Endometriosis is associated with aberrant metabolite profiles in plasma

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Objective: To identify metabolites that are associated with and predict the presence of endometriosis.
Design: Metabolomics study using state-of-the-art mass spectrometry approaches.
Setting: University hospital and universities.
Patient(s): Twenty-five women with laparoscopically confirmed endometriosis (cases) and 19 women with laparoscopically documented absence of endometriosis (controls). None of the women included in this study had received oral contraception or GnRH agonists for a minimum of 1 month before blood collection.
Intervention(s): Plasma collection.
Main Outcome Measure(s): Metabolite profiles were generated and interrogated using multiple mass spectrometry methods, that is, high performance liquid chromatography coupled with negative mode electrospray ionization tandem mass spectrometry, UPLC-MS/MS, and ultra performance liquid chromatography-electroSpray ionization-quadrupole time-of-flight (UPLC-ESI-Q-TOF). Metabolite groups investigated included phospholipids, glycerophospholipids, ether-phospholipids, cholesterol-esters, triacylglycerol, sphingolipids, free fatty acids, steroids, eicosanoids, and acylcarnitines.
Result(s): A panel of acylcarnitines predicted the presence of endometriosis with 88.9% specificity and 81.5% sensitivity in human plasma, with a positive predictive value of 75%. However, due to data limitations the outcome of the receiver operating characteristic curve analysis was not significant.
Conclusion(s): A diagnostic model based on acylcarnitines has the potential to predict the presence and stage of endometriosis. (Fertil Steril® 2017;107:699–706. ©2017 by American Society for Reproductive Medicine.)
Key Words: Diagnosis, endometriosis, metabolomics, acylcarnitines

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Endometriosis is an estrogen-dependent disease that is characterized by the presence of functional endometrial tissue outside of the uterine cavity. It affects 5%–10% of women of reproductive age and is characterized by inflammation, pelvic pain, and infertility (1–3). Endometriosis can appear as peritoneal lesions, ovarian superficial implants or endometriotic cysts (endometriomas), and/or deeply

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A.B.B. and T.M.D. should be considered similar in author order.
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infiltrating disease extending to bowel, bladder, and ureter, often associated with pelvic adhesions (4). Endometriosis is frequently associated with chronic abdominal pain, cyclic pelvic pain, dysmenorrhea, dyspareunia, dysuria, dyschezia, and impaired fertility (5, 6). Endometriosis-associated pain can be caused by peritoneal inflammation, adhesion formation, and specific innervation of the endometrium and endometriotic lesions and is correlated with the presence of deeply infiltrating disease (7–10). So far, it has not been possible to accurately predict the presence of endometriosis based on symptoms, clinical examination, imaging techniques, or blood tests, except laparoscopy. As a consequence it takes on average approximately 8–11 years (11) between onset of symptoms and diagnosis. At present, the gold standard for diagnosis of endometriosis is laparoscopic inspection with histologic confirmation (11). However, laparoscopy is a surgical procedure with rare but significant potential risks for the patient (12) and is only performed when endometriosis is suspected, which is often not the case.

According to our current understanding, endometriotic stromal cells are important drivers of endometriotic lesion growth and survival. At least two classes of small metabolites are known to be involved, that is, estrogens and prostaglandins (13). Estrogens are known to enhance the growth and invasion of endometriotic tissue, whereas prostaglandins and cytokines mediate pain, inflammation, and infertility. Numerous attempts have been made to elucidate whether any or a combination of these or other systemic biomarkers, metabolites, or cellular products can provide information regarding the presence and state of endometriosis (14).

Metabolomics is a promising approach to identify metabolite biomarkers in blood (15) as the metabolome is a reflection of phenotypic changes in an organism in response to the presence of disease, genetic changes, and nutritional, toxicological, environmental, and pharmacological influences (15). Several metabolomics studies employing nuclear magnetic resonance (NMR)-based approaches (metabolic fingerprinting) and mass spectrometry–based approaches (targeted metabolomics) have been performed in patients with endometriosis in an attempt to find disease biomarkers and gain more insight into the disease pathophysiology (16–22). Even though metabolites have been identified that show significant association with the presence of disease, none of the diagnostic candidates have been confirmed in independent metabolomics studies in other research labs nor have they been validated in prospective randomized clinical trials.

In the current pilot study, depending on the metabolites of interest, we used high performance liquid chromatography coupled with negative mode electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), ultraperformance LC (UPLC-MS/MS), and ultra performance liquid chromatography-electroSpray ionization-quadrupole time-of-flight (UPLC-ESI-Q-TOF) to interrogate a variety of metabolite classes in the plasma of patients with endometriosis that are known or suspected to have an association with the pathophysiology of endometriosis, that is, lipids, acylcarnitines, steroids, and eicosanoids. Vouk and coworkers (21) performed a similar study and evaluated 148 lipids and acylcarnitines, of which 109 passed measurement quality control, in the plasma of women with and without endometriosis. Eight lipid metabolites were identified as endometriosis-associated biomarkers. A model consisting of a combination of individual metabolites and ratios between pairs of metabolite concentrations showed the best diagnostic performance, 90% sensitivity and 84.3% specificity. In this study, we were able to enhance the scope of the metabolic diagnostic platforms by allowing the targeted analysis of 241 lipids and 43 acylcarnitines. Using nuclear magnetic resonance metabolomics, Vicente-Muñoz and coworkers (23) studied metabolites in the urine of women with endometriosis and observed that these women exhibited higher concentrations of N^1-methyl-4-pyridone-5-carboxamide, guanidinosuccinate, creatinine, taurine, valine, and 2-hydroxyisovalerate and decreased concentrations of lysine compared with healthy women.

Eicosanoids and steroids have not yet been thoroughly explored in the plasma of patients with endometriosis using mass spectrometry–based methods. Keski-Rahkonen and coworkers (24) developed LC-MS/MS methods for the quantitative analysis of seven steroid hormones in serum and analyzed serum samples in patients and controls for the purpose of validating the method but not to assess the predictive value of these metabolites. Using the same technology, Ray et al. (25) evaluated eicosanoids in the peritoneal fluid (PF) of patients with endometriosis in an attempt to find a plausible explanation for the chronic pelvic pain many women with endometriosis experience. While these investigators reported increased amounts of PGE_2, PGD_2, and 12- and 15-HETE in the PF of women with endometriosis, no inferences were made with regard to their diagnostic potential. In the present study, we report the development of two new platforms capable of detecting 17 different steroid hormones and 120 eicosanoids.

Using four high-performance targeted metabolomics platforms to search for (combinations of) metabolites with potential as diagnostic biomarkers, we report here that a selection of acylcarnitines was significantly associated with the presence of disease.

MATERIALS AND METHODS

Patient Information and Sample Collection

The women participating in the pilot study (n = 44) were recruited from the Leuven University Fertility Center of the University Hospital Gasthuisberg in Leuven, Belgium, and the Department of Obstetrics and Gynecology at the University Hospital Saint Luc in Brussels, Belgium. They were scheduled for laparoscopic surgery for the diagnosis and treatment of endometriosis or other gynecological diseases because of pelvic pain (n = 14/44 or 32%), infertility (n = 14/44 or 32%), or combined pelvic pain and infertility (n = 16 or 36%). None of these women had received oral contraceptives, GnRH analogues, or any other hormone treatment. Demographic, clinical, and menstrual cycle characteristics are shown in Table 1. All women gave their written informed consent to participate in this study, and the study was approved by the Commission for Medical Ethics of the Leuven University Hospital (approval number KU Leuven: ML6282) and of the