# Diagnostic Validity of Serum and Peritoneal CA125, CA19.9 and plasma Cell-Free Nuclear DNA (ccf nDNA) as Biomarkers of Pelvic Endometriosis:

## *A Case Control Study*

Submitted as Partial Fulfillment of MD Degree in Obstetrics and Gynecology


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# Contents

# List of Abbreviations

| Abb. | Meaning |
|---|---|
| **ASRM** | American Society of Reproductive Medicine |
| **AUC** | Area under the curve |
| **BMI** | Body mass index |
| **CA-125** | Cancer antigen 125 |
| **CA19.9** | Cancer antigen 19.9 |
| **ccf DNA** | Circulating cell fre DNA |
| **ccf nDNA** | Circulating cell free nuclear DNA |
| **CGRP** | Calcitonin gene-related protein |
| **COCs** | Combined oral contraceptives |
| **COX-1** | Cyclo oxygenase isoenzyme 1 |
| **COX-2** | Cyclo oxygenase isoenzyme 2 |
| **CPP** | Chronic pelvic pain |
| **CRP** | C-reactive protein |
| **CT** | Computed tomography |
| **DC** | Dendritic cells |
| **DIE** | Deeply infiltrating endometriosis |
| **DMPA** | Depot medroxyprogesterone acetate |
| **DNA** | Deoxyribo nucleic acid |
| **EC** | Endometrial cells |
| **ECM** | Extracellular matrix proteins |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **ELISA** | Enzyme linked immunosorbant assay |
| **EPF** | Endometriotic peritoneal fluid |
| **ERα** | Estrogen receptor alpha |
| **ERβ** | Estrogen receptor beta |

| Abb. | Meaning |
|---|---|
| **ESHRE** | European Society for Human Reproduction and Embryology |
| **EUS** | Endoscopic ultrasonography |
| **FasL** | Fas- ligand |
| **GAPDH** | Glyceraldehyde 3-phosphate dehydrogenase |
| **GI** | Gastrointestinal |
| **GnRH** | Gonadotrophin releasing hormone |
| **HS** | Highly significant |
| **hs-CRP** | High sensitive C reactive protein |
| **ICAM1** | Intercellular adhesion molecule-1 |
| **IFN-γ** | Interferon-γ |
| **IgG** | Immunoglobulin G |
| **IL** | Interleukin |
| **IUD** | Intrauterine device |
| **KAR** | Killer activating receptor |
| **KIR** | Killer inhibitory receptor |
| **LNG.IUS** | Levonorgestrel intrauterine system |
| **MCP1** | Monocyte chemotactic protein 1 |
| **MHC** | Major histocompatibility complex |
| **ml** | Millilitre |
| **MM** | Minimal-mild |
| **MMP** | Matrix metalloproteinases |
| **MRI** | Magnetic resonance imaging |
| **mRNA** | Messenger ribonucleic acid |
| **MS** | Moderate-severe |
| **mt DNA** | Mitochondrial DNA |

| Abb. | Meaning |
|---|---|
| **NF** | Neurofilament |
| **ng** | Nanogram |
| **NK** | Natural killer |
| **NPV** | Negative predictive value |
| **NPY** | Neuropeptide Y |
| **NS** | Non significant |
| **PBMs** | Peripheral blood monocytes |
| **PC** | Peritoneal cavity |
| **PF** | Peritoneal fluid |
| **Pg** | Picogram |
| **PGE2** | Prostaglandin E2 |
| **PGF2α** | Prostaglandin F2α |
| **PGP9.5** | Protein gene product 9.9 |
| **PGs** | Prostaglandins |
| **PID** | Pelvic inflammatory disease |
| **PlGF** | Placental growth factor |
| **PM** | Peritoneal macrophages |
| **PP14** | Placental protein 14 |
| **PPV** | Positive predictive value |
| **PRα** | Progesterone receptor alpha |
| **PRβ** | Progesterone receptor beta |
| **r-AFS** | Revised American fertility society |
| **RANTES** | Regulated on activation, normal T cell expressed and secreted |
| **r-ASRM** | Revised four staging scoring system of American society of reproductive medicine |

| Abb. | Meaning |
|---|---|
| **ROC** | Receiver operating characteristic |
| **ROS** | Reactive oxygen species |
| **RT-PCR** | Real time polymerase chain reaction |
| **S** | Significant |
| **S.ICAM1** | Soluble form of intercellular adhesion molecule-1 |
| **SD** | Standard deviation |
| **SLE** | Systemic lupus erythematosus |
| **SP** | Substance P |
| **SPRMS** | Selective progesterone receptor modulators |
| **TGF.B** | Transforming growth factor B |
| **TIMP** | Tissue inhibitor of metallo proteinases |
| **TNF-α** | Tumor necrosis factor alpha |
| **TVS** | Transvaginal sonography |
| **TVU** | Transvaginal ultrasound |
| **VEGF** | Vascular endothelial growth factor |
| **VIP** | Vasoactive intestinal peptide |

# Introduction

Endometriosis is a benign gynecological disease defined as the presence of endometrial-like glands and stroma outside the uterine cavity, most commonly implanted over visceral and peritoneal surfaces within the female pelvis ***(Van Gorp et al., 2004; Flores et al., 2007).***

Although the exact prevalence of endometriosis in the general population is not clear, the prevalence in women of reproductive age is estimated to range between 10 and 15%.

Endometriosis occurs mainly in women of reproductive age (16–50 years) and has a progressive character in at least 50%, but the rate and risk factors for progression are unknown ***(D'Hooghe et al., 2006)***. It is commonly associated with subfertility and a range of pelvic pain symptoms such as chronic dysmenorrhea, premenstrual abdominal and pelvic pain, back pain, dysuria, dyschezia and dyspareunia. However, the relationship between different pains and endometriosis is not well understood and there is poor correlation between the severity of pain symptoms and anatomical staging of the disease ***(Chapron et al., 2003)***.

The diagnosis of endometriosis can be suspected in women with pelvic pain and/or subfertility, although endometriosis may be completely asymptomatic ***(Kennedy et al., 2005)***. Clinical detection of abdominal or pelvic pain can

be suggestive of endometriosis. Vaginal ultrasound is an adequate diagnostic method to detect ovarian endometriotic cysts and deeply infiltrative endometriotic noduli, but does not rule out peritoneal endometriosis or endometriosis-associated adhesions. The gold standard for the diagnosis of endometriosis is laparoscopic inspection, ideally with histological confirmation ***(Kennedy et al., 2005)***

The diagnosis of endometriosis is a major stumbling block for both clinical management and research studies of this enigmatic disease. At the moment, there is no simple, reliable, non-invasive way to diagnose endometriosis, although there are a number of studies currently underway to try and identify 'biomarkers' of this disease ***(Kennedy et al., 2005)***.

Development of a non-invasive diagnostic test for endometriosis would have a groundbreaking impact on the patients' quality of life, on the efficacy of available treatment as well as on the cost of endometriosis. However, a recent survey completed in 7025 women with endometriosis ***(European Endometriosis Alliance, 2006)*** demonstrated that 65% of the women with endometriosis were first misdiagnosed with another condition, and 46% had to see five doctors or more before they were correctly diagnosed, resulting in an average delay of 8 years between the onset of symptoms and the diagnosis of endometriosis ***(Zondervan et al., 1999;***

***Ballard et al., 2006).*** A simple diagnostic test is urgently needed.

So far, non-invasive approaches such as ultrasound, magnetic resonance imaging or blood tests have not yielded sufficient power for the diagnosis of endometriosis ***(Chen et al., 1998; Mol et al., 1998; Zondervan et al., 1999; Harada et al., 2002; Somigliana et al., 2004; Kennedy et al., 2005; Ballard et al., 2006).*** However, most studies evaluating biomarkers for the diagnosis of endometriosis have shown various limitations: low patient number, mostly assessment of only one biomarker, univariate analysis only if multiple biomarkers were tested, or lack of consideration for biomarker variability according to menstrual cycle phase ***(O'Shaughnessy et al., 1993; Tabibzadeh et al., 1995a, b; Abrao et al., 1997; Bon et al., 1999; Harada et al., 2002; Somigliana et al., 2004; Xavier et al., 2005, 2006).***

Cancer antigen 125 (CA125), a high molecular weight glycoprotein, is widely used for differentiation of benign and malignant ovarian masses in gynecology. It is the most significant tumor marker for the diagnosis of epithelial ovarian carcinomas. It has been reported that more than 80% of patients with ovarian carcinoma have a CA125 concentration

above 35 IU/mL, compared to 1% of normal women. ***(Boyer et al. 1994).***

Elevated serum CA125 concentrations have been recognized in a variety of other gynecological malignancies such as tubal, endometrial, endocervical. Although the positive predictive value of CA125 >95 IU/mL for ovarian cancer is quite high (96%) in postmenopausal women with an adnexal mass, the specificity is lower in premenopausal women, as elevations may occur in the presence of adenomyosis, uterine fibroids, pelvic inflammatory disease, pregnancy, menstruation, or especially in endometriosis.

Serum CA125 concentrations in patients with endometriosis are seldom >100 IU/mL. Sometimes, serum CA125 concentrations in women with endometriosis can elevate rapidly as a consequence of peritoneal irritation as a result of acute rupture of an endometriomal cyst ***(Harada et al. 2002).*** In two different reports CA125 concentrations of 9300 IU/mL ***(Ye et al. 1994),*** and 6114 IU/mL ***(Ferrero et al. 2007)*** were demonstrated in association with ruptured endometriomas.

The human endometrium produces and secretes CA125, thus serum concentrations of CA125 during menstruation are approximately threefold higher than those before menstruation in women with endometriosis.

Another tumour marker is CA19-9 that is elevated in patients with malignant and benign ovarian tumours in gynecology ***(Ye et al. 1994).***

Serum CA19-9 concentrations are elevated in patients with gastrointestinal system malignancies, or malignant and benign ovarian tumours. However, there are some reports showing elevated serum CA19-9 concentrations in endometriosis ***(Harada et al. 2002).***

Increased concentrations of ccf DNA have been found in inflammatory conditions, such as systemic lupus erythematosus and rheumatoid arthritis ***(Galeazzi et al., 2003; Zhong et al., 2007a).***The discovery of circulating cell-free (ccf) DNA in circulation has opened up the possibilities of non-invasive diagnosis and monitoring of a wide variety of malignant diseases.

Additionally, there have been several recent studies demonstrating the existence of another species of circulating nucleic acids, i.e. mitochondrial DNA (mtDNA). Both ccf nDNA and ccf mtDNA in circulation have been found to be elevated in trauma ***(Lo et al., 2000; Lam et al., 2004)***, suggesting that cell death is the source of ccf DNA, including the proportion of histone-protein bound molecules and unbound part molecules ***(Seefeld et al., 2008).***