EndomKit

The novel tool in the diagnosis of endometriosis

A CE marked IVD test to shorten the time for diagnosis, allowing early and optimized disease management.

DISEASE PROFILE

Endometriosis is a chronic, debilitating disease defined as endometrial-like tissue outside the uterine cavity that leads to an oestrogen-dependent chronic inflammatory state, frequently associated with dysmenorrhea, non-menstrual pelvic pain and infertility[1-3]. There are three clinical manifestations of endometriosis:

- Superficial endometriosis
- Ovarian endometrioma
- Deeply infiltrating endometriosis (DIE).

Endometriosis can be classified based on a point system, into one of four progressive stages (Figure 1) depending on location, extent, and depth of endometriosis implants; presence and severity of adhesions; and presence and size of ovarian endometriomas. Most women have minimal or mild endometriosis, which is characterized by superficial implants and mild adhesions. Moderate and severe endometriosis are characterized by chocolate cysts in the ovaries and more severe adhesions. Although the stage of endometriosis does not always correlate with the presence or severity of symptoms, infertility is very likely with stage IV endometriosis[23].

It affects 5-10% of women of reproductive age. The prevalence of the disease is 50% among women with chronic pelvic pain and 24% among infertile women[4,16]. Despite this high prevalence, disease recognition is inadequate and diagnosis time ranges from 4 to 11 years, with 65% of women being initially misdiagnosed[18].

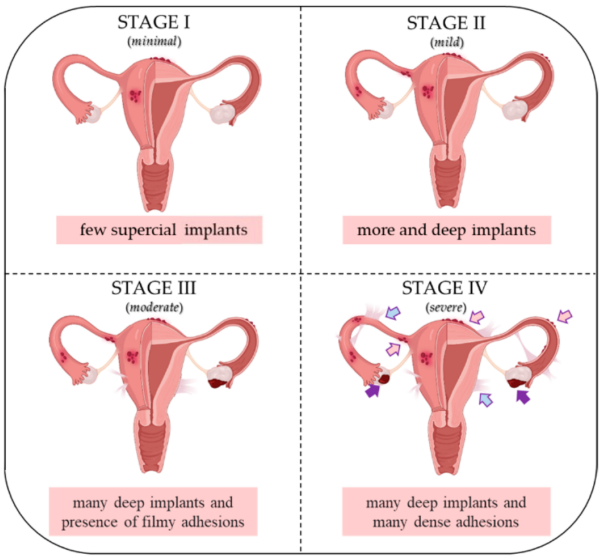

Figure 1: ASRM classification of endometriosis I–IV stages. Pink arrows indicate superficial implants; purple arrows indicate dense adhesions; blue arrows indicate filmy adhesions[24]

CURRENT METHOD OF DIAGNOSIS

At present, endometriosis is presumptively diagnosed as a result of patient symptomatology and imaging techniques (transvaginal ultrasound and magnetic resonance imaging). However, confirmation of the disease can only be obtained by a laparoscopic surgery followed by histopathological evaluation of the lesions[17]. Although being the gold standard for diagnosing the disease, laparoscopy being invasive contributes to this delay in diagnosis[5,16]. The diagnosis of women presenting only with superficial lesions is of special interest due to the limited value of existing imaging techniques for their identification, possibly leading to underdiagnosis and numerous misdiagnoses[16].

Earlier diagnosis with a simple assay could help provide timely treatment of young patients, thereby avoiding severe complications and improving quality of life while facilitating clinical management (medical treatment, surgery, assisted reproductive technologies).

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NEW DIAGNOSTIC TOOL – The EndomKIT

Given the tremendous unmet clinical need for a non-invasive diagnosis method for endometriosis, Exeltis has investigated a panel of biomarkers present in peripheral blood samples of women undergoing laparoscopy in combination with women's clinical parameters that have shown to be relevant to distinguish the presence of the disease. By providing added value in detecting cases of superficial lesions, this diagnostic tool addresses a crucial gap in current diagnostic capabilities.

The final model resulted in a diagnostic kit, now available as the EndomKIT, a test renowned for its high specificity, making it a simple and accurate rule-in test for early identification of the disease, even in the presence of non-specific symptoms. The EndomKIT combines the measurement of two serum markers (BDNF and CA125) by ELISA with patient's answers defining 6 clinical variables, resulting in 8 values as input for an algorithm made available online (https://endomkit.com). It allows automated and simultaneous measurement of 42 patient samples.

Why measuring BDNF?

BDNF (brain-derived neurotrophic factor) is a neurotrophin with a high affinity to neurotrophic tyrosine receptor kinase 2 (NTRK2)[6,16]. This ligand-receptor pair participates in some aspects of uterine physiology[7].

Estrogens strongly induce the production of BDNF in macrophages, which in turn binds to NTRK2 receptors on nerves, promoting neurogenesis. Estrogens also contribute to increasing the pain experienced from endometriosis by triggering the release of pro-inflammatory mediators from mast cells, which sensitize peripheral nerve endings in endometriotic lesions[8,9]. Protein expression for BDNF and NTRK2 were found to be greater in the eutopic endometrium of women with endometriosis compared to disease-free controls[10,11]. In addition, the expression of BDNF mRNA in ectopic lesions of women with endometriosis, has been found to be significantly elevated compared to eutopic endometrium of women without endometriosis[12].

Why measuring CA125?

The second biomarker measured in the EndomKIT is CA125 (cancer antigen 125), a glycoprotein expressed on cell surfaces of some derivatives of embryonic coelomic epithelium, believed to be precursors of endometriotic lesions[15]. Studies found CA125 levels to be higher in patients with endometriosis, indicating that CA125 can be a useful marker for diagnosing endometriosis, distinguishing the disease severity, monitoring the treatment effect, and identifying malignant transformation[19-21]. However, a negative CA125 test cannot reliably rule out the condition. It is particularly sensitive in diagnosing moderate to severe endometriosis (stages III and IV)[16].

On the other hand, BDNF shows better accuracy in diagnosing lower stages (I and II) of the disease[12,13,22]. Surgical removal of lesions reduces plasma BDNF levels in these patients[14].

Combining both biomarkers enhances diagnostic accuracy for endometriosis across all stages compared to using each biomarker alone[16].

Why including 6 clinical variables?

Initially 122 clinical variables were considered for inclusion in the multivariable diagnostic algorithm, extracted from psychometric questionnaires obtained from the Oxford Endometriosis CaRe Centre at Oxford University. Of the assessed clinical variables from the patients' medical histories, six were found to be significantly different between endometriosis cases and controls, i.e. record of previous surgery, painful periods as a symptom leading to referral for endometriosis, the severity of menstrual pain during last cycle, age at first experience of intercourse pain, age at first regular use of painkillers and age at first diagnosis of ovarian cyst[16].

The diagnostic medical software consists of an interface between the diagnostic data treatment algorithm and the user. The input parameters for the system are the concentrations of BDNF and CA125 obtained by the ELISA test and patient clinical information. The system provides a diagnosis (positive/negative) along with a diagnostic report. A rule-in test helps to diagnose the disease with high certainty when the result is positive. However, it's essential to acknowledge that a negative EndomKIT result cannot definitively rule out endometriosis. The diagnostic tool's performance remains robust even in the presence of confounding conditions like benign ovarian cysts or uterine fibroids, minimizing the risk of false positives[16].

Having the certainty that a positive test result corresponds to a woman with the disease, would allow for a personalized disease management as well as help general physicians or non-specialized gynecologists to refer patients to more specialized centers[17].

A testing strategy based on the EndomKIT and the described test characteristics is described in figure 2.

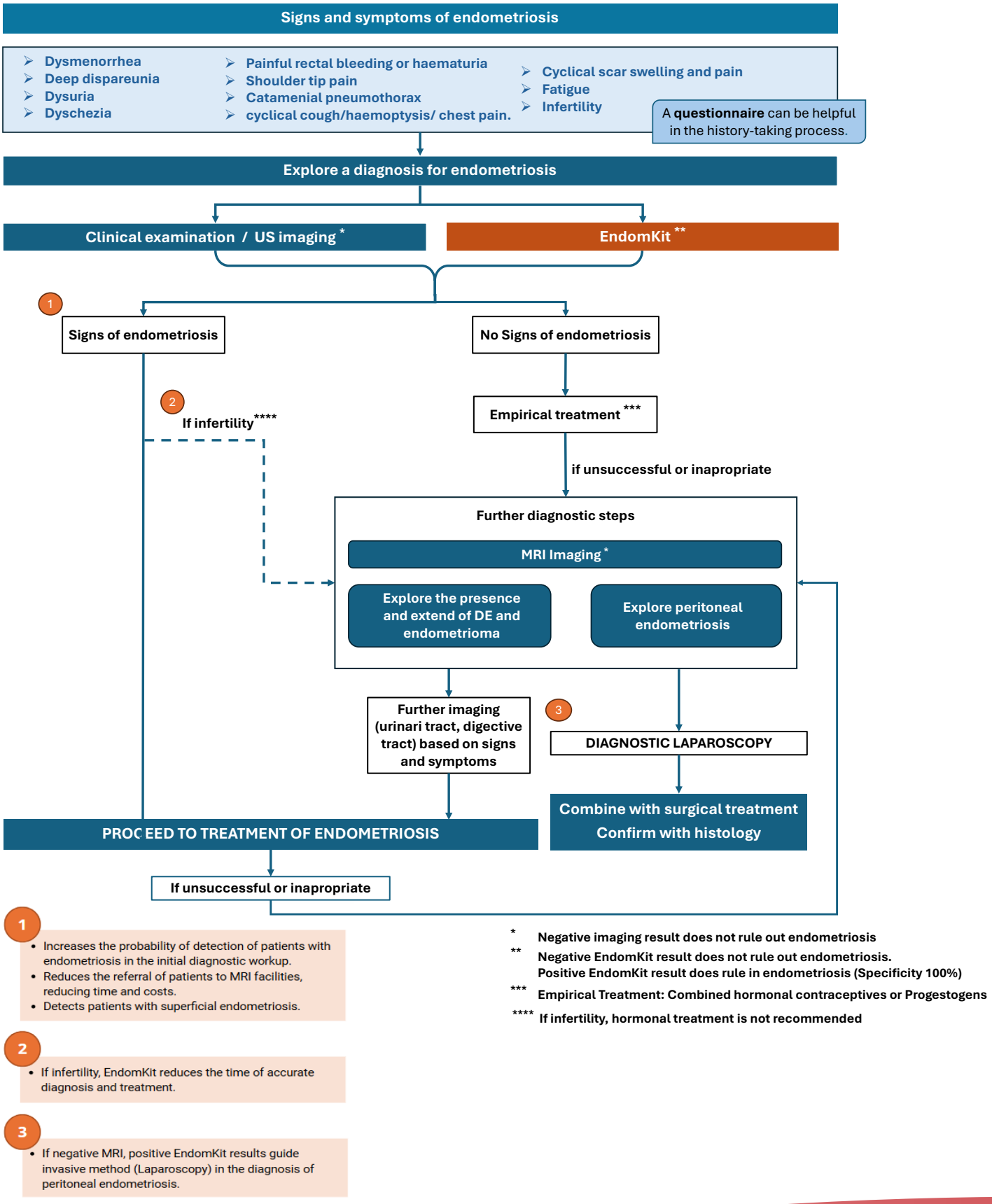

Figure 2: EndomKIT – use as an aid in diagnosing endometriosis (Rule-in test)

CLINICAL PERFORMANCE OF THE ENDOMKIT - Validation Study

Serum samples from n = 79 patients were included in the validation study: n = 52 patients with endometriosis, and n = 25 controls. Table 1 shows the demographic characteristics of these patients. In this study, low-stage (I–II) endometriosis patients represented 81% of the cases.

The validation study involved computing algorithm scores and corresponding outcomes using the IVD test software. A positive diagnosis was assigned when the score exceeded the predetermined cut-off, while a negative diagnosis was given when the score was below the cut-off.

The diagnostic performance of the IVD test had a diagnostic sensitivity (after weighting for disease stages) of 46.2% (95% CI: 25.5–66.8%) and a specificity of 100% (95% CI: 86.7–100%). The accuracy was 64.1% (95% CI: 50.4–77.8%), and the AUC was 0.758 (95% CI: 0.650–0.867). A good specificity was the primary objective because this assay is primarily intended to aid in identifying individuals with endometriosis. Interestingly, even in the presence of various confounding medical conditions, the diagnostic performance is not significantly affected[25].

The use of hormonal therapy and/or presence of potentially confounding medical conditions must be considered in the interpretation of the test results.

Hormone intake (COCP, GnRH agonists and POP) in the 3 months prior to blood extraction may increase the occurrence of a wrong diagnosis. Also, in the event that a patient is transitioning from one treatment to another, it is advisable to wait for a period of 3 months before conducting the test.

Table 1: Demographic characteristics of the patients in the external validation cohort

	Controls N=25	Cases N=52
Age years (mean ± SD)	35 (6.44)	35 (6.47)
BMI (mean ± SD)	26 (5.23)	26 (5.14)
rASRM classification		
I–II	-	42 (81%)
III–IV	-	7 (13%)
Missing information		3 (6%)
Endometriosis Classification		
Superficial	-	25 (48.1%)
Endometrioma	-	3 (5.8%)
DIE	-	14 (26.9%)
DIE + endometrioma	-	8 (15.4%)
Unclassified	-	3 (5.8%)
Other conditions		
Ovarian cysts	11	16
Ovarian cancer	0	4
Uterine fibroids	3	4
Adenomyosis	1	1

Note. BMI= Body Mass Index; rASRM= revised American Society for Reproductive Medicine, DIE = Deep Infiltrative Endometriosis.

Sensitivity	46%
Specificity	100%
Disease prevalence = 50% (Chronic pelvic pain)	
PPV50	100%
NPV50	65%
Disease prevalence = 24% (Infertility)	
PPV24	100%
NPV24	85%

CONCLUSION

The EndomKIT is now available as an excellent rule-in assay facilitating tremendously the diagnosis of endometriosis and providing significant value in the clinical management of this disease. It is simple to use and can be performed even in non-specialized settings[16]. The doctor should explain the EndomKIT test result and recommend the best disease management strategy according to the patient's clinical history.

For more information, contact us!

EndomKIT REF 780001
ELISA Kit containing 48 tests for measuring BDNF and CA125, combined with questionnaire for defining 6 clinical variables.

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