OXFORD

human reproduction

Human Reproduction, 2025, **40(2)**, 270–279
https://doi.org/10.1093/humrep/deae278
Advance Access Publication Date: December 24, 2024
Original Article

Gynaecology

Identification of plasma protein biomarkers for endometriosis and the development of statistical models for disease diagnosis

E.M. Schoeman [1], S. Bringans[1], K. Peters [1,*], T. Casey[1], C. Andronis[1], L. Chen[1], M. Duong[1], J.E. Girling[2,3], M. Healey[2], B.A. Boughton[4], D. Ismail[1], J. Ito[1], C. Laming[1], H. Lim[1], M. Mead[1], M. Raju[1], P. Tan[1], R. Lipscombe[1], S. Holdsworth-Carson[2,5], and P.A.W. Rogers[2]

[1]Proteomics International, Nedlands, WA, Australia
[2]Department of Obstetrics and Gynecology, University of Melbourne and Gynecology Research Centre, Royal Women's Hospital, Melbourne, VIC, Australia
[3]Department of Anatomy, School of Biological Sciences, University of Otago, Dunedin, New Zealand
[4]School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, VIC, Australia
[5]Julia Argyrou Endometriosis Centre, Epworth HealthCare, Melbourne, VIC, Australia

*Correspondence address. Proteomics International, 6 Verdun Street, Nedlands, Western Australia, 6009, Australia. E-mail: kirsten@proteomics.com.au https://orcid.org/0000-0003-1406-8797

ABSTRACT

STUDY QUESTION: Can a panel of plasma protein biomarkers be identified to accurately and specifically diagnose endometriosis?

SUMMARY ANSWER: A novel panel of 10 plasma protein biomarkers was identified and validated, demonstrating strong predictive accuracy for the diagnosis of endometriosis.

WHAT IS KNOWN ALREADY: Endometriosis poses intricate medical challenges for affected individuals and their physicians, yet diagnosis currently takes an average of 7 years and normally requires invasive laparoscopy. Consequently, the need for a simple, accurate non-invasive diagnostic tool is paramount.

STUDY DESIGN, SIZE, DURATION: This study compared 805 participants across two independent clinical populations, with the status of all endometriosis and symptomatic control samples confirmed by laparoscopy. A proteomics workflow was used to identify and validate plasma protein biomarkers for the diagnosis of endometriosis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A proteomics discovery experiment identified candidate biomarkers before a targeted mass spectrometry assay was developed and used to compare plasma samples from 464 endometriosis cases, 153 general population controls, and 132 symptomatic controls. Three multivariate models were developed: Model 1 (logistic regression) for endometriosis cases versus general population controls, Model 2 (logistic regression) for rASRM stage II to IV (mild to severe) endometriosis cases versus symptomatic controls, and Model 3 (random forest) for stage IV (severe) endometriosis cases versus symptomatic controls.

MAIN RESULTS AND THE ROLE OF CHANCE: A panel of 10 protein biomarkers were identified across the three models which added significant value to clinical factors. Model 3 (severe endometriosis vs symptomatic controls) performed the best with an area under the receiver operating characteristic curve (AUC) of 0.997 (95% CI 0.994–1.000). This model could also accurately distinguish symptomatic controls from early-stage endometriosis when applied to the remaining dataset (AUCs ≥0.85 for stage I to III endometriosis). Model 1 also demonstrated strong predictive performance with an AUC of 0.993 (95% CI 0.988–0.998), while Model 2 achieved an AUC of 0.729 (95% CI 0.676–0.783).

LIMITATIONS, REASONS FOR CAUTION: The study participants were mostly of European ethnicity and the results may be biased from undiagnosed endometriosis in controls. Further analysis is required to enable the generalizability of the findings to other populations and settings.

WIDER IMPLICATIONS OF THE FINDINGS: In combination, these plasma protein biomarkers and resulting diagnostic models represent a potential new tool for the non-invasive diagnosis of endometriosis.

STUDY FUNDING/COMPETING INTEREST(S): Subject recruitment at The Royal Women's Hospital, Melbourne, was supported in part by funding from the Australian National Health and Medical Research Council (NHMRC) project grants GNT1105321 and GNT1026033 and Australian Medical Research Future Fund grant no. MRF1199715 (P.A.W.R., S.H.-C., and M.H.). Proteomics International has filed patent WO 2021/184060 A1 that relates to endometriosis biomarkers described in this manuscript; S.B., R.L., and T.C. declare an interest in this patent. J.I., S.B., C.L., D.I., H.L., K.P., M.D., M.M., M.R., P.T., R.L., and T.C. are shareholders in Proteomics International. Otherwise, the authors have no conflicts of interest.

TRIAL REGISTRATION NUMBER: N/A.

Keywords: endometriosis / diagnosis / proteomics / statistical models / plasma protein biomarkers

Received: June 18, 2024. **Revised:** November 12, 2024. **Editorial decision:** November 28, 2024.

© The Author(s) 2024. Published by Oxford University Press on behalf of European Society of Human Reproduction and Embryology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

Introduction

Endometriosis is a chronic and progressive inflammatory disease characterized by the presence of estrogen-dependent endometrial-like tissue (or lesions) outside the uterus. Its symptoms include persistent pelvic pain and infertility. Endometriosis occurs in ∼11% of women and girls of reproductive age (Rowlands *et al.*, 2021) and has been observed in 35% of women using assisted reproductive technology procedures (Moss *et al.*, 2021). Disease severity does not always correlate with symptom severity, leading to diagnostic challenges and limited treatment options. In addition to the acute and chronic symptoms associated with the condition, endometriosis has been linked to long-term negative health consequences, including a higher risk of cardiovascular disease, ovarian cancer, and autoimmune diseases (Eisenberg *et al.*, 2022; Okoli *et al.*, 2023).

Based on the location and depth of lesions, the main types of endometriosis are superficial (peritoneal or other sites), deep endometriosis (DE), and ovarian (endometrioma). Disease stage is most commonly classified based on the revised American Society for Reproductive Medicine (rASRM) system, which considers the location, extent, and depth of lesions, as well as adhesions, all visualized at surgery (American Society for Reproductive Medicine, 1997). Despite its recognition for over a century, the exact cause of endometriosis remains elusive, resulting in delays in diagnosis and treatment. With the time for patient diagnosis averaging 7 years from symptom onset, there are negative impacts on physical, mental, and social well-being (Ellis *et al.*, 2022). Endometriosis also imposes a substantial economic burden due to productivity losses and healthcare costs (Ellis *et al.*, 2022).

Diagnosis involves medical history, physical examination, imaging, laparoscopy, and histopathology. The dependability of current diagnostic tools varies, owing to factors such as the location and severity of lesions, as well as the experience of the healthcare provider (Zondervan *et al.*, 2020). The gold standard for diagnosing endometriosis is via laparoscopy, but the procedure is invasive and costly, and carries risks including adverse events such as nerve damage, damage to pelvic organs or major blood vessels, and formation of post-surgical adhesions (Nisenblat *et al.*, 2016a). Imaging techniques such as transvaginal ultrasound and magnetic resonance imaging can identify ovarian endometriosis and DE, nonetheless, the non-invasive identification of endometriosis, particularly in superficial cases, continues to pose a challenge (Nisenblat *et al.*, 2016a).

Non-invasive diagnostic biomarkers would significantly improve early detection and management of endometriosis. Several potential blood biomarkers have been proposed, however, studies to date have been limited by cohort size or lacked validation studies (Nisenblat *et al.*, 2016b; Lipscombe *et al.*, 2021). This study aimed to identify and validate plasma protein biomarkers specific to endometriosis using a proteomics-based approach, which involved discovery, analytical, and clinical validation phases. The study hypothesized that people with endometriosis would have significantly different plasma concentrations of select proteins compared to the general population, or to those with similar pelvic symptoms but no endometriosis, and that such plasma biomarkers could be used for early diagnostic screening.

Materials and methods

Ethical approval

Recruitment and collection protocols were approved by the appropriate ethical review boards (Bellberry Human Research Ethics Committee (ref: 2016-05-383); Royal Women's Hospital Human Research Ethics Committee (Project No. 10-43, No. 11-24, and No. 16-43)), and all participants provided informed written consent.

Clinical and reference samples

Discovery phase

The discovery phase included samples from 22 endometriosis cases, 15 symptomatic controls, and 19 general population controls obtained from the Wesley Medical Research Institute Biobank (Brisbane, Australia). Samples were pooled in each of the clinical groups and differentially expressed proteins were compared between groups. All endometriosis and symptomatic control samples had their status confirmed by laparoscopy.

Analytical validation phase

Pooled reference plasma (from three donors, obtained from the Australian Red Cross Lifeblood) was used to design targeted assays to measure each biomarker peptide identified from the discovery phase and test the robustness of that measurement, providing analytical validation of the reproducibility of the biomarkers identified.

Clinical validation phase

To clinically validate the candidate protein biomarkers identified in the discovery phase each protein was measured in individual clinical samples from a separate cohort. Samples comprised those of endometriosis cases (n = 464 diagnosed via laparoscopy and confirmed with histopathology) and symptomatic controls without endometriosis (n = 132, confirmed with laparoscopy), obtained from the Royal Women's Hospital (RWH) (Melbourne, Australia). In addition, general population control samples (n = 153) were obtained from healthy volunteers in the Perth metropolitan area.

All RWH participants attended the Endometriosis and Pelvic Pain Clinic with pelvic, menstrual, and/or intercourse pain and underwent laparoscopy for treatment of endometriosis or suspected endometriosis with histopathology to confirm the presence or absence of endometriosis. Endometriosis severity was classified by the rASRM score including stage I/minimal (1–5), stage II/mild (6–15), stage III/moderate (16–40), and stage IV/severe (>40) (American Society for Reproductive Medicine, 1997). Exclusion criteria included menopause, positive pregnancy test or unknown pregnancy status, and malignancy. Comprehensive demographic and clinical information including age, BMI, age at menarche, gravidity, live births, problems conceiving, type of pelvic pain (menstrual/pelvic/intercourse), menstrual cycle length, smoking status, exogenous hormone medication use, family history of endometriosis, and ethnicity was available. All general population controls answered a comprehensive survey to exclude possible symptomatic endometriosis and other gynecological pathologies.

Sample collection

In all cases, whole blood was collected in EDTA-treated vacutainers (Becton Dickinson, USA) and plasma was prepared by centrifugation at 1500*g* for 10 min at 4°C. Plasma samples were stored at −80°C until biomarker analysis. For endometriosis cases and symptomatic controls (Wesley and RWH participants), plasma was collected on the day of admission for surgery. The median time for plasma processing was within one day of collection for all cohorts, with samples stored at 4°C between collection and centrifugation. The Wesley samples were collected between 2010 and 2017, the RWH samples between 2012 and

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

2022, and the general population controls between 2021 and 2022.

Participant characteristics

The clinical and demographic characteristics of participants in the discovery and clinical validation cohort are presented in Table 1. Associations between clinical variables and outcome (endometriosis vs symptomatic controls or general population), were tested using the chi-square test of independence for categorical clinical variables and the Point-biserial correlation test for continuous clinical variables.

Proteomics workflow

Discovery phase

This study analyzed plasma protein biomarkers using a proteomics workflow as previously described (Bringans *et al.*, 2017). In brief, quantitative biomarker discovery (iTRAQ labeling) was performed in quadruplicate experiments on pooled samples across the three groups: endometriosis cases, symptomatic controls, and general population. Each experiment involved immunodepletion of the pooled plasma sample to remove the 14 most abundant proteins. The immunodepleted fraction was then diafiltrated before reduction, alkylation, and enzymatic digestion with trypsin. The resulting peptide solutions were labeled with iTRAQ reagents (Sciex, USA) before mixing 1:1:1 for the three groups of pooled plasma. Desalted samples were then fractionated on a high-pH HPLC system with the resulting 12 fractions injected onto an LCMS system with analysis by a QE-HF Orbitrap (Thermo Fisher Scientific, USA) mass spectrometer.

Proteins observed to be differentially expressed (proteins required to have a *P*-value ≤0.05 with a relative ratio change of >10%) between endometriosis and symptomatic or general population groups were designated as candidate biomarkers if significant across the experiments. To this list, 12 putative biomarkers previously reported in the literature as having an association with endometriosis were added (see Fig. 1 and Supplementary Table S1).

Analytical validation phase

For analytical validation, targeted mass spectrometry assays using multiple reaction monitoring (MRM) were defined for each candidate protein biomarker as described in Bringans *et al.* (2017). Each assay measured changes in relative peptide abundances of individual plasma samples against an ^{18}O-labeled reference plasma to calculate peak area ratios for each of the candidate biomarkers. These ratios were normalized to the median value for each peptide. In brief, the analytical targeted assay was designed utilizing the following method. Each plasma sample was immunodepleted (removal of top 14 abundant proteins) before diafiltration, reduction, alkylation, and digestion of the plasma proteins. The reverse phase desalted sample was then injected along with a fixed amount of the internal standard ^{18}O labeled reference plasma digest onto a microflow (5 µl/min) HPLC system and analyzed on a Sciex 6500 Triple Quad mass spectrometer (Sciex, USA). Assays were assessed for robustness with analytical validation considered successful if the MRM signal for each peptide was individually verified to be unique and where the signal to noise (S/N) was >3.

Clinical validation phase

In clinical validation, a new cohort comprising individual samples (n = 464 endometriosis cases, n = 132 symptomatic controls, and n = 153 general population controls) was measured using the analytically validated targeted MRM mass spectrometry assay. Samples were randomized across plates before analysis to minimize batch effects and ensure consistency. Analysis of the mass spectrometry data was carried out in Skyline software (University of Washington, USA) with both unlabeled and ^{18}O labeled peptide peaks, integrated with peak areas exported to enable calculation of the relative peak ratios.

Statistical and data analyses

The peptide data presented reflect the relative concentration of a protein biomarker between samples. To maximize the likelihood of identifying biomarkers for the disease, changes in protein

Table 1. Demographics and clinical characteristics of participants (n = 805).

	Discovery cohort (N = 56)				
	Endometriosis (N = 22)	**Symptomatic controls (N = 15)**		**General population (N = 19)**	
Age (years) (mean ± SD)	35 ± 6	35 ± 7		36 ± 8	
	Clinical validation cohort (N = 749)				
	Endometriosis (N = 464)	**Symptomatic controls (N = 132)**	***P*-value (a)**	**General population (N = 153)**	***P*-value (b)**
Age (years) (mean ± SD)	30 ± 7	29 ± 8	0.051	28 ± 9	0.006
BMI (kg/m^2) (mean ± SD)	25 ± 5	26 ± 6	0.006	25 ± 6	0.594
Smoking status (% current or past)	41	37	0.771	30	0.001
Age at menarche (years) (mean ± SD)	13 ± 2	13 ± 2	0.421	13 ± 2	0.401
Family history of endometriosis (%)	27	25	0.571	3	<0.001
Pain (% menstrual/pelvic/intercourse)	93/80/75	94/85/82	0.608	0/0/0	<0.001
Cycle length (% 14–20/21–27/28/29+ days/other*)	33/8/29/21/9	45/11/20/16/8	0.071	0/19/50/31/0	<0.001
Cycle stage (% menses/prolif/secret/unknown)	4/25/23/48	2/22/23/53	0.447	9/44/39/8†	<0.001
Exogenous hormone medication (% oral/IUD/depo inj)	32/8/2	36/12/2	0.376	24/7/1	0.006
Gravidity (% 0/1/2/3+)	68/16/10/6	63/10/9/18	<0.001	73/9/8/10	0.709
Live births (% 0/1/2/3+)	81/10/7/2	71/11/9/9	0.001	81/5/12/2	0.270
Problems conceiving (% yes/no/not tried/unknown)	18/22/50/10	12/23/52/13	0.440	N/A	
Ethnicity (% AS/AMR/AFR/EUR/Other/Unknown)	11/1/1/77/4/6	5/1/0/85/8/1	0.148	17/5/1/62/6/9	<0.001

* Cycle length other, unknown, unsure, or not cycling; menses, menstruation; prolif, proliferative phase; secret, secretory phase; IUD, intrauterine device; depo inj, depot injection; AS, Asian; AMR, American; AFR, African; EUR, European; Other, mixed.

† Calculated from self-reported data. *P*-value (a) = endometriosis versus symptomatic controls; *P*-value (b) = endometriosis versus general population. N/A, data on pregnancy problems was not available for the general population cohort.

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

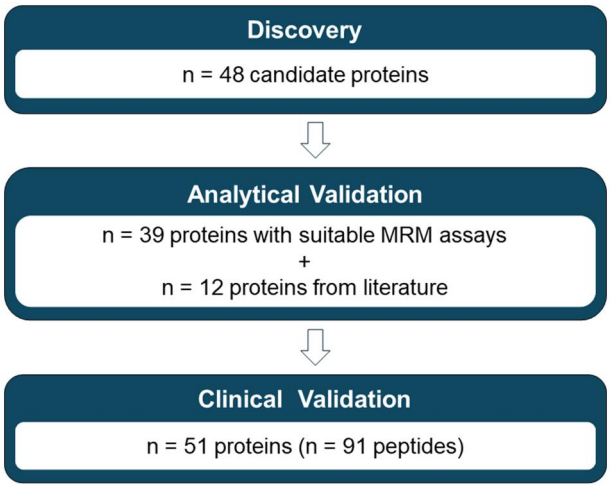

Figure 1. Flow diagram of biomarker identification proteomics workflow. MRM, multiple reaction monitoring.

concentration were initially assessed at the extremes of the disease spectrum, for example, symptomatic controls versus severe endometriosis or general population controls versus endometriosis. To improve the normality of the data, a natural logarithmic transformation was applied to all measurements. Candidate biomarkers were confirmed in bivariate analysis by two-way comparisons of medians using a Mann–Whitney *U*-test.

To evaluate the diagnostic relationship between clinical characteristics, biomarker concentration, and clinical groups, elastic-net logistic regression modeling was employed (R Statistical Software, v4.2.2; R Core Team, 2021). Clinical variables for inclusion in the models were restricted to age and BMI due to practical usability and accessibility. Repeated or nested cross-validation was performed (glmnet package v4.1-6; caret v6.0-93 (Kuhn, 2008); nestedcv.glmnet package v0.7.4). During the nested cross-validation approach, variables were filtered using a Wilcoxon *U*-test with a significance threshold of 0.2.

A series of multivariate logistic regression models containing both clinical factors and biomarker concentrations were developed to distinguish: (i) endometriosis cases from general population controls and (ii) endometriosis cases (stages II–IV) from symptomatic controls. To further evaluate the complex interactions and non-linear relationships between predictors, a random forest classifier was employed using the predictors identified during elastic-net logistic regression modeling. This third model was constructed by comparing stage IV endometriosis and symptomatic controls. The performance of Model 3 was then tested across the stages of endometriosis (stages I–IV, i.e. minimal to severe) to assess its effectiveness in diagnosing endometriosis at different disease levels. The randomForest package v4.6-14 (Liaw and Wiener, 2002) was used with 5-fold cross-validation and hyper-parameter tuning (mtry = 2, 3, 4, ntree = 100). Only participants with complete data were included in each model.

To assess the discriminative performance of each model, the area under the receiver operating characteristic curve (AUC) was assessed. DeLong's test was used to compare the AUC between biomarker models with and without clinical variables. The optimal predicted probability threshold was determined at the maximum Youden Index. Diagnostic performance metrics were computed based at this optimal threshold, including sensitivity (Sn) and specificity (Sp), and positive predictive value (PPV) and negative predictive value (NPV).

A power analysis was conducted to assess the study's power for subgroup analysis in different stages of endometriosis. The power analysis was performed using the pwr package version 1.3-0 (Champely, 2020) in R. The parameters for the power analysis included a sample size for the subgroup (stage I: n = 241, stage II: n = 65, stage III: n = 58, and stage IV: n = 89; only participants with complete data were included in this analysis), an effect size of 0.5 (Cohen's *d*), and a significance level of 0.05. The target statistical power was set at 0.8.

The interaction pathways of the proteins identified in the diagnostic models were examined to provide insights into the biological processes and molecular functions associated with these proteins (STRING database, v12.0; Szklarczyk *et al.*, 2019). Only interactions above a score of 4.0 (medium) were included in the predicted network.

Results

Participant demographics and clinical characteristics

Table 1 presents the demographics and clinical characteristics of the participants (n = 805) in both the discovery and clinical validation cohorts. Age was the only characteristic available for the discovery cohort and no significant difference was observed across the clinical groups. In the clinical validation cohort, BMI, gravidity, and live births were significantly different between endometriosis patients and symptomatic controls. Additionally, age, smoking status, family history of endometriosis, pain characteristics, cycle length, cycle stage, exogenous hormone medication use, and ethnicity were significantly different between the endometriosis patients and the general population.

The significant differences in the cycle stage between endometriosis patients and general population women may be largely explained by the higher proportion of endometriosis patients in the 'unknown or on hormones' group. A common management option for symptomatic endometriosis is hormone therapies, which are aimed at inducing amenorrhea and therefore hormone effects will be visualized on histology which do not permit grouping into menstrual/proliferative/secretory phases. It should also be noted that for the general population controls, the menstrual phase was calculated from self-reported data, whereas this was not the case for endometriosis participants (where menstrual dating was carried out using histological assessment by a pathologist).

Biomarker identification

The proteomics discovery experiment identified 48 candidate plasma protein biomarkers that were differentially expressed between endometriosis cases and both symptomatic controls and general population controls (Fig. 1 and Supplementary Table S1).

Targeted mass spectrometry assays were then built against all candidates, and well-defined assays were successfully developed for 39 of these, plus 12 putative biomarkers taken from the literature. Analytical validation was successful if analytically acceptable levels of reproducibility and signal to noise were achieved.

For the clinical validation phase, 51 protein biomarkers were analyzed. During two-way comparisons using a Mann–Whitney *U*-test, significant ($P \leq 0.05$) differences were observed for 41 of the 51 candidate proteins across one or both clinical group comparisons. Ten protein biomarkers (Table 2) were found to be independently associated with endometriosis after adjusting for age and BMI. These biomarkers were assessed for any correlation with the other available clinical information (e.g. menstrual cycle

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

Table 2. Protein associations: bivariate analysis (Mann–Whitney U-test) versus multivariate modeling (logistic regression).

		Bivariate analysis		Multivariate modeling	
Protein name	Accession number	P-value	Median difference (endometriosis vs control)	Model	Association direction (endometriosis)
Vitamin K-dependent protein S	P07225	<0.001	Decrease	1	Negative
Hemoglobin subunit beta	P68871	<0.001	Increase	1	Positive
Serum paraoxonase/arylesterase 1	P27169	<0.001	Increase	1	Positive
Afamin	P43652	<0.05	Decrease	2/3	Negative
Coagulation factor XII	P00748	<0.05	Decrease	2/3	Negative
Complement component C9	P02748	0.417	Increase	2/3	Positive
Neuropilin-1	O14786	<0.001	Decrease	2/3	Negative
Inter-alpha-trypsin inhibitor light chain	P02760	0.123	Decrease	2/3	Negative
Selenoprotein P	P49908	<0.05	Decrease	2/3	Positive
Proteoglycan 4	Q92954	0.066	Decrease	2/3	Negative

Model 1, endometriosis versus general population; Model 2, endometriosis (stages II–IV) versus symptomatic controls; Model 3, endometriosis (stage IV) versus symptomatic controls.

length), and no significant strong or moderate correlations were observed (maximum correlation coefficient of 0.26).

Model development and validation

Regression models were developed to discriminate between endometriosis cases and the general population (Model 1) or symptomatic controls (Model 2), as shown in Table 2. A random forest model (Model 3) was subsequently developed using the same biomarkers as Model 2 and constructed by comparing severe endometriosis and symptomatic controls, before being applied to all stages of endometriosis. No proteins provided utility in both models 1 and 2/3. For each model, the predicted probabilities for an endometriosis diagnosis were significantly higher ($P < 0.0001$) in the endometriosis group compared to the general population and symptomatic control groups.

Table 3 and the receiver operating characteristic (ROC) curves in Fig. 2 compare the outcomes predicted by the models against the observed diagnosis of endometriosis, along with the performance metrics (AUC, Sn, Sp, PPV, NPV) for each model. Three of the 10 protein biomarkers demonstrated excellent utility in distinguishing between the two clinical groups in Model 1 (AUC = 0.993, 95% CI 0.988–0.998) compared to age and BMI alone ($P < 0.001$). In Model 2, age and BMI were significant independent associates of endometriosis (stages II–IV) (AUC = 0.649, 95% CI 0.589–0.709). After adjusting for age and BMI, the remaining seven biomarkers provided significant incremental value to Model 2 (AUC = 0.729, 95% CI 0.676–0.783, $P < 0.01$). The same seven biomarkers demonstrated significant diagnostic accuracy in Model 3, with an AUC of 0.997 (95% CI 0.994–1.000) for discriminating stage IV endometriosis from symptomatic controls. Critically for clinical usage, Model 3 also showed strong diagnostic performance when applied to all stages of endometriosis (AUC for stage I: 0.852 (95% CI 0.811–0.893); stage II: 0.903 (95% CI 0.853–0.953); stage III: 0.908 (95% CI 0.852–0.965); stage IV: 0.997 (95% CI 0.994–1.000), respectively) (Fig. 3). Power analysis indicates that the study is well-powered for subgroup analysis in stage I, II, and IV endometriosis groups, with power levels of 100%, 80.8%, and 91.3%, respectively, however, the power for the stage III endometriosis subgroup was below the desired threshold at 76.1%.

Functional enrichment in the network of protein biomarkers for endometriosis

A network analysis of the 10 protein biomarkers associated with endometriosis revealed that most can be broadly categorized into three groups: coagulation cascade, complement system, and protein–lipid complex. Specific associations include: Coagulation factor XII, Complement component C9, and Vitamin K-dependent protein S with the complement and coagulation cascades ($P < 0.01$); and Afamin and Serum paraoxonase/arylesterase 1 with protein–lipid complex ($P < 0.001$).

Discussion

This study sought to develop a diagnostic blood test for endometriosis. A proteomics discovery workflow was used to identify and validate a novel panel of plasma protein biomarkers for the disease. Utilization of a large, clinically well-defined, independent cohort (n = 749) led to the development of three multivariate models, which demonstrated good to excellent performance for distinguishing endometriosis from both the general population and symptomatic controls. The models contained a panel of 10 protein biomarkers, which added significant value to clinical factors. This research contributes to the development of non-invasive diagnostic tools for endometriosis, which will have significant implications in reducing diagnostic delay and providing screening tools for surveillance of disease recurrence.

The primary objective of developing a diagnostic test for endometriosis was to distinguish symptomatic controls from endometriosis patients. To facilitate this, general population controls were included to allow investigation of the extremes of disease, thereby identifying potential protein biomarkers for the disease. A model distinguishing healthy women from those with endometriosis also has both biological and clinical relevance. The biology enables an understanding of disease pathophysiology, while clinically there is relevance in the context of fertility where a 3-fold increased incidence of endometriosis in women undergoing fertility treatments is observed (Moss et al., 2021). Infertility is a common consequence of endometriosis and a test to rule in or rule out endometriosis could help guide clinical decisions for assisted reproductive treatments. Model 1 (endometriosis vs the general population) demonstrates the biological association of these protein biomarkers with the disease state. Model 2 (stage II–IV endometriosis vs symptomatic controls) extends the utility of the test into a real-world scenario: differentiating the presence of endometriosis (lesions) from symptomatic pelvic pain in the absence of lesions. The inferior performance of Model 2 may reflect common symptom attributions between groups, and the marginal differences between patients with stage II endometriosis as compared to symptomatic patients, where no endometriosis is observed. Individuals with stage I endometriosis were specifically excluded to improve this, and further work is

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

Table 3. Predicted versus observed diagnosis of endometriosis and performance metrics.

				Performance				
	Endometriosis (predicted)	No endometriosis (predicted)	Total	AUC	Sn (%)	Sp (%)	PPV (%)	NPV (%)
Model 1 at max Youden Index cut-off								
General population controls (observed)	3	144	147	0.993	96	98	99	89
Endometriosis (observed)	426	17	443					
Total	429	161	590					
Model 2 at max Youden Index cut-off								
Symptomatic controls (observed)	43	87	130	0.729	73	67	78	60
Stage II–IV endometriosis (observed)	154	58	212					
Total	197	145	342					
Model 3 at max Youden Index cut-off								
Symptomatic controls (observed)	5	125	130	0.997	98	96	95	98
Stage IV endometriosis (observed)	87	2	89					
Total	92	127	219					
Model 3 applied to other Endometriosis stages using max Youden Index cut-off*								
Symptomatic controls (observed)	37	93	130	0.852	87	72	85	75
Stage I endometriosis (observed)	210	31	241					
Total	247	124	371					
Symptomatic controls (observed)	21	109	130	0.903	82	84	72	90
Stage II endometriosis (observed)	53	12	65					
Total	74	121	195					
Symptomatic controls (observed)	21	109	130	0.908	91	84	72	96
Stage III endometriosis (observed)	53	5	58					
Total	74	114	188					

Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value at the maximum Youden index. rASRM stage I=minimal, stage II=mild, stage III=moderate, and stage IV=severe endometriosis. Only participants with complete data were included in each model.

* Max Youden Index as defined for each specific group comparison.

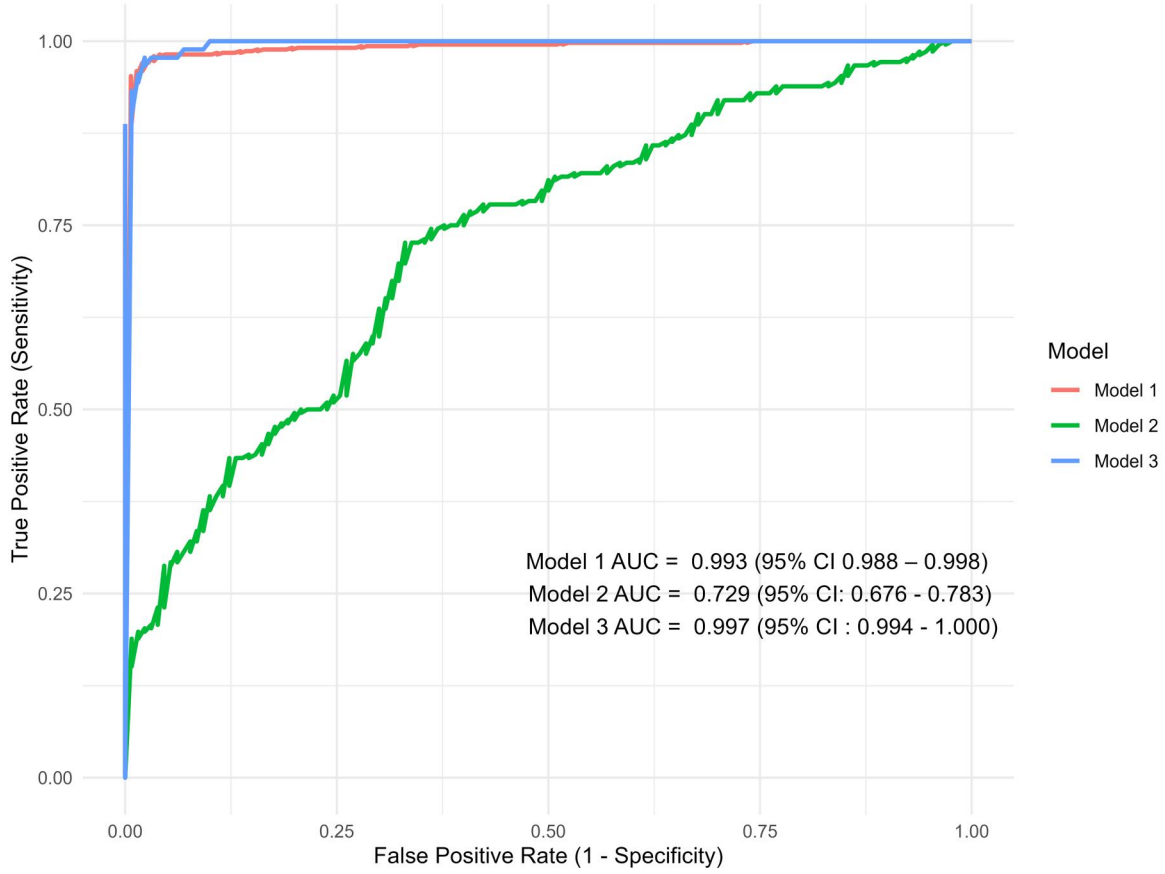

Figure 2. Receiver operating characteristic curves for Models 1, 2, and 3. Model 1: all endometriosis n = 443 versus general population controls n = 147, Model 2: endometriosis (stages II–IV) n = 212 versus symptomatic controls n = 130, and Model 3: endometriosis (stage IV) n = 89 versus symptomatic controls n = 130. Only participants with complete data were included in each model.

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

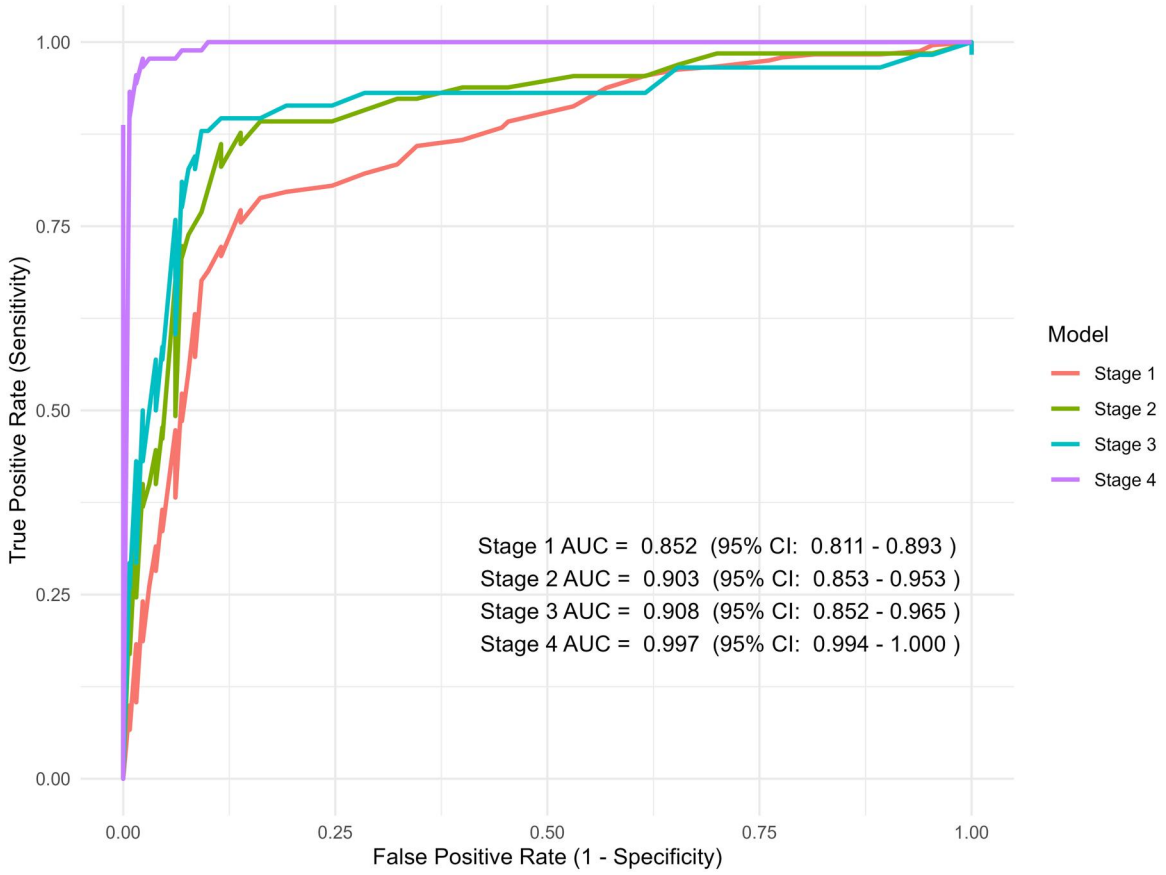

Figure 3. Receiver operating characteristic curves for Model 3: endometriosis (stage IV) versus symptomatic controls applied to all rASRM stages. Stage I: n = 241, stage II: n = 65, stage III: n = 58, and stage IV: n = 89. Only participants with complete data were included in this modeling.

required to examine this. Nonetheless, Model 3, which applied alternative statistical modeling to allow for the complex interactions and non-linear relationships between predictors in Model 2 (and was built by comparing the extremes of disease, namely stage IV endometriosis vs symptomatic controls), demonstrates strong performance for discriminating disease across all stages of endometriosis, suggesting a clear association of the biomarkers with disease state.

Laparoscopy is the gold standard for diagnosing endometriosis, but it is invasive and costly, carries risks, and is not readily accessible to all patients. Of known plasma biomarkers, CA-125 is sometimes used as a single biomarker for endometriosis. However, CA-125 has limited Sn and Sp, and elevated CA-125 levels can occur in multiple conditions such as ovarian cancer, pelvic inflammatory disease, and menstruation. A recent multicenter study showed that CA-125 differentiated endometriosis from symptomatic controls with Sn 61% at a pre-defined Sp of 60% (Burghaus *et al.*, 2024), with better performance for stage III/IV endometriosis compared to stage I/II (AUC 0.795 vs 0.583, respectively). A 2016 systematic review and meta-analysis reported a pooled Sn of 52% and Sp of 93% for CA-125, with significantly higher Sn for stage III/IV compared to stage I/II endometriosis (Sn 63% vs 25%, respectively) (Hirsch *et al.*, 2016). CA-125 can be effective for diagnosing stage IV endometriosis cases, such as those with dense pelvic adhesions or ovarian endometriomas but is less reliable for other forms of endometriosis, and its use may lead to potential false positives due to the presence of other conditions. Consequently, it is not widely recommended as a diagnostic or screening tool by major guidelines such as the ESHRE (Becker *et al.*, 2022).

The diagnostic models distinguishing endometriosis patients from symptomatic controls are particularly relevant for clinical practice. In comparison to known biomarkers, the multivariate biomarker models developed in the present study to distinguish endometriosis from symptomatic controls have sensitivities of 73% and 98% and specificities of 67% and 96%, for Models 2 and 3, respectively. Important to improving patient outcomes by enabling earlier and more accurate diagnosis, results indicate that Model 3 has potential utility across earlier stages of the disease, with AUCs of ≥0.85 for stage I–III endometriosis. These results compare favorably to the performance of known biomarkers in terms of AUC. In the present study, model cut-offs to assess performance metrics such as Sn and Sp were arbitrarily set at the maximum Youden Index, but further optimization should be considered before using in a clinical setting. By providing a non-invasive diagnostic method to differentiate endometriosis from other causes of pelvic pain, such tools can help clinicians make more informed decisions about which patients should undergo invasive procedures like laparoscopy, and facilitate more targeted and effective treatment plans, enhancing overall patient care.

The discrepancies observed between bivariate and multivariate results in Table 2 (Complement component C9, Inter-alpha-trypsin inhibitor light chain, Selenoprotein P, and Proteoglycan 4) can be attributed to two key factors: unmeasured confounders and the suppressor effect. Bivariate analysis assesses the association between two variables without considering other confounding factors. A suppressor variable, unlike a typical confounder, does not directly affect the outcome but interacts with the predictor, altering the strength or direction of the association.

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

Inclusion of a suppressor variable in a multivariate model, can reveal the true relationship between the predictor and the outcome (Woolley, 1997).

Biologically, each of the 10 protein biomarkers identified in the diagnostic models for endometriosis plays a role relevant to disease pathophysiology, including in the coagulation and complement cascades, lipid metabolism, oxidative defense, immune regulation, and tissue homeostasis and morphogenesis. Of the 10 proteins listed in Table 2, only three (Selenoprotein P, Neuropilin-1, and Serum paraoxonase/arylesterase 1) have previously been directly linked with endometriosis, as discussed below.

In the complement cascade, Complement component C9 is required for target cell lysis during complement activation (Noris and Remuzzi, 2013). In Model 2, Complement component C9 has a positive association with endometriosis. Complement dysregulation has been implicated in the pathophysiology of endometriosis (Rahal *et al.*, 2021).

For the coagulation cascade, Coagulation factor XII is crucial for fibrin clot formation (Renne *et al.*, 2012). Similarly, Vitamin K-dependent protein S has a role in regulating coagulation (Weizmann Institute of Science, 2024). In Model 1, Vitamin K-dependent protein S showed a negative association with endometriosis, whereas Coagulation factor XII had a negative association in Model 2. A previous small study failed to find a statistically significant difference in blood Vitamin K-dependent protein S levels between endometriosis patients and controls (Pretta *et al.*, 2007). Hemoglobin subunit beta, a component of hemoglobin, indirectly contributes to coagulation by contributing to overall blood function (Ross *et al.*, 2024). In Model 1, Hemoglobin subunit beta exhibited a positive association with endometriosis.

Afamin, Selenoprotein P, and Serum paraoxonase/arylesterase 1 play roles in lipid metabolism and oxidative defense (Dieplinger and Dieplinger, 2015; Shunmoogam *et al.*, 2018; Saito, 2021). While earlier work has found no significant difference in mean serum Afamin concentrations between endometriosis and control groups using ELISA (Seeber *et al.*, 2010), Afamin correlated negatively with endometriosis in Model 2. A positive correlation was observed for Selenoprotein P in Model 2, and the bivariate results for Selenoprotein P are consistent with previous findings from a small study ($n = 8$), where downregulated gene expression was reported in tissue samples from patients with endometriosis (Aghajanova *et al.*, 2011). Additionally, a positive correlation exists between Serum paraoxonase/arylesterase 1 and endometriosis in Model 1. Interestingly, another study reported reduced Serum paraoxonase/arylesterase 1 activity (not concentration) in women with endometriosis compared to controls (Verit *et al.*, 2008). This apparent discrepancy may arise from assessing different aspects of Serum paraoxonase/arylesterase 1 within distinct biological contexts or using varied measurement methods.

Neuropilin-1, Inter-alpha-trypsin inhibitor light chain, and Proteoglycan 4 each contribute significantly to immune regulation (Das *et al.*, 2019; Lord *et al.*, 2020; Chikh and Raimondi, 2024). Neuropilin-1 also plays important roles in vascular development (Chikh and Raimondi, 2024), while Inter-alpha-trypsin inhibitor light chain contributes to extracellular matrix organization (Lord *et al.*, 2020) and Proteoglycan 4 acts as a boundary lubricant (Das *et al.*, 2019). The negative association observed for Neuropilin-1 in Model 2 contrasts to previous findings of elevated serum Neuropilin-1 for endometriosis patients when compared to healthy controls by ELISA (Barberic *et al.*, 2020). Both Inter-alpha-trypsin inhibitor light chain and Proteoglycan 4 also showed negative associations with endometriosis in Model 2.

Further investigations are warranted to unravel the precise mechanisms underlying the associations of these protein biomarkers with endometriosis and potential therapeutic implications. The results presented here, which contrast with the literature, highlight the challenges of biomarker analysis in small cohorts and across different laboratories. Differences between published studies and the results in this manuscript may be also due to pre-analytical and analytical factors. Varying gene expression profiles across different tissues affected by endometriosis, along with post-transcriptional regulation and translation efficiency, may contribute to the divergent results.

The strengths of this study are in its robust sample size, independent cohorts, a well-defined clinical validation cohort, and high-performing models using simple components. The use of clinical variables in the models was deliberately limited to age and BMI because this information can be easily and precisely determined. In concert, the exclusion from the models of other clinical information such as menstrual cycle stage, exogenous hormone use, or family history of endometriosis avoids potentially imprecise variables, whilst also ensuring the test can be widely used. The robust sample size enhances statistical power, leading to more reliable conclusions. The utilization of independent cohorts strengthens the findings by validating the biomarkers across different populations, thereby minimizing bias and increasing generalizability. This study also benefits from a diagnosis method grounded in laparoscopy and histopathology, ensuring a more reliable assessment of disease absence or presence. The use of both elastic-net logistic regression and random forest algorithms in the analysis underscores the robustness and versatility of the models, providing a comprehensive evaluation of the diagnostic potential of the identified biomarkers. The random forest approach confirms the importance of the biomarkers, but may be dataset specific, and further validation is warranted.

The study also has potential limitations. The participants were mostly of European ethnicity, and the study was not powered to detect differences across ethnic groups. The use of minimal clinical variables in the models may reduce diagnostic performance, however, information on more complex clinical variables may not always be available or consistently measured. It is also possible that some general population controls might have endometriosis, which could potentially skew results. Given the nature of the condition, the prevalence of asymptomatic endometriosis at the general population level is difficult to ascertain, but has been reported to be as high as 11% (Buck Louis *et al.*, 2011). While the study was not specifically designed to stratify patients based on the stage of endometriosis, it is well-powered for subgroup analysis of stage I, II, and IV endometriosis. The experimental design used matched samples within each cohort, however, the delay in time between sample collection and processing and the difference in sample storage could affect biomarker concentrations observed in this study. Further analysis is required to enable generalizability of the findings to other populations or settings, including stratification of patients by type or stage of endometriosis.

This study represents an advancement toward precise non-invasive endometriosis diagnosis and personalized care, achieved through the integration of proteomics and clinical expertise. A panel of novel plasma protein biomarkers was identified that enabled the development of diagnostic models demonstrating strong discriminatory capabilities. The reported functions of these protein biomarkers offer potential insights into endometriosis pathogenesis. Further validation of these biomarkers will fortify the robustness and reliability of this diagnostic tool and

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

enable its integration into clinical practice, benefiting individuals affected by endometriosis and paving the way for improved patient care.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article cannot be shared publicly due to the privacy of individuals who participated in the study. The data will be shared on reasonable request to the corresponding author.

Acknowledgements

The authors thank the Wesley Research Institute (Queensland, Australia), the Royal Women's Hospital (Melbourne, Australia) for clinical samples, Linear Clinical Research (Perth, Australia) for the collection of healthy controls, and all of the participants who donated their blood samples. The authors also thank The Proteomics International laboratory staff for their precise contributions to sample preparation and analysis, and Dr Roop Judge, Sue Wong, and Dr Kerryn Garrett for their work in securing access to the clinical samples used in this study. Sections of this work have been presented previously at the 18th Human Proteome Organization World Congress in 2019 (Ito *et al.*, 2019), Fertility Society of AU & NZ Annual Conference 2023, 70th Annual Meeting of the Society for Reproductive Investigation in 2023, and 15th World Congress on Endometriosis in 2023.

Authors' roles

The study was designed by R.L., S.B., K.P., P.T., S.H.-C., and P.A.W.R., who were responsible for the overall direction and planning of the research. Sample collection and processing were conducted by J.E.G., M.H., and B.A.B. The laboratory work was carried out by C.A., T.C., L.C., M.D., D.I., J.I., C.L., H.L., M.M., and M.R. E.M.S. and K.P. were responsible for the statistical analysis and E.M.S., S.B., K.P., and R.L. were responsible for the interpretation of the results. The manuscript was written, reviewed, and prepared by E.M.S., K.P., S.B., and R.L., with S.H.-C. and P.A.W.R. Each author has approved the final version of the manuscript.

Funding

The subject recruitment at The Royal Women's Hospital, Melbourne, was supported in part by funding from the Australian National Health and Medical Research Council (NHMRC) project (GNT1105321, GNT1026033) and Australian Medical Research Future Fund (MRF1199715) (P.A.W.R., S.H.-C., and M.H.).

Conflict of interest

Proteomics International has filed patent WO 2021/184060 A1 that relates to endometriosis biomarkers described in this manuscript; S.B., R.L., and T.C. declare an interest in this patent. J.I., S.B., C.L., D.I., H.L., K.P., M.D., M.M., M.R., P.T., R.L., and T.C. are shareholders in Proteomics International. Otherwise, the authors have no conflicts of interest.

References

Aghajanova L, Tatsumi K, Horcajadas JA, Zamah AM, Esteban FJ, Herndon CN, Conti M, Giudice LC. Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. *Biol Reprod* 2011; **84**:801–815.

American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril* 1997;**67**:817–821.

Barberic M, Pavicic Baldani D, Rogic D, Kralik S. Serum concentrations of neuropilin-1 in women with endometriosis. *Scand J Clin Lab Invest* 2020;**80**:271–276.

Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, King K, Kvaskoff M, Nap A, Petersen K *et al.*; ESHRE Endometriosis Guideline Group. ESHRE guideline: endometriosis. *Hum Reprod Open* 2022;**2022**:hoac009.

Bringans SD, Ito J, Stoll T, Winfield K, Phillips M, Peters K, Davis WA, Davis TME, Lipscombe RJ. Comprehensive mass spectrometry based biomarker discovery and validation platform as applied to diabetic kidney disease. *EuPA Open Proteom* 2017;**14**:1–10.

Buck Louis G, Hediger M, Peterson C, Croughan M, Sundaram R, Stanford J, Chen Z, Fujimoto V, Varner M, Trumble A *et al.*; ENDO Study Working Group. Incidence of endometriosis by study population and diagnostic method: the ENDO study. *Fertil Steril* 2011; **96**:360–365.

Burghaus S, Drazic P, Wolfler M, Mechsner S, Zeppernick M, Meinhold-Heerlein I, Mueller MD, Rothmund R, Vigano P, Becker CM *et al.* Multicenter evaluation of blood-based biomarkers for the detection of endometriosis and adenomyosis: a prospective non-interventional study. *Int J Gynaecol Obstet* 2024;**164**:305–314.

Champely S. *pwr: Basic Functions for Power Analysis*. R Package Version 1.3-0. Vienna, Austria: The Comprehensive R Archive Network, 2020. https://cran.r-project.org/web/packages/pwr/pwr.pdf

Chikh AA-O, Raimondi CA-O. Endothelial Neuropilin-1: a multifaced signal transducer with an emerging role in inflammation and atherosclerosis beyond angiogenesis. *Biochem Soc Trans* 2024; **52**:137–150.

Das N, Schmidt TA, Krawetz RJ, Dufour A. Proteoglycan 4: from mere lubricant to regulator of tissue homeostasis and inflammation: does proteoglycan 4 have the ability to buffer the inflammatory response? *Bioessays* 2019;**41**:e1800166.

Dieplinger H, Dieplinger B. Afamin—a pleiotropic glycoprotein involved in various disease states. *Clin Chim Acta* 2015;**446**:105–110.

Eisenberg VH, Decter DH, Chodick G, Shalev V, Weil C. Burden of endometriosis: infertility, comorbidities, and healthcare resource utilization. *J Clin Med* 2022;**11**:1133.

Ellis K, Munro D, Clarke J. Endometriosis is undervalued: a call to action. *Front Glob Womens Health* 2022;**3**:902371.

Hirsch M, Duffy J, Davis CJ, Nieves Plana M, Khan KS; International Collaboration to Harmonise Outcomes and Measures for Endometriosis. Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis. *BJOG* 2016; **123**:1761–1768.

Ito J, Bringans S, Peters K, Casey T, Andronis C, Lipscombe R. *Biomarkers of Endometriosis*. Adelaide (Australia): 18th Human Proteome Organization World Congress, 2019.

Kuhn M. Building predictive models in R using the caret package. *J Stat Softw* 2008;**5**:1–26.

Liaw A, Wiener MC. Classification and regression by randomForest. *R News* 2002;**2**:18–221.

Lipscombe RJ, Bringans S, Casey TM. *Endometriosis Biomarkers (Patent WO2021184060A1)*. Geneva, Switzerland: World Intellectual Property Organization, 2021.

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

Lord MS, Melrose J, Day AJ, Whitelock JM. The inter-α-trypsin inhibitor family: versatile molecules in biology and pathology. *J Histochem Cytochem* 2020;**68**:907–927.

Moss KM, Doust J, Homer H, Rowlands IJ, Hockey R, Mishra GD. Delayed diagnosis of endometriosis disadvantages women in ART: a retrospective population linked data study. *Hum Reprod* 2021;**36**:3074–3082.

Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* 2016a;**2**:CD009591.

Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, Mol BW, Johnson N, Hull ML. Blood biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* 2016b;**2016**:CD012179.

Noris M, Remuzzi G. Overview of complement activation and regulation. *Semin Nephrol* 2013;**33**:479–492.

Okoli UA-O, Charoenngam N, Ponvilawan B, Jaroenlapnopparat A, Mettler S, Obiejesie O. Endometriosis and risk of cardiovascular disease: systematic review and meta-analysis. *J Womens Health (Larchmt)* 2023;**32**:1328–1339.

Pretta S, Remorgida V, Abbamonte LH, Anserini P, Ragni N, Del Sette M, Gandolfo C, Ferrero S. Atherosclerosis in women with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2007;**132**:226–231.

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, 2021. https://www.R-project.org/

Rahal D, Andrade F, Nisihara R. Insights into the role of complement system in the pathophysiology of endometriosis. *Immunol Lett* 2021;**231**:43–48.

Renne T, Schmaier AH, Nickel KF, Blomback M, Maas C. In vivo roles of factor XII. *Blood* 2012;**120**:4296–4303.

Ross JT, Robles AJ, Mazer MB, Studer AC, Remy KE, Callcut RA. Cell-free hemoglobin in the pathophysiology of trauma: a scoping review. *Crit Care Explor* 2024;**6**:e1052.

Rowlands IA-OX, Abbott JA, Montgomery GW, Hockey R, Rogers P, Mishra GD. Prevalence and incidence of endometriosis in Australian women: a data linkage cohort study. *BJOG* 2021;**128**:657–665.

Saito Y. Selenium transport mechanism via selenoprotein P—its physiological role and related diseases. *Front Nutr* 2021;**8**:685517.

Seeber BE, Czech T, Buchner H, Barnhart KT, Seger C, Daxenbichler G, Wildt L, Dieplinger H. The vitamin E-binding protein afamin is altered significantly in the peritoneal fluid of women with endometriosis. *Fertil Steril* 2010;**94**:2923–2926.

Shunmoogam N, Naidoo P, Chilton R. Paraoxonase (PON)-1: a brief overview on genetics, structure, polymorphisms and clinical relevance. *Vasc Health Risk Manag* 2018;**14**:137–143.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P *et al.* STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019;**47**:D607–D613.

Verit FF, Erel O, Celik N. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. *Hum Reprod* 2008;**23**:100–104.

Weizmann Institute of Science. *PROS1 Gene—Protein S*. GeneCards—The Human Gene Database. 2024. https://www.genecards.org/cgi-bin/carddisp.pl?gene=PROS1

Woolley KK. How variables uncorrelated with the dependent variable can actually make excellent predictors: the important suppressor variable case. ERIC 1997;ED407420. https://files.eric.ed.gov/fulltext/ED407420.pdf (11 March 2024, date last accessed).

Zondervan KT, Becker CM, Missmer SA. Endometriosis. *N Engl J Med* 2020;**382**:1244–1256.

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026

© The Author(s) 2024. Published by Oxford University Press on behalf of European Society of Human Reproduction and Embryology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Human Reproduction, 2025, 40, 270–279
https://doi.org/10.1093/humrep/deae278
Original Article

Downloaded from https://academic.oup.com/humrep/article/40/2/270/7932209 by guest on 30 July 2026