Validation of specific biomarkers for the diagnosis of endometriosis

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Current performance of biomarker signatures for non-invasive diagnosis, based on endometrial and blood samples combined with an algorithm, are the following (determined during previous studies): Sensitivity Specificity PPV NPV EndoSearch...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Uterine, pelvic and broad ligament disorders
Study type	Interventional

Summary

ID

NL-OMON49755

Source
ToetsingOnline

Brief title
EndoSearch

Condition

- Uterine, pelvic and broad ligament disorders

Synonym
pelvic pain and infertility caused by endometrial tissue outside of the uterus, uterine disorder

Research involving
Human

Sponsors and support

Primary sponsor: 1988
Source(s) of monetary or material Support: Endodiag is funded by H2020 program

Intervention

Keyword: biological samples, diagnosis, endometriosis

Outcome measures

Primary outcome

Primary endpoints

The analytical validation of biomarkers identified in the diagnosis of endometriosis will be based on the following results:

- Immunohistochemistry localization marking;

- Immunohistochemistry marking rate;

- Differential expression profile of microRNA.

The possibility, with biomarkers and an associated algorithm, to differentiate between endometriosis patients versus healthy control patients.

The primary endpoints will be determined from analysis results and are composed of sensitivity, specificity, NPV and PPV. These criteria will allow a conclusion on biomarker signature performance in diagnosing endometriosis.

Secondary outcome

Secondary endpoints

The analytical validation of biomarkers for the prognosis of endometriosis recurrence will be based on:

- Immunohistochemistry localization marking;

- Immunohistochemistry marking rate;

- Differential expression profile of microRNA.

The possibility, with biomarkers and an associated algorithm, to identify endometriosis patients in recurrence after 2 years of follow-up

The secondary endpoints will be determined from analysis results and are composed of sensitivity, specificity, NPV and PPV. These criteria will allow a conclusion on biomarker signature performance in predicting endometriosis recurrence for each patient.

Study description

Background summary

1. Context and current state of knowledge
1.1. Endometriosis
Endometriosis is a major and debilitating gynaecological disease, affecting 10% of all women of reproductive age, involving about 180 million patients worldwide. Disease diagnosis usually occurs more than nine years after disease manifestation, because of non-specific symptoms, severe chronic pelvic pain and infertility (in 40% of patients with endometriosis), and also by invasive diagnosis through surgery.
This is a debilitating and costly disease, costing 78 billion euros in major European countries and 79 billion dollars in the United States each year.

1.2. Aetiology and classification
Endometriosis is caused by tissue that lines the inside of the uterus which develops outside the uterus, in the abdominal cavity, resulting in lesions and/or adhesions in colonized organs and/or ovarian cysts, and sometimes affecting the brain and lungs.
Among the three main aetiological hypotheses for endometriosis are: (i) retrograde menstrual flow, (ii) embryogenesis and (iii) in situ development of endometriotic lesions. The preponderant hypothesis is retrograde flow of endometrial cells into the abdominal cavity during menstruation.
Endometriosis is a complex disease, with heterogeneous lesions due to their localization (peritoneal, deep and/or ovarian) or their color (red, white, black, red and black, etc.). Disease extent varies strongly between patients. Some patients have a few small lesions, while others have large ovarian endometriotic cysts (endometriomas) and/or extensive fibrosis and adhesions in various degrees of severity, leading to significant anatomical changes in the

pelvic area.

The principal classification used for endometriosis, from the American Fertility Society, revised in 1985, is used by surgeons to classify the severity of each degree of endometriosis by stage. This classification is based on intraoperative observation of endometriosis implantations in the peritoneum and/or ovarian area. The endometriosis stage depends on its extent and depth. Adhesions are also included in the score, which considers their density.
1. Stage I corresponds to rAFS score from 1 to 5.
2. Stage II corresponds to rAFS score from 6 to 15.
3. Stage III corresponds to rAFS score from 16 to 40.
4. Stage IV corresponds to rAFS score higher than 40.
It is important, however, to understand that this classification does not consider the progressive nature of the disease, and it has no predictive or prognostic value. Indeed, some small anatomical forms are very aggressive in terms of infertility, inefficacy of drug treatment, and persistence/recurrence despite surgery. By contrast, some advanced forms according to the classification (large cysts, for example), are easily accessible by laparoscopy and do not recur.

1.3. Endometriosis symptoms and impact
Patients with endometriosis present with severe, chronic pain (menstrual cramps, dysmenorrhoea), disabling, often unbearable pain, frequently in the lower back, the pelvis and abdomen, painful urination and irregular and excessive menstruation, and infertility in more than 40% of patients. Endometriosis interferes with the everyday life of patients, their social as well professional lives, and consequently their physical and emotional wellbeing. The disease impacts their quality of life as well, due to chronic pelvic pain, multiple surgeries, and hormonal treatments or IVF cycles which are doomed to fail.
Endometriosis is also characterised by a high recurrence rate: 50% within two years after surgery.
Despite its high incidence and prevalence, endometriosis is diagnosed with a mean delay of nine years, during which time the disease continues to progress. Societal costs of endometriosis are 79 billion dollars in the USA and 78 billion euros in the principal European countries per annum. These costs are comparable with the costs of other diseases; e.g., type II diabetes or Crohn’s disease. In France, the annual cost of endometriosis is about 21,000 euros per patient.

1.4. Current diagnosis and treatment of endometriosis
Endometriosis can be suspected during the clinical examination, combined with imaging techniques, such as ultrasound and MRI. However, these techniques are used only in first-line because of their significant limitations (operator dependent and non-specific). The gold standard of endometriosis diagnosis is laparoscopy under general anaesthesia, in order to perform an endometriotic lesion removal and tissue analysis by a pathologist. The diagnostic

intervention is also therapeutic. Generally, the diagnosis is made when pain is unbearable for the patient and/or when the abdominal cavity is massively invaded in severe forms of the disease.

Despite these alarming key facts, there is no definitive treatment. The American Society for Reproductive Medicine (ASRM), describes endometriosis as a chronic disease requiring medical monitoring throughout a patient's life. This active monitoring helps to avoid multiple surgical interventions in favour of hormonal treatment. Unfortunately, these treatments are not specific, have limited efficacy and are often associated with irreversible side effects (loss of bone density, virilization, acne, depressive disorder, etc.). Any recurrence of debilitating chronic pain is evidence of the recurrence of endometriosis.

In more detail, therapeutic options combine surgical and medical treatments.
Conservative surgery is an option frequently used:
• Operative laparoscopy (most frequent treatment). Some patients require over ten surgical procedures. These surgeries are often extreme and radical with organ ablation (bladder, terminal colon, hysterectomy, ovariectomy), and a greater risk of significant complications and morbidity. The chances of conception after a second surgery are two times less than after a first surgery because of decreased ovarian reserve.
• Hysterectomy with ovariectomy and excision of all the lesions in situ to prevent any risk of recurrence (often the "last resort" procedure).
Endometriosis drug therapies most commonly used:
• Non-steroidal anti-inflammatory drugs to relieve pain.
• Hormonal treatments to block the gonadotropic axis and inhibit lesion growth.

These hormonal treatments include:
• Progestogens and combined oral contraceptives (COC)
• LH-RH agonists. This treatment cannot be prescribed for more than one year because of significant adverse effects (loss of bone density)
• Danazol (serious virilization side effects)
To improve management of the endometriosis patient, it is essential to:
- Diagnose the disease earlier with a non-invasive and reliable method;
- Monitor the patient and her disease to assess treatment efficacy and early detection of recurrence;

This will allow detection, from the first symptoms, of women with endometriosis, and avoid therapeutic wandering and multiple visits and unsuitable treatments. The objective is also to improve individual patient monitoring to prevent recurrence and multiple surgeries. Finally, the preservation of fertility and the possibility of procreation are also a major challenge.

2. General description of the research

The study described in this protocol deals exclusively with specific biomarkers in the endometrium and in the blood in order to validate the analytical

specificity and sensitivity of endometriosis biomarkers in view of:
- early diagnosis of endometriosis;
- prognosis of disease recurrence for patients with endometriosis.

Endodiag will analyse several biomarkers using ImmunoHistoChemistry (IHC) and molecular biology techniques on human samples collected by investigators. These results, in correlation with clinical data from patients, will confirm the diagnostic and prognostic potential of the identified biomarkers in relation to endometriosis.

3. Summary of preclinical and clinical data
This project of, 1) diagnosis of endometriosis based on endometrial and/or blood samples and, 2) prognosis regarding disease recurrence, has already been investigated during a retrospective study on human samples in the context of which we have already obtained an authorization from the French Ministry of Higher Education and Research (date 02/12/2013, ref. No. DC-2013-1878) to store and prepare human samples for research purposes (named CODECOH).

3.1. Diagnosis from blood sample
Gold standard for the diagnosis of endometiosis is laparoscopy surgery, also allowing to treat by excision the visible endometriosis lesions. Currently, no signature of circulating biomarkers to diagnose endometriosis has been validated. Several research teams, including Endodiag, have studied the potential role of microRNAs in the development of the disease, because they have been described as regulating the expression of genes identified as implicated in the pathogenesis of endometriosis.
A study carried out on 51 serum samples (36 patients and 15 controls) allowed the identification of microRNAs of interest after clustering, some being low expressed (8) and others overexpressed (4) in patients. These biomarkers have been described as intervening in cellular functions potentially related to the ethiopathology of endometriosis (embryology, cell cycle, DNA replication, chromatic architecture, intracellular signalling, proteolysis and vesicular transport ...). These results have been patented.
Endodiag licensed the patent from this comparative study of blood samples from patients (aged 18-45 years) followed for endometriosis and controls (who had laparoscopic surgery for other indications and during which absence of endometriosis has been validated). Proteomic analyses and circulating biomarkers research allowed to identify microRNAs expressed differentially between the two groups (patients vs. controls).
A retrospective validation study on 60 serum samples, collected during the initial study and carried out in Endodiag laboratories, validated the performance of the licensed signature (sensitivity 90%, specificity 87%, PPV 88% and NPV 90%). The analytical validation of this signature will be carried out within the framework of this protocol and should result in the definition of a diagnostic kit, which will itself be validated clinically in a second study.

3.2. Endometriosis diagnosis from endometrial biopsy
Several publications have shown that there are biological differences between an endometriosis endometrium and a healthy endometrium. This information provides the perspective for non-invasive diagnosis from endometrial samples. We have conducted our own research program on endometriosis-specific endometrial biomarkers, and we have identified an 8 biomarker-signature with high specificity and sensitivity for endometriosis diagnosis. The current performances of our biomarker signatures are: sensitivity 95%, specificity 88%, PPV 85% and NPV 95%.
• Biomarker discovery: Endodiag is based on 20 years of endometriosis research conducted by its two founders to identify an initial panel of 35 biomarkers. They belong to signalling pathways: angiogenesis, immune and inflammatory system, hormonal, migration, proliferation and stem cells.
• Qualification of the identified biomarkers in 2015: these 35 biomarkers were studied in a first retrospective study on 55 endometrial biopsies embedded in paraffin. IHC analysis was performed and the correlation between biomarkers expression and results of diagnosis by laparoscopy were investigated. This study validated the analytical method, IHC, as robust and reliable. We then validated the identified biomarkers for diagnostic purposes.
• Verification of the biomarkers in 2016: based on the previous study results, we conducted a prospective study on 80 patients treated in 3 clinical centres (Saint Louis, United States, Fribourg, Switzerland and Paris, France) to validate biomarker diagnostic value. The analysis looked at the correlation between biomarkers expression and laparoscopic diagnosis. The results were analysed by an external data mining and biostatistics service provider to identify the most relevant biomarker combination, and to separate patients versus controls within a heterogeneous population with high sensitivity and specificity.

This combination of 8 biomarkers is associated to an interpretation algorithm to differentiate patients versus controls and diagnose endometriosis with certainty using an endometrial sample.
The validation study proposed in this protocol will analytically validate the identified biomarkers, the final aim being to develop a non-invasive diagnostic kit.

Study objective

Current performance of biomarker signatures for non-invasive diagnosis, based on endometrial and blood samples combined with an algorithm, are the following (determined during previous studies):
Sensitivity
Specificity PPV NPV
EndoSearch endometrium 95% 88% 85%
95%
EndoSearch blood 90% 87%
88% 90%

The objective, in this study, is to obtain at least the following performance in a large cohort:
Sensitivity
Specificity PPV NPV
EndoSearch endometrium 85% 85% 85%
85%
EndoSearch blood 85% 85%
85% 85%

Study design

1. Study type
This study is a multicentre, international, prospective, open-label study, conducted on two patient cohorts: one “with endometriosis” and the other “without endometriosis”.
Endodiag already has an authorization to import human samples and the list of foreign collaborators will be updated when the project is approved. The principal objective is the analytical validation of biomarkers identified for the diagnosis of endometriosis. The secondary objective is prognosis of disease recurrence after surgery for each patient.
No randomization and blinding will be performed because the study involves neither a medicinal product nor a medical device for human use.
Both patient and the health professional know the health status of the patient: having endometriosis or not.

2. Study duration, chronology and duration of each period
Total study duration: 2 years and 9 months
Inclusion period: for 9 months, until reaching 975 patients.
Patient participation period: 2 years: patient inclusion, surgery and patient follow-up for two years after the surgery.

3. Exclusion period from another clinical trial protocol
Patients can participate in other biomedical research protocols, except if the aim of the investigation is the assessment of an innovative therapy for endometriosis or another disease; e.g., a new drug. A treatment can change the nature of collected biological samples and consequently the results of analysis can be different and introduce a bias.

4. Experimental plan – practical process
In this study, the patient pathway starts at the preoperative consultation, when the doctor announces her eligibility to participate in the study. If the patient is interested in the study, her doctor gives her the information letter and informed consent to make her aware of all the research conditions.
The patient learning all the necessary information, she can immediately sign the consent form in duplicate copy (one kept by patient and the other by the centre), or take time to think about her participation. The consent form must necessarily be signed and returned to the centre prior to any personal data

collection, surgical intervention and sample collection. The patient will keep the information letter and a copy of the signed consent form. This information will be accessible on the dedicated web platform for patients.
Once the consent form is signed, the physician will complete the questionnaires “patient record” and “medical history” in the e-CRF from information provided by the patient. These data are collected during the consultation and the physician will tick a box “send platform login email to the patient” and an email will be sent automatically to the patient allowing connection and filling in of the questionnaires.
In this way, the patient can access, via the internet, dedicated online questionnaires. She will be able to fill in the sections “Patient record”, “Your Medical History”, “Your Health data” and “Quality of life”.
The final confirmation of patient inclusion in the study is done by her doctor, depending on the findings during laparoscopic surgery. This surgery is intended to diagnose or treat patients with endometriosis or another procedure for a non-endometriosis disorder. During this surgery, the doctor will collect samples intended to be used by the sponsor for endometriosis research purposes. Part of the sample is reserved for the pathology department to diagnose the presence or absence of endometriosis; the other part is sent to Endodiag for research purposes. The patient is officially included only after diagnostic confirmation of endometriosis for “endometriosis category of patient” and “non-endometriosis category for control patient”. Every patient for whom an endometrial biopsy was collected will be included in the study, in the “endometriosis patient” group or in the “control” group; there will be no sampling without patient inclusion in the study.
After surgery, the doctor will complete alone, without the patient, the “surgery” section of the questionnaire in the e-CRF.
The patient will follow the usual medical care pathway established by her doctor for post-op follow-up.
One year after surgery, the patient will be solicited by email to reply to the questionnaire section “1-year follow-up” on the dedicated web platform.
Two years after surgery, patient will be solicited by email to reply to the questionnaire section “2-year follow-up” on the dedicated web platform.

The end of patient participation in the study occurs 2 years after surgery.

The examinations, sampling and consultations usually are:
- Visits: 1st consultation, pre-operative visit, surgery, post-operative visit depending on physician-established timing;
- Medical procedures: laparoscopic surgery;
- Sampling: endometriotic lesions, ovarian cysts, if applicable, intended for histological analysis (1 cm), 1 tube of 5 mL peritoneal fluid or, lacking that, peritoneal lavage.

Medical examinations, sampling and additional visits specifically performed for research purposes are:
- Visits: no additional visit;

- Medical procedures: no additional procedures other than those already planned for usual patient medical care;
- Sampling: endometrial biopsy with Cornier Pipelle (max 2 cm) and 4 tubes of 5 ml of blood.

The biological samples are collected during laparoscopic surgery; endometrial sampling is performed with an intravaginal Cornier Pipelle (provided in the sampling kit) and blood sampling is performed before anaesthesia or during surgery by a nurse in the clinical centre.
Samples are collected in the sampling kit provided by the sponsor. This kit is composed of all the necessary containers for each type of sample:
- 2 tubes of 5 ml with EDTA to collect blood;
- 2 tubes of 5 ml free of blood to collect serum;
- 1 tube of 5 ml with heparin to collect peritoneal fluid;
- 2 containers of 5 ml formalin each to collect endometriotic lesions and an endometrial biopsy;
- 1 container of 20 ml formalin to collect ovarian cysts;
- 1 container with culture medium to collect endometriotic lesions;
- 1 container of 2 ml RNAlater for the endometrial biopsy.
It also contains the Cornier Pipelle required to perform endometrial tissue sampling.

Intervention

The intervention is a laparoscopy planned for endometriosis diagnosis or treatment (endo group) or other indication (control group). The surgery is not performed specially for the study needs.

Study burden and risks

Summary of predictable and known benefits and risks to study participants

1. Benefits to patients
There will be no direct clinical benefit for the participants involved in this study.
Samples and clinical data will be used for research purposes only. Laboratory analyses performed on samples (IHC and molecular biology) and on anonymized data (statistical studies and probability calculations) will not yield immediate information about patient health and are without consequence for their medical care.

Study results may lead to the development of marketed products, without financial benefit for patients.

2. Risks to patients
Known and foreseeable risks for research participants are essentially related

to surgical intervention performed as part of patient care (laparoscopic surgery under general anaesthesia, endometriotic lesion biopsy, peritoneal fluid sampling) and samples specific to the research study (blood samples and endometrial biopsy). As explained below, some collected tissues come from expanded sampling in the surgical context of usual medical care of patients and some others are done specifically for the research study.
The risks mentioned below do not differentiate specific medical procedures for research and procedure already planned in the patient's medical care.
1) Risks related to laparoscopy
• Post-operative pain may occur because of cutaneous incisions;
• Transitory phenomena: vertigo, insomnia, fatigue, concentration disorders, abdominal pain and/or back pain;
• Very rare but serious complications requiring emergency treatment: haemorrhage, fistula, organ or vessel lesions due to instrument insertion;
• Impossibility to perform the surgical operation (adhesions, consequences of previous surgery, anatomical anomaly, technical difficulties, unexpected complications, etc.), which require stopping surgery or switching to the traditional procedure, laparotomy (open abdomen).

2) Risks related to general anaesthesia
• Projected shoulder pain due to CO2 gas not evacuated at the end of the surgery;
• Nausea, vomiting after awakening;
• Sore throat, hoarseness, difficulties swallowing, dental lesions due to introduction of the respiratory assistance device;
• Nerve, muscle and skin damage: temporary numbness or reversible paralysis;
• Memory disorders, decreased concentration ability;
• Redness or pain at the anaesthesia injection area;
• Very rarely (once in several hundred thousand cases): serious reactions during anaesthesia: allergy to anaesthetics, cardiac arrest, asphyxia.

3) Risks related to blood drawing
• Incident: haematoma due to very tight tourniquet, difficult sampling (non-visible, too deep, very thin veins, mobile, repeated punctures, early sclerosis, agitated behaviour from the patient), dizziness (anxiety)
• Accident: needle puncture (contamination with hepatitis B, C or Delta or HIV), median nerve puncture (reversible nerve lesion, very rarely a defect in a motor or sensory nerve), hypocalcaemia: diabetic coma, syncope (total loss of consciousness for about 10 seconds)

4) Risks related to endometrial biopsy, in order of increasing likelihood
• Significant bleeding;
• Bleeding during more than two days after biopsy;
• Minor bleeding and discomfort;
• Fever or chills;
• Infection;
• Serious pain in lower abdomen;

• Abnormal and/or foul-smelling vaginal discharge

5) Risks related to peritoneal fluid collection
• Bleeding at the puncture area, even intraperitoneal haemorrhage;
• Secondary infection of the fluid;
• Intestinal lesion;
• Loss of protein and electrolytes;
• Fainting, even vasovagal shock especially when depletion is too rapid.

Contacts

Public
1988

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Scientific
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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Voluntary patients with endometriosis
* Women aged 18 to 45 years

* Patients with suspicion of endometriosis; associated adenomyosis is accepted
* Informed, voluntary signature of the consent form
* Patient requiring laparoscopy for endometriosis purposes (first procedure or recurrence) regardless of the endometriosis type (superficial, ovarian or deep)
* Possibility of follow-up for 2 years., Healthy voluntary patients
* Women aged 18 to 45 years
* Informed, voluntary signature of the consent form
* Patient requiring laparoscopy for an indication other than endometriosis, adenomyosis, uterine fibroma & other fibroid pathologies (example of possible surgeries: tubal sterilization, non-fibroid ovarian cyst, urinary incontinence requiring ureteral intervention).

Exclusion criteria

Voluntary patients with endometriosis
* Refusal or linguistic or psychic incapacity to sign informed consent
* No internet access or refusal to use new technologies
* Minors (under 18 years old)
* Pregnancy or breastfeeding
* Menopause
* Evidence of adenomyosis alone, without endometriosis
* Any metabolic, endocrine, chronic infectious or malignant pathology.
* Negative result for endometriosis on visual and histological examination by pathologist at the clinical centre, Healthy voluntary patients
* Refusal or linguistic or psychic incapacity to sign informed consent
* No internet access or refusal to use new technologies
* Minors (under 18 years old)
* Pregnancy or breastfeeding
* Menopause
* Any metabolic, endocrine, chronic infectious or malignant pathology.
* Positive result for endometriosis on visual and histological examination by pathologist at the clinical centre.

Study design

Design

Study type:	Interventional
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)

Control:	Active
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	14-10-2019
Enrollment:	65
Type:	Actual

Ethics review

Approved WMO

Date:	20-09-2019
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Approved WMO

Date:	11-06-2020
Application type:	Amendment
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
Other	2017-A01445-48
CCMO	NL68861.098.19