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;107:1191–99. ©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

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