latter did not analyze the effect of the menstrual cycle, Kuessel et al. (45) found no benefit in performing such method, and overall, it appears unlikely that CK19 has a future role in the diagnosis of endometriosis.

The urinary level of nonneuronal ENO1 was significantly higher in patients with endometriosis than in the control group after Cr correction (ELISA, $P = .026$) (51). The ENO1 level increased during the secretory phase compared with that during the follicular phase of the menstrual cycle in patients with endometriosis ($P = .043$); however, the difference in the ENO1 levels was not significant after correcting for Cr ($P = .909$; sensitivity, 56.4%; specificity, 72.2%) (Fig. 2A). Rokhgireh et al. (46) also showed that there was no difference in the ENO1 levels when corrected for Cr between patients with endometriosis and the control group (ELISA, $P = .106$; sensitivity, 97.8%; specificity, 13.5%) (Fig. 2A). The LR was 1.1, which currently demonstrates very little clinical utility. Further analysis (46) showed that women with confirmed endometriosis have higher ENO1 levels when corrected for Cr in the luteal phase than in the follicular phase ($P = .039$) and that the ENO1 levels increased in patients with advanced endometriosis in the later stages (III–IV). This observation together with combining ENO1 with CA125 (46, 51) showed improvements in the diagnostic value that warrants further investigation.

Potlog-Nahari et al. (43) observed no significant difference in the VEGF-A levels between patients with confirmed

FIGURE 2



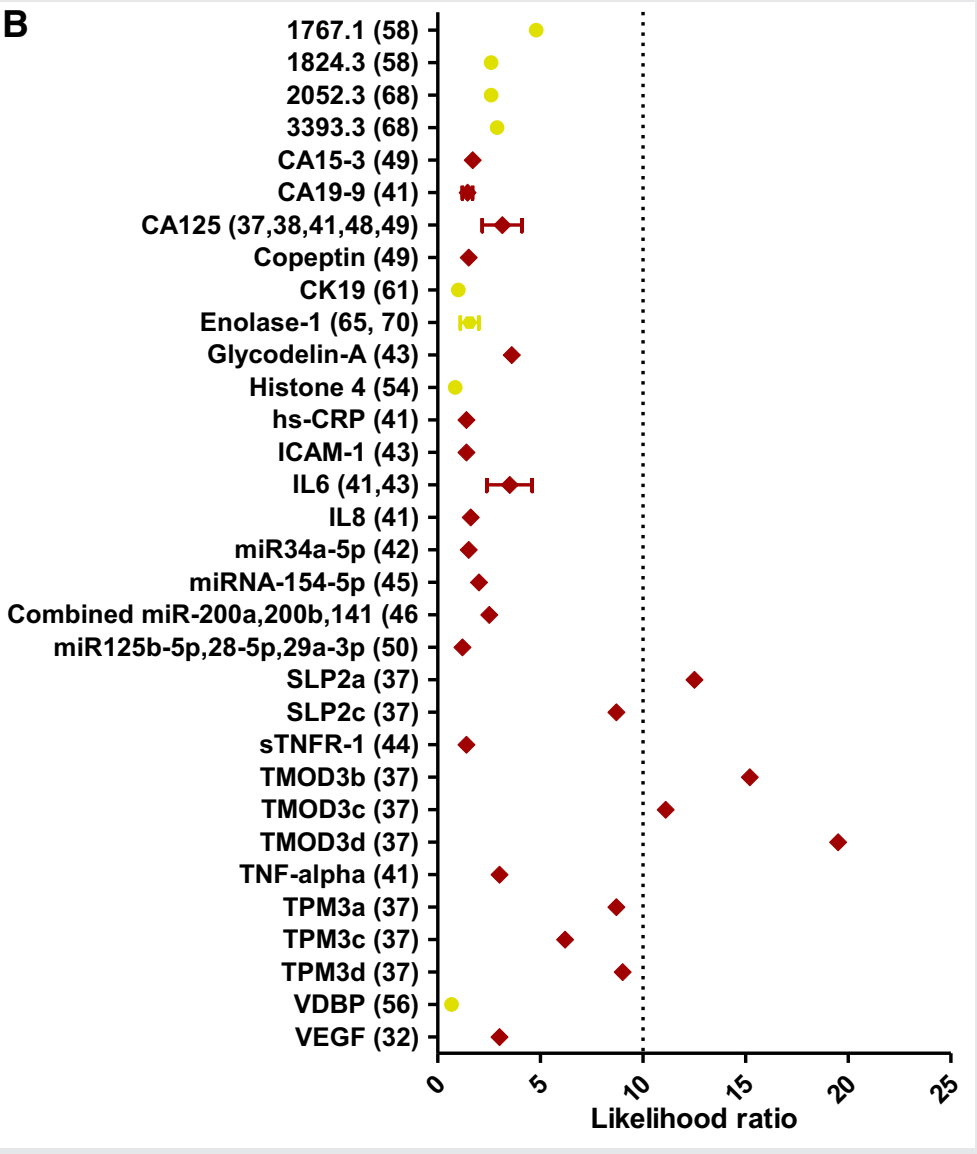
Sensitivity and specificity of each biomarker in selected articles. (A) The box and whisker graphs show the interquartile (box) and range (whisker) of sensitivities (upper section) and specificities (lower section) for pooled data on each biomarker and (B) likelihood ratios with standard error of the mean (SEM), for blood (pink) and urine (yellow) biomarkers on the selected studies. Numerical marker points indicate study reference. CA = cancer antigen; CK19 = cytokeratin-19; hs-CRP = high-sensitivity C-reactive protein; ICAM-1 = intracellular adhesion molecule-1; IL = interleukin; SLP = stomatin-like protein; sTNFR = soluble tumor necrosis factor receptor; TMOD = tropomodulin; TMP = tropomyosin; TNF-alpha = tumor necrosis factor-alpha; VDBP = vitamin D binding protein; VEGF = vascular endothelial growth factor.

Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

endometriosis and the control group ($P = .70$) after correction for Cr ($P = .77$) or when different menstrual cycle phases were considered ($P = .31$–$.51$) (43). The urinary VEGF levels were also not found to be significantly increased between patients with endometriosis and the control group by Cho et al. (74). Although any menstrual effect could be investigated, there appears to be little clinical utility of these markers. The group also analyzed other angiogenic structures, including TNF-α and fms-like tyrosine kinase (sFlt-1); however, only the urinary sFlt-1 level was significantly higher in patients with endometriosis than in the control group after correction for Cr ($P = .011$). The sFlt-1 level was significantly elevated when comparing patients with stage I-II endometriosis with those with stage III–IV endometriosis ($P = .015$) as well as between patients with endometriosis and the control group in the secretory phase of the menstrual cycle ($P = .021$).

Vicente-Muñoz et al. (48) showed a difference between the metabolomic profiles of patients with endometriosis and healthy controls. Seventy-nine of the 653 variables were statistically significant between the groups ($P < .05$). Those with the greatest statistically significant difference included N1-methyl-4-pyridone-5-carboxamide (4-Py), guanidinosuccinate, Cr, taurine, valine, 2-hydroxyisovalerate, and an unknown metabolite U2 that were each increased in level in patients with endometriosis, whereas the levels of lysine and 2 other unknown metabolites, U1 and U6, decreased. A power calculation was not provided; however, further investigation with a larger sample size particularly in early-stage disease would be of interest. Bostanci Durmus et al. (75)

FIGURE 2 Continued



Dolińska. Noninvasive biomarkers of endometriosis. Fertil Steril Rev 2023.

demonstrated a lack of a significant difference in the immune response as indicated by the urinary neutrophil gelatinase-associated lipocalin levels between patients with endometriosis and controls and between patients with mild and severe endometrioses. Previous suggestions that the serum soluble vascular adhesion molecule-1 was a promising biomarker for endometriosis (54) encouraged Proestling et al. (44) to investigate adhesion molecules including urinary soluble vascular adhesion molecule-1, soluble intracellular adhesion molecule-1, E-selectin, and P-selectin; however, they found no significant difference in levels between patients with endometriosis and controls. Othman et al. (42) studied estrogen metabolites and found that only 2OH1 was significantly higher in women with endometriosis than in the control group ($P = 043$). Only the investigation by Vicente-Muñoz et al. (48) showed that the stage of the menstrual cycle led to the detection of a difference in the level of a single urine biomarker, pseudouridine, whereas other studies failed to consider the menstrual cycle or did not show a significant difference in levels after urinalysis.

DISCUSSION

The reasons behind the delay in diagnosing endometriosis are complex and multifaceted with contributing factors including the absence of pathognomonic symptoms, limitations of imaging and the need for invasive testing, and an ongoing requirement for more menstrual health education for patients

and health care professionals. The implications of this should not be underestimated. Beyond the immediate impact on the physical and mental health of patients, this condition can have broad-reaching influences on relationships, work, education, and the ability of women to function on a daily basis. Simoens et al. (55) performed a multicenter, prospective, questionnaire-based survey to measure the costs and health-related quality of life of women with endometriosis-associated symptoms treated in referral centers. They demonstrated that the mean cost per woman was €9,579, of which two thirds of that cost was in productivity loss and the remainder was in health care costs.

Despite a substantial body of clinical research, there has not been an imaging modality (60) or biomarker (22–24, 57) that can replace laparoscopy in the diagnosis of endometriosis, and this remains the gold standard for several clinicians. Although there are undeniably advantages to diagnostic laparoscopy, such as the ability to obtain histology, offer concurrent surgical treatment, and provide photographic proof to patients, it is an invasive and expensive procedure with an inherent risk of complications that may account for part of the delay between symptom onset, diagnosis, and subsequent treatment (58). In several health care systems, the cost and availability remain barriers or present delays to diagnosis. It is plausible that obtaining a timely diagnosis and appropriate treatment will improve outcomes and patient quality of life. A noninvasive biomarker would reduce the requirement for surgery allowing for detection in earlier disease stages, enabling improvements in treatment outcomes and patient quality of life (24).

The studies identified here all had objectives and diagnosis and participant recruitment practices that were heterogeneous. The search yielded multiple possible biomarkers with diagnostic potential; however, none has been shown to be clinically useful yet, and thus, these require further research to investigate their potential to be used in clinical practice.

One biomarker that has been extensively investigated is CA125, which has widely been used in clinical practice as part of assessment tools in ovarian cancer (53). The results presented in 6 studies reviewed here demonstrate notable differences in both sensitivity and specificity (Fig. 2A). Mihalyi et al. (36) described differences in diagnostic performance between the secretory, proliferative, and menstrual phases of the cycle. Although 1 study excluded patients using exogenous hormones (34), Szubert et al. (38) excluded patients in the luteal phase, and a number of studies (34, 35, 36, 37, 39) did not consider the menstrual cycle in their analysis, which is likely to be a significant confounder. Furthermore, because CA125 is not endometriosis-specific, being a tumor marker whose level is also elevated in ovarian cancer (59), it has reduced specificity for endometriosis diagnosis.

Eighteen studies considered the menstrual cycle phase (30, 76, 68, 36, 40,77,63, 64, 70, 74, 71, 41, 43,44,46, 48, 49, 51). Although some studies showed that this had little impact on the level of measured biomarker (73, 71, 41, 43, 44, 50, 51), multiple studies noted cyclical differences that could adversely affect test efficacy. Mihalyi et al. (36) noted significant differences across the menstrual cycle and stages of endometriosis with sensitivity levels varying from 100% for stage III/IV during the menstrual phase to as low as 65.1% for all stages during the proliferative phase. Further prospective testing would be required to determine which cycle phase significantly outperforms the others and to assess its efficacy in a clinically applicable population.

Pateisky et al. (63) noted that miRNA-154-5p was regulated by the menstrual cycle in the control group but that this regulation was lost in endometriosis. Given this complex picture, it is likely that any menstrual and/or exogenous hormone effect needs to be addressed on an individual biomarker basis. It is possible that particularly in studies with small sample sizes, their limited ability to account for factors such as menstrual cycle and exogenous hormone use may obscure the interpretation of the results.

Single biomarkers can often provide useful information about a disease process but may not be specific or sensitive enough to be used in isolation. In the case of endometriosis, it is likely that more than 1 protein will be necessary to accurately predict its presence. Mihalyi et al. (36) investigated a variety of models and noted that multivariate methods, such as logistic regression and least-squares support vector machine, were significantly more effective than single protein models in predicting early-stage endometriosis. A diagnostic panel could combine multiple biomarkers or include clinical data that have been demonstrated in other conditions (63, 60–62).

Overall, the sensitivity of the biomarkers across the eligible studies ranged from 21% to 100%, and the specificity ranged from 11.1% to 100%, showing the heterogeneity between sensitivity and specificity for individual biomarkers. The only study that presented biomarkers with an LR suggesting strong evidence of indicating endometriosis was the study by Gajbhiye et al. (34) with SLP2a, TMOD3b, TOMD3c, and TMOD3d. The investigators acknowledge the need for further validation in a larger number of patients. In 4 of the eligible studies, the urine biomarkers were combined with blood markers, and in most cases, the combination resulted in an increase in the AUC, sensitivity, and specificity values. Considering these observations, there is a chance that the combined use of urinary and blood markers could facilitate the earlier detection of endometriosis.

One prominent finding in this review is that multiple studies had results that were not confirmed by other studies. The positive outcome of the investigation reported by Tokushige et al. (47) on CK19 was not supported either by Kuessel et al. (41) who revealed that the CK19 levels were not significantly elevated compared with those in controls or by Lessey et al. (45) who calculated a specificity below reasonable cutoff levels, which was corroborated by an LR of 1. Overall, the results of Kuessel et al. (41) and Lessey et al. (45) emphasize that CK19 has limited usefulness as an endometriosis biomarker in primary care. Another example was noted with ENO1 where Yun et al. (51) showed significant differences in the ENO1 levels between patients with endometriosis and their control groups; however, the outcomes of these studies were not supported by Rokhgireh et al. (46) who showed that the difference was not significant after Cr

correction. The inconsistencies of these results, especially in sensitivity and specificity, suggest that standardized techniques are essential for the direct comparisons of results. Some studies had a significant factor of difference, such as IL-33, where 2 studies independently reported mean serum levels of 2.48 ng/mL (66) and 7.5 pg/mL (67). This may, in part, be due to researchers choosing very specific patient populations to control confounders but makes the study both less reproducible and not a clinically representative population.

Choosing an appropriate control group for a clinically feasible diagnostic biomarker for endometriosis is challenging. It is tempting to choose a healthy population, such as women undergoing laparoscopic sterilization; however, it is likely that this may overestimate the efficacy of any test. The most clinically relevant control group would be those who are symptomatic of endometriosis because this is the population a test would be applied to; however, this would include a very heterogeneous group of conditions, such as several patients for whom their symptoms have no identifiable cause. Some studies circumvented this issue by including 2 control groups: those symptomatic of endometriosis with infertility and/or pelvic pain and self-reported healthy volunteers (63, 70). Because we know that some women may be asymptomatic of endometriosis and, therefore, remain undiagnosed, this approach is not infallible but aims to limit bias. Overall, these studies need careful interpretation within their current settings, and none represents a sufficient sample size to be generalized to a wider population.

One of the overarching themes of this SLR was the need to standardize methodology and study design. It was noteworthy that some investigators interrogated 1 variable in depth, such as time of day (65) or menstrual phase (30, 31, 76, 68, 36, 63, 64, 70, 45, 46, 48, 49, 78) while not appearing to consider others. A general limitation noted in all publications was the moderate number of samples used because of the substantial volume of potentially confounding variables including type of endometriosis, menstrual cycle stage, use of hormone treatment, and comorbidities. Only 5 of the included studies described their power calculations (76, 35, 68, 70, 45). Collaborative working is likely to be instrumental in any future biomarker development and will require cooperation between centers to facilitate studies on the basis of larger cohorts (63). The Endometriosis Phenome (and Biobanking) Harmonisation Project (79) aims to facilitate large-scale collaborative research by standardizing both data and sample collection and processing, and future studies should consider using such publicly available resources.

In conclusion, currently, there are no biomarkers with adequate sensitivity and specificity to be used in disease screening, highlighting the need for research into potential biomarkers either alone or in a panel for the rapid detection of endometriosis, decreasing diagnostic delay, and the requirement for invasive surgical procedures that have associated risks and costs. Gajbhiye et al. (34) presented 4 biomarkers—SLP2a, TMOD3b, TOMD3c, and TMOD3d—with promising LRs. There are multiple biomarkers described here that provide exciting avenues for further study particularly as part of diagnostic panels including microRNAs (37) and ILs (40, 67).

The diagnostic delay for women with endometriosis has not improved in a decade (80), which is often rationalized by describing endometriosis as a uniquely complicated disease rather than considering the factors that have contributed to this. One primary example is chronic underinvestment in research (81), with only 0.038% of the National Institutes of Health funding budget allocated to endometriosis in 2022, significantly less than other comparable diseases, such as Crohn's disease, receive (82). Despite having a significant social and economic disease burden, endometriosis has not been recognized as a public health priority in several countries (83). These political, social, and economic factors have hampered endometriosis research and have contributed to the cycle of small studies that do not translate well into clinical practice.

Endometriosis research and management have been characterized by a lack of consensus on even the classification of the disease and its subtypes. Professional bodies, such as the American College of Obstetricians and Gynecologists (84), Collège National des Gynécologues et Obstétriciens Français (85), European Society of Human Reproduction and Embryology (11), National Institute for Health and Care Excellence (86), Royal Australian and New Zealand College of Obstetricians and Gynaecologists (87), and Society of Obstetricians and Gynaecologists of Canada (88), have produced evidence-based guidelines for best clinical practice to standardize patient care. However, by their nature, they are designed to work within their own national health care systems. There is little doubt of the desire from professionals worldwide to attempt to resolve this long-standing problem. A recently published report shows strong support for a new unified descriptive system for the classification of endometriosis (89) to replace the currently available systems; however, the design and implementation of such a system will have its own challenges. The World Endometriosis Society convened a global consortium to address the issue of the classification of endometriosis, and a unanimous consensus could only be achieved in 10 of 28 statements (90). There is optimism to be found, and concerted efforts for international collaboration have resulted in guidelines such as the World Endometriosis Research Foundation that span the collection of detailed surgical, clinical, and epidemiologic phenotyping data together with standard operating procedures for collection, processing, and storage of biologic samples (91). This initiative provides a framework for future robust, epidemiologically sound, globally collaborative research. Only when we address these issues can we truly transform the diagnostic landscape for women with endometriosis.

REFERENCES

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med 2020; 382:1244–56.
2. Sarria-Santamera A, Orazumbekova B, Terzic M, Issanov A, Chaowen C, Asunsolo-Del-Barco A. Systematic review and meta-analysis of incidence and prevalence of endometriosis. Healthcare (Basel) 2020;9:29.
3. Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. Fertil Steril 2009;92:68–74.
4. Saunders PTK. Insights from genomic studies on the role of sex steroids in the aetiology of endometriosis. Reprod Fertil 2022;3:R51–65.

5. Machairiotis N, Vasilakaki S, Thomakos N. Inflammatory mediators and pain in endometriosis: a systematic review. Biomedicines 2021;9:54.
6. Davis AC, Goldberg JM. Extrapelvic endometriosis. Semin Reprod Med 2017;35:98–101.
7. Sinaii N, Plumb K, Cotton L, Lambert A, Kennedy S, Zondervan K, et al. Differences in characteristics among 1,000 women with endometriosis based on extent of disease. Fertil Steril 2008;89:538–45.
8. van Barneveld E, Manders J, van Osch FHM, van Poll M, Visser L, van Hanegem N, et al. Depression, anxiety, and correlating factors in endometriosis: a systematic review and meta-analysis. J Womens Health (Larchmt) 2022;31:219–30.
9. Hsu AL, Sinaii N, Segars J, Nieman LK, Stratton P. Relating pelvic pain location to surgical findings of endometriosis. Obstet Gynecol 2011;118:223–30.
10. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Vigano P. Endometriosis. Nat Rev Dis Primers 2018;4:9.
11. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE guideline: endometriosis. Hum Reprod Open 2022;2022:hoac009.
12. Vermeulen N, Abrao MS, Einarsson JI, Horne AW, Johnson NP, Lee TTM, et al. Endometriosis classification, staging and reporting systems: a review on the road to a universally accepted endometriosis classification. J Minim Invasive Gynecol 2021;28:1822–48.
13. Royal College of Obstetricians and Gynaecologists. Diagnostic laparoscopy (consent advice no. 2). Available at: https://www.rcog.org.uk/guidance/browse-all-guidance/consent-advice/diagnostic-laparoscopy-consent-advice-no-2/. Accessed October 28, 2022.
14. Bafort C, Beebeejaun Y, Tomassetti C, Bosteels J, Duffy JM. Laparoscopic surgery for endometriosis. Cochrane Database Syst Rev 2020;10:CD011031.
15. Ballard K, Lowton K, Wright J. What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. Fertil Steril 2006;86:1296–301.
16. Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: translational evidence of the relationship and implications. Hum Reprod Update 2011;17:327–46.
17. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10:261–75.
18. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol 2019;15:666–82.
19. Njoku K, Chiasserini D, Jones ER, Barr CE, O'Flynn H, Whetton AD, et al. Urinary biomarkers and their potential for the non-invasive detection of endometrial cancer. Front Oncol 2020;10:559016.
20. Jing J, Gao Y. Urine biomarkers in the early stages of diseases: current status and perspective. Discov Med 2018;25:57–65.
21. Grayson K, Gregory E, Khan G, Guinn BA. Urine biomarkers for the early detection of ovarian cancer - are we there yet? Biomark Cancer 2019;11:1179299X19830977.
22. Liu E, Nisenblat V, Farquhar C, Fraser I, Bossuyt PM, Johnson N, et al. Urinary biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2015;2015:CD012019.
23. Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, et al. Endometrial biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;4:CD012165.
24. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;2016:CD012179.
25. May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. Hum Reprod Update 2010;16:651–74.
26. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4:1.
27. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015;350:g7647.
28. Wohlin C. Guidelines for snowballing in systematic literature studies and a replication in software engineering. Proceedings of the 18th International Conference on Evaluation and Assessment in Software Engineering 2014;38:1–10.
29. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.
30. El-Kasti MM, Wright C, Fye HK, Roseman F, Kessler BM, Becker CM. Urinary peptide profiling identifies a panel of putative biomarkers for diagnosing and staging endometriosis. Fertil Steril 2011;95, 1261–1266.e1–6.
31. 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;105:988–96.
32. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. BMJ 2004;329:168–9.
33. Jaeschke R, Guyatt G, Lijmer J. Diagnostic tests. In: Guyatt G, Rennie D, editors. Uses' guides to the medical literature. A manual for evidence-based clinical practice. Chicago: AMA Press; 2002:121–40.
34. Gajbhiye R, Sonawani A, Khan S, Suryawanshi A, Kadam S, Warty N, et al. Identification and validation of novel serum markers for early diagnosis of endometriosis. Hum Reprod 2012;27:408–17.
35. Hirsch M, Duffy JMN, Deguara CS, Davis CJ, Khan KS. Diagnostic accuracy of Cancer Antigen 125 (CA125) for endometriosis in symptomatic women: a multi-center study. Eur J Obstet Gynecol Reprod Biol 2017;210:102–7.
36. 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;25:654–64.
37. Misir S, Hepokur C, Oksasoglu B, Yildiz C, Yanik A, Aliyazicioglu Y. Circulating serum miR-200c and miR-34a-5p as diagnostic biomarkers for endometriosis. J Gynecol Obstet Hum Reprod 2021;50:102092.
38. Szubert M, Suzin J, Wierzbowski T, Kowalczyk-Amico K. CA-125 concentration in serum and peritoneal fluid in patients with endometriosis - preliminary results. Arch Med Sci 2012;8:504–8.
39. 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;290:75–82.
40. Mosbah A, Nabiel 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;134:247–51.
41. Kuessel L, Jaeger-Lansky A, Pateisky P, Rossberg N, Schulz A, Schmitz AA, et al. Cytokeratin-19 as a biomarker in urine and in serum for the diagnosis of endometriosis–a prospective study. Gynecol Endocrinol 2014;30:38–41.
42. Othman ER, Markeb AA, Khashbah MY, Abdelaal II, ElMelegy TT, Fetih AN, et al. Markers of local and systemic estrogen metabolism in endometriosis. Reprod Sci 2021;28:1001–11.
43. Potlog-Nahari C, Stratton P, Winkel C, Widra E, Sinaii N, Connors S, et al. Urine vascular endothelial growth factor-A is not a useful marker for endometriosis. Fertil Steril 2004;81:1507–12.
44. Proestling K, Wenzl R, Yotova I, Hauser C, Husslein H, Kuessel L. Investigating selected adhesion molecules as urinary biomarkers for diagnosing endometriosis. Reprod Biomed Online 2020;40:555–8.
45. Lessey BA, Savaris RF, Ali S, Brophy S, Tomazic-Allen S, Chwalisz K. Diagnostic accuracy of urinary cytokeratin 19 fragment for endometriosis. Reprod Sci 2015;22:551–5.
46. 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 Res Int 2020;2020:5208279.
47. 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;95:46–9.
48. 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;104:1202–9.

49. Wang L, Liu HY, Shi HH, Lang JH, Sun W. Urine peptide patterns for non-invasive diagnosis of endometriosis: a preliminary prospective study. Eur J Obstet Gynecol Reprod Biol 2014;177:23–8.
50. Williams KE, Miroshnychenko O, Johansen EB, Niles RK, Sundaram R, Kannan K, et al. Urine, peritoneal fluid and omental fat proteomes of reproductive age women: endometriosis-related changes and associations with endocrine disrupting chemicals. J Proteomics 2015;113:194–205.
51. Yun BH, Lee YS, Chon SJ, Jung YS, Yim SY, Kim HY, et al. Evaluation of elevated urinary enolase I levels in patients with endometriosis. Biomarkers 2014;19:16–21.
52. Rekker K, Tasa T, Saare M, Samuel K, Kadastik U, Karro H, et al. Differentially-expressed miRNAs in ectopic stromal cells contribute to endometriosis development: the plausible role of miR-139-5p and miR-375. Int J Mol Sci 2018;19:3789.
53. Fassbender A, Burney RO, O DF, D'Hooghe T, Giudice L. Update on biomarkers for the detection of endometriosis. Biomed Res Int 2015;2015:130854.
54. 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;32:770–9.
55. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, et al. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. Hum Reprod 2012;27:1292–9.
56. Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;2:CD009591.
57. Nisenblat V, Prentice L, Bossuyt PM, Farquhar C, Hull ML, Johnson N. Combination of the non-invasive tests for the diagnosis of endometriosis. Cochrane Database Syst Rev 2016;7:CD012281.
58. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, et al. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol 2019;220:354.e1–12.
59. Scholler N, Urban N. CA125 in ovarian cancer. Biomark Med 2007;1:513–23.
60. Taneja I, Reddy B, Damhorst G, Dave Zhao S, Hassan U, Price Z, et al. Combining biomarkers with EMR data to identify patients in different phases of sepsis. Sci Rep 2017;7:10800.
61. Terracciano D, La Civita E, Athanasiou A, Liotti A, Fiorenza M, Cennamo M, et al. New strategy for the identification of prostate cancer: the combination of Proclarix and the prostate health index. Prostate 2022;82:1469–76.
62. Katzendorn O, von Klot CAJ, Mahjoub S, Faraj Tabrizi P, Harke NN, Tezval H, et al. Combination of PI-RADS score and mRNA urine test-a novel scoring system for improved detection of prostate cancer. PLoS One 2022;17:e0271981.
63. Pateisky P, Pils D, Szabo L, Kuessel L, Husslein H, Schmitz A, et al. hsa-miRNA-154-5p expression in plasma of endometriosis patients is a potential diagnostic marker for the disease. Reprod Biomed Online 2018;37:449–66.
64. Vanhie A, O D, Peterse D, Beckers A, Cuellar A, Fassbender A, et al. Plasma miRNAs as biomarkers for endometriosis. Hum Reprod 2019;34:1650–60.
65. Rekker K, Saare M, Roost AM, Kaart T, Sõritsa D, Karro H, et al. Circulating miR-200-family micro-RNAs have altered plasma levels in patients with endometriosis and vary with blood collection time. Fertil Steril 2015;104:938–46.e2.
66. Mbarik M, Kaabachi W, Henidi B, Sassi FH, Hamzaoui K. Soluble ST2 and IL-33: potential markers of endometriosis in the Tunisian population. Immunol Lett 2015;166:1–5.
67. Santulli P, Borghese B, Chouzenoux S, Vaiman D, Borderie D, Streuli I, et al. Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis. Hum Reprod 2012;27:2001–9.
68. Karakus S, Sancakdar E, Akkar O, Yildiz C, Demirpence O, Cetin A. Elevated serum CD95/FAS and HIF-1α levels, but not Tie-2 levels, may be biomarkers in patients with severe endometriosis: a preliminary report. J Minim Invasive Gynecol 2016;23:573–7.
69. DF O, Waelkens E, Peterse DP, Lebovic D, Meuleman C, Tomassetti C, et al. Evaluation of total, active, and specific myeloperoxidase levels in women with and without endometriosis. Gynecol Obstet Invest 2018;83:133–9.
70. Webster KE, Kennedy SH, Becker CM. Levels of circulating angiogenic cells are not altered in women with endometriosis. Hum Reprod 2013;28:651–7.
71. Cho S, Choi YS, Yim SY, Yang HI, Jeon YE, Lee KE, et al. Urinary vitamin D-binding protein is elevated in patients with endometriosis. Hum Reprod 2012;27:515–22.
72. Wang L, Liu HY, Shi HH, Lang JH, Sun W. Urine peptide patterns for non-invasive diagnosis of endometriosis: a preliminary prospective study. Eur J Obstet Gynecol Reprod Biol 2014;177:23–8.
73. 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.
74. Cho SH, Oh YJ, Nam A, Kim HY, Park JH, Kim JH, et al. Evaluation of serum and urinary angiogenic factors in patients with endometriosis. Am J Reprod Immunol 2007;58:497–504.
75. Bostanci Durmus A, Dincer Cengiz S, Yılmaz H, Candar T, Gursoy AY, Sinem Caglar G. The levels of matrix metalloproteinase-9 and neutrophil gelatinase-associated lipocalin in different stages of endometriosis. J Obstet Gynaecol 2019;39:991–5.
76. Fassbender A, D'Hooghe T, Mihalyi A, Kyama C, Simsa P, Lessey BA. Plasma C3a-des-Arg levels in women with and without endometriosis. Am J Reprod Immunol 2009;62:187–95.
77. Othman ER, Hornung D, Hussein M, Abdelaal II, Sayed AA, Fetih AN, et al. Soluble tumor necrosis factor-alpha receptors in the serum of endometriosis patients. Eur J Obstet Gynecol Reprod Biol 2016;200:1–5.
78. 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;98:281–9.
79. Becker CM, Laufer MR, Stratton P, Hummelshoj L, Missmer SA, Zondervan KT, et al. World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project: I. Surgical phenotype data collection in endometriosis research. Fertil Steril 2014;102:1213–22.
80. Ghai V, Jan H, Shakir F, Haines P, Kent A. Diagnostic delay for superficial and deep endometriosis in the United Kingdom. J Obstet Gynaecol 2020;40:83–9.
81. Horne AW, Saunders PTK, Abokhrais IM, Hogg L. Top ten endometriosis research priorities in the UK and Ireland. Lancet 2017;389:2191–2.
82. Ellis K, Munro D, Clarke J. Endometriosis is undervalued: a call to action. Front Glob Womens Health 2022;3:902371.
83. Hudson N. The missed disease? Endometriosis as an example of 'undone science'. Reprod Biomed Soc Online 2022;14:20–7.
84. Practice bulletin no. 114: management of endometriosis. Obstet Gynecol 2010;116:223–36.
85. Collinet P, Fritel X, Revel-Delhom C, Ballester M, Bolze PA, Borghese B, et al. [Management of endometriosis: CNGOF-HAS practice guidelines (short version)]. Gynecol Obstet Fertil Senol 2018;46:144–55.
86. Diagnosis and management of endometriosis: summary of NICE guidance. BMJ 2017;358:j4227.
87. Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Endometriosis clinical practice guideline. Available at: https://ranzcog.edu.au/resources/endometriosis-clinical-practice-guideline/. Accessed October 28, 2022.
88. Leyland N, Casper R, Laberge P, Singh SS. SOGC. Endometriosis: diagnosis and management. J Obstet Gynaecol Can 2010;32:S1–32.
89. Zondervan KT, Missmer S, Abrao MS, Einarsson JI, Horne AW, Johnson NP, et al. Endometriosis classification systems: an international survey to map current knowledge and uptake. J Minim Invasive Gynecol 2022;29:716–25.e1.
90. 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;32:315–24.
91. 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. Fertil Steril 2014;102:1223–32.
92. 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;94:2343–6.
93. Draj HA, Abbas AA, Abdullah TH. Serum and urine levels of cytokeratin-19 in endometriosis. Ann Trop Med Public Health 2020;23:SP231440.
94. Gjavotchanoff R. CYFRA 21-1 in urine: a diagnostic marker for endometriosis? Int J Womens Health 2015;7:205–11.