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CLINICAL ARTICLE

Gynecology

# Multicenter evaluation of blood-based biomarkers for the detection of endometriosis and adenomyosis: A prospective non-interventional study

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## Abstract

**Objective:** To evaluate blood-based biomarkers to detect endometriosis and/or adenomyosis across nine European centers (June 2014–April 2018).

**Methods:** This prospective, non-interventional study assessed the diagnostic accuracy of 54 blood-based biomarker immunoassays in samples from 919 women (aged 18–45 years) with suspicion of endometriosis and/or adenomyosis versus symptomatic controls. Endometriosis was stratified by revised American Society for Reproductive Medicine stage. Symptomatic controls were "pathologic symptomatic controls" or "pathology-free symptomatic controls". The main outcome measure was receiver operating characteristic-area under the curve (ROC-AUC) and Wilcoxon *P* values corrected for multiple testing (*q* values).

**Results:** CA-125 performed best in "all endometriosis cases" versus "all symptomatic controls" (AUC 0.645, 95% confidence interval [CI] 0.600–0.690, $q < 0.001$) and increased ($P < 0.001$) with disease stage. In "all endometriosis cases" versus "pathology-free symptomatic controls", S100-A12 performed best (AUC 0.692, 95% CI 0.614–0.769, $q = 0.001$) followed by CA-125 (AUC 0.649, 95% CI 0.569–0.729, $q = 0.021$). In "adenomyosis only cases" versus "symptomatic controls" or "pathology-free symptomatic controls", respectively, the top-performing biomarkers were sFRP-4 (AUC 0.615, 95% CI 0.551–0.678, $q = 0.045$) and S100-A12 (AUC 0.701, 95% CI 0.611–0.792, $q = 0.004$).

**Conclusion:** This study concluded that no biomarkers tested could diagnose or rule out endometriosis/adenomyosis with high certainty.

KEYWORDS
adenomyosis, blood-based biomarkers, CA-125, diagnosis, endometriosis, S100-A12, sFRP-4

## 1 | INTRODUCTION

The long interval between the onset of endometriosis and/or adenomyosis symptoms and receiving a diagnosis (4–12 years)[1–7] may result in disease progression, prolonged pain, and impaired fertility.[7,8] Endometriosis severity can be classified using the revised American Society of Reproductive Medicine (rASRM) system, into four stages.[9] The current gold standard for diagnosing endometriosis is direct visualization of lesions via laparoscopic surgery with or without subsequent histologic examination of the biopsies.[8,10–12] Such surgery carries inherent risks and extended patient recovery times;[13] moreover, detecting endometriosis during laparoscopy is often dependent on the surgeon's experience and lesion accessibility.[8,14] Adenomyosis is challenging to diagnose as it is based on the patient's symptoms combined with transvaginal ultrasound (TVUS) and/or magnetic resonance imaging (MRI);[15] accurate diagnosis requires highly experienced staff and appropriate equipment,[16] meaning that formal diagnosis often takes place via histologic evaluation of uterine tissue following hysterectomy.[17,18]

Blood-based biomarker testing may facilitate earlier endometriosis/adenomyosis diagnosis compared with current diagnostic techniques. Earlier detection may allow timely implementation of clinical management strategies and fertility procedures. It is unlikely that this testing would replace the need for diagnostic laparoscopy, but it could be implemented to help guide clinical decision making around further diagnostic procedures.

The primary objective was to evaluate suitable blood-based biomarkers, or biomarker combinations, for detection of suspected endometriosis and/or adenomyosis with high sensitivity.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

This multicenter, prospective, non-interventional study (June 2014–April 2018) was conducted at nine European centers. The study complied with the International Conference on Harmonization guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. Ethical approval was obtained for each study center (Table S1). All participants provided written informed consent.

### 2.2 | Participants

Eligible participants were recruited during routine clinical visits. Inclusion criteria were: women aged 18–45 years experiencing relevant symptoms (i.e. chronic pelvic pain and/or unexplained subfertility) with clinical suspicion of endometriosis and/or adenomyosis undergoing laparoscopy or laparotomy. Further enrolment criteria are described in the Methods S1. Surgical findings were recorded in accordance with the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project (EPHect) protocols, using standardized data and sample collection techniques.[19–21]

Participant disposition is shown in Figure 1. Participants were defined as "cases" (endometriosis, endometriosis and adenomyosis, or adenomyosis only) or as "all symptomatic controls" (no endometriosis or adenomyosis). Endometriosis diagnosis required histologic confirmation; adenomyosis diagnosis was made by either TVUS or visualization during surgery. Participants with endometriosis/adenomyosis ("cases") were stratified into two subgroups: the "all endometriosis" group, including participants with endometriosis with or without adenomyosis; and the "adenomyosis only" group. The "all endometriosis" group was further stratified by rASRM stage. The "all symptomatic controls" group was stratified into two sub-groups: "pathologic symptomatic controls", including participants with pelvic pain and/or unexplained subfertility with pathologic findings of a condition other than endometriosis/adenomyosis (e.g. fibroids); and "pathology-free symptomatic controls", which included participants with pelvic pain and/or unexplained subfertility, but no pathologic findings on laparoscopic investigation.

The following comparisons were undertaken: (1) "all endometriosis cases" versus "all symptomatic controls"; (2) "all endometriosis cases" versus "pathology-free symptomatic controls"; (3) "adenomyosis only cases" versus "all symptomatic controls"; and (4) "adenomyosis only cases" versus "pathology-free symptomatic controls".

### 2.3 | Measurement of blood-based biomarkers

Samples were combined into one large panel and subsequently randomized before measurement. For each biomarker, measurements for all samples were performed in one batch. A panel of biomarkers was examined, including 17 autoantibodies identified from previous internal discovery analysis, alongside seven selected clinical variables (Table S2). Protein biomarkers were chosen due to their association with endometriosis, adenomyosis, and/or pelvic inflammation, consistent with existing literature, or disease pathway analyses with a focus on the involvement of the neurogenic inflammation pathway.[22] Statistical analyses are described in the Methods S1.

### 2.4 | Statistical analysis

The highest-ranking single biomarkers for discriminating between cases and controls for each comparison were identified by plotting receiver operating characteristic curves for all biomarkers analyzed and calculating area under the curve (AUC) values. Biomarkers were assessed within menstrual cycle phase subgroups in a comparison between participants with confirmed hormone intake versus no hormone intake, described in the Methods S1. Wilcoxon *P* values were calculated for biomarkers to evaluate the statistical significance between cases and controls; *P* values were corrected for multiple testing by applying a false discovery rate correction (referred to as *q* values throughout).[23,24] To find the top-performing two- and three-biomarker combinations (including clinical variables) for distinguishing between cases and controls, all combinations were

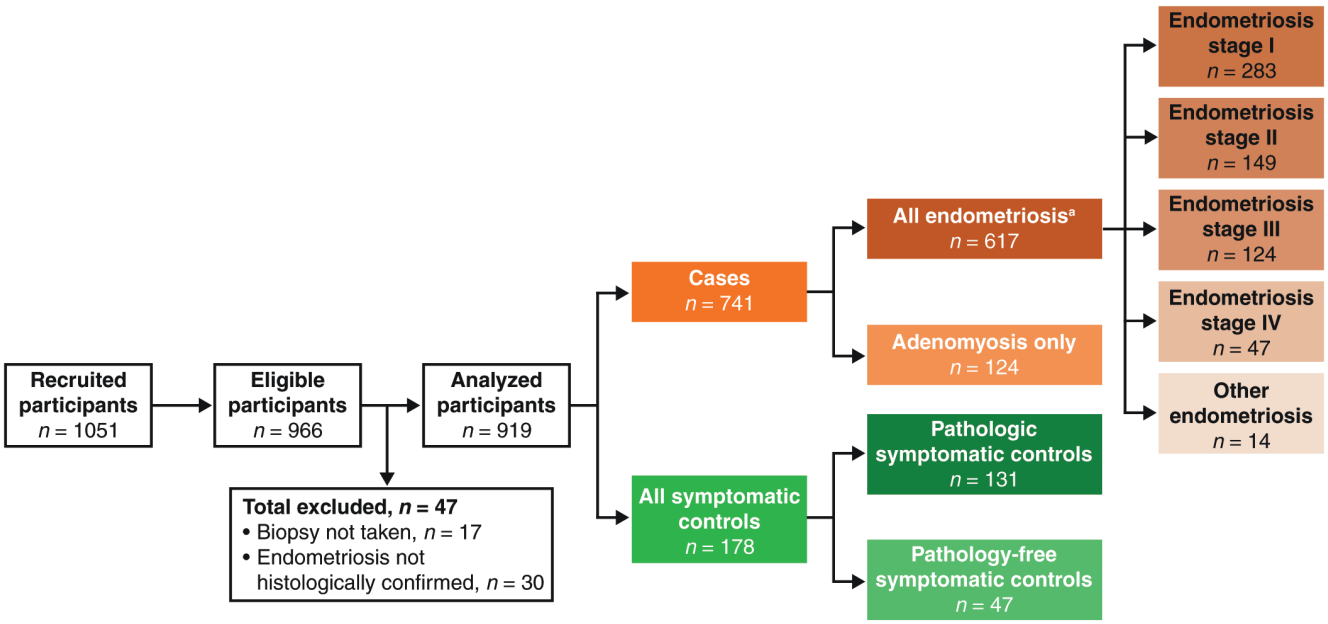


FIGURE 1 Participant disposition. [a]With or without adenomyosis.

systematically evaluated, and their performances were assessed in extensive double (two-tier) cross-validation employing logistic regression (Figure S1). The sensitivity of each top-performing biomarker or biomarker combination (set at 60% specificity, based on unpublished findings of an advisory board hosted by Roche Diagnostics International Ltd in October 2012) was also recorded. The statistical significance of the endometriosis stage dependency of each top-performing biomarker was assessed by linear regression, from which the *P* value of the *t* test (i.e. testing whether the stage regression coefficient is different from 0) was reported using an α of 0.05 for the cut-off for significance. For all analyses, R version 3.6.3 was used; multivariate analyses employed the mlr R-package.[25]

In rare cases, some biomarker measurements were not available due to measurement errors or insufficient sample volumes; see Tables S8–S12 for available biomarker measurements for each comparison. In such cases, participants with missing values were removed; no imputation was performed. The multivariate workflow was performed on the complete data matrix (i.e. the subset of participants with all biomarker values present).

## 3 | RESULTS

### 3.1 | Participant demographics and clinical characteristics

In total, 1051 women were recruited; 966 women were eligible to participate; data from 919 participants were analyzed; and 47 participants were excluded (Figure 1). Tables S3–S6 provide an overview of participant demographics and clinical characteristics by study center.

Of the 919 participants analyzed, 741 had confirmed endometriosis and/or adenomyosis ("cases") and 178 were symptomatic controls ("all symptomatic controls"; Figure 1). Of the participants with confirmed endometriosis and/or adenomyosis, 617 participants had endometriosis with or without adenomyosis ("all endometriosis cases") and 124 participants had adenomyosis only ("adenomyosis only cases"). Table S7 provides an overview of the surgical findings from participants with endometriosis and/or adenomyosis. Of the symptomatic controls ("all symptomatic controls"), 131 of the participants were symptomatic with pathologic findings other than endometriosis/adenomyosis ("pathologic symptomatic controls") and 47 were symptomatic controls without pathologic findings ("pathology-free symptomatic controls").

### 3.2 | Top-performing biomarkers

The highest-ranking single biomarkers, in terms of AUC, to distinguish between cases and controls are shown in Table 1; statistical analysis for all biomarkers and clinical variables in all comparisons are given in Tables S8–S11. For the comparison of "all endometriosis cases" versus "all symptomatic controls", cancer antigen 125 (CA-125; $q<0.001$) and secreted frizzled-related protein 4 (sFRP-4; $q=0.041$) were the top-performing biomarkers. For "all endometriosis cases" versus "pathology-free symptomatic controls", S100 calcium-binding protein A12 (S100-A12; $q=0.001$) and CA-125 ($q=0.021$) were the top-performing biomarkers. For "adenomyosis only cases" versus "all symptomatic controls", sFRP-4 ($q=0.045$) and hepatocyte growth factor ($q=0.141$) were the top-performing biomarkers. Finally, S100-A12 ($q=0.004$) and placental growth factor ($q=0.118$) were the top-performing biomarkers for "adenomyosis only cases" versus "pathology-free symptomatic controls". As the top-ranking biomarkers across each of the four comparisons, CA-125, S100-A12, and sFRP-4 were examined further.

Results from the hormone intake and menstrual cycle phase subgroup analyses are detailed in the Methods S1.

**TABLE 1** Top-performing biomarkers, autoantibodies, and clinical variables identified.

| Variable | Ranking[a] | *N* | Mean difference/fold change ($log_2$/linear) | Significance (Wilcoxon *q* value[b]) | AUC | Sensitivity at 60% specificity, % |
|---|---|---|---|---|---|---|
| "All endometriosis cases" versus "all symptomatic controls" | | | | | | |
| CA-125 | 1 | 793 | 0.537 | <0.001 | 0.645 | 60.6 |
| sFRP-4 | 2 | 783 | 0.444 | 0.041 | 0.579 | 51.0 |
| HDL:LDL cholesterol ratio | 3 | 795 | 0.153 | 0.048 | 0.575 | 50.2 |
| WorstPain12m | 4 | 662 | 0.168 | 0.159 | 0.569 | 40.1 |
| S100-A12 | 5 | 788 | 0.193 | 0.151 | 0.564 | 50.8 |
| Endoglin | 6 | 794 | 0.059 | 0.159 | 0.557 | 50.5 |
| "All endometriosis cases" versus "pathology-free symptomatic controls" | | | | | | |
| S100-A12 | 1 | 657 | 0.614 | 0.001 | 0.692 | 72.1 |
| CA-125 | 2 | 663 | 0.549 | 0.021 | 0.649 | 58.4 |
| PlGF | 3 | 664 | 0.169 | 0.035 | 0.638 | 54.3 |
| HE4 | 4 | 664 | 0.139 | 0.061 | 0.624 | 58.5 |
| PRL | 5 | 664 | −0.284 | 0.061 | 0.623 | 57.2 |
| hCALD | 6 | 657 | 0.126 | 0.265 | 0.600 | 54.1 |
| "Adenomyosis only cases" versus "all symptomatic controls" | | | | | | |
| sFRP-4 | 1 | 301 | 0.711 | 0.045 | 0.615 | 56.4 |
| HGF.aAB | 2 | 300 | 1.311 | 0.141 | 0.597 | 52.5 |
| Prokineticin-1 | 3 | 302 | −0.202 | 0.631 | 0.568 | 50.8 |
| NSE | 4 | 302 | 0.055 | 0.631 | 0.565 | 53.2 |
| S100-A12 | 5 | 298 | 0.193 | 0.631 | 0.564 | 47.5 |
| DNASE2.aAB | 6 | 300 | 0.547 | 0.749 | 0.556 | 49.2 |
| "Adenomyosis only cases" versus "pathology-free symptomatic controls" | | | | | | |
| S100-A12 | 1 | 167 | 0.613 | 0.004 | 0.701 | 74.6 |
| PlGF | 2 | 171 | 0.174 | 0.118 | 0.644 | 55.6 |
| HGF.aAB | 3 | 169 | 1.720 | 0.265 | 0.623 | 59.0 |
| hCALD | 4 | 167 | 0.126 | 0.265 | 0.617 | 59.0 |
| NSE | 5 | 171 | 0.113 | 0.265 | 0.614 | 57.3 |
| PRL | 6 | 171 | −0.243 | 0.265 | 0.611 | 53.2 |

*Note*: Not all biomarkers could be measured in all samples as there were measurement errors or insufficient sample volume remaining.

Abbreviations: AUC, area under the curve; CA-125, cancer antigen 125; DNASE2.aAB, deoxyribonuclease-2α; FDR, false discovery rate; hCALD, high-molecular-weight caldesmon; HDL, high-density lipoprotein; HE4, human epididymis protein 4; HGF.aAB, hepatocyte growth factor; LDL, low-density lipoprotein; NSE, neuron-specific enolase; PlGF, placental growth factor; PRL, prolactin; S100-A12, S100 calcium-binding protein A12; sFRP-4, secreted frizzled-related protein 4.

[a]Ranking order is based on the AUC of the biomarker for each comparison.

[b]FDR-corrected Wilcoxon *P* value; a *q*-value <0.05 was considered statistically significant.

### 3.3 | Biomarker analysis of "all endometriosis cases" versus "all symptomatic controls" and "pathology-free symptomatic controls", respectively

For the comparison versus "all symptomatic controls", CA-125 was the highest ranking univariate biomarker (AUC 0.645, 95% confidence interval [CI] 0.600–0.690, $q<0.001$; Figure 2a) and had a sensitivity of 60.6%; S100-A12 had an AUC of 0.564 (95% CI 0.518–0.611, $q=0.151$; Figure 2b) and a sensitivity of 50.8%; sFRP-4 had an AUC of 0.579 (95% CI 0.531–0.628, $q=0.041$; Figure 2c) and a sensitivity of 51.0%. No multivariate biomarker combination had a higher AUC than the univariate analysis for this comparison.

For the comparison versus "pathology-free symptomatic controls", S100-A12 was the top-performing univariate biomarker (AUC 0.692, 95% CI 0.614–0.769, $q=0.001$; Figure S2a) and had a sensitivity of 72.1%; CA-125 had an AUC of 0.649 (95% CI 0.569–0.729, $q=0.021$; Figure S2b) and a sensitivity of 58.4%; sFRP-4 had an AUC of 0.512 (95% CI 0.426–0.599, $q=0.975$; Figure S2c) and a sensitivity of 45.0%.

The top-performing bivariate biomarker combination for this comparison was CA-125 and S100-A12; there was an AUC improvement of ~2% (AUC 0.707, 95% CI 0.631–0.784; Figure S2d) and a sensitivity improvement of ~1% (sensitivity was 73.0%), when compared with S100-A12 or CA-125 alone. No trivariate biomarker combination had a higher AUC than this bivariate combination for this comparison.

### 3.4 | Biomarker analysis of "adenomyosis only cases" versus "all symptomatic controls" and "pathology-free symptomatic controls", respectively

For the comparison versus "all symptomatic controls", sFRP-4 was the top-performing univariate biomarker (AUC 0.615, 95% CI 0.551–0.678, $q=0.045$; Figure 3a) and had a sensitivity of 56.4%; CA-125 had an AUC of 0.507 (95% CI 0.441–0.573, $q=0.893$; Figure 3b) and a sensitivity of 48.4%; S100-A12 had an AUC of 0.564 (95% CI 0.498–0.631, $q=0.631$; Figure 3c) and a sensitivity of 47.5%.

For the comparison versus "pathology-free symptomatic controls", S100-A12 was the top-performing univariate biomarker (AUC 0.701, 95% CI 0.611–0.792, $q=0.004$; Figure S3a) and had a sensitivity of 74.6%. CA-125 had an AUC of 0.502 (95% CI 0.400–0.604, $q=0.983$; Figure S3b) and a sensitivity of 33.9%. sFRP-4 had an AUC of 0.547 (95% CI 0.448–0.647, $q=0.922$; Figure S3c) and a sensitivity of 44.4%.

### 3.5 | Biomarker analysis across endometriosis stage

The CA-125 concentration differences between "all endometriosis cases" and the "all symptomatic controls" and "pathology-free symptomatic controls" significantly increased ($P<0.001$) with endometriosis stage (Figure S4). For "all endometriosis cases" versus "all symptomatic controls", CA-125 had an AUC of 0.583 (95% CI 0.533–0.633) for detecting endometriosis Stages I/II and an AUC of 0.795 (95% CI 0.748–0.843) for Stages III/IV. For "all endometriosis cases" versus "pathology-free symptomatic controls", CA-125 had an AUC of 0.587 (95% CI 0.497–0.677) for detecting endometriosis Stages I/II and an AUC of 0.798 (95% CI 0.732–0.864) for Stages III/IV. For S100-A12 and sFRP-4, there was no significant trend in stage dependency (Figures S5 and S6). For "all endometriosis cases" versus "pathology-free symptomatic controls", CA-125 was a stronger predictor of endometriosis than S100-A12 in the Stages III/IV group, but weaker in the Stages I/II group (Figure S4).

## 4 | DISCUSSION

This study examined the diagnostic accuracy of selected blood-based biomarkers, alongside clinical variables, in samples from women with pelvic pain and/or unexplained subfertility to detect suspected endometriosis and/or adenomyosis. CA-125, S100-A12, and sFRP-4 were the top-performing statistically significant biomarkers across comparisons. The combination of CA-125 and S100-A12 performed better than either marker alone in distinguishing between "all endometriosis cases" and "pathology-free symptomatic controls". No other multivariate combination of biomarkers, or combination of biomarkers and clinical variables, outperformed the best univariate marker in all other comparisons. CA-125 showed a gradual and significant ($P<0.001$) increase by disease stage in "all endometriosis cases" versus "all symptomatic controls" and "pathology-free symptomatic controls".

Currently, this is the most robust multicenter, prospective study investigating biomarkers to identify endometriosis/adenomyosis. This contemporary analysis examined a well-characterized participant population attending routine clinical appointments and used standardized data and sample collection protocols. While a large panel of blood-based biomarkers was studied, it was not possible to conduct measurements for all autoantibodies potentially associated with endometriosis that have been identified in the literature.[26,27] This study supports the idea that endometriosis/adenomyosis are highly heterogeneous conditions encompassing a wide range of disease subgroups, making it difficult to identify a single biomarker to diagnose/rule out endometriosis and/or adenomyosis.

CA-125 has high accuracy for identifying severe endometriosis, but several studies report poor performance diagnosing other forms of endometriosis.[28–31] As such, the use of CA-125 as a diagnostic or screening tool is not recommended by National Institute for Health and Care Excellence or European Society of Human Reproduction and Embryology guidelines.[10,11,32] Nevertheless, several studies reported that CA-125 serum concentration increases with endometriosis stage and is particularly high in women with dense pelvic adhesions or ovarian endometriomas.[28,30,33,34] These studies support our findings that CA-125 showed moderate performance at detecting all stages of endometriosis, but performed better for Stage III/IV. Notably, the performance of CA-125 was not sufficient for diagnostic or screening purposes.[35]

To our knowledge, sFRP-4 and S100-A12 have not previously been investigated as blood-based biomarkers for the diagnosis of endometriosis/adenomyosis. In a small study, endometriotic cells collected from participants with endometriosis had significantly increased S100-A12 expression versus endometrial stromal cells from healthy participants.[36] Our results support these findings, S100-A12 showed a moderate performance at diagnosing adenomyosis/endometriosis in populations of women with symptoms but no pathologic findings. This is of biologic relevance because it suggests that altered S100-A12 serum levels may indicate pelvic inflammation associated with endometriosis/adenomyosis; however, the AUC values are insufficient for diagnostic/screening purposes.

In our study, sFRP-4 was the strongest predictor of adenomyosis in women with symptoms versus the other biomarkers. Our results support the findings of Delaney et al.,[37] who reported that, when

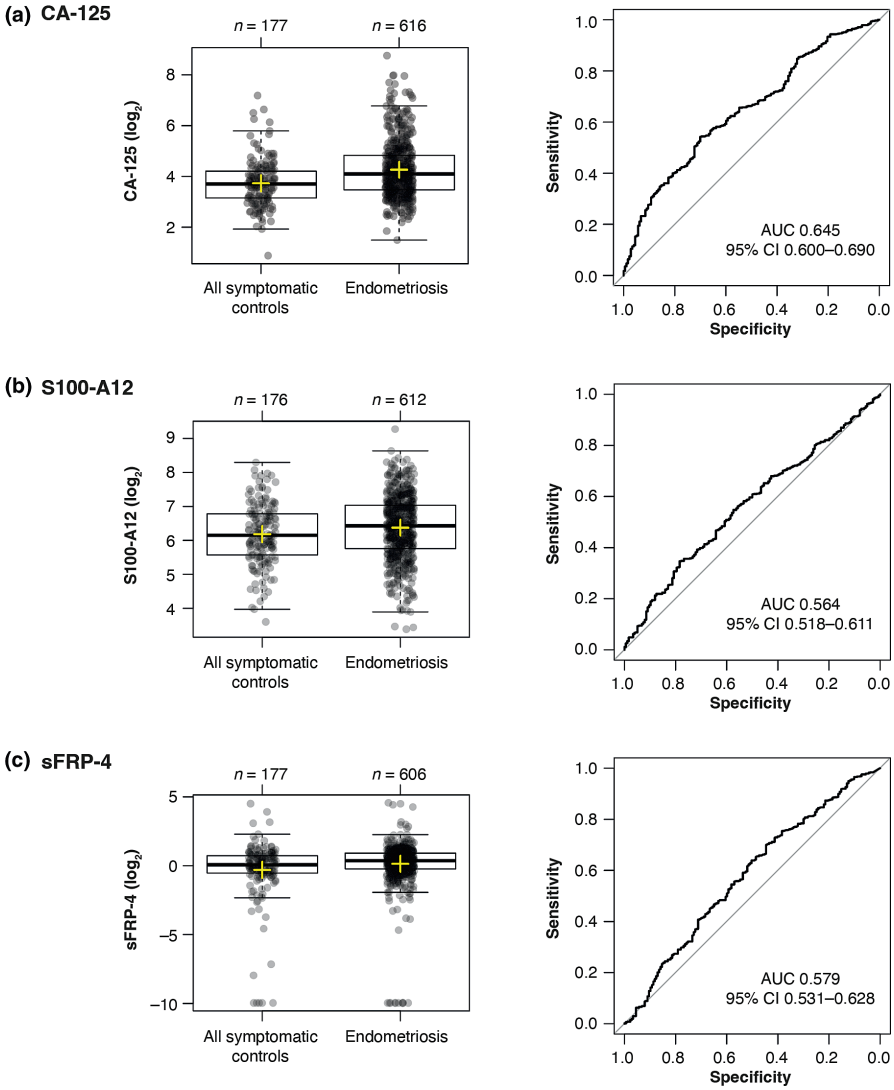


**FIGURE 2** Protein biomarker concentration differences and ROC curves of (a) CA-125, (b) S100-A12, and (c) sFRP-4 for "all endometriosis cases" versus "all symptomatic controls". "Other endometriosis" indicates when the surgeon did not/could not stage the endometriosis during laparoscopy, or the participant had deep endometriosis with or without adenomyosis. Measurement errors or insufficient sample volume meant that not all biomarkers could be measured in all samples. In the box plots: the black line shows the median value, the yellow cross shows the mean value, the box shows the interquartile range, and the whiskers show the range. AUC, area under the curve; CA-125, cancer antigen 125; CI, confidence interval; ROC, receiver operating characteristic; S100-A12, S100 calcium-binding protein A12; sFRP-4, secreted frizzled-related protein 4.

compared with healthy myometrium, sFRP-4 was overexpressed in uterine fibroids, which are benign myometrial lesions that commonly co-exist with adenomyosis.[38] Although these findings are of biologic relevance, the AUC values are insufficient for diagnostic or screening purposes.

For detecting superficial peritoneal endometriosis, MRI has a mean sensitivity/specificity of 79%/72% and TVUS has a mean sensitivity/specificity of 65%/95%.[39] For detecting deep endometriosis, MRI has a pooled sensitivity/specificity range of 66%–85%/93%–97%[40]; TVUS has a pooled sensitivity/specificity range of 59%–85%/86%–97% dependent upon lesion location.[40] For detecting adenomyosis, MRI has a sensitivity/specificity range of 88%–93%/67%–91%[15]; TVUS has an overall sensitivity/specificity of 83.8%/63.9%.[41] For comparison, the top-performing univariate biomarkers in this study had a sensitivity range of 56.4%–74.6% and the top-performing bivariate biomarker had a sensitivity of 73.0%; therefore, they have negligible diagnostic or screening utility.

The development of simple, non-invasive tools to improve diagnosis of endometriosis is currently a topic commanding a great deal of research interest. In addition to blood-based biomarkers, recent publications have reported investigations into the use of salivary microRNA signatures[42] and urinary biomarker measurements.[43] However, large-scale, confirmatory studies will be needed to better establish and validate the accuracy and utility of such tests before they can be considered for clinical use.

In conclusion, despite the high standard of data and sample collection and the large number of participants, a single biomarker or

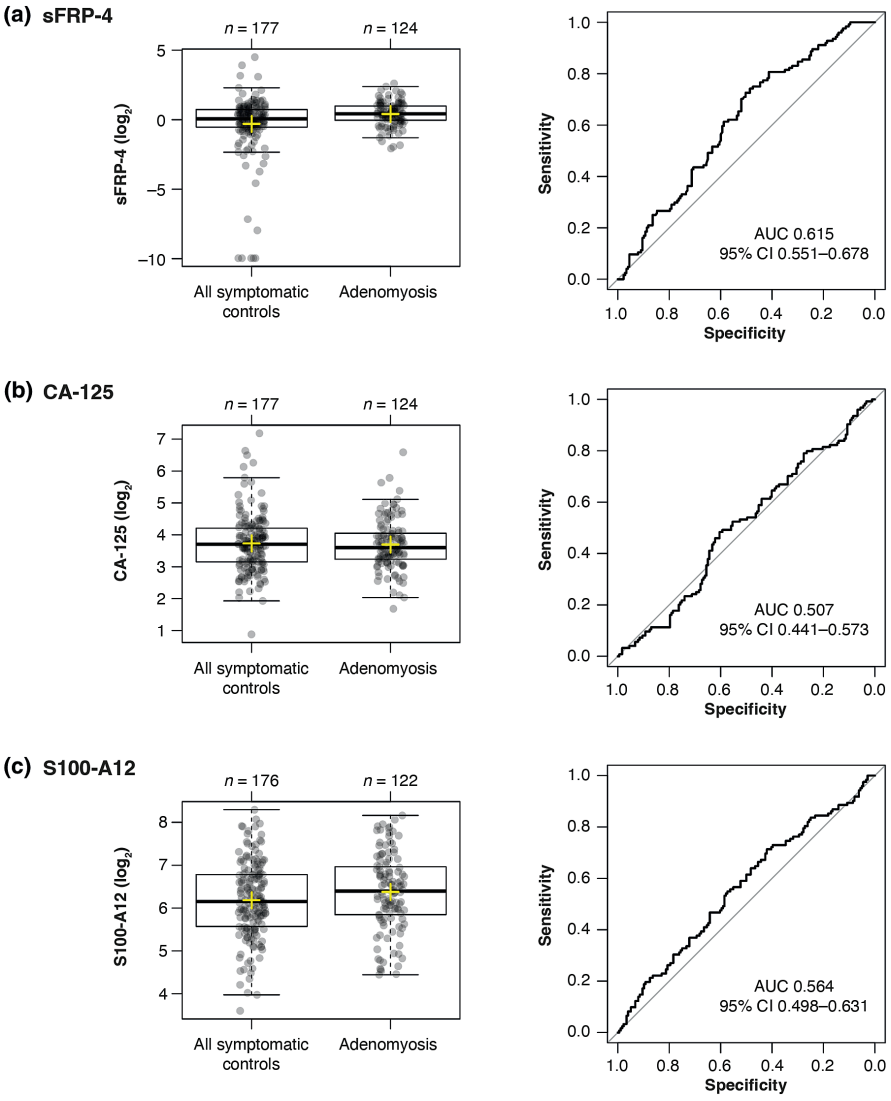


**FIGURE 3** Protein biomarker concentration differences and ROC curves of (a) sFRP-4, (b) CA-125, and (c) S100-A12 for "adenomyosis only cases" versus "all symptomatic controls". Measurement errors or insufficient sample volume meant that not all biomarkers could be measured in all samples. In the box plots: the black line shows the median value, the yellow cross shows the mean value, the box shows the interquartile range, and the whiskers show the range. AUC, area under the curve; CA-125, cancer antigen 125; CI, confidence interval; ROC, receiver operating characteristic; S100-A12, S100 calcium-binding protein A12; sFRP-4, secreted frizzled-related protein 4.

combination of biomarkers could not diagnose/rule out endometriosis and/or adenomyosis with a high certainty.

Further discovery studies of blood-based biomarkers in large, well-phenotyped sample sets with robust replication and validation opportunities are warranted.

## AUTHOR CONTRIBUTIONS

Monika Wölfler, Ralf Rothmund, Christian M. Becker, Krina T. Zondervan, Matthias W. Beckmann, Sibylle Berner-Gatz, Felix S. Grünewald, Martin Hund, Peter Kastner, Ruediger P. Laubender, Heike Wegmeyer, Ursula-Henrike Wienhues-Thelen, and Stefan P. Renner contributed to the study conception/design. Stefanie Burghaus, Predrag Drazic, Monika Wölfler, Sylvia Mechsner, Magdalena Zeppernick, Ivo Meinhold-Heerlein, Michael D. Mueller, Ralf Rothmund, Paola Vigano, Christian M. Becker, Krina T. Zondervan, Matthias W. Beckmann, Peter A. Fasching, Felix S. Grünewald, Peter Kastner, Ruediger P. Laubender, and Stefan P. Renner were involved in data acquisition. Predrag Drazic, Monika Wölfler, Ralf Rothmund, Christian M. Becker, Krina T. Zondervan, Felix S. Grünewald, Martin Hund, Peter Kastner, Martin Klammer, Ruediger P. Laubender, Heike Wegmeyer, and Stefan P. Renner contributed to data analysis and/or interpretation. All authors contributed to writing and/or critical review of the manuscript and approved the final version of the manuscript.

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Two pending patent applications have been submitted by Roche Diagnostics GmbH, F. Hoffmann-La Roche AG, for the use of S100 calcium-binding protein A12 (S100-A12; PCT/EP2020/070426) and secreted frizzled-related protein 4 (sFRP-4; 21174118.6-1118) as biomarkers for the early detection of endometriosis. For the S100-A12 pending patent, the inventors are listed as Aikaterini Georgopoulou, Felix S. Grünewald, Martin Hund, Martin Klammer, and Heike Wegmeyer. For the sFRP-4 pending patent, the inventors are listed as Sibylle Berner-Gatz, Manuel Dietrich, Felix S. Grünewald, Martin Hund, Peter Kastner, Martin Klammer, Ruediger P. Laubender, Heike Wegmeyer, and Ursula-Henrike Wienhues-Thelen. COBAS and ELECSYS are trademarks of Roche. All other product names and trademarks are the property of their respective owners.

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## CONFLICT OF INTEREST STATEMENT

SB, PD, MW, SM, MZ, IM-H, RR, PV, MWB, and SPR declare no conflicts of interest. CMB has acted as a consultant for Roche Diagnostics, Myovant, and Flo Health; has received research grants from MDNA Life Sciences and Bayer Healthcare; and is a member of the IDDM Board (ObsEva). MDM has acted as a paid speaker for Bayer AG. KTZ has received research grants from Roche Diagnostics GmbH, Bayer AG, MDNA Life Sciences, and Evotec; is a board member of the World Endometriosis Society and the World Endometriosis Research Foundation; and is a Research Advisory Board member of Wellbeing of Women, UK. PAF reports personal fees from Agendia, Astra Zeneca, Daiichi-Sankyo, Eisai, Gilead, Hexal, Lilly, Merck Sharp & Dohme, Novartis, Pierre Fabre, Roche, Sanofi Aventis, and SeaGen, grants from BioNTech and Cepheid, grants and personal fees from Pfizer during the time period of this study. SB-G, PK, and U-HW-T are employees of Roche Diagnostics GmbH and is named on the patent application filed for secreted frizzled-related protein 4 (sFRP-4). HW is an employee of Roche Diagnostics GmbH and is named on the patent applications filed for S100 calcium-binding protein A12 (S100-A12) and secreted frizzled-related protein 4 (sFRP-4). MH is an employee of Roche Diagnostics International Ltd and is named on the patent applications filed for S100 calcium-binding protein A12 (S100-A12) and secreted frizzled-related protein 4 (sFRP-4). FSG and MK are employees of Roche Diagnostics GmbH, hold shares in F. Hoffmann-La Roche, and are named on the patent applications filed for S100 calcium-binding protein A12 (S100-A12) and secreted frizzled-related protein 4 (sFRP-4). RPL is an employee of Roche Diagnostics GmbH; is named on the patent application filed for secreted frizzled-related protein 4 (sFRP-4); and is a visiting scientist at the Institute for Medical Information Processing, Biometry, and Epidemiology of the LMU, Munich, Germany.

## DATA AVAILABILITY STATEMENT

This study was conducted in accordance with the applicable regulations. There may be ethical, legal, or other restrictions on sharing the de-identified data set used for the analysis. Please contact the corresponding author (heike.wegmeyer@roche.com) in case of queries.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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