ORIGINAL ARTICLE: ENDOMETRIOSIS

Peritoneal fluid cytokines related to endometriosis in patients evaluated for infertility

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Objective: Our aim was to characterize peritoneal cytokine profiles in patients with infertility, with and without endometriosis, to illuminate potential differences in immune profiles that may reflect mechanistic differences between these two patient populations.
Design: Cross-sectional study.
Setting: University hospital and research center.
Patient(s): Women undergoing laparoscopy for infertility investigation (n = 107).
Intervention(s): Peritoneal fluid was collected during surgery. Clinical characteristics were registered preoperatively.
Main Outcome Measure(s): We determined the concentration of 48 different cytokines from the peritoneal fluid with multiplex immunoassays. Associations between cytokines and clinical findings were assessed with logistic regression and partial least squares discriminant analyses (PLS-DA).
Result(s): Concentrations of SCGF-β, IL-8, HGF, and MCP-1 were significantly higher, while IL-13 was significantly lower in the endometriosis group compared with the group without endometriosis. Multiple stepwise logistic regression identified a combination of SCGF-β, IL-13, and G-CSF concentrations that predicted the presence of endometriosis with 86% sensitivity and 67% specificity. PLS-DA identified a class of 11 cytokines (SCGF-β, HGF, IL-13, MCP-1, CTACK, MCP-3, M-CSF, LIF, IL-5, IL-9, and IFN-a2) that were more characteristic of endometriosis than nonendometriosis patients.
Conclusion(s): By combining univariate and multivariate analyses, profiles of cytokines more likely to be enriched or depleted in infertility patients with endometriosis compared with those without endometriosis were identified. These findings may inform future analyses of pathophysiological mechanisms of endometriosis in infertile patients, including dysregulated Th1/Th2 response and mobilization and proliferation of hematopoietic stem cells. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Cytokine, endometriosis, infertility, peritoneal fluid, PLS-DA

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Endometriosis is a common gynecologic disease affecting up to 10% of all women of reproductive age (1). The disease is characterized by the presence of endometrial tissue outside the uterine cavity. Since endometriosis currently requires surgery and preferably biopsy for a definite diagnosis, it is difficult to obtain a true estimate of the prevalence of the disease, and diagnosis is often delayed for many years after the first symptoms occur.

Apart from infertility, the most common symptoms associated with endometriosis are pelvic pain, dyspareunia, dysmenorrhea, dyschezia, and dysuria (2). In patients with endometriosis, the disease often has a significant negative impact on quality of life, work ability, and educational careers (3, 4).

Endometriosis has been described in different ways according to the degree of adhesions, distribution of lesions, and depth of invasion. The most widely used classification was developed by the American Society of Reproductive Medicine (ASRM) and is based on visual appearance during laparoscopy (5). Paradoxically, many women with endometriosis, independent of disease stage, have few or no symptoms (6). Several

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studies have failed to identify any strong correlation between symptom severity and stage (7).

According to Sampson's theory from 1927, endometrial cells expelled during menstruation are implanted on the peritoneal surface (8). The implants elicit an inflammatory response accompanied by angiogenesis, nerve sprouting, adhesions, fibrosis, and scarring (9, 10). The theory does not explain the fact that while 95% of women have retrograde menstrual flow, only 10% of women in reproductive age have the disease. Alteration in cellular immunity causing decreased clearance of endometrial implants or enhanced immunological reaction to such implants may contribute to establishment and progression of the disease (11, 12). Other recent theories implicate implantation and differentiation of stem cells migrating from the basal layer of the endometrium and bone marrow to the peritoneal cavity (13, 14).

Studies analyzing protein and cytokine composition of the peritoneal fluid (PF) of patients with endometriosis provide theories on the origins of adhesion formation, angiogenesis, and cellular invasion associated with endometriosis (15, 16). Cytokines are peptides secreted from a variety of cells involved in inflammation, cell proliferation, and differentiation. As these interact in networks, a multivariate approach may identify a common pattern reflecting key mechanisms in the pathogenesis of endometriosis (17–19). Network analyses will vary depending on different study samples and different study protocols (20).

A comprehensive approach combining studies from different countries and study populations may give a better understanding of the clinical diversity of endometriosis. Standardized collection of clinical data and biological samples from diverse patient samples and standardized analyses may allow comparison of data from several studies. The World Endometriosis Research Foundation is promoting this approach by its guidelines and protocols for collection of tissue samples and clinical data (21).

In a recent study, concentrations of 50 cytokines from the PF of women undergoing surgery for various gynecological conditions, including endometriosis, were measured simultaneously in multiplex assays (22). Multivariate analyses sorted the cases with endometriosis into two classes based on covariation in cytokine elevations. The cytokine profile was then linked to established protein-expression databases and related to clinical characteristics. Enrichment analyses revealed that activated macrophages were responsible for the cytokine signature and that specific kinase signaling was crucial for the propagation of the macrophage-driven network. Rakhila et al. studied cytokine patterns in a different patient population and identified 13 cytokines with mitogenic and angiogenic activities that were increased in late-stage endometriosis (23).

In this study, we examined the concentrations of 48 cytokines and growth factors in the PF of women undergoing diagnostic laproscopy with infertility as the primary symptom. The aim of the study was to test the hypothesis that distinct cytokine profiles may be associated with endometriosis in these patients, as such differences may yield insights into disease pathogenesis and aid efforts to develop therapies.

MATERIALS AND METHODS

Study Design and Patient Selection

Patients evaluated for inclusion were undergoing infertility assessment by laparoscopy between September 2013 and November 2014 at the Department of Gynecology at Oslo University Hospital, a setting where the majority of infertility patients undergo diagnostic laproscopy either in the general gynecology clinic associated with the Department of Gynecology (patients included in this study) or in a specialized endometriosis unit (patients not included in this study). The majority of the women were nulliparous and had infertility of unknown cause. All women signed informed consent, and the study protocol was approved by Oslo University Hospital and the regional ethical committee (REC) of South-East Norway (REC 2013/567). The REC is accredited by the Institutional Review Board (IRB00001871). The authors had no conflicts of interest. The study was performed as collaboration between the Department of Gynecology, Oslo University Hospital, Ullevål, and the Center for Gynepathology Research (CGR), Massachusetts Institute of Technology.

Other than infertility, there were no systematic inclusion criteria. Exclusion criteria were irregular cycles ($<$25 days or $>$35 days), hormonal therapy during the last 3 months, other intra-abdominal diseases, or inability to understand written consent or written questionnaires. Of the 107 patients recruited, four were excluded before processing: two because of irregular menstrual cycles, one due to ascites and liver cirrhosis, and one because a complication during surgery made collection of PF impossible. Of the remaining 103 patients, 94 had sufficient volume of PF collected for Luminex assays.

All patients had a gynecological examination and vaginal ultrasound before referral to surgery. No additional radiological examinations to evaluate extraperitoneal endometriosis were routinely performed. On the day of surgery, clinical data were collected from all patients. Cycle phase was calculated from the last menstrual period and average length of menstrual cycle.

Sample Collection and Processing

Before surgery and before the definitive diagnosis of endometriosis, the patients completed questionnaires about clinical characteristics and pain.

PF was collected from the cul-de-sac at the beginning of the laparoscopic surgery after insertion of trocars and before any manipulation of the pelvic organs. We used a thin suction cannula or a suction tube for aspiration of undiluted PF. Patients were evaluated for the presence of endometriosis and staged according to ASRM criteria. The diagnosis was confirmed with peritoneal biopsy in 73% of the cases. For the remaining 27%, the diagnosis was made by visual inspection of typical endometriotic lesions. The PF was stored on ice up to 45 minutes before it was centrifuged on 300 *g* for 5 minutes to pellet cells. The supernatant was transported to the laboratory on wet ice. The cell pellet was resuspended in phenol red-free Dulbecco's modified Eagle medium supplemented with 10% charcoal stripped bovine serum and

penicillin/streptomycin right after centrifugation. The fluid was stored in aliquots at −80°C. Fifty microliters of the cell suspension was saved for cell counting with the Scepter automated cell counter from Millipore (Sigma-Aldrich), using the 60 μm sensor tips. The suspension was then centrifuged again at 300 *g* and resuspended in the same medium supplemented with 10% dimethyl sulfoxide at a cell concentration of 1 million cells/mL. The cells were cryopreserved in 1 mL aliquots and stored at −80°C. The samples were subsequently shipped on dry ice from Norway to the CGR for centralized analyses and processing.

The sample collection protocol and analysis methods were developed and validated at CGR on different patient populations (22). To affirm consistency, 20 of the newly collected PF samples were analyzed in a pilot experiment for validation and compared with the samples collected locally in Newton, MA. The variation in PF volume, distribution of cycle phase, PF protein content, and leukocyte composition were similar in the two study groups. The total cytokine concentration in the PF was closely correlated ($r = 0.81$) between samples collected in the United States and Oslo, indicating that the protocol gave comparable results.

Multiplex Cytokine Immunoassay

The concentrations of 48 different cytokines, chemokines, and growth factors in the PF were measured with the Bio-Plex FlEXMAP 3D system (Bio-Rad Laboratories), and data were collected with xPONENT version 4.2 (Luminex Corporation). The cytokines were all included in human cytokine panels I and II from Bio-Rad. The samples were assayed in triplicate of undiluted PF samples.

The mean of median fluorescence intensity (MFI) in 10 parallel aliquots of standard diluents was used to establish the background MFI. The lowest limit of detection was defined as the background + 2 SD. Average MFI from three parallels of PF samples were converted to absolute concentrations above the lowest limit of detection via calibration to nine-point standard curves using the L5P function (Cardillo 2012) in MATLAB R2015b (The Mathworks).

Statistical Analysis and Modeling

A statistical power analysis, assuming $\beta = 0.1$ and $P=.05$, concluded that at least 60 patients were needed to detect a significant difference in cytokine levels between the endometriosis group and the control group. This power analysis is in agreement with a similar study (22) performed on a different patient population.

Patient characteristics were compared individually between the groups with and without endometriosis. The chi-square test was used for categorical variables, and the *t*-test for continuous variables.

Differences in cytokine concentrations between the groups with and without endometriosis were analyzed with univariate analysis and nonparametric Mann Whitney *U*-test. Data are presented as median and interquartile range. The individual cytokines were then tested for differences in median concentration according to the phase of the menstrual cycle. The association between concentration of individual cytokines and endometriosis was assessed with univariate logistic regression. Odds ratios (ORs) were estimated, and receiver-operating characteristics (ROC) curves were plotted to determine the diagnostic performance of the cytokines individually. The concentrations were log-transformed before the regression analyses to obtain normal distribution of the data. ORs are presented as standardized ORs by dividing the log-transformed concentrations with SD. The assessments were performed for all patients and for each cycle phase separately. Cytokines identified as significantly predictive of endometriosis in the univariate analysis were combined in multivariate forward-stepwise logistic regression to identify the final set of predictors for endometriosis with higher accuracy than that based on the single cytokines. For the multivariate models, standardized ORs for the individual cytokines in the model are presented. ROC curves were used to estimate sensitivity and specificity for each model. A two-sided $P<.05$ was considered statistically significant. SPSS version 22 (IBM) software was used for statistical analyses.

All the cytokines assessed in the univariate analysis are part of a complex system where individual cytokines interact and change in ways that are dependent on the other cytokines. By performing a multivariate analysis, the aim was to identify a set of chemical features or a profile that uniquely defines the system against a larger background.

PLS-DA (partial least squares discriminant analysis) is a supervised form of discriminant analysis that relies on class membership of each observation and is used to sharpen separation between groups. In this study, the method was used to identify cytokine combinations most strongly discriminating between the presence and absence of endometriosis. Validation of the class separation was performed by random permutation and the leave n-out method. ROC curves were evaluated to determine the minimal number of cytokines included in the final profile, by the following process: [1] the cytokine possessing the lowest current PLS-DA model weighting coefficient was removed; [2] a new, reduced PLS-DA model was determined with the remaining cytokines; [3] the area under the curve (AUC) of this new, reduced model was calculated; [4] the iteration process was continued until the smallest PLS-DA model (i.e., involving the lowest number of cytokines) still producing an AUC within 1 standard error of the maximal PLS-DA model was obtained.

To investigate whether particular cellular mediators could be associated with the class-separating cytokines, gene set enrichment analysis was performed with immune response in silico (IRIS) transcriptional compendia as reference (24).

RESULTS

Patient Characteristics

The clinical characteristics and PF composition of the 94 patients are summarized in Table 1. The primary diagnosis in all patients was infertility. The average age was 32.8 years (SD, 3.4). Patients were divided into two groups according to the absence or presence of endometriosis. In 38 women, there was no visual or histological sign of endometriosis. Endometriosis was diagnosed in 56 women. The disease was classified

TABLE 1

Patient and PF characteristics (n = 94).

Characteristics	Endometriosis present (n = 56)	Endometriosis absent (n = 38)	*P* value
Age	32 (24–39)	34 (27–40)	.08
Reproduction			
Pregnancies	0.4 (0–4)	0.5 (0–2)	.65
Infertility, mo	33 (12–100)	33 (12–100)	.96
Male factor	7 (13)	3 (8)	.18
Tubal factor	5 (9)	6 (16)	.31
Pain symptoms			
Dysmenorrhea	41 (73)	24 (63)	.30
Pelvic pain	18 (32)	12 (32)	.95
Dyspareunia	19 (34)	18 (47)	.19
Bowel symptoms	17 (30)	10 (26)	.67
Urinary symptoms	3 (5)	3 (8)	.62
PF aspirates			
PF volume, mL	12.1 (0.5–50)	11.6 (0.5–44)	.83
PF cells, $\times 10^6$	6.0 (1–14)	5.5 (1.5–9.9)	.16
Luteal phase	31 (55)	23 (61)	.62
Follicular phase	25 (45)	15 (39)	.62
I/II (minimal/mild)	43 (77)		
III/IV(moderate/severe)	13 (23)		

Note: Data are presented as mean (range) or proportion (%). *P* values represent significance of nonequivalence by independent samples *t*-test for continuous data and chi-square test for categorical data.

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as minimal to mild in 77% of the cases and moderate to severe in 23%.

The occurrence of endometriosis-associated symptoms, including dysmenorrhea, dyspareunia, dysuria, and bowel symptoms, was similar in women with and without endometriosis (Table 1). This was also observed when comparing the patients with minimal-to-mild to moderate-to-severe endometriosis. The age, parity, and duration of infertility were comparable. Similar proportions of women were tested during the follicular and luteal phase of the menstrual cycle. The cell count and volume of aspirated PF were not significantly different between the two groups.

Cytokines in the PF

We measured the concentration of 48 different cytokines in the undiluted PF using multiplex immunoassay. The cytokines included a variety of chemokines, growth factors, and inflammatory cytokines, and all except IL-17 and TNF-ß were quantified at concentrations above the lower limit of detection.

The remaining 46 cytokines were compared among women with and without endometriosis (Supplemental Table 1). The concentrations of SCGF-β ($P<.001$), IL-8 ($P=.001$), HGF ($P=.002$), and MCP-1 ($P=.023$) were significantly increased in the PF of women with endometriosis, while IL-13 ($P=.008$) was decreased (Table 2). The effect of cycle phase on cytokine concentrations was examined by comparing luteal phase and follicular phase pairwise for all the cytokines, and no significant difference was found except for with SDF1, which displayed significantly increased concentration in the luteal phase. The univariate associations between cytokines and endometriosis were analyzed after separation according to cycle phase (Table 2). When only patients in the luteal phase were considered, IL-8 ($P=.013$), HGF ($P=.010$), and SCGF-ß ($P=.021$) showed significantly increased concentrations in the infertile patients with endometriosis. In the follicular phase, IL-13 ($P=.040$), SCGF-ß ($P=.001$), IP-10 ($P=.003$), and IL-1ra ($P=.045$) were significantly differently expressed between patients with and without endometriosis.

Univariate logistic regression was used to quantify the association between cytokine concentrations and presence of endometriosis (Table 3, panel A). In addition, the discriminating ability of each cytokine was established by calculating sensitivity and specificity from the ROC curves. Cutoff values were chosen by the highest sum of sensitivity and specificity at a specificity $\geq 50\%$. In the complete data set, six of the cytokines (G-CSF, IL-13, SCGF-ß, IL-8, HGF, and MCP-1) were significantly associated with endometriosis. SCGF-ß was the only cytokine that was significantly predictive independantly of cycle stage and disease severity. The best statistical correlation was obtained for SCGF-ß during the follicular phase (AUC = 0.82). In the luteal phase, three cytokines (IL-8, SCGF-β, and HGF) were indicative of endometriosis, but the predictive power was low (Supplemental Fig. 1).

The six cytokines identified in the univariate analysis were combined in a forward conditional logistic regression analysis. A model with SCGF-β, IL-13, and G-CSF resulted in the highest predictive value, with an AUC of 0.81, a sensitivity of 86%, and a specificity of 66%. When excluding severe endometriosis, the same model had AUC = 0.80 with sensitivity of 83% and specificity of 66% (Table 3, panel B).

PLS-DA analyses on this data set of 46 cytokines identified a class of 11 cytokines (SCGF-β, IL-13, HGF, MCP-1, CTACK, IFN-a2, LIF, M-CSF, MCP-3, IL-5, and IL-9) characteristic of endometriosis compared with no endometriosis (Fig. 1). After calculating several permutations based on

TABLE 2

Comparison of cytokine concentrations (pg/mL) in patients with and without endometriosis.

Phase and cytokine	No endometriosis	Endometriosis	*P* value[a]
Any	n = 38	n = 56	
IL-8	10.03 (7.03–15.82)	16.07 (10.9–28.5)	.001
SCGF-ß	22,260 (13,821–28,828)	31,737 (23,741–42,207)	.001
HGF	363.7 (235.1–557.4)	494.8 (362.4–653.7)	.002
IL-13	8.85 (5.50–10.63)	6.46 (3.42–9.37)	.008
MCP-1	134.0 (105.0–170.4)	170.1 (116.9–273.8)	.023
Luteal	n = 23	n = 31	
IL-8	10.24 (6.49–19.02)	16.11 (11.51–28.34)	.013
HGF	386.0 (245.8–582.1)	521.8 (431.3–658.5)	.01
SCGF-ß	23,738 (14,476–31,156)	30,286 (23,781–41,708)	.021
Follicular	n = 15	n = 25	
IL-13	8.44 (5.47–12.36)	4.49 (3.26–9.67)	.04
SCGF-ß	21,578 (11,117–24,067)	34,013 (23,136–46,730)	.001
IP-10	3,292 (2,294–3,984)	5,198 (3,790–6,928)	.003
IL-1ra	18.49 (12.09–28.51)	31.08 (18.68–48.35)	.045

Note: Data are presented as median concentrations and interquartile range.
[a] Mann-Whitney *U*-test.

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random composition of cytokines, the ROC for the permutated profiles and the ROC curve for the observed data were compared. The best discriminative power was reached when 35 out of the 46 cytokines were removed. Five cytokines correlated negatively with endometriosis (IL-13, IL-5, CTACK, MCP-3, and M-CSF), and six showed positive covariation (SCGF-β, HGF, IFN-a2, MCP-1, IL-9, and LIF).

The 11 class-separating cytokines showed a discriminative power with a misclassification rate of 30%–40%. The discriminative power increased with increased disease severity. Four of the five cytokines that separated endometriosis from no endometriosis in the univariate analysis were included in the 11 cytokines identified from the PLS-DA analysis. This agreement strengthens the power of the results.

Through enrichment analyses, the 11 cytokines from the PLS-DA analyses were compared with expression profiles of immune cell populations described in the IRIS compendia that try to identify leukocyte phenotypes expressing the 11

TABLE 3

Logistic regression models with endometriosis as dependant variable.

A. Univariable logistic regression

Cycle phase	Stages compared	Variable (cytokine)	OR (CI)	*P* value	AUC	Sensitivity	Specificity
All	0/All	G-CSF	1.78 (1.03–3.05)	.038	0.61 (0.49–0.72)	61	60
		IL-13	0.50 (0.30–0.85)	.01	0.68 (0.56–0.79)	69	65
		SCGF-β	2.02 (1.34–3.04)	.001	0.72 (0.61–0.83)	77	58
		IL-8	2.45 (1.27–4.70)	.007	0.71 (0.61–0.82)	80	58
		HGF	2.39 (1.39–4.10)	.002	0.69 (0.58–0.80)	70	66
		MCP-1	2.09 (1.12–3.87)	.02	0.65 (0.53–0.76)	66	53
Luteal	0/All	IL-8	2.59 (1.06–6.34)	.038	0.70 (0.55–0.85)	94	52
		SCGF-β	2.42 (1.11–5.25)	.026	0.68 (0.53–0.84)	74	56
		HGF	2.83 (1.28–6.29)	.01	0.71 (0.56–0.85)	77	61
Follicular	0/All	SCGF-β	4.24 (1.49–12.1)	.007	0.82 (0.69–0.95)	72	79

B. Multiple forward conditional logistic regression

Cycle phase	Stage	Model	OR (CI)	*P* value	AUC (CI)	Sensitivity, %	Specificity, %
All	0/All	SCGF-β, IL-13, G-CSF			0.81 (0.72–0.90)	86	66
		SCGF-β	2.37 (1.23–4.54)	.01			
		IL-13	0.34 (0.17–0.72)	.005			
		G-CSF	2.23 (1.06–4.70)	.035			
All	0/I-II	SCGF-β, IL-13, G-CSF			0.80 (0.70–0.90)	83	66
		SCGF-β	2.04 (1.05–3.98)	.037			
		IL-13	0.37 (0.18–0.75)	.006			
		G-CSF	2.14 (1.02–4.48)	.045			

Note: Only cytokines with statistically significant standardized OR are listed.

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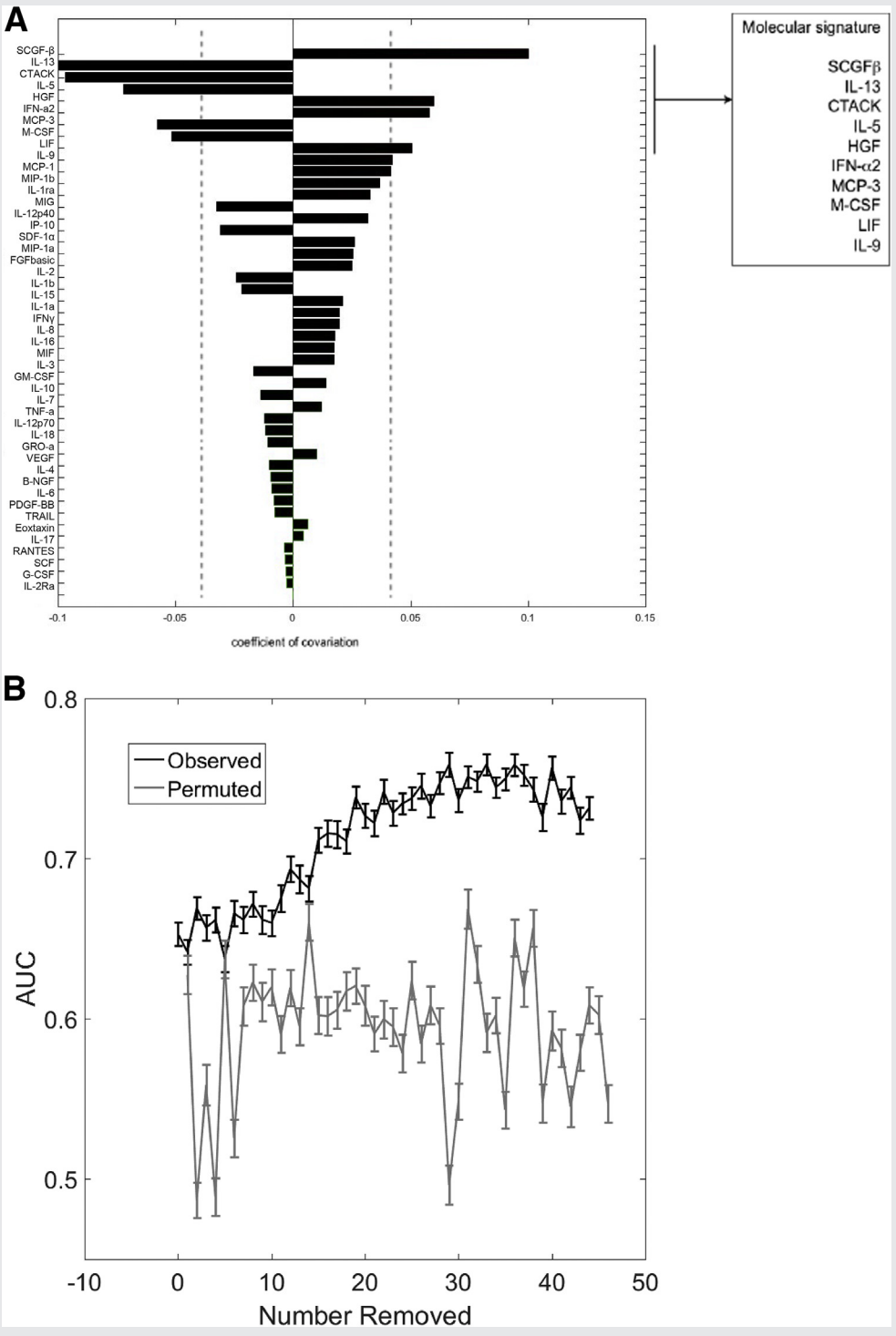
FIGURE 1



(**A**) PLS-DA analysis of all 94 patients identifying a minimum set of predictive cytokines separating endometriosis from no endometriosis. Numbers on the *x*-axis represent covariation between each cytokine and endometriosis. (**B**) AUC according to number of class-separating cytokines removed from the original 47 cytokines measured. AUCs for random permuted combinations and observed combinations of cytokines are shown for validation.

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cytokines and their cognate receptors. No specific cell lines could be identified from the profile.

DISCUSSION

Endometriosis is a heterogenous disease with diverse symptoms, visual appearance, and long-term outcome (25). Infertility is one of the most common symptoms of endometriosis, often in combination with pelvic pain. Among patients with unexplained infertility, the prevalence of endometriosis is reported to be up to 50% (26). We studied 94 patients with infertility as the primary diagnosis and found endometriosis in 60% of the cases. With multiplex assay, we measured a panel of 48 different cytokines from the PF collected during laparoscopy. The aim of the study was to test the hypothesis that infertility patients with endometriosis have different cytokine patterns than those without endometriosis, reflecting a possible difference in disease mechanism. If different patterns are observed in infertile patients with and without endometriosis, subsequent mechanistic analysis of the molecular networks of the different patient subgroups may yield insights into therapies or improved clinical management.

Symptoms and demographic characteristics were recorded before laparoscopy. The frequency of dysmenorrhea, chronic pelvic pain, dyspareunia, and bowel symptoms was high, but there was no significant difference between patients with and without endometriosis. In this study, as in other studies (27, 28), the presence or absense of pain reported preoperatively was not a reliable discriminator for endometriosis.

The majority of patients classified as having endometriosis present were stage I and II according to the ASRM classification (77% stage I/II, 23% stage III/IV), a distribution slightly more enriched in stage I/II (77% vs. 68%) than a prior study from the IVF clinic at Oslo University Hospital (29). There was no difference in the frequency of the different pain symptoms according to disease stage, but the relatively small number of patients with advanced-stage disease and the binary yes/no pain metric, rather than a pain scale, reduced statistical discriminatory power.

Of all the 48 cytokines, four (MCP-1, IL-8, HGF, and SCGF-ß) were significantly elevated, and one (IL-13) decreased in patients with visual or histological proven endometriosis, compared with patients without endometriosis. MCP-1, IL-8, and HGF have been recognized as increased in endometriosis in several other studies (19, 30). The serum level of IL-13 in mild endometriosis was significantly reduced in another recently published study (31). IL-13 can act as an anti-inflammatory cytokine by antagonizing the activity of activated macrophages and thereby reducing the production of other cytokines like MCP-1; hence, its suppression is mechanistically consistent with an elevation in proinflammatory cytokines (NBC Gene. ID:3596). SCGF-ß has to our knowledge not been recognized as associated with endometriosis before. When stratifying into follicular and luteal phase, SCGF-ß was the only cytokine that was significantly differently expressed in both cycle phases. The difference was more pronounced in the follicular phase.

To investigate whether any of the measured cytokines were more strongly associated with endometriosis, we first performed a univariate logistic regression and then combined the significant cytokines in different multivariate logistic regression models. Similar methods have been used in other studies with varying results, with respect both to predictive power and to the selected cytokines (32–34). Borrelli and coworkers evaluated a model with PF IL-8, MCP-1, and MIP-1β as markers and found a positive predictive value of 89% (32). Vida Kocbek et al. established a model where a ratio of PF fiocolin2/glycocodein-A in combination with IL-8 and age predicted endometriosis with a sensitivity of 72.5% and a specificity of 91.2%. In these studies, the percentage of advanced endometriosis was 42% and 76%, respectively (33). In our study, the best statistical power was obtained with a model including SCGF-ß, IL-13, and G-CSF (sensitivity of 86% and specificity of 66%). The difference between our results and previous reports may reflect that we were studying different patient populations or that the methods used to detect the proteins were different, with different detection sensitivity and calibration biases. Further, the objective of this study was mechanistic in nature and not aimed at developing a diagnostic for endometriosis. Although blood biomarkers for endometriosis have not yet shown clinical diagnostic value (35) and PF is in closer approximation to the endometrial lesions and better reflects the inflammatory mechanisms with endometriosis, it is not reasonably accessible for the diagnosis of endometriosis. A test of this model would be to analyze the cytokines in the PF of an independent patient group to see how well it discriminates between presence and absence of endometrisis.

As endometriosis is a complex disease with polygenic nature and environmental influence, multivariate data-driven biostatistical methods are neccessary to obtain reliable and robust discrimination between patient groups. Logistic regression has limitations when the predictor variables are not independent: it tends to overestimate the predictive power of the variables, and it is hard to select the best predictors among many independent variables. Elucidating a multivariate set of key biological factors (i.e., cytokines in this case) within phenotypically discriminitory groups is a more powerful approach for identifying biological mechanisms because pathophysiological processes generally involve integrated operation of multiple factors. We chose PLS-DA modeling for this purpose, and the result was a set of 11 covarying cytokines, which collectively generated the strongest discriminative power for endometriosis. Two of these overlapped with the three identified by the multiple logistic regression analysis. Taken together, we identified 13 cytokines that discriminate the presence or absence of endometriosis, which can be broadly considered as belonging to three subgroups with overlapping biological functions, such as chemokines (IL-8, MCP-1, MCP-3, and CTACK), hematopoietic growth factors (IL-5, IL-13, IL-9, M-CSF, and G-CSF), and general growth factors (HGF and LIF).

Among the chemokines, IL-8 and MCP-1 showed positive correlation and MCP-3 and CTACK showed negative correlation with endometriosis. IL-8 and MCP-1 are chemokines involved in attracting leukocytes to sites of inflammation. Both have been found to be elevated in the PF with endometriosis in other studies (19, 23). IL-8 is a well studied

proinflammatory chemokine, is produced in several tissues as response to inflammation and trauma, attracts neutrophils, and promotes angiogenesis (36, 37). MCP-1 is produced by a variety of cells and is a key chemoattractant for monocytes, T-helper cells, and natural killer cells. MCP-1 is involved in many inflammatory diseases involving monocytic infiltrates (38). Positive correlation with endometriosis and MCP-3 has been seen in another recent study (23). How MCP-1 is affected by other diseases in the pelvis is not known. MCP-3 has many similar characteristics with MCP-1: both stimulate Th2 polarization of T-cells and are elevated in asthma and other chronic inflammatory diseases (39). We found a negative association with endometriosis. CTACK is a chemokine mainly expressed in the skin and is involved in attracking specialized T-cells. Increased levels have been measured in serum and ectopic endometrial samples from patients with endometriosis (40). We found CTACK to be negatively correlated with endometriosis when measured in the PF.

IL-5, IL-13, IL-9, M-CSF, G-CSF, and SCGF-ß are hematopoietic growth factors. IL-5, IL-9, and IL-13 are all produced by Th2 T-helper cells and are involved in many types of allergic reactions and inflammation, which lead to enhanced Ig-E production of B-cells and proliferation and maturation of mast cells. IL-5 and IL-13 are located on the same gene locus and are highly coregulated and act as key regulators of eosinophile production. Studies on other inflammatory diseases like obesity indicate that IL-5, IL-13, and IL-9 also can have anti-inflammatory properties (41). In our study, IL-5 and IL-13 were inversely correlated to endometriosis and IL-9 was positively correlated. M-CSF stimulates hemotopoietic stem cells to differentiate into macrophages. Studies indicate that M-CSF also has a direct effect on endometrial cell proliferation and attachment to peritoneal mesothelial cells (42, 43). G-CSF stimulates proliferation and maturation of neutrophile precursors into mature neutrophils. It is also a potent inducer of hematopoietic stem cell mobilization from the bone marrow.

In combination with GM-CSF, G-CSF, and erythropoietin, SCGF-ß promotes proliferation of erythroid and myeloid progenitor cells in the bone marrow. This cytokine has been studied in serum samples from patients with dilated cardiomyopathy (44) and silent brain infarction (45), examples of inflammatory disease. Serum concentrations were increased in both conditions, indicating that SCGF-ß may be a marker for inflammation. Increased serum levels have also been demonstrated after stem cell transplantation (46). Beste et al. (22) also analyzed SCGF-ß in the PF in a cohort of patients whose main symptom was pain, and they found no difference between patients with endometriosis and controls, although proteins were measured by a different method. The concentration of SCGF-ß was overall high in our population and most pronounced in the PF from patients with endometriosis collected during the follicular phase. This is to our knowledge the first time SCGF-ß has been studied in relation to endometriosis. Among the different theories on the pathogenesis of endometriosis is the contribution of endometrial stem cells and bone marrow–derived stem cells as the origin for ectopic lesions (47, 48). If SCGF-ß elevation in endometriosis can be reproduced in other studies, it may give more insight into this theory.

HGF is a growth factor, secreted by mesenchymal cells, which was increased in endometriosis in our study. It acts on endothelial, epithelial, and hematopoietic cells in addition to T-cells. HGF plays a central part in angiogenesis and tissue regeneration, matrix invasion, and neovascularization. It has been described in relation to adenomyosis (49) and endometriosis (18). LIF is a growth factor involved in many different biological activities in different tissues, such as implantation of the embryo and development of placenta. It serves as a coordinator between the nervous and the immune system and can have both inflammatory and antinflammatory properties. LIF acts on stem hematopietic stem cells by keeping them in their pluripotential state.

IFN-a2 has antiviral and antitumor activities. This interferon has been associated with autoimmune diseases such as lupus erythematosus. It has not been previously associated with endometriosis. In our study, IFN-a2 was positively correlated with endometriosis.

The profile of 13 cytokines identified by univariate and multivariate analysis of cytokines in the PF may reflect some important pathogenic mechanisms (50). Angiogenesis is required for the endometrial implants to grow and invade. The abundance of growth factors and hematopoietic factors controlling the growth of endometrial and leukocyte progenitor cells indicates increased hematopoietic proliferative activity and recruitment. Dysregulated Th1/Th2 response reflected by the altered cytokines produced by Th2 cells may be an important contributor to reduced clearance of endometrial cells in the peritoneal cavity and hypersensitive immune reactions in this patient population.

Conclusion

Using a systems biology approach, we have gained a better understanding into the pathogenesis of inflammation associated with endometriosis in an infertile patient population. Of 48 cytokines measured, four cytokines, MCP-1, IL-8, HGF, and SCGF-ß, showed significantly higher concentrations in patients with endometriosis compared with patients without the disease, while IL-13 had a lower concentration in patients with endometriosis. A model with a combination of SCGF-β, G-CSF, and IL-13 was able to identify endometriosis with 86% sensitivity and 67% specificity. Multivariate analyses identified a class of 11 cytokines that were different in infertile patients with and without endometriosis. There was considerable agreement between the two methods of classifying endometriosis from no endometriosis. The profile identified in this study supports theories of aberrant Th1/Th2 activity and increased angiogenesis and hematopoietic proliferative activity as mechanisms of importance in the pathogenesis of endometriosis in infertility patients. These insights may be helpful in future developement of targeted therapy and improved diagnostic methods for infertility patients with and without endometriosis. The high prevelance of endometriosis and comorbidity with other conditions motivate further studies to classify patients according to molecular mechanisms that might correlate to clinical symptomology.

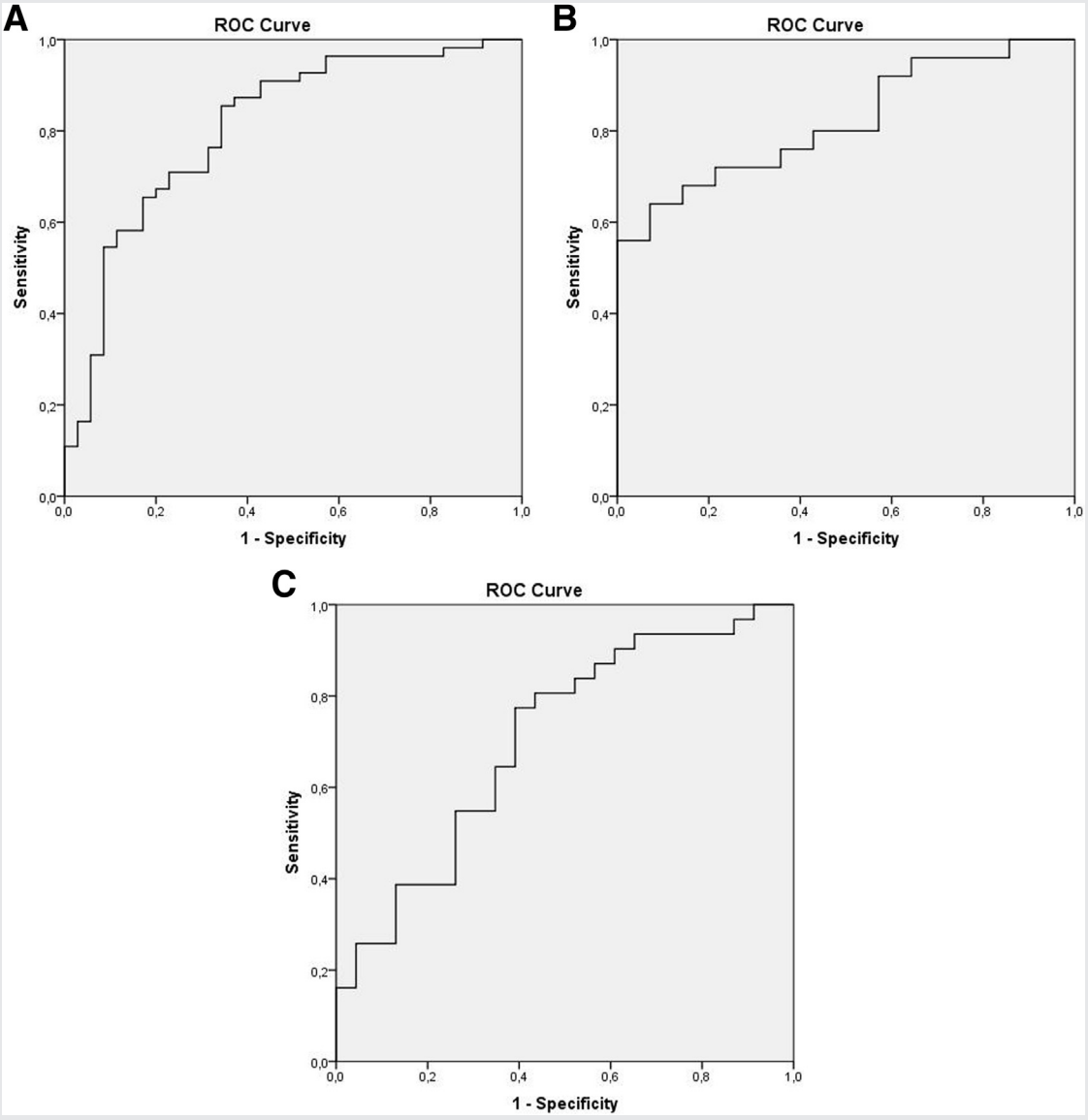
REFERENCES

1. Guidice LC. Endometriosis. N Engl J Med 2010;362:2389–98.
2. Burney RO, Guidice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012;98:511–9.
3. Culley L, Law C, Hudson N, Denny E, Mitchell H, Baumgarten M, et al. The social and psychological impact of endometriosis on women's lives: a critical narrative review. Hum Reprod Update 2013;19:625–39.
4. De Graff AA, Dirksen CD, Simoens S, De Bie B, Hummelshoj L, D'Hooghe TM, et al. Quality of life outcomes in women with endometriosis is highly influenced by recruitment strategies. Hum Reprod 2015;30:1331–41.
5. American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997;5:817–21.
6. Sinaii N, Plumb K, Cotton L, Lambert A, Kennedy S, Zondervan K, et al. Differences in characteristics among 1,000 women with endometriosis based on extent of disease. Fertil Steril 2008;89:538–45.
7. Chapron C, Fauconnier A, Dubuisson JB, Barakat H, Vieira M, Beart G. Deep infiltrating endometriosis: relation between severity of dysmenorrhea and extent of disease. Hum Reprod 2003;18:760–6.
8. Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3:93–110.
9. Herington JL, Bruner-Tran KL, Lucas JA, Osteen KG. Immune interactions in endometriosis. Expert Rev Clin Immunol 2011;7:611–26.
10. Ahn SH, Monsanto SP, Miller C, Sinh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. Biomed Res Int 2015; 2015:795976.
11. Bianco B, André GM, Vilarino FL, Peluso C, Mafra FA, Christofolini DM, et al. The possible role of genetic variants in autoimmune-related genes in the development of endometriosis. Hum Immunol 2012;283:306–15.
12. Jeung I, Cheon K, Kim MR. Decreased cytotoxicity of the peritoneal natural killer cells in endometriosis. Biomed Res Int 2016;2016:2916070.
13. Brosens I, Gargett CE, Puttemans P, Gordts S, Brosens JJ, Benagiano G. Origins and progression of adoloscent endometriosis. Reprod Sci 2016;23:1282–8.
14. Hufnagel D, Li F, Cosar E, Krikun G, Taylor HS. The role of stem cells in the ethiology and pathophysiology of endometriosis. Semin Reprod Med 2015; 35:333–40.
15. May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. Hum Reprod Update 2010;16:651–74.
16. Stilley JA, Birt JA, Sharpe-Timms KL. Cellular and molecular basis for endometriosis-associated infertility. Cell Tissue Res 2012;349:849–62.
17. Fassbender A, Burney RO, Dorian FO, D'Hoodhe T, Guidice L. Update on biomarkers for the detection of endometriosis. Biomed Res Int 2015;2015:130854.
18. Fassbender A, Vodolazkaia A, Saunders P, Lebovic D, Waelkens E, De Moor B, et al. Biomarkers of endometriosis. Fertil Steril 2013;99:1135–45.
19. Borrelli GM, Abrao MS, Mechsner S. Can chemokines be used as biomarkers for endometriosis? A systematic review. Hum Reprod 2014;29:253–66.
20. Hirsch M, Duffy GM, Kusznir JO, Davis CJ, Plana MN, Khan KS. Variations in outcome reporting in endometriosis trials: a systematic review. Am J Obstet Gynecol 2016;14:02587.
21. Becker CM, Laufer MR, Stratton P, Hummelshoj L, Missmer SA, Zondervan KT, et al. World endometriosis research foundation endometriosis phenome and biobanking harmonisation project. I. Surgical phenotype data collection in endometriosis research. Fertil Steril 2014;102:1213–22.
22. Beste MT, Pfaffle-Doyle N, Prentice EA, Morris SN, Lauffenburger DA, Isaacson KB, et al. Molecular network analysis of endometriosis reveals a role of c-Jun-regulated macrophage activation. Sci Transl Med 2014;6:1–17.
23. Rakhila H, Al-Akoum M, Bergeron ME, Leboeuf M, Lemyre M, Akoum A, et al. Promotion of angiogenesis and proliferation cytokines patterns in peritoneal fluid from women with endometriosis. J Reprod Immunol 2016;116:1–6.
24. Abbas AR, Baldwin D, Ma Y, Ouyang W, Gurney A, Martin F, et al. Immune response in silici (IRIS): immune-specific genes identified from compendium of microarray expression data. Genes Immun 2005;6:319–31.
25. Greene AD, Lang S, Kendziorski JA, Sroga-Rios JM, Hertzog TJ, Burns KA. Endometriosis: where are we and where are we going? Reproduction 2016;152:63–78.
26. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet 2010;27:441–7.
27. Ballard K, Lane H, Hudelist G, Banerjee S, Wright J. Can specific pain symptoms help in the diagnosis of endometriosis? A cohort study of women with chronic pelvic pain. Fertil Steril 2010;94:20–7.
28. Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: translational evidence of the relationship and implications. Hum Reprod Update 2011; 17:327–46.
29. Opøien HK, Fedorcsak P, Omland AK, Åbyholm T, Bjerche S, Ertzeid G, et al. In vitro fertilization is a successful treatment in endometriosis-associated infertility. Fertil Steril 2012;59:912–8.
30. Kahn KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, et al. Immunopathogenesis of pelvic endometriosis: role of hepatocyte growth factor, macrophages and ovarian steroids. Am J Reprod Immunol 2008;60:383–404.
31. Lee YH, Cui L, Fang J, Chern BS, Tan HH, Chan JK. Limited value of pro-inflammatory oxylipins and cytokines as circulating biomarkers in endometriosis—a targeted 'omics study. Sci Rep 2016;6:26117.
32. Borrelli GM, Kaufmann AM, Abrao MS, Mechsner S. Addition of MCP-1 and MIP-3β to the IL-8 appraisal in the peritoneal fluid enhances the probability of identifying women with endometriosis. J Reprod Immunol 2015;109:66–73.
33. Kocbek V, Vouk K, Bersinger NA, Mueller MD, Lanisnik Rizner T. Panels of cytokines and other secretory proteins as potential biomarkers of ovarian endometriosis. JMDI 2015;17:325–34.
34. Vodolazkaia A, El-Aalamat Y, Popovic D, Mihalyi A, Bossuyt X, Kyama CM, et al. Evaluation of 28 biomarkers for the non-invasive diagnosis of endometriosis. Hum Reprod 2012;27:2698–711.
35. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Schefters CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016:CD012179.
36. Taylor HS, Lebovic DI, Mueller MD. Angiogenetic factors in endometriosis. Ann N Y Acad Sci 2002;995:89–100.
37. Hsiao KS, Chang N, Lin SC, Li YH, Wu MH. Inhibition of dual specificity phosphatase-2 by hypoxia promotes interleukin-8-mediated angiogenesis in endometriosis. Hum Reprod 2014;29:2747–55.
38. Karimian MS, Pirro M, Majeed M, Sahebkar A. Curumin as a regulator of monocyte chemoattractant protein-1. Cytokine Growth Factor Rev 2016;16:30131–9.
39. Lampinen M, Carlson M, Håkansson LD, Venge P. Cytokine-regulated accumulation of eosinophils in inflammatory disease. Allergy 2004;59:793–805.
40. Ruan F, Ma J, Zhou J. Depletion of CCL27 inhibits cell proliferation, metastasis and adhesion in ectopic endometrial stromal cells. Int J Clin Exp Med 2016;9:19074–83.
41. Chalubinski M, Luczak E, Wojdan K, Gorzelak-Pabis P, Broncel M. Innate lymphoid cells type 2—emerging immune regulators of obesity and atherosclerosis. Immunol Lett 2016;179:43–6.
42. Aligeti S, Kirma NB, Binkley PA, Schenken RS, Tekmal RR. Colony-stimulating factor-1 exert direct effects on the proliferation and invasiveness of endometrial epithelial cells. Fertil Steril 2011;95:2464–6.
43. Budrys NM, Nair HB, Liu YG, Kirma NB, Binkley PA, Kumar S, et al. Increased expression of macrophage colony-stimulating factor and its receptor in patients with endometriosis. Fertil Steril 2012;97:1129–35.
44. Wang Y, Khan A, Heringer-Walther S, Schultheiss HP, Moreira Mda C, Walther T. Prognostic value of circulating levels of stem cell growth factor beta (SCGFbeta) in patients with Chagas'disease and dilated cardiomyopathy. Cytokine 2013;61:728–31.
45. Sarchielli P, Nardi K, Chiasserini D, Eusebi P, Tantucci M, De Piero V, et al. Immunological profile of silent brain infarction and lacunar stroke. PLoS One 2013;8:e68428.
46. Ito C, Sato H, Ando K, Watanabe S, Yoshiba F, Kishi K, et al. Serum stem cell growth factor for monitoring hematopoietic recovery following stem cell transplantation. Bone Marrow Transplant 2003;32:391–8.
47. Du H, Taylor H. Contribution of bone marrow derived stem cells to endometrium and endometriosis. Stem Cells 2007;25:2082–6.
48. Sourial S, Tempest N, Hapangame DK. Theories on the pathogenesis of endometriosis. Int J Reprod Med 2014;2014:9.

49. Khan KN, Kitajima M, Fujishita A, Nakashima M, Masuzaki H. Involvement of hepatocyte growth factor–induced epithelial-mesenchymal transition in human adenomyosis. Biol Reprod 2015;92:1–11.

50. Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol 2003;1:123.

SUPPLEMENTAL FIGURE 1



ROC curves for the best models for discrimination of endometriosis versus no endometriosis. (**A**) The model includes all patients. SCGF-β, IL-13, and G-CSF were significantly predictive of endometriosis; AUC = 0.81. (**B**) Patients in follicular phase. SCGF-β was the only significantly predictive factor; AUC = 0.82. (**C**) Patients in luteal phase. HGF was the only significantly predictive factor in the multivariate model; AUC = 0.71.

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SUPPLEMENTAL TABLE 1

Concentrations (pg/mL) of 46 cytokines in the PF.

	Endometriosis absent (n = 38, 40%)	Endometriosis present (n = 56, 60%)	*P* value[a]
BNGF	11.7 (10.4–13.9)	12.18 (11.0–14.23)	.422
CTACK	649.7 (500.7–814.0)	579.6 (472.6–693.6)	.061
Eotaxin	134.1 (86.9–172.8)	139.0 (102.6–181.5)	.765
HGF	363.7 (235.1–557.4)	493.8 (362.4–653.7)	.002[a]
IL-16	616.6 (507.2–793.3)	729.4 (573.4–930.4)	.077
IL-18	40.2 (30.3–50.5)	38.55 (31.1–56.9)	.698
IL2Ra	82.8 (51.3–123.0)	96.6 (73.4–125.6)	.128
IL-6	36.2 (21.6–75.3)	39.9 (28.1–103.7)	.183
IL-8	10.0 (7.03–15.8)	16.1 (10.9–28.5)	.001[a]
IP-10	3,442.1 (2,773.1–7,966.7)	4,718.2 (3,669.4–6,959.1)	.165
MCP-1	134.0 (105.0–170.4)	170.1 (116.9–273.8)	.023[a]
MIF	169.8 (87.7–222.3)	166.3 (114.6–311.5)	.115
MIG	468.8 (369.8–734.7)	613.7 (428.5–813.6)	.089
SCF	177.8 (145.6–219.3)	177.1 (152.3–207.3)	.863
SCGF-ß	22,260.1 (13,821.2–28,827.5)	31,736.9 (23,741.1–42,207.0)	<.001[a]
SDF-1α	1,002.9 (732.2–1,225.2)	1,082.7 (849.9–1,338.2)	.216
LIF	4.45 (2.42–6.22)	4.13 (2.01–7.07)	.931
FGFbasic	21.9 (12.1–59.6)	26.74 (15.16–59.61)	.696
G-CSF	74.8 (53.7–118.4)	95.27 (62.41–196.5)	.067
GM-CSF	14.01 (10.28–22.0)	16.32 (11.5–21.77)	.405
IFN-g	21.94 (7.21–29.28)	39.11 (2.73–137.8)	.316
IL-10	5.57 (4.20–8.66)	6.62 (3.80–10.62)	.387
IL-12p70	7.48 (5.84–12.54)	8.93 (6.93–23.4)	.108
IL-13	8.85 (5.50–10.63)	6.60 (3.42–12.7)	.008[a]
IL-15	38.8 (31.1–48.9)	40.8 (31.42–50.8)	.698
IL-1b	0.30 (0.20–0.74)	0.28 (0.16–0.59)	.593
IL-1ra	17.05 (10.95–29.22)	24.4 (13.0–39.4)	.170
IL-2	4.16 (2.45–5.15)	3.32 (1.71–5.71)	.428
IL-4	0.15 (0.13–0.20)	0.16 (0.12–0.22)	.568
IL-5	2.11 (1.26–4.02)	1.17 (0.98–3.40)	.147
IL-7	4.75 (3.32–6.11)	4.48 (2.60–6.18)	.701
IL-9	4.20 (2.46–8.17)	7.76 (4.77–10.12)	.659
MIP-1a	1.57 (1.10–1.88)	1.49 (1.25–2.17)	.313
MIP-1b	24.0 (16.85–31.48)	24.42 (17.7–39.6)	.604
PDGF-BB	4.73 (2.40–7.06)	4.37 (4.33–27.3)	.857
RANTES	12.23 (6.10–30.8)	17.68 (7.31–47.7)	.188
TNF-a	5.35 (2.97–14.3)	9.94 (2.81–20.8)	.653
VEGF	136.8 (104.7–196.2)	160.9 (119.2–226.7)	.319
GRO-a	94.6 (79.7–137.0)	95.7 (74.8–153.3)	.774
IFN-a2	31.83 (24.7–35.1)	35.82 (27.6–41.5)	.098
IL-12p40	197.5 (138.2–265.0)	208.8 (113.7–283.9)	.842
IL-1a	0.59 (0.34–1.07)	0.79 (0.47–1.25)	.083
IL-3	91.4 (67.8–127.1)	89.9 (73.1–157.3)	.511
M-CSF	8.16 (6.47–9.85)	8.26 (6.23–10.1)	.851
MCP-3	63.2 (38.7–99.2)	67.9 (45.3–99.2)	.715
TRAIL	29.2 (20.7–51.4)	37.6 (22.6–54.4)	.475

Note: Data are medians and interquartile range.
[a] Mann-Whitney *U*-test.

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