

Review

Laboratory testing for endometriosis

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Abstract

Background: Typically, endometriosis is diagnosed surgically by laparoscopy. CA-125 is the principal serum marker used in the diagnosis and management of late-stage endometriosis. The search for a body fluid marker of early stage disease has included studies of serum, peritoneal fluid (PF), and/or tissue levels of secretory proteins, cell adhesion molecules, cytokines, tumor necrosis and vascular endothelial growth factors (VEGFs), chemokines, antiendometrial antibodies, autoantibodies to oxidized lipoproteins, aromatase P-450 expression, cytokeratins, and hormone receptors. We compared the diagnostic accuracy and clinical utility of these various types of substances in the non-surgical identification of patients with endometriosis. **Method:** We reviewed the MEDLINE database for all publications on serum, peritoneal fluid and tissue markers of endometriosis. **Results:** Except for serum interleukin (IL)-6 and peritoneal fluid tumor necrosis factor (TNF)- α levels, the diagnostic accuracy of other markers of endometriosis was either similar or worse than that of CA-125 (sensitivity 24–94%; specificity 83–93%). The diagnostic accuracy of IL-6 and TNF- α was 90–100% (sensitivity) and 67–89% (specificity). **Conclusion:** CA-125 has limited diagnostic accuracy in the identification of early stage endometriosis and none of the other markers we reviewed dramatically outperformed CA-125 in this regard with the possible exception of serum IL-6 and peritoneal fluid TNF- α levels.

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1. Introduction

Endometriosis is a common gynecologic disorder that presents with chronic pelvic pain or infertility. It represents one of the most common admitting diagnoses in women of reproductive age. Endometriosis is defined as the presence of endometrial tissue outside

of the uterus. It is typically present in the pelvis such as on the ovaries and pelvic peritoneum. It may also involve the bowel, ureter or bladder. Extrapelvic endometriosis has been reported in many areas such as the sciatic nerve and lung. The histologic diagnosis requires the presence of endometrial glands and stroma from a tissue sample.

The pathogenesis of endometriosis is complex. However, research into the mechanisms of disease formation has allowed the discovery of several potential diagnostic markers. Most theories of the pathogenesis of this disease involve some retrograde flow of endometrium with subsequent implantation. The

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Table 1
Markers for endometriosis

Tumor markers and polypeptides

- (A) CA-125, CA-19-9
(B) SICAM-1 (soluble forms of the intercellular-adhesion molecule-1)
(C) Glycodelin-A (PP 14)

Immunological markers

- (A) Cytokines: IL-6, TNF
(B) Autoantibodies
(1) Antiendometrial
(2) Autoantibodies to markers of oxidative stress

Genetic markers

- Early growth response (EGR)-1 gene
P450 aromatase
Placental Protein 14 (PP14)

Tissue markers

- (A) Aromatase P 450
(B) Cytokeratins
(C) Hormone receptors
-

process of implantation requires secretion of growth factors that allows neovascularization. Immune dysfunction has been identified in these patients either as a result of the disease or as a consequence of disease. Some of these immunologic abnormalities have been implicated in the disruption of the reproductive system causing infertility.

The “gold” standard for the diagnosis of endometriosis is a surgical intervention, a laparoscopy. The severity of disease is variable and patients are usually categorized according to the American Fertility Society classification of disease into four groups that represent mild to severe disease, stages I to IV (1). There is a poor correlation between the severity of disease and the patient’s symptoms. Furthermore, the disease can be found in asymptomatic patients. This heterogeneity in clinical presentation has contributed to the difficulties in identifying a marker. Since some women are asymptomatic, clinical trials require a control group of women that require a surgical procedure to exclude the presence of endometriosis. Considerable effort has been invested in searching for non-invasive methods of diagnosis. Many reports have suggested that various serum, peritoneal fluid (PF) and tissue markers are associated with endometriosis (Table 1). This review will discuss the potential use of serum, PF and tissue

markers as diagnostic tools in symptomatic patients with endometriosis.

2. Tumor markers and polypeptides

2.1. Serum CA-125

Serum CA-125, a 200,000 Da glycoprotein, concentration has been associated with the presence of many gynecologic disorders (Table 2), including endometriosis [2]. The CA-125 antigen is expressed in many normal tissues such as the endometrium, endocervix and peritoneum. Serum levels of CA-125 may change with age [2]. However, the reports have been contradictory; some authors have reported that levels decrease with age, whereas others have reported that levels increase or do not change with age [2]. In some women, CA-125 levels increase

Table 2
Benign and malignant gynecologic diseases associated with increased CA-125 levels

Malignant ovarian tumors

- Serous adenocarcinoma
Mucinous adenocarcinoma
Undifferentiated adenocarcinoma
Endometrioid adenocarcinoma
Papillary carcinoma
Dysgerminoma
Clear cell carcinoma

Neoplasia of low malignant potential (borderline tumors) and benign ovarian tumors

- Adenoma
Cysts
Benign cystic teratomas
Granulosa cell tumor
Thecoma

Cervical, endometrial and tubal malignancy

- Cervical carcinoma
Endometrial carcinoma
Tubal carcinoma

Benign gynecologic conditions

- Uterine leiomyoma
Adenomyosis
Endometriosis
Cervical polyps
Pelvic Inflammatory disease
Ectopic pregnancy
-

during menstruation, possibly because the menstrual endometrium refluxes into the peritoneal cavity [2]. Pittaway and Fayed [3] showed that mean CA-125 levels were higher during menses in patients with and without endometriosis. It is therefore recommended that CA-125 levels not be drawn during a menstrual period.

The most important clinical use of this serum marker has been in monitoring the course of ovarian cancer in response to treatment. In a study at the Cleveland Clinic Foundation [4], 213 consecutive patients with a CA-125 greater than 65 IU/ml were assessed. In this group, gynecologic cancers accounted for 74% of the diagnoses, non-gynecologic cancers accounted for 7% and non-malignant conditions accounted for 13%. Most patients in the non-malignant gynecologic disorder group had endometriosis. When patients with a pelvic mass were excluded, 90% of patients in the group with non-malignant conditions had a CA-125 greater than 65 U/ml. Malkasian et al. [5] found that the positive predictive value for malignancy was only 49% in premenopausal patients with a pelvic mass and a CA-125 level of greater than 65 U/ml. The cut-off value for all these studies was 65 U/ml and not 35 U/ml, which is considered abnormal in standard laboratory reference values.

Extremely high levels have been reported in women with endometriosis, tubo-ovarian abscess and multivisceral tuberculosis [2]. One plausible explanation for such an increase is that the CA-125 membrane concentration is higher in ectopic cells than in eutopic endometrial epithelial cells. Moreover, the endometriosis-associated inflammatory response increases CA-125 shedding into the peritoneal cavity [1].

Many studies have assessed the role of serum CA-125 measurement in the detection of endometriosis. [6–8]. The main confounding variable in determining the sensitivity and specificity of serum CA-125 is the stage of the disease. Typically, most patients with advanced endometriosis (and few patients with early stage disease) will have elevated serum CA-125 levels, which is similar to what occurs in ovarian cancer.

A recent meta-analysis was performed to assess the diagnostic performance of serum CA-125 in detecting endometriosis [9]. Twenty-three studies were included in the initial analysis; 16 were cohort studies and

seven were case-control studies. The studies included women with infertility or pelvic pain. The sensitivity and specificity were presented as receiver operating characteristic (ROC) curves. Data were reported for the diagnosis of any form of endometriosis as well as advanced stages only. The sensitivity ranged from 4% to 100% and the specificity ranged from 38% to 100% for the diagnosis of any stage of disease. The ROC curve showed a poor diagnostic performance. At a specificity of 90%, a sensitivity of 28% was reported. If the sensitivity was increased to 50%, the specificity dropped to 72%.

For advanced disease, the sensitivity ranged from 0% to 100% and the specificity ranged from 44% to 95%. In this case, the ROC curve showed a better diagnostic performance. For a specificity of approximately 90%, the sensitivity was 47%. If the sensitivity was increased to 60%, the specificity dropped to 81%.

The main limitation of this meta-analysis was that the analysis did not consider the patients' history (such as dysmenorrhea) or their physical examination results both of which may increase the sensitivity or specificity of the test. Also, studies that included patients who had a pelvic mass on sonography were excluded from this analysis. If the purpose of a test is to identify the majority of patients with a disease, then the diagnostic accuracy of a serum CA-125 is inadequate. According to the authors of this study, a negative result would delay the diagnosis in 70% of patients with endometriosis. The routine use of serum CA-125 cannot be advocated as a diagnostic tool to exclude the diagnosis of endometriosis in patients with chronic pelvic pain or infertility.

CA-125 may be more useful in evaluating recurrent disease or the success of a surgical treatment. In a study to evaluate the prognostic value of serial CA-125 determinations, 342 women having a laparoscopy for infertility were evaluated. One hundred and twenty three (36%) had endometriosis and were surgically treated [10]. Fifty-six of 123 (46%) infertile women with endometriosis had preoperative CA-125 values that were more than or equal to 16 U/ml. These women were followed for 12 months with serial CA-125 determinations. The main outcome measure was the proportion of women achieving a pregnancy within 12 months from surgery. The results showed that preoperative CA-125 concentrations were not

statistically different, but postoperative CA-125 values were significantly lower in the women who achieved a pregnancy. Univariate analyses indicated that preoperative CA-125 values between 16 and 25 U/ml and postoperative CA-125 values less than 16 U/ml were associated with significantly higher pregnancy rates. Multivariate analyses of confounding factors indicated that only postoperative CA-125 concentrations were associated with pregnancy even after controlling for all covariables. This study suggested that CA-125 levels have prognostic value for pregnancy in infertile women with surgically treated endometriosis [10].

CA-125 levels may also be useful in patients with initially elevated levels and advanced endometriosis. Several centers have reported high diagnostic accuracy for recurrent disease when elevated levels of CA-125 were observed after treatment [11]. This may be useful in symptomatic patients in whom repeat laparoscopy cannot be performed. Measures of serum CA-125 accuracy in diagnosing endometriosis are shown in Table 3.

2.2. Serum CA 19-9

CA 19-9 is a high-molecular-weight glycoprotein [12]. Serum CA19-9 levels are elevated in patients

Table 3
Diagnostic accuracy of serum CA-125 \geq 20 U/ml in diagnosing patients with endometriosis

| | No. of patients | Sensitivity, % | Specificity, % |
|----------------------------|-----------------|----------------|----------------|
| Collacuri et al. [130] | 28 | 44 | 90 |
| Fedele et al. [11] | 154 | 85 | 100 |
| Fisk and Tan [30] | 48 | 43 | 04 |
| Gurgan et al. [31] | 38 | 0 | 100 |
| Homstein et al. [127] | 123 | 16 | 91 |
| Ismail et al. [133] | 30 | 50 | 38 |
| Kruitwagen et al. [32] | 74 | 20 | 82 |
| Lanzone et al. [134] | 119 | 53 | 86 |
| Medl et al. [132] | 368 | 33 | 91 |
| Molo et al. [126] | 35 | 100 | 93 |
| Moloney et al. [128] | 60 | 34 | 100 |
| Moretuzzo et al. [7] | 40 | 20 | 90 |
| Muscatello et al. [131] | 119 | 51 | 86 |
| O'Shaughnessy et al. [129] | 100 | 27 | 100 |
| Ozaksit et al. [135] | 86 | 80 | 90 |
| Palton et al. [125] | 113 | 13 | 93 |
| Pittaway and Fayed [6] | 385 | 17 | 93 |

Modified from Mol et al., *Fertil. Steril.* 70 (1998) p. 1104.

with malignant and benign ovarian tumors [13] and in those with ovarian chocolate cysts [14]. Serum CA19-9 levels in women with endometriosis fell significantly after treatment for endometriosis when compared with the basal levels before treatment [15]. There are a limited number of reports on the significance of serum CA19-9 levels in the diagnosis of endometriosis.

In a recent study, 34 of 101 patients with endometriosis (34%) had elevated serum CA19-9 levels (>37 IU/ml) but it was not elevated in all of the 22 control patients [16]. Serum CA19-9 levels were not elevated in 38 patients with stage I and II endometriosis but were elevated in 34 of the 63 patients (54%) with stage III and IV endometriosis. On comparing the sensitivities of the CA19-9 and CA-125 tests for the diagnosis of endometriosis, the authors found that the sensitivity of the CA19-9 test was significantly lower than that of the CA-125 test (0.34 and 0.49, respectively). Thus, the observed sensitivity of 0.34 limits the diagnostic value of the CA19-9 test, especially in the early stages of disease [16].

The same study showed that using a cutoff value of 37 IU/ml, the mean serum CA19-9 level in patients with endometriosis, increased in accordance with the stage of the disease. On the other hand, if a new cutoff value was used that ranged from 20 to 25 IU/ml, the sensitivity of the CA19-9 test improved without a change in specificity or in the positive and negative predictive values. However, this study concluded that the clinical utility of the CA19-9 measurement is not superior to that of the CA-125.

2.3. Serum-soluble intercellular adhesion molecule-1

Soluble forms of the intercellular-adhesion molecule-1 (sICAM-1) are secreted from the endometrium and endometriotic implants [17]. Moreover, endometrium from women with endometriosis secretes a higher amount of this molecule than tissue from women without the disease. Consequently, a strong correlation exists between levels of sICAM-1 shed by the endometrium and the number of endometriotic implants in the pelvis [17]. With this in mind, it has been hypothesized that sICAM-1 may be useful in the diagnosis of endometriosis. Many investigators have reported a significant increase in serum concentration of sICAM-1 in patients with endometriosis [18–21].

In a recent prospective cohort study to evaluate the utility of sICAM-1 as a potential serum marker of endometriosis, Somigliana et al. included a series of 120 consecutive women of reproductive age who underwent laparoscopy for benign gynecologic conditions. They found that serum levels of sICAM-1 were only slightly but not significantly higher in women with endometriosis [22] than in women without the disease. However, serum concentrations of sICAM-1 in the 21 women who were found to have deep peritoneal endometriosis were significantly higher than concentrations in women without the disease and in those with superficial endometriosis. The sensitivity and specificity of sICAM-1 in detecting deep peritoneal endometriosis were 0.19 and 0.97, respectively. On comparing that to CA-125, Somigliana found that the sensitivity and specificity were 0.14 and 0.92, respectively. When both markers were used concomitantly, the sensitivity and specificity were 0.28 and 0.92, respectively. They concluded that measurement of CA-125 and sICAM-1 may be helpful in specifically identifying women with deep infiltrating endometriosis [22].

2.4. Other serum polypeptides

Serum placental protein 14 (PP 14)—currently known as glycodelin-A [23]—was found to be significantly higher in endometriosis patients than in healthy controls [24]. Levels were significantly lowered by conservative surgery as well as by treatment with danazol and medroxy progesterone acetate. The ability of serum PP 14 levels to diagnose of endometriosis is limited because of a low sensitivity (0.59). Typically, the PF concentrations of PP 14 are low. The levels are elevated in the luteal phase of endometriosis patients. Whether this is of any diagnostic importance is controversial [25].

2.5. Peritoneal fluid CA-125

Peritoneal fluid is often seen in the vesicouterine cavity or the cul-de-sac during gynecologic surgery. It bathes the pelvic cavity, uterus, fallopian tubes and ovaries and is believed to be a major factor controlling the peritoneal microenvironment, which influences the development and progression of endometriosis and endometriosis-associated infertility. Peritoneal fluid is

formed in part by follicular activity, corpus luteum vascularity and hormonal production. The volume of PF is dynamic and phase dependent, and it peaks at the time of ovulation [26]. The PF components vary from cycle to cycle and with different pathologic entities [27,28]. Women with endometriosis have a greater volume of PF than fertile controls, patients with tubal disease and those with unexplained infertility. Moreover, an increased volume of PF may be commonly associated not only with endometriosis but also with idiopathic infertility.

Many investigators have measured levels of CA-125 in the PF of patients with and without endometriosis [29–31]. Although PF levels of CA-125 were almost 10 times higher than serum levels, no differences were found between women with and without endometriosis [32]. CA-125 levels have also been measured in other body fluids such as menstrual discharge [33] and uterine fluid [34] but were not found to be useful in clinical practice.

3. Immunological markers

The immune system has been shown to play a significant role in the pathogenesis of endometriosis [35]. Based on these recent findings, endometriosis is starting to be treated as an autoimmune disease [36]. Accumulating evidence suggests that systemic T cell activity influences the pathogenesis of endometriosis [37,38]. Research has shown that the T-helper to T-suppressor ratio and concentration of both cells are altered in serum, PF [39] and endometriotic tissue [40] in endometriosis patients. Moreover, such differences could be detected between eutopic endometrium from women with and without the disease. There is lack of consistency, however, regarding the alterations in T-cells and their role in the pathophysiology of endometriosis.

Natural killer (NK) cells are also altered in endometriosis. Both peripheral and PF NK cells from women with endometriosis have different characteristics than those of healthy controls [41]. Additionally, NK cell cytotoxicity has been shown to be inversely correlated with the stage of the disease [42]. Consequently, altered NK cytotoxicity to endometrial tissue may be partly responsible for the initiation, propagation and establishment of pelvic endometriosis. Sera

and PF from women with endometriosis have been shown to reduce NK cell activity [43]. This reduction in activity is probably caused by monocyte or macrophage secretions that modulate immune and non-immune cells.

In addition to alterations in T cell function, many recent findings have shown that B-cell function is altered in endometriosis patients as evidenced by abnormal antigen–antibody reaction and increased B-cell function [44–48]. Decreased C3 deposition in the endometrium and a corresponding reduction in the serum total complement levels have been found in endometriosis patients [44]. Antiendometrial antibodies, particularly IgG and IgA, have been detected in the sera and vaginal and cervical secretions of endometriosis patients [45]. The presence of antiphospholipids and antihistones of IgG, IgM and IgA have been documented by some investigators [46] and questioned by others [47]. The exact correlation between the stage of endometriosis and autoantibodies ranges from positive [48] to negative [49] to no relationship at all [50]. These observations of immune alterations have lead investigators to believe that markers of immune reactivity, particularly cytokines, may be potentially used as a diagnostic aid for endometriosis.

3.1. Cytokines: chemistry

Cytokines are polypeptides or glycoproteins that are secreted into the extracellular compartment mainly by leukocytes. Upon secretion, they exert autocrine, paracrine and sometimes endocrine effects. Moreover, cytokines may exist in cell-membrane-associated forms where they exert juxtacrine activity on adjacent cells. They are essential mediators of cell–cell communication in the immune system and affect a wide variety of target cells exerting proliferative, cytostatic, chemoattractant or differentiative effects. Their biological activities are mediated by their ability to couple with intracellular signaling and second-messenger pathways via specific high-affinity receptors on target cell membranes. The cytokine nomenclature reflects the historical description of these biological activities.

3.2. Cytokines: sources

The main source of cytokines is macrophages, which originate in bone marrow, circulate as mono-

cytes and migrate to various body cavities. Chemoattractant cytokines, particularly RANTES (Regulated on Activation, Normal T-Cell Expressed and Secreted) and interleukin (IL)-8, facilitate macrophage recruitment into the peritoneal cavity. The second major source of cytokines is T lymphocytes. Helper T cells can be classified into two subsets: type 1 (Th1) and type 2 (Th2). Th1 cells produce IL-2, IL-12 and interferon- γ , which are potent inducers of cell-mediated immunity. Th2 cells produce mainly IL-4, IL-5, IL-10 and IL-13, which suppress cell-mediated immunity. In patients with endometriosis, the cytokines secreted by Th1 and Th2 cells favor those produced by Th2 cells. This alteration may be partly responsible for the impaired immunologic defense in endometriosis [51].

Tsuda et al. [52] hypothesized that cytokines are not only produced by immune competent cells but by endometriotic implants as well. They demonstrated that endometriotic cells constitutively express IL-6 messenger RNA and produce IL-6 protein and that adding TNF- α stimulates IL-6 gene and protein expression in a dose-dependent manner. On comparing IL-6 production by macrophages and endometriotic stromal cells in patients with endometriosis, they found that similar levels of IL-6 were produced in stromal cells derived from an endometrioma and by macrophages under basal and TNF- α stimulated conditions. This finding supports the hypothesis that endometriotic tissue is another important source of cytokines [52].

3.3. Peritoneal fluid cytokines

Peritoneal fluid is rich with variable cellular components including macrophages, mesothelial cells, lymphocytes, eosinophils and mast cells. The normal concentration of PF leukocytes is 0.5 to $2.0 \times 10^6/\text{ml}$, of which approximately 85% are macrophages [27,28]. It has been hypothesized that peritoneal macrophage activation is a pivotal step in disease initiation and progression [53]. Activated macrophages in the peritoneal cavity of women with endometriosis are potent producers of cytokines [54]. Thus, PF contains a rich mixture of cytokines. Iron overload was also observed in the cellular and PF compartments of the peritoneal cavity in patients with endometriosis, suggesting that the iron overload plays a role in the pathogenesis of this disease as well [55].

3.4. Individual cytokines

3.4.1. Tumor necrosis factors

The tumor necrosis factors (TNF) are pleiotropic cytokines that play an essential role in the inflammatory process. TNF is believed to be seminal in many physiological and pathological reproductive processes and to have beneficial and hazardous effects. The quantity of TNF produced is the main factor that controls its role in the disease process. The main TNF is TNF- α , which is produced by neutrophils, activated lymphocytes, macrophages, NK cells and several non-hematopoietic cells. Little is known about TNF- β , which is produced by lymphocytes. The primary function of the TNFs is to initiate the cascade of cytokines and other factors associated with inflammatory responses. TNF- α helps activate helper T cells.

In the human endometrium, TNF- α is a factor in the normal physiology of endometrial proliferation and shedding. TNF- α is expressed mostly in epithelial cells, particularly in the secretory phase [56]. Stromal cells stain for TNF- α mostly in the proliferative phase of the menstrual cycle. These data suggest that this cytokine is influenced by hormones [57].

TNF- α concentrations in PF are elevated in patients with endometriosis, and some studies show that higher concentrations correlate with the disease stage [58]. However, our study did not observe any relationship between levels of TNF- α and disease stage [54]. The source of the elevated TNF- α concentration in the PF of endometriosis patients varies. Some *in vitro* studies suggest that peritoneal macrophages [59] and peripheral blood monocytes [60] from these patients have up-regulated TNF- α protein secretion. Activated macrophages play a critical role in the pathogenesis of endometriosis. Secreted TNF- α may play an important role in the local and the systemic manifestations of the disease. Because of its importance in other inflammatory processes, it is likely that this cytokine plays a central role in the pathogenesis of endometriosis [61]. Moreover, measuring its level in the PF can be used as a foundation for non-surgical diagnosis of endometriosis [54]. The concept of using TNF- α blockers in treating endometriosis has recently gained popularity [36].

3.4.2. Interleukin-6

IL-6 is a regulator of inflammation and immunity, which may be a physiologic link between the endocrine and the immune systems. It also modulates secretion of other cytokines, promotes T-cell activation and B-cell differentiation and inhibits growth of various human cell lines [36]. IL-6 is produced by monocytes, macrophages, fibroblasts, endothelial cells, vascular smooth-muscle cells and endometrial epithelial stromal cells and by several endocrine glands, including the pituitary and the pancreas [62].

The role of IL-6 in the pathogenesis of endometriosis has been extensively studied. IL-6 response was dysregulated in the peritoneal macrophages [63], endometrial stromal cells [64] and peripheral macrophages [60] in patients with endometriosis. The level of IL-6 detected in the PF of patients with endometriosis was inconsistent. Some investigators have demonstrated elevated concentrations [65,66], whereas others have found no elevation [67]. Some studies failed to demonstrate statistically significant differences in IL-6 levels between controls and endometriosis patients [68]. These inconsistent findings likely are related to the antibody specificity of the assay. In our recent study, we found that IL-6 was significantly elevated in the sera of endometriosis patients but not in their PF as compared with patients with unexplained infertility and tubal ligation/reanastomosis [54].

3.4.3. Vascular endothelial growth factor

Many studies have focused on the proliferation and neovascularization of the endometriotic implants. Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors. When VEGF binds to its targeted receptor, the VEGF-receptor activation leads to a rapid increase in intracellular Ca²⁺ and inositol triphosphate concentrations in endothelial cells [69,70]. The basic physiological function of VEGF is to induce angiogenesis, which allows the endometrium to repair itself following menstruation. It also modulates the characteristics of the newly formed vessels by controlling the microvascular permeability and permitting the formation of a fibrin matrix for endothelial cell migration and proliferation [71]. This modulation may be responsible for local endometrial edema, which helps prepare the endometrium for embryo implantation [72].

In endometriosis patients, VEGF is localized in the epithelium of endometriotic implants [73], particularly in hemorrhagic red implants [74]. Moreover, the concentration of VEGF is increased in the PF of endometriosis patients. The exact cellular sources of VEGF in PF have not yet been precisely defined. Although evidence suggests that endometriotic lesions themselves produce this factor [73], activated peritoneal macrophages also can synthesize and secrete VEGF [75]. Antiangiogenic drugs are potential therapeutic agents in endometriosis.

3.4.4. RANTES

Regulated on Activation, Normal T-Cell Expressed and Secreted (RANTES) belongs to the β or “C-C” chemokine family. It attracts monocytes and memory T-cells. RANTES is a secretory product of hematopoietic, epithelial and mesenchymal cells and a mediator in both acute and chronic inflammation [76].

RANTES protein distribution in ectopic endometrium is similar to that found in a eutopic endometrium [77]. However, *in vitro* secretion of RANTES by endometrioma-derived stromal cell cultures is significantly greater than in eutopic endometrium. In this way, PF concentrations of RANTES may be increased in patients with endometriosis [78].

3.4.5. Interleukin-1

Interleukin-1 (IL-1) is a key cytokine in the regulation of inflammation and immune responses. IL-1 affects the activation of T-lymphocytes and the differentiation of B-lymphocytes. There are two receptors for IL-1, namely IL-1 α and IL-1 β , which share only 18–26% amino acid homology. Both receptors are encoded by different genes but have similar biological activities. Research has shown that the administration of exogenous IL-1 receptor antagonist blocks successful implantation in mice. This illustrates its important role in the implantation of the ectopic endometrium [79]. IL-1 has been isolated from the PF of patients with endometriosis. Results have been inconsistent, with some investigators demonstrating elevated concentrations in patients with endometriosis [80] and others finding no elevation [54,59,81].

3.4.6. Other cytokines

Highly sensitive ELISA kits have made it easy to measure the entire battery of cytokines in the serum

and PF of endometriosis patients. Other PF cytokines have been identified and include IL-4 [51]; IL-5 [65]; IL-8 [54,82]; IL-10 [83]; IL-12 [54,84]; IL-13 [85]; interferon- γ [67]; monocyte chemotactic protein-1 (MCP-1) [86]; macrophage colony stimulating factor (MCSF) [87] and transforming growth factor (TGF)- β [88]. All of these cytokines may regulate the actions of leukocytes or may act directly on ectopic endometrium, where they may play various roles in the pathogenesis and pathophysiology of endometriosis. However, they have not been extensively investigated as a diagnostic tool.

3.5. Cytokines as a screening tool

The role of cytokines and growth factors in the pathophysiology of endometriosis is evident as previously discussed. They are probably responsible for endometrial cell proliferation [89,90] and implantation of endometrial cells or tissue [91]. Moreover, cytokines increase tissue remodeling through their influence on the matrix metalloproteinases [92]. Increased angiogenesis of the ectopic endometrial tissue and neovascularization of the affected region is probably the most important effect of cytokines on ectopic endometrial tissue. Consequently, cytokines play a major role in the initiation, propagation and regulation of immune and inflammatory responses. Immune cell activation results in a burst and cascade of inflammatory cytokines.

Besides their role in the pathogenesis of endometriosis, they might have a diagnostic role as well. To evaluate this hypothesis, we conducted a prospective controlled trial to investigate the ability of a group of serum and PF markers to non-surgically predict endometriosis [54]. Serum and PF from 130 women were obtained while they underwent laparoscopy for pain, infertility, tubal ligation or sterilization reversal. We measured the concentrations of 6 cytokines (IL-1 β , IL-6, IL-8, IL-12, IL-13 and TNF- α) in serum and PF and levels of reactive oxygen species (ROS) in PF and compared the levels among the women who were divided into groups according to their postsurgical diagnosis. Fifty-six patients were diagnosed with endometriosis, 8 were diagnosed with idiopathic infertility, 27 had undergone tubal ligation or reanastomosis (control group) and 39 were excluded due to bloody PF.

Only serum IL-6 and PF TNF- α could discriminate between patients with and without endometriosis with a high degree of sensitivity and specificity. The PF TNF- α had an exceptional 99% area under the curve (95% CI: 97% to 100%), suggesting that it has a very high discrimination ability. A cut-off of 15 pg/ml resulted in 100% sensitivity and 89% specificity (positive likelihood ratio of 9.1 and negative likelihood ratio of 0). A cut-off of 20 pg/ml yielded 96% sensitivity and 95% specificity (positive likelihood ratio of 19.2 and negative likelihood ratio of 0.04).

The serum IL-6 achieved a relatively high diagnostic value with an area under the curve of 87% (95% CI: 75% to 99%). A serum IL-6 cut-off of 2 pg/ml provided a sensitivity of 90% and specificity of 67% (positive likelihood ratio of 2.7 and negative likelihood ratio of 0.14). A cut-off of 4 pg/ml provided sensitivity of 85% and specificity of 80% (positive likelihood ratio of 4.3 and negative likelihood ratio of 0.19), and a cut-off of 7.5 pg/ml provided sensitivity of 80% and specificity of 87% (positive likelihood ratio of 6.2 and negative likelihood ratio of 0.23).

Because the positive and negative likelihood ratios of PF TNF- α are excellent, it is possible that ultrasonographically guided transvaginal aspiration of PF from the cul-de-sac may serve as a basis for the non-surgical diagnosis of endometriosis. However, our study had two main limitations. First, there may not be enough serum and PF to measure the cytokines. In some patients, there was not enough serum and PF to measure the cytokines. Second, all bloody PF samples were excluded because cytokine levels could have been affected by blood contamination. Consequently, the study conclusions are not applicable to patients with blood-contaminated PF. However, our study showed that serum IL-6 and PF TNF- α might be potential markers for endometriosis, thereby allowing for non-surgical diagnosis.

3.6. Autoantibodies

A variety of autoantibodies have been detected in endometriosis patients. The most commonly reported types are antiendometrial antibodies [44,93] and autoantibodies against oxidatively modified lipoproteins [94].

3.6.1. Antiendometrial antibodies

The antigens used to induce antiendometrial antibodies include sonicated endometrium of women with normal menstrual cycles, endometrial tissue of patients with endometriosis, endometriosis tissue, human endometrial carcinoma cells line, epithelial monolayers or endometrial glands and stromal cells. Moreover, the exact antigen is not known. Consequently, there is no simple antigen–antibody assay that is currently available [49].

3.6.1.1. Serum antiendometrial antibodies. Some investigators have postulated that antiendometrial antibodies are a probable cause of infertility in endometriosis patients [44,93] while others have disagreed with this hypothesis [95]. Besides the inconsistency of the assay techniques used [96], the nature of the antigens used in those studies to elicit an immune response are inconsistent as well.

The sensitivity and the specificity of serum antiendometrial antibody screening were reported by some investigators to be 0.84 and 1.00, respectively [45]. When comparing infertile women with those with endometriosis and unexplained infertility, Wild and Shiver [50] found a sensitivity of 0.71 and a specificity of 1.00. Similarly, Meek et al. [47] found a sensitivity of 0.75 and a specificity of 0.90 while in another study, the values were 0.85 and 0.67, respectively [97]. Although the sensitivity and specificity of serum antiendometrial antibody matches that of CA-125, this assay is not generally used in the diagnosis of endometriosis. This is most likely due to the availability of the CA-125 testing to gynecologists compared to test for antiendometrial antibodies. Despite this limitation, antiendometrial antibody was proposed not only as a screening marker but also as a follow-up marker of treatment results and recurrence [98].

3.6.1.2. Peritoneal fluid antiendometrial antibodies.

Although antiendometrial antibodies were found in the PF of endometriosis patients, their sensitivity and specificity are variable. Halme and Mthur [99] found a sensitivity of 0.23 and a specificity of 0.96 using a passive haemagglutination assay, whereas the results were 0.75 and 0.90 using Ouchterlony immune diffusion [47].

3.6.2. Autoantibodies to markers of oxidative stress

Increasing evidence suggests that oxidative stress occurs in the PF of women with endometriosis and that oxidatively modified lipoproteins exist in the PF [54,100,101]. In addition, oxidation-specific epitopes and macrophages are present in the endometrium of healthy women and in endometriosis patients [94]. Lipid peroxides interact with proteins, resulting in several types of alterations. Such oxidatively modified proteins are themselves antigenic. Antigenicity is attributed to specific modified epitopes and not to the protein backbone.

In a study to measure autoantibodies to oxidatively modified proteins in the sera of women with surgically proven endometriosis, Murphy et al. [94] included women undergoing surgery for endometriosis or tubal ligation. They measured serum and PF autoantibody titers to malondialdehyde-modified low-density lipoprotein, oxidized low-density lipoprotein (Ox-LDL) and lipid peroxide-modified rabbit serum albumin determined by ELISA. They correlated the autoantibody titers with the disease stage, symptoms and morphologic type of endometriosis.

They found that autoantibodies to markers of oxidative stress were significantly increased in women with endometriosis without any correlation with the stage, symptoms or morphologic type of the disease. Peritoneal fluid did not contain autoantibodies to any of the three antigens. Given the fact that autoantibodies to Ox-LDL have been long considered as a screening tool for atherosclerosis [102], a similar role might be claimed in endometriosis.

4. Genetic markers of endometriosis

Given the fact that the etiology of endometriosis is complex and multifactorial, endometriosis is an ideal target for genome-wide scanning. Familial inheritance plays a role, and multiple candidate genes are involved [103]. Many technological approaches can help identify possible genetic markers of endometriosis. A gene-based diagnostic test for endometriosis may be the long sought after ideal screening test [104]. A number of technologies have emerged to facilitate progress in this direction. Gene based technologies that may be a suitable foundation for genetic markers of the disease include subtractive cDNA

hybridization [105,106] and cDNA microarray techniques [107–110].

Based on recent DNA technologies, an on-going study in our center is attempting to create a cDNA library from normal endometrial tissue as well as endometriotic implants. Based on the refined library, potential specific serum antibodies may be identified and subsequently used as a non-surgical screening tool for endometriosis (personal communication).

5. Endometrial tissue biochemical markers

5.1. Aromatase P450

Aromatase P450 is a catalyst of the conversion of androstenedione and testosterone to estrone and estradiol, respectively. This enzyme is expressed in both eutopic and ectopic endometrium of endometriosis patients but not in eutopic endometrium of healthy controls [111]. Although endometrial aromatase P450 expression does not correlate with the disease stage, a recent study demonstrated that detection of aromatase P450 transcripts in the endometrium of endometriosis patients may be a potential qualitative marker of endometriosis [112].

Another retrospective, case-controlled study reported that 7 (25%) of 28 women without detectable levels of endometrial aromatase P450 protein, determined by immunohistochemical analysis, had either endometriosis, fibroids, adenomyosis or a combination of these disorders [111]. The potential use of such marker as a clinically useful diagnostic tool of pelvic disease is limited by the observation that large numbers of women with endometriosis do not express aromatase P450 in their eutopic endometrium. Nevertheless, the use of aromatase inhibitors as a potential treatment of endometriosis is gaining momentum based on these molecular facts [113–116].

5.2. Cytokeratins

In culture, endometriosis cell lines have been shown to exhibit an epithelial-like morphology and immunoreactivity for cytokeratins 8, 18, 19, vimentin and human leukocyte class I antigens [117]. However, the cultured cells were negative for an entire set of haematopoietic cell markers, including the lymphoid

cell antigens CD3, CD20 and CD45, von Willebrand factor, carcinoembryonic antigen and CA-125 [117]. In another study, endometriotic tissues from various locations were immunostained for estrogen receptor, vimentin, Ber-EP-4 and cytokeratins [118]. Using immunofluorescence with monoclonal antibodies against cytoskeletal components and epithelial mucins, Matthew and associates studied the staining patterns for cytokeratins 18 and 19, vimentin and three different epithelial mucins in endometriotic cell cultures. They found that cytokeratins were located in epithelial cells and that vimentin was expressed in both stromal and epithelial cells [119]. No studies have evaluated the use of these molecular markers as a potential diagnostic/screening tool in endometriosis.

5.3. Hormone receptors

Given the fact that endometriosis is an estrogen-dependant condition, the quantification of estrogen and progesterone receptors in the endometrium could be potentially useful in screening for this disease. The content of estrogen and progesterone receptors is phase dependent and cyclic [120]. However, the ectopic endometrium of patients with endometriosis is very different from normal endometrium in regards to apoptosis [121], cytokines and other characteristics [64]. Although cyclic changes were also detected in ectopic endometrium, different patterns of receptor expression suggested a difference in hormonal regulation between the two sites. The concentrations of steroid receptors in ectopic endometrium increased gradually as the cycle progressed. Compared with eutopic endometrium, estrogen and progesterone receptor concentrations were significantly lower in the proliferative phase, similar in the early secretory phase and significantly higher in the late secretory phase [122]. The different patterns of receptor expression suggested different hormonal regulations between eutopic and ectopic endometrium [120].

There are two isoforms for estrogen (ER) and progesterone (PR) receptors—ER- α and ER- β , PR-A and PR-B. These isoforms exist in the endometrium, and their function and content are different from one another [123]. The different concentrations and biological activity of steroid receptor isoforms might lead to various hormonal responsiveness of ectopic endometrium. High concentrations of E and P recep-

tors in the ectopic endometrium during the secretory phase could explain the high proliferative activity of endometriotic tissue in this phase. Conversely, a decrease in E and P receptor expression in ectopic implants during the secretory phase might lead to diminished proliferation [122]. The expression of estrogen and progesterone receptors may be regarded as an index of differentiation of the endometriotic implant. Consequently, E and P receptors may be used as markers of the activity of all subtypes of endometriotic lesions [49].

6. Conclusion

One of the major challenges facing gynecologists is the inability to diagnose endometriosis without the need for laparoscopy or laparotomy. At present, there are no reliable markers of this disease. Measuring serum concentrations of tumor markers, particularly CA-125, which is the most extensively studied and used serum marker of endometriosis, has limited diagnostic utility. This poses a particular problem for those who are investigating the presence of early stages of the disease. The detection of endometriosis is critical in infertile women with stage I or II disease because laparoscopic treatment of the lesions associated with early disease has been reported to almost double the rate of a spontaneous pregnancy. The high specificity of CA-125 results indicates its potential usefulness in disease monitoring and follow-up. Since medical treatment has been associated with temporary suppression of disease activity, CA-125 may be important only in long-term monitoring of surgical therapy. Randomized clinical trials on the use of surgery for infertility or pain associated with endometriosis have shown a clear benefit [124]. Thus early

Table 4
Comparison of the diagnostic accuracy of various serum markers and peritoneal fluid TNF- α

| Marker | Sensitivity, % | Specificity, % |
|--------------------------------|----------------|----------------|
| Serum CA-125 | 0–85 | 4–100 |
| Serum glycodelin | 59 | – |
| Serum endometrial antibodies | 71–85 | 67–100 |
| Serum interleukin-6 | 90 | 67 |
| Peritoneal fluid TNF- α | 100 | 89 |

detection is a critical part of the diagnosis and treatment of this disease.

Immunological markers have gained importance with the accumulating evidence regarding the immunological changes that occur during the evolution of the disease. The most promising diagnostic test is the use of PF and serum cytokines (Table 4). Large clinical trials will be needed to validate this hypothesis. Tests for autoantibodies detect circulating antibodies to a variety of antigens. Their use as a screening tool however is limited by their low diagnostic sensitivity. The combination of recent immunological discoveries and recent advances in DNA technologies may provide the long sought screening tool with the desirable diagnostic accuracy for this puzzling disorder.

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