

# Current Insights and Future Advances in Endometriosis Diagnostics

Michele Cioffi and Maria Teresa Vietri

*Department of General Pathology,  
Faculty of Medicine and Surgery of Second University of Naples, Naples,  
Italy*

## 1. Introduction

Endometriosis is a benign gynaecological disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. This condition is mainly found in women of reproductive age, from all ethnic and social groups and it is associated with pelvic pain and infertility. Endometriosis is typically present in the pelvis such as on the ovaries and pelvic peritoneum, but may also involve the bowel, ureter or bladder. It regresses after menopause or ovariectomy, suggesting it could depend on the production and metabolism of sex steroids: high concentrations of estrogens were found in the endometriotic lesions, which grow and regress in an oestrogen-dependent way. Nevertheless, the pathogenesis and the molecular mechanism that underlie the development of endometriosis have troubled the investigators through many years, remaining an enigma. The disease is widely accepted to result from the ectopic implantation of refluxed menstrual tissues. In addition, immunologic changes, environmental, hormonal and genetic factors contribute to the multifactorial etiology of endometriosis.

Many studies are therefore focusing on identifying markers for the diagnosis and follow-up of endometriosis. Although the “gold standard” for the diagnosis of endometriosis is the laparoscopy, many reports have suggested that various serum, peritoneal fluid and tissue markers might be associated with endometriosis. In fact, the identification of more sensitive and specific markers of endometriosis should facilitate the development of accurate and non-invasive techniques for diagnosis and prognosis (Table 1). Furthermore, the inheritable susceptibility to endometriosis justifies the growing interest in identifying genes and/or genetic polymorphisms that could lead to an increased risk of disease. Identifying these polymorphisms may open to their use as genetic biomarkers of endometriosis.

Over the last 20 years, several proteomics technologies have been used to research novel proteins with a potential etiological role in endometriosis, and to identify candidate serum markers for this condition. While some molecules identified by proteomics technologies may have a relevant role in the pathogenesis of endometriosis, the research of potential serum markers for this condition is still far from any clinical application.

The early diagnosis of endometriosis could prevent the possible progression of endometriosis, resulting in more pain, infertility and in a declining quality of life.

For a clinical purpose, the identification of highly sensitive and specific diagnostic test of endometriosis should facilitate the development of accurate and non-invasive test diagnosis and prognosis.

PERITONEAL FLUID AND/OR SERUM MARKERS	Glycoproteins Growth factors Cytokines Autoantibodies Hormones Proteolytic enzymes and their inhibitors Soluble adhesion molecules Environmental contaminant
ENDOMETRIAL MARKERS	Cell adhesion molecules (CAMs) Proteolytic enzymes
ENDOMETRIAL TISSUE BIOCHEMICAL MARKERS	Aromatase P450 Hormone receptors
GENETIC MARKERS	Survivin gene expression p53 mutations Polymorphisms

Table 1. Markers for endometriosis

## 2. Peritoneal fluid and/or serum markers

Many serum and peritoneal fluid markers can be used to discriminate between patients with or without endometriosis (Table 2). Using markers with a high degree of sensitivity and specificity for endometriosis it is possible the development of peritoneal fluid and /or serum based diagnostics tools, therapeutic strategies and prognosis markers.

GLYCOPROTEINS	CA125 CA19-9
GROWTH FACTORS	Hepatocyte growth factor (SF/HGF) Fibroblast growth factor (FGF) Epidermal growth factor (EGF) Transforming growth factor-alpha (TGF- $\alpha$ ) Transforming growth factor-beta (TGF- $\beta$ ) Vascular endothelial growth factor (VEGF) Epidermal growth factor receptor (EGF-R) Insulin-like growth factor I (IGF-I)
CYTOKINES	TNF- $\alpha$ IL-1 IL-6 IL-8 Monocyte chemoattractant protein (MCP)-1 Interferon- $\gamma$

Table 2. Peritoneal fluid (PF) and/or serum markers for endometriosis

AUTOANTIBODIES	Antiendometrial antibodies Autoantibodies to oxidized lipoproteins Thyroid peroxidase antibodies IgG anti-laminin-1 antibodies Anti-phospholipid antibodies
HORMONES	Luteinizing hormone (LH) Progesterone Estradiol Thyroid stimulating hormone (TSH) Follicle stimulating hormone (FSH) Leptin
PROTEOLYTIC ENZYMES AND THEIR INHIBITORS	Matrix metalloproteinases (MMPs) Tissue inhibitors for MMPs (TIMPs)
SOLUBLE ADHESION MOLECULES	Intercellular adhesions molecule-1 (sICAM-1) Human leukocyte class I antigens (sHLA-I) E-cadherin
ENVIRONMENTAL CONTAMINAT	Dioxin-like chemicals

Table 2. Peritoneal fluid (PF) and/or serum markers for endometriosis. (Continuation)

## 2.1 Glycoproteins

Some serum glycoproteins, more commonly known for its use in the diagnosis or monitoring of cancers, might also serve as a marker for endometriosis, although levels are usually elevated only in advanced stages and are therefore not suitable for routine screening.

### 2.1.1 CA125

CA125 is a 200,000 Da glycoprotein expressed on the surface of the coelomic epithelium, including the epithelium of the endocervix, endometrium, fallopian tube, pelvic peritoneum and placental tissues. Serum CA125 levels increase in patients with malignant and benign gynaecologic diseases, including endometriosis.

Despite the most important clinical use of CA125 is the monitoring of patients with ovarian cancer, high levels can be found in women with endometriosis. Many studies have assessed the role of CA125 serum levels in women affected with endometriosis. The sensitivity and specificity of serum CA125 assay varies with the stage of disease. Usually, high CA125 serum levels can be found both in most patients with advanced endometriosis and in few patients with early-stage disease. Therefore, the routine use of serum CA125 cannot be used as a diagnostic tool for endometriosis. Serum CA125 may be more useful in evaluating recurrent disease or the outcome of a surgical treatment. CA125 levels may also be useful in patients with advanced endometriosis and several studies have suggested the use of this marker in the preoperative diagnosis of endometriosis.

The patients with endometriosis often undergo repeated laparoscopic examinations to assess the progress during and after therapy or to determine the recurrence of disease. Therefore, CA125 may be useful in the management of endometriosis and some authors

suggest its assessment in women with suspected endometriosis, in association with laparoscopy and biopsy. In addition, in literature was reported that measurement of serum CA125 levels might be useful in identifying patients with infertility that may have severe endometriosis and could benefit from early surgical treatment.

CA125 levels were assessed in the PF of patients with and without endometriosis. Although levels of CA125 in the PF were almost 10 times higher than serum levels, no differences were found between women with and without endometriosis. In addition, CA125 levels are measured also in other body fluids, such as menstrual discharge and the uterine fluid, but are not useful in clinical practice.

### 2.1.2 CA19-9

CA19-9 is a high-molecular-weight glycoprotein that was initially thought to be an oncofetal antigen. Serum CA19-9 levels were elevated in patients with some malignant tumour, such as gastrointestinal adenocarcinoma, pancreatic carcinoma, or lung carcinoma; thus, the measurement of serum CA19-9 levels is useful in the diagnosis of these tumours. In gynaecology, the serum CA19-9 levels are elevated in patients with malignant and benign ovarian tumours. Furthermore, a case of an ovarian chocolate cyst with a markedly elevated serum CA19-9 level has been reported. In addition, it has been reported that serum CA19-9 levels in women with endometriosis are significantly reduced during therapy compared with the basal levels before treatment. Serum CA19-9 levels in patients with endometriosis are significantly higher than those in patients without endometriosis and that serum CA19-9 levels increase in accordance with the advancement of the clinical stage of endometriosis. CA19-9 was also detected in the endometrial glandular epithelium in ovarian chocolate cysts by immunohistochemistry. These results reveal that the measurement of the serum CA19-9 levels, as well as the serum CA125 level, may prove to be a valuable tool for predicting the severity of endometriosis as diagnosed by laparoscopy.

### 2.2 Growth factors

The endometrium in endometriosis behaves like tumorous tissue and the growth factors involved in tumour proliferation, angiogenesis and invasiveness have been investigated for their expression in endometriosis. Indeed, the degree of endometriosis is positively correlated with the concentration in peritoneal fluid and serum of *Hepatocyte Growth Factor/Scatter Factor (HGF/SF)*, a multifunctional polypeptide that has been implicated in embryo development, tissue repair, and cancer growth, produced mainly by mesenchymal cells with activity mediated through the c-met receptor found principally on epithelial and endothelial cells (Zong et al., 2003).

Inflammatory macrophages and the inflammatory mediators they release could be related to the ectopic implantation of endometriosis: *Fibroblast Growth Factor (FGF)*, *Epidermal Growth Factor (EGF)*, *Transforming Growth Factor-alpha (TGF- $\alpha$ )*, *Transforming Growth Factor-beta (TGF- $\beta$ )* and *Tumor Necrosis Factor-alpha (TNF- $\alpha$ )*. It is been shown that these growth factors stimulate in vitro proliferation of endometrial stromal cells, suggesting that they could improve the implantation of endometrial cells.

Elevated serum levels of *Epidermal Growth Factor Receptor (EGF-R)*, involved in angiogenesis, suggest an active role for EGF in the development of endometriosis (Matalliotakis et al., 2003a, 2003b).

*Insulin-like Growth Factor I (IGF-I)* serum levels in patients with early stage endometriosis, and in healthy control, were significantly lower than the levels in patients with late stage endometriosis, suggesting that IGF-I is an important mediator in the development and/or maintenance of endometriosis or progression to late stage disease (Gurgan et al., 1999).

Many studies report that angiogenesis is probably involved in the pathogenesis of endometriosis. *Vascular endothelial growth factor (VEGF)*, also known as vascular permeability factor, is one of the most potent and specific angiogenic factors. VEGF has emerged as an important regulator of normal angiogenesis and pathological neovascularisation.

VEGF levels in both peritoneal fluid and serum were higher in women with endometriosis compared with controls. The cellular source of VEGF in peritoneal fluid has not yet been precisely defined. Some evidence suggests that the endometriotic lesions themselves produce this factor and that the activated peritoneal macrophages are able to synthesize and secrete VEGF (Matalliotakis et al., 2003a, 2003b).

## 2.3 Immunological markers

The immune system plays an important role in the pathogenesis of endometriosis, which begins, therefore, to be treated as an autoimmune disease. T-helper, T-suppressor and natural killer (NK) cells concentrations are altered in serum and peritoneal fluid of patients with endometriosis (Lebovic et al., 2001; Nothnick, 2001). In addition, IgG and IgA anti-endometrial antibodies have been detected in the sera and vaginal and cervical secretions of endometriosis patients. The presence of anti-phospholipids and anti-histones antibodies has been documented by some authors and questioned by others. These observations would believe that markers of immune reactivity, particularly cytokines, might be used as a diagnostic aid for endometriosis.

### 2.3.1 Cytokines

Macrophages are a major source of many cytokines involved in immune response, haematopoiesis, inflammation and many other homeostatic processes. Upon stimulation by microorganisms, microbial products or endogenous factors including cytokines, macrophages can *de novo* synthesize and release a large variety of cytokines (i.e. IL-1, IL-1ra, IL-6, IL-8, IL-10, IL-12, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , MCP-1, MCP-3, MIF, M-CSF, G-CSF, GM-CSF, MIP-1, MIP-2, LIF, OSM, TGF- $\beta$ ). Some cytokines can up-regulate the production of cytokines by macrophages (IL-3, GM-CSF, IFN- $\gamma$ ) while others can inhibit it (IL-4, IL-10, IL-13, TGF- $\beta$ ). In addition, these cytokines can modulate most of the macrophage functions and cell surface marker expression. Other cytokines (chemokines such as MCP-1, 2, 3, MIP-1,2 and RANTES) contribute to the recruitment of circulating monocytes within tissues.

T lymphocytes are important regulatory cells that secrete several cytokines and participate actively in this inflammatory response. According to the pattern of cytokines secreted, the immune response is classified as cytotoxic or type 1 (IFN- $\gamma$ , IL-2, IL-12) and humoral or type 2 (IL-4, IL-5, IL-10 and IL-13) (Barcelò et al., 2006).

The role of cytokines and growth factors in the pathophysiology of endometriosis is evident. They are probably responsible for the proliferation of endometrial cells and implantation of endometrial cells or tissue. In addition, cytokines increase the tissue remodelling through their influence on matrix metalloproteinases. Probably the most important effect of cytokines on ectopic endometrial tissue is an increase in angiogenesis of ectopic endometrial tissue and neovascularisation of the affected region. Therefore, cytokines play an important role in the initiation, propagation and regulation of immune and inflammation responses. The activation of immune cells results in a burst and cascade of inflammatory cytokines.

ELISA kits are available to assess the cytokines in the serum and peritoneal fluid (PF) of endometriosis patients. PF is rich with variable cellular components including macrophages, mesothelial cells, lymphocytes, eosinophils and mast cells. Approximately 85% of PF leukocytes are macrophages. It has been hypothesized that peritoneal macrophage activation is a key step in disease initiation and progression. Activated macrophages in the peritoneal cavity of women with endometriosis are potent producers of cytokines (Bedaiwy et al., 2002). Thus, PF contains a rich mixture of cytokines. Cytokines, such as TNF- $\alpha$ , IL-1, IL-6, IL-8, monocyte chemoattractant protein (MCP)-1 and IFN- $\gamma$ , are elevated in the PF of women with endometriosis, suggesting that they are involved in the progression of the disease. The level of IL-1 in PF is positively correlated with the progression of endometriosis, but the serum level of IL-1 seems to have no correlation with endometriosis.

*The Tumor Necrosis Factors (TNF)* superfamily of cytokines represents a multifunctional group proinflammatory cytokines, which activate signalling pathways for cell survival, apoptosis, inflammatory responses, and cellular differentiation. Induction of cellular responses to TNF occurs through two receptors, TNFR1 (TNF Receptor-1 or CD120a) and TNFR2 (TNF Receptor-2 or CD120b). TNFR1 is activated in most human tissues by the binding of TNF $\alpha$ . On the other hand, TNFR2 is primarily expressed in immune cells and is activated by both TNF $\alpha$  and TNF $\beta$  (Kawasaki et al., 2002).

The main TNF is TNF- $\alpha$ , which is produced by neutrophils, activated lymphocytes, macrophages, NK cells and several non-hematopoietic cells. The TNF- $\alpha$  is involved in the normal physiology of the endometrial proliferation in the human endometrium. TNF- $\alpha$  is expressed predominantly in epithelial cells, especially in the secretory phase. The stromal cells stain for TNF- $\alpha$  mostly in the proliferative phase of the menstrual cycle. These data suggest that hormones influence the role of this cytokine.

Some reports found that concentrations of TNF- $\alpha$  in both serum and PF were very high at the early stage of the disease and decreased with the severity of the endometriosis. Moreover, the assessment of TNF- $\alpha$  levels in the PF can be used as a basis for non-surgical diagnosis of endometriosis.

The role of IL-6 in the pathogenesis of endometriosis has been widely studied. IL-6 is a regulator of inflammation and immunity, which may represent a physiological link between the endocrine and immune systems. IL-6 also modulates the secretion of other cytokines, promotes T-cell activation, differentiation of B cells and inhibits the growth of several human cell lines (Nothnick, 2001).

The data about IL-6 levels in the PF of patients with endometriosis are controversial. In fact, no statistically significant differences are reported between controls and endometriosis

patients. In contrast, serum levels of IL-6 were significantly higher in women with endometriosis than in controls and the highest levels were found in women with chocolate cysts (Wieser et al., 2003; Iwabe et al., 2003).

### 2.3.2 Autoantibodies

Endometriosis is supposed to be an autoimmune disorder and many autoantibodies have been proposed as a diagnostic test. A variety of autoantibodies have been detected in endometriosis patients (thyroid peroxidase antibody, IgG anti-laminin-1 antibodies, anti-phospholipid antibodies and the novel anti-PDIK1L antibodies). The most commonly reported types are antiendometrial antibodies, autoantibodies against the oxidative-stress-induced, antigens to malondialdehyde-modified low-density lipoprotein (LDL) and oxidized low-density lipoprotein (Ox-LDL).

Some investigators have hypothesized that antiendometrial antibodies may cause infertility in some women with endometriosis by preventing the fertilized embryo from implanting in the uterus. In addition, increasing evidence suggests that oxidative stress occurs in the PF of women with endometriosis and oxidatively modified lipoproteins were found in the PF.

### 2.4 Hormones

Serum and PF hormones levels vary in patients affected with endometriosis. Luteinizing hormone (LH) levels are significantly higher in both serum and PF in patients with endometriosis than in normal controls (Illera et al., 2001). However, levels of prolactin, thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) in the serum were no different between the different groups.

Recently it was reported that serum concentrations of leptin are increased in patients with endometriosis. This increase may play an anti-apoptotic role in activated endometrial stromal cells into the peritoneal cavity, stimulating endometrial cell implantation and cause infertility (Tanaka et al., 2003). Furthermore another study measured the serum concentration of leptin using a radioimmunoassay method, showing a significant association between leptin concentrations and stage of endometriosis (Viganò et al., 2002).

### 2.5 Proteolytic enzymes and their inhibitors

The physiological changes in endometriosis involve multiple steps of matrix remodelling. Endometriosis associated to abnormal matrix remodelling is affected by several molecular factors including proteolytic enzymes and their inhibitors, which mediate tissue turnover, and ovarian steroids, which normally regulate reconstruction of endometrium in the menstrual cycle.

The extracellular matrix (ECM) constitutes a well-organized network structure that surrounds the cells. The tissue remodelling involving ECM turnover is regulated by the pooled action of proteolytic enzymes, matrix metalloproteinases (MMP) and tissue inhibitors for MMP (TIMP). The inappropriate expression of MMP and TIMP is associated with tumorigenesis and metastasis, as well as with endometriosis.

Several MMP have been implicated in the development of endometriosis (Sillem et al., 2001). The levels of MMP and TIMP in patients with endometriosis are different depending on the method of measurement and collection of samples of different tissues at different stages of endometriosis. The values of TIMP-1 is determined by radioimmunoassay measurement in serum of patients with PF in endometriosis are lower than in controls. In contrast, the concentration of TIMP-1 was restored after treatment with gonadotropin releasing hormone. Another study reported that there was no significant difference in levels of cathepsin D, a proteolytic enzyme thought to promote digestion of ECM proteins in endometriosis, in serum from women with and without endometriosis.

## 2.6 Soluble adhesion molecules

It is thought that the retrograde flow of the menstrual debris to the peritoneal cavity plays an important role in the origin of endometriosis but the mechanism of endometrial cells implantation remains unknown. Recently many studies have reported the importance of adhesion molecules in this process.

Several adhesion molecules (CAM) are expressed in the human endometrium: i.e. integrins, cadherins and immunoglobulin superfamilies. These adhesion molecules show cyclical changes during the menstrual cycle. The major cell surface receptors of the ECM are the *integrins* that contain large ( $\alpha$ ) and small ( $\beta$ ) subunits.

$\beta$ 1-integrins are known to mediate the interaction between the cell-cell and cell-extracellular matrices and are represented by very late activation (VLA) antigen molecules. It is well known that endometriosis is frequently associated with immunological abnormalities. However, only a few studies have been conducted on the adhesion molecules, particularly on  $\beta$  1-integrins, in endometriosis. It has reported that integrins are expressed in the endometrium in endometriosis. The ability of endometriotic tissues to express integrins may explain the high recurrence rates in patients with endometriosis, as these samples retain their adhesion potency after retrograde menstruation and are thus able to establish cell-cell and cell-matrix interactions with the surrounding peritoneum.

The soluble forms of the *intercellular-adhesion molecule-1* (sICAM-1) are secreted from the endometrium and endometriotic implants. 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 and the number of endometriotic implants in the pelvis. Therefore, it has been hypothesized that sICAM-1 may be useful in the diagnosis of endometriosis (Leng et al., 2002). Many investigators have reported a significant increase in serum concentration of sICAM-1 in patients with endometriosis. The sICAM-1 concentrations were higher in patients with stage I-II endometriosis, suggesting that studies on these soluble adhesion molecules can help clarify the pathogenic mechanisms of endometriosis. Elevated ICAM-1 levels were found in patients with severe endometriosis, but its sensitivity is not high and the concomitant use of the CA125 marker increases the sensitivity and specificity of detection (Somigliana et al., 2002).

The **E-cadherin** mediates cell-cell interaction and cells adhere preferentially to cells that express the same cadherin. Cadherins are distributed widely among animals and play a potentially significant role in morphogenetic events during embryogenesis. Cadherin is also



expressed in the cell-to-cell boundaries of the endometrium. It is reported that E-cadherin expression on the endometrium was higher in the secretory phase than in the proliferative phase, although there is one report that the expression was unchanged during the cycle. Furthermore, the level of E-cadherin in serum of endometriosis patients was significantly higher than that of control group.

The level of E-cadherin in the serum of both III stage and IV stage endometriosis patients was higher than that of I and II stage patients. However, the difference between them was not statistically significant. E-cadherin may play a role on the morbidity of endometriosis and the serum E-cadherin assay might be helpful as a serum marker for the diagnosis and management of endometriosis (Fu & Lang, 2002).

## 2.7 Environmental contaminant

Environmental toxins, such as dioxins and polychlorinated biphenyls are some of the factors that have been suggested to play a significant role in the development of endometriosis. In fact, detection of environmental contaminant residues in serum and ovarian follicular fluid confirms this hypothesis. Dioxin-like chemicals, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polyhalogenated aromatic hydrocarbons (PHAHs), may exert effects on the pathophysiology of endometriosis through a number of pathways: (1) activation of pro-carcinogens; (2) altered synthesis and metabolism of estradiol; (3) altered production of pro-inflammatory growth factors or cytokines and (4) alterations in tissue remodelling processes.

Exposure to HAHs and TCDD seems to be associated with a dose-dependent increase in the incidence and severity of endometriosis. TCDD may target peripheral blood and peritoneal and endometrial leukocyte populations inducing chronic expression of TNF- $\alpha$  and other inflammatory mediators resulting in increased adhesion, vascularization and proliferation of endometriotic cells. It has been suggested that an elevated concentration of TNF- $\alpha$  might participate in TCDD-mediated toxicity and contribute to the pathogenesis of endometriosis.

Dioxins may affect the expression of TNF- $\alpha$  via the induction of an inflammatory cytokine network, since the region of DNA that recognize the ligand-activated AhR, the dioxin-response element of DRE, is present in the genes of potent inducers of TNF- $\alpha$  including IL-1b, IL-6 and IFN- $\gamma$  (Rier & Foster, 2003).

## 3. Endometrial markers

The adhesion of endometrial cells to the extracellular matrix (ECM) would be expected to play a central role in the pathogenesis of endometriosis. Various cell adhesion molecules (CAMs) have been investigated for their expression in endometriotic endometrium. Each cell type expresses a distinct pattern of integrins and other CAMs, including the cadherins, selectins and members of the immunoglobulin family that determines cell shape, maintains cell position and polarity and affects hormonal responsiveness. In addition, apoptosis might be mediated through loss of appropriate signals from the ECM through alternations in the integrin expression. Based on cell adhesion and the genes involving in adhesion and invasion aspects, cell adhesion molecules (CaMs) and proteolytic enzymes were investigated for their mechanisms in association with the progression of endometriosis.

## 4. Endometrial tissue biochemical markers

### 4.1 Aromatase P450

Aromatase P450 is the key enzyme for biosynthesis of oestrogen, which is an essential hormone for the establishment and growth of endometriosis. There is no detectable aromatase enzyme activity in normal endometrium; therefore, oestrogen is not locally produced in endometrium. Endometriosis tissue, however, contains very high levels of aromatase enzyme, which leads to production of significant quantities of oestrogen (Dheenadayalu et al., 2002). Moreover, one of the best-known mediators of inflammation and pain, prostaglandin E2 (PGE2), was found to be the most potent known inducer of aromatase activity in endometriotic stromal cells. The clinical significance of local aromatase activity that is induced strikingly by PGE2 in endometriotic tissue was exemplified recently by the successful use of an aromatase inhibitor to treat an unusually aggressive case of recurrent postmenopausal endometriosis that was resistant to any other surgical or hormonal modalities of treatment. Therefore, the aberrant expression of aromatase P450 in endometriotic tissue, in contrast to eutopic endometrium, justifies the local biosynthesis of estrogen that promotes the growth of these lesions and possibly mediates the resistance to conventional hormonal treatments, which is observed in a number of women with endometriosis. The molecular mechanisms that are responsible for aberrant aromatase P450 expression may provide insights into the etiology of endometriosis and lead to identification of molecular targets for the development of novel treatment strategies. 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 might be a potential qualitative marker of endometriosis.

### 4.2 Hormone receptors

The expression of receptors for the ovarian steroid hormones oestrogen and progesterone was studied immunohisto-chemically using monoclonal antibodies. The quantification of these receptors in the endometrium could be potentially useful in screening for this disease.

The eutopic endometrium of patients with endometriosis is different from endometrium of fertile controls regarding apoptosis, cytokines and other characteristics. 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, oestrogen 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. The different patterns of receptor expression suggested different hormonal regulations between eutopic and ectopic endometrium.

There are two isoforms for oestrogen (ER) and progesterone (PR) receptors-ER- $\alpha$  and ER- $\beta$ , PRA and PR-B. These isoforms exist in the endometrium and their function and content are different from one another. The different concentrations and biological activity of steroid receptor isoforms might lead to various hormonal responsiveness of ectopic endometrium. High concentrations of ER and PR in the ectopic endometrium during the secretory phase

could explain the high proliferative activity of endometriotic tissue in this phase. Conversely, a decrease in ER and PR expression in ectopic implants during the secretory phase might lead to diminished proliferation. The expression of oestrogen and progesterone receptors may be regarded as an index of differentiation of the endometriotic implant. Consequently, ER and PR receptors may be used as markers of the activity of all subtypes of endometriotic lesions.

## **5. Genetic markers**

Several genetic abnormalities or mutations have been suggested that might be related to endometriosis. Many technological approaches can help identify possible genetic markers of endometriosis. A number of technologies have emerged to facilitate progress in this direction (Taylor et al., 2002). Gene based technologies includes subtractive cDNA hybridization and cDNA microarray techniques.

### **5.1 Survivin gene expression**

In endometriotic lesions, although derived from normal endometrium, decreased expression of adhesion molecules and increased expression of proteolytic enzymes may contribute to establishment of endometrial glands and stroma at ectopic sites, likely as behaviour of cancer cells. Normal epithelial cells undergo apoptosis when they separate from their primary tissue. However, spontaneous apoptosis of ectopic endometrial tissue is impaired in women with endometriosis, and its decreased susceptibility to apoptosis might participate in the growth, survival, and invasion of endometriotic tissue. Although there have been some reports on the induction of apoptosis in endometriotic lesions, there is no consensus on the mechanism of escape from apoptosis in endometriosis, and little is known on the correlation between survival activity and invasive phenotype in endometriotic cells. Among the regulators of cell death, inhibitor of apoptosis (IAP) proteins has recently emerged as modulators of an evolutionarily conserved step in apoptosis, which may potentially involve the direct inhibition of terminal effector caspases 3 and 7.

Survivin is a novel inhibitor of apoptosis and is expressed during fetal development and in cancer tissues, but its expression has not been reported in normal adult tissues or benign diseases. Survivin gene and protein expression was detected in normal human endometrium and that survivin could play an important role in physiological homeostasis during the normal menstrual cycle (Konno et al., 2000). The survivin is also expressed in ectopic endometriotic tissue; however, there has been no report on the biological significance of survivin in endometriosis, an aggressive tumour-like benign disease.

### **5.2 p53 mutations**

Genomic alterations may represent important events in the development of endometriosis. Tumour suppressor genes play a role in the regulation of cell growth and prevention of carcinogenesis. The altered tumour suppressor genes might be related with the development of endometriosis. p53, a representative tumour suppressor, is involved in cell proliferation and progression of various tumour types (Akahane et al., 2007).

High frequency of p53 locus deletion was observed in the endometriosis specimens (Bischoff et al., 2002). The p53 protein abnormalities and chromosomal aberrations may be involved in malignant transformation of ovarian endometriosis (Mhawech et al., 2002). In contrast, some investigators have demonstrated the undetectable expression of p53 in the endometriosis specimens.

Although the real role of p53 polymorphism has not been clarified, it deserves more attentions in the study of endometriosis and the development of gene therapy. However, the real roles of these p53 gene polymorphisms upon endometriosis remain to be clarified. Larger cohort recruitment is request for its further clarification. After the elucidation of these issues, some tumour suppressor gene polymorphisms might become useful markers to predict the future development of endometriosis as well as the development and intervention of genetic therapy.

### 5.3 Polymorphisms

Some genetic polymorphisms, involved in sex steroids biosynthesis and metabolism, may be reasonably associated with an increased risk of endometriosis. Specific genes with polymorphisms have been investigated for an association with endometriosis. Some association studies implicated GALT (a gene involved in galactose metabolism) and GSTM1 and NAT2 (genes encoding for the detoxification enzymes) as possible disease susceptibility genes. Recent finding have added to the evidence for the involvement of GSTM1 and NAT2, but have cast doubt on the role of GALT. The CDKN1A gene codon 31-arginine/serine polymorphism is not associated with endometriosis. Polymorphisms of the arylhydrocarbon receptor (AHR) gene and related genes were examined, and in at least one study, no association was found.

The endometriosis regresses after menopause or ovariectomy, suggesting it could depend on the production and metabolism of sex steroids (Kitawaki et al., 2002): high concentrations of estrogens were found in the endometriotic lesions, which grow and regress in an oestrogen-dependent way.

The inheritable susceptibility to endometriosis justifies the growing interest in identifying genes and/or genetic polymorphisms that could lead to an increased risk of disease. Identifying these polymorphisms may open to their use as genetic biomarkers of endometriosis (Vietri et al., 2007a, 2007b). Some genetic polymorphisms, involved in sex steroids biosynthesis and metabolism, may be reasonably associated with an increased risk of endometriosis, like progesterone receptor (PR), AR, oestrogen receptor (AR), 17beta-hydroxysteroid dehydrogenase type 1 (HSD17B1), cytochrome P450 subfamily 17 (CYP17) and cytochrome P450 subfamily 19 (CYP19) (Guo, 2006). No doubt this list is likely to increase over the years. The most widely used approach for the identification of endometriosis-predisposing genetic polymorphisms are the genetic association studies, by which genetic susceptibility polymorphisms are identified through the identification and assessment of the difference in allele/genotype frequencies between patients and control subjects.

The **CYP17 genotype** contains a single nucleotide T>C polymorphism situated 34bp upstream of the translation initiation site. C allele may have great promoter activity, increasing the transcription of P450c17 alpha enzyme. This effect amplifies the production of

precursor androgens that are subsequently converted to estrogens. In fact, C allele is associated with high levels of estradiol in young women. CYP19 gene lies on chromosome 15 and encodes cytochrome P450, a major component of aromatase. Aromatase is a key enzyme in the conversion of androgens to estrogens, and mediates the rate-limiting step in the metabolism of C<sub>19</sub> androgens to estrogens. Different polymorphisms of CYP19 are present in the gene and have been related to variations of aromatase activity (Gennari et al., 2004). A silent SNP, C1558T, corresponding to the 3' untranslated region of the mRNA, has been correlated to the level of aromatase mRNA in breast tumour cells. Another polymorphism, GA at Val80, has been previously associated with breast cancer risk. Few studies have been published on the association between CYP17 T>C polymorphism with risk of endometriosis, showing controversial data. Some studies connected C1558T polymorphism with endometriosis risk (Huber et al., 2005), while no report relates Val80 SNP to endometriosis.

The **CYP19 genotype** may play a role in increased risk of endometriosis lying on an environmental and genetic background. The polymorphisms of CYP19 are significantly represented in Val80 and C1558T in patients affected with endometriosis. Despite endometriosis is a multifactorial disease, identifying Val80 and C1558T polymorphisms of CYP19 could help to comprehend the mechanisms of endometriosis. The assessment of these polymorphisms could help to anticipate the diagnosis or expect it in asymptomatic women to elaborate a follow up program. Other than that, a follow up by ultrasound and blood markers could be proposed in these patients, in order to define unclear symptoms such as dysmenorrhoea and chronic pelvic pain.

## 6. Future scope

Endometriosis is associated with genetic and immunological influences and exposure to environmental factors. It seems to result from a complex sequence of events in which multiple gene loci interact with each other and the environment to produce the disease phenotype, but thus far little is known about the candidate genes involved (Balow & Kennedy 2005). Because of this complexity, endometriosis is ideally suited as a target for genome wide scanning. Mutations and single-nucleotide polymorphisms (SNPs) have been identified in a number of genes that might confer susceptibility to endometriosis, but their precise role remains to be determined.

**Proteome analysis** is now widely accepted as a complementary technology to genetic profiling and together enables a better understanding of diseases and the development of new treatments. Proteomics allows the simultaneous observation of alterations in protein expression that may be either a precursor to or causative in disease development or a consequence of the disease. These techniques check and identify proteins that are expressed differently in patients with endometriosis versus normal controls. More recently, protein arrays using antibodies enable the screening of thousands of proteins against one sample. In future, such arrays could measure the expressions of multiple proteins to reveal changes in their regulation and expression in disease states. Furthermore, by using protein chip arrays, differential analysis of protein expression in women with and without differential protein profiling technology can be developed into a powerful tool for endometriosis research.

The study of protein function and protein-protein interaction can clarify the biology of the disease more so than the application of genomics. This is because gene expression and biological effects are linked via complex protein synthesis and gene interaction pathways.

**Genomics** includes hybridization techniques (e.g. differential colony hybridization), subtractive techniques (e.g. hybridization and representational difference analysis), gel-based techniques (e.g. RNA arbitrarily primed or differential display), and sequencing based techniques (e.g. expression sequence tags and serial analysis of gene expression). Furthermore, the use of DNA microarrays allows the search for new gene expression markers of endometriosis by identifying differentially expressed genes in endometriosis implants compared with endometrial tissue. The aim of the technique is to identify changes in gene expression characterizing the disease state so that we can understand the disease's progression and identify novel therapeutic targets.

Apart from the better understanding of the pathophysiology and the metabolic pathways that lead to potential biomarkers for endometriosis, there are still issues to be clarified and applications to be achieved. Once a protein or small number of proteins have been shown to be differentially expressed in endometriosis, the next step will be to use this information to try to develop a non-invasive diagnostic test for endometriosis. This diagnostic test should ideally have good sensitivity and specificity as well as satisfactory positive and negative predicative values for the detection of endometriosis, and also be cost effective and readily available.

Genetic markers that are prognostic for endometriosis can be genotyped early in life and could predict individual response to various risk factors and treatment. Genetic predisposition revealed by genetic analysis for susceptibility genes can provide an integrated assessment of the interaction between genotypes and environmental factors, resulting in synergistically increased prognostic value of diagnostic tests. Thus, pre-symptomatic and early symptomatic genetic testing is expected to be the cornerstone of the paradigmatic shift from late surgical interventions to earlier preventative therapies. Thus, there is an urgent need for novel genetic markers that are predictive of endometriosis and endometriosis progression, particularly in treatment decisions for individuals who are recognized as having endometriosis.

Such genetic markers may enable prognosis of endometriosis in much larger populations compared with the populations that can currently be evaluated by using existing risk factors and biomarkers.

The availability of a genetic test may allow, for example, early diagnosis and prognosis of endometriosis, as well as clinical intervention to mitigate progression of the disease. The use of these genetic markers will also allow selection of subjects for clinical trials involving novel treatment methods.

The discovery of genetic markers associated with endometriosis will further provide novel targets for therapeutic intervention or preventive treatments of endometriosis and enable the development of new therapeutic agents for treating endometriosis.

## 7. Conclusions

One of the main objectives of the gynaecologist is to diagnose endometriosis without the use of laparoscopy or laparotomy. Currently, laparoscopy offers the most specific and sensitive

technique for evaluating and monitoring endometriosis. Even so, microscopic or occult endometriosis may be misdiagnosed because of the inability to visualize some lesions. Attempts for early diagnosis and treatments of endometriosis have been weighed down by lack of proper methods to study and manage the disease. Furthermore, the need for non-invasive diagnostic methods is evident because the laparoscopy is a surgical procedure with potentially dangerous risks.

At present, there are no reliable markers for the diagnosis and prognosis of endometriosis and identification of serum and endometrial markers is decisive for disease diagnosis and follow-up of patients.

The diagnostic laboratories are using new genomic and proteomic technologies to develop novel diagnostic and therapeutic approaches for endometriosis. These technologies will facilitate the generation of molecular expression profiles and then identifying potential gene and protein targets. This will lead to available markers with high sensitivity and specificity for screening of endometriosis, then to the development of serum diagnostic tools, therapeutic strategies and prognosis markers.

The combination of immunological discoveries and recent advances in DNA technologies may provide the long sought screening tool with the desirable diagnostic accuracy for this puzzling disorder.

The identification of specific genetic alterations and protein profiles associated with endometriosis offers a unique opportunity to develop assays for early diagnosis and/or treatment. By identifying proteins in biological samples, a minimally invasive tool should be feasible to assess the presence of disease and monitor response to treatment and/or disease progression.

The promise for gene-based diagnostic tests for endometriosis and rational development of genetically targeted and molecular therapeutic strategies is, in principle, excellent. The evolving genomic and proteomic technologies remain poised to revolutionize the diagnosis and treatment of endometriosis, but have not yet lead to a single new therapy or tested biomarker. Many problems remain to be resolved and, while some of these are technical in nature, the most intractable ones have mainly to do with the complex and multifactorial character of the disease itself.

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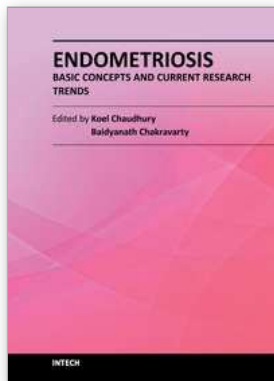
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This book provides an insight into the emerging trends in pathogenesis, diagnosis and management of endometriosis. Key features of the book include overviews of endometriosis; endometrial angiogenesis, stem cells involvement, immunological and hormonal aspects related to the disease pathogenesis; recent research reports on infertility, endometrial receptivity, ovarian cancer and altered gene expression associated with endometriosis; various predictive markers, and imaging modalities including MRI and ultrasound for efficient diagnosis; as well as current non-hormonal and hormonal treatment strategies This book is expected to be a valuable resource for clinicians, scientists and students who would like to have an improved understanding of endometriosis and also appreciate recent research trends associated with this disease.

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Fax: +86-21-62489821

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