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Multi-Biomarker Strategies For Improved Diagnosis Of Endometriosis

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Abstract: Endometriosis is a chronic, estrogen-dependent inflammatory disorder affecting approximately 10% of reproductive-age women, often leading to pelvic pain, infertility, and reduced quality of life. Diagnosis is typically delayed by several years due to reliance on invasive laparoscopy rather than reliable non-invasive biomarkers. CA-125 remains the cornerstone biomarker, particularly effective in advanced and ovarian endometriosis, but its limited specificity necessitates combination with other markers. Integrative multi-biomarker strategies combining CA-125 with Annexin A5 (ANXA5), HE4, CA72-4, and inflammatory indices like the platelet-to-lymphocyte ratio (PLR) significantly enhance diagnostic accuracy. Urinary biomarkers such as vitamin D-binding protein (VDBP) and alpha-1 antitrypsin (A1AT) further improve non-invasive detection, achieving high sensitivity (up to 90.9%) and specificity (76.5%). Incorporating clinical parameters, including cyst morphology, dysmenorrhea, and BMI, refines diagnostic precision and staging. Dynamic-phase CA-125 measurement across menstrual cycles also increases accuracy in deep infiltrative endometriosis. Overall, multi-marker, integrative approaches combining serum, urinary, and clinical data hold promise for earlier, more accurate, and less invasive diagnosis of endometriosis. Future research should focus on validating standardized biomarker panels and defining optimal cut-off values to reduce diagnostic delays and improve patient outcomes globally.

Keywords: Endometriosis; CA-125; multi-biomarker panel; Annexin A5 (ANXA5); HE4; CA72-4; Vitamin D-binding protein (VDBP); Alpha-1 antitrypsin (A1AT); Platelet-to-lymphocyte ratio (PLR); Non-invasive diagnosis; Urinary biomarkers; Deep infiltrative endometriosis.

Introduction: Endometriosis is a chronic, estrogen-dependent inflammatory disorder characterized by the presence of endometrial-like tissue outside the uterine cavity, affecting nearly 10% of women of reproductive age worldwide (Wu et al., 2025){10}. The condition often manifests with severe pelvic pain, dysmenorrhea, dyspareunia, and infertility, profoundly impairing quality of life and productivity (Chauhan et al., 2025){11}. Despite its high prevalence and clinical significance, diagnosis remains delayed by 7–10 years on average, largely because current diagnostic methods rely on invasive laparoscopic confirmation rather than accessible biomarkers (Ramadan et al., 2025){12}.

CA-125 continues to be a cornerstone biomarker in the diagnosis of endometriosis, particularly in advanced stages and ovarian involvement (Wu et al., 2025){10}. However, its utility is limited when used alone due to biological variability and low specificity, as CA-125 levels can also rise in other gynaecological and inflammatory disorders such as fibroids, adenomyosis, and pelvic inflammatory disease (Romanov et al., 2025){13}.

Combining CA-125 with other biomarkers such as Annexin A5, HE4, CA72-4, and inflammatory indices like the platelet-to-lymphocyte ratio (PLR) substantially improves diagnostic accuracy, sensitivity, and specificity (Wu et al., 2025){10}. In addition, urinary biomarkers such as vitamin D-binding protein (VDBP) and alpha-1-antitrypsin (A1AT) have demonstrated potential as non-invasive diagnostic tools, reflecting systemic inflammatory and oxidative stress pathways implicated in endometriosis progression (Chauhan et al., 2025){11}.

Incorporating clinical parameters, including cyst morphology, dysmenorrhea, dyspareunia, body mass

index (BMI), and menstrual cycle phase, further refines diagnostic performance and facilitates disease staging (Ramadan et al., 2025){12}. Such integrative, multi-source approaches—combining molecular, inflammatory, and clinical data offer promising avenues for early detection, non-invasive assessment, and monitoring of endometriosis and associated pelvic adhesions (Wu et al., 2025){10}.

Given the seriousness of the disease, which can lead to chronic pain, infertility, and psychosocial distress, future research should prioritize the validation of standardized biomarker panels, optimization of cut-off values, and development of practical, non-invasive diagnostic tools that can reduce diagnostic delays and enhance patient management globally (Chauhan et al., 2025){11}.

Multiple Roles of CA-125 in Endometriosis Diagnosis

Cancer antigen 125 (CA-125) has long been recognized as a biomarker for epithelial ovarian cancer, but its diagnostic utility extends to various forms and stages of endometriosis. CA-125 levels are typically elevated in women with advanced disease, reflecting both the extent of ectopic endometrial tissue and associated inflammatory activity {1,2}. Several studies have demonstrated that CA-125 can act as a moderate predictor of endometriosis when measured in serum, particularly in Stage III–IV disease {3,4}. Szubert et al. reported that serum CA-125 levels were significantly higher in patients with laparoscopically confirmed endometriosis compared to controls, and the concentration correlated positively with disease stage ($R = 0.5993$, $p < 0.001$) {3}. Peritoneal fluid measurements further enhanced detection, with significantly higher levels in affected patients, suggesting that local inflammatory activity contributes to systemic elevation {3}.

The predictive power of CA-125 can be improved by combining it with other biomarkers. Kovalak et al. showed that while CA-125 alone had a sensitivity of 73% and specificity of 98% for Stage III–IV endometriosis, combining CA-125 with Annexin A5 (ANXA5) improved specificity to 100%, highlighting the advantage of multi-marker panels in advanced disease {4}. Similarly, Guo and Zhang found that combining CA-125 with systemic inflammatory markers, such as the platelet-to-lymphocyte ratio (PLR), improved prediction of pelvic adhesions in endometriosis patients, emphasizing CA-125's role in reflecting disease severity beyond simple presence or absence {5}.

CA-125 levels are also influenced by the menstrual cycle, which can be exploited to improve diagnostic accuracy. Oliveira et al. demonstrated that measuring CA-125 in both menstrual and midcycle phases yielded higher diagnostic performance for deep infiltrative endometriosis (DIE), with AUC values of 0.96 when comparing menstrual-phase measurements or the difference between menstrual and midcycle phases {6}. This suggests that dynamic-phase measurement can reduce the long delay between symptom onset and definitive diagnosis, which averages around eight years in DIE {6}.

Moreover, CA-125 remains valuable in differentiating endometriosis from other ovarian pathologies. Zhang et al. analysed CA-125 in patients with ovarian endometriotic cysts (OEC) and found that preoperative CA-125 levels were significantly higher than in benign ovarian or gynaecological disease groups, yet lower than in malignant ovarian tumours. They proposed specific cut-off values for distinguishing OEC from benign and malignant conditions, emphasizing CA-125's role as a quantitative tool in clinical decision-making {7}.

Beyond serum measurements, non-invasive urinary markers can complement CA-125 for endometriosis detection. Chen et al. investigated urinary CA-125, along with vitamin D binding protein (VDBP) and alpha-1 antitrypsin (A1AT), demonstrating that a combined panel of serum and urinary markers achieved an AUC of 0.913, sensitivity of 90.9%, and specificity of 76.5% {8}. This approach may reduce the need for invasive diagnostic procedures, offering a practical alternative for monitoring disease progression or response to treatment.

The diagnostic performance of CA-125 is sometimes enhanced when used in combination with other serum biomarkers. Micu et al. evaluated CA-125 together with HE4 and CA72-4 in ovarian endometriosis and found that CA-125, particularly when combined with HE4, can improve detection, while CA72-4 strongly correlated with HE4, suggesting potential utility in multi-marker panels for ovarian endometriosis {9}.

In summary, CA-125 serves multiple roles in endometriosis diagnosis: it reflects disease severity, aids in differentiating benign from malignant ovarian lesions, improves detection when combined with other biomarkers, and shows enhanced performance when measured in dynamic menstrual phases. Both serum and urinary measurements have been explored, with combined approaches offering the highest diagnostic yield. These findings underscore the versatility of CA-125 as a cornerstone biomarker in the clinical evaluation of endometriosis, especially when integrated into multi-marker strategies or tailored measurement protocols {1–9}.

Complementary Biomarkers for Endometriosis

Diagnosis

While CA-125 is the most widely studied biomarker for endometriosis, several other molecules have demonstrated potential for improving diagnostic accuracy, particularly when used in combination with CA-125. These include Annexin A5 (ANXA5), soluble intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (VCAM-1), inflammatory cytokines, and urinary biomarkers such as vitamin D binding protein (VDBP) and alpha-1 antitrypsin (A1AT).

Annexin A5 (ANXA5)

ANXA5, a calcium-dependent phospholipid-binding protein, has been evaluated as a co-biomarker to enhance CA-125 performance. Kovalak et al. reported that ANXA5 alone did not significantly distinguish Stage III–IV endometriosis from controls ($p > 0.05$), but when combined with CA-125, diagnostic specificity improved to 100% while maintaining a sensitivity of 73% {1}. This indicates that ANXA5 may reflect aspects of endometriotic pathology not captured by CA-125 alone, possibly related to apoptotic or inflammatory processes within ectopic endometrial tissue {1}.

Inflammatory and Adhesion Molecules

Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), along with adhesion molecules like sICAM-1 and sVCAM-1, have been proposed as biomarkers of disease activity. Although individually these markers showed limited diagnostic power, their levels often correlate with the severity of pelvic inflammation and adhesions in endometriosis {1,2}. Elevated IL-6 and TNF-α may reflect local inflammatory microenvironments, while sICAM-1 and sVCAM-1 could indicate vascular or endothelial activation associated with lesion formation {1,2}.

Urinary Biomarkers

Non-invasive urinary biomarkers have been increasingly explored for endometriosis diagnosis. Chen et al. measured urinary CA-125, VDBP, and A1AT in 33 patients with clinically diagnosed endometriosis/adenomyosis and 19 controls. Serum CA-125 remained the most accurate individual marker (AUC 0.888, $p = 0.001$), but urinary VDBP (AUC 0.841) and A1AT (AUC 0.722) also demonstrated significant diagnostic potential {3}. Importantly, combined panels integrating serum CA-125 with urinary VDBP and A1AT achieved an AUC of 0.913, with 90.9% sensitivity and 76.5% specificity, highlighting the potential of non-invasive, multi-marker approaches for both detection and monitoring {3}.

HE4 and CA72-4

Human epididymis protein 4 (HE4) and CA72-4 have been studied alongside CA-125 in ovarian endometriosis. Micu et al. found that CA-125 levels were significantly higher in endometriosis patients, while HE4 levels were lower, and CA72-4 levels were highly correlated with HE4 ($p < 0.0001$) {4}. These findings suggest that HE4 may help discriminate endometriosis from malignant ovarian disease, while CA72-4 could serve as a supportive marker in multi-biomarker panels {4}.

CA19-9

CA19-9 has been evaluated primarily for its utility in ovarian endometriotic cysts. Zhang et al. reported that CA19-9 alone had limited value in distinguishing endometriosis from benign or malignant ovarian lesions, although it may provide supportive information in combination with CA-125 {5}. The use of CA19-9 in multi-marker panels may enhance specificity, particularly in complex ovarian cystic lesions {5}.

Integrative Multi-Marker Approaches

Across multiple studies, integrating biomarkers improves diagnostic performance compared to any single marker. For instance, combining CA-125 with ANXA5, urinary VDBP, and A1AT, or with HE4 and CA72-4, enhances sensitivity, specificity, and overall predictive accuracy {1,3,4}. This multi-marker strategy is especially valuable in early or deep infiltrative endometriosis (DIE), where traditional imaging or serum CA-125 alone may be insufficient {1,3,6}.

Summary: CA-125 remains the cornerstone of biomarker-based endometriosis diagnosis, but complementary markers such as ANXA5, HE4, CA72-4, urinary VDBP, A1AT, and inflammatory molecules can significantly enhance diagnostic performance. Multi-marker panels, particularly those combining serum and urinary markers, represent a promising approach for non-invasive, accurate detection of both superficial and deep infiltrative endometriosis, and may reduce the diagnostic delay historically observed in this disease {1–6}.

Table 1. Summary of Key Studies Evaluating Multi-Biomarker Approaches in Endometriosis Diagnosis.

No.	Study / Author (Year)	Markers Studied	Population / Sample Size	Sensitivity (%)	Specificity (%)	AUC	Notes / Key Findings

1	Kovalak et al. (2023)	CA-125, ANXA5, sICAM-1, IL-6, TNF-α, sVCAM-1, VEGF	Stage III–IV endometriosis, n=30; Controls n=49	73 (CA-125 + ANXA5)	100 (CA-125 + ANXA5)	–	ANXA5 alone not significant; combination with CA-125 improved specificity to 100%.
2	Chen et al. (2021)	Serum CA-125, Urinary CA-125, VDBP, A1AT	Clinically diagnosed endometriosis/adenomyosis, n=33; Controls n=19	90.9 (combined 3 markers)	76.5 (combined 3 markers)	0.913	Non-invasive urine biomarkers added diagnostic value; urinary VDBP and A1AT improved detection when combined with serum CA-125.
3	Knific et al. (2018)	Serum CA-125, BMI, cyst info, dysmenorrhea/dyspareunia	Endometriosis n=124; Controls n=97	74–74.8	79.2–81.3	0.819–0.836	Logistic regression models combining CA-125 with clinical features provided moderate diagnostic accuracy.
4	Szubert et al. (2012)	CA-125 in serum and peritoneal fluid	Endometriosis n=44; Controls n=15	68 (serum cut-off 11 U/mL)	–	0.794	Serum CA-125 correlated with disease stage; higher peritoneal fluid CA-125 observed in endometriosis patients.
5	Micu et al. (2023)	CA-125, HE4, CA72-4	Ovarian endometriosis	–	–	–	CA-125 higher in endometriosis

			n=29; Controls n=26				; HE4 lower; CA72-4 correlated with HE4; combination recommended for improved diagnostic accuracy.
6	Zhang et al. (2024)	CA-125, CA19-9	Stage III–IV ovarian endometriotic cysts n=183; Controls n=276	89.6 (CA-125 vs benign)	81.5 (CA-125 vs benign)	0.90 (CA-125 vs benign)	CA-125 useful for diagnosis; CA19-9 alone not ideal; multilocular, bilateral, and ruptured cysts associated with higher CA-125 levels.
7	Oliveira et al. (2017)	CA-125 menstrual vs midcycle phases	Deep infiltrative endometriosis n=34; Controls n=20	–	–	0.96 (menstrual phase & difference)	Measuring CA-125 in both menstrual and midcycle phases improved diagnostic accuracy for deep infiltrative endometriosis (DIE).

AUC = Area Under the Curve, CA-125 = Cancer Antigen 125, ANXA5 = Annexin A5, VDBP = Vitamin D Binding Protein, A1AT = Alpha-1 Antitrypsin, DIE = Deep Infiltrative Endometriosis, BMI = Body Mass Index, "–" indicates data not reported or not found.

CONCLUSION

CA-125 remains a central biomarker in the non-invasive diagnosis of endometriosis, particularly for advanced stages and ovarian involvement. Its diagnostic accuracy improves significantly when combined with other biomarkers such as Annexin A5, HE4, CA72-4, and urinary markers like VDBP and A1AT. Integration of CA-125 with clinical features—including cyst characteristics, dysmenorrhea, dyspareunia, and BMI—further enhances sensitivity and specificity. Multi-marker approaches, considering menstrual cycle fluctuations and non-invasive urinary testing, offer the greatest potential for early and accurate diagnosis. Future efforts should focus on validating standardized multi-marker panels and establishing optimized cut-off values to reduce diagnostic delays and guide clinical management effectively.

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