CHAPTER TWO

New biomarkers in endometriosis

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Abstract

Endometriosis is a benign gynecological disorder which presents significant challenges in terms of diagnosis and management. Despite decades of research, there are no sufficiently sensitive and specific signs and symptoms nor blood tests for the clinical confirmation of endometriosis, which hampers prompt diagnosis and treatment. The huge majority of potential biomarkers has been discarded in research stage and very few have been translated to clinical practice. Serum CA-125 is the most studied and used one, but studies have shown its poor diagnostic performance. Several factors involved in the chronic inflammatory process of endometriosis, such as hormones, cytokines, chemokines, angiogenic factors, oxidative stress markers and others, have been implicated in the disease's pathogenesis and have been extensively studied, but not a single one has successfully been able to accurately identify the disease. MicroRNAs have emerged more recently but their utility to detect endometriosis remains uncertain. The search for a biomarker or a set of biomarkers is still open and may benefit from novel molecular biology and bioinformatics approaches to mine and uncover molecular signatures specifically associated with the disease.

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1. Introduction

Endometriosis is a common gynecologic disease characterized by the presence of endometrial-like tissue outside the uterine cavity. Endometriotic implants may contain both epithelium and/or stroma, and are responsive to estrogen stimulus. The disease can affect up to 10% of women in reproductive age worldwide, and in symptomatic women (symptoms as: dysmenorrhea, dyspareunia and infertility) this number can raise to 30–50% [1,2].

Despite its high cost to the health care system and negative impact in patients' quality of life, many endometriosis related topics still lack elucidation. Underlying mechanisms implicated in the pathophysiology, more effective and less invasive diagnostic methods and better treatment options are some of the issues that are worth discussing in this field [2–4].

2. One disease or multiple diseases?

Endometriosis is a disorder with a broad spectrum of clinical presentation. It can be manifested, more often, as superficial implants in the peritoneum, ovarian cysts (endometriomas), and deep infiltrating lesions typically located in the rectovaginal septum, uterosacral ligaments, and pelvic portions of the urinary and gastrointestinal tracts. More rarely, the disease may be found outside the pelvis [5,6].

The most common symptoms associated with endometriosis are infertility, acyclic pelvic pain, dysmenorrhea and deep dyspareunia. Symptoms may vary depending on the anatomical site affected [7]. For instance, urinary and intestinal impairment may cause, respectively, dysuria and dyschezia. Endometriotic implants can also be incidentally found in asymptomatic patients during surgical procedures [4].

Direct visualization of endometriotic foci during surgery remains the gold standard currently recommended for the diagnosis of the disease. Histopathological analysis must be associated to confirm the surgical findings, even though a negative biopsy cannot exclude the diagnosis in a positive laparoscopy [8]. Owing to the invasive and costly diagnostic approach method currently available and the nonspecific clinical presentation of the disease, reaching a definitive diagnosis of endometriosis is not a simple task. Moreover, there is an estimated gap of approximately 7 years between the onset of symptoms and the diagnosis confirmation [9], which is distressing [10] and may add to the severity of the disease [11].

In the last decade, imaging methods have gained growing importance in the diagnosis and surgical planning. Thus, in the hands of experienced professionals, transvaginal ultrasound and pelvic magnetic resonance imaging (MRI) can perform better than routine laparoscopy in the identification of deep lesions. However, they cannot effectively diagnose superficial forms endometriosis [12–14].

Until now, there is no ideal single way to classify the disease. One of the staging methods currently used is the revised American Society for Reproductive Medicine (ASRM) Classification of Endometriosis, but it has some limitations. It is based on surgical findings and it classifies endometriosis in four stages: minimal, mild, moderate and severe. However, it fails to establish a good correlation between these stages and pain symptoms or fertility impairment [15,16]. Therefore, other systems have been proposed taking into consideration the anatomical distribution of deep lesions, in an attempt to improve the correlation of disease stages with pain severity, and also to help surgical planning [17,18]. Likewise, a new classification system was developed to better reflect the fertility prognosis [19].

In spite of the magnitude of its clinical implications, the pathogenesis of endometriosis is not fully understood. Several theories have been presented over the years but none has been able to explain the full spectrum of the disease. Retrograde menstruation, as proposed by Sampson in 1927, remains the most accepted theory so far, but it is not able to explain the whole process, since the retrograde flow of menses appears to be an almost universal event, even in women without endometriosis. Moreover, it cannot clarify the origin of extrapelvic manifestations and the widespread phenotype of endometriosis. Other theories such as metaplastic or lymphovascular derivation of ectopic foci, iatrogenic direct implantation, genetic and epigenetic influences also have been postulated over the years and it is possible that more than one mechanism may be involved in the pathogenesis of endometriosis [4,6,20].

In the past few decades, the intrinsic molecular pathways related to endometriosis have been exhaustively studied and new theories are being put forward. In their pioneer study with rhesus monkeys, Dmowski et al., in 1981, suggested that endometrial cells translocated from the uterus would implant only in women with specific immunity alterations [21]. Further studies have demonstrated that patients with endometriosis may have an altered immune response in both cell-mediated and humoral immunity, which predisposes to the implantation and progression of endometrial cells in ectopic areas [22].

A close relationship between endometriosis and inflammation has been established and the disorder is now considered a chronic inflammatory condition. There are elevated levels of pro-inflammatory cytokines in the peritoneal fluid, endometriotic tissue, eutopic endometrium and blood in patients with endometriosis, when compared with controls, demonstrating the systemic scope of the disease [23–25]. In particular, endometriotic tissues and cells have an excessive activation of the mitogen-activated protein kinase (MAPK) signaling pathways that amplify the local inflammatory response through the enhanced production of proinflammatory cytokines and prostaglandins [26].

It would be, at the very least, reasonable to say that endometriosis represents more than a simple disease. It could be considered a heterogeneous entity that might be related to more than one underlying mechanism [6]. Beyond the focal endometriotic lesions, endometriosis has a high extent of systemic repercussions and several molecules are altered in the peripheral blood of women with the disorder. These findings challenge the search of new specific biomarkers for endometriosis that could be used to identify the different phenotypes of the disease [27].

3. From mechanisms to biomarkers

More than to understand the real pathophysiology of endometriosis, studies intend to discover non or minimally invasive biomarkers to be used as diagnostic tools, with less risks and lower cost when compared to surgical diagnosis. Besides early diagnosis, biomarkers could also be useful in the screening and follow-up of endometriosis and even contribute to the development of new therapeutic options [28].

Several factors involved in the typical chronic inflammatory process of endometriosis, such as hormones, cytokines, chemokines, angiogenic factors, oxidative stress markers and others have been implicated in the disease's pathogenesis and some of them are expected to perform as endometriosis biomarkers (Fig. 1).

Notwithstanding hundreds of studies in this field, data remain inconsistent [4,29]. A Cochrane systematic review published in 2016 evaluated blood biomarkers in patients with endometriosis and controls, in order to elucidate whether they could be regarded as replacement tests to laparoscopy or screening tests to be used before surgery. The predetermined criteria for considering a clinically useful blood test to replace the gold standard diagnostic method were a sensitivity of 0.94 and a specificity

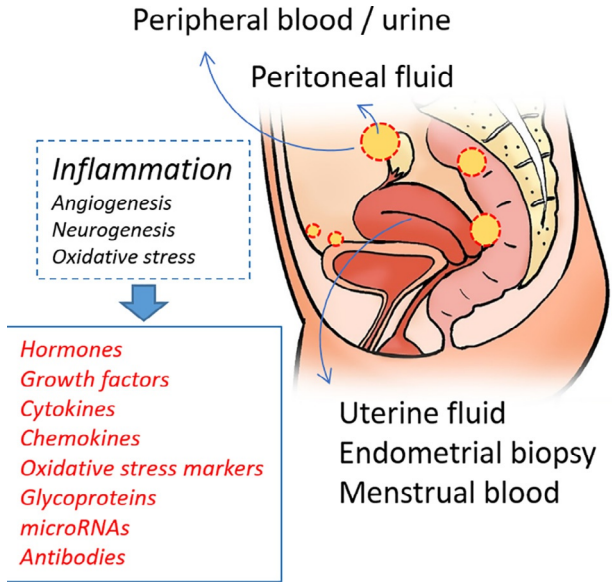

Fig. 1 Several potential biomarkers of endometriosis (box) are produced by the endometriotic implants themselves, by the invaded tissues and/or by the immune system and have been studied in the peritoneal fluid (obtained during surgery), in uterine materials or in peripheral blood or urine. The mechanism behind increased or decreased levels of endometriosis biomarkers is often unknown. Small superficial lesions usually do not rise biomarkers in peripheral blood, whereas many conditions other than endometriosis may give false positive test results.

of 0.79. The criteria defined for an accurate triage test were a sensitivity $\geq$0.95 and a specificity $\geq$0.50 [30].

More than 15,000 patients were included in the review and a total of 122 biomarkers were assessed. However, most of the 141 studies enrolled were small and presented poor methodological quality. Other sources of inconsistence between studies were the different cutoffs adopted by the researchers and the lack of uniformity about the menstrual cycle phases. Thus, meta-analyses could only be performed for four potential biomarkers. The mean sensitivities and specificities were, respectively, for anti-endometrial antibodies: 0.81 (95% CI 0.76–0.87) and 0.75 (95% CI 0.46–1.00); for interleukin-6 (IL-6), considering cutoff >1.90–2.00 pg/mL: 0.63 (95% CI 0.52–0.75) and 0.69 (95% CI 0.57–0.82); for cancer antigen 19-9 (CA-19-9), considering cutoff >37.0 UI/mL: 0.36 (95% CI 0.26–0.45) and 0.87 (95% CI 0.75–0.99); for CA-125, considering cutoff >35.0–36.0 U/mL: 0.40 (95% CI 0.32–0.49) and 0.91 (95% CI 0.88–0.94). None of these markers have met the parameters to be considered a replacement diagnosis tool or a triage diagnostic test [30].

Unfortunately, until the present moment, the available evidence failed to determine biomarkers with enough accuracy to be considered for clinical use either isolated or in panels [31]. No specific antigen or molecule for endometriosis has been discovered yet. Biochemical signs and putative endometriotic products that have been studied and thought to perform as endometriosis biomarkers are, in their majority, unspecific and can be altered in many conditions, such as other systemic inflammatory diseases and physiological process, generating false positive results.

On the other hand, the small size of endometriotic implants and consequent reduced cellular content may not provide sufficient amounts of inflammatory markers to outweigh other sources of the same molecules, justifying some cases of false negatives in patients with confirmed disease [27]. Another possible reason of false negative results in biomarkers application could be related to the endometriosis heterogeneity. It is possible that different phenotypes of the disease may have particular etiopathogenic mechanisms and, thus, distinct mediators involved. As a consequence, a specific biomarker could be able to identify one but not all phenotypes of the disorder [32].

In this chapter, some of the factors known to be involved in endometriosis pathophysiology will be summarized and their putative role as biomarkers will be discussed.

3.1 Hormones

It is well known that endometriosis is an estrogen-dependent disease. Endometriotic cells contain receptors for sexual hormones and they also synthetize P450 aromatase, an enzyme that converts androgens into estrogens [33–35]. When compared to intrauterine endometrium, the estrogen metabolism and clinical hormonal responses seem to be altered in endometriotic implants. Studies have demonstrated that there are some differences in expression of estrogen and progesterone receptors in ectopic tissue, such as higher levels of estrogen receptor type beta than alpha and progesterone resistance, resulting in an estrogenic environment [4]. Since these alterations in sex steroid synthesis and action are circumscribed to the endometriotic lesion and the eutopic endometrium, serum levels of estradiol and progesterone are not affected by the presence of endometriosis.

Other hormones like prolactin [36], leptin, LH and TSH have been targeted by endometriosis studies but their role as biomarkers could not

be defined [32]. Likewise, we have evaluated activin A, follistatin, and urocortin 1 in women with endometriosis, starting from the observation that these hormones are produced within endometriotic lesions and have effects on the differentiation of endometrial cells [37,38]. Plasma urocortin 1 levels were increased in the presence of endometriosis in symptomatic women, but no cutoff level combined high sensitivity with high specificity because the marker was also altered, although to a lesser extent, in symptomatic women with other gynecologic diseases [37]. Serum activin A and follistatin had limited accuracy to detect ovarian endometrioma and did not help to detect superficial peritoneal or deep endometriosis [38].

3.2 Cytokines

Cytokines are a wide range of small proteins that take part in cell signaling. They are produced by macrophages, lymphocytes, endometrial and mesothelial cells. In endometriosis, cytokines seem to contribute to the development of the disease. They are supposed to play a key role in the decreased immunological surveillance, recognition and destruction of endometrial cells, contributing for implantation of endometriotic foci [39].

Altered levels of cytokines have been identified in patients with endometriosis and their diagnostic value has been quantified. In the last decades, the most studied ones were IL-6 and TNF-alpha and, in 2002, Bedaiwy et al. suggested that they might be used as endometriosis biomarkers. In their study, increased levels of serum IL-6 (threshold of 2 pg/mL) and peritoneal fluid TNF-alpha (threshold of 15 pg/mL) were able to discriminate women with and without the disease, with a high sensitivity and fair specificity (respectively, 90% and 67% for IL-6; 100% and 89% for TNF-alpha) [40]. Despite these encouraging results, further studies did not consolidate the role of these cytokines as endometriosis biomarkers. Two systematic reviews found inconsistent results in the literature and could not establish a link between elevated serum levels of IL-6 and endometriosis. The heterogeneity among the studies may justify these findings, as many different cutoff values and control groups had been used. Moreover, the stage of the disease and menstrual cycle phase variations could have influenced the levels of IL-6, contributing to the discrepant findings [30,32].

Some cytokines have been found altered in specific endometriosis phenotypes. IL-33, a member of the IL-1 family that associates with fibrotic disorders, was increased in the serum and peritoneal fluid of patients with deep infiltrating endometriosis and correlated with the severity of symptoms and

with the extension of the lesions [25]. Conversely, low serum levels of the antiinflammatory cytokines IL-19 and IL-22 were found in women with ovarian endometrioma and were associated with the occurrence of severe deep dyspareunia [41].

To date, no cytokine has been proved to be good enough to perform as a biomarker for endometriosis, which can be justified, at least in part, by the lack of specificity, as these molecules have a pleiotropic capacity and can be involved in various biological pathways, either physiological (e.g., menstrual cycle) or pathological. Moreover, recent studies have demonstrated that patients with endometriosis have a heightened risk of other diseases related to autoimmune or hormonal dysregulation, such as fibromyalgia, infections, chronic fatigue syndrome, irritate bowel syndrome, thyroiditis, systemic lupus erythematosus and multiple sclerosis [42–44]. So, it might be difficult to correlate altered levels of cytokines with a single disease.

3.3 Chemokines

Chemokines are a family of small cytokines that induce directed chemotaxis in nearby responsive cells. Some of them are considered homeostatic, taking part in physiological processes. Others have a pro-inflammatory role, recruiting immunological cells during an immune response [45].

Khorram et al. first described in 1993 the relationship between increased levels of a chemokine and endometriosis [46]. Although the exact mechanisms involved are still under investigation, chemokines seem to be related to the aggressiveness, invasiveness and progression of endometriosis [47].

Two decades later, Borelli et al. reviewed 62 studies that had measured chemokine levels in patients with endometriosis and controls, in order to evaluate whether they could be considered as endometriosis biomarkers. A total of 27 chemokines or their receptors were evaluated. The most studied and, probably, the most relevant chemokine was CXCL8 (IL-8), followed by CCL2 (MCP-1) and CCL5 (RANTES). The first one appeared to have the better results among all the other chemokines evaluated, but the studies enrolled had many limitations and the authors could not define whether chemokines are useful diagnostic or prognostic biomarkers of endometriosis [48]. Being unspecific inflammatory proteins, chemokines can be enhanced in other chronic diseases, leading to false positive results; therefore, combination with non-inflammatory markers could be an option to improve their diagnostic utility [48].

3.4 Oxidative stress

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms. ROS are intermediate products derived from oxygen metabolism. Although their formation represents a physiological event, they are considered inflammatory mediators and can cause cellular damage. To maintain redox homeostasis inside the cellular environment, there are antioxidant systems responsible for combating the formation and the action of ROS, preventing their harmful effects [28].

Several studies have indicated that oxidative stress may be involved in endometriosis pathophysiology [49,50]. Macrophages, erythrocytes and apoptotic endometrial tissue derived from retrograde menstruation seem to increase the production of ROS [6]. The enhanced levels of ROS induce a general inflammatory response favoring adhesion and growth of desquamated endometrial cells in the peritoneal cavity [51].

However, data are still incipient about the possible use of oxidative stress markers as diagnostic tests for endometriosis [30]. Paraoxonase-1 had good accuracy in a preliminary study that still needs confirmation [52], whereas carbonyls and thiols had low specificity and/or sensitivity at the several cut-offs tested [53].

3.5 Angiogenic factors

Angiogenesis is an essential part of inflammatory response. In endometriosis, the development of new blood vessels takes a central role in the growth of endometrium in ectopic areas, specially, in the peritoneum [54]. Vascular endothelial growth factor (VEGF) is responsible for stimulating angiogenesis and increasing vessel permeability. Studies have already demonstrated increased levels of VEGF in peritoneal fluid from patients with endometriosis, mainly in those with advanced disease. Although studies have demonstrated that VEGF has a key role in the development of endometriotic implants, its value as a biomarker has not been proven [4,29].

3.6 Glycoproteins

Cancer antigen 125 (CA-125) is a well-established tumor marker yielded in coelomic epithelia during embryonic development. It is often used in the diagnosis of ovarian cancer, but it can also be altered in endometriosis. A meta-analysis, performed two decades ago, has demonstrated increased levels of CA-125 in endometriosis patients and a possible correlation with the most advanced stages of the disease [55]. Further studies have shown,

though, that CA-125 levels fluctuate across the menstrual cycle and can vary with the clinical type of endometriosis presentation [56,57].

Hirsch et al. recently reviewed the accuracy of serum CA-125 for endometriosis diagnosis. Data of more than 1500 patients with endometriosis and about 1300 controls were meta-analyzed. With a cutoff of 30 units/mL, the sensitivity was 52.4% (95% CI 37.9–66.4%) and the specificity was 92.7% (95% CI 89.4–95.1%). Higher sensitivity was shown in stages III/IV. According to the authors, serum CA-125 (threshold $\geq$30 units/mL) could be used as a rule-in test in symptomatic patients, whereas it is not recommended to be used as a screening test [58]. Many physiological and pathologic conditions and some medications can yield enhanced levels of CA-125, increasing the false positive results [29,30,32]. Therefore, CA-125 fails in meeting ideal criteria for an adequate diagnostic test in endometriosis.

CA-19-9, also a tumor marker used in the investigation of ovarian cancer, has been tested in patients with endometriosis, and only a few studies detected a tendency of elevated levels in patients with the disease. A significant decrease of CA-19-9 levels has been noted during treatment with danazol [59]. However, no data has validated this glycoprotein as a biomarker for endometriosis.

4. From molecular signatures to biomarkers

The investigation of biomarkers in endometriosis research has been classically made from a "hypothesis-driven" model. In other words, based on a prior hypothesis related to pathophysiological events, a theory is postulated and research is carried out, allowing the study of just one or few markers at once [31].

More recently, molecular biology techniques associated with bioinformatics analysis have enabled a massive production and quick processing of data in the study of biological samples. By these new technologies, even without a hypothesis in advance of which marker could be altered in a particular condition, a large amount of molecules can be evaluated at the same time [60,61]. The so-called omic sciences (genomics, transcriptomics, proteomics, metabolomics, etc.) integrate this set of new techniques that have been widely used by researchers in the last years, mainly in the investigation of complex diseases. They provide information for identifying the molecular signatures, that can be defined as specific panels of altered molecules, or any particular profile of gene or protein expression in patients with the disease [62].

Endometriosis has been considered as an ideal target for omic sciences. The heterogeneity, multiple phenotypes, obscure pathophysiology, association with other immune diseases and lack of an ideal diagnostic tool hamper the discovery of biomarkers from a reliable hypothesis. The wide scanning provided by omics techniques, on the other hand, makes possible a generic approach of innumerous molecules and can be regarded as a promising tool for the discovery of biomarkers for endometriosis. In spite of that, the processes of discovering molecular signatures still have some pitfalls and limited reproducibility. Until now, omic sciences could not identify any specific biomarker for clinical use in patients with endometriosis [62,63].

Studies have demonstrated that endometriosis is a condition influenced by innumerous genetic and environmental factors. Thus, the characterization and knowledge of genetic risk factors is essential for understanding the disease. Genome wide association studies (GWAS) are used to detect variations in genome, called single nucleotide polymorphisms (SNP), that can be associated to a higher risk of a particular disease or condition. GWAS can identify thousands of SNPs at the same time, guiding researchers to unravel genetic implications in complex chronic conditions [64,65].

A meta-analysis of GWAS identified SNPs in six genomic regions possibly involved in endometriosis pathophysiology, including 7p15.2, WNT4, VEZT, CDKN2B-AS1, ID4, and GREB1 across European, American, and Japanese women with stage III/IV endometriosis. Further studies were replicated in other populations corroborating the correlation of SNPs with high risk to develop endometriosis. Preliminary studies, though heterogeneous and lacking standardization, have provided a groundwork to future investigation in this field [65,66].

Still considering the close relation of endometriosis with genetic factors, it is worth mentioning the role of MicroRNAs (miRNAs) as an emergent technology in this area. They are small non-coding RNAs that bind target messenger RNA (mRNA) and repress translation, regulating the degree of gene expression [42]. Studies have found miRNA signatures representative of many diseases obtainable from diseased tissues or from urine, serum, plasma and other body fluids. Several miRNAs are tissue specific and the identification of their systemic dysregulation points toward a particular disease [67].

A dysregulation of miRNAs has been suggested to take part in endometriosis pathophysiology and they have been investigated as potential biomarkers [68]. Studies have identified distinct circulating miRNAs signatures but their origin and role are still inconclusive. In a recent systematic

review, Agrawal et al. critically analyzed studies of circulating miRNAs in endometriosis. They found 42 different dysregulated miRNAs, but only one common miRNA (miR-20) was differentially expressed in more than one study. Hence, there is no miRNA, single or in panel, that can be used as an endometriosis biomarker so far [42].

Metabolomic analysis holds a particularly advantageous potential, given the fact that metabolytes can be derived from specific pathological pathways of the endometriotic tissue. Thus, this approach can potentially track the pathophysiology of the lesions and reveal specific abnormal cellular products. Moreover, the availability of biological samples in different clinical presentations of endometriosis can provide a comprehensive panel of biomarkers. High plasma levels of valine, fucose, choline-containing metabolites, lysine/arginine, and lipoproteins have been found in women with endometriosis, and these molecules may be related to the severity or spread of the disease [69]. A panel of plasma acylcarnitines also represents a potential diagnostic approach [70].

In the context of infertility and in vitro fertilization, ovarian follicular fluid can be a valuable sample for diagnostics and other pathophysiological applications. Studies have found elevated levels of lactate, β-glucose, pyruvate, and valine in the follicular fluid of women with endometriosis in comparison to controls [71], as well as carnitine, phosphatidylcholine, and sphingomyelin metabolites [72]. Eutopic endometrium of endometriosis patients has also been tested by metabolomic science and revealed differences in comparison to control women, promising to reveal a practical diagnostic tool [73–75].

The heterogeneity of endometriosis is a critical aspect that should be carefully addressed in the exploratory analyses of potential biomarkers with omics technologies. Many studies have used the ASRM classification that mixes under the same stage patients with different disease phenotypes, or have just pooled all cases of endometriosis without phenotype distinction. The influence of confounders such as coexisting diseases and drug treatments should also be appreciated.

5. The uterus as a diagnostic target

Evidence has shown several differences in eutopic endometrium between women with and without endometriosis. Gene expression, regulatory intrinsic mechanisms and hormonal response seem to have a peculiar

behavior in both eutopic and ectopic endometrium of women with endometriosis. In patients with the disease, intrauterine endometrial cells exhibit aberrant molecular regulatory mechanisms. Therefore, it would be reasonable to search biomarkers through endometrial samples and intrauterine fluid [3,76].

Assessing intrauterine content, studies have tested biomarkers either in menstrual or aspirate fluid, in whole endometrial tissue or in separate endometrial components. The most used method is endometrial biopsy that can be performed with curette or aspirative device in a minimally-invasive way. However, the reliability of the biopsy testing depends on the skills of the professional to obtain a representative sample, the instrument used, the menstrual cycle phase and the quality of laboratories and protocols applied [77].

A Cochrane review evaluated the biomarkers obtained from menstrual fluid and endometrial tissue. Some factors evaluated were discarded as potential endometriosis markers, to quote: CYP19, TIMP-1 and VEGF. Only the immunohistochemical identification of small nerve fibers in the functional layer of the endometrium had promising results. Indeed, the expression of PGP 9.5, a small nerve fiber marker, appeared to meet the criteria for a replacement diagnostic test, but it could not be validated because of the poor quality and high heterogeneity of the studies included in the review. The presence of other gynecological diseases such as adenomyosis and fibroids can represent another limitation for the use of uterine samples, once they could produce false positive results [77].

6. From bench to bedside

Despite the great efforts made in the search of new biomarkers for endometriosis, no candidate has reached the sensitivity and specificity required for a useful diagnostic tool. Indeed, the huge majority of potential biomarkers has been discarded in research stage and very few have been translated to clinical practice. Serum CA-125 has been the most studied and used one, but studies have shown its poor diagnostic performance. Indeed, discovering an accurate non-invasive diagnostic test remains one of the major priorities in endometriosis research [2,78]. Although omic sciences may offer hope to identify specific biomarkers for clinical use, there is still a long winding road from bench to bedside.

7. Perspectives

While the search continues for new biochemical markers, with omics science, GWAS studies and miRNA dysregulation research, much attention has been given to imaging methods.

In spite of not being able to diagnose all types of endometriotic lesions or to provide functional information, imaging with transvaginal ultrasonography and MRI has become an essential step toward better recognition of all the compromised sites and resolutive surgical approach. Systematization of sonographic pelvic examination for endometriosis mapping has been published recently [79].

As these strategies cannot reliably diagnose or exclude superficial peritoneal endometriosis, molecular imaging techniques have attracted attention of scientists. Positron Emission Tomography with different radiotracers has been attempted. The first approaches used ^{18}fluordeoxyglucose (^{18}FDG), without any specific uptake pattern in different types of lesions [80,81]. Estrogen receptor and somatostatin receptor ligands have also been studied in animal models and humans, with some encouraging preliminary results [82,83].

Recently, ^{18}F-Fluorcholine, a marker of cell proliferation, has been used in an experimental animal model of endometriosis [84]. Further studies are warranted to better evaluate these methods and establish their value in clinical settings.

8. Conclusions

Endometriosis is a benign gynecological disorder which presents significant challenges in terms of diagnosis and management. Despite decades of research, there are no sufficiently sensitive and specific signs and symptoms nor blood tests for the clinical confirmation of endometriosis, which hampers prompt diagnosis and treatment. Factors known to be involved in endometriosis pathophysiology have been extensively studied but not a single one has successfully been able to accurately identify the disease. The search for a biomarker or a set of biomarkers is still open and may benefit from novel molecular biology and bioinformatics approaches to mine and uncover molecular signatures specifically associated with the disease.

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