Biomarkers in endometriosis: challenges and opportunities

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Endometriosis is a debilitating gynecologic disease affecting millions of women across the world, with symptoms including dysmenorrhea, chronic pelvic pain, and infertility. Theorized to stem from the phenomenon of retrograde menstruation, the diagnosis of endometriosis is typically delayed by 8–10 years owing to misinterpretation of symptoms as common menstrual cramps in adolescent girls and young women. With increased incidence of endometriosis in young girls correlated with earlier menarche, the development of diagnostic biomarkers is imperative for diagnosing and treating women afflicted with endometriosis as early as we can. In the past few years, multiple reviews highlighted the list of potential diagnostic candidates in peritoneal fluid, blood, urine, and endometrial biopsies from endometriosis patients in different stages of disease and menstrual cycle. In this review, we explore the opportunities and challenges facing the field of diagnostic biomarkers for endometriosis. We highlight the importance of eutopic endometrium as a source of potential diagnostic biomarkers by looking at the expression levels of noncoding RNA in tissue as well as in blood. Finally, we discuss some of the challenges that hinder our efforts in validating candidate diagnostic biomarkers for endometriosis. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, biomarkers, challenge

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Endometriosis is a debilitating gynecologic disease characterized by the presence of uterine epithelial and stromal tissues outside of the uterine cavity (1–5). Theorized to arise from the endometrial fragments escaping into the peritoneal cavity through the process of retrograde menstruation (6), endometriosis patients experience significant reduction in quality of life owing to increase in symptoms including nonmenstrual pelvic pain (38.7% vs. 14.3%), dyspareunia (29.5% vs. 13.4%), and infertility (11.6% vs. 3.4%) compared with women without endometriosis (7). Indeed, epidemiologic data show that in Canada, the inability of women to contribute to society because of disease amounts to the economic burden of $1.8 billion (8), which is increased to $18–22 billion in the United States (9, 10). Although decades of research into the pathogenesis of endometriosis have led to insightful elucidations into the hormonal and nonhormonal mechanisms involved in disease development and persistence, the therapeutic regimens to treat endometriosis and the methods for early diagnosis of endometriosis are still lacking.

The economic impact of endometriosis is compounded by the latency in the diagnosis of endometriosis, especially in young women that delay seeking treatment. Owing to the common misinterpretation of endometriosis-induced pain as menstrual-related abdominal pain, the diagnosis of endometriosis is typically delayed by 8–10 years: Adolescent girls who suffer from the symptoms of endometriosis delay seeking medical attention by 4.6 years, and by the time they seek medical attention, it takes another 4.7 years until diagnosis (11). In 2004, Ballweg reported an increase of endometriosis-like symptoms in girls before the age of 15 years as well as menarche occurring earlier (12), indicating the potential need to screen adolescent and younger girls as early as they display symptoms of endometriosis for confirmatory diagnosis. Laparoscopic surgery remains the current criterion-standard diagnostic tool; however, it is unlikely that women of reproductive age would subject themselves to such an invasive surgery when they can opt to temporarily diminish pain symptoms by means of other therapeutic mechanisms.

Indeed, to diminish the disease burden and minimize symptoms of pain, nonsteroidal antiinflammatory drugs, GnRH agonists, progestins, and oral contraceptive pills are mainstream therapeutic options (13). Because estrogen is the primary driver of endometriosis lesion development, most of the established therapeutics are targeted

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to create a hypoestrogenic state that offers temporary relief. One of the major disadvantages of all present drug treatments for endometriosis is they prevent women from pursuing pregnancy. Women are forced to decide whether to improve quality of life by diminishing pelvic pain symptoms, or forgo chances of pregnancy for the sake of minimizing endometriosis-associated pain.

Endometriosis is a complex disease with variable phenotypic and symptomatic presentation in women (14, 15). Aside from estrogen dependence, we know that immune dysfunction and inflammation play a role in its pathobiology (16–20). In addition, we are beginning to elucidate the genetic variants associated with endometriosis risk by means of genome-wide assessment studies (21–26), which have demonstrated that the endometrium of endometriosis patients displays aberrant molecular expression patterns that give it the ability to implant, invade, and develop into endometriotic foci (27–30). We are also beginning to map out the epigenetics of endometriosis by identifying aberrantly methylated genes (e.g., *TNFRSF1B*, *IGSF21*, and *TP73* [26]) involved in the pathogenesis of endometriosis (31). It is now well established that endometriosis thrives in an inflammatory environment. Researchers have documented elevated levels of proinflammatory cytokines in the peritoneal fluid, eutopic endometrium and ectopic lesion samples, and blood in women with endometriosis (32–36), which can decrease significantly on laparoscopic removal of the lesions (37). However, it remains unclear whether inflammation contributes to the pathogenesis of endometriotic lesion establishment or is a by-product of the process. Intriguingly, the only aspect of its pathogenesis targeted for therapeutic approach focus on the dependence of endometriosis on estrogen for growth, the suppression of which remains perhaps the single scientifically and clinically proven therapy with some success.

The hunt for a noninvasive biomarker for endometriosis has been an ongoing and challenging issue. The World Endometriosis Research Foundation, in a series of guidelines published in 2014 (38, 39), has prompted physicians, gynecologists, and researchers to standardize methods of sample collection and analysis of data, which in 2016 were further highlighted as research priorities for endometriosis (10). Indeed, the methods of tissue excision, body fluids collection, storage, and transportation need to be congruent in all facilities dedicated to endometriosis research to streamline data analysis. As a step forward, in 2016, an online multicenter documentation system across five hospitals and outpatient facilities was launched in Europe to optimize data collection and sharing and to improve molecular and clinical assessment of noninvasive biomarker discoveries (40). That study, however, did not specify whether the pain symptoms were included in patient characterization. Truly, the description of pain, including dyspareunia, chronic pelvic pain, and other related symptoms, needs to be systemically recorded to potentially identify biologic mechanisms underpinning the generation of pain (12). These potential confounding factors hinder our progress of noninvasive biomarker discovery because they affect the analysis of scientific findings. With improved characterization of patient history and standardized means of collection, storage, and interpretation of data, we will have the tools to identify noninvasive diagnostic biomarkers for endometriosis.

This review will elucidate the realistic opportunities and challenges in the verification of noninvasive diagnostic biomarkers for endometriosis. Potential biomarkers, including microRNA (miRNA) and long noncoding (lnc) RNA from blood, endometrial biopsies, and epigenetic markers of endometrium, will be evaluated. We will also assess their potentials as biomarkers in an attempt to guide our collective efforts into defining a noninvasive diagnostic biomarker or a group of markers that can precisely distinguish women with endometriosis from those without.

THE CHALLENGE

Heterogeneous Nature of Endometriosis and Endometriosis-Associated Symptoms

Endometriosis is often described as occurring in three areas in the pelvis—in the peritoneum, on the ovary, and in the rectovaginal pouch—which are referred to as peritoneal implants, ovarian endometrioma, and deep infiltrating endometriosis, respectively. However, endometriosis has been documented to manifest outside of the pelvic region (41). As such, the disease can be categorized into endopelvic and extrapelvic manifestations (42). The endopelvic manifestations include deep infiltrating endometriosis involving the posterior wall of the vagina and anterior wall of the rectum and ovarian endometrioma, which present as cystic growth on the surface of the ovary, where endometrial stroma and glands are found within the wall of the cyst (43). The extrapelvic manifestations include the typical peritoneal or abdominal wall endometriosis, including endometriosis found on surgical scars on the peritoneum (44, 45), the urinary and gastrointestinal tract, the thorax, and the nasal mucosa (42). Other extrapelvic locations of endometriosis include rare cases of vesical, or bladder, endometriosis, which occurs in 1% of women, especially during pregnancy (46). Twenty-two cases of hepatic endometriosis have also been cited in the medical literature (47). In addition, endometriotic foci have been found on the uterine ligaments, cervix, labia, and vagina (48). The widespread phenotype of endometriosis in patients is truly reminiscent of the metastatic characteristic of cancer, to which endometriosis is often compared. The ability of endometriosis to be found not only in the abdominopelvic cavity, but also in the thoracic cavity and even in nasal mucosa is an example that mechanisms aside from retrograde menstruation are involved in the pathogenesis of endometriosis. Such heterogeneity of disease phenotype also increases the potential of false-negative laparoscopic surgery in symptomatic women, wherein a “clean” pelvic cavity might not necessarily indicate the absence of endometriosis in other areas of the body (Table 1).

Unless accompanied by explorative laparoscopic surgery for complete diagnosis, accurate differential diagnosis in potential endometriosis patients based on symptoms proves to be a difficult challenge. In this regard, patients with