

Serum and peritoneal abnormalities in endometriosis: potential use as diagnostic markers

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Endometriosis is an ambiguous disease and its exact pathogenesis still remains elusive to clinicians and scientists. Local and systemic aberrations in immune response are associated with endometriosis. This article reviews the literature regarding various immunological factors such as cytokines, growth factors, adhesion molecules and angiogenic factors involved in the etiopathogenesis of this disease. Our review summarizes the literature regarding biomarkers, which may be reliable nonsurgical tools used in the diagnosis of endometriosis. Superior biomarkers characterized by high sensitivity, specificity and predictive value can help in the early detection and monitoring of disease progression as well as its response to therapeutic treatments critical for its management. A combination predictive model utilizing multiple biomarkers rather than individual markers alone is proposed to improve the diagnostic performance for identifying women with a high likelihood of having endometriosis. Immunomodulators and angiogenic factor blockers have a potential for endometriosis treatment and also to alleviate the pain or infertility associated with the disease. Potential new therapeutic agents include modulators, such as cytokine receptor blockers and angiogenic receptor blockers, presently used for treating endometriosis.

Key words: Endometriosis - Biological markers - Cytokines - Immunomodulators.

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Endometriosis is a complex disease characterized by the extrauterine presence of endometrial glands and stroma. It is multifactorial in origin and associated with pain and infertility. Endometriosis affects and impacts the lives of millions of women all over the world. Early diagnosis of the disease is still not possible, as laparoscopy remains the diagnostic gold standard. Hence, the average delay in diagnosis is 9.3 years. The prevalence of endometriosis has been reported to vary from 8-10% among women in the reproductive age group. Essential links are identified in the chain of the pathogenesis of endometriosis. One of these links includes regurgitated endometrium which is established through a series of processes such as peritoneal tissue remodeling, invasion, angiogenesis and proliferation. Failure of immune clearance and apoptosis of the regurgitated endometrium are both associated with the establishment of endometriosis.

Many literature reports highlight that both the serum and peritoneal fluid of women with endometriosis have elevated numbers of activated macrophages, cytokines and growth factors. There are currently no acceptable mark-

ers that can differentiate infertile from fertile patients with endometriosis. There is also a lack of well-designed human epidemiologic studies, thus far concerning noninvasive diagnostic markers in endometriosis. Current studies generally suffer from disease misclassification, patient selection bias, and detection bias. Activated macrophages present in elevated concentrations in the peritoneal fluid are known to elaborate cytokines, growth factors, and enhance free radical activity. A localized pelvic inflammatory response results due to the functional derailment of the immune homeostasis.

This article reviews current knowledge of peritoneal fluid and serum biomarkers in endometriosis, although the markers may not be highly sensitive and specific. Future studies are needed to investigate markers specific for infertility or pain associated with endometriosis. Larger studies with adequate groups of patients may also be able to delineate specific serum and peritoneal markers for the three different subtypes of endometriosis.

Peritoneal fluid biomarkers of endometriosis

Cytokines: chemistry and sources

Cytokines are a diverse family of proteinaceous signaling compounds used extensively for cell-cell communication. They are critical to the function of both innate and adaptive immune responses. Apart from their importance in immune system development and function, cytokines play a major role in a variety of immunological, inflammatory and infectious diseases. They are also implicated in several developmental processes throughout embryogenesis.

Cytokines found in the peritoneal fluid of women with endometriosis are potentially produced by activated macrophages and T-lymphocytes. They are excreted in response to inflammatory stimuli and are pleiotropic, impacting a variety of cells in an autocrine, paracrine, or endocrine manner. Cytokine effects are varied and include the following: immune cell proliferation and differentiation, in-

duction of hormone release enzymes and acute phase proteins, enhancement of various cytotoxic activities, regulation of immunoglobulin secretion, and chemotaxis. These actions are often dependent on the presence of other chemicals and cytokines. The cytokine family is mostly comprised of smaller water-soluble proteins and glycoproteins, many of which are functionally redundant. Interleukin (IL)-8 and regulated on activation, normal T cell expressed and secreted (RANTES) promote macrophage recruitment and activation in the peritoneal cavity. Cytokine production has also been demonstrated by endometriotic implants.¹ Endometriotic cells constitutively express genes for IL-6 and also produce the IL-6 protein.

Peritoneal fluid is richly colonized by a cellular apparatus composed of macrophages, lymphocytes, eosinophils and mast cells. Normally, the peritoneal fluid contains $0.5-2 \times 10^6$ leucocytes per milliliter.^{2,3} Approximately 80% of cells in peritoneal fluid are macrophages. Macrophage activation is a key step in the development of endometriosis.⁴⁻⁶ The increased number of activated macrophages elaborates elevated concentrations of cytokines, prostaglandins, complement components, and growth factors such as tumor necrosis factor- β (TNF- α), IL-6 and transforming growth factor- β (TGF- β). Normally, endometrial cells that gain access to the peritoneal cavity are removed by macrophages. Aberrant immune mechanisms in endometriosis result in the ineffectiveness of the disposal systems. Activated macrophages and elevated levels of cytokines result in the initiation, progression, and growth of endometrial cells as well as neovascularization.

Individual immunological markers

Tumor necrosis factor- α

TNF- α is a pleiotropic cytokine released by white blood cells such as neutrophils, activated macrophages, leucocytes, endothelium and several other tissues in the course of damage such as infection.⁷ Its release is stimulated by several other mediators including IL-1 and bacterial endotoxin. It has potent inflammatory,

angiogenic, and cytotoxic effects on target cells, generally working together with IL-1 and IL-6. TNF- α strongly attracts neutrophils and facilitates their adhesion to endothelial cells for migration. On macrophages, it stimulates phagocytosis as well as the production of IL-1, oxidants and prostaglandin E2. With activated macrophages playing a central role in the pathogenesis of endometriosis, the effects of secreted TNF- α may give rise to local and systemic demonstrations of the disease.

Many studies have implicated TNF- α in the pathogenesis and progression of endometriosis as well as associated infertility. TNF- α concentrations have been shown to exhibit significant value as a qualitative diagnostic measure of endometriosis in women.^{8,9} TNF- α levels in the peritoneal fluid of women with endometriosis were demonstrated to be significantly higher when compared with measurements in controls. Some studies have taken this observation further by asserting that peritoneal fluid TNF- α concentrations in women with endometriosis correlate with the stage of disease. Conversely, the findings of Bedaiwy *et al.* demonstrate that a significant relationship exists between TNF- α levels and endometriosis, but not with the severity of the disease.¹⁰ At a cut-off peritoneal fluid concentration of 20 pg/mL, a 96% sensitivity and a 95% specificity (positive likelihood ratio [LR] of 19.2 and negative LR of 0.04) were reported (Table I).¹⁰⁻²⁰ This lack of consensus obligates further investigation of TNF- α as a quantitative measure of the progression of endometriosis.

Another recent area of study with regards to TNF- α and its involvement in endometriosis focuses on the receptors for TNF- α : soluble TNF receptor (sTNFR)-I and sTNFR-II. These receptors are bioactive substances that play a regulatory role to specifically antagonize TNF- α 's activity. It is assumed that both receptors use similar regulatory mechanisms since their individual peritoneal fluid concentrations in women with and without endometriosis have proven to strongly correlate.²¹ Derived from extracellular membrane, sTNFR-I and sTNFR-II are proteolytically cleaved to their soluble form found in various bodily fluids, such as peritoneal fluid.

A significant relationship between soluble

receptor concentrations in the peritoneal fluid and the presence of endometriosis was elucidated by Koga *et al.*²¹ The peritoneal fluid of women with endometriosis has shown significantly higher concentrations of sTNFR-I and sTNFR-II when compared with those of healthy subjects. Excessive receptor concentrations in the peritoneal fluid may attenuate the apoptotic effects of TNF- α on refluxed endometrial cells in the peritoneal cavity, contributing to the pathogenesis of endometriosis. However, it should be noted that soluble TNF- α receptor levels were not further elevated in patients with moderate to severe endometriosis and would, therefore, not be suitable measures of disease progression and severity in patients.

TNF- α and its regulatory counterparts (sTNFR-I and sTNFR-II) are strong candidates for use as non-invasive biomarkers that may indicate the presence of endometriosis in patients. Recombinant human TNF binding protein-1 (r-hTBP-1) has been demonstrated to inhibit the development of endometriosis in rats and baboons.²² In a randomized placebo controlled trial, baboons receiving the r-hTBP-1 had lower endometriosis revised American Fertility Society (rAFS) scores, a lower surface area and estimated volume of peritoneal endometriotic lesions as seen on laparoscopy.¹² However, a case report in humans that utilized TNF- α blocker etanercept reported that there were no noticeable reductions in endometriosis severity.²³

Interleukin-6

IL-6 is a multifunctional proinflammatory cytokine derived from T cells and macrophages to stimulate an immune response to tissue trauma. It induces immunoglobulin production by B lymphocytes, promotes T cell activation, and at high levels is associated with increased autoantibody production. Its production is up-regulated by IL-1, found in the peritoneal fluid, leading to the amplification inflammatory response triggers.

Impaired regulation of IL-6's activation of peritoneal macrophages and promotion of endometrial stromal cell proliferation is thought to play a role in the pathogenesis of endometriosis. In a study conducted by Punnonen

et al., IL-6 concentrations in the peritoneal fluid of women with endometriosis were reported to be significantly higher than in the peritoneal fluid of control subjects.²⁴ These findings establish that increased macrophage activity leading to disturbed immune function contributes to endometriosis.

Furthermore, Mahnke *et al.* demonstrated that the peritoneal fluid of endometriosis patients with a high degree of endometriotic implants had significantly higher IL-6 levels than those patients with a low degree of endometriotic implants.²⁵ These results may signify the endometrial implant's role in the vicious cycle of neovascularization which promotes the development of new implants. Also, peritoneal fluid IL-6 concentrations were not seen to correlate with the varying stages of endometriosis and, therefore, may not be indicative of disease severity.

IL-6 functions in the regulation of ovarian steroid production and early implantation events. Therefore, the role of this cytokine and its levels in peritoneal fluid in distinguishing endometriosis-associated infertility from pelvic pain was investigated. IL-6 concentrations in peritoneal fluid did not significantly differ between women with endometriosis-associated symptoms of infertility and endometriosis patients suffering with pelvic pain. Therefore, IL-6 measurements would not be helpful in a quantitative assessment of disease symptoms.

Interestingly, the Mahnke *et al.* study reported that IL-6 concentrations were significantly depressed in the peritoneal fluid of healthy women taking oral contraceptives in contrast to the control group.²⁵ From this, one might deduce the potential for oral contraceptives to be used therapeutically in order to subdue local inflammation and alleviate the symptoms of endometriosis.

IL-6 may play a role in the pathogenesis of endometriosis, but the findings that support this supposition have been contradicted by many previous investigations. These differences may possibly be attributed to factors such as varying study designs, but nevertheless, they require that IL-6 be further explored concerning its definite capability as a non-invasive peritoneal fluid biomarker in the diagnosis of endometriosis.

Regulated on activation, normal T cell expressed and secreted

RANTES is a cytokine chemotactic for T cells, eosinophils and basophils. RANTES is secreted by hemopoietic, epithelial and mesenchyme cells and acts as a mediator of acute and chronic inflammation by actively recruiting leukocytes into inflammatory sites.

Multiple experiments have shown RANTES concentrations in peritoneal fluid as elevated in the endometriotic state.²⁶ This observation was validated by the findings of Bersinger *et al.* who demonstrated that RANTES levels were increased significantly in the peritoneal fluid of women with endometriosis when compared with the RANTES levels in the peritoneal fluid of the control subjects (Table I).¹⁴

RANTES has also been correlated positively with the severity of endometriosis in patient studies. The study by Bersinger *et al.* confirmed this as RANTES concentrations in peritoneal fluid were increased significantly ($P < 0.001$) in moderate to severe stages of the disease, compared with the measurements obtained from patients with mild endometriosis and controls.¹⁴

Although the exact mechanism of action has yet to be uncovered in detail, exaggerated levels of RANTES in peritoneal fluid are somehow incriminated in the pathogenesis and progression of endometriosis. The consistent correlation between RANTES concentrations and the presence of and degree of disease support RANTES as a suitable peritoneal fluid biomarker candidate to be used in the non-invasive diagnosis of endometriosis.

Interleukin-1

IL-1 is a cytokine that is produced and secreted mainly by macrophages and monocytes. It increases the expression of adhesion factors on endothelial cells to enable the migration of leukocytes to sites of infection. IL-1 up-regulates the expression of other proinflammatory cytokines, namely IL-6, IL-8, epithelial neutrophil activating peptide-78 (ENA-78), and TNF- α . IL-1 also potently stimulates matrix metalloproteinases (MMPs). IL-1 is a promoter of T cell and B cell proliferation, prostaglandin synthesis, collagen deposition, and fibrinogen formation.

TABLE I.—*Select serum and peritoneal markers in endometriosis: correlation with disease severity.*

Marker	Assay specifics	Patient groups	Detected value (p value)	Study	
Peritoneal fluid Tumor necrosis factor- α	Cut-off: 20 pg/mL; Sensitivity: 96% and specificity: 95% Interassay variation coefficient: <5% Intra-assay variation coefficient: <3%	Control (median)	0 pg/mL (P<0.001)	Bedaiwy <i>et al.</i> ¹⁰	
		Endometriosis (median)	54.83 pg/mL (P<0.001)		
		Control (median)	0 pg/mL (P<0.05)	Skrzypczak <i>et al.</i> ¹¹	
		No endometriosis+infertility (median)	0 pg/mL (P<0.05)		
		Endometriosis+fertility (median)	0 pg/mL (P<0.05)		
Lowest limit of sensitivity: 180 fg/mL	Superficial endometriosis (median)	1.3 pg/mL	D'Hooghe <i>et al.</i> ¹²		
		Deep endometriosis (median)		3.6 pg/mL	
Serum tumor necrosis factor- α	Kit Sensitivity: 4.4 pg/mL Lowest limit of sensitivity: 180 fg/mL	Control (median)	1.37 pg/mL (P=0.002)	Bedaiwy <i>et al.</i> ¹⁰	
		Endometriosis (median)	8.33 pg/mL (P=0.002)		
		Superficial Endometriosis (median)	2.5 pg/mL	D'Hooghe <i>et al.</i> ¹²	
Peritoneal fluid interleukin-6	Kit Sensitivity: 0.7 pg/mL Interassay variation coefficient: <5% Intra-assay variation coefficient: <3%	Deep endometriosis (median)	2.6 pg/mL	Bedaiwy <i>et al.</i> ¹⁰	
		Control (median)	24.88 pg/mL (P=NS)		
		Endometriosis (median)	39.32 pg/mL (P=NS)	Skrzypczak <i>et al.</i> ¹¹	
		Control (median)	0 pg/mL (P<0.05)		
		No endometriosis + infertility (median)	0 pg/mL (P<0.05)		
Kit Sensitivity: 0.7 pg/mL	Superficial endometriosis (median)	3.3 pg/mL	D'Hooghe <i>et al.</i> ¹²		
		Deep endometriosis (median)		21.6 pg/mL	
		Control (median)		1 pg/mL (P=0.23)	Somigliana <i>et al.</i> ¹³
		Endometriosis I/II (median)		0.7 pg/mL (P=0.23)	
Endometriosis III/IV (median)	0.6 pg/mL (P=0.23)				
Serum interleukin-6	Kit Sensitivity: 0.7 pg/mL; area under curve: 87%	Control (median)	0 pg/mL (P<0.001)	Bedaiwy <i>et al.</i> ¹⁰	
		Endometriosis (median)	21.58 pg/mL (P<0.001)		
		Superficial endometriosis (median)	0.9 and 14.7 pg/mL	D'Hooghe <i>et al.</i> ¹²	
Deep endometriosis (median)	1.3 pg/mL				
Peritoneal fluid RANTES	Kit Sensitivity: 2 pg/mL	Control (median)	11 pg/mL	Bersinger <i>et al.</i> ¹⁴	
		Endometriosis stages I and II (median)	22.5 pg/mL (P>0.1)		
		Endometriosis stage III and IV (median)	35.5 pg/mL (P<0.001)		
Peritoneal fluid interleukin-1 α	Kit Sensitivity: 0.5 pg/mL	Control (mean)	<0.5 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵	
		Early endometriosis: stages I and II (mean)	7.8 pg/mL (P<0.0001)		
		Advanced endometriosis: stages III and IV (mean)	16.6 pg/mL (P<0.0001)		

Table I continued

Table I continued.

Marker	Assay specifics	Patient groups	Detected value (p value)	Study
Serum interleukin-1 α	Kit Sensitivity: 0.5 pg/mL	Control (mean)	<0.5 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵
		Early endometriosis: stages I and II (mean)	6.7 pg/mL (P<0.0001)	
		Advanced endometriosis: stages III and IV (mean)	10.9 pg/mL (P<0.0001)	
Peritoneal fluid interleukin-1 β	Kit Sensitivity: 1 pg/mL	Control (median)	4.3 pg/mL (P=NS)	Bedaiwy <i>et al.</i> ¹⁰
		Endometriosis (median)	3.53 pg/mL (P=NS)	
	Interassay variation coefficient: <5%	Control (median)	0.9 pg/mL	Skrzypczak, <i>et al.</i> ¹⁰
		No endometriosis + infertility (median)	3.5 pg/mL	
Serum interleukin-1 β	Kit Sensitivity: 1 pg/mL	Control (median)	9.7 pg/mL (P=NS)	Bedaiwy <i>et al.</i> ¹⁰
		Endometriosis (median)	10.98 pg/mL (P=NS)	
Peritoneal fluid interleukin-1 sRII	Kit Sensitivity: 10 pg/mL	Control (mean)	131.1 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵
		Early endometriosis: stages I and II (mean)	57.3 pg/mL (P<0.0001)	
		Advanced endometriosis: stages III and IV (mean)	116.4 pg/mL (P<0.0001)	
Serum interleukin-1 sRII	Kit Sensitivity: 10 pg/mL	Control (mean)	150.1 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵
		Early endometriosis: stages I and II (mean)	77.4 pg/mL (P<0.0001)	
		Advanced endometriosis: stages III and IV (mean)	122.2 pg/mL (P<0.0001)	
Peritoneal fluid interleukin-1 Ra	Kit Sensitivity: 22 pg/mL	Control (mean)	263.8 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵
		Early endometriosis: stages I and II (mean)	181.6 pg/mL (P<0.0001)	
		Advanced endometriosis: stages III and IV (mean)	620.8 pg/mL (P<0.0001)	
Serum interleukin-1 Ra	Kit Sensitivity: 22 pg/mL	Control (mean)	287.3 pg/mL (P<0.0001)	Kondera-Anasz <i>et al.</i> ¹⁵
		Early endometriosis: stages I and II (mean)	965.1 pg/mL (P<0.001)	
		Advanced endometriosis: stages III and IV (mean)	503.1 pg/mL (P<0.001)	
Peritoneal fluid interleukin-8	Kit Sensitivity: 10 pg/mL	Control (median)	6.06 pg/mL (P=0.01)	Bedaiwy <i>et al.</i> ¹⁰
		Endometriosis (median)	14.59 pg/mL (P=0.01)	
	Interassay variation coefficient: <5%	Control (median)	1 pg/mL (P<0.01)	Skrzypczak <i>et al.</i> ¹¹
		No endometriosis + infertility (median)	0 pg/mL (P<0.01)	
	Intra-assay variation coefficient: <3%	Endometriosis + fertility (median)	4 pg/mL (P<0.01)	Bersinger <i>et al.</i> ¹⁴
		Endometriosis + infertility (median)	16 pg/mL (P<0.01)	
Kit Sensitivity: 2 pg/mL	Control (median)	11.2 pg/mL	Bersinger <i>et al.</i> ¹⁴	
	Endometriosis stages I and II (median)	15.2 pg/mL (P>0.1)		
	Endometriosis stage III and IV (median)	17.6 pg/mL (P<0.001)		

Table I continued

Table I continued.

Marker	Assay specifics	Patient groups	Detected value (p value)	Study
Peritoneal fluid interleukin-10	Lowest limit of sensitivity: 2 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	3.2 pg/mL 7.9 pg/mL	D'Hooghe <i>et al.</i> ¹²
Serum interleukin-10	Lowest limit of sensitivity: 2 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	8.1 pg/mL 3.3 pg/mL	D'Hooghe <i>et al.</i> ¹²
Peritoneal fluid interleukin-13	Kit Sensitivity: 32 pg/mL	Control (median) Endometriosis (median)	0 pg/mL (P=NS) 1.2 pg/mL (P=NS)	Bedaiwy <i>et al.</i> ¹⁰
Serum interleukin-13	Kit Sensitivity: 32 pg/mL	Control (median) Endometriosis (median)	44.57 pg/mL (P=NS) 44.57 pg/mL (P=NS)	Bedaiwy <i>et al.</i> ¹⁰
Peritoneal fluid IFN- γ	Lowest limit of sensitivity: 3 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	0 pg/mL 6.7 pg/mL	D'Hooghe <i>et al.</i> ¹²
Serum IFN- γ	Lowest limit of sensitivity: 3 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	0 pg/mL 0 pg/mL	D'Hooghe <i>et al.</i> ¹²
Peritoneal fluid interleukin-15	Kit Sensitivity: 0.5 pg/mL	Control (mean) Endometriosis stage I (mean) Endometriosis stage II (mean) Endometriosis stage III (mean) Endometriosis stage IV (mean)	2.1 pg/mL (P<0.001) 2.9 pg/mL 2.6 pg/mL 2.4 pg/mL (P=0.01) 2.4 pg/mL (P=0.006)	Arici <i>et al.</i> ¹⁶
Peritoneal fluid interleukin 16	NA	Control (median) Endometriosis (median)	778.1 pg/mL (P=NS) 539.4 pg/mL (P=NS)	Zhang <i>et al.</i> ¹⁷
Serum interleukin-16	NA	Control (median) Endometriosis (median)	296.8 pg/mL (P=NS) 290.5 pg/mL (P=NS)	Zhang <i>et al.</i> ¹⁷
Peritoneal fluid interleukin-18	Kit Sensitivity: 8.0 pg/mL	Control (median) Endometriosis (median)	653.4 pg/mL (P=0.016) 144.8 pg/mL (P=0.016)	Zhang <i>et al.</i> ¹⁸
Serum interleukin-18	Kit Sensitivity: 8.0 pg/mL	Control (median) Endometriosis (median)	37.6 pg/mL (P=NS) 20.6 pg/mL (P=NS)	Zhang <i>et al.</i> ¹⁸
Peritoneal fluid TGF- β -1	Lowest limit of sensitivity: 5 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	0 pg/mL 0 pg/mL	D'Hooghe <i>et al.</i> ¹²
Serum TGF- β -1	Lowest limit of sensitivity: 5 pg/mL	Superficial endometriosis (median) Deep endometriosis (median)	49.2 pg/mL 28.2 pg/mL	D'Hooghe <i>et al.</i> ¹²
Peritoneal fluid Fas L	Intra-assay coefficient of variation: 5.5% Interassay coefficient of variation: 6.9%	Control (mean) Endometriosis stages I and II (mean) Endometriosis stages III and IV (mean)	81 pg/mL 80.5 pg/mL (P=NS) 166.2 pg/mL (P<0.05)	Garcia-Velasco <i>et al.</i> ¹⁹
Serum Fas L	Intra-assay coefficient of variation: 5.5% Interassay coefficient of variation: 6.9%	Control (mean) Endometriosis stages I and II (mean) Endometriosis stages III and IV (mean)	87.2 pg/mL 88.2 pg/mL (P=NS) 162.3 pg/mL (P<0.05)	Garcia-Velasco <i>et al.</i> ¹⁹
Peritoneal fluid leptin	Assay minimum detection limit: 0.2 ng/mL	Control (mean) Endometriosis (mean)	17.5 ng/mL (P=0.005) 35.9 ng/mL (P=0.005)	Matarese <i>et al.</i> ²⁰
Serum leptin	Assay minimum detection limit: 0.2 ng/mL	Control (mean) Endometriosis (mean)	15.6 ng/mL (P=0.007) 30.3 ng/mL (P=0.007)	Matarese <i>et al.</i> ²⁰

Past studies have indicated that in women with endometriosis levels of macrophage-derived factors, such as IL-1, are elevated in peritoneal fluid. This was evidenced in the study of Kondera-Anasz *et al.*, in which IL-1 was found in the peritoneal fluid of women with endometriosis, whereas minimally detectable levels of IL-1 could be found in the peritoneal fluid of control subjects.¹⁵ Furthermore, concentrations of the cytokine were severity-dependent with peritoneal fluid IL-1 levels being significantly higher in the advanced stages of endometriosis than in the peritoneal fluid of women with early stages of the disease (Table D).

Essentially, the two major forms of IL-1, IL-1 α and IL-1 β interact with the same receptor and carry out similar biological activities. IL-1's soluble receptor Type I (IL-1sRI) mediates cell activations in response to the cytokine. IL-1 soluble receptor Type II (IL-1sRII), however, acts as an imitation target of IL-1. IL-1sRII is shed from the cell surface as a soluble molecule and regulates IL-1 activity in inflammatory sites by inhibiting binding to IL-1sRI. The IL-1 receptor antagonist, IL-1Ra, is an agent that regulates the biological activities of IL-1 in inflammatory sites by binding to the same cell surface receptor as IL-1, thus preventing IL-1 from sending a signal to that cell.

The study conducted by Kondera-Anasz *et al.* addressed the immunoregulatory effects of IL-1sRII and IL-1Ra.¹⁵ It was determined that mean IL-1sRII levels were significantly higher in controls and advanced endometriosis cases when they were compared with levels in the peritoneal fluid of women with mild endometriosis (Table D). This defective IL-1sRII gene expression during the early stages of disease may contribute to the cause of endometriosis. Also, mean IL-1Ra concentrations were seen to be significantly elevated in the peritoneal fluid of women with endometriosis when compared with control subjects. Since the main sources of IL-1 are monocytes and macrophages, not ectopic endometrial tissue, increased IL-1Ra expression in individuals with endometriosis might suggest an elevated activation of these cells.

IL-1 and the agents that modulate this cytokine play a key role in the body's inflam-

matory immune response and are hypothesized to be causative of endometriosis. However, results correlating the cytokine levels with incidence and severity of the disease have been inconsistent. Therefore, further study is required to assess the potential of IL-1 and its agents of regulation as peritoneal fluid biomarkers for use in the non-invasive diagnosis of endometriosis.

Interleukin-8

IL-8 is a soluble chemokine and potent angiogenic factor produced by monocytes and fibroblasts as well as other cell types such as endothelial, mesothelial and endometrial stromal cells. Released by macrophages, this cytokine serves as a chemoattractant that recruits neutrophils to sites of inflammation, potentially leading to their activation. These neutrophils are present in ectopic endometrial lesions and are accompanied by inflammation and neovascularization, features often characteristic of IL-8's biological actions.²⁷ Acting as an autocrine growth factor in the endometrium, IL-8 can induce endometrial stromal cell proliferation.²⁸

Calhaz-Jorge *et al.* investigated IL-8's role in endometriosis and found that its levels in the peritoneal fluid of women with endometriosis were slightly more elevated than levels measured in the peritoneal fluid of control subjects.²⁸ It should be noted that this difference in IL-8 measurements between both groups was more pronounced during the luteal phase of the menstrual cycle.

In the endometriosis patients studied by Calhaz-Jorge *et al.*, IL-8 concentrations were significantly higher in more advanced stages of the disease when compared with early stage cases of endometriosis. In accordance with this trend, patients with mild endometriosis had peritoneal fluid levels of IL-8 that were significantly elevated compared with the levels in control subjects. These results suggest that IL-8 stimulates the development of endometriosis in a dose-dependent manner.

Calhaz-Jorge *et al.* also assessed the relationship between the presence of endometriotic red lesions with IL-8 concentrations in peritoneal fluid. Red lesions, which are highly

vascularized and proliferative, are usually representative of the early stages of endometriosis. During the luteal phase of the menstrual cycle, IL-8 levels were significantly higher in the peritoneal fluid of endometriosis patients with red lesions, when compared with the levels in control subjects or endometriosis patients without red lesions. Sex steroid receptors, whose cyclical variations influence IL-8 expression, are expressed by lesions in endometriosis. This relationship may underlie the association between IL-8 in peritoneal fluid with the development and maintenance of endometriosis.

There is little doubt that IL-8's physiological function as an angiogenic, chemoattractant cytokine is related to the proliferation and neovascularization seen in ectopic endometrial tissue. However, more research to establish a consensus on its definite effect in the pathogenesis of endometriosis is needed to establish IL-8 as a peritoneal fluid biomarker to diagnose endometriosis in a non-invasive manner.

Interleukin-10

IL-10 is an anti-inflammatory cytokine that down-regulates the synthesis of proinflammatory cytokines, such as Interferon- γ , IL-2, IL-3, TNF- α and granulocyte macrophage colony stimulating factor. Conversely, it can act to stimulate certain T cells, mast cells and B cells. It is mainly expressed in macrophages as well as Type 2 T helper cells and B cells. IL-10 may partly account for the high degree of autoantibody production in endometriosis patients.²⁴ Increased peritoneal fluid IL-10 levels cause deviations in immune system function that might contribute to the pathogenesis of endometriosis.

Many conflicting reports regarding the correlation of IL-10 concentration in peritoneal fluid with the presence of endometriosis are present. Punnonen *et al.* found an association in which IL-10 levels were significantly higher in the peritoneal fluid of patients with endometriosis in comparison with the levels seen in control subjects.²⁴ These results support the theory that macrophage activity is increased, thereby disturbing immune function, in endometriosis. It is worth noting, however, that IL-10 concentrations in peritoneal fluid were

not correlated with the severity of endometriosis. Also, the proposed down-regulatory mechanism of action of IL-10 was not evidenced due to the fact that IL-10 levels in peritoneal fluid could not be correlated with levels of other inflammatory cytokines.

IL-10 is also thought to alter immune function by way of reducing peritoneal T cell activation, especially during the advanced stages of endometriosis. Lee *et al.* conducted a study in which they treated T helper cells and monocytes with the peritoneal fluid of both women with and without endometriosis. The group observed the effects of such treatment on major histocompatibility complex-II (MHC-II) expression.²⁹ MHC-II expression plays a central role in the processing of antigens by macrophages to helper T cells. This measure was, therefore, used as a relative index of immune function. Cells treated with the peritoneal fluid of endometriosis patients were found to have greatly reduced MHC-II expression when compared with control cells treated with normal peritoneal fluid. The modulation of MHC-II expression by IL-10 was significant because the endometriotic peritoneal fluid contained levels of IL-10 which were inversely correlated with MHC-II expression levels on cells treated with the same fluid. IL-10's role in disturbing T cell activation and overall immune function was further emphasized by the fact that IL-10-neutralizing antibody was seen to attenuate the down-regulatory action of endometriotic peritoneal fluid on MHC-II expression.

It is conceivable that increased peritoneal fluid levels of IL-10 are accountable for the immune system deregulation observed in patients with endometriosis. Theoretically, its measurement should then be useful in distinguishing endometriosis patients within a given population. However, conflicting results of past studies hinder this cytokine from being established as a definite peritoneal fluid biomarker in non-invasive diagnosis.

Interleukin-13

IL-13 is secreted by many cell types including the helper T type 2 cells. It is a central mediator of the physiological changes induced by allergic inflammation in many tissues as

well. IL-13 shares many pleiotropic effects with the closely related cytokine, IL-4. IL-13 functions to inhibit the activation of macrophages and lymphocytes, increase endothelial adhesion molecule production, and suppress estradiol and prostaglandin synthesis. Therefore, decreased IL-13 in the peritoneal fluid could be implicated in uninhibited macrophage activation that may contribute to the overall pathogenesis of endometriosis.²⁷

A study by Gallinelli *et al.* explored the relationship between immune cell subsets and IL-13 levels in the peritoneal fluid of women with and without endometriosis.³⁰ Subjects with endometriosis, irrespective of the stage of the disease were found to have significantly lower levels of IL-13 in their peritoneal fluid in comparison with the levels in the peritoneal fluid of healthy control subjects ($P < 0.01$). In accordance with previous work related to IL-13 and endometriosis, this macrophage-inhibiting cytokine's anti-inflammatory effects were found to be greatly diminished when its concentrations in peritoneal fluid were lower.

Also, both control patients and patients with endometriosis demonstrated an inverse relationship between activated T cells and IL-13 concentrations in peritoneal fluid. This is indicative of IL-13's down-regulatory effect on the inflammatory immune actions, which play a critical role in the initial development of endometriosis. Despite the established association between IL-13 and the presence of disease, these findings need to be confirmed and complemented by other studies before they can be considered for use as a non-invasive diagnostic biomarker for endometriosis.

Osteoprotegerin

Osteoprotegerin (OPG) is an anti-apoptotic survival factor and a member of the TNF receptor superfamily. It regulates apoptosis through binding to the TNF-related apoptosis-inducing ligand (TRAIL). This inhibits TRAIL from interacting and binding with the DR4 and DR5 apoptotic receptors, which normally induce cell death. Since the predominant theory concerning the causes of endometriosis implicates the decreased apoptosis of refluxed endometrial cells, it is postulated that the

TRAIL/OPG scheme is involved in the pathogenesis of endometriosis.

Yoshino *et al.*³¹ assessed the involvement of OPG in endometriosis pathogenesis and progression.³² OPG was significantly increased in the peritoneal fluid of women with endometriosis when compared with OPG levels in the peritoneal fluid of healthy control subjects. The degree of severity of endometriosis was correlated to the OPG concentration found in the peritoneal fluid of patients as well.

In accordance with the findings of Yoshino *et al.*,³¹ Harada *et al.*³² confirmed that levels of OPG in the peritoneal fluid of women with endometriosis were indeed significantly greater than those of the control group.¹⁴ Furthermore, the concentrations of OPG in women with advanced stage endometriosis were significantly elevated in contrast with the peritoneal fluid level measurements of OPG in women with early stages of the disease.

TRAIL, which down-regulates T cell activation and proliferation without causing T cell death, was found in higher concentrations in the peritoneal fluid of women with early stage endometriosis than in those with advanced stage endometriosis. Elevated concentrations of TRAIL, relative to OPG levels, may reflect the physiological actions of the body during efforts to stop the progression of the disease beyond its early stages. In addition, Harada *et al.* demonstrated that mRNA of the apoptotic DR5 receptor was expressed in all endometriotic tissue.³² Therefore, the regulation of apoptosis is most likely influenced by the relative concentrations of OPG and TRAIL.

OPG is a relevant factor at play in the pathogenesis of endometriosis, which has proven to be positively correlated with the incidence and increasing severity of the disease. The previously mentioned studies from which these conclusions were drawn are hampered by reasons which failed to distinguish OPG concentrations as related to cause or effect of the disease, as well as having statistical overlap. Therefore, OPG alone is not an effective peritoneal fluid biomarker when used for the non-invasive diagnosis of endometriosis due to its lack of specificity and sensitivity. However, its use as a biomarker in conjunction with the measurement of other, more reliable bio-

markers could be helpful in confirming a diagnosis.

Epithelial neutrophil-activating peptide-78

ENA-78 is a chemokine involved in local inflammatory reactions by way of its recruitment and activation of neutrophils.³³ Inflammatory mediators, such as IL-1 and TNF- α , can induce the expression of ENA-78 in monocytes, neutrophils, alveolar and intestinal epithelial cells, as well as endometrial cells. ENA-78 may also be induced by progesterone levels in human endometrial cells, exerting both direct and indirect angiogenic effects, as vascular endothelial growth factor (VEGF) is expressed by the mobilized neutrophils. Present in the peritoneal fluid of women, it may help facilitate the implantation, growth, and neovascularization of ectopic endometrial implants.³⁴

Mueller *et al.* demonstrated that the peritoneal fluid of women with endometriosis exhibited elevated levels of epithelial neutrophil-activating peptide-78 in women with endometriosis compared with controls.³³ IL-8 and ENA-78 have been demonstrated to exert angiogenic effects mediated through the same receptor. In addition to mean ENA-78 levels being significantly higher in the peritoneal fluid of women with endometriosis compared with controls, relatively high levels of ENA-78 protein were detected in the culture medium of inactive macrophages. This showcases that ENA-78 transcripts can be actively expressed by macrophages and other supplementary components of endometriotic lesions. Therefore, elevated ENA-78 concentrations in the peritoneal fluid of women with endometriosis may be a consequence of the greater number of angiogenic macrophages present in the endometriotic peritoneal environment.

Suzumori *et al.* correlated peritoneal fluid concentrations of ENA-78 with the severity of endometriosis.³⁴ Their study validated the findings of Mueller *et al.* and demonstrating that ENA-78 levels were indeed significantly increased in the peritoneal fluid of women with endometriosis.³³ Women with stage III or stage IV endometriosis had significantly higher peritoneal fluid ENA-78 concentrations when compared with the measurements obtained from

women with earlier stages of the disease. The most significant and pronounced increase was seen in stage IV, advanced endometriosis patients. Assessing the levels of ENA-78 in women with endometriosis might prove a valuable technique to assess the progression of the disease throughout its different stages.

Recent studies have examined ENA-78 in the context of therapeutic targets for the treatment of endometriosis rather than as an indicative biomarker in the diagnosis of disease. The potential for using ENA-78 as a peritoneal fluid biomarker in the non-invasive diagnosis of endometriosis exists, but must be thoroughly researched in future investigations to elucidate the chemokine's specific disease contributions.

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that participates in inflammatory and immune responses and is involved in macrophage functions, such as phagocytosis and self-distribution. This cytokine is copiously expressed and accumulates within the cytoplasm.³⁵ MIF is produced by activated T cells and upregulated by TNF- α . It is implicated in the development of endometriosis and endometriotic implants through its regulation of endothelial cell proliferation and angiogenesis. MIF's angiogenic capacity may be illustrated by the fact that anti-MIF antibody has been shown to significantly inhibit the endothelial cell growth promoting activity of peritoneal fluid.³⁶ MIF facilitates the invasion of endometriosis by modulating the production and release of MMPs. It may also inhibit natural killer (NK) cells' defensive apoptotic activity against refluxed endometrial cells.³⁷

The findings of Kats *et al.* were elementary in displaying the presence of elevated MIF levels in the peritoneal fluid of women with endometriosis.³⁶ MIF's pathophysiological role in the initial stages of endometriosis might be deduced by the fact that peritoneal fluid of early stage endometriosis patients had significantly higher levels of MIF than peritoneal fluid of patients with advanced stages of the disease.³⁶ The increased concentrations of MIF in the peritoneal fluid of endometriosis patients were

more pronounced during the secretory phase of the menstrual cycle. Hence, MIF is thought to recruit macrophages and maintain their inflammatory effects throughout the course of the menstrual cycle. Furthermore, the elevation of peritoneal fluid MIF concentrations in endometriosis was found to be more pronounced in patients with endometriosis-associated infertility. This could be due to the macrophage-mediated inhibition of early embryonic development and the secretion of various embryotoxic cytokines.

Results of a study by Mahutte *et al.* confirmed that MIF levels were higher in the peritoneal fluid of women with endometriosis than in the peritoneal fluid of control subjects.³⁷ However, contrary to the findings of Kats *et al.*, the degree of increased MIF concentration in endometriotic peritoneal fluid was not found to differ significantly when comparing patients in early stages with those experiencing advanced stage endometriosis.³⁶ In addition, the levels of MIF in peritoneal fluid did not differ between endometriosis patients when present with superficial implants, deep implants, or endometriomas. Also, MIF concentrations in the peritoneal fluid of women with endometriosis were not significantly related with the incidence of endometriosis-associated infertility or pelvic pain.

In the context of peritoneal fluid biomarkers, macrophage MIF may, at best, suspect the presence of disease. There is a lack of consensus concerning correlations between MIF and different aspects of endometriosis as well as ambiguity regarding the exact role of MIF in the pathogenesis of the disease. Therefore, further studies are necessary to uncover the sources of increased peritoneal MIF levels, explain the mechanisms involved in the up-regulation of MIF secretion, and understand the role of MIF in endometriosis using experimental models.³⁶

Additional cytokines

Adiponectin

Adiponectin is an adipocytokine that oversees the function of a number of metabolic processes, including glucose regulation and

fatty acid catabolism. This cytokine with hormone action is mainly secreted from adipose tissue into the bloodstream. Levels generally correlate negatively with body mass index. Adiponectin suppresses angiogenesis, tumor growth and fibrosis. In addition to these protective effects, it also expresses anti-inflammatory characteristics through the inhibition of TNF- α and phagocytic activity. Takemura *et al.* demonstrated lower levels of adiponectin in the peritoneal fluid of women with endometriosis.³⁸ Takemura *et al.* ascertained that a deficiency in peritoneal fluid adiponectin may contribute to the development of fibrotic adhesions associated with endometriosis.

Fractalkine

Fractalkine is a membrane-bound chemokine expressed on endothelial cells. It is significantly involved in leukocyte recruitment from the blood. Induced by inflammation, it mediates leukocyte adhesion to activated endothelium *via* a specific receptor located on both T cells and macrophages. The results of a study by Shimoya *et al.* show that women with endometriosis have significantly decreased levels of fractalkine in their peritoneal fluid when compared with controls.³⁹ However, fractalkine levels were not found to be correlated with the severity of endometriosis. The authors put forth the suggestion that decreased fractalkine levels in the peritoneal cavity might have a role in the precipitation of endometriosis-associated infertility.

Interferon- γ -induced protein-10

Interferon- γ -induced protein-10 (IP-10) is a chemokine induced in various cells in response to interferon γ and lipopolysaccharide. IP-10 has anti-angiogenic properties and is produced by activated lymphocytes, endothelial cells and fibroblasts. IP-10 is chemoattractant to NK cells, stimulating them to carry out cytolytic functions. IP-10 has the ability to inhibit endothelial cell proliferation. An investigation by Yoshino *et al.* described experimental results in which IP-10 concentrations were significantly depressed in cases of advanced stage endometriosis, as compared with incidence of early stage endometriosis.³¹ These results show

that a deficiency of IP-10 in the peritoneal fluid might render the peritoneal environment more favorable to enhanced angiogenesis, therefore contributing to the pathogenesis of endometriosis.

Interleukin-15

IL-15 is a cytokine with a variety of biological functions including the activation and maintenance of cellular immune reactions. IL-15 interacts with IL-2 components to stimulate the proliferation and migration of T-lymphocytes. This cytokine inhibits neutrophil apoptosis, stimulates MMPs, and up-regulates VEGF secretion by NK cells. Arici *et al.* found an inverse correlation between IL-15 concentrations in peritoneal fluid and the stages of endometriosis, thus suggesting its involvement in the pathogenesis of the initial stages of the disease (Table I).¹⁶

Interleukin-17

IL-17 is a potent pro-inflammatory cytokine produced by activated T cells. IL-17 up-regulates the peritoneal macrophage production and secretion of IL-6 and TNF- α , which are strongly implicated in the pathogenesis of endometriosis. It is a cytokine worth mentioning as it induces many factors related to the development and progression of endometriosis, such as ICAM-1, prostaglandin E2, cyclooxygenase-2, nitric oxide (NO) synthase-2 and MMP-3. Zhang *et al.* showed that IL-17 levels in peritoneal fluid were elevated most significantly during early stages of endometriosis in comparison to controls or the subjects with advanced stages of the disease.¹⁷ As IL-17 is pro-angiogenic, its increased concentrations during the initial stages of endometriosis may facilitate the implantation, proliferation, and formation of early endometriotic lesions.

Interleukin-18

IL-18 is a cytokine of interest with regard to endometriosis as it regulates innate and acquired immune responses and is produced locally within the peritoneal cavity. Conflicting results from various studies attempting to establish a relationship between IL-18 levels in

peritoneal fluid and the pathogenesis of endometriosis exist. Zhang *et al.* observed decreased levels of IL-18 in peritoneal fluid of patients with endometriosis (Table I).¹⁸ Conversely, the findings of Arici *et al.* showed that elevated IL-18 levels in the peritoneal fluid were most significant in the early stages of disease.⁴⁰

Some additional ILs hypothesized to contribute to the development, maintenance and progression of endometriosis, but found to be of little or no relevance include IL-2, IL-4, IL-12, and IL-16.

Proteolytic enzymes

Proteolytic enzymes are biological agents that break peptide bonds between amino acids. Proteolytic cleavage is a common mechanism of activation or inactivation of enzymes in physiological reactions and highly regulated cascades. Increased concentrations of some naturally-occurring proteolytic enzymes have been implicated in the pathogenesis of endometriosis.

Pregnancy associated plasma protein A

Pregnancy associated plasma protein A (PAPP-A) is large zinc-binding protein produced by the endometrium, ovary and placenta. It exhibits protease activity toward insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4), which contributes to increased free levels of potentially mitogenic IGF in the peritoneal fluid.

Bersinger *et al.* showed that PAPP-A levels in peritoneal fluid were increased significantly in women with endometriosis.¹⁴ Also, PAPP-A concentrations were positively correlated with the disease's severity. These findings ascertained that PAPP-A on its own may not be an ideal biomarker for diagnosis as its levels fluctuate under the influence of different menstrual cycle phases. This additional variable could mask indications of the presence of endometriosis.

Cathepsin D

Cathepsin D is an aspartyl acid protease prevalent in animal and human cells. The con-

centration of this proteolytic enzyme is correlated with a high risk of metastasis and proteolytic events resulting in the degradation of basement membrane and extracellular matrix components. Cathepsin D might, therefore, be implicated in creating a peritoneal cavity environment that welcomes the invasion of ectopic endometrial cells.

The peritoneal fluid of women with endometriosis had significantly higher concentrations of cathepsin D compared with control subjects, according to a study by Suzumori *et al.*⁴¹ Furthermore, cathepsin D levels were significantly higher in the peritoneal fluid of patients with advanced stage endometriosis in contrast with the levels in patients with early stage endometriosis. These findings insinuate that actively spreading endometriotic lesions may produce cathepsin D at excess levels that may be detected in the peritoneal fluid during the later stages of endometriosis.

Matrix metalloproteinases

Endometriosis is a disease characterized by its ability to invade normal tissue, requiring proteolytic activity, activation of the plasminogen activator pathway and MMPs. MMPs are zinc-dependent endopeptidases secreted by endometrial cells that degrade extracellular matrix proteins and process a number of bioactive molecules. They are known to be involved in the regulation of chemokine activity and the release of apoptotic ligands, such as the FAS ligand. MMPs are linked to the pathogenesis of endometriosis as they are thought to also play a major role in cell proliferation, migration, differentiation, adhesion, angiogenesis, and apoptosis. Activated MMPs can be inhibited by their tissue inhibitors.

Huang *et al.* demonstrated that MMP-2 levels were significantly increased in the peritoneal fluid of patients with endometriosis-associated infertility compared with control subjects.⁴² This implies that the significant deviations of MMP-2 from its normal level in peritoneal fluid may trigger infertility in patients with early stages of endometriosis. Also, the increase of MMP-2 concentrations in patients with endometriosis-associated infertility was found to be more pronounced during the pro-

liferative phase of the menstrual cycle. Presently, MMP-2 concentrations are positively correlated with estrogen levels, implying that estrogen has an up-regulatory effect on MMP-2 production in patients with endometriosis. During the secretory phase, MMP-2 concentrations are inversely correlated with progesterone levels, suggesting that progesterone has a down-regulatory effect on the production of MMP-2 in endometriosis patients. Although MMPs are definitely implicated in the pathogenesis of endometriosis, more research is needed to elucidate their exact role in the development and maintenance of endometriosis. The variation in peritoneal fluid MMP concentration over the course of the menstrual cycle limits its potential for use as an accurate biochemical marker in the non-invasive diagnosis of endometriosis.

Secretory leukocyte protease inhibitor

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor produced by epithelial cells that is concentrated in bodily fluids. As a part of the body's natural defense system, SLPI acts to potently inhibit proteolytic enzymes such as leukocyte elastase and cathepsin. SLPI produces anti-inflammatory effects within the body that protect against the further development of endometriosis.

The findings of Shimoya *et al.* show that SLPI concentrations are significantly higher in the peritoneal fluid of women with endometriosis than compared with control subjects.⁴³ This difference may be perceived as a result of either the elevated macrophage concentrations or the selective expression of protease inhibitors in response to inflammation. The ratio of SLPI to elastase, a proteolytic enzyme involved in the pathogenesis of endometriosis, was significantly higher in the peritoneal fluid of women with variable stages of endometriosis compared with control subjects. Therefore, SLPI may exert its anti-inflammatory effect by inhibiting macrophage cytokine production, a process often induced by elastase. In addition, macrophages are thought to be one of the main sources from which SLPI is derived from in the peritoneal fluid.

Hormones

Leptin

Leptin is an adipocyte-derived protein hormone belonging to the helical cytokine group. It plays a key role in regulating energy intake and energy expenditure in the body, including the regulation of appetite and metabolism. The elaboration of leptin has been proposed to originate from both the peritoneal fluid fat and also the endometriotic foci. The pro-inflammatory cytokines TNF- α and IL-1 β induce leptin expression and secretion. Leptin stimulates angiogenesis synergistically with VEGF and induces the expression of MMPs, thought to facilitate the initial invasion of ectopic endometrial cells. Leptin is a modulator of CD4+ T cell activities, cytokine production, and is also associated with endometrial stromal cell production. The pro-inflammatory and neoangiogenic effects of leptin have led many investigators to explore the association of leptin levels with the pathogenesis of endometriosis.

Several studies have documented that increased leptin concentrations exist in the peritoneal fluid and serum of women with endometriosis. This observation signifies that leptin is catabolized more slowly in patients with endometriosis compared with controls. In some investigations, leptin levels were found to be significantly higher in patients with early stage endometriosis compared to patients with advanced stages of the disease.^{20, 44} Conversely, Bedaiwy *et al.* demonstrated peak leptin concentrations in the advanced stages of the disease.⁴⁵

Leptin concentrations have been inversely correlated with the stage and degree of endometriosis invasion. Unlike a study by Gogacz *et al.*, which found no difference in leptin levels between patients with endometriosis and those with idiopathic infertility, Bedaiwy *et al.* compared the peritoneal fluid of both groups and reported significantly increased levels of leptin in endometriosis patients.^{45, 46} According to De Placido *et al.* and Bedaiwy *et al.*, higher concentrations of leptin were observed in endometriosis patients presented with peritoneal implants compared with those endometriosis patients who did not have implants.^{44, 45}

Furthermore, mean leptin levels were identified to be significantly higher in the peritoneal fluid of women with superficial peritoneal implants than in the peritoneal fluid of women with deep implants or endometriomas.⁴⁷ This may necessitate distinguishing peritoneal endometriosis and endometriomas as two distinct phenotypic entities and disease processes.

Leptin concentrations in the peritoneal fluid of women with endometriosis have also been explored in terms of their relationship with the presence of major symptoms associated with the disease. The study of Bedaiwy *et al.* showed that peritoneal fluid measurements of leptin were positively correlated with the incidence of chronic pelvic pain in endometriosis patients.⁴⁵ However, Mahutte *et al.* found no difference in leptin levels between women with endometriosis-associated complaints of infertility and those with chronic pelvic pain.⁴⁷

The therapeutic capacity of gonadotropin-releasing hormone (GnRH) agonists to suppress endometriotic implants in endometriosis might be validated by the fact that endometriosis patients treated with GnRH agonists and control subjects had comparable levels of leptin in their peritoneal fluid.⁴⁷

Leptin and other angiogenic cytokines, such as IL-8, are reported to correlate with disease activity. A combination predictive model combining the various angiogenic factors may provide better predictive probability of the active disease.

Luteinizing hormone

Luteinizing hormone (LH) is a hormone synthesized and released by gonadotropes in the anterior lobe of the pituitary gland. In conjunction with the pituitary's gonadotropin follicle stimulating hormone, it works to regulate proper reproductive function. In the female, LH regulates the menstrual cycle, steroidogenesis, folliculogenesis, and ovulation.

LH levels are much higher in the peritoneal fluid than in the serum of women with or without endometriosis. LH may diffuse from the ovarian vasculature into peritoneal fluid, where it is present at levels shown to fluctuate.

ate during various phases of the menstrual cycle.⁴⁸ LH concentrations in the peritoneal fluid reach their peak during the proliferative phase of the menstrual cycle and plummet to their lowest levels during the early proliferative phase. In the Illera *et al.* study, the peritoneal fluid of women with endometriosis had significantly higher amounts of LH than the peritoneal fluid of healthy control subjects.⁴⁸ Elevated LH concentrations might explain the altered endometrial protein patterns exhibited in the peritoneal fluid of women with endometriosis.

Cell adhesion molecules

Soluble intercellular adhesion molecule-1

Soluble intercellular adhesion molecules (sICAMs) are molecules that promote adhesion between cells and interfere with immunosurveillance.⁴⁹ During the acute stages of inflammation, cytokines stimulate an increase and release of sICAMs, which promote the adherence of leukocytes to endothelium. ICAM-1 is expressed by the vascular endothelium, macrophages and lymphocytes. It is continuously present in low concentrations in the membranes of leukocytes and endothelial cells. Despite the fact that sICAM-1 is thought to be induced by IL-1 and TNF- α , its levels were not significantly correlated with IL-1 β according to a study by Calhaz-Jorge *et al.*⁵⁰ This may suggest that IL-1 β is in fact irrelevant in influencing sICAM-1 activity in the peritoneal fluid.

Calhaz-Jorge *et al.* experimentally demonstrated that sICAM-1 levels in the peritoneal fluid of women with endometriosis did not significantly differ from those of healthy control subjects.⁵⁰ The levels of sICAM-1 did not differ between endometriosis patients with different stages of the disease. Peritoneal fluid measurements of sICAM-1 were shown to distinguish symptoms of endometriosis. Women with endometriosis-associated infertility had significantly higher levels of sICAM-1 in their peritoneal fluid compared with fertile women with endometriosis.¹¹ sICAMs should be further investigated for their potential use in dis-

criminating the types of symptoms associated with one's condition.

Growth factors

Insulin-like growth factors

IGFs are potent mitogenic polypeptides with a similar genetic sequence as that of insulin. IGFs are utilized by cells communicating with their physiological environment and may act to mediate the activity of estrogen and other growth factors. Kim *et al.* found that IGF-1 levels in peritoneal fluid were significantly higher in endometriosis patients compared with control subjects.⁵¹ However, no difference in peritoneal fluid IGF-II levels was found between the two groups. Also, the IGF concentrations in peritoneal fluid were not affected by each patient's stage of endometriosis.

IGF-1 and IGF-II are regulated by a family of proteins known as the IGFBPs. These proteins help modulate IGF action in complex ways that involve both inhibiting IGF by preventing its binding to the IGF-1 receptor and promoting IGF through aiding in its delivery to the correct receptor and increasing IGF half-life. Currently, there are 6 characterized IGFBPs (IGFBP1-6). Kim *et al.* showed that the relative levels of IGFBP-3 in peritoneal fluid were significantly lower in patients with endometriosis when compared with controls.⁵¹ A decrease in binding proteins could lead to the increased bioavailability of IGFs in the peritoneal fluid of women with endometriosis.

Angiogenin

Angiogenin (Ang) is a single-chain non-glycosylated polypeptide implicated in the formation of new blood vessels by its activation of capillary endothelial cells. Ang may be produced by peritoneal macrophages and endometriotic tissues. It plays a role in the maintenance and development of endometriosis.

Suzumori *et al.* demonstrated that the peritoneal fluid samples of women with endometriosis contained significantly higher concentrations of Ang than the peritoneal fluid of control subjects.⁵² Concentrations positively

correlate with the degree of severity of endometriosis.

Vascular endothelial growth factor

VEGF is an important signaling protein, which potently stimulates both the *de novo* formation of the embryonic circulatory system and the development of blood vessels from pre-existing vasculature. As its name suggests, VEGF activity is restricted mainly to cells of the vascular endothelium and works to stimulate monocytes and macrophage migration. VEGF stimulates endothelial cell mitogenesis, cell migration, and enhances microvascular permeability. There are numerous literature reports linking VEGF to neovascularization and the proliferation of endometrial implants.

The known effects of VEGF on the peritoneal environment have led to its postulated involvement in the pathogenesis of endometriosis. Mahnke *et al.* demonstrated that women with a high degree of endometriotic peritoneal implants had significantly elevated concentrations of VEGF in their peritoneal fluid when compared with women with a low degree of peritoneal implants.²⁵ This may suggest that endometriosis implants are involved in a vicious cycle of VEGF-induced neovascularization, perpetuating the growth and development of additional implants.

Transforming growth factor- β

TGF- β receptors are serine/threonine kinase receptors with potent pleiotropic and immunomodulatory effects. TGF- β inhibits the growth and stimulates the proliferation of various cell types.¹² TGF- β stimulates angiogenesis and regulates the synthesis of extracellular matrix proteins. It also up-regulates Fas ligand expression in a dose-dependent manner. Triggering the Fas-mediated cell death of activated immune cells allows ectopic endometrial cells in the peritoneal fluid to escape the immune system, implant and grow.

Despite the function and effects of TGF- β , often thought to contribute to the pathogenesis of disease, D'Hooghe *et al.* did not find any statistically significant differences in TGF- β concentration in the peritoneal fluid of women with and without endometriosis.⁵³ The study's

sample size was too small to detect any significant relationships between TGF- β concentration, the presence of endometriosis, and its progression.

Proteins

Mannose binding lectin

Mannose binding lectin (MBL) is an important immune system soluble factor. MBL function appears to be pattern recognition and its binding to an unwanted antigen results in the activation of the lectin pathway of the complement system. MBL also effects complement-independent opsonization and phagocytosis, regulates inflammation, and binds to apoptotic cells of ectopic endometrium to enhance their clearance. Decreased MBL levels have been said to render cells susceptible to attack by autoimmune diseases.

Despite the inferred link between MBL and endometriosis, Kavoussi *et al.* did not find statistically significant differences between the MBL levels in the peritoneal fluid of patients with endometriosis and the peritoneal fluid levels of MBL observed in healthy control subjects.⁵⁴ Also, no differences in MBL concentration were found between the peritoneal fluid of women with early stage endometriosis and those with the advanced stages of the disease. The Kavoussi *et al.*'s study, which was limited by the small sample size used in its analyses, failed to demonstrate any relationship between MBL levels with the presence or stage of the disease. There seems to be a lack of biologic plausibility for MBL to be considered for use as a biomarker for endometriosis.

Placental growth factor

Placental growth factor (PlGF) is a type of VEGF as well as a molecular marker for inflammation. PlGF is angiogenic glycoprotein expressed in both placental cells and non-placental endothelial cells. It works to stimulate tissue factor production and chemotaxis in monocytes and may play a role in the pathogenesis of endometriosis.

Suzumori *et al.* elucidated that women with endometriosis have increased levels of PlGF

in their peritoneal fluid.⁵⁵ Patients with varying stages of endometriosis had significantly different concentrations of PlGF in their peritoneal fluid than controls. The PlGF concentration was positively correlated with the severity of endometriosis. PlGF's role in the neovascularization of endometriotic tissue was established by the observation that patients with peritoneal "red lesion" implants had significantly higher amounts of PlGF in their peritoneal fluid compared with endometriosis patients who did not have any "red lesions".

PlGF is significantly related to the severity of disease and the depth of endometrioma invasion. Suzumori *et al.* show that in both the control and endometriosis groups, significantly greater levels of PlGF are exhibited during the secretory phase of the menstrual cycle.⁵⁵ Consequently, the potential for using PlGF, as a reliable biomarker in the non-invasive assessment of endometriosis development and progression, is limited due to its cyclic variation.

Fas ligand

Fas ligand is a type II transmembrane protein that belongs to the TNF family. It is a trimeric molecule predominantly expressed in T cells causing apoptotic death in Fas-bearing cells that interact with its receptor. Activity of Fas ligand is up-regulated by TGF- β , platelet derived growth factor, IL-8, and TNF- α . Defective Fas mediated apoptosis may lead to the destruction of immune cells that eliminate retrograde menstruation, thereby down-regulating the pro-apoptotic activity against ectopic endometrial cells.

Garcia-Velasco *et al.* explored the case of elevated levels of soluble Fas ligand in the peritoneal fluid and serum of women with endometriosis (Table I).¹⁹ Increased levels of soluble Fas ligand could possibly originate from endometriotic lesions and/or peritoneal fluid leukocytes. Women with moderate to severe stage endometriosis had significantly higher peritoneal fluid concentrations of soluble Fas ligand compared with the peritoneal fluid of women with earlier stages of the disease. Elevated levels of Fas ligand cause apoptotic dysregulation and proliferation of endometri-

al cells, resulting in a higher disease burden in the peritoneal cavity.

Autoantibodies

Eosinophils

Eosinophil granulocytes are white blood cells that combat infection by parasites in the body. Eosinophils may participate in the general inflammatory response of the immune system by regulating tissue remodeling, fibroblast growth, and collagen synthesis. In endometriosis, eosinophils contribute to fibrosis and the secretion of MMPs, which are hypothesized to play a role in the pathogenesis of endometriosis.

Eidukaite *et al.* found that there were higher amounts of eosinophils in the peritoneal fluid of women with endometriosis than in the peritoneal fluid of control subjects.⁵⁶ Macrophage levels in the peritoneal fluid of endometriosis patients were also heightened and could modulate the activity of immunocompetent cells as well as induce endometrial cell implantation and proliferation. Allergic reactions associated with endometriosis may account for the detectable elevations in eosinophil count in the peritoneal fluid.

Oxidative stress biomarkers

Reactive oxygen species

Reactive oxygen species (ROS) include oxygen ions, free radicals and peroxides. They are small molecules that are highly reactive due to the presence of unpaired valence shell electrons. ROS are a natural byproduct of the metabolism of oxygen and have important roles in cell signaling. In response to environmental stress, however, ROS levels can drastically increase, resulting in significant damage to cell structures. This situation is termed 'oxidative stress' and is usually opposed by cells using superoxide dismutase and catalase enzymes.²

ROS influence the programmed cell death and apoptosis of cells and are also involved in the induction of host defense genes. Platelets release ROS to recruit additional platelets to

sites of injury. ROS recruit leukocytes and are active in a variety of inflammatory responses.²

Reactive nitrogen species

NO, an endothelium-derived relaxing factor, is an anti-oxidant molecule synthesized from arginine and oxygen by NO synthase enzymes. NO is produced by macrophages to kill cellular targets and protects tissues by scavenging small amounts of ROS.²

Ho *et al.* found no obvious oxidant or antioxidant imbalance in the peritoneal fluid of women with endometriosis.² This could be attributed to the fact that small molecules like NO and their metabolites in the bodily fluids decay too rapidly to be easily detected. Also, the indirect effects of one's diet may cause NO levels to fluctuate within the peritoneal environment. NO secretion can be altered by increased TNF- α and decreased IFN- γ in the peritoneal fluid of patients with endometriosis.

Small molecular antioxidants, such as glutathione, play important roles as cellular antioxidants.⁵⁷ Glutathione is a tripeptide hypothesized to protect cells against oxidation by free radicals. The Polak *et al.*'s study is a pioneer in demonstrating the presence of glutathione in the peritoneal fluid of women with endometriosis.⁵⁷ However, no significant differences in GSH levels were found to exist between the peritoneal fluid of women with endometriosis and the peritoneal fluid of control subjects.

NO levels in the peritoneal fluid and other measures of oxidant/antioxidant balance are hypothesized to play a role in the pathogenesis of endometriosis. Nevertheless, due to a lack of control for numerous variables and factors that influence their detectable concentrations, they should not be considered for use as biomarkers in the non-invasive diagnosis of endometriosis.

Miscellaneous

L-carnitine

L-carnitine (levocarnitine) is an essential intracellular ammonium compound derived from the amino acid lysine. L-carnitine is necessary in the transport of long chain fatty acids from

the cytosol and across the mitochondrial membrane into the mitochondria. L-carnitine can modify immune responses by affecting humoral and cellular immune responses. Maintenance of the cell membrane's structure and viability, and reductions in the apoptotic levels of cytotoxic cells are effects delivered by L-carnitine suggesting its involvement in the pathogenesis of endometriosis.⁵⁸

L-carnitine levels were shown to correlate with the cytokine and cellular profile of endometriosis. Dionyssopoulou *et al.* treated mouse peritoneal fluid with L-carnitine and found that it produced significantly higher amounts of IFN- γ and somewhat higher levels of TNF- α , IL-6, IL-2, VEGF, and IGF-1.⁵⁸ The regulation of cytokines by L-carnitine may contribute to endometriosis since increased IL-6 in the peritoneal fluid has been established as a factor related to the progression of endometriosis. Elevated IGF-1 levels may play a role in the growth of ectopic endometrium. L-carnitine treatment also decreased angiogenic factors such as VEGF and granulocyte macrophage colony stimulating factor. Patients with advanced stages of endometriosis exhibited a significant decrease in acyl-L-carnitine levels in the peritoneal fluid.

Dionyssopoulou *et al.* also assessed the effect of L-carnitine on cytokine release in mouse serum. The serum of mice receiving L-carnitine treatment has elevated levels of IFN- γ , TNF- α , IL-6, IL-2, VEGF, and IGF-1 compared with controls. Conversely, this same L-carnitine treatment caused serum levels of GM-CSF to decrease.⁵⁸ These results may be suggestive of L-carnitine's role in producing a localized inflammatory response *via* its influence on cytokine levels in endometriosis.

Interleukin-8

IL-8 is an autocrine growth factor in the endometrium that contributes to the pathogenesis of endometriosis by encouraging endometrial cell attachment, invasion, cell growth and proliferation, immune protection, and IL-8 secretion in ectopic sites.⁵⁹ IL-8 is also known to increase the expression of surface adhesion molecules on neutrophils and stimulate angiogenesis as well as the mitogenesis of epi-

dermal and vascular smooth muscle cells. IL-8 levels were earlier described in the peritoneal fluid, but significant studies investigating it in endometrium are described in this section.

IL-8 is expressed in endometrial endothelial cells, glandular cells, and perivascular stroma. Human endometrial endothelial cells regulate the transport of leukocytes into the endometrium and the tissue-specific changes that result from this process. Luk *et al.* conducted a study that demonstrated the differential regulation of chemokine expression in the endometrial endothelial cells by sex steroids in women with or without endometriosis.⁶⁰ Estradiol treatment suppressed IL-8 mRNA levels in women without endometriosis and induced an increase in IL-8 mRNA levels in women with endometriosis. Treating women without endometriosis with progesterone suppresses IL-8 mRNA levels, whereas in women with endometriosis it induced an increase in IL-8 mRNA levels. A combined treatment of estradiol plus progesterone decreased the IL-8 mRNA levels in endometrial endothelial cells from women without endometriosis, yet this same treatment was found to increase the levels of IL-8 mRNA in endometrial endothelial cells from women with endometriosis.

The findings of Luk *et al.* assert that human endometrial endothelial cells' differential regulation of the immune response in the endometrium is modulated by sex steroids.⁶⁰ Therefore, increased IL-8 expression in endometrial endothelial cells of women, during their reproductive age, may pathophysiologically provoke the development of endometriosis.

IL-8 binds to its membrane receptors: CXCR1 and CXCR2 to exert its effects, which are modulated by these receptors themselves. CXCR1 and CXCR2 respond to IL-8 binding by affecting intracellular calcium concentrations, the release of enzyme granules, and chemotaxis. CXCR1 is particularly involved in IL-8-mediated O₂⁻ release and phospholipase D activation.⁵⁹ A study by Ulukus *et al.* showed that there is a higher expression of IL-8 receptors in endometriosis.⁵⁹ The affinity of CXCR1 for IL-8 was demonstrated as low compared to that of CXCR2, but its recovery rate was rapid after being desensitized by IL-8. The rapid recovery rate of CXCR1 hints that it may play a sig-

nificant role at regions of high IL-8 concentrations, such as at sites of inflammation. However, CXCR2-with its high affinity, might therefore work most efficiently at potentiating IL-8's effects where the chemokine is found at lower concentrations. Women with endometriosis were found to have increased levels of both types of receptor in both epithelial and stromal cells, demonstrating that IL-8 may have a more pronounced effect on the eutopic endometrium of women with the disease. The results of the Ulukus *et al.* study indicate that the eutopic endometrium of women with endometriosis differs from that of women without endometriosis. Therefore, the leading defect in endometriosis may lie within the eutopic endometrium of women.

Serum markers

There is continuing interest in identifying serum markers for the prediction of endometriosis with high diagnostic probability. Cytokines such as IL-6 have been proposed to be able to discriminate between patients with endometriosis and without. Newer markers, such as angiogenesis promoters, fibroblast growth factor (FGF) and Ang, have been investigated as they correlate with the proliferative activity of the disease. The serum marker CA-125, a high molecular weight glycoprotein has been discussed. A tumor-associated antigen lacks a high predictive probability as elevated levels may also indicate carcinoma, pelvic inflammatory disease, uterine fibroids.

Interleukin-6

IL-6 in the serum has a high diagnostic accuracy with an area under the curve of receiver operating curves (ROC) of 87% (95% confidence interval: 75-99%).¹⁰ A sensitivity of 90% and specificity of 67% were reported when a cut off value of 2 pg/mL was used in a randomized controlled trial from our center.¹⁰

Cancer antigen 125

Serum levels of cancer antigen 125 (CA-125) increase as women age.⁶¹ CA-125 is most com-

monly thought of as a marker for ovarian cancer and is utilized for monitoring the course of ovarian cancer, but tends to be elevated in the presence of any peritoneal inflammatory condition, cancerous or benign.^{62, 63} CA-125 may also be elevated in, endometriosis. Serum CA-125 is the most extensively investigated and used marker for endometriosis. CA-125 is produced by the endometrium, is a 200 000 Da glycoprotein and exudes into the serum *via* the endothelial lining of capillaries in response to inflammation. CA-125 has a very high specificity for endometriosis, but cannot be used for initial diagnosis of endometriosis because of its low sensitivity and positive predictive value. A meta-analysis of 23 studies, both case control and cohort, reported sensitivities varying from 4% to 100% and specificity ranging from 38% to 100% for the diagnosis of endometriosis. ROCs showed better diagnostic performance of CA-125 in the advanced stages of the disease.

A study by Matalliotakis *et al.* demonstrated that women with endometriosis had significantly higher levels of CA-125 in their peritoneal fluid, regardless of the phase of menstrual cycle.⁶⁴ Advanced stage endometriosis patients had higher, but not statistically significant, concentrations in their peritoneal fluid compared with endometriosis patients with early stages of the disease. It should be noted that CA-125's diagnostic value was proven limited by its numerous overlapping values between the studied patients and control subjects in this study. A meta-analysis showed poor diagnostic performance of CA-125 on the ROC at a specificity of 90% with a sensitivity drop to 28%.⁶⁵

The key problem with using CA-125 for early detection is its lack of specificity. This could lead to many inaccurate diagnoses. Also, CA-125 has not been shown to elevate early enough in the disease process to justify its use for early intervention.

Fibroblast growth factor-2

FGF promotes endothelial cell proliferation and angiogenesis. It is more potent, as an angiogenic factor, than VEGF or platelet-derived growth factor. As well as stimulating blood

vessel growth, FGF plays an important role in wound healing. It stimulates the proliferation of fibroblasts that give rise to granulation tissue, used to fill wound cavities early in the healing process. The angiogenic and mitotic activities mediated by FGF-2 take place in human ectopic and eutopic endometrium.

Bourlev *et al.* found that levels of FGF-2 in the serum of women with endometriosis scored as stage II to III were significantly higher than levels in healthy controls.⁶⁶ In addition, higher concentrations of FGF-2 were found in patients with lesions showing high mitotic activity.⁶⁶ Therefore, FGF-2 might be a useful measure of the proliferative activity of endometriotic lesions.

Endoglin (cd105) and S100A13

Endoglin and S100A13 are known angiogenic factors that may be involved in the pathogenesis of endometriosis. Endoglin is a membrane glycoprotein located on cell surfaces as part of the TGF- β receptor. Endoglin plays a vital role in vascular remodeling and angiogenesis during early development. Expressed in human endothelial cells, macrophages, stromal cells, and vascular smooth muscle cells, endoglin is implicated as an angiogenic marker in endometriosis. S100A13 is a member of the S100 protein family and is involved in multiple signaling pathways in endothelial cells, suggesting its possible use as a marker of endothelial cell activation.

Hayrabyan *et al.* assessed the expression of endoglin and S100A13 in human endometrial tissues.⁶⁷ Endoglin was positively expressed not just in endometriotic lesions, but also in the eutopic endometrium of women with endometriosis. In addition, S100A13 was found to be overexpressed in endometriotic tissues compared to normal endometrium. Although S100A13 was expressed in the normal endometrium of healthy control subjects, endoglin was not. Endoglin and S100A13 both stained the activated microvessel endothelia in endometriosis. Therefore, the potential of endoglin and S100A13, to be used as markers to monitor the progression of endometriosis and its treatment, should be further investigated.

Soluble intercellular adhesion molecule-1

ICAMs are molecules that promote adhesion between cells and interfere with immunosurveillance. During the initial stages of inflammation, cytokines increase the production and release of ICAMs, which promotes the adherence of leukocytes to endothelium. ICAM-1 is expressed at the surface of endometrial cells, endothelial cells and leukocytes. This expression is highly regulated by cytokines. A soluble form of ICAM-1 (sICAM-1) is shed from the cell surface and during inflammation can compete with ICAM-1 for receptor binding involved in antigen recognition by T cells. Therefore, sICAM-1 may have an inhibitory effect on ICAM-1 function. Ectopic endometrial cell modification of ICAM-1 expression could prevent their clearance by the immune system, playing a role in the development of endometriosis.

Several literature reports have demonstrated increased levels of the soluble form of the molecule (sICAM-1) in the peritoneal fluid of patients with endometriosis. Steff *et al.* compared the sICAM-1 levels in the serum of women with and without endometriosis.⁶⁸ The study was carefully controlled for cofounders such as infertility and exhibited no difference in sICAM-1 levels between controls and patients with endometriosis.

Follicular fluid markers

Macrophages and macrophage derivatives play diverse roles in intra-ovarian events, including folliculogenesis, tissue restructuring at ovulation and corpus luteum formation and regression. Many literature reports on various chemokines (IL, RANTES, TNF- α , and growth-regulated oncogene- α in the follicular fluid) have reported discordant results. Cunha-Filho *et al.* in a cross-sectional prospective study of infertile endometriosis patients going through assisted reproductive technologies (*in vitro* fertilization, IVF), reported moderate/severe endometriosis stage patients had lower follicular fluid levels of IGFBP (IGFBP-1) compared with controls.⁶⁹ VEGF levels are significantly higher in endometriosis patient follicular fluid

versus tubal factor infertility patents. Attar *et al.* in their study reported that the elevated follicular fluid VEGF levels did not adversely affect the IVF-embryo transfer outcomes in women with endometriosis.⁷⁰ ENA-78 has role in angiogenesis associated with inflamed tissue and was demonstrated significantly higher in follicular fluid of endometriosis than controls.⁷¹

Endometrial and genetic markers

Endometrium from women with endometriosis is characterized by inherent abnormalities. The essential abnormalities lead to allow the endometrium to be able to attach, remodel the peritoneum, persist, grow and develop new vasculature. A collaborative study from our group with University of Toronto has reported that the polymorphisms observed in plasminogen activator-1 gene are an important determinant of persistence of the fibrin matrix and associated hypofibrinolysis.⁷²

Endometrial and genetic markers have an appeal for the development of a marker in the future, which is predictive of endometriosis.

Conclusions

The diagnosis of endometriosis continues to be a challenge to gynecologists because definitive diagnosis requires visualization and or peritoneal tissue sampling obtained at laparoscopy or laparotomy. Early detection of endometriosis is important as randomized controlled trials have demonstrated the therapeutic results with medical or surgical treatments are significantly better in stages I and II compared with stages III and IV. An early diagnosis of endometriosis with a peritoneal fluid or serum diagnostic marker having a high diagnostic accuracy, sensitivity and specificity may avoid unwarranted testing of patients with pelvic pain and or infertility. It may also avoid empirical treatment for these patients. The severity and activity of endometriosis has been correlated with levels of some biologic markers. Future studies with adequate groups of patients should be designed to validate serum and peritoneal markers, which are specific to

infertility and pain associated with endometriosis.

Riassunto

Anormalità sieriche e peritoneali nell'endometriosi: marcatori diagnostici potenzialmente utilizzabili

L'endometriosi è una patologia ambigua la cui esatta patogenesi resta elusiva per i clinici e i ricercatori. Aberrazioni locali e sistemiche della risposta immunitaria sono associate all'endometriosi. Quest'articolo rivede la letteratura scientifica relativa a vari fattori immunologici, quali le citochine, i fattori di crescita, le molecole d'adesione e i fattori angiogenici coinvolti nell'eziopatogenesi di questa malattia. La nostra *review* riassume i lavori scientifici relativi a biomarcatori che possono essere utilizzati come mezzi affidabili, non chirurgici, per la diagnosi di endometriosi. I biomarcatori ideali sono caratterizzati da elevata sensibilità, specificità e valore predittivo e possono essere di aiuto per il riconoscimento precoce e il monitoraggio della progressione della malattia, così come della sua risposta ai trattamenti e degli aspetti critici per la sua gestione. Per migliorare le possibilità diagnostiche, che consentono di identificare le pazienti con alta probabilità di avere un'endometriosi, viene proposto un modello predittivo di combinazione che utilizza diversi biomarcatori, piuttosto che singoli marcatori individuali. Gli immunomodulanti e i bloccanti dei fattori angiogenici sono potenzialmente utilizzabili per il trattamento dell'endometriosi e anche per alleviare il dolore o contrastare la sterilità associata a questa malattia. I nuovi, potenziali, agenti terapeutici comprendono modulatori quali i bloccanti dei recettori citochinici e quelli dei recettori angiogenici, che attualmente vengono utilizzati per il trattamento dell'endometriosi.

Parole chiave: Endometriosi - Marker biologici - Citochine - Immunomodulatori.

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