



Turun yliopisto  
University of Turku

# MODERN METHODS OF EVALUATING ENDOMETRIOSIS

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Pia Suvitie



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*This work is dedicated to all teenagers and women  
with endometriosis*

## **ABSTRACT**

**Pia Suvitie**

### **Modern methods of evaluating endometriosis**

University of Turku, Faculty of Medicine, Institute of Clinical Medicine, Department of Obstetrics and Gynaecology, Doctoral Programme in Clinical Research, and Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology/Physiology, Turku, Finland

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Endometriosis is a common chronic disease, affecting women of reproductive age. Typical symptoms include severe menstrual pain, other pelvic pain symptoms as well as infertility. Adolescent onset of symptoms is common, and the delay between the onset of symptoms and the diagnosis is typically several years. Presently, the diagnosis can be confirmed only by laparoscopy. The symptoms can be alleviated with hormonal medications or surgery, but there is no curable treatment.

This study aimed to evaluate the prevalence of symptoms suggestive of endometriosis among adolescent girls. Furthermore, the value of a panel of 29 serum cytokines in the diagnosis of endometriosis, the usefulness of serum HE4 (Human Epididymis Secretory Protein 4), a novel biomarker for ovarian cancer, in discriminating ovarian endometriotic cysts from ovarian cancer, as well as the long-term effects of surgery on pain were assessed. These studies were based on two prospective cohorts: The ENDOMET study, including 137 endometriosis patients scheduled for surgery and 62 healthy women, and the TEENMAPS questionnaire study that included 1103 adolescent girls aged 15–19 years.

The study showed that dysmenorrhea was prevalent among teenagers, while other pain symptoms were less common. Importantly, approximately 5–10% of adolescent girls had symptoms suggestive of endometriosis. Among the potential diagnostic markers, the serum concentrations of five cytokines were significantly different between endometriosis patients and healthy controls, but these markers did not significantly improve the diagnostic accuracy of that obtained with the biomarker CA-125 alone. Interestingly, serum levels of HE4 were not increased in endometriosis, and thus, this biomarker is useful in differentiating ovarian endometriosis from ovarian cancer. Surgery was found to result in significant long-term alleviation of pain during 5-year follow-up, and women with deep infiltrating endometriosis benefitted the most.

**Keywords:** endometriosis, diagnosis, biomarker, HE4, CA-125, cytokine, dysmenorrhea, abdominal pain, dyspareunia, dyschezia, dysuria, adolescent, surgery

## TIIVISTELMÄ

**Pia Suvitie**

### **Uusia menetelmiä endometrioosin diagnostiikkaan ja hoitoon**

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen laitos, Synnytys- ja naistentautioppi, Turun kliininen tohtorihjelma, ja Biolääketieteen laitos, Integratiivisen fysiologian ja farmakologian tutkimusyksikkö ja Turun yliopiston Tautimallinnuskeskus, Turku, Suomi

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Endometrioosi on yleinen krooninen hedelmällisessä iässä olevien naisten sairaus, joka aiheuttaa voimakkaita kuukautis- ja lantiokipuja sekä lapsettomuutta. Oireet alkavat usein jo teini-iässä, mutta viive oireiden alusta diagnoosiin on tyypillisesti useita vuosia. Tällä hetkellä diagnoosi voidaan varmistaa vain laparoskopialla. Oireita hoidetaan hormonilääkkein ja/tai leikkauksella, mutta parantavaa hoitoa ei ole.

Tutkimuksessa selvitettiin endometrioosiin viittaavien kipuoireiden esiintyvyyttä teini-ikäisillä. Lisäksi arvioitiin 29 seerumin sytokiinin hyödyllisyyttä endometrioosin diagnostiikassa, uuden munasarjansyövän merkkiaineen HE4:n (Human Epididymis Secretory Protein 4) käyttökelpoisuutta munasarjan endometriosikystojen ja munasarjasyövän erotusdiagnoosiin sekä leikkaushoidon pitkäaikaisvaikutusta kipuun. Tutkimus koostui kahdesta prospektiivisestä aineistosta: ENDOMET-tutkimukseen rekrytoitiin 137 endometriosileikkauspotilasta sekä 62 tervettä naista ja TEENMAPS-kyselytutkimukseen osallistui 1103 15–19-vuotiasta tyttöä.

Tutkimuksen mukaan kuukautiskipu oli yleinen oire teini-ikäisillä, mutta muita endometrioosiin viittaavia kipuja esiintyi harvemmin. Noin 5–10%:lla teini-ikäisistä tytöistä oli endometrioosiin viittaavia oireita. Tutkituista merkkiaineista viiden seerumin sytokiinin pitoisuus erosi merkitsevästi endometriosipotilailla verrattuna terveisiin naisiin, mutta sytokiinit eivät lisänneet diagnostiikan tarkkuutta verrattuna pelkän CA-125-merkkiaineen käyttöön. Merkittävä löydös oli, että endometrioosi ei nostanut HE4-pitoisuutta ja merkkiainetta voidaan käyttää munasarjasyövän ja endometriosikystojen erotusdiagnoosiin. Endometrioosin leikkaushoito lievitti merkittävästi kipuja viiden vuoden seurannassa ja erityisesti syvää endometrioosia sairastavat naiset hyötyivät toimenpiteestä.

**Avainsanat:** endometrioosi, diagnostiikka, merkkiaine, HE4, CA-125, sytokiini, kuukautiskipu, vatsakipu, yhdyntäkipu, ulostamiskipu, virtsaamiskipu, teini-ikäinen, leikkaushoito

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## **ABBREVIATIONS**

|                |   |
|----------------|---|
| AUC            | Area under curve  |
| BENS           | Bowel Endometriosis Syndrome  |
| BFLUTS         | Bristol Female Lower Urinary Tract Symptoms   |
| CA-125         | Cancer antigen 125  |
| CA-19-9        | Cancer antigen 19-9   |
| CD40L          | Soluble CD40 ligand   |
| CI             | Confidence interval   |
| CPP            | Chronic pelvic pain   |
| DIE            | Deep infiltrating endometriosis   |
| DRG            | Dorsal root ganglion  |
| EDCs           | Endocrine disrupting chemicals  |
| E2             | Estradiol   |
| EGF            | Epithelial growth factor  |
| EHP-30         | Endometriosis Health Profile-30   |
| ENDOMET        | Novel diagnostic tools for endometriosis and their exploitation for prognosis and prevention of complications |
| EOC            | Epithelial ovarian cancer   |
| FSFI           | Female Sexual Function Index  |
| G-CSF          | Granulocyte colony-stimulating factor   |
| GIQLI          | Gastrointestinal Quality of Life Index  |
| GM-CSF         | Granulocyte–macrophage colony-stimulating factor  |
| HE4            | Human epididymal secretory protein E4   |
| HRQoL          | Health-related Quality of Life  |
| HSD            | Hydroxysteroid dehydrogenase  |
| IBS            | Irritable bowel syndrome  |
| IFN- $\gamma$  | Interferon gamma  |
| IL             | Interleukin   |
| IL-1Ra         | Interleukin-1 receptor antagonist   |
| IP-10          | IFN- $\gamma$ -induced protein-10   |
| IPSS           | International Prostate Symptom Score  |
| IVF            | In vitro fertilization  |
| KESS           | Knowles-Eccersley-Scott Symptom Questionnaire   |
| LNG-IUS        | Levonorgestrel-releasing intrauterine system  |
| MCP            | Monocyte chemotactic protein  |
| MIP-1 $\alpha$ | Macrophage inflammatory protein 1-alpha   |

## *Abbreviations*

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|               |   |
|---------------|---|
| miRNA         | microRNA  |
| MMP           | Matrix metalloproteinase                                      |
| MRI           | Magnetic resonance imaging                                    |
| NGF           | Neural growth factor  |
| NNE           | Enolase 1   |
| NRS           | Numerical rating scale  |
| NSAID         | Non-steroidal anti-inflammatory drug                          |
| OC            | Combined oral contraceptive                                   |
| OMA           | Endometrioma  |
| OR            | Odds ratio  |
| PCOS          | Polycystic ovary syndrome                                     |
| PF            | Peritoneal fluid  |
| PG            | Prostaglandin   |
| POD           | Pouch of Douglas  |
| PR            | Progesterone receptor   |
| RANTES        | Regulated in Activation, Normal T Cell Expressed and Secreted |
| ROC           | Receiver operating characteristics                            |
| ROS           | Reactive oxygen species                                       |
| RR            | Relative risk   |
| RVS           | Rectovaginal septum   |
| SF-MPQ-2      | Short-Form McGill Pain Questionnaire                          |
| SF-36         | Medical Outcomes Study Short Form 36                          |
| sICAM-I       | Soluble intercellular adhesion molecule-I                     |
| SOP           | Standard operating procedure                                  |
| sVCAM-I       | Soluble vascular cell adhesion molecule-I                     |
| TEENMAPS      | Teenage menstrual and abdominal pain symptoms                 |
| TGF- $\alpha$ | Transforming growth factor alpha                              |
| TNF           | Tumor necrosis factor   |
| TVUS          | Transvaginal ultrasound                                       |
| USL           | Uterosacral ligament  |
| USP           | Urinary Symptom Profile                                       |
| VAS           | Visual analogue scale   |
| VDBP          | Vitamin D binding protein                                     |
| VEGF          | Vascular endothelial growth factor                            |

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–IV. The original publications have been reprinted with the kind permission of the copyright holders.

- I. P. Suvitie, M. Hallamaa, J. Matomäki, J.I. Mäkinen, A. Perheentupa: Prevalence of pain symptoms suggestive of endometriosis among Finnish adolescent girls (TEENMAPS study): *J Pediatr Adolesc Gynecol* 2016 Apr;(2):97-103
- II. P. Suvitie, K. Huhtinen, T. Vahlberg, H. Kujari, M. Setälä, J.I. Mäkinen, M. Poutanen, A. Perheentupa: Performance of 28 serum cytokines and CA-125 as markers of endometriosis. *Manuscript*
- III. K. Huhtinen, P. Suvitie, J. Hiissa, J. Junnila, J. Huvila, H. Kujari, M. Setälä, P. Härkki, J. Jalkanen, J. Fraser, J. Mäkinen, A. Auranen, M. Poutanen, A. Perheentupa: Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br J Cancer*. 2009 Apr 21;100 (8):1315-9
- IV. P. Suvitie\*, T. D. Laajala\*, M. Setälä, P. Härkki, K. Huhtinen, T. Aittokallio, M. Poutanen, A. Perheentupa, Effect of Complete Endometriosis Surgery on Pain – A Prospective 5-year Follow-up. *Manuscript*

*\*These two authors have equally contributed to the manuscripts*

# 1 INTRODUCTION

Endometriosis is a common estrogen-dependent chronic inflammatory disease associated with lesions of functional endometrial-like tissue at ectopic sites of the pelvic cavity (Giudice 2010). Though the disease was microscopically discovered already in the 19<sup>th</sup> century, the underlying pathophysiology is very complex and still only partly understood (Nezhat et al. 2012). It affects millions of women worldwide at their childbearing age with an estimated prevalence of 1–10% (Giudice 2010, Eisenberg et al. 2018).

Endometriosis causes pelvic pain and infertility compromising the physical, mental and social wellbeing of the affected women (De Graaff et al. 2013). Dysmenorrhea is the key symptom, and it is often combined with noncyclic abdominal pain, dyspareunia, dyschezia and dysuria. Commonly patients experience their first symptoms already before the age of 20 years, but adolescents wait longer than adults before seeking for help (Greene et al. 2009).

No definitive cure exists for endometriosis and treatments aim to relieve symptoms or enhance fertility. The first line treatment of pain is hormonal suppression, typically with progestins or combined oral contraceptives (OC), aiming to decrease ovarian estrogen production and to induce amenorrhea or to reduce the frequency of menstrual bleedings (Dunselman et al. 2014). When medication is ineffective, laparoscopic removal of all visually detected lesions is the golden standard (Johnson et al. 2013). Surgical treatment of severe endometriosis is difficult and includes marked risk of complications. Thus, such operations should be performed in centers of expertise (Dunselman et al. 2014). Many patients, especially women with severe disease, need long-term hormonal medication, commonly need assisted reproductive treatments to achieve a pregnancy and typically undergo multiple surgeries. Treatments and impaired working productivity due to pain lead to significant societal and economic burden (Soliman et al. 2016).

One of the key challenges is the long delay, typically several years, between the onset of symptoms and the diagnosis (Nnoaham et al. 2011). Reasons for the delay are multifactorial and partly due to unawareness of the disease among women and the healthcare providers (Nnoaham et al. 2011). Typical symptoms can raise suspicion of endometriosis. Moreover, ovarian endometriomas and most deep nodules can be detected with modern imaging modalities, if available. In some patients, ovarian endometriomas show atypical ultrasound features and need to be distinguished from malignant ovarian tumors.

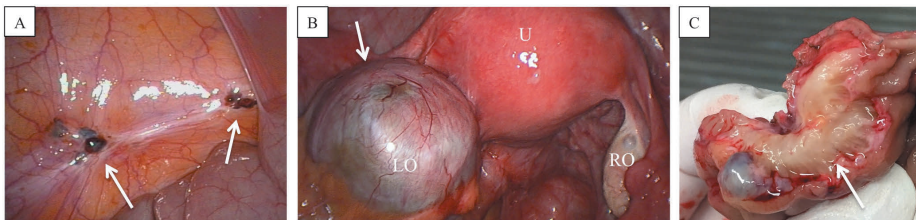
At present, the only way to confirm the diagnosis of endometriosis is laparoscopy. Despite active research, no non-invasive diagnostic methods, such as a blood

test, have been discovered to diagnose endometriosis. An important future goal is to shorten the diagnostic delay by raising the awareness of endometriosis and by developing non-invasive diagnostic methods. In addition, it is of importance to discover new medical treatment options, and more actively and at earlier age treat women with medications to avoid repeated surgical procedures and to preserve fertility.

## 2 REVIEW OF LITERATURE

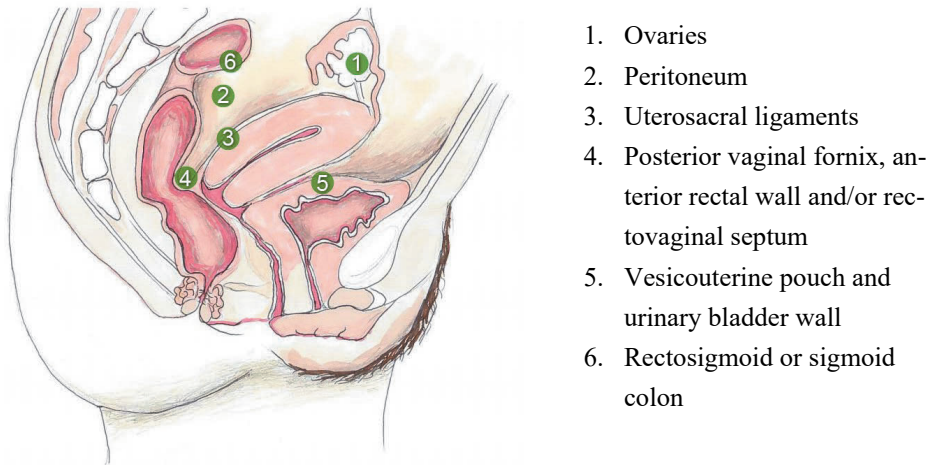
### 2.1 Definition and clinical presentations of endometriosis

Endometriosis is histologically defined by the presence of endometrial-like tissue, glands and stroma, outside the uterine cavity (Giudice 2010). Clinically it forms macroscopically detectable lesions of three types: peritoneal i.e. superficial lesions, ovarian endometriotic cysts i.e. endometrioma (OMA) and deep lesions i.e. deep infiltrating endometriosis (DIE) (**Figure 1**). Endometriosis lesions are usually located in the pelvis, they are typically multifocal, and all lesion types can be present in the same patient (Redwine 1999). Lesions are more common in the posterior pelvic compartment and in the left side independent of the disease type (Chapron et al. 2006, Scioscia et al. 2011). The typical anatomical locations of endometriosis are shown in **Figure 2**.



**Figure 1.** Laparoscopic appearance of black peritoneal endometriotic lesions (A), left ovary endometrioma (B) and deep infiltrating lesion of the resected sigmoid colon (C). Lesions are marked with arrows. U, uterus; LO, left ovary with endometrioma; RO, right healthy ovary.

**Peritoneal endometriosis** is a common lesion type (Redwine 1999). Forty per cent of patients presented with peritoneal endometriosis at their first surgery in a large Finnish nationwide register study (Saavalainen et al. 2018). The typical appearances include red, black and white lesions, and all types may occur in the same woman. Lesions vary in size from a few millimeters to several centimeters. The appearance reflects the age of the lesion beginning from a red “fresh” lesion that has recently adhered to the peritoneum (Giudice et al. 2012). Further on, it turns into a black lesion due to intralesional bleeding and hemosiderin deposits (**Figure 1A**) and finally evolves to a white lesion as it is accompanied with fibrosis induced by chronic inflammation.



1. Ovaries
2. Peritoneum
3. Uterosacral ligaments
4. Posterior vaginal fornix, anterior rectal wall and/or rectovaginal septum
5. Vesicouterine pouch and urinary bladder wall
6. Rectosigmoid or sigmoid colon

**Figure 2.** Typical locations of endometriotic lesions. Reprinted with permission (Tarjanne et al. 2017).

In addition to typical superficial lesions, adolescents may present with subtle lesions (clear, non-pigmented or light brown vesicles or as reddish polyps) that are more difficult to recognize in laparoscopy, leading to underdiagnosis in this specific age group. Further on, two thirds of pelvic peritoneal pockets, often detected in laparoscopy performed due to chronic pelvic pain, contain endometriosis either around the rim or at the bottom of the pocket (Giudice et al. 2012). Peritoneal pockets are also referred as Allen-Masters syndrome. Finally, occult microscopic lesions are detected in 6–15% of blind biopsies of normal looking peritoneum of symptomatic patients, women with infertility as well as of healthy control women (Nisolle et al. 1990, Balasch et al. 1996, Evers et al. 2005, Khan et al. 2014). These lesions are biologically active but their natural course and clinical significance is not yet clear (Khan et al. 2014).

**Ovarian endometriosis** i.e. endometrioma (OMA) is present in 17–46% of patients (Chapron et al. 2002, Saavalainen et al. 2018). The cyst can vary in size from a few millimeters to over 20 cm, and it contains old blood, giving it the nickname “chocolate cyst”. OMAs are often bilateral and commonly adherent to the lateral pelvic sidewall, fallopian tubes, sacrouterine ligaments, the uterus or the rectosigmoid colon. The clinical presentation of bilateral OMAs adhered to each other behind the uterus is called “kissing ovaries”. The inner layer of the cyst wall is formed of ectopic endometrium, and surrounded by a fibrotic pseudo capsule. Inflammation and pressure around the cyst has a negative impact on the ovarian reserve and surgical removal can cause further damage (Benagiano et al. 2016, Endometriosis Treatment Italian Club 2014).

**Deep infiltrating endometriosis (DIE)** is defined as the invasion of the nodule deeper than 5 mm from the peritoneal lining or as a lesion involving or distorting the bowel, the bladder, the ureters or the vagina (Johnson et al. 2017). Some authors prefer to use the term adenomyosis externa instead of DIE (Gordts et al. 2017). DIE is most commonly found in the posterior compartment of the pelvis and in the left side (Chapron et al. 2006). It commonly involves the uterosacral ligaments (**USL**; 52.7% of the cases) and the bowel (22.7%), and less often the vagina (16.2%), the bladder (6.3%) and the ureters (2.1%) (Chapron et al. 2006). Urinary tract endometriosis is considered rare, affecting only ~1% of all endometriosis patients but 19.53% of patients with DIE (Maggiore et al. 2017a). Rectovaginal endometriosis (**RVE**) is defined as DIE located in the rectovaginal area involving the vagina, the rectum and/or the rectovaginal septum (**RVS**) (Guerriero et al. 2016a). Rectovaginal septum is an anatomic structure below the Pouch of Douglas (POD), and isolated DIE of RVS is rare (Reid et al. 2014). Usually DIE of RVS is considered an extension of the posterior vaginal wall or the anterior rectum DIE (Guerriero et al. 2016a). However, the definition of RVE is inconsistent in the literature.

The estimated prevalence of DIE is 1% among fertile aged women (Koninckx et al. 2012). Among Finnish women receiving their first endometriosis diagnosis at surgery between years 1996–2012, 8.2% presented with DIE (Saavalainen et al. 2018). Commonly, patients have multiple DIE lesions, severe pelvic adhesions and distorted anatomy. Half of the patients with DIE also have OMA (Chapron et al. 2009). DIE is considered the most severe disease form in terms of symptom severity and the complexity of the treatment needed (Ferrero et al. 2015).

## 2.2 Epidemiology and impact

### 2.2.1 Prevalence

The prevalence of endometriosis among the general female population is unknown because presently the only reliable method to confirm the diagnosis is laparoscopy. The reported prevalence is much higher in studies based on hospital records compared with studies consisting of general populations (6–15% vs. ~1.5%) (Hickey et al. 2014). The higher estimates are based on selected surgical populations mostly from 1980's (Eskenazi et al. 1997) and may overestimate the prevalence. In **general adult populations**, the reported prevalence of clinical or surgical diagnosis is much lower, ranging from 1.5% in the UK (Ballard et al. 2008) to 0.8% in Germany (Abbas et al. 2012) and 1.1% in Israel (Eisenberg et



al. 2018). As these estimates are not based on surgical diagnosis, they may well under- or overestimate the prevalence.

According to a meta-analysis, endometriosis can be detected in 75% of **symptomatic adolescents** undergoing laparoscopy due to chronic pelvic pain (CPP) resistant to medical treatment (Janssen et al. 2013), and a similar finding (79%) has been recently reported (Ragab et al. 2015). In **adult women undergoing laparoscopy due to CPP**, endometriosis is detected in one third of all women and in 78–84%, if endometriosis is preoperatively suspected (Howard 2000).

Endometriosis is found in 21% of adult women undergoing **hysterectomy** due to CPP, while the rate of unexpected endometriosis is 8% in hysterectomies performed for other indications (Mowers et al. 2016). High rates of unexpected endometriosis (3–44%) have also been reported in asymptomatic women seeking for **laparoscopic sterilization**, and in most cases, minimal disease is found (Vercellini et al. 2015, Tissot et al. 2017).

In one study including **infertile women** with ovulatory menstrual cycles and normospermic partners, the prevalence of surgically verified endometriosis was as high as 47%, and surprisingly high (40%) even in a subgroup of painless infertile women (Meuleman et al. 2009). In other studies, the prevalence among infertile women has ranged between 5–50% (McLeod et al. 2010, Cranney et al. 2017).

### **2.2.2 Risk factors and comorbidities**

Endometriosis presents with a **complex heritable component**, and several endometriosis-related genes have been identified in linkage and genome-wide association studies (Rahmioglu et al. 2015). Many studies have shown a higher risk of endometriosis in relatives of affected women compared with controls (Rahmioglu et al. 2015). The risk ratio for sisters is 5.2 (Stefansson et al. 2002), and a twin study suggested a genetic influence as high as 51% to the liability to endometriosis (Treloar et al. 1999).

Several **characteristics of the menstruation and reproductive factors** are related to the risk of endometriosis. Early menarche, severe dysmenorrhea, short menstrual cycle, heavy menstrual bleeding and obstructive Müllerian anomalies are associated with a higher risk of endometriosis, while multiple pregnancies and long breastfeeding are protective (McLeod et al. 2010, Cramer et al. 2002, Smarr et al. 2016). In addition, in utero exposure to diethylstilbestrol or to endo-

crine disrupting chemicals (EDCs), such as dioxin, is reported to increase the risk.

Certain **markers in clinical history, especially during adolescence**, have been linked to the risk of severe endometriosis later in life (**Table 1**) (Chapron et al. 2011a). This cross-sectional study calculated the predictive value of three adolescent factors on later risk for DIE compared with the risk for other lesion types among 229 patients operated on for endometriosis.

**Table 1.** The odds ratio (OR) of selected markers in clinical history of adolescence associated with an increased risk for DIE in adulthood. Modified from Chapron et al. 2011a.

| Risk factors of adult DIE                   | OR  | 95% CI   |
|---|-----|----------|
| Positive family history of endometriosis    | 3.2 | 1.2–8.8  |
| Absenteeism from school during menstruation | 1.7 | 1–3      |
| OC use for severe primary dysmenorrhea      | 4.5 | 1.9–10.4 |

The risk of endometriosis related to **OC** use is controversial, and likely dependent on the indication for use and the study setting (Vercellini et al. 2011a, Chapron et al. 2011b, Kavoussi et al. 2017). A meta-analysis of 18 studies concluded that current OC use decreases the relative risk (RR) of endometriosis (RR 0.63; 95% CI 0.47–0.85), while past or never users do not show a statistically significant increase in the relative risk (RR 1.21; 95% CI 0.94–1.56 and 1.19; 95% CI 0.89–1.60, respectively) (Vercellini et al. 2011a). The analysis did not take into account the indications for OC use. In contrast to the meta-analysis, a large cross-sectional study reported comparable prevalences of endometriosis between current OC users and never users (logistic regression; adjusted OR 1.22; 95% CI 0.6–2.52) (Chapron et al. 2011b). This study consisted of 410 endometriosis patients and 566 women without endometriosis at the time of surgery performed for benign indications. Interestingly, past OC use due to severe primary dysmenorrhea was significantly associated with an increased risk of endometriosis (adjusted OR 5.6, 95% CI 3.2–9.8) and women with DIE had the strongest association (adjusted OR 16.2; 95% CI 7.8–33.3). In a recent case–control study, women with peritoneal endometriosis and a history of hormonal contraceptive use (mostly OC) had a much lower risk of concurrent endometrioma compared with women without past hormonal contraceptive use (18% vs. 49%,  $p < 0.001$ ) (Kavoussi et al. 2017).

OCs are typically prescribed as the first treatment option for women with dysmenorrhea, the most common symptom of endometriosis. The reported preventive effect of current OC use compared with past or no use might be due to alleviation of pain symptoms of undiagnosed endometriosis and postponement of surgery (Vercellini et al. 2011a). Moreover, OC use could potentially prevent *de*

*novo* endometriosis, especially OMA, due to long-term ovulation inhibition and smaller volume of retrograde menstruation (Kavoussi et al. 2017).

Certain patient **phenotypes** are reported to correlate with the risk of endometriosis. Lean women have an increased risk of laparoscopically confirmed endometriosis (Shah et al. 2013, Farland et al. 2017). Obesity may decrease the risk due to long-term effects of ovulation failure, leading to fewer menstrual bleedings and less retrograde menstruation during the reproductive life, or PCOS-related hyperandrogenism inhibiting lesion growth (Shah et al. 2013). On the other hand, the likelihood of laparoscopy being suggested to obese women may be lower compared with lean women. Furthermore, this association may be biased in many ways: due to inverse relationship of obesity and low socioeconomic status, weight gain due to pregnancies and loss of appetite due to endometriosis symptoms. Affected women may also be taller (Farland et al. 2017), and, according to Italian studies, more attractive (Vercellini et al. 2013a) and have blue eyes (Vercellini et al. 2014a). Two studies have suggested that black women may have a lower risk and oriental women may have a higher risk of endometriosis than white women (Cramer et al. 1986, Sangi-Haghpeykar et al. 1995).

**Lifestyle factors** may contribute to the risk as well: smoking, high vegetable and fruit consumption and regular exercise are proposed to be preventive while alcohol and caffeine consumption and high amount of red meat and fat in the diet seem to increase the risk (McLeod et al. 2010). However, these associations may be biased because endometriosis symptoms and treatments might influence lifestyle.

Endometriosis slightly increases the risk of epithelial ovarian cancer (**EOC**), especially endometrioid and clear cell carcinoma types (Thomsen et al. 2017, Poole et al. 2017). The overall lifetime risk for ovarian cancer is approximately 1.3% among general female population, and the chance of dying due to it is 1% (Reid et al. 2017). In Nurses' Health Study, women with laparoscopically confirmed endometriosis had an adjusted RR of 2.28 (95% CI 1.54–3.38) for ovarian cancer, and in women with self-reported endometriosis, the adjusted RR was 1.94 (95% CI 1.35–2.78) (Poole et al. 2017).

Women with endometriosis have an increased risk of having certain **concomitant autoimmune diseases and painful comorbidities**. Several autoimmune disorders, including ulcerative colitis, Crohn's disease, celiac disease, autoimmune rheumatoid diseases and multiple sclerosis, are more common in women with surgically confirmed endometriosis, and these conditions may share partly a similar pathophysiological background (Stephansson et al. 2011, Jess et al. 2012, Kvaskoff et al. 2015). Endometriosis patients also more commonly suffer from coexisting abdominal/pelvic pain syndromes, such as irritable bowel syndrome

(IBS), painful bladder, or abdominal myofascial pain with overlapping symptoms with endometriosis (Smorgick et al. 2013, Maroun et al. 2009, Jarrell 2011, Hansen et al. 2014). In addition, patients are reported to have an increased risk of cutaneous melanoma, asthma and atopic disease, cardiovascular diseases and migraine, decreased risk of cervical cancer and unchanged risk of endometrial cancer (Yang et al. 2012a, Farland et al. 2016, Kvaskoff et al. 2015, Poole et al. 2017).

### **2.2.3 *Impact on women and society***

Endometriosis is a chronic disease that causes a significant burden on patients, their families, healthcare systems and economies (Nnoaham et al. 2011, Jia et al. 2012, De Graaff et al. 2013, Soliman et al. 2016). It impacts negatively women's physical, mental and social wellbeing and significantly decreases the HRQoL (Nnoaham et al. 2011, Jia et al. 2012, De Graaff et al. 2013). Pain is the main reason for the impaired quality of life (Jia et al. 2012). Even appropriate treatments cannot fully erase the negative consequences of endometriosis (De Graaff et al. 2013).

The early signs of the negative impact preceding later diagnosis can possibly be foreseen already among adolescents with symptoms suggestive of endometriosis. One-third to half of teenage girls with severe primary dysmenorrhea report absenteeism from school, and severe menstrual pain interferes negatively with social activities, sports, sexuality, relationships and completing schoolwork (Banikarim et al. 2000, Parker et al. 2010, Esen et al. 2016).

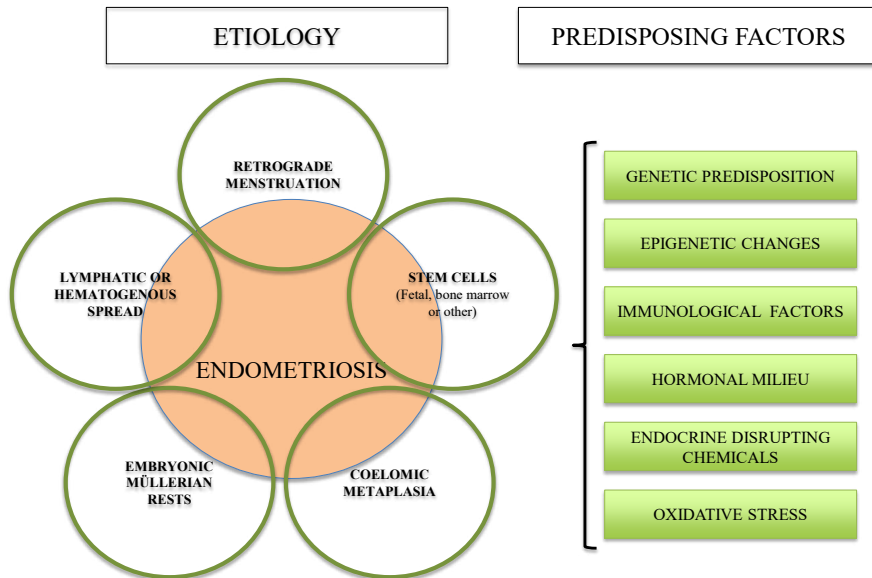
Endometriosis causes a marked economic burden on society (Soliman et al. 2016, Simoens et al. 2007). The economic impact consists of direct costs due to surgical, medical, psychological and infertility treatments and indirect costs due to the loss of working days, impaired working ability or even unemployment (Soliman et al. 2016). Endometriosis is associated with markedly higher healthcare costs compared to an average woman, and the direct annual costs per patient range from \$1109 in Canada to \$12118 in USA (Mirkin et al. 2007, Soliman et al. 2016). A prospective European multicenter survey proposed an average annual total costs to be 10000€ per patient with endometriosis (Simoens et al. 2007). The indirect costs to patients, employers and society are understudied and difficult to quantify (Klein et al. 2014).

## 2.3 Origin and pathogenesis

Endometriosis is a complex and mysterious disease. It is unknown why and when it begins, whether different disease types share a common origin and what caused the wide individual variation in pain symptoms, disease severity and tendency to progress. Even the nature of endometriosis as being a chronic and progressive disease is being debated (Evers 2013, Vercellini et al. 2015, Canis et al. 2016). The limited evidence suggests that in 42% of patients the lesions resolve spontaneously, in 29% they are stable and in 29% the disease is progressive (Evers 2013). To date, no studies have compared the progressive tendency of different forms of endometriosis.

### 2.3.1 Origin of lesions

The **origin of endometriosis lesions** is unknown, but many theories have been proposed (**Figure 3**). In 1927, Sampson introduced his theory of **retrograde menstruation** as the etiology, and his theory is widely accepted as the most probable pathogenic mechanism (**Figure 4**) (Sampson 1927a, Burney et al. 2012, Vercellini et al. 2014b). According to Sampson's theory, viable pieces of eutopic endometrium enter the pelvic cavity during menstruation via fallopian tubes, adhere to the peritoneum and start growing. Scientific evidence including anatomical distribution of the lesions, primate models and increased risk for endometriosis in adolescents with congenital obstructed uterine outflow support this theory (D'Hooghe et al. 2002, Scioscia et al. 2011, Burney et al. 2012). However, most women (90%) have retrograde menstruation, and it is believed that affected women have multiple predisposing factors of genetic, epigenetic, immunological, hormonal and environmental origin (**Figure 3**) (Burney et al. 2012).



**Figure 3.** Suggested theories on the origin of endometriosis and the predisposing factors.

During normal menstruation, only the functional layer of the endometrium bleeds, while the basal endometrium stays mostly intact. Leyendecker et al. (Leyendecker et al. 2002) postulated that both endometriosis and adenomyosis originate from the basal endometrium, which is found more commonly in menstrual blood of endometriosis patients compared to controls. They proposed that chronic or cyclic uterine hyperperistalsis in early reproductive life leads to micro trauma between the basal endometrium and the inner myometrium (Leyendecker et al. 2009). This in turn leads to dislocation of basal endometrial fragments into the myometrium and the pelvic cavity, leading to development of adenomyosis and endometriosis. A physiological wound healing process called “tissue injury and repair” (TIAR), demonstrated *in vitro*, is linked to this theory of cyclic trauma. TIAR includes similar patterns as the pathogenesis of endometriosis; interleukin-1 $\beta$  (IL-1 $\beta$ ) induced cyclo-oxygenase-2 (COX-2) activation, prostaglandin (PG) formation, aromatase expression and local estradiol (E2) production (Leyendecker et al. 2009). Similarly, TIAR may be induced in endometriotic lesions due to intralesional bleedings or mechanical trauma.

Recent **stem cell** theories have given new perspective to the origin of endometriosis. These theories propose that potent **endometrial stem cells of menstrual blood or bone marrow stem cells** enter the pelvic cavity and form endometriotic lesions due to predisposing hormonal, genetic and environmental factors (Gargett et al. 2010, Djokovic et al. 2014). Interestingly, these stem cells may enter the pelvis already in newborn girls (Brosens et al. 2013). Newborn menstruation is a well-recognized phenomenon and newborn retrograde menstruation

is assumed to be even more common (Brosens et al. 2013). It has been suggested that potent fetal endometrial stem cells enter the pelvis and implant to the peritoneum. These cells may then proliferate in pre-menarcheal and adolescent girls under the influence of estrogen and lead to early onset endometriosis.

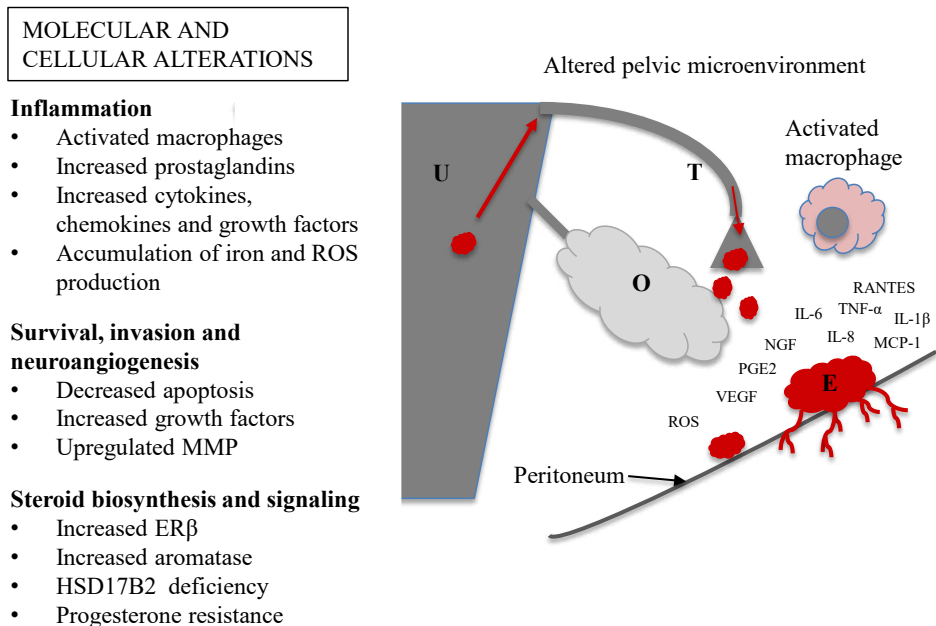
One theory proposes that lesions are benign metastasis of endometrial cells due to **lymphatic or hematogenous spread** (Halban 1924, Sampson 1927b). Indeed, histologically proven endometriosis has been found in 6–7% of women undergoing lymphadenectomy, and lesions have also been detected in bone, lung and brain (Burney et al. 2012). Other theories propose origin from **coelomic metaplasia** (Merrill 1966, Levander et al. 1955) or embryonic **Müllerian rests** (Russell 1899). Coelomic metaplasia refers to the possible transformation of healthy peritoneum to ectopic endometrium and endometriosis. Müllerian origin suggest that embryonic cells from Müllerian ducts, i.e. fetal cells migrating to the pelvis and forming the uterus, tubes and upper vagina, remain along the way and maintain their proliferative capacity. Both of these theories include induction of the disease by hormonal or other stimulus. Case reports of ectopic endometrium in fetuses, in women with uterine aplasia or in men undergoing hormonal treatment for prostate cancer support these theories (Signorile et al. 2010, Burney et al. 2012).

To date, the origin of different disease types is not fully understood. Already two decades ago, it was postulated that peritoneal, ovarian and deep endometriosis are three separate entities (Nisolle et al. and Donnez 1997). Sampson's implantation theory and related endometrial stem cells may explain peritoneal and ovarian endometriosis, but the origin of DIE remains controversial (Donnez 2017). Importantly, it is debated if subtle or minimal peritoneal endometriosis is a true disease or rather a physiological phenomenon occurring intermittently in all women (Gordts et al. 2017). The onset of true endometriosis is suggested to require genetic and epigenetic changes, and these may vary between different disease types (Gordts et al. 2017).

Three theories have been proposed to explain the origin of endometrioma: 1) invagination of ovarian cortex secondary to a bleeding superficial lesion, 2) invagination of ovarian cortex secondary to metaplasia of coelomic epithelium in an inclusion cyst or 3) transformation of a functional ovarian cyst to an endometrioma (Maggiore et al. 2017b). DIE is suggested to develop at a later age compared to peritoneal and ovarian lesions, and the repeated TIAR related to intraleisional bleeding may cause epigenetic modifications favoring the development of DIE (Donnez 2017). However, the progression of superficial implant to DIE has never been demonstrated. Müllerian rest theory may also explain DIE, but no consensus exists.

### 2.3.2 Pathogenesis

The key features in the pathogenesis of endometriosis are **chronic inflammation, local estrogen synthesis, progesterone resistance, angiogenesis and neurogenesis (Figure 4)** (Burney et al. 2012). These molecular and cellular alterations create a favorable pelvic microenvironment for endometrial cells to survive, invade and grow.

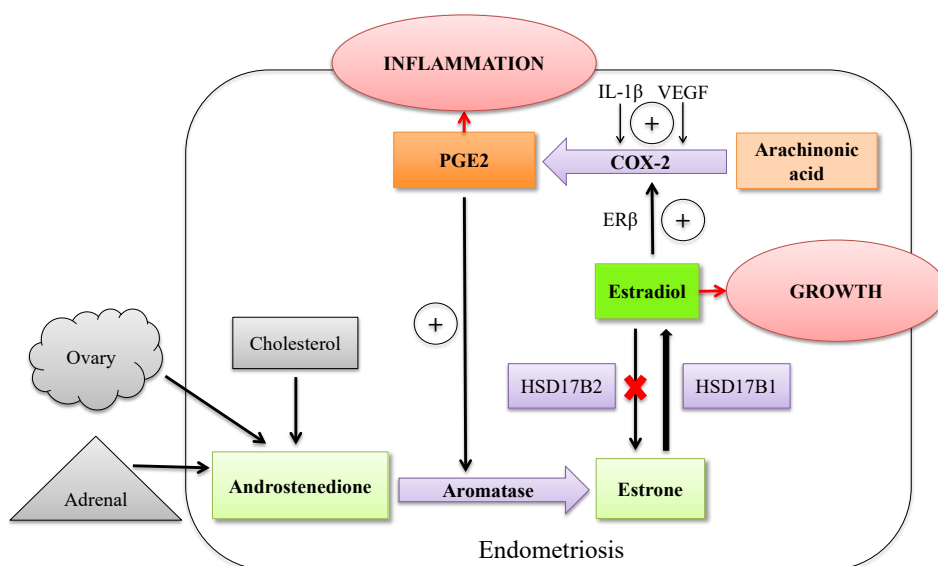


**Figure 4.** Pathogenesis of endometriosis according to the retrograde menstrual theory, and the key alterations in the molecular and cellular microenvironment. Activated macrophages and, to a lesser extent, endometrial lesions produce prostaglandins, cytokines, chemokines and growth factors (not all included in the illustration) enhancing lesion growth, vascularization, formation of *de novo* nerves and inflammation. Steroid action is altered in eutopic endometrium and endometriotic lesions. ER $\beta$ , estrogen receptor  $\beta$ ; HSD17B2, 17 $\beta$ -hydroxysteroid dehydrogenase 2; MMP, matrix metalloproteinase; ROS, reactive oxygen species; U, uterus; T, fallopian tube; O, ovary; E, endometrial lesion; RANTES, Regulated in Activation, Normal T Cell Expressed and Secreted; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; IL, interleukin; MCP, monocyte chemo attractant protein; NGF, nerve growth factor; PG, prostaglandin; VEGF, vascular endothelial growth factor. Modified from Vercellini et al. 2014b.

**Endometrial alterations** play a central role in the pathogenesis: numerous studies have shown changes in gene expression and hormonal environment of endometriotic tissue and eutopic endometrium of patients as compared with eutopic endometrium of healthy women (Burney et al. 2012, Huhtinen et al. 2012a, Benagiano et al. 2014).



**Estrogen** is the fuel for endometriosis, and the ovaries are the major source of estradiol (E2) in premenopausal women. In addition, circulating androstenedione is converted into estrone by the aromatase enzyme in adipose tissue and skin (Bulun 2009). Healthy endometrium does not produce E2, owing to lack of aromatase activity. However, in endometriosis, several alterations in the local steroid metabolism favor increased E2 production (**Figure 5**). In patients with endometriosis, both eutopic and ectopic endometrium show local E2 production due to aromatase activity, as well as decreased conversion of E2 to less potent estrone due to deficient 17 $\beta$ -hydroxysteroid dehydrogenase 2 enzyme (HSD17B2) activity (Bulun 2009, Huhtinen et al. 2012b). Estradiol has proinflammatory effects and it inhibits apoptosis in endometrium (Reis et al. 2013).



**Figure 5.** Mechanisms of altered local steroid metabolism and inflammation in endometriotic tissue. Modified from Bulun 2009.

The role of progesterone in endometrium and endometriosis is even more complex compared with the role of E2 (Reis et al. 2013). In human endometrium, progesterone modulates apoptosis-related genes in favor of induced apoptosis (Reis et al. 2013). Endometriotic tissue is **progesterone (P) resistant** and many steps in P signaling are altered (Patel et al. 2017). Importantly, in endometriosis, progesterone lacks the ability to upregulate HSD17B2 enzyme, resulting in decreased conversion of E2 to estrone. The supposed reason is the decreased progesterone receptor B (PR-B) level. Furthermore, P resistance may be caused by PR gene polymorphism, altered microRNA (miRNA) expression or epigenetic changes of PR receptors and their targets. These changes may originate already

from fetal “preconditioning” (Gargett et al. 2014). Decreased progesterone signaling results in a proinflammatory state, and conversely, chronic inflammation can promote P resistance (Patel et al. 2017). Importantly, P resistance may explain why hormonal therapy is ineffective in some patients.

Endometriosis presents with a strong **inflammatory microenvironment** in the pelvic cavity and in the endometrial lesions (Vetvicka et al. 2016). The peritoneal fluid of the patients contains an increased number of **activated macrophages** that overproduce prostaglandins, cytokines, chemokines and growth factors (**Figure 4**) (Tran et al. 2009, Burney et al. 2012, McKinnon et al. 2015). **Activated macrophages** of the patients overexpress COX-2 enzyme and release high amounts of PGs compared with macrophages from healthy controls (Wu et al. 2002). In endometriotic lesions, high local E2 level increases COX-2 activity and stimulates PG production, and high PG further stimulates estrogen production (**Figure 5**). This self-feeding E2–PG cycle is also stimulated by several inflammatory cytokines and growth factors (Burney et al. 2012, Benagiano et al. 2014). The repetitive cyclic retrograde menstruation and intralesional bleedings further induce pelvic inflammation via **TIAR**, and may lead to scarring and adhesion formation (Burney et al. 2012).

It has been suggested that erythrocytes from retrograde menstrual blood and intralesional bleeding release heme and free iron molecules, leading to local iron overload and **oxidative stress** (Donnez et al. 2016). The formed **reactive oxygen species (ROS)** may damage peritoneal tissue and cause adhesions and chronic inflammation. Similarly, an endometrioma contains high amounts of free iron, ROS, proteolytic enzymes and inflammatory molecules, and these may damage the surrounding ovarian tissue (Sanchez et al. 2014).

**Angiogenesis** is a physiological phenomenon involved in the monthly growth and remodeling of eutopic endometrium. Sufficient vascular supply is also essential in the survival and growth of ectopic endometrium. High concentrations of vascular endothelial growth factor (**VEGF**) have been detected in peritoneal fluid of endometriosis patients (McLaren et al. 1996). In addition, lesions develop their own sensory and sympathetic **innervation**, and neural growth factor (**NGF**) is the key mediator in neurogenesis (Kobayashi et al. 2014, McKinnon et al. 2015). De novo nerves have been found in all lesion types and are suggested to contribute to pain symptoms (Wang et al. 2009, Zhang et al. 2009, Tokushige et al. 2010, Kobayashi et al. 2014). Women with endometriosis also show increased nerve density in eutopic endometrium as compared with endometrium of healthy women (Tokushige et al. 2006).

## 2.4 Classification

Classification of endometriosis is challenging due to the complexity of the disease. Recently, World Endometriosis Society proposed that until better systems are developed, the revised American Society for Reproductive Medicine (**r-ASRM**) classification, **Enzian** classification for DIE and the Endometriosis Fertility Index (**EFI**) may be used when surgery is performed (ASRM 1997, Tuttlies et al. 2005, Adamson et al. 2010, Johnson et al. 2017). These classifications aim to predict fertility and to categorize the anatomical locations, appearance and size of the lesions and the extent of pelvic adhesions. However, none of these predict pain severity, response to treatment, recurrence risk, HRQoL or other measures important to individuals or health care providers (Johnson et al. 2017).

The **r-ASRM** classification is the oldest and widely used surgical classification of endometriosis (ASRM 1997, Johnson et al. 2017). It is divided into four stages (I–IV) with IV being the most severe category. This system was primarily created for estimating postoperative fertility, and it rates the severity of adnexal adhesions, endometrioma size, pouch of Douglas obliteration and the area covered by the peritoneal implants. However, r-ASRM classification does not adequately describe DIE or include the involvement of other pelvic organs than the uterus and the adnexa. Furthermore, it correlates poorly with the severity of pelvic pain or QoL and does not predict fertility, the risk of recurrence or treatment outcomes (Vercellini et al. 2006, Vercellini et al. 2007, Johnson et al. 2017).

**Enzian classification** describes in detail the operative findings of DIE. It was introduced in 2005 as a supplement to the r-ASRM score (Tuttlies et al. 2005) and further revised in 2010 and 2011 (Haas et al. 2013b). The Enzian system focuses on DIE involvement on the posterior pelvic compartment because DIE lesions are most commonly located there (Chapron et al. 2006, Haas et al. 2011). It divides the posterior compartment into three parts: A) anterior (rectovaginal septum and vagina), B) lateral (sacrouterine ligaments and pelvic sidewall) and C) posterior (rectum and sigmoid colon). The extent or infiltration of these lesions is further divided into grades 1–3 (i.e. 1–3 cm).

In addition to the posterior compartment, other locations are listed (uterine adenomyosis (FA), bladder (FB), intrinsic involvement of the ureter (FU), bowel disease cranial to the rectosigmoid junction (FI) and extra pelvic locations FO)). One study demonstrated a significant correlation between the highest Enzian score and the presence of dysmenorrhea, general abdominal pain and bowel symptoms ( $p < 0.001$ ,  $p = 0.002$  and  $p = 0.002$ , respectively) (Haas et al. 2013a). However, the value of Enzian score in predicting fertility and other outcome measures has not yet been demonstrated.

**EFI classification** was introduced in 2009 to predict spontaneous pregnancy rate after surgery (Adamson et al. 2010). The EFI includes functional description of the fallopian tubes, fimbria and ovaries at the end of surgery in addition to measures from r-ASRM classification. It also takes into account the patient's age, length of infertility and prior pregnancies. This score helps clinicians in estimating the need for in vitro fertilization (IVF) after surgery.

## 2.5 Symptoms

### 2.5.1 *Pelvic pain and dysfunction*

Dysmenorrhea is the most common and debilitating pain symptom, but the majority of patients also report acyclic/chronic pelvic pain, dyspareunia (pain during intercourse), dyschezia (pain during defecation) or dysuria (pain during urination) (Sinaii et al. 2008, Johnson et al. 2013, Vitonis et al. 2014). Patients may also be asymptomatic (Tissot et al. 2017). All five pain symptoms should be included in the history taking of a woman with suspected endometriosis (Guerriero et al. 2016a, Rogers et al. 2017). Commonly, the first pain symptoms occur already during adolescence before the age of 20 (Greene et al. 2009, DiVasta et al. 2018) or in the early adulthood before the age of 25 (Klein et al. 2014). Adolescents present with similar symptoms as adults, typically dysmenorrhea and/or chronic pelvic pain (CPP) (Janssen et al. 2013, Yeung et al. 2011, Benagiano et al. 2018, DiVasta et al. 2018), and all r-ASRM stages and lesion types can be detected in teenagers (Andres et al. 2014, Audebert et al. 2015).

Pain severity correlates poorly with the extent of the disease when measured with r-ASRM stage (Chapron et al. 2003, Vercellini et al. 2007). The lesion type correlates better with pain severity compared with r-ASRM stage. Some studies have reported that women with DIE typically have more severe pain symptoms compared with non-DIE women (Chapron et al. 2012, Dai et al. 2012, Vercellini et al. 2007, Lafay Pillet et al. 2014). Furthermore, women with DIE have a longer history of pain (Dai et al. 2012, Lafay Pillet et al. 2014).

#### 2.5.1.1 *Dysmenorrhea*

Dysmenorrhea or painful menstruation is defined as painful, abdominal, cramping sensation of uterine origin, and it appears to be the most common gynecological complaint irrespective of the nationality and age (Iacovides et al. 2015). The

prevalence of dysmenorrhea in women of reproductive age has been reported to range from 16% to 91% in large cohort studies published between 2002 and 2011, and 2–29% of women report severe menstrual pain (Ju et al. 2014).

**Primary dysmenorrhea** refers to menstruation-related pain of 8–72 hours' duration starting during adolescence shortly after menarche (6–24 months) and without any underlying pathological condition (Iacovides et al. 2015). The suggested reason for pain is overproduction of PGs in the luteal phase endometrium of the ovulatory cycles (Iacovides et al. 2015). High local PG levels result in uterine hyper contractility, myometrial ischemia and sensitization of local uterine nerves to pain. Primary dysmenorrhea affects majority of teenage girls (68–93%) worldwide, and the prevalence of severe dysmenorrhea ranges from 6 to 43% (**Table 2**) (Banikarim et al. 2000, Agarwal et al. 2009, Parker et al. 2010, Zannoni et al. 2014, Ragab et al. 2015, Esen et al. 2016).

**Table 2.** List of studies reporting the prevalence of adolescent dysmenorrhea and severe dysmenorrhea published between 2000 and 2017.

| Author <sup>a</sup>     | Country   | N    | Age Range (mean) | Dysmenorrhea (%) | Severe dysmenorrhea (%) |
|-------------------------|-----------|------|------------------|------------------|-------------------------|
| Esen 2016               | Turkey    | 879  | 13–19 (16.2)     | 92               | 28                      |
| Ragab 2015 <sup>b</sup> | Egypt     | 506  | NA (15.2±3.5)    | 49               | 43                      |
| Zannoni 2014            | Italy     | 250  | 14–20 (17.7)     | 68               | 6                       |
| Parker 2010             | Australia | 1052 | 15–19 (16.8)     | 93               | 21                      |
| Agarwal 2009            | Singapore | 5561 | 12–19 (16±1.1)   | 83               | 10                      |
| Banikarim 2000          | USA       | 706  | NA (16±1.4)      | 85               | 33                      |

<sup>a</sup> Esen and Parker defined severe dysmenorrhea as NRS 8–10, other authors have not defined severe pain, <sup>b</sup>The authors excluded 148 girls with irregular cycle from the study and 506 girls with regular cycle were included. NA, not applicable.

**Secondary dysmenorrhea** means exacerbation of primary dysmenorrhea or menstrual pain occurring later in the adulthood. It is usually caused by an underlying pathology such as endometriosis, adenomyosis or uterine fibroids. Endometriosis-related menstrual pain is typically severe, lasts for many days and starts several days before the onset of the bleeding. Often NSAIDs offer only limited or no relief, and commonly patients are absent from school or work during the worst pain.

Altogether 68–80% of endometriosis patients undergoing surgery for stage I–IV disease report preoperative dysmenorrhea (Porpora et al. 1999, Sinaii et al. 2008, Coccia et al. 2011) and moderate/severe dysmenorrhea (Visual analogue scale, i.e. VAS 51–100) in 57% (Vercellini et al. 2007). Women with DIE report severe dysmenorrhea (Numerical rating scale i.e. NRS 7–10) in 54% (Fauconnier et al.

2002). Patients with OMA with or without related DIE report severe dysmenorrhea in 46%–62% depending on the study and the definition of severe pain (NRS 8–10 or NRS 7–10) (Porpora et al. 2010, Chapron et al. 2012).

### **2.5.1.2 Chronic pelvic pain (CPP)**

CPP is defined as intermittent or constant pain in the lower abdomen or pelvis of at least 6 months' duration, not occurring exclusively with menstruation or intercourse and not associated with pregnancy (RCOG 2018). The overall prevalence in women ranges from 6% to 27% (Ahangari 2014), and it is as common as asthma and chronic back pain (Brawn et al. 2014). Adolescent girls report recurrent weekly abdominal pain in up to 18–27% depending on the age; 27% at the age of 11–12 and 18% at the age of 15–16 (Kristjánssdóttir 1996). Endometriosis is the leading cause for CPP with ~50% prevalence in diagnostic laparoscopy, but in at least a third of women with CPP, no organic cause is detected (Daniels et al. 2010). In the prospective EndoCost Study initiated by the World Endometriosis Research Foundation, 60% of women diagnosed with endometriosis and treated in 12 tertiary centers in ten countries worldwide reported having CPP (De Graaff et al. 2013).

### **2.5.1.3 Other symptoms**

Endometriosis patients commonly experience **gastrointestinal symptoms**, such as pain during defecation (dyschezia), bloating, diarrhea, constipation, nausea, vomiting, feeling of incomplete stool evacuation and rectal bleeding (Maroun et al. 2009, Roman et al. 2012, Hansen et al. 2014, Ek et al. 2015). These symptoms are typically more intense during menstruation (Fauconnier et al. 2002, Roman et al. 2012, Moore et al. 2017a) and correlate poorly with the presence of bowel endometriosis (Maroun et al. 2009, Roman et al. 2012, Ek et al. 2015). These symptoms are likely to be partly related to cyclic inflammatory phenomena leading to irritation of the gastrointestinal system (Roman et al. 2012, Ferrero et al. 2015). This mechanism explains why women without endometriosis may also have similar complaints during menstruation (Nnoaham et al. 2012a).

**Dyschezia** (pain during defecation) during menstruation correlates strongly with the risk of endometriosis irrespective of the r-ASRM stage (validation cohort; any stage endometriosis OR 3.47; 95% CI 1.4–8.57;  $p=0.007$  and stage III–IV OR 3.09; 95% CI 1.39–6.87;  $p=0.006$ ) (Nnoaham et al. 2012a). Endometriosis patients (diagnosed with laparoscopy or MRI) more commonly present with

dyschezia that disturbs work compared general reference population (16% and 3%, respectively) (Hansen et al. 2014). Dyschezia is very common among women with colorectal DIE (68%), but also a marked proportion of patients with DIE without bowel involvement and women with superficial endometriosis only report dyschezia (43% and 38%, respectively) (Roman et al. 2012). In a cohort of 17 adolescent endometriosis patients dyschezia was present in 77% (Yeung et al. 2011).

Limited data exists on **urinary symptoms** in endometriosis. In one study, 9% of the endometriosis patients reported **dysuria** (pain during urination) that disturbed at work compared with 2% of the reference women (Hansen et al. 2014). Dysuria is commonly related to bladder lesions as compared with disease without urinary tract endometriosis (69% vs. 6%) (Knabben et al. 2015). Patients with posterior DIE, especially parametrial endometriosis, have an altered urinary function compared with the controls: International Prostate Symptom Score questionnaire (IPSS) for evaluating the impact of lower urinary symptoms detected significant difference between these groups in voiding symptoms ( $p=0.002$ ), quality of life ( $p=0.003$ ) and total IPSS score ( $p=0.002$ ) (Ballester et al. 2010). One study showed that urodynamic detrusor over activity is commonly related to DIE compared with ovarian endometriosis (92% vs. 8%, respectively) (Serati et al. 2013).

Women with RVE have a high risk of ureteral involvement and the risk increases with increasing size of the nodule (Knabben et al. 2015). Ureteral endometriosis does not cause any specific symptoms (Knabben et al. 2015), and the risk of **hydronephrosis** and silent loss of kidney function is as high as 25–50% in patients with severe ureteral stenosis (Stepniewska et al. 2011). Thus, ultrasonographic evaluation of the kidneys should be routinely performed in women with suspected endometriosis (Pateman et al. 2015).

**Sexual health** forms a fundamental part of quality of life, and endometriosis may negatively affect many aspects of sexuality and partnership (Pluchino et al. 2016, Barbara et al. 2017). Limited evidence suggests that endometriosis causes sexual dysfunction and distress in 70–75% of patients (Pluchino et al. 2016). Women with endometriosis have a high risk of dyspareunia compared with general female population (OR 9.4, 95% CI 8.0–11.1) (Ballard et al. 2008). **Deep dyspareunia** is commonly associated to all types of endometriosis and especially to posterior compartment DIE (Ferrero et al. 2005, Fauconnier et al. 2005, Vercellini et al. 2007, Pluchino et al. 2016). Mechanical pressure and traction of fibrotic, adhesive and inelastic tissue in posterior DIE may trigger deep pain during intercourse (Vercellini et al. 2012).

Endometriosis-related sexual dysfunction is multidimensional and includes much more than just pain during intercourse (Pluchino et al. 2016, Barbara et al. 2017).

Recurrent severe dyspareunia may result in fear towards intercourse, a feeling of being an incomplete woman and guilt of disappointing the partner (Fritzer et al. 2013). More than half of women with endometriosis (66%) are afraid of pain during or after intercourse and 46% are willing to suffer pain to satisfy their partner (Fritzer et al. 2013). The fear of pain may in turn have a negative impact on desire, arousal, lubrication, genital congestion and sexual satisfaction (Pluchino et al. 2016). In addition to dyspareunia, hormonal medications, surgery, physical and mental comorbidities, life situation, infertility, personality and women's and partners' expectations influence sexual health.

### 2.5.2 *Infertility*

Endometriosis is associated with impaired fertility (de Ziegler et al. 2010, Prescott et al. 2016). While at least half of the patients conceive normally, 30–50% have some degree of infertility (Evans et al. 2017). In the Nurses' Health Study, a history of endometriosis was related to an age-adjusted 2-fold increase in the risk of infertility (Prescott et al. 2016). The prevalence of endometriosis in infertile women is estimated to be 20–50% (Meuleman et al. 2009, Cranney et al. 2017).

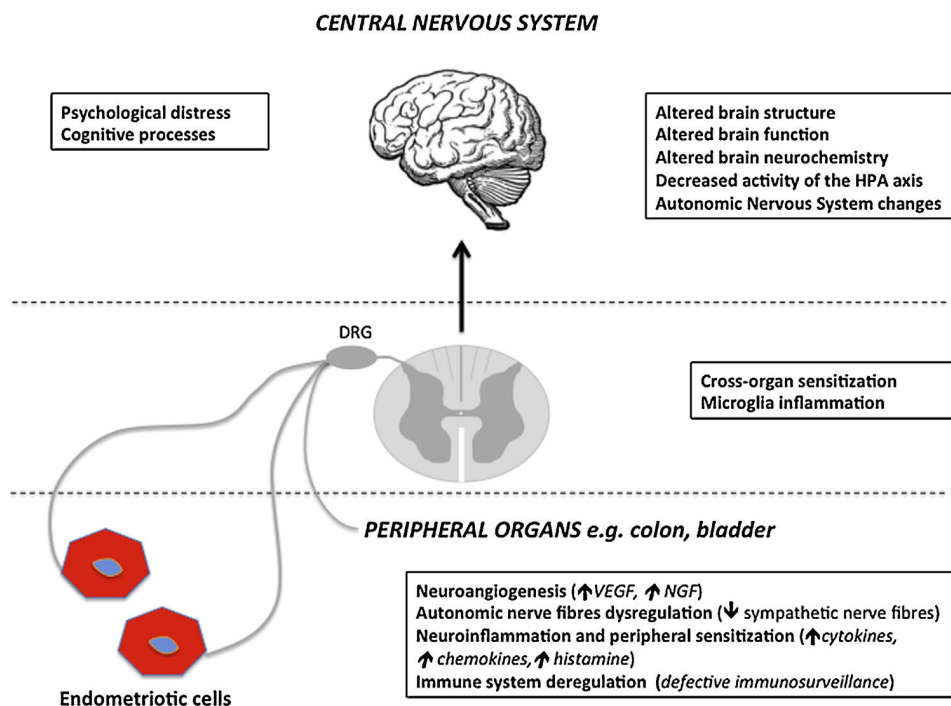
### 2.5.3 *Mechanisms and origin of pelvic pain*

Endometriosis-associated pain is a complex combination of **nociceptive, inflammatory and neuropathic pain**, and it includes **alterations in peripheral and central nervous system (Figure 6)** (Morotti et al. 2017). Growing evidence suggests that *de novo* nerves detected in endometriotic lesions and in the eutopic endometrium of endometriosis patients may contribute to the generation of endometriosis-associated pain (Tokushige et al. 2006, McKinnon et al. 2012, Morotti et al. 2014). Furthermore, estrogen action supports inflammation in endometriotic lesions, may promote neuroangiogenesis by stimulating VEGF and NGF production and modulate nociceptive responses (Morotti et al. 2014). At the spinal cord level, intersegmental connections may lead to pelvic–lower abdomen cross-organ sensitization between the gastrointestinal, urinary and gynecological organs and broaden the pain sensation (Malykhina 2007).

Nociceptive pain is caused by the activation of the peripheral nociceptors due to non-neural tissue damage such as distension, ischemia or inflammation. Pelvic nociceptors are primary afferent neurons whose cell bodies are located in the dorsal root ganglia (DRG; **Figure 6**). Neuroinflammation, i.e. inflammatory irri-



tation of pelvic nerves or *de novo* nerves of the endometriotic lesions, may promote peripheral nerve sensitization and cause neuropathic pain (McKinnon et al. 2015). Nerve fibers play an active role in this process by secreting proinflammatory neuromediators. Furthermore, entrapment or injury of pelvic nerves due to endometriotic lesions or their surgical removal may also cause neuropathic pain (Morotti et al. 2017).



**Figure 6.** Different levels of peripheral and central nervous system and related factors involved in the generation of endometriosis-associated pain. The extent of sensitization to pain is dynamically modulated by estrogen and cytokines. Peritoneal fluid immune cells secrete growth factors like neural growth factor (NGF) and numerous immune mediators that can promote the growth of nerve fibers and sensitize or excite the terminals of sensory nerve fibers of peripheral organs or endometriosis lesions. These nerve fibers enter the same dorsal root ganglion (DRG) and may lead to cross-organ sensitization. Normally, multiple intersegmental spinal connections exist and they may cause cross-sensitization at spinal level. Many factors influence how each individual's brain processes pain. Figure reprinted with permission (Morotti et al. 2017)

Chronic pain, including dysmenorrhea without endometriosis, is associated with several structural and functional changes in the central nervous system (Brawn et al. 2014, Rogers et al. 2017). Peripheral and central sensitization to pain stimuli may intensify or even generate painful sensations (Brawn et al. 2014, Morotti et al. 2014). Furthermore, each woman's individual psychological and physical stress, hormonal factors and various coping mechanisms further modulate the

perception of pain. Interestingly, women with endometriosis have lower pain thresholds throughout the body compared with controls (Brawn et al. 2014). Genetic predisposition may also increase the risk for chronic pain (Morotti et al. 2017).

Pelvic pain may originate from **visceral** or **somatic** nerves depending on the location of the lesions or the tissue trauma (Lamvu et al. 2006). Visceral pain originates from intra-abdominal visceral organs or visceral peritoneum and somatic pain from the musculoskeletal pelvic system or from the parietal peritoneum lining the abdominal wall and the pelvic sidewalls.

**Visceral pain** is transmitted through the sympathetic nerve fibers of the autonomic nervous system, and it is typically poorly localized, described as dull or crampy and felt in the regions of the lower abdominal wall, low back, inguinal region and anterior thigh (Lamvu et al. 2006, Vercellini et al. 2009b). Autonomic symptoms, such as nausea, vomiting, diarrhea and sweating are typically associated with visceral pain (Lamvu et al. 2006, Hansen et al. 2014).

Hypogastric nerve is the most important visceral nerve structure in endometriosis. It transmits nociceptive stimuli from the uterus, broad ligament, upper vagina, rectum, bladder, urethra and distal ureters to the central nervous system (Lamvu et al. 2006). The ovaries are innervated by a network of visceral nerves from T10 and T11 (Lamvu et al. 2006). During extensive endometriosis surgery for DIE, the hypogastric nerve may be damaged accidentally or even purposely to enable complete surgery, and nerve damage can induce neuropathic pain.

**Somatic pain** is transmitted via sensory afferent fibers of the somatic nerves and it is better localized than visceral pain. In endometriosis, it can originate from lesions or related adhesions affecting the parietal peritoneum. The nerve supply of the parietal peritoneum is dependent on the anatomical location: in the pelvic region, it is innervated by the pudendal nerve, in the area of the abdominal wall above the pelvis by the first lumbar and lower six thoracic nerves of the corresponding skin dermatomes, in the central part of the diaphragm by the phrenic nerve and in the peripheral diaphragm by the lower six thoracic nerves. In rare cases, endometriosis irritates or infiltrates somatic pelvic nerves; typically sciatic nerve roots, leading to invalidating pain.

#### **2.5.4 Evaluation of pain, dysfunction and HRQoL**

**The intensity and the type of pain** as well as the QoL should be measured in studies assessing the outcome of an endometriosis treatment (Vincent et al. 2010,

Bourdel et al. 2015, Morotti et al. 2017). Visual analogue scale (**VAS**) and numerical rating scale (**NRS**) are valid, precise and reliable methods to measure **pain intensity** (Breivik et al. 2008, Bourdel et al. 2015). VAS is a 100 mm line and NRS is an 11-point scale from 0 to 10. In both scales, 0 means “no pain” and the opposite end of the scale means “worst pain you can imagine”. Of these scales, NRS is preferable because patients find it easier to use and it has more power to identify differences (Breivik et al. 2008, Bourdel et al. 2015).

Each pain type should be evaluated separately instead of a single pain measure, and at least dysmenorrhea, dyspareunia and acyclic pelvic pain but preferably also dyschezia and dysuria should be included (Vincent et al. 2010, Bourdel et al. 2015, Hirsch et al. 2016b, Rogers et al. 2017). Optimally, daily pain should be measured for one month prior to the treatment and then at months 3, 6 and 12 afterwards, and follow-up should be continued as long as possible (Bourdel et al. 2015). Revised version of the Short-Form McGill Pain Questionnaire (**SF-MPQ-2**) can be used to characterize the **type of pain**, i.e. aching, stabbing, burning etc., more precisely (Dworkin et al. 2009).

In clinical studies, NRS or VAS scales are often divided into four categories: no, mild, moderate and severe pain. However, there is no consensus on the cut-offs for these categories in endometriosis pain, and comparison of studies is difficult because many variations are used. A significant response to endometriosis treatment is defined as a relative degree of reduction of pain intensity from the baseline pain levels by >30% or >50% (Vincent et al. 2010).

Endometriosis has a negative impact on **HRQoL**, and QoL assessment should be included in studies evaluating the effectiveness of surgical or medical treatments. Endometriosis Health Profile-30 Questionnaire (**EHP-30**) and its short form **EHP-5** are validated QoL questionnaires developed for endometriosis care and research (Jones et al. 2001, Jones et al. 2004). These questionnaires grade disease-specific symptomatology and its influence on daily life. Medical Outcomes Study Short Form 36 (**SF-36**) is a more general QoL tool that allows comparison to other diseases, and it has been also validated in endometriosis (Stull et al. 2014). Furthermore, several other QoL tools have been used in endometriosis studies (Hirsch et al. 2016b). Collection of data on mental health is recommended as it may interfere with outcome (Rogers et al. 2017).

Endometriosis-related **sexual dysfunction** can be evaluated with specific questionnaires such as Female Sexual Function Index (**FSFI**), which has been most widely used in endometriosis studies (Barbara et al. 2017). FSFI has been proposed as a valid tool to evaluate sexual function in endometriosis patients (Vanhie et al. 2016).

**Bowel symptoms** can be assessed with various questionnaires including Knowles-Eccersley-Scott Symptoms Questionnaire (**KESS**), the Gastrointestinal Quality of Life Index (**GIQLI**) or **Wexner** scale (Roman et al. 2018). Recently, a new scoring system, Bowel Endometriosis Syndrome (**BENS**), was introduced to measure pelvic organ dysfunction and QoL related specifically to bowel endometriosis (Riiskjaer et al. 2016). BENS attempts to estimate the severity of bowel symptoms, but the usefulness in clinical studies is to be demonstrated. In **urinary tract** endometriosis, the modified International Prostate Symptom Score (**modified IPSS**), the Bristol Female Lower Urinary Tract Symptoms (**BFLUTS**) questionnaire or the Urinary Symptom Profile (**USP**) may be useful in preoperative screening of symptoms and in monitoring surgical outcome (Maggiore et al. 2017a, Roman et al. 2018).

## 2.6 Diagnosis of endometriosis

### 2.6.1 Diagnostic delay

It is well documented that there is a long diagnostic delay worldwide between the onset of symptoms and the diagnosis (Nnoaham et al. 2011, Dunselman et al. 2014). These studies consider diagnosis as a surgically verified disease. The average median delay is 6.7 years and it varies between 2 and 10.7 years depending on the country (Nnoaham et al. 2011, Hudelist et al. 2012, Klein et al. 2014, Staal et al. 2016, DiVasta et al. 2018). The long delay causes unnecessary suffering and is associated with reduced HRQoL (Nnoaham et al. 2011). Presently, the mean age at first surgery ranges between 29 and 36 years (Liu et al. 2008, Gylfason et al. 2010, Nnoaham et al. 2011, Klein et al. 2014, Staal et al. 2016), while symptoms typically start during adolescence or early adulthood (Greene et al. 2009, Klein et al. 2014, DiVasta et al. 2018).

Several factors influence the length of the diagnostic delay. According to some studies, delay is longer when symptoms begin during adolescence compared with adult onset of symptoms (Greene et al. 2009, Klein et al. 2014, Staal et al. 2016), but also opposite findings have been documented (DiVasta et al. 2018). Teenagers more likely first see a general practitioner, and women first visiting primary health care take longest to be diagnosed (Greene et al. 2009, Nnoaham et al. 2011, Staal et al. 2016). Similarly, if the healthcare is government-funded, the delay is longer compared with individual or insurance-funded healthcare (8.3 vs. 5.5. years) (Nnoaham et al. 2011). Higher BMI and, surprisingly, also more nu-

merous pain symptoms are related to a longer delay (Nnoaham et al. 2011), while infertility shortens it (Dmowski et al. 1997, Klein et al. 2014, Staal et al. 2016).

The problem of studies evaluating diagnostic delay is that they measure the time interval between the onset of symptoms and the surgical diagnosis. Instead, future studies should measure the delay until clinical diagnosis to show clinically relevant delay.

### 2.6.2 *Surgical and clinical diagnosis*

For decades, the diagnosis of endometriosis has been based on **visual and/or histological detection at laparoscopy**. A meta-analysis of four studies evaluating the accuracy of laparoscopy found that visual confirmation alone had 94% sensitivity and only 79% specificity compared with the reference method of histology (Wykes et al. 2004). In 2015, a group of key opinion leaders stated that presently the diagnosis should be based on symptoms, clinical presentation and transvaginal ultrasound (TVUS), and laparoscopy should be intended only for surgical treatment with few exceptions (Vercellini et al. 2015). In most women, endometriosis is first suspected due to **typical pelvic pain symptoms**. In addition, unexplained infertility or, rarely, imaging for evaluation of other conditions raise the suspicion. Many other diseases and conditions hamper the symptom-based diagnosis because of overlapping symptoms. For example, irritable bowel syndrome (IBS), pelvic inflammatory disease (PID), painful bladder syndrome or inflammatory bowel diseases cause similar complaints (Smorgick et al. 2013, Jarrell 2011, Maroun et al. 2009, Hansen et al. 2014). In **gynecological examination**, focal deep pelvic tenderness, palpable nodules in the rectovaginal septum or retrocervical area, adnexal mass or immobile pelvic organs are suggestive of the disease. However, especially in cases with superficial endometriosis, the only sign can be general tenderness in pelvic palpation.

**Symptom-based screening** tools may help to predict the risk of endometriosis. A multicenter study including 1396 symptomatic adult women evaluated the diagnostic power of a 25-item screening questionnaire published by World Endometriosis Research Foundation (Nnoaham et al. 2012a). A model of best predicting questions acted poorly in diagnosing any stage endometriosis (AUC = 68.3) but found advanced endometriosis (stage III–IV) with good accuracy (AUC = 84.9, sensitivity 82.3%, specificity 75.8% with a cut-off of 0.24). Of all questions included, dyschezia during menstruation and a history of benign ovarian cyst had the strongest correlation with the risk of endometriosis irrespective of disease severity (any stage endometriosis/validation cohort; OR 3.47; 95% CI 1.40–8.57;  $p=0.007$  and 4.15; 95% CI 2.19–7.86;  $p<0.001$ , respectively). Recently, a screen-

ing questionnaire was introduced to identify adolescents at risk of endometriosis for pilot testing and validation (Geysenbergh et al. 2017). The authors based the selection of questions on a systematic literature review, and included age at menarche, cycle duration, frequency of dysmenorrhea, pain characteristics, presence of dyschezia and urinary symptoms.

One study evaluated the power of a predictive score to detect women with DIE among women with OMA (Lafay Pillet et al. 2014). A model of four best-predicting variables could define a high-risk group where the risk of DIE was 88%. This model included deep dyspareunia (NRS >5), gastrointestinal symptoms (NRS  $\geq$ 5), severe dysmenorrhea (OC use for primary dysmenorrhea of worsening of secondary dysmenorrhea) and primary or secondary infertility.

### 2.6.3 Imaging

TVUS and magnetic resonance imaging (MRI) have high sensitivity and specificity to diagnose OMA and DIE in experienced hands, but neither can detect peritoneal lesions (Table 3) (Nisenblat et al. 2016a, Rogers et al. 2017). TVUS, combined with clinical examination, is the preferred diagnostic imaging method because it is cost-effective and widely available (Noventa et al. 2015, Guerriero et al. 2016a, Nisenblat et al. 2016a). When surgery is planned, an accurate pre-operative mapping of DIE with TVUS, or optionally MRI, should be carried out with expertise to estimate the extent of surgery and to avoid discovering unexpectedly severe disease at laparoscopy, and, importantly, to enable sufficient patient information (Hudelist et al. 2011, Guerriero et al. 2016a, Nisenblat et al. 2016a).

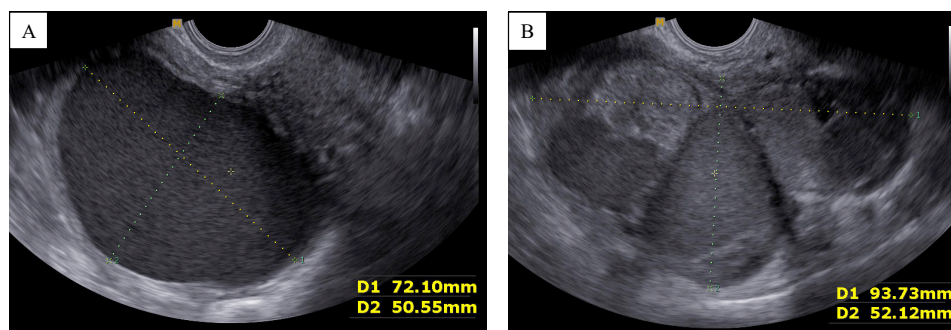
**Table 3.** Diagnostic power of transvaginal ultrasound (TVUS) and magnetic resonance imaging (MRI) in detecting ovarian endometrioma (OMA) and deep infiltrating endometriosis (DIE) in experienced hands. Modified from Nisenblat et al. 2016a.

| Disease type                             | TVUS        | MRI         |
|--|-------------|-------------|
| <b>OMA, number of studies (patients)</b> | 8 (765)     | 3 (179)     |
| Sensitivity, % (95% CI)                  | 93 (87–99)  | 95 (90–100) |
| Specificity, % (95% CI)                  | 96 (92–99)  | 91 (86–97)  |
| <b>DIE, number of studies (patients)</b> | 9 (934)     | 6 (266)     |
| Sensitivity, % (95% CI)                  | 79 (69–89)  | 94 (90–97)  |
| Specificity, % (95% CI)                  | 94 (88–100) | 77 (44–100) |

In less experienced settings, the “**sliding sign**” in TVUS can be used as a screening method for severe endometriosis (Reid et al. 2014, Guerriero et al. 2016a). The sliding sign determines whether the anterior rectosigmoid wall glides freely behind the posterior surface of the uterus, cervix and upper vagina, and free movement is considered a positive sign. A negative sliding sign has been reported to predict pouch of Douglas (POD) obliteration with 83% sensitivity and 97% specificity (Reid et al. 2014) and DIE with 85% sensitivity and 96% specificity (Hudelist et al. 2013). One study demonstrated that patients with obliterated POD have three times higher risk of requiring bowel surgery compared with women with open POD (58% versus 20%;  $p < 0.001$ ), when undergoing laparoscopic endometriosis surgery for pelvic pain or infertility (Khong et al. 2011).

### 2.6.3.1 *Imaging features of ovarian endometrioma*

The most common (51%) ultrasound appearance of an OMA is a unilocular cyst with ground glass echogenicity, but also multilocular (<5) OMAs without papillary structures with detectable blood flow are considered typical (**Figure 7**) (Van Holsbeke et al. 2010, Dunselman et al. 2014). An atypical OMA means a unilocular-solid adnexal mass with ground glass content and with papillary projection, which does not show blood flow (Van Holsbeke et al. 2010). A solid vascularized component in the endometriotic cyst is susceptible of borderline or malignant ovarian tumor (Testa et al. 2011). Previous ovarian surgery, severe adhesions and the presence of other benign ovarian cysts or hydrosalpinx may complicate the ultrasound evaluation. In premenopausal women, the rate of atypical OMA increases with age as cysts are more often multilocular and have papillary or solid components (Guerriero et al. 2016b).



**Figure 7.** Transvaginal ultrasound appearance of (A) a typical unilocular endometrioma with ground glass content and (B) an atypical endometrioma complex with multilocular/bilateral cysts (“kissing ovaries”) with ground glass content and “solid looking” component. No blood flow was detected in the solid part.

An expert ultrasound examiner can reliably distinguish between an OMA and malignancy (Van Holsbeke et al. 2010, Testa et al. 2011). In a large International Ovarian Tumor Analysis (IOTA) Study of 3511 ovarian masses, the examiners' subjective evaluation misclassified only 0.9% of malignancies as an OMA (Van Holsbeke et al. 2010). IOTA simple ultrasound rules (including shape, size, solidity and color Doppler examination) targeted to predict benignity/malignity of an adnexal mass did not improve the diagnostic power of the subjective evaluation.

To date, none of the biomarkers for ovarian cancer, including HE4, have been proven superior to the subjective ultrasound evaluation by an expert sonographer in differentiating malignant and benign ovarian masses (Stukan et al. 2015). Similarly, none of the common diagnostic algorithms for ovarian cancer, i.e. Risk of Malignancy Index (RMI) based on CA-125, menopausal status and ultrasound criteria, Risk of Ovarian Malignancy Algorithm (ROMA) based on CA-125, HE4 and menopausal status or IOTA ultrasound rules, have been proven better compared with an expert ultrasound evaluation in distinguishing benign and malignant ovarian tumors (Stukan et al. 2015).

#### **2.6.4 Biomarkers**

There are numerous studies assessing potential blood, urine and endometrial biomarkers for endometriosis, but none has yet been proven useful in clinical practice (Nisenblat et al. 2016b, Gupta et al. 2016, Liu et al. 2015). Blood, urine and menstrual blood samples are considered non-invasive, while endometrial sampling is a semi-invasive but clinically accepted diagnostic method. **Table 4** shows selected original studies or meta-analyses of interest assessing single or combined non-invasive biomarkers chosen from the excessive literature. Laparoscopy with visual diagnosis is presented as a reference method, and CA-125 and screening questionnaire are shown for comparison.



**Table 4.** Selection of original studies or meta-analyses of interest evaluating the diagnostic accuracy of single or combined non-invasive biomarkers for endometriosis. Laparoscopic visualization is presented as a reference methods and screening questionnaire and CA-125 are shown for comparison. Methods are listed according to the level of sensitivity.

| Marker/panel                                       | Sample/method                       | Number of studies (participants) | Stage, cycle phase               | Sensitivity (95% CI) | Specificity (95% CI) | Reference               |
|--|-------------------------------------|----------------------------------|----------------------------------|----------------------|----------------------|-------------------------|
| miR-199a   | Blood                               | 1 (80)                           | I-IV, NA                         | 100 (NA)             | 100 (NA)             | Maged 2018              |
| miR-125b-5p  | Blood                               | 1 (48)                           | III-IV                           | 100 (NA)             | 96 (NA)              | Cosar 2016              |
| IL-6 + PGP 9.5                                     | Blood + endometrium                 | 1 (78)                           | I-II, proliferative              | 100 (91-100)         | 93 (80-98)           | Elgator el Sharkwy 2013 |
| miR-122  | Blood                               | 1 (80)                           | I-IV, NA                         | 96 (NA)              | 91 (NA)              | Maged 2018              |
| PGP 9.5  | Endometrium                         | 7 (361)                          | I-II or I-IV, varied             | 96 (91-100)          | 86 (70-100)          | Gupta 2016              |
| VIP+PGP 9.5 + SP                                   | Endometrium                         | 1 (40)                           | I-II, secretory                  | 95 (NA)              | 100 (NA)             | Bokor 2009              |
| CA-125 + syntaxin-5 + laminin-1                    | Blood                               | 1 (80)                           | I-IV, NA                         | 95 (86-99)           | 70 (46-88)           | Ozhan 2014              |
| Galectin-9   | Blood                               | 1 (135)                          | I-IV, varied                     | 94 (NA)              | 94 (NA)              | Brubel 2017             |
| Visual evaluation at surgery                       | Laparoscopy                         | 4 (433)                          | NA                               | 94 (80-98)           | 79 (67-87)           | Wykes 2004              |
| miR-199a + miR-122 + miR-145* + miR-542-3p         | Blood                               | 1 (85)                           | I-IV, secretory or proliferative | 93 (84-98)           | 96 (80-100)          | Wang 2013               |
| CA-125 +/-or CCR1 miRNA +/-or MCP-1*               | Blood                               | 1 (151)                          | I-IV, proliferative              | 92 (85-97)           | 82 (68-91)           | Agic 2008               |
| sVCAM-1/sICAM-1 ratio <sup>a</sup>                 | Blood                               | 1 (61)                           | I-IV, secretory or proliferative | 90 (NA)              | 87 (NA)              | Kuessel 2017            |
| IL-6 + TNF- $\alpha$ + CA-125                      | Blood                               | 1 (294)                          | I-IV, secretory                  | 90 (NA)              | 71 (NA)              | Mihalvi 2009            |
| IL-6 + TNF- $\alpha$                               | Blood                               | 1 (85)                           | I-II, secretory                  | 87 (NA)              | 71 (NA)              | Mihalvi 2009            |
| VEGF + annexin V + CA-125 + glycodelin             | Blood                               | 1 (40)                           | Ultrasound negative, menstrual   | 82-90 (NA)           | 63-68 (NA)           | Vodolazkaia 2012        |
| Symptom screening                                  | Questionnaire                       | 1 (625)                          | All (no ultrasound), NA          | 85 (80-89)           | 44 (36-51)           | Nnoaham 2012            |
| IgG endometrial antibodies                         | Blood                               | 4 (759)                          | I-IV, varied/NA                  | 81 (76-87)           | 75 (46-100)          | Nisenblat 2016          |
| Six autoantibodies**                               | Blood                               | 1 (237)                          | I-II, secretory or proliferative | 79 (NA)              | 80 (NA)              | Gajbhiye 2017           |
| CYP19 (Aromatase)                                  | Endometrium                         | 8 (444)                          | Varied, varied                   | 77 (70-85)           | 74 (65-84)           | Gupta 2016              |
| IL-6 + CRP   | Blood                               | 1 (95)                           | I-IV, proliferative              | 75 (63-85)           | 100 (88-100)         | Foda 2012               |
| IL-6+TNF- $\alpha$                                 | Blood                               | 1 (96)                           | I-IV, proliferative              | 70 (57-80)           | 100 (88-100)         | Foda 2012               |
| Menstrual length + CA-125 + endometrial leucocytes | Blood + endometrium + questionnaire | 1 (368)                          | I-IV, luteal                     | 61 (41-81)           | 95 (91-99)           | Gagne 2003              |
| CA-125 $\geq$ 30U/ml                               | Blood                               | 14 (2920)                        | Varied, varied                   | 52 (38-66)           | 93 (89-95)           | Hirsch 2016             |

Note: NA = not applicable, miR = microRNA, PGP 9.5 = gene product 9.5, VIP = vasoactive intestinal polypeptide, SP = substance P, sVCAM = soluble vascular cell adhesion molecule-1, sICAM-1 = soluble intercellular adhesion molecule-1, IL = interleukin, TNF = tumor necrosis factor, CA = cancer antigen, VEGF = vascular endothelial growth factor, \*Test was consider positive if at least one marker above cut-off level, \*\*anti-TMOD3b-autoAb, anti-TMOD3c-autoAb, anti-TMOD3d-autoAb, anti-TPM3c-autoAb, and anti-TPM3d-autoA

### **2.6.4.1 Blood biomarkers**

A recent Cochrane Review of blood biomarkers included 141 studies that evaluated 122 biomarkers (Nisenblat et al. 2016b). These included angiogenesis factors, growth factors, apoptosis markers, cell adhesion molecules, high-throughput markers (biomarkers searched from the proteome of metabolome), hormones, immune system or inflammatory markers, oxidative stress markers, microRNAs (miRNA), tumor markers and other proteins. While most studies assessed the diagnostic performance of single markers, 30 included combined tests of 3–6 biomarkers. The majority of studied biomarkers did not differentiate endometriosis patients from healthy controls. A meta-analysis could be performed on anti-endometrial antibodies, interleukin-6 (IL-6), cancer antigen 19-9 (CA-19-9) and CA-125, and none of these were considered clinically useful.

#### **2.6.4.1.1 CA-125**

Cancer antigen 125 (CA-125) is a glycoprotein expressed by MUC16 gene in endometrium, endocervix, fallopian tubes, pleura and peritoneum, but not in the healthy ovarian epithelium (Kabawat et al. 1983). CA-125 is a well-established biomarker for detecting and monitoring epithelial ovarian cancer (EOC), and values <35 U/ml are considered normal (Sundar et al. 2015). However, CA-125 has low sensitivity in early stage (I-II) EOC (Bast et al. 2005). Furthermore, its expression depends on cancer histology (Høgdall et al. 2007), and it is also elevated in many other malignant and benign diseases and conditions including endometrium, lung, breast, pancreas and colon cancers (Bast et al. 2005), endometriosis, adenomyosis, fibroids, pregnancy and pelvic inflammatory disease (Meden et al. 1998, Park et al. 2012, Hirsch et al. 2016a).

Endometriosis is a common benign reason for elevated CA-125 concentrations. Thus, CA-125 has been extensively studied also in endometriosis (Nisenblat et al. 2016b). Among healthy fertile aged women, median serum CA-125 concentrations of 15.2 U/ml (Park et al. 2012) and 16.0 U/ml (Santulli et al. 2015) have been recently reported, and levels decrease after menopause (Park et al. 2012). Especially ovarian endometriosis, but also DIE, increase CA-125 levels (Fassbender et al. 2015, Santulli et al. 2015). In a large study of 406 endometriosis patients, the mean serum CA-125 was 60.8 U/ml in OMA, 55.2 U/ml in DIE but only 23.2 U/ml in superficial endometriosis (Santulli et al. 2015). Extremely high values have been reported in patients with unruptured or ruptured OMAs (7900 U/ml and >10000 U/ml, respectively) (Kahraman et al. 2007, Park et al. 2014).

CA-125 performs well as a rule-in test for endometriosis, i.e. endometriosis is highly likely if serum levels of CA-125 are elevated (Hirsch et al. 2016a). In a meta-analysis of 14 studies, the cut-off level of  $\geq 30$  U/ml had 93% specificity but only 52% sensitivity (**Table 4**) (Hirsch et al. 2016a). The major flaw of CA-125 is its insufficiency to detect women with minimal or superficial endometriosis only, and to rule out endometriosis (Hirsch et al. 2016a, Santulli et al. 2015). CA-125 fluctuates during menstrual cycle in both endometriosis patients and healthy women, and levels are highest during menstruation (Oliveira et al. 2017, Kafali et al. 2004). Menstrual phase CA-125 level has a good diagnostic performance (AUC 0.96) in DIE (Oliveira et al. 2017), but its usefulness in ultrasound-negative or superficial endometriosis is unknown. Treatment of endometriosis with levonorgestrel-releasing IUS (LNG-IUS) or GnRH agonist decreases CA-125 levels significantly (Petta et al. 2009), and OCs are likely to have a similar effect.

#### **2.6.4.1.2 HE4**

Human Epididymis Secretory Protein 4 (HE4), encoded by WFDC2 gene, is a promising blood biomarker for ovarian cancer with higher sensitivity in early stage disease compared with CA-125 (Havrilesky et al. 2008). However, ovarian cancer subtypes differ in their HE4 expression. Serous, clear cell and endometrioid ovarian carcinomas commonly express HE4, while it is rarely expressed in mucinous, germ cell and sex cord–stromal tumors (Galgano et al. 2006). HE4 is also expressed to a lesser extent in endometrioid adenocarcinoma (Moore et al. 2008).

HE4 is highly expressed in several normal human tissues including trachea, salivary glands, kidney, breast, epididymal and spermatic ducts, prostate, endometrium, fallopian tubes, endocervical and Bartholin's glands, but not in normal ovarian epithelium (Drapkin et al. 2005). In fertile-aged healthy women, upper 97.5% reference ranges of 34 pmol (Park et al. 2012), 51.5 pmol (Bolstad et al. 2012) and 89.1 pmol (Moore et al. 2012) have been reported. In clinical practice, levels  $< 70$  pmol are considered normal in premenopausal women and levels  $< 140$  pmol in postmenopausal women (Abbott Diagnostics). Renal failure and chronic heart failure are the most common benign conditions increasing serum HE4 above the normal values (Escudero et al. 2011, Piek et al. 2017). In addition, smoking, ageing, fibroids and adenomyosis increase (Bolstad et al. 2012, Park et al. 2012), and pregnancy decreases HE4 level (Moore et al. 2012), while menstrual cycle phase or hormonal medication have no significant effect (Moore et al. 2017b, Hallamaa et al. 2012).

### 2.6.4.1.3 Cytokines and other blood biomarkers

A wide variety of **inflammatory and immunological markers** have been examined as possible biomarkers for endometriosis among women with surgically verified or excluded endometriosis (Fassbender et al. 2015, Nisenblat et al. 2016b). However, data are partly limited and presently none of the evaluated inflammatory cytokines or chemokines have been proven useful as single biomarkers (Borrelli et al. 2014, Nisenblat et al. 2016b, Ahn, et al. 2017). However, the Cochrane Review stated that VEGF, IL-6, some oxidative markers, urocortin, high-throughput markers (proteome or metabolome) and follistatin need further studies (Nisenblat et al. 2016b). In a recent study, serum **galectin-9** was reported to have excellent sensitivity and specificity in differentiating endometriosis patients from healthy controls (94% and 94%, respectively; **Table 4**) (Brubel et al. 2017).

Numerous studies have tested various combinations of blood biomarkers with only few showing promise (Nisenblat et al. 2016b, Agic et al. 2008, Foda et al. 2012, Wang et al. 2013, Ozhan et al. 2014). Recently, the ratio of serum soluble vascular cell adhesion molecule-I (sVCAM-I) and soluble intercellular adhesion molecule-I (sICAM-I) (**sVACM-I/sICAM-I ratio**) was reported as a promising biomarker with an AUC of 0.93, 90% sensitivity and 87% specificity (**Table 4**) (Kuessel et al. 2017). In addition, several serum **microRNAs** or their combinations have also shown promise as biomarkers, but results are not uniform and further studies are needed (Nisenblat et al. 2016b, Ahn et al. 2017). Two new studies showed high sensitivity and specificity of miR-122, miR199a and miR-125b-5p (**Table 4**) (Maged et al. 2018, Cosar et al. 2016). A combination of four miRNAs (miR-199a, miR-122, miR-145\* and miR-542-3p) had an AUC of 0.99, sensitivity of 93% and specificity of 96% (**Table 4**) (Wang et al. 2013). A meta-analysis of four studies assessing **IgG anti-endometrial antibodies** in detecting endometriosis with varying methodology, had a mean sensitivity and specificity of 81% (95% CI 76-87%) and 75% (95% CI 46-100%), respectively (**Table 4**) (Nisenblat et al. 2016b).

Some studies have tried to identify blood biomarker combinations especially for minimal–mild or ultrasound negative endometriosis (**Table 4**). In one study, a panel of **six auto-antibodies** (anti-TMOD3b-autoAb, anti-TMOD3c-autoAb, anti-TMOD3d-autoAb, anti-TPM3a-autoAb, anti-TPM3c-autoAb, and anti-TPM3d-autoAb) had an AUC of 0.87, sensitivity of 79% and specificity of 80% in detecting minimal–mild endometriosis (Gajbhiye et al. 2017). In another study evaluating a panel of six plasma biomarkers, minimal–mild endometriosis was best detected in proliferative phase with a combination of **IL-6** and tumor necrosis factor- $\alpha$  (**TNF- $\alpha$** ; AUC 0.85, sensitivity 87% and specificity 71%) (Mihaljiy et

al. 2010). A third study assessed the diagnostic performance of 28 plasma biomarkers, and ultrasound negative endometriosis was best diagnosed with a combination of **VEGF, annexin V, CA-125 and glycodelin or sICAM-I** taken during the menstrual phase (AUC of 0.78-0.86, sensitivity of 81–90% and specificity of 63–81%) (Vodolazkaia et al. 2012).

#### **2.6.4.2 Urine and endometrial biomarkers**

A urine sample is easy and cheap to obtain, and it would be an ideal method for non-invasive diagnostics. However, limited data exist on urinary biomarkers for endometriosis. **Urinary enolase 1 (NNE), vitamin D binding protein (VDBP) and urinary peptide profiling** have been reported to distinguish between women with and without endometriosis, however without sufficient power for clinical diagnosis (Liu et al. 2015).

**Menstrual fluid and specimens of endometrial tissue** have been investigated in 54 diagnostic studies of endometriosis according to a Cochrane Review (Gupta et al. 2016). The assessed biomarkers have included angiogenesis and growth factors, cell adhesion and DNA-repair molecules, endometrial and mitochondrial proteome as well as hormonal, inflammatory, myogenic, neural and tumor markers. Of these, **endometrial CYP19 (aromatase) and gene product 9.5 (PGP 9.5)**, a marker of nerve fibers in the functional layer of the endometrium, have been more widely studied and included in a meta-analysis (**Table 4**). Pooled analysis of eight studies found PGP 9.5 accurate in diagnosing endometriosis (sensitivity 96% and specificity 86%), but there was wide heterogeneity between the studies (sensitivity 19–100 and specificity 5–100). Women in the included studies were not using hormonal treatment. Importantly, hormonal medication is reported to decrease endometrial nerve fiber density, and medication may hamper testing in clinical practice (Tokushige et al. 2008). A combined test of three endometrial neural markers, i.e. **vasoactive intestinal polypeptide (VIP), PGP 9.5 and substance P (SP)**, obtained from Pipelle samples could differentiate women with minimal–mild endometriosis from healthy women with 95% sensitivity, 100% specificity and 98% accuracy (**Table 4**) (Bokor et al. 2009).

CYP19 studies included in a meta-analysis revealed a sensitivity of 77% (95% CI 70-85) and specificity of 74% (95% CI 65-83) (Gupta et al. 2016). Several additional endometrial markers including endometrial proteome, HSD17B2, IL-1R2, caldesmon and other neural markers, have also shown promise as biomarkers for endometriosis (Gupta et al. 2016).

### 2.6.4.3 Combination of diagnostic methods

Presently, only a few studies have combined biomarkers obtained with different measurements from different body fluids and tissue specimens with some showing promise (Nisenblat et al. 2016c). The most interesting study combined **serum IL-6** (cut-off >15.4 pg/ml) **with endometrial PGP 9.5** (metallic suction curette sample) and reported 100% sensitivity and 93% specificity (**Table 4**) (Elgafor El Sharkwy 2013). Another study combined **the duration of menstruation, CA-125** (cut-off >35U/ml) **and endometrial leukocytes** and detected 61% sensitivity and 95% specificity (**Table 4**) (Gagné et al. 2003). One study used proteomic technique to analyze urine samples, and found twenty-two proteins differentiating patients with and without endometriosis (Cho et al. 2012). **A multiplication of creatinine corrected urine vitamin D binding protein (VDBP-Cr) and CA-125** (VDBP-Cr x CA-125; cut-off >2755) had 74% sensitivity and 97% specificity. However, the authors stated that CA-125 alone (cut-off >35 U/ml) was equal to the tested combination.

## 2.7 Treatment of endometriosis

Endometriosis-related symptoms can be treated with analgesics, hormonal medications and surgery, but none of these is curative. IVF is the preferred option in endometriosis-related infertility. Long-term hormonal therapy forms the basis for the treatment and it has several aims: to alleviate symptoms and reduce the need for analgesics, to avoid surgery, to prevent disease progression or to decrease the risk of postoperative recurrence (Vercellini et al. 2015). Pain relapse is expected at discontinuation, and effective medication should not be stopped without a meaningful reason such as attempting a pregnancy or reaching menopause. However, medical therapy is ineffective or poorly tolerated in approximately 20% of patients (Vercellini et al. 2011b).

Current guidelines encourage offering medical treatment without surgical confirmation if the woman presents with typical symptoms (Johnson et al. 2013, Schleedoorn et al. 2016). If empirical medical therapy fails or cannot be used, a “see-and-treat” laparoscopy should be offered to symptomatic adolescents and adults (Rogers et al. 2017, Vercellini et al. 2015). In some women, surgery is indicated as an investigation of unexplained infertility or prior to IVF due to large OMAs or invalidating pain. In rare cases, procedures are necessary due to life-threatening situations such as bowel occlusion or hydronephrosis. Surgical treatment should be individually tailored depending on the symptoms, patient’s age, current or future desire for pregnancy and the disease status, and carried out

in centers of expertise. In cases with pain resistant to treatment or recurrent chronic pain without clinically significant findings, the patient care should be shifted from surgical and medical treatment-oriented to a life with acceptance, self-management and cognitive-behavioral management of pain (Jarrell 2011).

### **2.7.1 Medical therapy**

Non-steroidal anti-inflammatory drugs (**NSAIDs**) are the primary analgesics recommended in endometriosis, as inflammation is essential in the pathogenesis. NSAIDs inhibit COX-1 and COX-2 enzymes, hence reducing PG production. Different NSAIDs have comparable efficacy in primary dysmenorrhea (Iacovides et al. 2015), but limited evidence exists on endometriosis-related pain (Brown et al. 2017). NSAIDs can be combined with paracetamol, but opioids should be avoided in the long-term medical treatment. Neuropathic or centralized pain component is resistant to NSAIDs but may respond to adjunctive analgesics including gabapentin, pregabalin, amitriptyline and duloxetine (Morotti et al. 2017).

**Progestins or OCs**, including vaginal ring and dermal patch, should be offered as the first line therapy for women presenting with typical pain symptoms (Dunselman et al. 2014, Schleedoorn et al. 2016, Vercellini et al. 2016). They offer a good long-term option as they are cost-effective and have a good safety profile and acceptable side effects (Vercellini et al. 2016). OCs and most progestins also act as reliable contraceptives if needed. Progestins can be administered orally, intramuscularly/subcutaneously or by intrauterine route by using levonorgestrel-releasing IUS (LNG-IUS). Strongest evidence on efficacy exists on norethisterone acetate (NETA) and dienogest (Vercellini et al. 2016). Available commonly used hormonal therapies are shown in **Table 5**. Most hormonal medications have approximately the same efficacy for pain alleviation, and at least two-thirds of patients respond to OCs or progestins (Vercellini et al. 2015, Vercellini et al. 2016).

**Table 5.** Commonly used progestins or combinations of estrogen and progestin with evidence of efficacy for endometriosis. Modified from Vercellini et al. 2016.

| Hormonal product  | Administration route               |
|---|------------------------------------|
| <b>Progestins</b>                                       |                                    |
| Norethisterone acetate (NETA) 2.5–5 mg/day              | Oral                               |
| Lynesterol 5–20 mg/day                                  | Oral                               |
| Medroxyprogesterone acetate 10–50 mg/day                | Oral                               |
| Dienogest 2 mg/day                                      | Oral                               |
| Desogestrel 75 µg/day                                   | Oral                               |
| Levonorgestrel IUS 20 µg/24h                            | Intrauterine                       |
| Etonogestrel implant 68 mg                              | Subcutaneous implant               |
| <b>Combined contraceptives i.e. estrogen–progestins</b> | Oral, vaginal ring or dermal patch |

Progestins have been used for decades in endometriosis care. They inhibit ovulation and suppress ovarian estrogen synthesis in a dose-dependent manner. They also have anti-inflammatory effects and can inhibit implantation of eutopic endometrium by inhibition of matrix metalloproteinases and angiogenesis (Vercellini et al. 2003a). Furthermore, progestins inhibit endometrial growth, induce apoptosis in endometrial cells and reduce nerve fiber density in endometriotic lesions (Tokushige et al. 2009, Reis et al. 2013). Contraindications are rare, but irregular bleedings often compromise the treatment compliance. Progestin effect is prevalent also in OCs, which offer a better bleeding profile compared with progestins preparations. However, contraindications and increased risk for thromboembolism hamper their use (Vercellini et al. 2016). OCs can be administered cyclically, with a prolonged cycle or continuously. Continuous use offers good dysmenorrhea relief when cyclic treatment is ineffective (Vercellini et al. 2003b). Furthermore, postoperative OC use markedly reduces the risk for OMA recurrence and continuous use is most favorable (Vercellini et al. 2013b).

Endometriosis is an estrogen-dependent disease, and menstruation and ovulation likely play a major role in the pathophysiology. Thus, all current hormonal treatments aim at decreasing ovarian estrogen synthesis, suppressing ovulation and/or controlling bleedings (Vercellini et al. 2016). From patients' perspective, a prolonged menstrual cycle or amenorrhea is preferable because pelvic pain is typically at its worst during menstruation. Furthermore, the decrease or absence of retrograde menstrual bleeding will likely inhibit cyclic pelvic inflammation and prevent *de novo* formation of peritoneal lesions. Ovulation is considered crucial in the formation of OMA, and anovulation is preferable in women with ovarian endometriosis.

Second line medical therapies include **GnRH agonists** (commonly leuprolide 3.75 mg/28d) and **aromatase inhibitors** (letrozole 2,5 mg/day or anastrozole 1 mg/day) (Johnson et al. 2013, Ferrero et al. 2015). GnRH agonists strongly sup-



press ovarian function and induce a temporary menopause-like status, and they have a favorable effect on pain. However, menopausal side effects and bone loss limit their use to 6 months, but additional add-back treatment with hormonal replacement therapy enables longer use. Furthermore, high costs limit the usefulness of GnRH agonists. Aromatase inhibitors suppress local estrogen synthesis in endometrium and endometriosis. They induce ovulation, and are thus usually combined with OCs or progestins. Options for future medical therapy may include gonadotropin-releasing hormone receptor antagonists, selective progesterone receptor modulators (SPRM), selective estrogen receptor modulators (SERM), anti-angiogenic medications and immunomodulators (Bedaiwy et al. 2017).

### 2.7.2 *Surgical treatment*

Laparoscopy is currently the preferred approach for endometriosis surgery with substantial benefits, although open surgery is equally effective in the treatment of pain (Dunselman et al. 2014). Complete removal of all visually detectable endometriotic lesions results in best alleviation of pain (Hidaka et al. 2012, Angioni et al. 2015, Cao et al. 2015). However, the peritoneal or bowel wall lesions may be microscopic in size (Khan et al. 2014, Roman et al. 2016), and surgery can likely never be truly complete.

**Superficial endometriotic lesions** can be treated with laparoscopic excision (surgical removal of the peritoneal implants) or ablation (electrocoagulation or vaporization of the lesions). A recent meta-analysis concluded that surgical excision shows a greater relief of dysmenorrhea, dyschezia and CPP at 12 months after surgery compared with ablation (Pundir et al. 2017). Surgery on **OMAs** is more controversial. Laparoscopic removal of an OMA reduces pain, and excision of the cyst wall, i.e. stripping technique, results in better outcome in terms of pain relief and risk of recurrence compared with the ablative techniques (Dunselman et al. 2014). However, both the OMA per se and the surgical excision impair the ovarian reserve, and the indication and timing of OMA surgery should be critically considered (Uncu et al. 2013, Endometriosis Italian Treatment Club 2014).

Surgical removal of **DIE** lesions reduces endometriosis-associated pain and improves HRQoL and sexual function (For review see Dunselman et al. 2014, Ferrero et al. 2015). However, surgery for DIE carries a significant risk of intra- and postoperative complications, such as bleeding, infection, bowel or ureter injuries, bowel anastomosis leakage, rectovaginal fistula, post-anastomotic rectal stenosis or dysfunctional symptoms of the bladder or the bowel (Vercellini et al.

2009a, De Cicco et al. 2011, Donnez et al. 2017), and not all patients are willing to accept these risks. Large or multiple bowel nodules are usually treated with segmental bowel resection, but otherwise the tendency is towards more conservative methods, i.e. discoid resection or shaving of the bowel nodule, as they include a lower risk of complications (Donnez et al. 2017).

Short-term effect of endometriosis surgery on pain symptoms is good, but the beneficial effect decreases with time (Duffy et al. 2014, Abbott et al. 2003, Seracchioli et al. 2010a). A Cochrane Review concluded that surgical excision or ablation of minimal–moderate endometriosis offers a good short-term pain relief for up to 12 months (Duffy et al. 2014). However, surgery is also associated with 22–32% placebo effect, and thus only 30–40% of patients could be considered benefitting from surgery (Vercellini et al. 2009a).

Long-term (~3 years or longer) data on surgical pain outcomes are limited and very heterogeneous. These studies have mainly focused on DIE, and such patients seem to accomplish a long-term improvement in both pain and QoL (Vercellini et al. 2009a, Meuleman et al. 2011, Ferrero et al. 2015). A few studies have included patients with only peritoneal and/or ovarian endometriosis or patients with all r-ASRM stages, and outcomes have been favorable (Vercellini et al. 2009a, Porpora et al. 2010, Coccia et al. 2011, Vercellini et al. 2006, Abbott et al. 2003). However, comparisons of pain outcomes between women with and without DIE have not been published.

### **2.7.3 Risk of recurrence after surgery**

High risk of pain or disease recurrence is the main challenge after the first-line surgery. Recurrent pain or disease is a complex of dynamics between several factors, and pain recurrence does not automatically indicate recurrent disease and vice versa. Several factors may influence the surgical outcome: the type of endometriosis, extent of the surgery and skills of the surgeon, postoperative hormonal medication, fertility issues, other comorbid pains conditions, personality and centralization of pain may have an effect on the outcome (Guo 2009). Postoperative medical treatment with cyclic or continuous OCs or LNG-IUS is recommended to reduce the risk of recurrence (Vercellini et al. 2015).

Disease types and patient groups vary in their risk of recurrence and reoperation. High overall reoperation rates have been reported in studies including women with all disease types or r-ASRM stages; 36% at 3 years (Abbott et al. 2003), 34% at 5 years (Tandoi et al. 2011) and 46% at 7 years (Shakiba et al. 2008). Among adolescent patients with all r-ASRM stages, the reported symptom or

disease recurrence has been as high as 46–56% (Audebert et al. 2015, Tandoi et al. 2011, Yang et al. 2012b). OMA recurrence has been shown to affect 10–50% of patients after a 2–5 year long follow-up (Porpora et al. 2010, Vercellini et al. 2013b, Seracchioli et al. 2010b). After surgery for DIE with colorectal involvement, the recurrence rates (>2 years), have mostly been near 10% (5–25%) (Meuleman et al. 2011).

### **3 AIMS OF THE STUDY**

This study had several aims. The first aim was to explore the prevalence and severity of symptoms suggestive of endometriosis among adolescent girls, as most patients report the onset of pain symptoms already during adolescence. Little is known about adolescent symptoms and endometriosis. The second aim was to discover novel methods for non-invasive diagnosis and differential diagnosis of endometriosis. The rationale is the long diagnostic delay noticed worldwide and the lack of a non-invasive diagnostic method. New tools would shorten the diagnostic delay, could help to avoid unnecessary surgery and improve the differential diagnosis between ovarian endometriosis and ovarian cancer. A wide panel of serum cytokines was chosen for diagnostic testing because previous studies have shown increased levels of various cytokines in the serum and in the peritoneal fluid of the patients. A novel ovarian cancer marker HE4 was explored, as its performance in endometriosis was unknown. The last aim was to evaluate whether surgery relieves pain symptoms in the long run, and to identify the risk of reoperation in the study setting.

The specific aims were:

1. To evaluate the prevalence and severity of pain symptoms suggestive of endometriosis among adolescent girls.
2. To explore 29 serum cytokines as biomarkers for endometriosis and to compare them with serum CA-125.
3. To analyze the usefulness of serum HE4 in differentiating ovarian endometriosis from ovarian cancer.
4. To estimate the long-term effect of complete endometriosis surgery on pain symptoms.

## **4 MATERIALS AND METHODS**

Detailed description of materials and methods used in this academic dissertation are included in the original publications I–IV. The original tables and figures are cited with italics in parenthesis.

### **4.1 TEENMAPS data (I)**

Study I was a cross-sectional questionnaire study organized at the Department of Obstetrics and Gynecology at Turku University Hospital, Finland. The Ethics Committee of Hospital District of Southwest Finland approved the study protocol. The study name is an abbreviation constructed from the words “**T**eenage **M**enstrual and **A**bdominal **P**ain **S**ymptoms”. The target population was the 15–19 year old girls attending elementary school, high school or vocational institute in three municipalities in Southwest Finland. In Turku, the data was collected during spring 2010, and in Lieto and Kaarina during spring 2011. The estimated study population size was 3814, and the figure was based on personal information from the school nurses.

A 49-point questionnaire was developed for this study, and it included details on menstrual characteristics, contraception, pain medication, absenteeism from school or hobbies, comorbidities and presence and severity of five pain symptoms (dysmenorrhea, acyclic abdominal pain, dyspareunia, dyschezia and dysuria). Participants evaluated the severity of each pain type at its worst by using numerical rating scale (NRS 0–10). The recall period at which the most severe pain symptoms were experienced was not defined in the question. However, other questions included data on durations and frequency of pain as well as the relationship of pain to menstruation. The school nurses distributed the questionnaire to girls attending school at the time of the study, and participation was voluntary and anonymous.

### **4.2 ENDOMET data (II, III, IV)**

Studies II, III and IV are part of the ENDOMET study (Novel diagnostic tools for endometriosis and their exploitations for prognosis and prevention of complications), and they were organized at the Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku, and at the Research Centre for Integrative Physiology and Pharmacology/Physiology, University of Turku, Turku, Finland.

#### 4.2.1 *Endometriosis patients and healthy controls (II, III, IV)*

Altogether, **137 endometriosis patients** (II, III, IV) scheduled for surgical treatment in two University Hospitals (Turku and Helsinki) and two Central Hospitals (Pohjois-Karjala and Päijät-Häme) and **62 healthy control women** (II, III) seeking laparoscopic sterilization in Turku University Hospital were prospectively recruited between October 2005 and November 2007. The Ethics Committee of Hospital District of Southwest Finland approved the study protocol, and a signed informed consent was required from all participants prior to surgery.

Participants were aged between 18 and 48, and they were allowed to use hormonal contraception or hormonal treatment for endometriosis before and after the surgery if appropriate. The exclusion criteria were acute pelvic infection, pregnancy, suspicion of malignancy and other significant diseases and medications that could interfere with the study protocol. Preoperative diagnostic protocol was carried out according to clinical practice, and a minimum of gynecological examination combined with TVUS was performed for all patients.

Seven gynecological surgeons experienced with the treatment of endometriosis carried out the operations in the participating hospital with a multidisciplinary team, if needed. Three gynecologists carried out most procedures. Surgical treatment was performed by laparoscopy or laparotomy, and endometriosis was confirmed by histopathological evaluation performed by one pathologist. Endometriosis was classified into r-ASRM stages I–IV. The operations were carried out irrespective of the menstrual cycle phase. Additional pathology, such as fibroids or benign ovarian tumors, was recorded during endometriosis surgery. In control women, endometriosis was visually excluded during laparoscopic tubal ligation. Women with suspected endometriosis but no observed lesions in laparoscopy (n=3) were considered as healthy controls in study III and excluded from studies II and IV. Women with unexpected asymptomatic endometriosis in sterilization (n=8) were included as patients in study III and excluded from studies II and IV.

Serum samples were collected prior to surgery, and for study III altogether nine control women had blood samples taken twice in different menstrual cycle phases. Endometrial cancer was excluded and the phase of the menstrual cycle was defined by endometrial sample (Pipelle de Cornier; Laboratoire CCD, Paris, France, [www.ccd-international.com](http://www.ccd-international.com)) taken during surgery from all participants. The phase of the menstrual cycle was defined as proliferative, secretory, atrophic, inactive, menstrual or insufficient. Study population size and r-ASRM stage in studies II, III and IV are shown in **Table 6**.

**Table 6.** Number of participants and their disease, and disease status in studies II, III and IV.

| Patient group               | Study II | Study III | Study IV |
|-----------------------------|----------|-----------|----------|
| <b>Endometriosis*</b>       | 124      | 129       | 100      |
| Stage I–II                  | 28       | 33        | 28       |
| Stage III–IV                | 93       | 96        | 72       |
| Stage not recorded          | 3        |           |          |
| <b>Healthy controls,</b>    | 53       | 66        |          |
| <b>Ovarian cancer**</b>     |          | 14        |          |
| Stage I–II                  |          | 5         |          |
| Stage III–IV                |          | 9         |          |
| <b>Endometrial cancer**</b> |          | 16        |          |
| Stage I–II                  |          | 14        |          |
| Stage III–IV                |          | 2         |          |

\* r-ASRM stage, \*\* FIGO stage

Specific study questionnaires were designed for the ENDOMET study. Patients completed a detailed questionnaire preoperatively and annually for five years after surgery. This questionnaire evaluated menstrual history, family history, pain symptoms (dysmenorrhea, dyspareunia, dyschezia, noncyclic abdominal pain and dysuria), fertility issues, current hormonal medication, reoperations and the current main problem. Patients evaluated the severity of each pain type at its worst by using numerical rating scale (NRS 0–10). In addition, they were asked for how long time they had experienced each pain type (<1 year, >1 year, always, how many years?), how frequently they felt each pain and whether the pain was related to the menstrual cycle phase. The operating gynecologists recorded detailed surgical data including r-ASRM stage, endometriosis lesion types, location and size, procedures performed and intraoperative complications. Postoperative complications were retrospectively searched from patients' hospital records and classified according to the Clavien–Dindo system (Dindo et al. 2004). According to the Clavien–Dindo classification, grade I–II complications do not require any significant intervention or can be treated with medication, blood transfusion or total parenteral nutrition. In contrast, grade III–IV complications require endoscopic, surgical or radiological intervention and/or treatment in the intensive care unit. Grade V complication results in death of the patient.

#### 4.2.2 Patients with ovarian or endometrial cancer (III)

Serum samples of 14 women with diagnosed ovarian cancer (*OvCa*) and 16 women with endometrial cancer (*EmCa*) were included (**Table 6**). Malignancies were confirmed using histopathological samples collected in laparoscopy or laparotomy, and disease stage was recorded according to FIGO classification (Benedet et al. 2000). Ovarian cancer cases included seven serous, three mucin-

ous, two clear cell, one endometrioid and one small cell carcinoma. All endometrial cancers were endometrial adenocarcinomas.

#### **4.2.3 Serum biomarker analysis (II, III)**

The serum samples were collected from all participants within 24 hours prior to surgery into non-heparinized tubes and centrifuged for 15 min at 800 g after being kept at room temperature for 30 minutes. The serum was stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  until analyzed, and the storage time did not exceed 3 years. HE4 and CA-125 concentrations were evaluated using ELISA analysis according to the manufacturer's instructions (Fujirebio Diagnostics Inc, Malvern, PA, USA).

Serum concentrations of 29 cytokines were measured using Human Cytokine/Chemokine Pre-mixed LINCoplex Kit according to the manufacturer's instructions (HCYTO-60K-PMX29; LINCO Research Inc., St. Charles, Missouri, USA). This panel was chosen, as it was the widest commercial panel available when this study was initiated. This multiplex assay kit enables the simultaneous quantitative determination of the following proteins: EGF, eotaxin, fractalkine, G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN- $\gamma$ ), interleukin-1alpha (IL-1 $\alpha$ ), IL-1 $\beta$ , IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN- $\gamma$ -induced protein-10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1 $\alpha$ ), macrophage inflammatory protein 1-beta (MIP-1 $\beta$ ), soluble CD40 ligand (CD40L), transforming growth factor alpha (TGF- $\alpha$ ), TNF- $\alpha$  and VEGF. According to the manufacturer, the intra-assay variation is between 1.6% and 14.6%, and the inter-assay variation is from 5.0% to 15.6%, depending on the analyte. Measurements of serum CD40L were excluded because of constantly high values in all participants. Thus, 28 of the 29 measured cytokines were included in the statistical analyses. Whenever the cytokine concentration was outside the upper or lower detection limits, the closest detectable value of each marker was used instead.

### **4.3 Statistical methods**

#### **4.3.1 Study I**

Analyses were performed using PASW Statistics 19 software (SPSS Inc., IBM, Chicago, USA). The baseline clinical characteristics were compared using Chi-



Square Test or Two-sample T-test, as appropriate. Chi-Square Test for Trend was used to analyze dysmenorrhea prevalence and severity according to both age and time since menarche. T-test was used to compare pain severities (NRS) between different subgroups, since the variables were normally distributed. P-value <0.05 was considered statistically significant. Correlations between variables were analyzed by Spearman's correlation coefficient. OR was computed to evaluate the effect size of selected variables. The prevalence of each parameter was calculated based on the answers received for each question (valid %).

#### **4.3.2 Study II**

Statistical analyses were performed using PASW Statistics 19 software (SPSS Inc., IBM, Chicago, USA) and SAS System for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA). The categorical baseline characteristics were compared using Chi-Square Test, Two-sample T-test or Mann-Whitney U-test, as appropriate. The effect of age and BMI on markers was tested using Spearman rank correlation coefficients. Rank analysis of covariance adjusted for age was used for comparison of each marker concentration between patients and controls. The comparisons between stage I/II, stage III/IV and the control group were done with rank analysis of covariance adjusted for age, and Bonferroni's adjusted p-values were used in pairwise comparisons.

ROC analysis was used to evaluate the significance of each marker in distinguishing patients from controls. AUCs between different models were compared using nonparametric approach. Multivariate analysis of a marker panel was done using age-adjusted stepwise logistic regression model, including markers with significant p-values in both age-adjusted rank analysis of covariance and ROC analysis. The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of selected markers were calculated. The optimal cut-off values were estimated by choosing the value with the highest sum of sensitivity and specificity. A p-value of <0.05 was considered statistically significant.

#### **4.3.3 Study III**

HE4 and CA-125 concentrations separately and in combination were analyzed using Tukey's multiple comparisons of means with 95% family-wise confidence level. The classification capability of HE4 and CA-125 as single markers and together, were assessed and sensitivity at 95% specificity and accuracy were cal-

culated. The receiver operating characteristics (ROC) curves were constructed and the area under curve (AUC) was used to summarize the overall performance of the regression model.

#### 4.3.4 Study IV

Baseline patient characteristics were analyzed using PASW Statistics 23 software (SPSS Inc., IBM, Chicago, USA). The categorical variables between the subpopulations were compared using Chi-Square test or Fisher's exact test and the numerical variables were compared with Mann–Whitney U-test. Data are given as numbers (n) and percentages (%) or medians with interquartile range (IQ range), and ORs are presented with 95% CI. These tests were two-sided and unadjusted for confounding factors, and  $p < 0.05$  was considered statistically significant.

Pairwise associations of pain severity (NRS) between the baseline and individual follow-up time points in the whole study population were analyzed with generalized linear mixed-effect models with the logistic link function. P-values were corrected for multiplicity but otherwise unadjusted. Subjects were grouped as a random effect. Response variables were the ordinal NRS pain scales and the only predictor variable was the time point. These analyses were carried out using SAS version 9.4 (SAS Institute INC., Cary, USA).

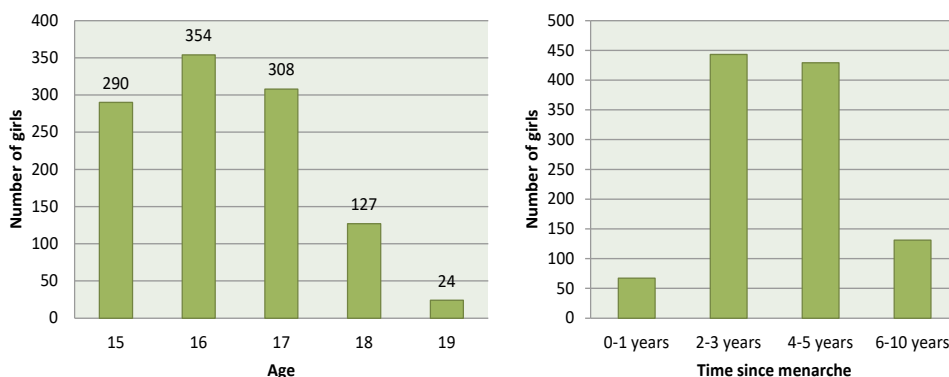
After exploring the longitudinal effects in a pairwise manner, a more comprehensive linear mixed-effects modeling of all the follow-up pain types was conducted to model their longitudinal relationships with a multivariable model separately fit for each pain type. A greedy forward-selection model building strategy (Guyon et al. 2003) was utilized by testing a single potential new fixed effect at a time. The set of tested fixed effect hypotheses included a year-specific change in pain severity due to hormonal medication, wish for pregnancy, given birth, and self-reported pain problem. Tested baseline variables included patient's age and BMI, presence of severe endometriosis (r-ASRM III/IV-disease), hysterectomy and DIE. In addition to having these indicators alone, further longitudinal hypotheses were introduced by multiplying these with the follow-up year variable. The baseline and time-dependent covariates were chosen based on the literature on potential risk factors and protective factors for pain or disease recurrence. The comprehensive modelling was conducted using R.3.2.2 (36) with the R-packages lme4 (version 1.1-12), lmerTest (v. 1.0) and Hamlet (v. 0.9.4-2), all publicly available in the Comprehensive R Archive Network (CRAN) repository (R Core Team 2018).

## 5 RESULTS

### 5.1 Prevalence and severity of pain symptoms suggestive of endometriosis among Finnish adolescent girls (I)

The study questionnaire was distributed to 2582 girls, 1117 envelopes were returned and 1103 eligible answers were included in the analysis. The overall response rate was 43% (1117/2582), and a great variation was detected between the schools (0–100%) and the municipalities (Lieto 24%, Turku 41% and Kaarina 91%). However, no statistically relevant differences were detected in the main variables of interest (age, BMI, prevalence and intensity of pain, OC use and smoking) between the participating municipalities.

The mean age of girls was 16.8 years and the mean age at menarche was 12.6 years (range 9–16). **Figure 8** shows the distribution of calendar age and time since menarche among participants.



**Figure 8.** Distribution of age and time since menarche among 1103 girls participating in the TEENMAPS study.

The prevalence and severity of each pain symptoms is presented in **Table 7**. Dysmenorrhea was commonly reported (67.6%) while other pain symptoms were less frequent. One-third of all participants (33%,  $n=355/1089$ ) had severe dysmenorrhea (NRS 8–10) and these girls had a 10-fold risk of regular absenteeism from school or hobbies due to pain compared with girls with mild or moderate dysmenorrhea (*I*, *Table 1*). These girls also showed an increased risk of concomitant acyclic abdominal pain (OR 2.67; 95% CI 1.96–3.64) or dyschezia (2.56; 95% CI 1.62–3.93) compared with all other participants (*I*, *Table 2*).

**Table 7.** Prevalence and severity of pain symptoms suggestive of endometriosis among 1103 girls aged 15–19 years. Pain is assessed at its worst with numerical rating scale (NRS 0–10).

| Symptom                         | Prevalence (%) | Severity <sup>a</sup> (SD) |
|---------------------------------|----------------|----------------------------|
| Dysmenorrhea (n=735)            | 67.6           | 7.0 (2.0)                  |
| Acyclic abdominal pain (n=207)  | 19.1           | 6.3 (2.3)                  |
| Dyspareunia (n=53) <sup>b</sup> | 11.6           | 5.6 (2.3)                  |
| Dyschezia (n=85)                | 8.0            | 4.9 (2.4)                  |
| Dysuria (47)                    | 4.6            | 5.0 (1.5)                  |

<sup>a</sup> Mean NRS (standard deviation), <sup>b</sup> only girls who had intercourse more than once were included

The participants commonly used OC (30.5%; n=333/1091), and 61.4% of users (151/246) reported dysmenorrhea as one indication. Most girls with dysmenorrhea used pain medication, mostly NSAIDs, for their complaint (80%; n=588/735). However, in 43% the medication offered little or no pain relief (*I, Figure 1*). By including different characteristics chosen based on the literature we could identify girls at risk of having endometriosis (**Table 8**).

**Table 8.** Proportions of girls with symptoms suggestive of endometriosis estimated using different characteristics among 1103 Finnish girls aged 15–19 years. Modified from Suvitie et al. 2016.

| Characteristics   | n   | % <sup>a</sup> |
|---|-----|----------------|
| Dysmenorrhea, dyschezia and acyclic abdominal pain  | 28  | 2.5            |
| Pain medication 3 ≥ days for dysmenorrhea with inadequate relief <sup>b</sup>                   | 59  | 5.3            |
| Severe dysmenorrhea <sup>c</sup> lasting three or more days                                     | 64  | 5.8            |
| Severe dysmenorrhea <sup>c</sup> combined with acyclic abdominal pain                           | 106 | 9.6            |
| Severe dysmenorrhea <sup>c</sup> , using OC and pain medication, inadequate relief <sup>b</sup> | 53  | 4.8            |
| Severe dysmenorrhea <sup>c</sup> and absent from school monthly or most months                  | 49  | 4.4            |
| Severe dysmenorrhea <sup>c</sup> , using OC, absent from school monthly or most months          | 21  | 1.9            |

Note: OC = oral contraceptive pill, <sup>a</sup> Prevalence among all 1103 participants, <sup>b</sup> No or minimal help of pain medication, <sup>c</sup> Severe dysmenorrhea = numerical rating scale 8–10

## 5.2 Performance of 28 serum cytokines in the diagnostics of endometriosis and in comparison to CA-125 (II)

The study consisted of 124 patients with endometriosis and 53 healthy control women. The patients were younger than the controls (mean age 31.4 vs. 39.2,  $p < 0.001$ ; *II, Table 1*). Age was included as a confounding factor, because of small but statistically significant correlation with age in some of the analyzed markers. Twenty-eight patients (23%) had stage I–II and 93 (75%) had stage III–IV disease, while in three patients (2%) the stage was not recorded. Patients and con-

trols used hormonal medication, mostly OCs or LNG-IUS, as often (in 44% and 42%, respectively;  $p=0.06$ ).

Interestingly, the use of hormonal medication did not affect the biomarker values in endometriosis patients, while in the control women, the levels of IL-1ra, TGF $\alpha$  and VEGF were slightly but significantly higher among women using hormonal medication ( $p=0.03$ ,  $0.04$  and  $0.045$ , respectively). These findings were not considered interfering with the results, as patients and control women used hormonal medication as often.

The biomarker concentrations were compared between proliferative and secretory phase separately in the patient and control groups. Most participants using hormonal medication were excluded from this analysis because their endometrial sample was defined as atrophic, inactive or insufficient. Among patients, only serum fractalkine showed significant cycle-dependent difference, and the median concentration was 10-fold higher during proliferative phase compared with secretory phase (41.9 vs. 4.3,  $p=0.02$ ). Among the controls, TGF $\alpha$  and VEGF were significantly higher during secretory phase as compared with proliferative phase (median 23.8 vs. 4.0,  $p=0.02$  and 128.7 vs. 65.1,  $p=0.03$ , respectively). These findings were not considered important in the study, as the concentrations of these three markers were equal between patients and controls (*II, Table 2*).

The median serum concentrations of five cytokines (G-CSF, IL-1Ra, EGF, IP-10, IL-17) and CA-125 were significantly different in patients compared with controls by using age-adjusted rank analysis of covariance (**Table 9**). The most significant difference was observed in the concentration of CA-125 ( $p<0.001$ ).

**Table 9.** Median serum concentrations of biomarkers differentiating patients with endometriosis from healthy controls.

| Marker <sup>a</sup> | Patient group (n=124) |             | Control group (n=53) |            | P-value <sup>b</sup> |
|---------------------|-----------------------|-------------|----------------------|------------|----------------------|
|                     | Median                | IQ-range    | Median               | IQ-range   |                      |
| CA125               | 25.8                  | 13.0–45.5   | 6.7                  | 5.1–10.5   | <0.0001              |
| G-CSF               | 68.3                  | 4.9–124.4   | 12.6                 | 4.9–78.2   | 0.008                |
| IL-1Ra              | 192.8                 | 104.1–447.2 | 122.6                | 60.2–224.3 | 0.011                |
| EGF                 | 148.2                 | 78.1–204.4  | 82.9                 | 50.1–178.5 | 0.013                |
| IP-10               | 52.3                  | 39.1–86.6   | 57.6                 | 46.0–92.2  | 0.025                |
| IL-17               | 9.7                   | 3.7–36.1    | 3.7                  | 0.5–18.9   | 0.037                |

Note: IQ = interquartile, <sup>a</sup> Cytokines expressed in pg/ml and CA-125 in U/ml, <sup>b</sup> Rank analysis of covariance adjusted for age

The serum levels of markers were further compared between the subgroups of patients with stage I–II or stage III–IV and controls (*II, Table 3*), and CA-125 was the only biomarker with significant difference in both comparisons ( $p<0.001$ ). Interestingly, IL-1Ra was the only cytokine that differed significantly

in stage I–II as compared with controls ( $p=0.017$ ), and G-CSF and EGF could distinguish stage III–IV from controls ( $p=0.007$  and  $p=0.017$ , respectively).

Five biomarkers with significant difference between patients and controls in both age-adjusted rank analysis of covariance and ROC analysis (CA-125, IL-1Ra, IL-17, EGF and G-CSF) were chosen to a panel for multivariate analysis. The AUC of the panel was no better than that of CA-125 alone (0.87 vs. 0.86,  $p=0.42$ ; *II, Table 4*) in differentiating endometriosis patients from controls. The optimal cut-off value for CA-125 was 13.3 U/ml, and it distinguished all patients from controls with 75% sensitivity and 91% specificity and stage I-II from controls with 57% sensitivity and 91% specificity.

### 5.3 The use of serum HE4 in differentiating ovarian endometriosis from ovarian cancer (III)

The median serum HE4 concentration was normal ( $<70$  pmol) and similar in both healthy controls and in women with endometriosis irrespective of the presence of OMA (**Table 10**). In contrast, women with ovarian cancer had a highly elevated HE4 level compared with healthy controls ( $p<0.0001$ ), and the median concentration was above the normal range also in women with endometrial cancer. Women with endometriosis and healthy controls were significantly younger than women with a malignancy.

The median CA-125 level was significantly higher, although considered normal ( $<35$  U/ml), both in women with OMA (**Table 10**) and in women with stage IV non-ovarian endometriosis (*III, Table 2*) compared with healthy controls ( $p<0.0001$ ). However, it was highly elevated in women with ovarian cancer.

**Table 10.** Serum HE4 and CA-125 concentrations in healthy women and in patients with endometriosis, ovarian and endometrial cancer. Modified from Huhtinen et al. 2009.

| Diagnosis                 | n   | Age <sup>a</sup> | HE4 (pmol)           |                          | CA-125 (U/ml)      |                          |
|---------------------------|-----|------------------|----------------------|--------------------------|--------------------|--------------------------|
|                           |     |                  | Median (range)       | p-value                  | Median (range)     | p-value                  |
| <b>Healthy women</b>      | 66  | 38.5             | 38.6 (27.0–80.7)     | –                        | 6.7 (2.2–31.2)     | –                        |
| <b>Endometriosis</b>      |     |                  |                      |                          |                    |                          |
| All stages                | 129 | 31.8             | 43.5 (15.2–111.0)    | –                        | 25.3 (0.8–182.0)   | –                        |
| Endometrioma              | 69  | 31.6             | 44.0 (15.2–111.0)    | 0.894 <sup>b</sup>       | 33.7 (0.9–182.0)   | $<0.0001$ <sup>b</sup>   |
| <b>Ovarian cancer</b>     | 14  | 63.8             | 268.3 (46.5–10250.0) | $<0.0001$ <sup>b,c</sup> | 240.0 (6.6–6890.0) | $<0.0001$ <sup>b,c</sup> |
| <b>Endometrial cancer</b> | 16  | 60.5             | 73.3 (26.5–330.5)    | $<0.0001$ <sup>b</sup>   | 15.5 (9.6–106.0)   | $<0.0001$ <sup>b,c</sup> |

<sup>a</sup> Mean, <sup>b</sup> compared with healthy women, <sup>c</sup> compared with patients with endometrioma

Serum HE4 distinguished OMA from ovarian cancer with 92% accuracy and ovarian cancer from healthy controls with 94% accuracy at 95% specificity (**Table 11**). The accuracy to distinguish OMA from ovarian cancer further increased slightly when HE4 and CA-125 were combined.

**Table 11.** The diagnostic power of HE4 and CA-125 to distinguish ovarian cancer from ovarian endometriosis and ovarian cancer from healthy controls at 95% specificity. Modified from Huhtinen et al. 2009.

| Markers                                | Accuracy (%) | ROC-AUC (%) | Sensitivity (%) |
|--|--------------|-------------|-----------------|
| <b>Ovarian cancer vs. endometrioma</b> |              |             |                 |
| HE4                                    | 91.6         | 91.9        | 71.4            |
| CA-125                                 | 92.8         | 77.0        | 64.3            |
| CA-125 + HE4                           | 94.0         | 91.3        | 78.6            |
| <b>Ovarian cancer vs. controls</b>     |              |             |                 |
| HE4                                    | 93.8         | 95.5        | 78.6            |
| CA-125                                 | 96.3         | 91.7        | 78.6            |
| CA-125 + HE4                           | 96.3         | 91.1        | 92.9            |

#### 5.4 Long-term effect of complete endometriosis surgery on pain (IV)

Altogether 100 women underwent complete surgery for endometriosis, 80 by laparoscopy and 20 by laparotomy including four conversions. Median age of patients was 30 years (range 20–48; *IV, Table 2*), and two-thirds (68%) had deep endometriosis, 55% had endometrioma and 82% had peritoneal endometriosis. r-ASRM stage was I–II in 28% and III–IV in 72%. For 86% of the women, pain was one of the indications for surgery, and women with and without DIE were similar in this respect ( $p=0.36$ ; *IV, Table 2*). Nearly half (49/100) had previously undergone endometriosis surgery.

In the majority of patients (80%), the index surgery was conservative (the uterus and at least one ovary remaining). Hysterectomy was performed in 20% of all operations; for 25% ( $n=17/68$ ) of women with DIE and for 9.4% ( $n=3/32$ ) of non-DIE women ( $p=0.07$ ). Surgical procedures included 31 segmental bowel resections and 24 of those were rectal resections (*IV, Table 4*). Altogether 41 women (41%) had a postoperative complication according to the Clavien–Dindo classification. In most cases the complication was grade I–II (30/41), and infection was the most common complication (16/41; *IV, Supplemental table 1*). A grade III–IV complication occurred in 11%, and segmental bowel resection included the highest risk compared with other procedures (26% vs. 4%;  $p=0.004$ ).

At baseline, the prevalences of dysmenorrhea, dyspareunia, dyschezia, noncyclic abdominal pain and dysuria were 95%, 76%, 67%, 62% and 35%, respectively (**Table 12**). Most women (93%) had more than one pain symptom and 72% had at least three pain symptoms. Women with DIE had more often at least three pain symptoms compared with non-DIE women (81% versus 59%, respectively;  $p=0.03$ ; OR 2.9; 95% CI 1.1–7.3). Additionally, noncyclic abdominal pain was more common in women with DIE compared with non-DIE women (69% vs. 47%;  $p=0.04$ ; OR 2.6; 95% CI 1.1–6.2).

Fifteen women were reoperated during the follow-up, and all data after repeat surgery were excluded. In these cases, the follow-up time was counted up to the date of the repeat surgery. After this adjustment, the median follow-up time was 59 months (range 12–65), and the annual response rates at years 1–5 were 94%, 84%, 89%, 87% and 80% (**Table 12**).

After surgery, the severity of dysmenorrhea (among women with intact uterus), dyspareunia, dyschezia and noncyclic abdominal pain was significantly lower in every postoperative time point compared with the baseline (**Table 12**; linear mixed-effects model for time point comparisons). The median NRS of these four pain symptoms remained constantly decreased at years 1–5 with 28–50% depending on the symptom. Dysuria was not common among all participants, and thus the outcome was evaluated among those 35 women who had this symptom at baseline. The postoperative decrease in dysuria was highly significant throughout the follow-up period ( $p<0.001$ ). The distributions of NRS scales of each pain symptom during follow-up are shown in **Figure 9**.

The longitudinal multivariable modeling of the whole post-surgery follow-up was controlled for potential baseline and time-dependent confounders, such as hormonal medication or delivery as mentioned in the Statistics section. Three different subpopulations were tested: all patients ( $n=100$ ; Group A), women with DIE ( $n=68$ ; Group B) and women with conservative surgery ( $n=80$ ; Group C). Surgery consistently reduced dysmenorrhea in all subpopulations (**Figure 10; A–C**). In subgroups including both DIE and non-DIE patients (**Figure 10; A, C**), only women with DIE benefitted from the surgery with respect to dyschezia, dyspareunia and non-cyclic abdominal pain, with the exception of dyspareunia relief being only age-dependent instead of DIE-status-dependent in the whole study population (**Figure 10; A**). Hysterectomy resulted in an extremely effective dysmenorrhea relief (**Figure 10; Hyst; A-B**). However, no other beneficial pain outcomes were related to hysterectomy.

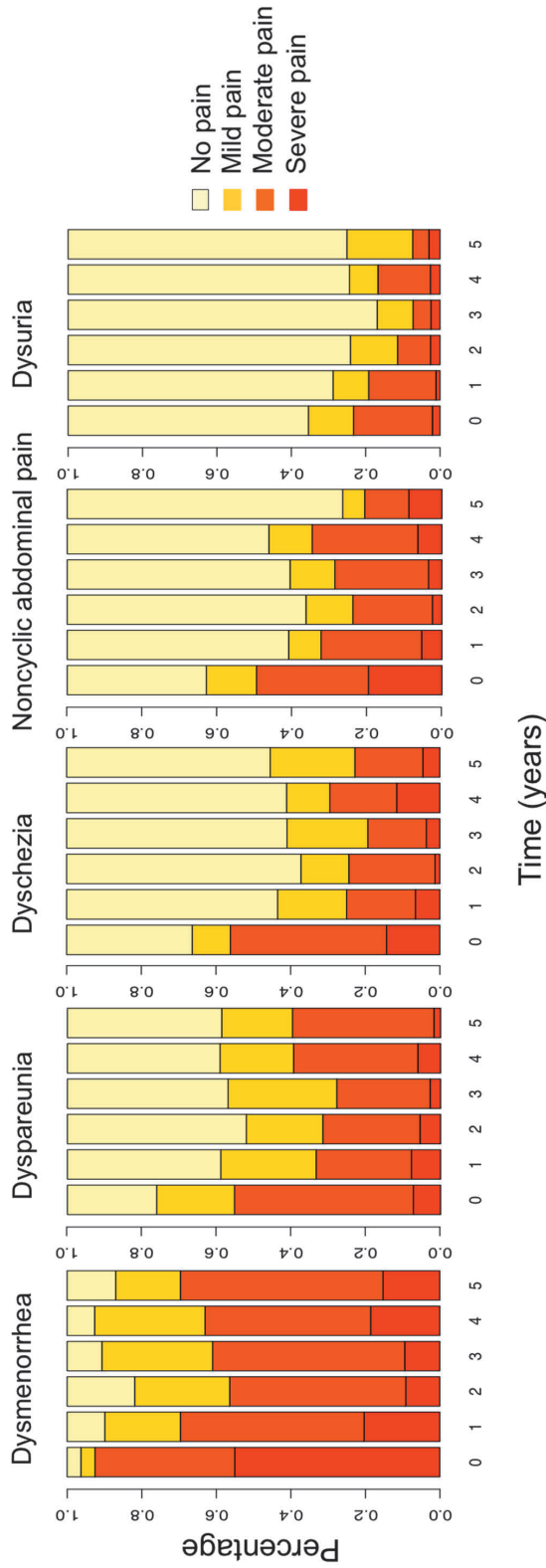


**Table 12.** Clinical characteristics and pain symptoms at baseline and during follow-up among 100 women operated on for endometriosis.

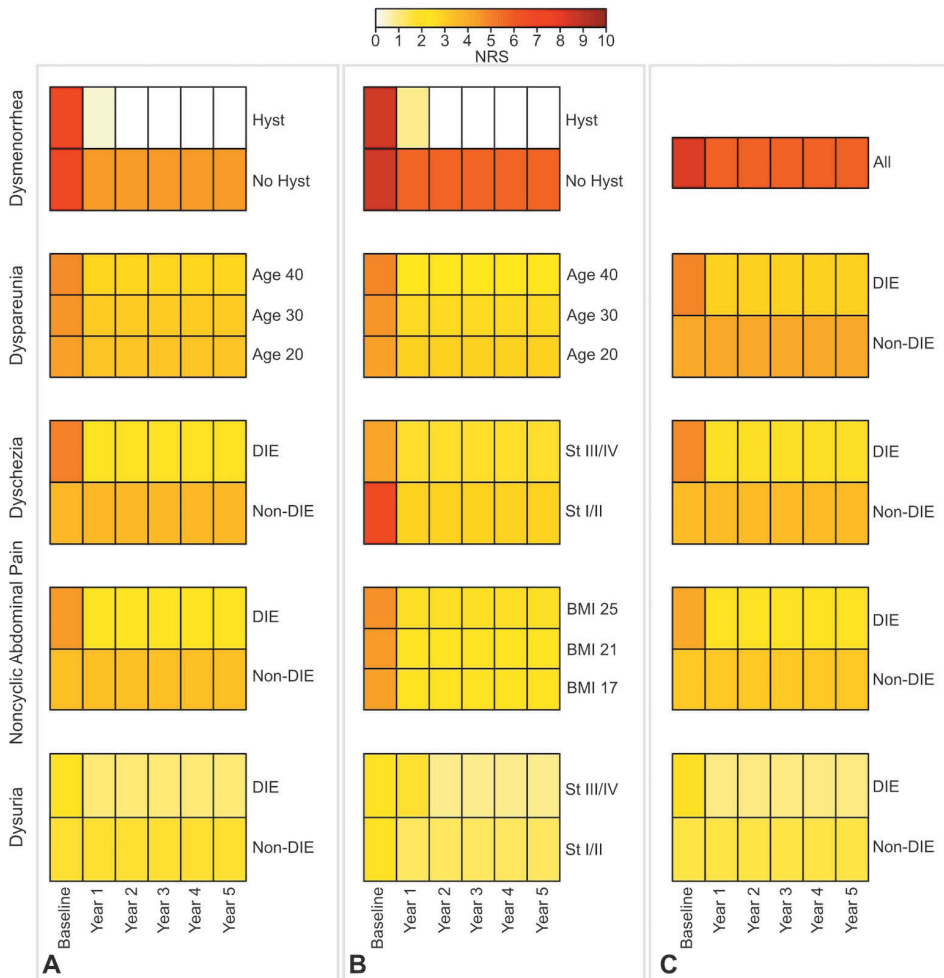
| Characteristic or symptom          | Baseline    | First year               | 2nd year                 | 3rd year                 | 4th year                 | 5th year                 |
|------------------------------------|-------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Response rate <sup>†</sup>         | NA          | 94/100 (94)              | 80/95 (84)               | 84/94 (89)               | 78/90 (87)               | 68/85 (80)               |
| Cumulative number of reoperations* | NA          | 0/96 (0)                 | 5/96 (5)                 | 6/96 (6)                 | 10/96 (10)               | 15/96 (16)               |
| Current wish for pregnancy         | 40/96 (42)  | 30/87 (35)               | 19/80 (24)               | 27/82 (33)               | 20/74 (27)               | 11/65 (17)               |
| Hormonal medication                | 46/100 (46) | 28/92 (30)               | 25/80 (31)               | 22/83 (27)               | 23/76 (30)               | 16/68 (24)               |
| <b>Current main problem</b>        |             |                          |                          |                          |                          |                          |
| Pain only                          | 66/99 (67)  | 24/88 (27)               | 24/80 (30)               | 22/82 (27)               | 23/73 (32)               | 25/66 (38)               |
| Infertility only                   | 5/99 (5)    | 13/88 (15)               | 12/80 (15)               | 13/82 (16)               | 5/73 (7)                 | 5/66 (7.5)               |
| Pain and infertility               | 19/99 (19)  | 13/88 (15)               | 5/80 (6)                 | 10/82 (12)               | 9/73 (12)                | 5/66 (7.5)               |
| No pain or infertility             | 9/99 (9)    | 38/88 (43)               | 39/80 (49)               | 37/82 (45)               | 36/73 (49)               | 31/66 (47)               |
| <b>Dysmenorrhoea<sup>‡</sup></b>   |             |                          |                          |                          |                          |                          |
| Prevalence                         | 95/100 (95) | 62/69 (82) <sup>§</sup>  | 45/55 (82) <sup>  </sup> | 58/64 (91) <sup>§</sup>  | 50/54 (93) <sup>§</sup>  | 40/46 (87) <sup>§</sup>  |
| Severity <sup>†</sup>              | 9 (8–10)    | 6 (3–8) <sup>†</sup>     | 6 (2–8) <sup>  </sup>    | 5.5 (3–8) <sup>†</sup>   | 6 (3–8) <sup>  </sup>    | 6.5 (4–8) <sup>  </sup>  |
| <b>Dyspareunia</b>                 |             |                          |                          |                          |                          |                          |
| Prevalence                         | 72/95 (76)  | 52/89 (59) <sup>  </sup> | 38/73 (52) <sup>  </sup> | 41/72 (57) <sup>  </sup> | 40/67 (60) <sup>  </sup> | 32/58 (55) <sup>  </sup> |
| Severity                           | 5 (1.5–7)   | 3 (0–6) <sup>  </sup>    | 2 (0–5) <sup>†</sup>     | 2.5 (0–5) <sup>†</sup>   | 3 (0–7) <sup>  </sup>    | 3 (0–6) <sup>  </sup>    |
| <b>Dyschezia</b>                   |             |                          |                          |                          |                          |                          |
| Prevalence                         | 67/100 (67) | 41/94 (44) <sup>†</sup>  | 29/79 (37) <sup>†</sup>  | 33/83 (40) <sup>†</sup>  | 32/78 (41) <sup>†</sup>  | 30/68 (44) <sup>  </sup> |
| Severity                           | 5 (0–8)     | 0 (0–4.5) <sup>†</sup>   | 0 (0–4) <sup>  </sup>    | 0 (0–4) <sup>†</sup>     | 0 (0–6) <sup>†</sup>     | 0 (0–4) <sup>†</sup>     |
| <b>Chronic abdominal pain</b>      |             |                          |                          |                          |                          |                          |
| Prevalence                         | 61/98 (62)  | 38/93 (41) <sup>†</sup>  | 29/80 (36) <sup>†</sup>  | 34/84 (41) <sup>  </sup> | 35/77 (46) <sup>  </sup> | 17/68 (25) <sup>†</sup>  |
| Severity                           | 4 (0–8)     | 0 (0–6) <sup>†</sup>     | 0 (0–4) <sup>  </sup>    | 0 (0–5) <sup>†</sup>     | 0 (0–6) <sup>  </sup>    | 0 (0–3) <sup>†</sup>     |
| <b>Dysuria</b>                     |             |                          |                          |                          |                          |                          |
| Prevalence                         | 35/99 (35)  | 25/92 (27) <sup>§</sup>  | 18/80 (23) <sup>§</sup>  | 14/84 (17) <sup>  </sup> | 17/78 (22) <sup>§</sup>  | 15/68 (22) <sup>§</sup>  |
| Severity                           | 0 (0–4)     | 0 (0–3) <sup>§#</sup>    | 0 (0–0) <sup>§#</sup>    | 0 (0–0) <sup>†#</sup>    | 0 (0–0) <sup>§#</sup>    | 0 (0–0.5) <sup>§#</sup>  |

Note: NA, not applicable. Prevalence data are n (%) and pain severity data (numerical rating scale 0–10) are medians (inter quartile range). Statistical analyses between baseline and postoperative pain data were performed with generalized linear mixed-effect models with multinomial distribution and cumulative logit link function.

<sup>†</sup> Calculated annually among women not reoperated, <sup>\*</sup> Data missing n=4, <sup>‡</sup> Follow-up data and comparisons include 80 women with preserved uterus of whom 77/80 had dysmenorrhoea at baseline, <sup>§</sup> Not significant, <sup>||</sup> Multiplicity corrected p<0.05–0.001, <sup>††</sup> Multiplicity corrected p<0.001, <sup>‡‡</sup> Multiplicity corrected p<0.001 if severity compared among 35 women who had dysuria at baseline



**Figure 9.** Distribution (percentage) of the severity of endometriosis related pain symptoms at baseline and during follow-up (years 1–5) among 100 women operated on for endometriosis. Pain severity was evaluated with numerical rating scale (NRS 0–10) and further divided into four categories: no (0), mild (1–4), moderate (5–7) and severe (8–10) pain.



**Figure 10.** Heatmap of numerical rating scales (NRS) of dysmenorrhea, dyspareunia, dyschezia, noncyclic abdominal and dysuria (5 rows, respectively) of 100 women at baseline and during follow-up years 1–5 after complete endometriosis surgery. Only statistically significant effects, after adjusting for confounding factors, are displayed. The columns indicate specific findings of subpopulations: A) all patients (n=100), B) patients with deep infiltrating endometriosis (DIE; n=68) and C) patient with conservative surgery (No Hyst; n=80).

## 6 DISCUSSION

The complexity of endometriosis as a disease challenges researchers, clinicians and patients worldwide. To date, much remains unknown about the etiology and the nature of endometriosis, origin of pain and disease phenotypes as well as the optimal treatment options.

The World Endometriosis Society has recently updated recommendations for future research priorities (Rogers et al. 2017). Amongst other topics, the discovery of non-invasive biomarkers for diagnosis was further recognized as an important future goal to accelerate diagnosis. Furthermore, the recommendation encouraged combining biomarkers, imaging modalities and clinical characteristics to improve the diagnostic accuracy. Adolescent endometriosis patients were recognized as an underserved group, and future research should focus on adolescents with symptoms suggestive of endometriosis. In addition, it was acknowledged that short- and long-term surgical outcomes should be compared to non-surgical treatment option.

### 6.1 Screening of adolescent symptoms suggestive of endometriosis

The TEENMAPS study discovered three notable findings (I). The first and most important of these was that 5–10% of adolescent girls presented with symptoms suggestive of endometriosis (I, Table 4). Only limited previous data exist on adolescent pelvic pain symptoms other than dysmenorrhea, and this study was the first with the specific aim at screening adolescent endometriosis symptoms. One previous Australian study with a similar target population and sample size aimed at exploring menstrual disturbance in teenagers (Parker et al. 2010). They reported rates of dysmenorrhea, dyspareunia, dyschezia and dysuria with comparable results to ours (Table 13). However, they did not include acyclic abdominal pain, and they only reported the severity (NRS) of menstrual pain. Nevertheless, they noticed equal prevalence of girls at risk of endometriosis with the criteria of severe dysmenorrhea despite of using OCs and pain medication (Table 13).

**Table 13.** Comparison of the participants and the results of the TEENMAPS study and the MDOT study. Modified from Suvitie et al 2016 and Parker et al. 2010.

|   | Study I | Parker et al. 2010 |
|---|---------|--------------------|
| Number of participants                                  | 1103    | 1051               |
| Median age (years)                                      | 16.8    | 16.8               |
| Using OCs (%)   | 31      | 22                 |
| Using OCs for dysmenorrhea (%)                          | 61      | 61                 |
| Dysmenorrhea (%)  | 68      | 93                 |
| Severe dysmenorrhea <sup>a</sup>                        | 33      | 21                 |
| Acyclic abdominal pain (%)                              | 19      | Not reported       |
| Dyspareunia (%)   | 12      | 13                 |
| Dyschezia (%)   | 8       | 12                 |
| Dysuria (%)   | 5       | 10                 |
| Severe dysmenorrhea while using OCs and pain medication | 5       | 5                  |

<sup>a</sup> numerical rating scale 8–10

Second, we detected that medical treatment of primary dysmenorrhea was insufficient in the studied region. While most girls with dysmenorrhea used pain medication, in 43% the medication offered little or no pain relief (*I, Figure 1*). Unfortunately, we did not record the timing or dosage of pain medication, and these may have been suboptimal. Another finding suggesting insufficient treatment was that 14% of the girls with severe dysmenorrhea were regularly absent from school or hobbies due to pain (*I, Table 1*).

The third interesting detail in study I was the co-existence of pain symptoms, as girls with severe dysmenorrhea had an increased risk of concomitant acyclic abdominal pain and dyschezia (OR 2.67 and OR 2.56, respectively). This finding may partly indicate early onset of endometriosis. Especially treatment-resistant severe dysmenorrhea and menstrual dyschezia have been linked to an elevated risk of endometriosis (Nnoaham et al. 2012a, Janssen et al. 2013). Alternatively, this clustering of pain may indicate early sensitization to pain. Chronic pain induces alterations in the central nervous system, and it is hypothesized that women with repetitive or chronic menstrual or pelvic pain are prone to centralization and increased pain sensitivity (Brawn et al. 2014).

Dysmenorrhea was common among the study population (68%), and recent studies have demonstrated that primary dysmenorrhea is prevalent across the world (68–93%; **Table 2**). A previous older Finnish study reported a 54% prevalence among 5155 adolescents, and in global studies from 1950s and 1960s the prevalence varied between 5–50% (Widholm 1979). It's thrilling to speculate that the dysmenorrhea rate has increased in half a century for some reason, but it is by no means possible to prove it.

## 6.2 Performance of serum cytokines as biomarkers for endometriosis

Study II evaluated the usefulness of 29 serum cytokines as biomarkers for endometriosis. Many of these cytokines had not been previously reported in endometriosis at the time of the analysis, offering the possibility to discover novel biomarkers for endometriosis. However, although five cytokines (G-CSF, IL-1Ra, EGF, IP-10, IL-17) could differentiate endometriosis patients from healthy controls, the most significant difference was observed in the concentration of CA-125 (**Table 9**). Furthermore, a panel of the best performing cytokines combined with CA-125 was no better than CA-125 alone (AUC 0.87 vs. 0.86,  $p=0.42$ ; *II, Table 4*) in differentiating endometriosis from controls.

Endometriosis is an inflammatory disease, and serum cytokines have been widely studied as biomarkers for endometriosis (Nisenblat et al. 2016b). However, presently none of the evaluated cytokines or growth factors has been proven useful as single biomarkers (Borrelli et al. 2014, Nisenblat et al. 2016b, Ahn et al. 2017). Thus, our results are in line with previous data. Nevertheless, VEGF, IL-6 and TNF- $\alpha$  may have a role in a future biomarker panel (**Table 4**) (Nisenblat et al. 2016b).

Interestingly, IL-1Ra showed some promise in distinguishing minimal–mild endometriosis from controls (AUC 0.69; *II, Table 3, Table 4 and Figure 1*), but it had no role in the advanced stages. Similarly, one study has reported elevated serum IL-1Ra concentrations in endometriosis, and the serum level was higher in early stages compared with advanced endometriosis (Kondera-Anasz et al. 2005). However, also conflicting results exist (Zhang et al. 2007).

IL-1Ra is a receptor antagonist with anti-inflammatory action. The secretory form is produced by macrophages, monocytes, neutrophils, endometrial cells, liver and other cells (Arend 2002). Blood levels of IL-1Ra are elevated in patients with a variety of inflammatory, infectious, and post-surgical conditions (Arend et al. 1998). Local tissue production of IL-1Ra has been proposed to block the effect of IL-1 (Arend 2002), which is an important mediator of inflammation and tissue damage. Furthermore, the imbalance between IL-1 and IL-1Ra is considered to predispose to the development of inflammatory disease (Arend 2002). In the present study, the elevated serum IL-1Ra in minimal–mild endometriosis may indicate an attempt of the inflammatory system to fight against emerging disease.

### 6.3 Role of HE4 in endometriosis

A novel and clinically relevant finding in study III was that serum HE4 was not elevated in endometriosis compared with healthy controls (**Table 10**). Most importantly, the median HE4 level was comparable between patients with endometrioma and healthy women contrary to serum CA-125. The combination of HE4 and CA-125 could distinguish ovarian endometriosis from epithelial ovarian cancer (EOC) with 94% accuracy and 79% sensitivity at 95% specificity (**Table 11**). These findings have been further confirmed in other prospective studies (**Table 14**) (Anastasi et al. 2013, Nikolova et al. 2017).

**Table 14.** Diagnostic performance of HE4 in differentiating ovarian endometriosis (OMA) from epithelial ovarian cancer (EOC) (Huhtinen et al. 2009, Anastasi et al. 2013, Nikolova et al. 2017).

| Author               | N <sup>a</sup> | HE4 <sup>b</sup> (p/l) |                | Accuracy (%) | AUC (%) | Sensitivity (%) | Specificity (%) |
|----------------------|----------------|------------------------|----------------|--------------|---------|-----------------|-----------------|
|                      |                | OMA                    | EOC            |              |         |                 |                 |
| <b>Study I</b>       | 69/14          | 44 (15–111)            | 268 (47–10250) | 91.6         | 92      | 71              | 95              |
| <b>Anastasi 2013</b> | 57/39          | 53 (26–98)             | 426 (48–850)   | NA           | 99      | 87              | 100             |
| <b>Nikolova 2017</b> | 37/11          | 36 (14–57)             | 997 (31–6488)  | 95.8         | 93      | 82              | 100             |

Note: NA=Not applicable, <sup>a</sup> Number of patients with ovarian endometriosis/ovarian cancer, <sup>b</sup> Median serum concentration (range)

Endometriosis increases the risk of EOC, especially endometrioid and clear cell carcinoma types (Thomsen et al. 2017, Poole et al. 2017). An expert ultrasound examiner rarely (0.9%) misclassifies ovarian malignancies as OMA (Van Holsbeke et al. 2010), but nevertheless in clinical practice the differential diagnosis is occasionally challenging. CA-125 is a non-specific ovarian cancer biomarker often elevated in endometriosis, and it is commonly included in the diagnostic protocol of ovarian masses. Elevated levels give rise to concern and normal levels do not rule out malignancy. In these situations, measuring both HE4 and CA-125 could facilitate the decision-making.

Adnexal surgery can readily be performed in women with completed family. A relevant question in these cases is where and by whom the operation should be carried out. The setting of ovarian cancer surgery impacts patient survival (Earle et al. 2006), and similarly, endometriosis surgery is highly dependent on surgical expertise (Vercellini et al. 2009a). In endometriosis, patients wishing to preserve their fertility it is often essential to postpone surgery and avoid unnecessarily radical treatment. A normal HE4 concentration combined with moderately elevated CA-125 is suggestive for endometriosis in women presenting with symptoms suggestive of endometriosis. Indeed, HE4 measurement has been incorporated

into clinical practice when differentiating ovarian endometriosis from malignant ovarian tumors.

#### 6.4 Do patients benefit from surgical treatment of endometriosis?

Complete endometriosis surgery resulted in a clinically significant long-term alleviation of all measured pain symptoms among the whole study population (IV, **Table 12**). The median NRS of pain symptoms remained constantly decreased by ~30–50% after surgery depending on the symptom. This degree of alleviation is suggested to define a favorable response to the treatment (Vincent et al. 2010). Women with DIE had the most favorable pain outcome when compared with non-DIE women, but both benefitted from surgery.

In line with these findings, favorable long-term pain outcomes have been reported in previous studies that have included patients with all disease types or r-ASRM stages (Vercellini et al. 2009a, Porpora et al. 2010, Coccia et al. 2011, Vercellini et al. 2006, Abbott et al. 2003). Similarly, earlier studies focusing on women with DIE have reported a long-term improvement in pain and quality of life (Vercellini et al. 2009a, Meuleman et al. 2011, Ferrero et al. 2015). On the whole, comparison of the present results to previous publications on surgical outcome is difficult because of their very heterogeneous patient selection and methodology to assess pain outcome. Interestingly, comparisons of pain outcomes after surgery between women with and without DIE have not been previously published.

Dysmenorrhea was the most severe symptom, and it was alleviated irrespective of the disease type in women with fertility-sparing surgery (**Figure 10**). Hysterectomy was a definitive cure for dysmenorrhea, and there is seldom need to spare the uterus during complete endometriosis surgery if the woman has completed her family.

The 5-year reoperation rate was 16%, and a true surgical recurrence was detected in only 10% of the patients. Even if missing data were counted as a recurrence, the 5-year reoperation rate and the confirmed surgical recurrence rates were still acceptable (19% and 14%, respectively). Hysterectomy did not decrease the risk of reoperation compared with conservative surgery (11% vs. 17%,  $p=0.73$ ). Reoperated women had more often noncyclic abdominal pain prior to surgery, and this subgroup of women may have centralized pain, which may be resistant to surgical treatment. DIE status or other comorbid conditions did not influence the risk of reoperation.



In an attempt to define a more individualized and realistic long-term outcome, we calculated the “complete benefit rate” among those women (85%) who had pain as one of the indications for surgery. Participants were asked in the follow-up questionnaire whether they considered themselves as having a “pain problem” or not. This question took into account that mild pain might not be bothersome, and, on the contrary, not all women with severe pain are reoperated. At each time point, individuals were defined as “completely benefitted” if they had not been reoperated and did not report “pain problem”. Missing data were considered as failure. Complete benefit rate diminished from 46% at year one to 36% five years after surgery, and this trend was expected. Due to missing data the “benefit” outcome was unknown in 11–20% at years 1–5, and thus the true complete benefit rate may have been near 50%. Preoperatively, 85% of participants reported having “pain problem” compared with 42% one year after surgery. Thus, there are quite a few patients with residual postoperative pain, though improvement was noted from surgery.

It is well recognized that surgical treatment carries a marked risk of postoperative symptom or disease recurrence (Vercellini et al. 2009a). Furthermore, it is not realistic to expect surgery to totally erase pain symptoms. In an international multicenter questionnaire study, a notable proportion of patients treated in tertiary care centers had pain symptoms and impaired quality of life (De Graaff et al. 2013). In clinical practice, risks and benefits of endometriosis surgery must be clearly explained to patients.

The value of this study was mostly its usefulness in serving as an internal quality evaluation. Although the patient selection is likely biased, women who underwent complete endometriosis surgery in the participating hospitals seemed to have good pain outcomes and low risk of reoperation.

## **6.5 Methodological limitations and strengths**

All studies were prospective with some concerns in the patient selection, sample size and the methodology of data collection. The TEENMAPS study had a large sample that gives strength to the findings (I). Furthermore, no previous study has specifically targeted the prevalence and severity of symptoms suggestive of endometriosis among general adolescent population. Thus, our results serve as valuable reference data for the future studies on adolescents at risk of endometriosis. Unfortunately, data were collected anonymously, and do not allow further evaluation and follow-up of the girls presenting with symptoms suggestive of endometriosis.

One limitation in study I was the low response rate (43%) which may have biased the results. Possibly asymptomatic adolescents may have been less motivated to answer the questionnaire. However, no statistically relevant differences were detected in the main parameters of interest between the municipality with high response rate (91%) and the municipalities with low response rate (24% and 42%), giving confidence to our results.

The primary aim of the ENDOMET study (II, III and IV) was to recruit endometriosis patients and healthy control women (altogether 230 participants) to obtain blood and tissue samples for molecular evaluation and for discovery of novel diagnostic and prognostic biomarkers. No power calculations were performed, and the final sample size was relatively small for studies II and IV. Furthermore, the patient selection was biased because not all operated endometriosis patients of the participating hospitals were included during the recruitment period. The gynecologists were allowed to recruit patients when possible.

The distribution of endometriosis stage or disease type was not optimal for studies II and IV. The majority of patients had DIE and advanced disease (stage III–IV). This reflects the indications for surgery, the role of the participating hospitals serving as referral centers for endometriosis and the role of participating gynecologist as having expertise for DIE surgery. However, patients with minimal–mild endometriosis or peritoneal disease only are of special interest in diagnostic studies (II). These patients would perhaps most benefit from novel non-invasive diagnostic methods, as they commonly have no findings in TVUS. Due to low number of women with stage I–II endometriosis, study II was likely underpowered to demonstrate the diagnostic power of cytokines in this subgroup.

Recently, the World Endometriosis Research Foundation has given a recommendation to the use of standardized patient questionnaires and detailed standard operating procedures (SOPs) for sample collection and storage (Vitonis et al. 2014, Fassbender et al. 2014, Rahmioglu et al. 2014). As such recommendation did not exist in 2005, they were not used in studies II, III and IV. However, our research group considers the included questionnaire as well as the used sampling methods and sample storage appropriate. Furthermore, studies I and IV assessed all five endometriosis-associated pain symptoms recommended to be included in endometriosis research (Rogers et al. 2017), although the quality of pain (burning, aching, stabbing etc.) was not evaluated. In addition, pain intensity was measured with a validated and recommended method i.e. numerical rating scale (Bourdel et al. 2015).

In study II, the control women were not optimal. Women undergoing laparoscopic sterilization were chosen as healthy controls to the ENDOMET study due to several reasons, although symptomatic women without endometriosis at laparos-

copy would have served as optimal controls in study II. First, one aim in the ENDOMET study was to evaluate gene expression profiles of endometrium of patients and healthy women as well as endometriotic tissue, and molecular changes in symptomatic women without endometriosis might have biased these results. Furthermore, laparoscopic tubal ligations were commonly performed during the time the study was initiated (2005), and thus, the possibility to recruit an adequate number of control women was considered realistic.

In study II, one limitation was that blood and tissue samples were collected throughout the menstrual cycle, and subgroup sizes of women in different phases were relatively small (II). Importantly, patients were allowed to use hormonal medication during the sample collection reflecting the real life situation, and this is not typical in biomarker studies. However, it allowed us to examine the effect of hormonal medications on serum biomarkers.

Study III had a novel study question and appropriate sample size. However, due to limited number of women with stage I–II ovarian cancer ( $n=5$ ), the comparison between OMA and early stage ovarian malignancy was not possible. A methodological flaw was the accidental inclusion of nine control women twice in different menstrual cycle phases. However, this is likely not a clinically significant error as the comparison of women with endometriosis to women with ovarian cancer formed the basis for conclusions.

The strengths of study IV are the long follow-up time, high response rate and utilization of modern statistics to analyze the results. However, the sample size was relatively small. Furthermore, inclusion of clinical evaluation during the follow-up and a quality of life instrument such as EHP-30, would have been valuable additions to the evaluation of the long-term surgical outcome. In addition, study IV would have benefitted from a larger sample size of women with minimal–mild endometriosis, as it would have enabled detection of significant changes in the less common pain symptoms after surgery. Furthermore, the study population was very heterogeneous in terms of radicality of surgery, the use of hormonal medication and fertility issues, limiting the conclusions. Finally, the study did not include a control group to allow comparison of long-term outcome between combined treatment modalities and medical treatment only.

## **6.6 Timely diagnosis of endometriosis – future perspectives**

There is no easy and quick solution to reach timely diagnosis. Currently, long diagnostic delay and unawareness of endometriosis result in unnecessary suffering, and may enable endometriosis to progress to a more advanced stage and

compromise fertility. Endometriosis symptoms are underrecognized among the general population and health care providers, and increasing the awareness of endometriosis is one method to facilitate early diagnosis and initiation of medical treatment (Chapron et al. 2011a, Steenberg et al. 2013, Geysenbergh et al. 2017, Bush et al. 2017). Likely, the timely pain alleviation soon after the onset of symptoms and mental support to cope with pain will reduce the risk of centralization of pain.

Presently, the diagnosis is based on typical symptoms, clinical examination and TVUS, and no non-invasive diagnostic biomarkers exist (Vercellini et al. 2015). TVUS and MRI are highly accurate in detecting OMA and DIE in experienced hands as compared with surgical diagnosis (Nisenblat et al. 2016a, Rogers et al. 2017), but the true diagnostic challenge is to confirm or exclude peritoneal endometriosis. Furthermore, modern imaging modalities performed with expertise are not widely available, and novel non-invasive diagnostic methods are required in general health care.

Endometriosis symptoms commonly occur during adolescence, but some data indicate that teenagers do not actively search for help (Greene et al. 2009). Thus, tools are needed to identify such girls. The screening and sufficient treatment of severe primary dysmenorrhea is of high importance, as it is common and has a harmful impact on many aspects of teenagers' well-being and social life (Banikarim et al. 2000, Parker et al. 2010, Esen et al. 2016). Furthermore, guidelines encourage to actively treat adolescents with symptoms suggestive of endometriosis (Johnson et al. 2013, Schleedoorn et al. 2016). However, population-wide screening of endometriosis irrespective of symptoms is not considered preferable (Vercellini et al. 2015).

Presently, no validated screening tool exists for adolescents, and there is no data showing benefits or harms of such screening (Vercellini et al. 2015). Recently, a screening questionnaire was introduced to identify adolescents at risk of endometriosis for pilot testing and validation (Geysenbergh et al. 2017). As an extension to that questionnaire, **Table 15** shows selected markers in clinical history linked to an increased risk of endometriosis according to literature. These markers could be included in screening questionnaires for adolescents or adults, as appropriate.

**Table 15.** Selected markers in clinical history related to an increased risk of endometriosis based on literature (Treloar et al. 1999, Stefansson et al. 2002, Chapron et al. 2011a, Nnoaham et al. 2012a, Nnoaham et al. 2012b, Janssen et al. 2013, Lafay Pillet et al. 2014).

| Marker                                      | Reference                                     |
|---|---|
| Early age at menarche ( $\leq 12$ )         | Nnoaham 2012b                                 |
| Positive family history                     | Chapron 2011a, Stefansson 2002, Treloar 1999  |
| History of benign ovarian cysts             | Nnoaham 2012a                                 |
| Primary or secondary infertility            | Lafay Pillet 2014 <sup>a</sup>                |
| Dysmenorrhea limiting work/daily activities | Nnoaham 2012a                                 |
| OC use due to severe primary dysmenorrhea   | Chapron 2011a, Lafay Pillet 2014 <sup>a</sup> |
| Dysmenorrhea resistant to NSAID and OC      | Janssen 2013                                  |
| Absenteeism from school during periods      | Chapron 2011a                                 |
| Painful defecation during menstruation      | Nnoaham 2012a                                 |
| Deep dyspareunia NRS $>5$                   | Lafay Pillet 2014 <sup>a</sup>                |
| Gastrointestinal symptoms $\geq 5$          | Lafay Pillet 2014 <sup>a</sup>                |

<sup>a</sup> Increased risk of deep infiltrating endometriosis among women with endometrioma

Non-invasive biomarkers are actively developed, and these could simplify and accelerate diagnosis and serve the primary health care. It is likely that instead of a single biomarker, a combination of biomarkers (obtained from blood, endometrium, menstrual blood or urine) combined with clinical symptoms, will provide the best diagnostic power (Rogers et al. 2017). Presently, neural markers of the endometrium and microRNAs have shown greatest promise as biomarkers for endometriosis (**Table 4**). However, technical difficulties of adequate endometrium sampling hamper the introduction of neural markers into clinical practice (May et al. 2011). As the symptoms typically start during adolescence or early adulthood, diagnostic research should be extended to symptomatic adolescents. Furthermore, a collaborative effort to build large databases of samples and data collected with validated SOPs and questionnaires may enable identification and validation of biomarkers in the future (Rogers et al. 2017).

## 7 CONCLUSIONS

1. Primary dysmenorrhea is a common complaint among 15–19-year old Finnish girls, while acyclic abdominal pain, dyschezia, dyspareunia and dysuria are less commonly reported. Girls with severe dysmenorrhea have significantly increased risk for concomitant acyclic abdominal pain and dyschezia as compared with girls with no or less intense menstrual pain. Approximately five per cent of teenage girls suffer from severe dysmenorrhea resistant to treatment with oral contraceptives and pain medication or leading to regular absenteeism from school or hobbies. These girls are considered at risk of having endometriosis (I).
2. The evaluated 29 serum cytokines were not useful as single or combined biomarkers for endometriosis. While there was a significant difference in the serum concentrations of five cytokines (G-CSF, IL-1Ra, EGF, IP-10, IL-17), a combination of these cytokines with CA-125 did not improve the diagnostic accuracy of CA-125 alone (II).
3. Serum HE4, a modern biomarker for epithelial ovarian cancer, is not elevated in majority of endometriosis patients irrespective of disease type in contrast to serum CA-125. This novel finding is useful in clinical practice, when distinguishing atypical endometriomas from malignant ovarian tumors (III).
4. Complete surgical removal of all visible endometriosis lesions results in significant long-term alleviation of dysmenorrhea, acyclic abdominal pain, dyspareunia, dyschezia and dysuria. Especially women with deep infiltrating endometriosis have a favorable pain outcome after surgery. Before surgery, women with DIE present with more numerous pain complaints and have more commonly acyclic abdominal pain as compared with non-DIE women. Five years after surgery, a minimum of one-third of patients has neither bothersome pain nor been reoperated (IV).

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