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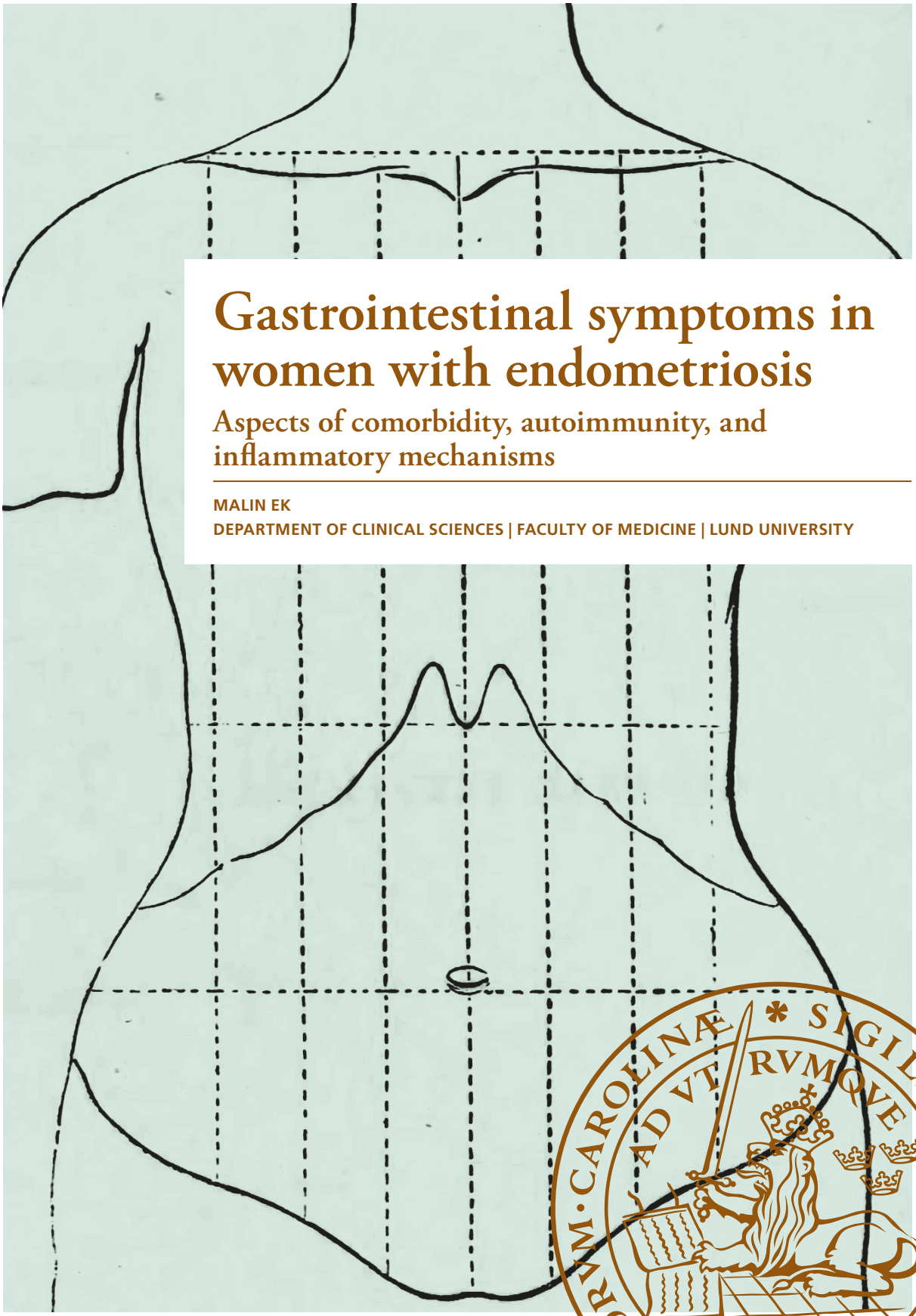

Gastrointestinal symptoms in women with endometriosis

Aspects of comorbidity, autoimmunity, and inflammatory mechanisms

MALIN EK
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY

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Malin Ek

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Abstract

Gastrointestinal (GI) symptoms are commonly associated with endometriosis; however, the mechanisms underlying these symptoms have not yet been elucidated. Endometriosis is considered to be an inflammatory condition, and comorbidity with irritable bowel syndrome (IBS) has been reported. Antibodies against gonadotropin-releasing hormone (GnRH) have been associated with bowel pathology and have been found in increased prevalence in functional GI disorders. This thesis sought to explore GI symptoms associated with endometriosis and connections with comorbidity, autoantibodies and molecular inflammatory mechanisms. In total, 188 women, including 172 women with surgically confirmed endometriosis, were recruited to this study. The participants answered questionnaires regarding socioeconomic and lifestyle factors, GI symptoms, and medical history and provided fecal and blood samples. Women from the general population and healthy controls served as controls, and women with microscopic colitis, IBS, or enteric dysmotility were used for comparisons of GI symptoms.

In women with endometriosis, a high prevalence of GI symptoms was reported. Bloating and flatulence was the most impairing symptom. Increasing age correlated with reduced GI symptoms, and patients who were currently being treated with opioids or GnRH analogs experienced aggravated bowel symptoms. Endometriosis was associated with IBS, the current use of antidepressants, and impaired psychological well-being, whereas alcohol intake, physical activity, BMI, and asthma were negatively associated with endometriosis. Furthermore, impaired psychological well-being correlated positively with all GI symptoms measured but negatively with age. Elevated levels of thyroid-stimultating hormone receptor antibodies (TRAb) were associated with endometriosis; however, thyroid disease was not associated with endometriosis. Among the studied endometriosis patients, 35% met the Rome III criteria for IBS. Women with IBS experienced aggravated GI symptoms and impaired psychological well-being compared with women with endometriosis. However, the GI symptoms were similar between patients with IBS and endometriosis patients who met the Rome III criteria. In patients with endometriosis, antibodies against GnRH, luteinizing hormone (LH) and their receptors, antibodies associated with celiac disease, and antibodies against tenascin C and matrix metalloproteinase 9 were found at similar levels as those in healthy controls. Changes in the levels of several inflammatory proteins were observed in the sera of endometriosis patients compared with healthy controls and women with microscopic colitis. AXIN1 and sulfotransferase 1A1 (ST1A1) were significantly increased, whereas the C-X-C motif chemokine 9 (CXCL9) was significantly decreased. Furthermore, the elevation of AXIN1 was confirmed in a larger cohort compared with controls from the general population. Sparse correlations between inflammatory proteins and GI symptoms were identified. In patients with endometriosis, P-AXIN1 levels were negatively correlated with fecal calprotectin (F-calprotectin), B-hemoglobin, B-erythrocytes and B-platelets levels, but no correlation was observed for high sensitivity C-reactive protein levels. P-AXIN 1 levels also correlated with the duration of endometriosis and some GI symptoms and were found to be elevated in patients recieving progestin treatment.

Taken together, these results showed a high prevalence of GI symptoms in endometriosis patients, and a large proportion of endometriosis patients met the diagnostic criteria for IBS. However, we were unable to determine if comorbidity between endometriosis and IBS exists. The questionnaires available and the definition of the Rome criteria may not be sensitive enough to distinguish between the two conditions. GI symptoms associated with impaired mental health, but not with autoantibodies or serum inflammatory proteins. The inflammatory protein AXIN1 appears to be an interesting protein whose role should be further evaluated in endometriosis.

Key words: Endometriosis, gastrointestinal symptoms, IBS, GnRH, AXIN1, microscopic colitis

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To my family

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Paper I: Characteristics of endometriosis: A case-cohort study showing elevated IgG titers against the TSH receptor (TRAb) and mental comorbidity

Malin Ek, Bodil Roth, Peter M. Nilsson, and Bodil Ohlsson. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2018 Dec; 231:8-14.

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Paper II: Gastrointestinal symptoms in women with endometriosis and microscopic colitis in comparison to irritable bowel syndrome – A cross-sectional study

Malin Ek, Bodil Roth, Mariette Bengtsson, and Bodil Ohlsson. Submitted.

Paper III: Autoantibodies common in patients with gastrointestinal diseases are not found in patients with endometriosis: A cross-sectional study

Malin Ek, Bodil Roth, Lil Valentin, Johanna Nordengren, and Bodil Ohlsson. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2019 Sep; 240:370-374.

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Paper IV: AXIN1 in Plasma or Serum is a potential new biomarker for Endometriosis

Malin Ek, Bodil Roth, Gunnar Engström, and Bodil Ohlsson. International Journal of Molecular Sciences. 2019 Jan 7; 20.

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Paper V: Plasma AXIN1 is correlated with inflammatory biomarkers and gastrointestinal symptoms in endometriosis

Katharina Dihm, **Malin Ek,** Bodil Roth, and Bodil Ohlsson. Submitted.

Related publications by the author:

Gastrointestinal symptoms among endometriosis patients – A case-cohort study

Malin Ek, Bodil Roth, Per Ekström, Lil Valentin, Mariette Bengtsson, and Bodil Ohlsson. BMC Women's Health. 2015 Aug 13; 15:59.

Abbreviations

5-HT3	5-hydroxytryptamine type 3
APC	Adenomatous polyposis coli gene
BMI	Body mass index
BSA	Bovine serum albumin
CA-19-9	Cancer antigen 19-9
CA-125	Cancer antigen 125
CC	Collagenous colitis
CD	Celiac disease
CK1	Casein kinase 1
CXCL-9	C-X-C motif chemokine 9
CXCL-10	C-X-C motif chemokine 10
DPG	Deamidated gliadin
ED	Enteric dysmotility
ELISA	Enzyme-linked immunosorbent assay
ENS	Enteric nervous system
FSH	Follicle-stimulating hormone
GI	Gastrointestinal
GnRH	Gonadotropin-releasing hormone
GnRH-R	Gonadotropin-releasing hormone receptor
GSK3	Glycogen synthase kinase 3
HRP	Horseradish peroxidase
Hs-CRP	High sensitivity C-reactive protein
IBS	Irritable bowel syndrome
IL	Interleukin
IQR	Interquartile range
IUD	Intrauterine device
LC	Lymphocytic colitis
LH	Luteinizing hormone
LH-R	Luteinizing hormone receptor

MC	Microscopic colitis
MRI	Magnetic resonance imaging
MCP-1	Monocyte chemoattractant protein 1
MMP	Matrix metalloproteinase
NPX	Normalized protein expression
NSAID	Non-steroidal anti-inflammatory drugs
OVA	Ovalbumin
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline containing 0.05% Tween-20
PEA	Proximity extension assay
PCR	Polymerase chain reaction
PPI	Proton pump inhibitors
RU	Relative units
SD	Standard deviation
SSRI	Selective serotonin reuptake inhibitors
ST1A1	Sulfotransferase 1A1
TCF	T-cell factor
TMB	3,3′,5,5′-tetramethylbenzidine
TN-C	Tenascin C
TNF-α	Tumor necrosis factor α
TPO	Thyroid peroxidase
TRAb	Thyroid-stimulating hormone receptor antibodies
tTG	Tissue transglutaminase
VAS-IBS	Visual analog scale for irritable bowel syndrome
VEGF	Vascular endothelial growth factor

Introduction

Both gynecological and gastrointestinal (GI) symptoms are commonly reported by young women (1-3), and in endometriosis, GI symptoms have been reported occur almost as frequently as gynecological symptoms, irrespective of bowel involvement of the disease (4). The mechanisms underlying GI symptomatology in endometriosis have not yet been fully elucidated; however, possible contributing factors include inflammatory activity, mechanical obstruction, visceral hypersensitivity, and psychiatric comorbidity (5-8). Several studies have suggested an epidemiological association between endometriosis and irritable bowel syndrome (IBS) (8, 9); however, whether these two conditions coexist or if symptoms related to endometriosis are often misdiagnosed as IBS remains under debate.

The gold standard for the diagnosis of endometriosis involves surgery and histopathological confirmation, which, combined with the occurrence of non-specific symptoms, often results in considerable diagnostic delay (10). No independently validated biomarkers currently exists for endometriosis, despite extensive research in the field (11-13).

This doctoral thesis has attempted to investigate the occurrence of GI symptoms in women with endometriosis. The prevalence of GI symptoms was investigated and compared with the GI symptoms reported by women with IBS and women from the general population. Socioeconomic factors, lifestyle habits, comorbidity and associations with GI symptoms were examined. Furthermore, serum antibodies associated with bowel pathology and serum inflammatory proteins were analyzed, and calculations were performed to examine associations between inflammatory proteins and GI symptoms.

Female sex hormones and their influences on the gastrointestinal tract

Gonadotropin-releasing hormone (GnRH) regulates female reproductive function, and is synthesized by hypothalamic neurons and is secreted in a pulsatile manner by the hypothalamus into the hypophyseal portal circulation. GnRH activates GnRH receptors (GnRH-Rs) in the anterior pituitary gland and stimulates the synthesis and

secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (14). In females, FSH and LH stimulate the synthesis and secretion of the gonadal steroid hormones estrogen and progesterone from the ovaries. In turn, circulating estrogen and progesterone regulate the secretion of GnRH through a negative feedback mechanism (15) (Figure 1).

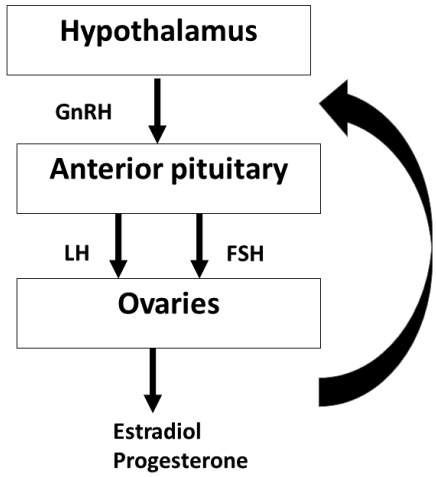

Figure 1
A simplified schematic showing the hypothalamo-pituitary-gonadal axis in women.

In mammals, two types of GnRH have been detected, GnRH1 and GnRH2, where GnRH1 is secreted from the hypothalamus, and both GnRH1 and GnRH2 are widely distributed in the brain. Several types of GNRH-Rs have been described, but only the GnRH1 receptor is expressed in mammals (15). GnRH analogs are a potential treatment option for endometriosis; during chronic treatment, GnRH-Rs becomes desensitized, and the secretion of LH and FSH decline (16, 17), resulting in the suppression of ovarian function, which limits the growth and activity of endometriosis (18).

GnRH and LH receptors (LH-Rs) have also been shown to be present in submucosal and myenteric neurons of the human enteric nervous system (ENS) (19, 20). Severe GI dysmotility has been described in some women following treatment with GnRH analogs, with histopathological examinations revealing decreased numbers of enteric neurons, reductions in the numbers of GnRH-expressing enteric neurons, and the presence of IgM antibodies against GnRH1 in plasma (21). Elevated titers of IgM antibodies against GnRH1 have also been identified in patients with IBS, GI dysmotility, posterior laryngitis, Sjögren's syndrome, and diabetes mellitus, irrespective of treatment with GnRH analogs, but not in patients with celiac disease

(CD), inflammatory bowel disease (IBD), or microscopic colitis (MC) (16). These findings suggest that enteric neuropathy may be a possible mechanism underlying GI symptoms in functional bowel diseases.

The menstrual cycle is characterized by cyclic changes in estrogen and progesterone levels (Figure 2).

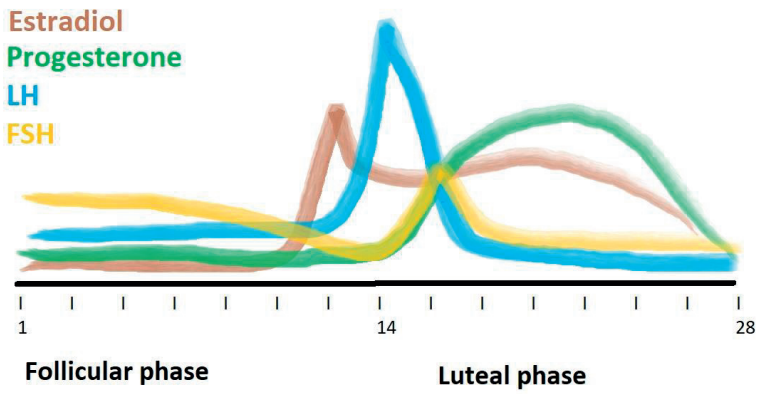

Figure 2
A simplified schematic showing hormone levels in the menstrual cycle

A review article concluded that visceral hypersensitivity increases during menstruation compared with other phases of the menstrual cycle in women with IBS (22). Increased GI symptoms during menstruation and early menopause have been hypothesized to be associated with the declining or low levels of ovarian hormones (23). Meurs-Szodja (24) reported that 80% of endometriosis patients with IBS experience aggravated GI symptoms during menstruation. During menstruation, rectal sensitivity increases in women with IBS and stools become looser (22, 25). Both estrogen and progesterone receptors are present in the human GI tract and impact GI function, including relaxing the lower esophageal sphincter and decreasing colonic transit (26). Estradiol has been shown to decrease colonic permeability, through the estrogen-receptor-β-mediated upregulation of occludin and junctional adhesion molecule-A in epithelial cells (27), which may contribute to the gender differences that have been observed in the prevalence of functional GI diseases.

Endometriosis

Endometriosis is a benign gynecological condition with a prevalence of 6-10% in the general female population (18, 28). The disease is defined by the presence of endometrial glands and stroma outside the uterine cavity, most commonly on the pelvic peritoneum, the ovaries, and the rectovaginal septum (18) (Figure 3). Endometriosis is the most common cause of chronic pelvic pain in women and can also cause dysmenorrhea, deep dyspareunia, dysuria (29, 30), and GI symptoms (4, 31, 32). Symptomatic endometriosis has been shown to negatively affect the quality of life, work productivity (29), and mental health (7, 33).

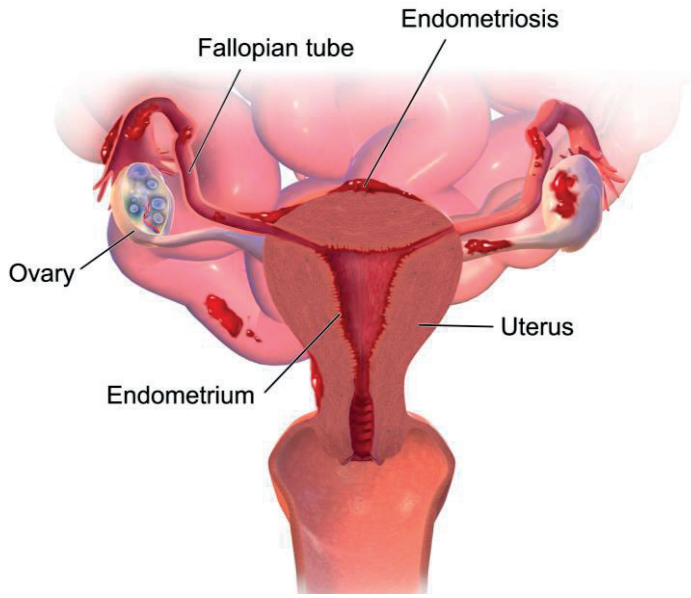

Figure 3
The anatomy of endometriosis. Picture by Bruce Blaus is licensed under CC by 3.0 (https://creativecommons.org/licenses/by/3.0/) Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436. Changes were made from the original format.

History

The first distinct description of endometrial tissue in an ovary was reported by Russel in 1899, who reported the case of a woman who underwent surgery for adenocarcinoma of the left ovary. The right ovary of this woman was described to have been “enveloped in adhesion of the posterior face of the broad ligament”. The macroscopic appearance was normal, but microscopically, Russel noticed regions

containing uterine glands and interglandular connective tissue within the ovary (34). In publications from 1921 and 1922, John A Sampson described ovarian hematomas and “chocolate cysts” found during surgeries for pelvic diseases (35, 36).

Pathogenesis

The first theory regarding the pathogenesis of endometriosis, the theory of retrograde menstruation, was proposed by John A. Sampson in 1927. According to Sampson’s theory, menstrual blood containing endometrial cells passes backward through patent fallopian tubes and into the peritoneal cavity during menstruation (37). However, blood in the peritoneal fluid has been reported in up to 90% of women with patent fallopian tubes during the perimenstrual period, indicating that retrograde menstruation is a common physiological phenomenon in menstruating women (38).

Since not all women who experience retrograde menstruation develop endometriosis, the establishment of the disease has been hypothesized to be dependent on other mechanisms, allowing the attachment and invasion of endometrial fragments, the establishment of blood supply, and an impaired immune response that inhibits the clearance of endometrial implants (18, 37, 39). Immunological, genetic, and environmental factors are believed to affect these processes (18, 39). Large twin studies have estimated additive genetic factors to be approximately 50% (40, 41), and evidence of epigenetic aberrations in endometriosis have been reported (42). Metaplasia of coelomic epithelium in situ into endometrial tissue has been proposed to contribute to the development of the disease (37), and stem/progenitor cells derived from bone marrow have been hypothesized to be capable of differentiation into endometrial tissue (43). The benign metastasis theory suggests that the lymphatic or hematogenous dissemination of endometrial cells may lead to endometrial implants in local areas, such as the ovaries, and distant regions, including the lung and brain (37). The theory of embryonic Müllerian rests proposes that cells from embryologic Müllerian duct migration may develop into endometriosis lesions under the influence of estrogen during puberty (37).

Ectopic endometrial growth is estrogen-dependent, and aberrant estrogen signaling is associated with endometriosis. The estrogen receptor β is present at higher levels in ectopic tissue compared with eutopic tissue (44). Progesterone resistance in the endometrium of endometriosis patients has been described, inducing decreased inhibition of the estrogen-dependent proliferation of epithelial cells and the secretory maturation of glands (37, 44, 45). Exposure to environmental contaminants that disrupt steroid hormone activities, such as dioxin and polychlorinated biphenyl (PCB), has been suggested to increase the risk of endometriosis (46, 47).

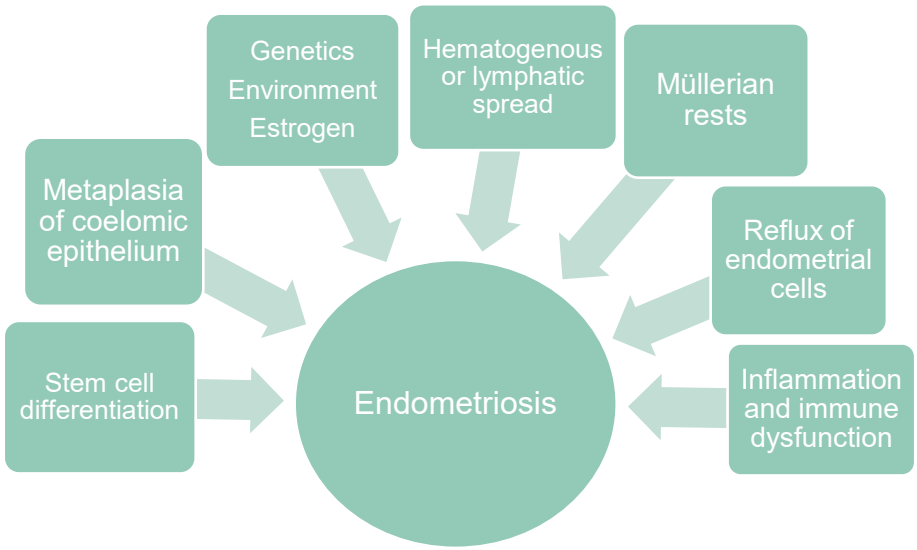

Figure 4
A simplified overview of theories regarding endometriosis pathogenesis

Endometriosis is widely considered to be an inflammatory condition, and immune dysfunction has been implicated in the pathobiology of the disease. Elevated levels of cytokines have been reported in both the peritoneal fluid and peripheral blood of women with endometriosis (11, 13, 37, 48). In the peritoneal fluid, increased concentrations of cytokines including interleukin (IL) 1, 6, 8 and 10, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1), growth factors, and angiogenic factors, such as vascular endothelial growth factor (VEGF), have been reported (18, 39, 49). An increased activation of peritoneal macrophages has been reported, but with decreased phagocytic abilities, and also a defect natural killer cell activity (50-52). In the peripheral blood, the concentrations of several cytokines, including IL 4, 6 and 8, TNF-α, MCP-1, RANTES, and YKL-40, have been shown to be elevated in women with endometriosis (11, 53, 54).

Endometriosis shares similar features with autoimmune diseases, including increased levels of cytokines and antibodies and an aberrant immune system response (55, 56). Increased levels of IgG and IgA antibodies against endometrial and ovarian tissue and IgG, IgM, and IgA autoantibodies against cellular antigens, including histones and phospholipids, have been reported in endometriosis (13, 56). Associations between endometriosis and several autoimmune diseases, including hypothyroidism, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, and multiple sclerosis, have also been described (57, 58).

The gut microbiota has been suggested to be involved in the onset and progression of endometriosis (59), and the increased presence of Proteobacteria, Enterobacteriaceae, Streptococcus, and *Escherichia coli* at various microbiome sites has been described (60).

Tenascin C and matrix metalloproteinase 9

Tenascin is a matrix glycoprotein, involved in cell differentiation, proliferation, and migration, and elevated expression levels of tenascin have been reported in endometriosis and have been linked to the development of the disease (61-63). Estrogen induces the upregulation of tenascin levels in endometriotic stromal cells in culture (63). Tenascin C (TN-C) is a member of the tenascin family that is upregulated during inflammation, is expressed in human cancers, and has been associated with migration, angiogenesis, and proliferation, which are factors that have also been associated with endometriosis (64, 65). Although TN-C expression in human adults is sparse, expression has been identified in the human endometrium (63, 66). In addition, TN-C has been linked with GI pathology, and the upregulation of TN-C has been associated with ulcerative colitis disease activity (67).

Matrix metalloproteinases (MMPs) are a family of endopeptidases, enzymes which are involved in the degradation of the extracellular matrix (68). MMP-9 has been shown to be upregulated in ectopic endometrium tissue and in the plasma, peritoneal and follicular fluid, during endometriosis (68, 69). TN-C may be cleaved by MMPs, which may impact TN-C function (64).

AXIN1 and the Wnt pathway

Signaling by the Wnt family of glycoproteins is a mechanism associated with cell proliferation, cell polarity, and cell fate determination during tissue homeostasis and embryonic cell development (70). Mutations and the dysregulation of the Wnt pathway have been linked to cancers, birth defects, and various other diseases, including intestinal inflammation, bacterial infections, and autoimmune disorders (71-74). The aberrant activation of the Wnt pathway has been implicated in the pathophysiology of endometriosis, and has been suggested to underlie the increased migration and invasion of menstrual endometrial cells (75). Gaetje et al. (76) reported the significantly increased expression of Wnt7a mRNA in human endometriosis lesions compared with eutopic endometrium tissue. The activation of the Wnt pathway has also been suggested to be involved in mechanisms underlying the development of fibrosis in endometriosis (77).

One of the most studied Wnt pathways, the canonical Wnt pathway, functions by regulating β-catenin. β-catenin is a transcriptional coactivator that controls key developmental gene expression programs (71). Casen kinase 1 (CK1) phosphorylates β-catenin, which is then ubiquitinated and degraded by the proteasome (78). Wnt signaling suppresses the ubiquitination of β-catenin, resulting

in an accumulation and nuclear translocation of β-catenin, which engages nuclear T-cell factor (TCF) transcription factors that activate the Wnt transcriptional program (78) (Figure 5).

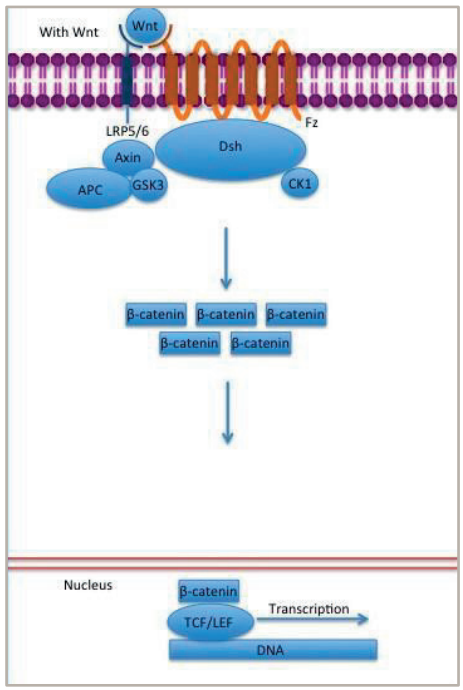

Figure 5
"Canonical Wnt pathway with Wnt" (79) by Gpruett2 is licensed under CC BY 3.0 (https://creativecommons.org/licenses/by/3.0/)

Furthermore, an association between estrogen and Wnt signaling has been reported, and estradiol has been shown to enhance β-catenin expression. However, the precise regulation mechanisms are not clear (80). Increased levels of MMP-9 in menstrual endometrial epithelial and stromal cells in endometriosis have been reported, and MMP-9 is one of the TCF/β-catenin target genes. Treatment with an antagonist against the TCF/β catenin complex decreased the levels of both total and active MMP-9 in the stromal and epithelial cells of patients with endometriosis (81).

AXIN1 is considered to be a down-regulator of the Wnt pathway (82). In the absence of Wnt, cytoplasmic β-catenin is degraded by the Axin complex, which consists of the scaffolding protein Axin, adenomatous polyposis coli gene product (APC), CK1, and glycogen synthase kinase 3 (GSK3) (71). AXIN1 is the rate-limiting factor of the Axin complex and interacts directly with all core components of the complex (78).

Comorbidity and lifestyle factors

Menarche at an early age, short menstrual cycles, and nulliparity have all been associated with endometriosis (83, 84). Furthermore, studies have suggested positive associations between endometriosis and high socioeconomic status, high alcohol consumption levels, and low body mass index (BMI) values, whereas regular physical activity has been negatively associated with the disease (84, 85).

Several other conditions have been associated with endometriosis, such as CD (86, 87). Furthermore, in a large Danish cohort study, increased risks of ulcerative colitis and Crohn's disease diagnoses were identified in women with endometriosis (88). A small case-control study concluded that endometriosis patients had a significantly higher prevalence of food intolerance and altered nutrient intake compared with controls (89). The prevalence of IBS has been shown to be higher in women with endometriosis (8, 90), and atopic diseases, fibromyalgia, hypothyroidism, and chronic fatigue syndrome are more common in women with endometriosis compared with the general female population (55, 57). Endometriosis is a risk factor for the development of epithelial ovarian cancer (91, 92), and the prevalence of psychiatric disorders, such as depression and anxiety disorders, is increased in endometriosis patients compared with the general population (7, 33).

Symptoms

The symptoms associated with endometriosis vary, and the most common symptoms include chronic pelvic pain, dysmenorrhea, deep dyspareunia, lower abdominal pain, back pain, bladder pain, and GI symptoms (18, 29). However, approximately 20-25% of endometriosis patients are asymptomatic, and the disease may be diagnosed during infertility investigations (93).

Gastrointestinal symptoms in endometriosis

GI symptoms are common in women with endometriosis and have been reported to be almost as frequent as gynecological symptoms (4). The most common GI symptoms associated with endometriosis include bloating, nausea, abdominal pain, constipation, vomiting, painful bowel movements, and diarrhea, and these symptoms may increase in intensity during menstruation (4, 6, 94, 95). Maroun et al. (4) reported that 90% of women with endometriosis experienced GI symptoms, but only 7.5% had intestinal involvement of endometriosis, indicating that GI symptoms are largely independent of bowel involvement.

The mechanisms underlying GI symptoms in endometriosis have not been fully elucidated; however, hypotheses include that inflammatory activity caused by endometriosis lesions and prostaglandin release alter bowel function (6, 8). Visceral hypersensitivity may worsen abdominal pain in endometriosis (5). Bowel or

rectovaginal lesions can cause GI symptoms due to inflammation and micro-hemorrhages in the rectal wall (6, 96), and rectal stenosis or rectal fixations on the uterine cervix or vaginal fornix or pelvic adhesions may also disturb bowel functions (6). However, GI symptoms are also frequently present without established bowel involvement of endometriosis (4), although bowel endometriosis may be difficult to diagnose, resulting in the underdiagnoses of bowel involvement in endometriosis patients (97).

Associations between endometriosis and organic bowel diseases (87, 88), and also IBS (8, 24, 98, 99) has been reported, which may render GI symptoms in some patients. GnRH analogs are a pharmacological treatment option for endometriosis, and treatment with GnRH analogs has been shown to be associated with the development of GI symptoms and dysmotility (21, 100). Furthermore, antibodies against GnRH are more common in patients with IBS and enteric dysmotility (ED) compared with controls (101), why a link between GnRH and GI function has been hypothesized. Opioids are sometimes used to treat painful endometriosis symptoms, but are also known to induce bowel dysfunction (102). Hypothetically, treatment with GnRH analogs and opioids may impact GI functions in endometriosis.

Diagnosis

Endometriosis often has a considerable diagnostic delay due to the variability among symptoms and symptom overlap with other diseases (18).

Biomarkers

Numerous biomarkers for the diagnosis of endometriosis have been investigated. A large Cochrane systematic review, published in 2016, summarized blood biomarkers associated with endometriosis (13). More than 15,000 patients were included in the study, which evaluated 122 biomarkers. Meta-analyses could only be performed for four biomarkers: anti-endometrial antibodies, IL6, cancer antigen 19-9 (CA-19-9), and cancer antigen 125 (CA-125). Of these biomarkers, none met the parameters to be considered as replacement diagnostic tools or triage diagnostic tests. Biomarkers are not currently recommended for the diagnosis of endometriosis (103).

Ultrasonography

A systematic review concluded that transvaginal ultrasonography, which is recommended for the diagnosis of endometriomas (103), can reliably identify these lesions. However, this method does not reliably detect small endometriosis lesions or infiltration depths (29).

Magnetic resonance imaging (MRI)

MRI may be used to detect subperitoneal endometriotic deposits and endometriomas. However, MRI is suggested to be less accurate than transvaginal ultrasonography for the detection of suspected endometriosis (29).

Laparoscopy

The gold standard for diagnosing endometriosis is the visualization of lesions through surgery, and the diagnosis may be confirmed by histopathological examination (18). Indications for laparoscopy include infertility in asymptomatic women or suspected endometriosis when symptoms are severe, or when symptoms are persistent despite pharmacological treatment (29).

Endometriosis can be divided into three subtypes; superficial peritoneal endometriosis lesions, deep infiltrating endometriosis, and cysts (29). The disease can be classified according to the American Society for Reproductive Medicine revised system into minimal, mild, moderate, or severe endometriosis, based on the type of lesion, location, appearance, depth of invasion, the extent of the disease, and adhesions. However, classification according to this system has a poor correlation with symptoms (29).

Treatment

The treatment of endometriosis aims to suppress ovarian hormone secretion to reduce disease activity and improve pain symptoms, and fertility (18) (Table 1). First-line treatments include combined oral contraceptives and progestin, and second-line treatments include GnRH analogs and intrauterine devices (IUDs) containing progestin (29). Analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and opioids, can be used to relieve endometriosis-related pain (29), and tricyclic antidepressants and anti-epileptic drugs can also be used to treat painful symptoms (104).

Surgical treatments include the excision or ablation of endometriosis lesions, ovarian cystectomy, the ablation or electrocoagulation of endometriomas (29, 105), the lysis of adhesions, and the interruption of nerve pathways (18). Hysterectomy, with or without bilateral salpingo-oophorectomy, is an alternative surgical strategy, but the latter results in surgical menopause. The recurrence of symptoms after surgery is estimated to be 10-50% after one year and increases over time, and the pain associated with endometriosis may remain chronic, even after surgery (29).

Table 1
Pharmacological and surgical treatments for endometriosis

Ovarian suppression	First line: - Combined oral contraceptives - Continuous progestin Second line - GnRH agonists - Progestin-releasing IUD
Analgesia	NSAIDs Paracetamol Tricyclic antidepressants Anti-epileptic drugs Opioids
Surgical treatment	Excision or ablation of lesions Cystectomy, ablation or electrocoagulation of endometriomas Hysterectomy, with or without salpingo-oophorectomy

Irritable bowel syndrome

IBS is a functional bowel disorder, with a worldwide prevalence of 9-23% (106). The prevalence of the disease is higher in women than in men and higher in individuals younger than 50 years old (107). The Rome IV criteria is the current standard for diagnosing IBS, which is revised criteria compared with the previous Rome III criteria (108, 109) (Table 2). The current basis for diagnosing IBS is chronic abdominal pain that occur at least one day a week, on average, during the previous 3 months, with an onset of at least 6 months before diagnosis. Abdominal pain should be associated with at least two of the following symptoms: pain related to defecation, change in frequency of stool, or change in the appearance of stool (108, 109)

IBS can be categorized into four subtypes: diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), IBS with mixed symptoms of constipation and diarrhea (IBS-M), and unspecified IBS (110).

The disease has multiple associated comorbidities, including depression, anxiety, somatization, fibromyalgia, chronic fatigue syndrome, chronic pelvic pain, gastroesophageal reflux disease, and dyspepsia (111). Due to the great symptom burden, IBS has negative impacts on work productivity and quality of life (110).

Table 2
Rome III and Rome IV criteria for the diagnosis of IBS

Rome III	Rome IV
Recurrent abdominal pain or discomfort at least 3 days per month during the last 3 months associated with two or more of the following criteria: Improvement with defecation Onset associated with a change in frequency of stool Onset associated with a change in stool form	Recurrent abdominal pain at least 1 day per week, on average, during the last 3 months, Abdominal pain is associated with two or more of the following criteria: Related to defecation Associated with a change in stool frequency Associated with a change in stool form
Criteria should be fulfilled for the last 3 months, with symptom onset at least 6 months prior to diagnosis	

Pathogenesis

IBS is classified as a functional disorder, since no clear structural abnormalities have been detected; however, it is a heterogeneous disorder, and evidence has suggested several mechanisms that may be of importance in the pathophysiology. Therefore, IBS may encompass several diseases with different underlying pathophysiological mechanisms that have similar clinical presentations.

Environmental factors associated with IBS include early life stressors, food intolerance, exposure to antibiotics, and enteric infections (111). The odds of developing IBS increase following acute bacterial, viral, or protozoal GI infections (112), and patients with post-infectious IBS are more likely to display low-grade intestinal inflammation (110).

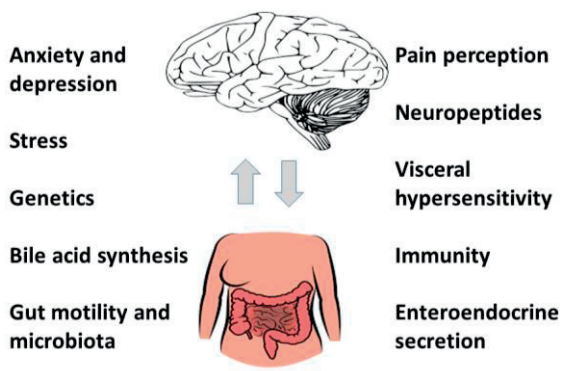

Figure 6
A simplified overview of pathophysiological mechanisms in IBS

Host factors that may contribute to IBS include altered gut microbiota and dysbiosis, visceral hypersensitivity, altered brain-gut interactions, increased intestinal permeability, and increased gut mucosal activation (111) (Figure 6). IBS has been considered to be a gut-brain axis disorder due to associations with psychiatric disorders, childhood trauma, and increased levels of corticotrophin-releasing factor; however, causation remains unclear (110). The intestinal microbiome has been suggested to be altered in IBS, and reduced microbial diversity has been associated with the severity of symptoms (113); however, studies regarding gut microbiota composition have been conflicting (114). Intestinal permeability is altered in some IBS patients, which may cause immune activation (110). A mutation in a sodium channel gene (SCN5A) has been identified and is estimated to explain 2% of IBS cases (110).

A proportion of patients with IBS experience food as a trigger for GI symptoms and a diet low in FODMAPs has been shown to successfully improve bowel symptoms in these patients (115). Congenital sucrase-isomaltase deficiency is a rare genetic form of disaccharide malabsorption, characterized by symptoms similar to those associated with IBS. Sucrase-isomaltase gene variants with defective or reduced enzymatic activity have recently been described to predispose carriers to IBS (116).

Management of IBS

The treatment of IBS is directed towards easing symptoms, and several pharmacological treatment options are available. Antidiarrheals, such as Loperamide, can prolong transit time and decrease fecal volume. Bulking agents and osmotic laxatives are also treatment options, particularly for IBS-C. Antidepressants, including selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants, are believed to act on centrally mediated antinociceptive pathways to decrease abdominal pain but may also affect gut transit times. Antispasmodics relax smooth muscle, affecting GI motility to reduce abdominal pain (117). Other treatments include prosecretory agents, antibiotics, peppermint oil, probiotics, and 5-hydroxytryptamine type 3 (5-HT3) receptor antagonists (111).

Additionally, increased physical exercise has been shown to improve overall IBS symptoms (118), and a low-FODMAP diet has been shown to reduce IBS-related symptoms (111). Fecal microbiota transplantation to reverse dysbiosis associated with IBS is a promising treatment; however, larger studies are necessary to verify the efficacy of this treatment for IBS (119).

Connections between gastrointestinal symptoms experienced by endometriosis patients and irritable bowel syndrome

Several studies have reported an increased prevalence of IBS in women with endometriosis and an increased risk of presenting with IBS-like symptoms compared with controls (5, 8, 24, 32, 98, 99, 120, 121). In a large case-control study, women with endometriosis were 3.5-fold more likely to be diagnosed with IBS compared with controls and 2.5-fold more likely to receive an IBS diagnosis, even after being diagnosed with endometriosis (8). Lee et al. (98) reported the prevalence of IBS in endometriosis patients to be as high as 52%. In addition, women who are diagnosed with endometriosis without bowel involvement have been shown to be more likely to be diagnosed with IBS compared with controls (90).

Several potential shared pathophysiological mechanisms exist between endometriosis and IBS, such as chronic low-grade inflammation, increased mast cell numbers, increased mast cell activation, visceral hypersensitivity, altered gut microbiota and increased intestinal permeability, which has been described in relation to both endometriosis and IBS (5, 9, 39, 59, 122-127). IBS is more prevalent in women, and both diseases are associated with psychiatric conditions (7, 128). Psychiatric comorbidities are common for both endometriosis and IBS, and anxiety and depression have been associated with GI symptoms among the general population (7, 129-131). In women with endometriosis also diagnosed with IBS, presence of mood disorders and sleep disturbances, but also a lower stage of endometriosis, was associated with severity of GI symptoms (98). Psychiatric symptoms may therefore potentially represent factors that affect the prevalence of GI symptoms in these diseases.

Visceral hypersensitivity is discussed in relation to both endometriosis and IBS (5, 123, 132) and has been shown to be present irrespective of the extent and severity of endometriosis (5), which may explain why the endometriosis stage correlates poorly with symptoms. Visceral hypersensitivity involves both central and peripheral sensitization and may be an important factor for both painful bowel symptoms during endometriosis and abdominal pain during IBS. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) cause intestinal luminal distension, resulting in pain symptoms in patients with visceral hypersensitivity (115). The low-FODMAP diet has been shown to be successful for the management of bowel symptoms in a majority of patients with IBS (115) and has also been shown to improve GI symptoms in women with endometriosis (99).

Pharmacological treatments commonly used for endometriosis, such as opioids to manage pain and hormonal treatments, have been shown to aggravate GI symptoms

(100, 133). The menstrual cycle phase has also been shown to impact GI symptoms in patients with both endometriosis and functional bowel disorders, with worsened symptoms occurring during menstruation (24, 134).

Thus, several studies have found an increased prevalence of IBS in women with endometriosis, and the two diseases share several potential pathophysiological mechanisms that may cause GI symptoms.

Microscopic colitis

MC is a relatively common cause of chronic, non-bloody diarrhea, especially among older patients. The disease has two subtypes, collagenous colitis (CC) and lymphocytic colitis (LC). The incidence rate of MC among patients undergoing investigation for chronic diarrhea is 10-20% (135), and the disease is more common in women than in men (136). Diarrhea is not the only symptom of MC; abdominal pain, weight loss, and arthralgia are reported by up to 50% of patients (137). The etiology of MC remains unknown. Associations between MC and autoimmune diseases, including CD, diabetes mellitus, thyroid disorders, and rheumatoid arthritis have been reported (135, 136). Smoking is a risk factor for the development of MC (138). Drug exposure has been implicated as a potential cause of MC, and NSAID, proton pump inhibitor, SSRI, and beta-blocker use have been strongly associated with MC (136).

Substantial overlap exists between the clinical presentations of CC and LC, and the natural histories of the two disease subtypes vary greatly (135). Diagnosis is determined by the results of a colonoscopy, with a corresponding histopathological examination. Colonoscopies generally display normal colonic mucosa, but colonic biopsies reveal the classic histological features of either LC (>20 intraepithelial lymphocytes per 100 epithelial cells) or CC (10-20 μm of a thickened subepithelial collagen band) (136). Other possible etiologies that may cause inflammation should be excluded, including infectious colitis, IBD and CD (136).

The initial management strategy for MC treatment is to eliminate any factors that may exacerbate symptoms, including medications and dairy products in lactose-intolerant patients. However, a majority of patients require pharmacological treatment. In patients with mild symptoms, antidiarrheal medication may be used (137). For the induction of clinical remission, evidence strongly supports the use of the corticosteroid budesonide (136). Other treatment options include bismuth subsalicylate, cholestyramine, and mesalamine, and immunomodulators may be considered for refractory symptoms (135).

Gastrointestinal dysmotility

Motility disorders encompasses numerous symptoms that can occur anywhere throughout the GI tract. These diseases are often chronic and can have dramatic effects on quality of life. No universally accepted definition of GI dysmotility currently exists, but the term is broad and includes a spectrum of disorders that are defined by the dysregulation or disruption of the enteric neuromuscular coordination (139). Motility disorders are often characterized by nausea, vomiting, distension, defecation difficulties, and pain, but limited correlations between symptoms and clinical findings or investigations have been reported (140).

Among patients with chronic, severe, GI dysmotility, approximately 80% are characterized as idiopathic. Autonomic neuropathies may underlie the syndromes that present with GI dysmotility, including diabetic gastroenteropathy, postural orthostatic tachycardia syndrome, and pseudo-obstruction. Other diseases that may present with dysmotility include Parkinson's disease, multiple sclerosis, myasthenia gravis, mitochondriopathies, and Guillain-Barre syndrome. Spinal cord injuries can also lead to dysmotility. Dysmotility can also be caused by paraneoplastic, autoimmune, endocrine, inflammatory, or infectious phenomena. Iatrogenic dysmotility may be caused by opioids or other drugs or radiation enteropathy (140, 141).

Patients with less severe dysmotility, compared to intestinal pseudo-obstruction, is referred to as ED. Clinically, these patients have the findings of pathological motility by intestinal manometry but lack the features of dilated small intestine. Symptoms similar to those associated with IBS are often present in ED (141).

Currently, no cure exists for most causes of GI dysmotility. Treatments primarily focus on symptom management through pharmacological, nutritional, and surgical approaches (139).

Aims

The overall aim of this thesis was to investigate the prevalence of GI symptoms in patients with endometriosis and to investigate factors associated with GI symptoms. The aims of the individual studies were as follows.

Paper I

The primary aim was to examine socioeconomic factors, lifestyle habits, comorbid conditions, and antibodies associated with thyroid disease in women with endometriosis compared with women from the general population. The secondary aim was to investigate associations between GI symptoms, comorbid conditions, and antibodies in endometriosis.

Paper II

The primary aim was to investigate the characteristics of GI symptoms in women with IBS compared to women with endometriosis and women with MC, to compare the clinical expression of the diseases.

Paper III

The primary aim was to measure the prevalence of antibodies associated with GI diseases in endometriosis patients compared with both healthy female controls and women with IBS or ED. The secondary aim was to investigate associations between antibodies and GI symptoms.

Paper IV

The primary aim was to investigate serum inflammatory profiles in women with endometriosis compared with women with organic bowel disease (MC), healthy female controls, and women from the general population. The secondary aim was to correlate protein levels with GI symptoms.

Paper V

The primary aim was to investigate the correlation between plasma AXIN1 levels and blood and fecal inflammatory biomarker levels in endometriosis patients. The secondary aim was to correlate inflammatory biomarker levels with clinical characteristics and GI symptoms in endometriosis patients.

Materials and Methods

Ethics

All studies were approved by the Ethics Review Board of Lund University, approval numbers 320-03, 510-02, 2009/565, 2010/386, 2011/44, 2011/209, 2012/564, 2012/594, and 2016/56. All subjects gave their written, informed consent before participation in the studies.

Patients

Patients with endometriosis were recruited at the Department of Gynecology at Skåne University Hospital, in Malmö, Sweden. Patients were identified by the International Statistical Classification of Diseases and Related Health Problems, ICD-10, N 80.

The inclusion criteria were a definite diagnosis of endometriosis, confirmed by laparoscopy or laparotomy, and an ability to comprehend the Swedish or English languages. For paper II, patients who were diagnosed with endometriosis by ultrasonography were also included. Exclusion criteria were an uncertain diagnosis of endometriosis, living too far away from the geographical area of Skåne University Hospital, multiple and severe somatic or psychiatric comorbidities, IBD, and current pregnancy. For paper II, current opioid use was an additional exclusion criterion.

During the first inclusion period, which was conducted continuously between March 2013 and July 2014, 307 patients who fulfilled the inclusion criteria and did not fulfill the exclusion criteria were identified. Of these, 198 patients were excluded for the following reasons: unwilling to participate (N = 145), moved from the geographical area (N = 49), and denying the diagnosis (N = 4). Thus, 109 women with endometriosis were identified for inclusion in the study. Nine of these women were further excluded because of a non-surgically confirmed diagnosis, resulting in 100 women being included in the study.

During the second inclusion period, conducted between September 2016 and March 2017, 266 women who fulfilled the inclusion criteria and did not fulfill the exclusion

criteria were identified. Of these, 187 women were excluded for the following reasons: unwilling to participate (N = 162) and moved from the geographical area (N = 23). Two women were excluded because of an uncertain diagnosis. Furthermore, seven of these women were excluded because of a non-surgically confirmed diagnosis of endometriosis, resulting in the inclusion of 72 women in the study (Figure 7).

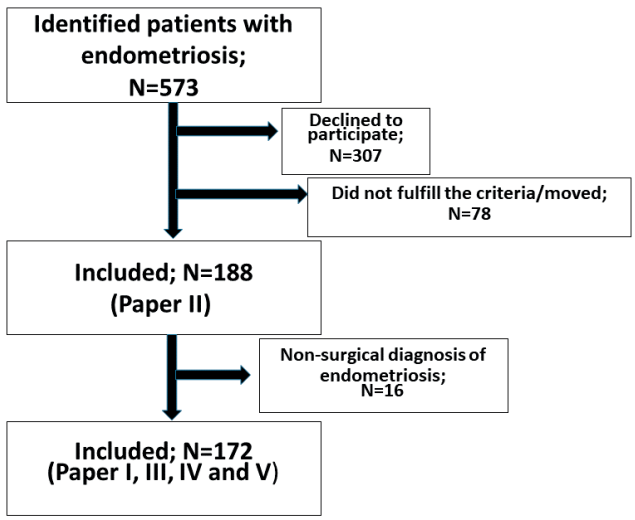

Figure 7
A flow-chart showing the inclusion procedure for endometriosis patients

Dropout analysis

A total of 307 women with endometriosis were excluded due to an unwillingness to participate in the study. The mean age of the included patients was 37.3 ± 7.3 years, compared with a mean age of 35.8 ± 6.9 years for the patients who declined to participate, which were not significantly different ($p > 0.05$). The reasons for their unwillingness to participate are not known. The patients who did not agree to participate were not contacted again for response analyses, since this is not allowed by the ethical review board.

Controls

An overview of the patients, the healthy controls, and the controls from the general population who were used for comparisons with endometriosis patients are presented in Table 3.

Paper I

The Malmö Offspring Study (MOS) is a prospective cohort that consists of the offspring of participants in the Malmö Diet and Cancer cardiovascular cohort (MDCS) (142). A randomly selected cohort from the MOS has previously been used to study GI symptoms in the general population (143). Women with an age < 60 years (N = 158) served as controls for this study. Because questionnaires and blood samples were not available for all of the controls, 117 women served as controls for analyses of socioeconomic factors, lifestyle habits, psychological well-being, GI symptoms, and comorbidity [median age 42 (28–52) years], and 114 women [median age 31 (25–42) years] served as controls for chemical analyses.

Paper II

Patients with IBS were both identified retrospectively according to the ICD classifications of functional GI disorders (K58, K59.0, K59.1, and K59.9) and recruited consecutively when visiting the Department of Gastroenterology at Skåne University Hospital Malmö. All patients were examined, including the collection of blood and fecal samples, endoscopies, antibody analyses, and tests for bacterial overgrowth or malabsorption, according to clinical indications to exclude organic diseases that may cause GI symptoms. Between 2005 and 2010, 304 patients who fulfilled the Rome III criteria for IBS diagnoses (109) were identified. Of the identified patients, 109 women, with a mean age of 37.3 ± 12.3 years, agreed to participate in this study (144, 145).

Patients with MC were identified retrospectively using the local register at the Department of Pathology at Skåne University Hospital in Malmö, between 2002 and 2010, according to the ICD-10 classifications for CC and LC (K52.8). Women who were diagnosed at any of the Departments of Gastroenterology in Skåne were also included. In total, 240 women with diagnoses verified by colonic biopsies and histopathological examinations were identified. Of those, 159 agreed to participate. One patient was excluded because of another IBD diagnosis. Of the patients who agreed to participate, 70 patients with transient symptoms were excluded, leaving 88, patients with a mean age of 60.7 ± 9.7 years, who were included in the study (146, 147).

To perform age-standardizations for the variables included in the Visual Analog Scale for Irritable Bowel Syndrome (VAS-IBS), using a linear regression model, data were obtained from a study by Hammar et al. (100). The Swedish Population Registry was used, and 248 female subjects from this registry were randomly selected. The subjects were contacted and after one reminder, only 29 questionnaires were returned. Due to the low response rate, controls were also acquired from among hospital staff. A total of 65 women (mean age 38.4 ± 7.4 years) were recruited and completed the VAS-IBS.

Paper III

The control group for chemical analyses consisted of 100 healthy female blood donors, with a median age of 42.5 (30.0–53.0) years.

To serve as comparisons for chemical analyses, 29 women with IBS or ED were used (148). Of these, 11 had IBS-M, 6 had IBS-C, 2 had IBS-D, and 10 suffered from ED. The criterion for ED was an abnormality in the small bowel manometry, without sub-occlusion episodes (141). Their median age was 34.0 (25.5–51.5) years.

Paper IV

Women diagnosed with MC at the Department of Gastroenterology at Skåne University Hospital in Malmö were used as controls for proximity extension assay (PEA) analyses. The controls were obtained from a study by Roth et al. (149). Because not all subjects could be included, the controls were selected randomly. Women with histopathological changes and chronic GI symptoms were classified as MC (N = 50). Of those, 3 were excluded due to technical problems, leaving 47 women with MC in the study, with a mean age of 59.4 ± 9.9 years. Women who had suffered a transient episode of MC and had no current symptoms or signs of disease were categorized as healthy controls (N = 31). The healthy controls had no GI symptoms and did not have any severe somatic or psychiatric comorbidities. Of these women, three were excluded due to technical problems, leaving 28 healthy controls, with a mean age of 64.1 ± 5.5 years.

From the MOS cohort, 100 female controls were used as controls for enzyme-linked immunosorbent assay (ELISA) analyses of AXIN1, with a mean age of 36.2 ± 8.1 years.

Table 3
An overview of the patients and controls included in Papers I-V

Paper	Patients	Diagnosis	Controls
I	N = 172, median age 38.0 (32.0 –43.0) years	Endometriosis confirmed surgically	Women from MOS, For chemical analyses: N = 114, median age 31.0 (25.0–42.3) years For questionnaires: N = 117, median age 42.0 (28.0–52.0) years
II	N = 158, mean age 37.4 ± 6.6 years (30 patients with opioid treatment were excluded)	Endometriosis confirmed surgically or by ultra-sonography	Women with IBS, N = 109, mean age 37.3 ± 12.3 years Women with MC, N = 88, mean age 60.7 ± 9.7 years For calculation of Z scores, Women from the general population, N = 65, mean age 38.4 ± 7.4 years
III	N = 100, median age 38.0 (32.3–43) years In analyses of TN-C IgM and MMP-9 IgG: N = 172, median age 38.0 (32.0–43.0) years	Endometriosis confirmed surgically	Healthy female blood donors, N = 100, median age 42.5 (30.0–53.0) years IBS/ED patients N = 29, median age 34.0 (25.5–51.5) years
IV	For the PEA: N = 94, mean age 36.7 ± 7.4 years For ELISA: N = 172, mean age 37.3 ± 7.3 years	Endometriosis confirmed surgically	For the PEA: MC, N = 47, mean age 59.4 ± 9.9 years and healthy controls, N = 28, mean age 64.1 ± 5.5 years. For ELISA: Women from MOS, N=100, mean age 36.2 ± 8.1 years
V	For AXIN1 and hs-CRP analyses, N = 172, mean age 37.3 ± 7.3 years For F-calprotectin and remaining blood samples, N = 64, mean age 37.6 ± 7.0 years	Endometriosis confirmed surgically	

MOS = Malmö Offspring Study, IBS = irritable bowel syndrome, MC = microscopic colitis, ED = enteric dysmotility, PEA = proximity extension assay, TN-C = tenascin C and MMP-9 = matrix metalloproteinase 9.

Study Design

This study used a cross-sectional design. Patients were contacted through an information letter via mail, and after one week, they were also contacted via telephone. When patients agreed to participate, two questionnaires, the VAS-IBS, and a clinical data survey, were sent via mail for completion within a week of the appointment. At the hospital visit, patients were interviewed, questionnaires were collected, and blood samples were drawn. Blood samples were frozen at -20°C and -80°C. Of the last included patients, 64 provided fecal samples that were frozen at -80°C.

The first patients included (N = 18) were followed prospectively, were interviewed, completed the VAS-IBS, and had blood samples drawn 3 and 6 months after their initial visits.

The medical journals of the included patients were scrutinized regarding the localization of endometriosis lesions, current pharmacological treatments, comorbid medical conditions, and the methods used to confirm the diagnosis of endometriosis.

Questionnaires

Clinical Data Survey

The clinical data survey included questions regarding socioeconomic factors, including education and occupation, and questions regarding lifestyle habits, including smoking, alcohol consumption, and physical activity. The data survey also included questions addressing medical history, pharmacological treatments, and GI- and endometriosis-associated symptoms, including the onset of symptoms, the pharmacological treatment of symptoms, and potential triggering factors associated with complaints.

The Visual Analogue Scale for Irritable Bowel Syndrome

The VAS-IBS was used to measure the GI symptoms in patients and controls. The VAS-IBS is a psychometrically validated questionnaire that measures the most common GI symptoms in patients with non-organic, functional bowel disease (150). The VAS-IBS has been validated for the prospective measurement of GI symptoms (151) and has also been validated in Asian settings (152). The symptoms measured by the VAS-IBS include abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, psychological well-being, and intestinal symptoms

influence on daily life activities. The symptoms are measured on a scale from 0 to 100, where 0 represents very severe symptoms and 100 represents a complete lack of symptoms. In papers I and III-V, the VAS-IBS was inverted, and on the inverted scale, 100 represents very severe symptoms. Two additional questions, regarding the experience of defecation urgency and the experience of incomplete evacuation when defecating, are answered with a yes or no.

The Malmö Offspring Study Questionnaire

Participants in the MOS answered a questionnaire regarding education, occupation, marital status, alcohol and nicotine habits, physical activity, medical conditions, and pharmacological treatments (143).

Laboratory Methods

Paper I

Analyses of antibodies against the TSH receptor (TRAb) and thyroid peroxidase (TPO)

Antibodies against the TSH receptor (TRAb) and thyroid peroxidase (TPO) were analyzed in serum samples from both patients and controls by the Department of Laboratory medicine at Skåne University Hospital in Malmö, using routine clinical protocols. A competitive electrochemiluminescence immunoassay detection technique, based on a Ruthenium derivative, was used (153, 154). The detection limit for TRab IgG was 0.3 IE/L. Titers > 1.7 IE/L were considered to be positive, whereas titers between 1.2 and 1.7 were considered to be grey-zone values, according to the laboratory protocol. For anti-TPO antibodies, the detection limit was 5.0 kIE/L, and titers >34 kIE/L were considered to be positive (155).

Paper III

Analyses were performed using in-house ELISA methods. To develop a color reaction, a phosphatase substrate kit or a 3,3′,5,5′-tetramethylbenzidine (TMB) peroxidase substrate system was used. Absorbance was measured by an ELISA reader. Antibody levels are presented as relative units (RU). RU values > 97.5th percentile of the control group, which consisted of 100 healthy, female, blood donors, was defined as the cut-off value for determining the presence of antibodies.

Analyses of antibodies against gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor

Analyses were conducted as previously described, by Hammar and Roth et al. (100, 147). The wells of microtiter plates were coated with human GnRH or N-terminal GnRH-R peptides conjugated with ovalbumin (OVA). After overnight incubation, the wells were blocked with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-T). Appropriately diluted serum samples from patients and blood donors, mouse anti-human GnRH antibody, or rabbit anti-human GnRH-R antibody were then added to the microtiter plates. Following incubation and rinsing, the deposition of antibodies was detected with biotinylated rabbit anti-human IgM and IgG, biotinylated anti-rabbit IgG or biotinylated anti-mouse IgG (for the standard curves). Following incubation and washing, the bound biotinylated antibodies were detected using alkaline phosphatase-conjugated streptavidin.

Analyses of antibodies against luteinizing hormone and luteinizing hormone receptor

Analyses were conducted as previously described (100). Microtiter plates were coated with intact, purified, native, human LH in PBS or with N-terminal LH-R peptide conjugated with OVA in 100 mM carbonate buffer pH 9.2. After incubation, the plates were washed and blocked with 0.5% BSA in PBS-T. Appropriately diluted serum samples from patients and blood donors or rabbit anti-human LH antibody in serial dilution, with BSA in PBS-T were then added to the plates. After incubation and washing, the deposition of antibodies against LH was detected with biotinylated rabbit anti-human IgM and IgG or goat anti-rabbit IgG. The deposition of antibodies against LH-R was detected using biotinylated rabbit anti-human IgM or IgG.

Analyses of antibodies against matrix metalloproteinase-9

The microtiter plates were coated with a recombinant MMP-9 in PBS. After overnight incubation, the plates were washed and blocked with 0.5% BSA in PBS-T. Appropriately diluted serum samples from patients and blood donors or rabbit IgG anti-human MMP-9 antibody in serial dilution with BSA in PBS-T were then added to the plates and incubated. The washing procedure was repeated, and the deposition of autoantibodies was detected using horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG, IgM, or IgA, or goat anti-rabbit IgG, appropriately diluted.

Analyses of antibodies against tenascin C

The microtiter plates were coated with recombinant TN-C in 50 mM carbonate buffer, pH 9.2, or carbonate buffer only. After overnight incubation, the microtiter

plates were washed with PBS-T and blocked with 1.0% BSA in PBS-T. Dilutions of serum samples from patients and blood donors or rabbit anti-human TN-C antibody with BSA in PBS-T were then added to the microtiter plates and incubated. The washing procedure was repeated, and the deposition of antibodies against TN-C was detected using HRP-conjugated rabbit anti-human IgG, rabbit anti-human IgM or goat anti-rabbit IgG.

Analyses of antibodies against tissue transglutaminase (tTG) and deamidated gliadin (DPG)

Analyses of tTG and DPG antibodies were conducted using the Celiac Fusion™ test (Immco Diagnostics Inc., Buffalo, NY, USA). Celiac Fusion is an ELISA for the detection of tTG and DPG IgA and IgG antibodies in serum. Analyses were performed according to the manufacturer's instructions as a solid phase immunoassay (156). Micro-wells were coated with recombinant antigen containing tTG and DPG epitopes, followed by a blocking step. Then, serum samples from patients and controls were incubated in the antigen-coated wells. Unbound antibodies were removed by washing, and bound antibodies were detected by adding a HRP conjugated anti-human IgA/IgG conjugate. A specific enzyme-substrate (TMB) was then added to the wells, and the intensity of the color reaction was determined using a spectrophotometer. The results were expressed as ELISA units per milliliter and were reported as either positive or negative (qualitative determination).

Paper IV

Proximity extension assay

Inflammatory proteins were analyzed in serum samples from patients and controls using a multiplex proximity extension assay (PEA). The Inflammation 1 kit (Olink, Bioscience, Uppsala, Sweden) was used, and analyses were conducted at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden. During these analyses, 92 inflammation-related proteins are simultaneously analyzed (157, 158). Briefly, DNA oligonucleotide-labeled antibody pairs bind pairwise with their target proteins in plasma samples in a homogenous assay. Due to a proximity-dependent DNA polymerization event, a polymerase chain reaction (PCR) target sequence is formed. The amplification of this newly created piece of DNA barcode is conducted using a standard real-time PCR method. The amount of each barcode DNA is quantified by microfluidic PCR. Information concerning data validation, limits of detection, specificity, and reproducibility are available via the company's website (159). Data are presented as normalized protein expression (NPX) values, an arbitrary value on a log2 scale.

Analyses of human AXIN1

Plasma AXIN1 was analyzed using a sandwich ELISA method, according to the manufacturer's instructions. Standards and human ethylenediamine tetraacetic acid (EDTA) plasma, either undiluted or diluted 1:2 in dilution buffer, were incubated in a plate that was pre-coated with an anti-AXIN1 antibody. After incubation and washing, biotin-labeled AXIN1 antibodies were added to the micro-wells. The unbound antibodies were washed off, and HRP-streptavidin conjugate was added. The incubation and wash procedures were repeated, and the TMB substrate was added to form an HRP enzymatic reaction. The absorbance was measured at 450 nm.

Paper V

Hemoglobin, erythrocytes, leukocytes, and platelets in blood and high-sensitivity C-reactive protein (hs-CRP) in plasma were analyzed, according to routine procedures at the Department of Clinical Chemistry at Skåne University Hospital in Malmö, Sweden (160).

Analyses of fecal calprotectin

Fecal calprotectin (F-calprotectin) was analyzed using an ELISA method by the Department of Clinical Chemistry in Malmö according to routine clinical procedures. The lowest detection value was 25 mg/kg. Values < 50 mg/kg were considered to be normal, values between 50-100 mg/kg were considered grey zone values, and values >100 mg/kg were considered to be pathological (161).

Analyses of human AXIN1

Human AXIN1 in plasma was analyzed by a sandwich ELISA technology, described for Paper IV.

Statistical Analyses

Statistical analyses were performed by SPSS© for Windows (Statistical Package for the Social Sciences) (release 23.0; IBM). Variables were analyzed for normal distribution using the Kolmogorov-Smirnov test and by visualization in a histogram. To perform comparisons between groups, when variables were normally distributed, the Student's T-test was used (Paper IV), and when the distribution was skewed, the Mann-Whitney U-test was used (Papers I, II, III, IV, and V). For correlations, Spearman's rank test was used (Papers I, IV, and V). For categorical variables, Fischer's exact test was used (Papers II, III, IV, and V). A logistic regression,

adjusted for confounders, was used in Paper I to calculate associations. In Paper II, a linear regression model was used, including age as a covariate, and the parameters were then expressed as Z-scores. A linear regression model adjusted for age was used for comparisons of proteins levels between groups in Paper IV because the ages differed between patients and controls. Receiver operating characteristics (ROC)-curves, with an area under the curve (AUC) and 95% confidence interval (CI), were calculated for the two proteins that differed the most between patients and controls. A principal component analysis (PCA) was conducted to identify uncorrelated factors among the plasma proteins (Paper IV). Values are presented as the median [interquartile range (IQR)], mean ± standard deviation (SD) or number [percentages (%)].

Results

Basal characteristics of the endometriosis cohort

In total, 188 women with endometriosis were recruited and were included in the analyses performed for Paper II. After the exclusion of 16 who were diagnosed by ultrasonography, 172 women with surgically confirmed endometriosis were included in all other analyses performed for this cohort. The mean age of the 172 included patients was 37.3 ± 7.3 years, and the median BMI was 24.3 (21.8–27.1) kg/m^2. Socioeconomic and lifestyle factors are described in Table 4.

Table 4
Socioeconomic factors and lifestyle habits among the endometriosis patients (N = 172)

Education		**Smoking habits**	
Primary school	5 (2.9)	Never smoked	109 (63.4)
Secondary school	31 (18.0)	Former smoker	36 (20.9)
University or college	135 (78.5)	Current smoker	26 (15.1)
Missing	1 (0.6)	Missing	1 (0.6)
Occupation		**Alcohol consumption**	
Working/student	152 (88.4)	No consumption/< 1 sd/week	108 (62.8)
Sick leave/unemployed	19 (11.0)	1-4 sd/week	52 (30.2)
Missing	1 (0.6)	5-14 sd/week	12 (7.0)
Marital status		**Physical activity**	
Single/living alone	51 (29.7)	Sedentary leisure time	56 (32.6)
Married/cohabitation	113 (65.7)	Moderate exercise	30 (17.4)
Missing/other	8 (4.7)	Regular/regular intense exercise	85 (49.7)
		Missing	1 (0.6)

SD = standard glass of alcohol. Values are presented as number and percentages.

A majority of this cohort had either a university or college education and were working or studying. Most of the patients were non-smokers and reported a low alcohol intake.

Of the patients, a majority (84%), reported having suffered from GI complaints during the past year. The GI symptoms reported for the past two weeks are described in Table 5. Bloating and flatulence was estimated to be the most impairing symptom, followed by abdominal pain and the sensation of incomplete evacuation.

Table 5
Gastrointestinal symptoms measured by the inverted VAS-IBS scale in endometriosis patients (N = 172)

Abdominal pain (mm) *Reference value*	40 (10–72) *5 (1-15)*
Diarrhea (mm) *Reference value*	15 (0–55) *3 (0-10)*
Constipation (mm) *Reference value*	28 (0–70) *9 (1-22)*
Bloating and flatulence (mm) *Reference value*	55 (18–80) *14 (1-29)*
Vomiting and nausea (mm) *Reference value*	9 (0–45) *2 (0-3)*
Psychological well-being (mm) *Reference value*	30 (8–64) *4 (0-16)*
Influence on daily life (mm) *Reference value*	40 (9–75) *2 (0-18)*
Defecation urgency (n,%)	61 (36%)
Incomplete evacuation (n,%)	95 (56%)
Missing value	3 (1.7%)

Mm = millimeter. On the inverted VAS-IBS scale, 100 represents very severe symptoms and 0 a complete lack of symptoms. Values are presented as the median (IQR) and the number (percentage). Yes/no answers are indicated by the number (percentage) of yes answers.

Increasing age in patients correlated with less diarrhea (rs = -0.23, p = 0.002), less bloating and flatulence (rs = -0.20, p = 0.009), and less vomiting and nausea (rs = -0.21, p = 0.008) and with better psychological well-being (rs = -0.21, p = 0.005).

Most commonly, the GI symptoms had gradual onsets (61%) and were intermittent (37%). Only 28% of patients reported a trigger for GI symptoms, and the most frequently reported trigger was menstruation. Almost half of the patients had been prescribed pharmacological treatments for GI symptoms, including opioids, NSAIDs, bulking agents or laxatives, PPIs, and paracetamol. Some patients had undergone diagnostic examinations due to GI symptoms, including colonoscopy (10%), gastroscopy (8%), proctoscopy (5%), unspecified endoscopy (2%), ultrasonography (8%), MRI (4%) and unspecified radiological imaging (6%).

Almost all (90%) patients had received hormonal treatments for endometriosis at some point during the disease course. Of these, 69% had received combined oral contraceptives, 55% had received GnRH analogs, 43% had received progestin, and 5% had received estrogen. Furthermore, 10% reported ever being treated with a hormonal IUD. Almost half of the patients, 46.5%, were currently using systemic hormonal treatment for endometriosis. Current hormonal treatments included combined oral contraceptives (23%), progestin (17 %), GnRH analogs (9%), and estrogen (1%). Furthermore, 3% reported that they were currently using a hormonal IUD. The patients who reported current use of GnRH analogs experienced

aggravated abdominal pain compared with the remaining patients [74 (35–90) vs. 40 (5–70) mm, p=0.006].

The most common other pharmacological treatments currently being used by patients included antidepressants (19%), NSAIDs (19%), opioids (17%), paracetamol (16%), and levothyroxine (11%). Patients currently using opioids reported more severe GI symptoms than the remaining patients for all parameters measured on the VAS-IBS (all $p < 0.05$), except for diarrhea (Figure 8).

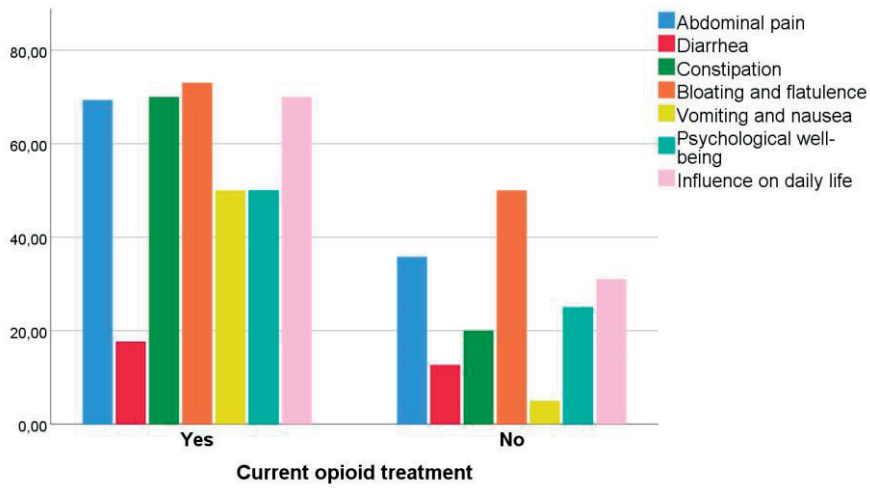

Figure 8
Gastrointestinal symptoms measured by the inverted VAS-IBS scale in patients currently treated with opioids compared with patients not currently treated with opioids (N=172). The Y-axis represent the median value on the inverted VAS-IBS scale.

The most common localization of endometriosis lesions were ovarian endometriomas (73%, of which 38% had isolated ovarian lesions). One-fifth (20%) of the patients had bowel lesions (of these, 10% had unspecified bowel lesions, 8% had rectal lesions, 3% had colon lesions, and 0.5% had lesions in the ileum). Other localizations included peritoneal lesions/small pelvic lesions (unspecified by the medical journals) (35%), Pouch of Douglas (12%), sacro-uterine ligaments (10%), vesico-uterine pouch (9%), urine bladder or the urethra (5%), fallopian tubes (3%), the vagina (3%), rectovaginal septum (2%), and the cervix (1%). Uncommon localizations included the groin, the liver, the diaphragm, rectus musculature, an abdominal surgical scar, and the umbilicus (all N = 1). In addition, 2% had lesions in unspecified locations. The localization of endometriosis lesions did not affect GI symptoms.

All patients had undergone surgery for endometriosis, since the diagnoses for all patients were surgically confirmed. Of these patients, 8 (5%) had undergone hysterectomy and 4 (3%) hysterectomy with bilateral salpingo-oophorectomy.

Paper I

All socioeconomic factors were similar between patients and controls. Alcohol consumption, leisure-time physical activity, BMI, and asthma were negatively associated with endometriosis. Smoking habits did not differ between patients and controls.

A diagnosis of IBS was associated with endometriosis. Of the endometriosis patients, 42% fulfilled the Rome III criteria for IBS (109). Among the women with endometriosis, 28.5% had been diagnosed with depression, or anxiety disorder, or both, during the past five years. Abdominal pain and constipation were aggravated and psychological well-being was impaired among patients with depression or anxiety compared with the remaining patients. The current use of antidepressant medications ($p = 0.020$) and impaired psychological well-being (p for trend = 0.003) were both associated with endometriosis. Impaired psychological well-being correlated positively with all of the GI symptoms included on the VAS-IBS scale (all $p < 0.001$), but correlated negatively with age.

Hypothyroidism was common in endometriosis patients with a prevalence of 11%; therefore, antibodies against thyroid tissue were analyzed. The presence of elevated TRAb titers was associated with endometriosis (p for trend < 0.001); however, titers of TRAb above the cut-off value were not associated with endometriosis. TRAb titers did not correlate with age, BMI or GI symptoms. The presence of TPO antibodies was not associated with endometriosis.

Paper II

Approximately one-third of endometriosis patients fulfilled the Rome III criteria for IBS (35%), whereas more than half of MC patients experienced IBS-like symptoms (55%).

A majority of GI symptoms, including abdominal pain, diarrhea, bloating and flatulence, vomiting and nausea, defecation urgency, and a sensation of incomplete evacuation, were aggravated in the IBS cohort compared with the endometriosis cohort, with impaired psychological well-being and more profound impacts on daily life activities in the IBS cohort.

When comparing IBS patients with patients who have endometriosis and met the Rome III criteria for IBS, the only significant difference was a more profound impact on daily life activities for IBS patients ($p = 0.005$).

Women with IBS experienced aggravated abdominal pain, bloating and flatulence, and worse psychological well-being compared with women with MC. Women with MC who experienced IBS-like symptoms experienced aggravated diarrhea compared with women with IBS ($p = 0.005$).

Paper III

In endometriosis patients, all analyzed antibodies had low prevalence. IgM antibodies against TN-C tended to be more prevalent in endometriosis patients compared with controls (7.6% vs. 2.0%); however this difference was non-significant ($p = 0.06$). The prevalence of IgM antibodies against TN-C was not associated with basal characteristics, pharmacological treatments, or GI symptoms in endometriosis patients.

Women with IBS or ED had a higher prevalence of IgM antibodies against GnRH1 compared with both women with endometriosis and controls. They also had a higher prevalence of IgM antibodies against TN-C compared with controls (Table 6).

Table 6
Prevalence of antibodies in women with endometriosis, women with IBS and ED, and controls

	Endo-metriosis	Controls	IBS/ED	P-value, Endo vs. Controls	P-value Endo vs. IBS/ED	P-value IBS/ED vs. controls
GnRH1 IgM	3 (3.0)	2 (2.0)	6 (20.7)	1.00	**0.004**	**0.002**
GnRH-R IgM	4 (4.0)	2 (2.0)	3 (10.3)	0.68	0.19	0.08
TN-C IgM	13 (7.6)	2 (2.0)	5 (17.2)	0.06	0.15	**0.006**

Values are given as number (percentage).

Paper IV

Several protein levels were altered in sera of women with endometriosis compared with women with MC and healthy controls, and the proteins with the most significant elevations in endometriosis were AXIN1 ($p < 0.001$ and 0.001, respectively), and sulfotransferase 1A1 (ST1A1) ($p = 0.001$ and 0.010, respectively). ROC curves for these proteins showed high AUC values; 0.830 for AXIN1 and 0.839 for ST1A1. C-X-C motif chemokine 9 (CXCL-9) had the most

significantly decreased level in women with endometriosis compared with both women with MC and healthy controls (both $p < 0.001$).

In a principal component analysis (PCA), four factors were identified which explained 44% (factor 1), 9.8% (factor 2), 4.7% (factor 3), and 3.8% (factor 4) of the total variance. Only factor 2 differed significantly when patients with endometriosis were compared with patients with MC ($p < 0.001$) or with healthy controls ($p = 0.001$). AXIN1 had the highest correlation with factor 2 ($r = 0.886$).

Oncostatin M was the only protein that tended to correlate with several GI symptoms in endometriosis.

Levels of AXIN1 were further analyzed in plasma samples for the whole cohort of 172 women with endometriosis and compared with the levels in plasma from 100 female controls from the general population. In this larger cohort, AXIN1 levels were increased in women with endometriosis compared with controls ($p < 0.001$) (Figure 9).

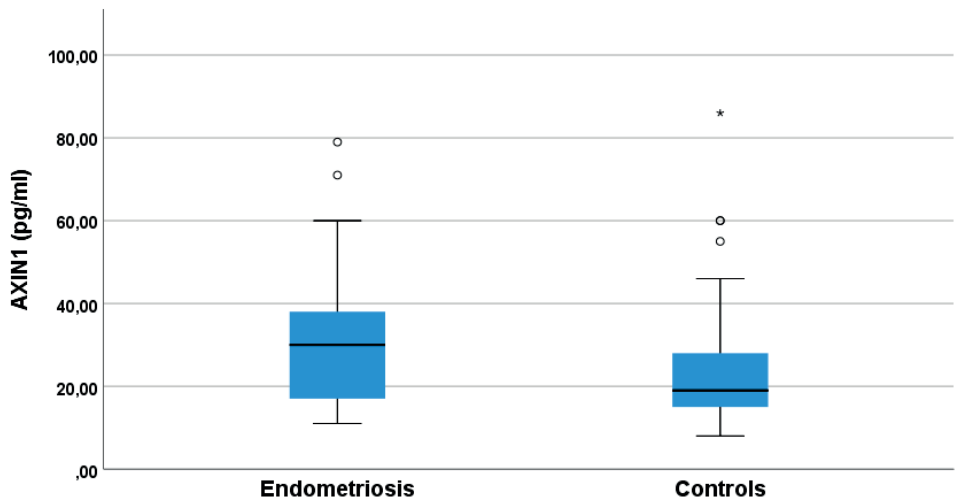

Figure 9
Plasma AXIN 1 values in patients with endometriosis and controls

Paper V

Among a subgroup of patients who provided both blood and fecal samples (N = 64), P-AXIN1 was negatively correlated with F-calprotectin, B-hemoglobin, B-erythrocytes, and B-platelets, whereas hs-CRP was positively correlated with F-calprotectin and B-leukocytes (Table 7).

Table 7
Biomarkers and their correlation with AXIN1 in plasma

Biomarker	Median (IQR)	AXIN1	
		Correlation Coefficient	P-value
P-AXIN1	39.0 (35.8–42.0)	1.00	-
F-calprotectin	25.0 (25.0–29.5)	-0.37	0.003
B-hemoglobin	124.0 (118.0–127.3)	-0.28	0.030
B-erythrocytes	4.3 (4.1–4.5)	-0.27	0.033
B-leukocytes	7.8 (6.5–9.1)	-0.18	0.168
B-platelets	265.5 (218.8–324.8)	-0.30	0.017
P-hs-CRP	1.1 (0.6–3.2)	-0.05	0.758

Values are presented as median (IQR).

Of the endometriosis patients, 18 women (28%) had measurable F-calprotectin levels (>25 mg/kg). Of these, 6 women (9%) had levels in the grey zone (50-100 mg/kg) while 5 women (8%) had high levels (>100 mg/kg). Patients with unmeasurable F-calprotectin levels had higher AXIN1 values ($p = 0.002$) and lower hs-CRP values ($p = 0.029$) compared with patients with measurable F-calprotectin levels. Patients with measurable F-calprotectin levels suffered from more constipation ($p = 0.048$) and more frequently experienced incomplete evacuation when defecating ($p = 0.009$) compared with patients with unmeasurable F-calprotectin.

In the larger cohort of 172 women with endometriosis, AXIN1 had a median value of 30.00 (17.00–38.00) pg/ml and hs-CRP had a median value of 1.10 (0.60–3.20) mg/L. In this cohort, AXIN1 and hs-CRP levels were not correlated. AXIN1 levels were correlated with the duration of endometriosis ($r = 0.172$, $p = 0.047$) and with the duration of GI symptoms ($r = 0.244$, $p = 0.009$). The GI symptoms of vomiting and nausea and intestinal symptoms influence on daily life were correlated with AXIN1 levels, whereas no GI symptoms correlated with hs-CRP levels. Women who were receiving current hormonal treatments (46.5%) had higher levels of AXIN1 and hs-CRP compared with untreated patients. Women who were receiving progestin treatment had higher levels of AXIN1 ($p = 0.029$), whereas women who were receiving combined oral contraceptive treatment had higher levels of hs-CRP ($p<0.001$).

Discussion

The main findings reported in this thesis were the widespread presence of GI symptoms in women with endometriosis, with a majority of endometriosis patients reporting GI complaints. Psychiatric comorbidities was also common, and impaired psychological well-being correlated with all GI symptoms. Elevated titers of TRAb were associated with endometriosis (Paper I). Women with IBS experienced aggravated GI symptoms compared with endometriosis patients, but similar symptoms as endometriosis patients who fulfilled the Rome III criteria (Paper II). Antibodies that have been reported to be elevated in functional GI disorders were not found in increased prevalence in endometriosis (Paper III). The levels of several inflammation-related proteins were increased or decreased in women with endometriosis, and the levels of AXIN1 were increased in endometriosis compared with both women with MC, healthy controls, and controls from the general population (Paper IV). P-AXIN1 was negatively correlated with several inflammatory biomarkers, including F-calprotectin and B-platelets, and positively correlated with some clinical characteristics and GI symptoms of endometriosis (Paper V) (Figure 11).

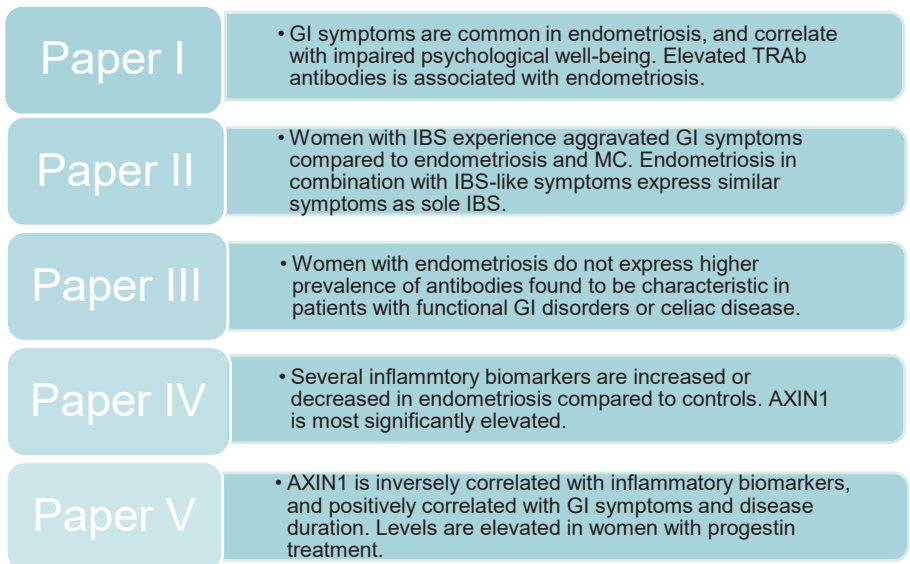

Figure 11
The main findings of this thesis

The finding of increased GI symptoms in women with endometriosis is in agreement with previous studies (4, 94, 95). Bloating was the most common symptom, followed by abdominal pain. Bowel endometriosis may result in GI symptoms, but GI symptoms have been shown to be present irrespective of bowel involvement (4), and in the present cohort, bowel endometriosis was not associated with aggravated GI symptoms. Menstruation-exacerbated symptoms have been described in several studies (24, 94, 95); however, in the present thesis, only a minority of patients reported menstruation to be a trigger for GI symptoms. We did not collect data regarding the menstrual cycle phase for all patients; therefore, the relationship between GI symptoms and the menstrual cycle could not be examined in this thesis. However, almost half of the patients were currently receiving hormone treatment, which impact the menstrual cycle. An increase in GI symptoms during menstruation has not only been described for women with endometriosis but has also been described for women with functional bowel diseases and for healthy women (22-25, 134). Women treated with GnRH analogs experienced aggravated abdominal pain but did not show an increased prevalence of GnRH antibodies. The patients treated with opioids experienced significantly aggravated GI symptoms, with the exception of diarrhea. These patients may have already experienced severe symptoms, resulting in opioid treatments. However, treatment with opioids is well-known to induce GI symptoms through effects on motility, sphincter function, and secretion. Opioid-induced bowel dysfunction manifests with the symptoms of constipation, abdominal cramping, and bloating (133, 162). Thus, many endometriosis patients may not benefit from opioid treatments, and the initiation of opioid treatments in endometriosis patients must be carefully evaluated.

Endometriosis is widely considered to be an inflammatory condition, and both antibodies, and several inflammatory cytokines are elevated in the peripheral blood and peritoneal fluid in endometriosis patients (11, 53, 54, 56). Immune dysfunction has been hypothesized to be involved in the pathogenesis of the disease, and in the present thesis, several inflammation-related proteins were found to be increased in patients with endometriosis compared with the levels in women with organic bowel disease and healthy controls; however, several inflammation-related proteins were also found to be decreased in endometriosis patients. Of proteins previously reported elevated, MCP-1 was decreased compared to controls. However, a systematic review of MCP-1 reported that 50% of studies detected increased MCP-1 levels in the peripheral blood of endometriosis patients and 50% found no statistically significant differences (12). Decreased MCP-1 may be a compensatory mechanism. Both CXCL 9 and 10 were decreased in endometriosis patients, which is in agreement with previous findings (163, 164). MMP-9 has been reported to be elevated in the endometrium, plasma and peritoneal fluid in endometriosis (68, 69) and MMP-9 cleaves CXCL 9 at three different sites and degrades CXCL 10 (165), which may result in the decreased levels. Elevated IL-8 levels have been reported

in both peripheral blood and peritoneal fluid in endometriosis patients (12, 166); however, our study was unable to analyze IL-8 levels due to technical difficulties. VEGF, IL-6, and IL-10 were not elevated in endometriosis patients compared with controls in the present thesis. However, the control groups were small in size, which is a limitation for detecting differences.

AXIN1, which is involved in and considered to be a negative regulator of the Wnt pathway (82), was the most interesting protein identified during these analyses and was elevated compared to both MC patients, healthy controls, and controls from the general population. The Wnt pathway is involved in biological processes, including cellular proliferation and tissue regeneration (71, 72). Wnt stabilizes cytoplasmic β-catenin, which stimulates the expression of genes, including c-myc, c-jun, and cyclin D1 (82). The dysregulation of the Wnt pathway has been implicated in the pathogenesis of various autoimmune diseases (72), as well as ovarian cancer (167), intestinal inflammation (73), and endometriosis (75, 168). However, the extent that Wnt signaling contributes to inflammatory injury repair remains unclear, as it is interconnected with several other signaling cascades (73). Aberrant activation of the Wnt pathway causing increased cell migration and invasion has been implicated in the pathogenesis of endometriosis (75, 168).

There are two isoforms of AXIN, AXIN1 and AXIN2, which are considered functionally equivalent (169). AXIN plays the role of a scaffolding protein by building a destruction complex through interactions with APC, GSK3, CK1α, and β-catenin, which enables the down-regulation of β-catenin via phosphorylation (71, 73, 82). β-catenin expression has been shown to be increased in endometriosis lesions (168), and the increased levels of AXIN1 may, therefore, reflect a response to the increased β-catenin levels in endometriosis. Elevated levels of F-calprotectin and B-platelets and decreased levels of hemoglobin, may be due to an inflammatory response (170-172). The findings of negative correlations between AXIN1 and inflammatory biomarkers could indicate that the downregulation of the Wnt pathway by AXIN1 decreases inflammation and could represent a response to the inflammatory state associated with endometriosis. However, AXIN1 was not correlated with hs-CRP, and AXIN1 levels were increased in patients treated with progestin. Whether the increase in AXIN1 levels and the downregulation of the Wnt pathway represents an initial step in the development of endometriosis or whether elevated AXIN1 represents a compensatory mechanism in response to increased β-catenin levels remains unclear. To our knowledge, AXIN1 has not previously been analyzed in peripheral blood samples from endometriosis patients; therefore, this result represents a novel finding that must be further validated. Further studies are warranted to investigate the connection between AXIN1 and endometriosis and the potential influences of hormonal treatments on plasma AXIN1 levels. AXIN1 has the potential to be an endometriosis biomarker and may also provide increased insight into the pathophysiology of endometriosis.

ST1A1, which catalyzes sulfur conjugation of substances such as hormones and bile acids (173), but also different xenobiotics (174), were also found in increased levels in sera of the endometriosis patients. ST1A1 plays a role in estrogen-metabolism (173), and its activity can be affected by inflammation (175). Sulfotransferase 1E1 mRNA has been reported in increased levels in deep infiltrating endometriosis and in decreased levels in ovarian endometriosis, while other studies have not found any significant differences in expression (176). OSM was the only protein that tended to correlate with several GI symptoms in endometriosis, but serum levels were not elevated. OSM is produced by activated T-cells and monocytes (177), and is expressed in high levels in inflamed intestinal tissue in IBDs and correlated with disease severity (178, 179). The role of ST1A1 and OSM in endometriosis must be further evaluated.

F-calprotectin is a neutrophil protein that is released during inflammation and can be measured in fecal samples and used to differentiate between inflammatory intestinal disorders and functional bowel diseases, clinically (180). Studies have suggested that the probability of IBD in patients with F-calprotectin ≤ 40 mg/kg is ≤ 1% (181). Among the patients in this study, 28% displayed measurable levels of F-calprotectin (values >25 mg/kg), and 17% women had values ≥ 50 mg/kg. A case study reported elevated F-calprotectin (over 400 mg/kg) in a patient with endometriosis on the appendix, causing intussusception (182). Endometriosis bowel lesions may cause elevated F-calprotectin levels; however, only one patient with elevated F-calprotectin levels had confirmed bowel lesions. Patients with measurable F-calprotectin levels experienced more severe GI symptoms compared with those with unmeasurable levels of F-calprotectin. We could not find any other studies, aside from the case study describing endometriosis on the appendix, that investigated the levels of calprotectin in fecal or in blood samples from endometriosis patients. Hypothetically, low-grade inflammation in the bowel may underlie the GI symptoms in a subgroup of endometriosis patients. The findings of elevated levels of F-calprotectin and inflammation-related proteins in some patients further strengthens the view that endometriosis represents an inflammatory condition.

Psychiatric symptoms, such as depression, anxiety, and impaired quality of life, has been reported to be overrepresented among endometriosis patients (7, 33), which is in alignment with the findings of this thesis. Furthermore, the prevalence of depression has been shown to be more common among endometriosis patients with chronic pelvic pain compared with patients without pain symptoms (183). Thus, pelvic pain in endometriosis is associated with psychological distress. In the present thesis, impaired psychological well-being was positively correlated with all GI symptoms in endometriosis patients, which demonstrates a connection between psychological well-being and all bowel symptoms, not only pelvic pain. Furthermore, pharmacological treatments for endometriosis including GnRH

analogs, have been associated with psychiatric symptoms, and GI pathology (16, 184, 185). However, whether psychiatric comorbidity represents a consequence of the symptoms associated with endometriosis, a factor that negatively impacts the severity of symptoms or both, remains unclear. GI symptoms may improve following the treatment of anxiety and depression; however, psychological symptoms may also improve if painful endometriosis symptoms and bowel symptoms are treated. IBS is considered to be a brain-gut axis disorder (110), and similar mechanisms may be present in endometriosis. The treatment of endometriosis should involve a multidisciplinary approach, taking into consideration both pain symptoms and psychiatric symptoms, as they seem to be interconnected. One positive finding from this study was that, although impaired psychological well-being and the use of antidepressants were associated with endometriosis, a majority of patients were still able to attain a high education level and a majority of patients were capable of working or studying. This finding indicates that some patients may have found coping strategies for managing endometriosis-related symptoms.

Age appears to be a factor in how patients perceive symptoms, and older endometriosis patients have been shown to experience fewer anxiety symptoms than younger patients (186). A study by Lövkvist et al. (187) concluded that Swedish women with endometriosis experienced a significantly lower quality of life compared with the general female population. Furthermore, younger endometriosis patients experienced more symptoms and reported a lower quality of life compared with older endometriosis patients. In the present study, increasing age correlated with reduced GI symptoms and better psychological well-being. The correlation between age, psychological well-being, and GI symptoms may have several explanations. Older patients may have had more time to psychologically cope with their disease and the associated symptoms or they may have had the time to try several different pharmacological and surgical treatment options. Women who were diagnosed with endometriosis early in life may have more severe symptoms than women diagnosed later in life, resulting in these women seeking medical attention and receiving diagnoses earlier. In addition, older women may have altered female sex hormones which may affect symptoms.

Several similarities exist between IBS and endometriosis. Both diseases have a high prevalence in the general population, and IBS is predominantly diagnosed in women compared with men (2, 84, 107). Pathophysiological mechanisms discussed in IBS including altered gut microbiota, chronic low grade inflammation, increased intestinal permeability, increase in mast cells and visceral hypersensitivity, are also discussed in relation to endometriosis (5, 9, 59, 111, 122, 123, 188). GI symptoms differed between the IBS and endometriosis patients, with more severe GI symptoms being reported by IBS patients. However, the women with endometriosis who fulfilled the Rome III criteria reported similar symptoms as women with sole

IBS. Since the prevalence of IBS is approximately 10 % in the general population, it is likely that some cases of IBS exist within the endometriosis cohort. Based on these findings, we cannot determine whether the high prevalence of IBS-like symptoms in endometriosis patients is due to the coexistence between endometriosis and IBS or due to endometriosis-related GI symptoms. However, the high prevalence of bowel complaints in endometriosis patients indicates that other mechanisms likely contribute to the occurrence of GI symptoms. Symptoms related to endometriosis may be misdiagnosed as IBS, in some cases. IBS is a heterogeneous disorder, and evidence suggests that several different underlying mechanisms may be of importance in the pathophysiology of the disease (123, 124, 126). This proposes challenges in clinical trials, especially because the diagnostic criteria for IBS have changed over time. The Rome-IV-positive IBS patients constitute a subgroup of the Rome-III-positive IBS patients, with more severe GI symptoms and a lower quality of life (189). Wide variability among the symptoms experienced by patients and large placebo effects also exist, and clear pathophysiological targets or endpoints are lacking when assessing treatments (190). These factors result in difficulties comparing studies and drawing valid conclusions from such comparisons. Therefore, the IBS concept must be refined, instead of representing a catchall term for GI symptoms without obvious organic bowel pathologies. The Rome criteria may be too vague to differentiate between endometriosis and IBS, and further diagnostic tools that are capable of differentiating the symptoms of these and similar diseases are necessary. The management strategies for IBS and endometriosis are different; therefore, identifying methods capable of differentiating between these two diseases is paramount.

The increased prevalence of thyroid disease in endometriosis patients has been described (55, 57, 191, 192). In this thesis, hypothyroidism had a prevalence of 11% among the endometriosis patients, which was not significantly elevated compared with controls. However, the serum levels of thyroid hormones were not analyzed, and cases of thyroid disease may remain undiagnosed in patients and controls. In view of this, thyroid antibodies were analyzed in this study, and TRAb antibodies were found to be significantly elevated in endometriosis patients compared with controls, which is a novel finding that strengthens the connection between thyroid disease and endometriosis. Studies have demonstrated that endometrial cells express TSH receptor mRNA (193, 194). In addition, endometrial Ishikawa cells represent a site for extra thyroidal hormone production, where secretion of thyroid hormones occurs in response to TSH (193). Thus, endometriosis implants may also express TSH receptor mRNA, inducing TRAb formation. Endometriosis shares several similar features with autoimmune diseases, including increased antibody formation, which is supported by these findings (55).

The role of GnRH has been implicated in the ENS and GI pathophysiology (16, 19, 21, 195). IgM antibodies against GnRH1 have been described in IBS patients, but not in patients with CD or IBD (16, 101). Enteric neuropathy may represent a potential mechanism underlying GI symptoms in at least a subgroup of IBS patients, but not in patients with organic bowel diseases. Abdominal pain was aggravated in patients receiving current GnRH treatment. In line with previous studies, GnRH1 IgM antibodies were significantly elevated in IBS/ED patients compared with both controls and endometriosis patients. A low prevalence of GnRH1 antibodies was observed in endometriosis patients, implying that enteric neuropathy with secondary antibody formation against GnRH is not involved in bowel dysfunction in endometriosis. Other effects of GnRH on sensation and motility may be of importance, however, because GnRH treatment was associated with aggravated GI symptoms. However, these aggravated symptoms may be the result of the most severely affected patients being treated with these drugs. Interestingly, IgM antibodies against TN-C were elevated in both endometriosis and IBS patients; however, this elevation was non-significant in endometriosis patients ($p = 0.06$). TN-C is a protein that is upregulated during inflammation and in ulcerative colitis, where it is also associated with disease severity (61, 64, 67). Tenascin is expressed in endometrial stromal cells but has the highest expression levels in endometriosis implants (62, 63). The protein has therefore been hypothesized to play a role in the development of endometriosis (62, 63). Although TN-C antibodies tended to be elevated in endometriosis, they were not associated with GI symptoms or disease characteristics. Since TN-C antibodies were not related to disease characteristics, they may solely reflect the increased levels of TN-C observed in endometriosis. In addition, the strong expression of TN-C had been described in other inflammatory conditions, such as rheumatoid arthritis (64), suggesting that it might be an expression of inflammation. Because the prevalence of antibodies in endometriosis patients was low, they do not represent clinically useful biomarkers and they do not appear to be related to GI symptoms. TN-C would be interesting to further investigate in relation to IBS and ED. MMP-9 cleaves TN-C and has been shown to be elevated in ectopic endometrium, the plasma, and the peritoneal fluid in endometriosis and has been associated with disease severity (68, 69). MMP-9 IgG had a higher prevalence in endometriosis patients; however, this increase was also non-significant.

In summary, in this thesis we showed that women with endometriosis experience GI symptoms at high frequencies, and treatments with opioids and GnRH analogs are associated with these symptoms. Psychiatric comorbidity is common in endometriosis, and impaired psychological well-being is correlated with aggravated GI symptoms. TRAb antibodies are elevated in endometriosis; however, none of the antibodies that have been shown to be present in patients with functional bowel diseases are elevated in endometriosis patients. Finally, serum inflammation protein

profiles differ in endometriosis patients compared with controls, and the protein AXIN1 represents the most promising biomarker for further examination.

Strengths and limitations

One strength of this study is that it encompasses approaches from both internal medicine and gynecology to study a gynecological condition. This approach facilitates the inclusion of different views and novel ideas for the investigation of GI symptoms.

The papers included in this thesis have several limitations. The study design is cross-sectional, which prevents causality from being inferred. The data were obtained from patients who had already received endometriosis diagnoses, and a majority were currently being treated pharmacologically. A proportion of the patients had also already undergone surgical procedures for the management of endometriosis. Obtaining native data from patients prior to treatment would have been preferable. With native samples, antibody analyses and inflammatory biomarkers could have been interpreted without the interference of pharmacological treatments. In addition, patients were included from a secondary center, and the possibility exists that our study includes a selection of endometriosis patients with the most severe symptoms. Patients with less severe symptoms can be managed at primary care facilities or by private gynecologists, and these patients may not receive endometriosis diagnoses confirmed by surgery. However, for all of the papers in this thesis, except Paper II, the inclusion criteria included a surgically confirmed diagnosis of endometriosis, to ensure that the diagnosis was definite.

Information regarding patient eligibility for this study was obtained from the Department of Gynecology. Some patients with severe comorbidities and pregnancy were never referred for potential inclusion in this study. This exclusion was unfortunately not documented, and we do not have exact data regarding those patients that were excluded due to severe mental or somatic comorbidities or pregnancy.

A relatively large proportion of patients asked to participate in the study declined. The ethical review board does not allow patients that decline to be contacted; therefore, we do not know the reasons why they opted not to participate. Therefore, selection bias is a potential risk that may affect the results of the present thesis.

Patients and controls were not age-matched in any of the papers included in this thesis. However, we have adjusted for age in the statistical analyses. The use of age-matched controls would have improved the validity of the present thesis.

The menstrual cycle is known to impact GI symptoms, with reports of aggravated symptoms during menstruation. We did not obtain data regarding the menstrual cycle phases of all patients; therefore, we could not adjust for the menstrual cycle phase during statistical calculations. However, the menstrual cycle phases are likely to be evenly distributed among both patients and controls. Furthermore, almost half of the patients were currently using hormonal treatments, which can affect the menstrual cycle.

Information regarding diagnostic procedures conducted to assess endometriosis, pharmacological treatments, and comorbid conditions was obtained from the clinical data survey. Patients are not always reliable reporters for medical data; therefore, a review of the patients' medical journals was also performed. However, we only had access to the Skåne University Hospital medical records, and not medical records from all primary care facilities, private caregivers, or hospitals outside Skåne that are not in the same system. As a result, we may have underestimated the pharmacological treatments and comorbid conditions for these patients. A relatively small proportion of patients were currently using systemic hormonal treatment, although this number may have been underestimated as some patients may have been prescribed systemic hormonal treatments or hormonal IUDs by private practice gynecologists.

Data regarding endometriosis localization was obtained from medical records; however, some localizations may not have been described. Endometriosis can be divided into stages and correlating clinical data, GI symptoms, and biochemical findings with endometriosis stage would have been interesting. However, we did not have data regarding the disease stage because this data was not described in the medical journals.

To characterize IBS and IBS-like symptoms, the Rome III criteria were used because these criteria were being used by clinical practice at the time the study was initiated. Rome III was used as the diagnostic criteria for IBS between 2006 and 2016. The Rome IV criteria were published in 2016. A review of patients who fulfilled the Rome III criteria in Sweden found that 85% also fulfilled the Rome IV criteria (196). Consequently, patients who fulfill both the Rome III and Rome IV criteria constitute a more severe group than patients who only fulfill the Rome III criteria. Therefore, some IBS patients who were used as controls in this study may not fulfill the new IBS criteria.

Several questionnaires can be used to estimate GI symptoms and psychological symptoms. The Gastrointestinal Symptom Rating Scale (GSRS), the IBS severity scoring system (IBS-SSS), and the Psychological General Well-Being Index (PGWB) are commonly used questionnaires. In the present thesis, the VAS-IBS was used. The main advantage of the VAS-IBS is that it represents a continuous scale, in contrast with the GSRS and PGWB, and the VAS-IBS has been validated against

both the GSRS and PGWB (150, 151). IBS-SSS does not measure separate bowel symptoms, making the VAS-IBS is to preferable in this setting.

Future perspectives

To further study GI symptomatology associated with endometriosis, obtaining native blood samples and assessments of bowel symptoms will be necessary before pharmacological and surgical treatments for endometriosis are initiated, which would facilitate analyses free from the interference of these treatments. Understanding the relationship between GI symptoms and the menstrual cycle would also be of value, since female sex hormones are known to affect bowel symptoms. Longitudinal studies would provide us with further knowledge regarding whether treatments for endometriosis also can alleviate GI symptoms, and patients should be examined both before and after the initiation of these treatments.

Further explorations regarding the connections among impaired psychological well-being, psychiatric comorbidities, and GI symptoms would be valuable, to determine the relationships between mental health and bowel symptoms. Other treatment options, such as psychotherapy and the development of coping skills, might be valuable for this patient category.

The present thesis identified the inflammation-related protein AXIN1 to be elevated in endometriosis patients and to be negatively correlated with other inflammatory biomarkers, which are both novel findings. The Wnt pathway, in which AXIN1 is a key-player, has been connected with endometriosis (75, 168). The relationship between AXIN1 and endometriosis should be validated in future studies. Additional knowledge regarding the connection between AXIN1 and endometriosis could contribute to improved understanding of the pathophysiological mechanisms underlying the disease.

The gut microbiota has been linked to both IBS and endometriosis (59, 60, 188). It has been hypothesized to be involved in the onset and progression of endometriosis (59); however, few human studies have been conducted examining the gut microbiome in endometriosis patients (60). Further explorations of the connections between gut microbiota and GI symptoms in endometriosis patients would be valuable.

In conclusion, verifying the findings of the present study in other patient cohorts and using other study designs represent the most important next steps.

Conclusions

A high prevalence of GI symptoms was observed in the endometriosis patients. The most impairing symptom was bloating and flatulence, followed by abdominal pain and sensation of incomplete evacuation. Increasing age correlated with reduced GI symptoms. Patients currently treated with opioids or GnRH analogs experienced aggravated bowel symptoms.

Endometriosis was positively associated with IBS, the current use of antidepressants, and impaired psychological well-being, whereas alcohol intake, physical activity, BMI, and asthma were negatively associated. Impaired psychological well-being correlated significantly with all GI symptoms. Elevated levels of TRAb antibodies were associated with endometriosis; however, they were not correlated with clinical characteristics or GI symptoms.

Of the patients with endometriosis, 35% fulfilled the Rome III criteria. Women with IBS generally experienced aggravated GI symptoms and impaired psychological well-being compared with women with endometriosis and MC. However, women with endometriosis who also fulfilled Rome the III criteria reported bowel symptoms similar to those reported by women with IBS.

Women with endometriosis did not express a higher prevalence of autoantibodies found to be characteristic in other patient groups with GI symptoms, compared to controls.

AXIN1 and ST1A1 were the most significantly increased inflammation-related proteins in serum, and CXCL9 was the most significantly decreased serum protein in women with endometriosis compared to women with MC and healthy controls. The ROC curve for AXIN1 had a large AUC. There were few correlations between inflammation-related proteins in serum and GI symptoms in endometriosis.

Plasma levels of AXIN1 were negatively correlated with inflammatory biomarkers routinely used in clinical practice, except for hs-CRP. AXIN1 was also correlated with the duration of endometriosis and GI symptoms, and levels were elevated in patients currently treated with progestin.

Overall, GI symptoms are very common in endometriosis patients, and inflammatory factors are of importance for the disease. However, we were unable to determine the genesis underlying GI symptoms in endometriosis patients in this

thesis. We were unable to determine whether comorbidity between endometriosis and IBS exists or whether the available questionnaires and the Rome criteria are too insensitive to distinguish between the two conditions.

The most important finding, which has direct clinical implications, is that endometriosis must be considered in women with GI symptoms and IBS. Furthermore, the analgesic effects of opioids must be evaluated before the treatment is continued. Psychological well-being is strongly correlated with GI symptoms in endometriosis patients.

Populärvetenskaplig sammanfattning

Sjukdomen endometrios är vanlig bland kvinnor i fertil ålder och man uppskattar att mellan 6-10% är drabbade. Endometrios definieras av förekomst av livmoderslemhinna utanför livmodern, vanligen i form av cystor på äggstockarna eller sjukdomshärdar i lilla bäckenet. Vanliga symptom är bäckensmärtor, menstruationssmärtor och samlagssmärtor, men även besvär från magtarmsystemet och urinvägarna. Ungefär en femtedel har inga symptom, och hos en del av dessa kvinnor upptäcks sjukdomen vid fertilitetsutredningar. Då symptomen kan variera, och en definitiv diagnos ställs genom operation, kan det dröja lång tid från det att symptomen börjar tills att man får en diagnos, inte sällan flertalet år.

Magtarmbesvär, såsom buksmärtor, uppspändhet, gaser, illamående, förstoppning och diarré är vanligt förekommande hos kvinnor med endometrios. Det är i nuläget oklart om det är sjukdomen i sig som orsakar magtarmbesvären, eller om symptomen också kan bero på att kvinnor med endometrios oftare drabbas av den funktionella tarmsjukdomen irritable bowel syndrome (IBS). Endometrios kan behandlas med en syntetisk variant av hormonet gonadotropinfrisättande hormon (GnRH), ett hormon som reglerar bildande av kvinnliga könshormoner. Behandling med syntetiskt GnRH har i vissa fall kopplats till magtarmbesvär och produktion av antikroppar mot hormonet. Psykisk ohälsa är associerat med magtarmbesvär och vid endometrios har man sett en ökad frekvens av depression och ångest. Det finns idag ingen markör i blodet för sjukdomen, något som skulle kunna underlätta en tidigare diagnosticering av endometrios.

Denna avhandling syftar till att karakterisera magtarmbesvär vid endometrios och undersöka eventuella kopplingar till samsjuklighet, inflammatorisk aktivitet och antikroppar i blodet. 188 kvinnor med endometrios, varav 172 diagnosticerade kirurgiskt, rekryterades till studien och fick besvara frågeformulär samt lämna avförings- och blodprover. Resultaten jämfördes med friska kvinnliga kontroller, kvinnliga kontroller från den allmänna befolkningen samt även kvinnliga patienter med magtarmsjukdomarna IBS, mikroskopisk kolit och enterisk dysmotilitet.

I delarbete 1 karakteriseras magtarmbesvär, livsstilsfaktorer och samsjuklighet vid endometrios. Majoriteten av patienterna hade haft magtarmbesvär de senaste 2 veckorna. Gaser och uppspändhet var de mest uttalade symptomen följt av buksmärta. Patienter som behandlades med syntetiskt GnRH eller morfinlikande preparat hade förvärrade magtarmbesvär. Nästan en tredjedel hade blivit

diagnosticerade med ångest eller depression, och användning av antidepressiva läkemedel och förvärrat psykologiskt mående var associerat med endometrios. Förvärrat psykologiskt mående var kopplat till förvärrade magtarmbesvär, samtidigt som ökande ålder var kopplat till minskade besvär. Sköldkörtelsjukdomar har kopplats till endometrios, varför antikroppar associerade dessa sjukdomar undersöktes. TRAK, en antikropp man kan finna vid överfunktion i sköldkörteln, återfanns i ökade halter i blodet vid endometrios jämfört med kontroller.

Studier har visat att IBS är vanligare vid endometrios än hos den generella befolkningen, därför jämfördes magtarmbesvär vid IBS och endometrios i delarbete 2. Ungefär en tredjedel (35 %) av endometriospatienterna uppfyllde diagnoskriterierna för IBS. När magtarmbesvär jämfördes mellan IBS patienter och samtliga endometriospatienter visade det sig att kvinnor med IBS generellt hade förvärrade magtarmbesvär. Valde man dock ut kvinnorna med endometrios som också uppfyllde diagnoskriterier för IBS, så var det i princip inga skillnader mellan grupperna avseende magtarmbesvär.

GnRH hormonet är kopplat till magtarmbesvär. Endometrios behandlas med syntetiskt GnRH, vilket potentiellt skulle kunna bidra till magtarmsymptom. Därför undersöktes förekomsten av antikroppar mot GnRH och dess receptor i delarbete 3. Hormonet LH är kopplat till GnRH, varför detta också undersöktes. Vidare undersöktes även förekomst av antikroppar mot Tenascin C (TN-C), MMP-9 och antikroppar som återfinns vid celiaki, då dessa också är kopplade till magtarmbesvär. Det fanns ingen ökad förekomst av dessa antikroppar jämfört med friska kontroller vid endometrios. Dock fanns det ökad förekomst av antikroppar mot GnRH och GnRHs receptor, samt TN-C vid IBS och enterisk dysmotilitet, vilket är tidigare känt.

Då endometrios ses som en inflammatorisk sjukdom analyserades flertalet inflammatoriska proteiner i blodet i delarbete 4 för att undersöka halten av dessa samt om det fanns kopplingar till magtarmbesvär. Det protein som var mest förhöjt jämfört med friska kontroller och kvinnor med tarmsjukdomen mikroskopisk kolit var proteinet AXIN1. Flera andra proteiner återfanns också i förhöjda, men även sänkta halter jämfört med kontroller. Proteinet Oncostatin M var i viss grad kopplat till magtarmbesvär.

För att vidare undersöka betydelsen av proteinet AXIN1 vid endometrios, mättes flera andra inflammationsmarkörer i blodet och avföringen vid endometrios i delarbete 5. Det fanns ett omvänt samband mellan nivåer av AXIN1 och nivåer av feces kalprotektin (en markör för inflammation i tarmen), blodplättar, blodvärde och röda blodkroppar. Nivåer av AXIN1 var också kopplat till durationen av endometrios och magtarmsymptom, samt magtarmsymptomen illamående och kräkningar. Högkänsligt CRP, en markör för inflammation i blodet, var inte förhöjt vid endometrios, och inte heller kopplat till nivåer av AXIN1.

Sammanfattningsvis återfanns utbredda magtarmbesvär vid endometrios, där uppspändhet och gaser var de vanligaste symptomen. Psykiatrisk samsjuklighet och försämrat psykiskt mående var överrepresenterat vid endometrios, och kopplat till ökade magtarmbesvär. Antikroppar som man funnit vid magtarmsjukdomar såsom IBS var inte förhöjda vid endometrios, men ökad förekomst av TRAK antikroppar var kopplat till sjukdomen. Halten av det inflammatoriska proteinet AXIN1 var ökat i blodet vid endometrios och hade ett omvänt samband med andra inflammatoriska markörer.

Denna studie visade en stark koppling mellan psykisk hälsa och magtarmbesvär. Vi kan dock inte säga något om orsakssambandet; är det symptomen vid endometrios som leder till psykisk ohälsa, eller förvärrar den psykiska ohälsan symptomen? Det är dock viktigt att inte bara fokusera på att behandla smärtsamma symptom vid endometrios, utan även fokusera på den psykiska hälsan. En relativt stor andel av patienterna fick morfinliknande preparat, och dessa hade förvärrade magtarmsymptom. Det är därför viktigt att noggrant utvärdera all insättning av morfinliknande preparat och avsluta behandlingen om de inte har någon effekt eller till och med förvärrar besvären.

Det finns flera likheter mellan endometrios och IBS. Man vet dock inte om den ökade förekomsten av IBS vid endometrios beror på ett äkta samband mellan sjukdomarna, eller om symptomen vid endometrios misstolkas som IBS. Denna studie visar att man inte kan skilja på tillstånden genom att använda IBS kriterierna. En andel av IBS patienterna har antikroppar mot GnRH, men detta återfinns inte vid endometrios. Vi fann en ökning av proteinet AXIN1 i blodet hos endometriospatienter, vilket är ett nytt fynd. Det behövs ytterligare forskning för att klargöra sambandet mellan endometrios och magtarmbesvär, men även för att fastställa om AXIN1 är relevant vid endometrios.

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References

1. Altman G, Cain KC, Motzer S, Jarrett M, Burr R, Heitkemper M. Increased symptoms in female IBS patients with dysmenorrhea and PMS. Gastroenterology Nursing : the Official Journal of the Society of Gastroenterology Nurses and Associates. 2006;29(1):4-11.
2. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clinical Epidemiology. 2014;6:71-80.
3. Ju H, Jones M, Mishra G. The prevalence and risk factors of dysmenorrhea. Epidemiologic Reviews. 2014;36:104-13.
4. Maroun P, Cooper MJ, Reid GD, Keirse MJ. Relevance of gastrointestinal symptoms in endometriosis. The Australian & New Zealand Journal of Obstetrics & Gynaecology. 2009;49(4):411-4.
5. Issa B, Onon TS, Agrawal A, Shekhar C, Morris J, Hamdy S, et al. Visceral hypersensitivity in endometriosis: a new target for treatment? Gut. 2012;61(3):367-72.
6. Roman H, Ness J, Suciu N, Bridoux V, Gourcerol G, Leroi AM, et al. Are digestive symptoms in women presenting with pelvic endometriosis specific to lesion localizations? A preliminary prospective study. Human Reproduction (Oxford, England). 2012;27(12):3440-9.
7. Pope CJ, Sharma V, Sharma S, Mazmanian D. A Systematic Review of the Association Between Psychiatric Disturbances and Endometriosis. Journal of Obstetrics and Gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2015;37(11):1006-15.
8. Seaman HE, Ballard KD, Wright JT, de Vries CS. Endometriosis and its coexistence with irritable bowel syndrome and pelvic inflammatory disease: findings from a national case-control study--Part 2. BJOG : an International Journal of Obstetrics and Gynaecology. 2008;115(11):1392-6.
9. Vigano D, Zara F, Usai P. Irritable bowel syndrome and endometriosis: New insights for old diseases. Digestive and Liver disease : Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2018;50(3):213-9.
10. Ghai V, Jan H, Shakir F, Haines P, Kent A. Diagnostic delay for superficial and deep endometriosis in the United Kingdom. Journal of Obstetrics and Gynaecology : the Journal of the Institute of Obstetrics and Gynaecology. 2019:1-7.
11. Fassbender A, Burney RO, O DF, D'Hooghe T, Giudice L. Update on Biomarkers for the Detection of Endometriosis. BioMed Research International. 2015;2015:130854.

12. Borrelli GM, Abrao MS, Mechsner S. Can chemokines be used as biomarkers for endometriosis? A systematic review. Human Reproduction (Oxford, England). 2014;29(2):253-66.
13. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. The Cochrane Database of Systematic Reviews. 2016(5):Cd012179.
14. Perrett RM, McArdle CA. Molecular mechanisms of gonadotropin-releasing hormone signaling: integrating cyclic nucleotides into the network. Frontiers in Endocrinology. 2013;4:180.
15. Naor Z. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. Frontiers in Neuroendocrinology. 2009;30(1):10-29.
16. Ohlsson B. Gonadotropin-Releasing Hormone and Its Physiological and Pathophysiological Roles in Relation to the Structure and Function of the Gastrointestinal Tract. European Surgical Research Europaische Chirurgische Forschung Recherches Chirurgicales Europeennes. 2016;57(1-2):22-33.
17. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science (New York, NY). 1978;202(4368):631-3.
18. Giudice LC, Kao LC. Endometriosis. Lancet (London, England). 2004;364(9447):1789-99.
19. Hammar O, Ohlsson B, Veress B, Alm R, Fredrikson GN, Montgomery A. Depletion of enteric gonadotropin-releasing hormone is found in a few patients suffering from severe gastrointestinal dysmotility. Scandinavian Journal of Gastroenterology. 2012;47(10):1165-73.
20. Hammar O, Veress B, Montgomery A, Ohlsson B. Expression of Luteinizing Hormone Receptor in the Gastrointestinal Tract in Patients with and without Dysmotility. Drug Target Insights. 2012;6:13-8.
21. Ohlsson B, Veress B, Janciauskiene S, Montgomery A, Haglund M, Wallmark A. Chronic intestinal pseudo-obstruction due to buserelin-induced formation of anti-GnRH antibodies. Gastroenterology. 2007;132(1):45-51.
22. Houghton LA, Lea R, Jackson N, Whorwell PJ. The menstrual cycle affects rectal sensitivity in patients with irritable bowel syndrome but not healthy volunteers. Gut. 2002;50(4):471-4.
23. Heitkemper MM, Chang L. Do fluctuations in ovarian hormones affect gastrointestinal symptoms in women with irritable bowel syndrome? Gender Medicine. 2009;6 Suppl 2:152-67.
24. Meurs-Szojda MM, Mijatovic V, Felt-Bersma RJ, Hompes PG. Irritable bowel syndrome and chronic constipation in patients with endometriosis. Colorectal Disease : the Official Journal of the Association of Coloproctology of Great Britain and Ireland. 2011;13(1):67-71.
25. Jackson NA, Houghton LA, Whorwell PJ, Currer B. Does the menstrual cycle affect anorectal physiology? Digestive Diseases and Sciences. 1994;39(12):2607-11.
26. Eliakim R, Abulafia O, Sherer DM. Estrogen, progesterone and the gastrointestinal tract. The Journal of Reproductive Medicine. 2000;45(10):781-8.

27. Braniste V, Leveque M, Buisson-Brenac C, Bueno L, Fioramonti J, Houdeau E. Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. The Journal of Physiology. 2009;587(Pt 13):3317-28.
28. Eskenazi B, Warner ML. Epidemiology of endometriosis. Obstetrics and Gynecology Clinics of North America. 1997;24(2):235-58.
29. Hickey M, Ballard K, Farquhar C. Endometriosis. BMJ (Clinical research ed). 2014;348:g1752.
30. Ballard KD, Seaman HE, de Vries CS, Wright JT. Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study--Part 1. BJOG : an International Journal of Obstetrics and Gynaecology. 2008;115(11):1382-91.
31. Kumar D. Irritable bowel syndrome, chronic pelvic inflammatory disease and endometriosis. European Journal of Gastroenterology & Hepatology. 2004;16(12):1251-2.
32. Lea R, Bancroft K, Whorwell PJ. Irritable bowel syndrome, chronic pelvic inflammatory disease and endometriosis: a comparison of symptomatology. European Journal of Gastroenterology & Hepatology. 2004;16(12):1269-72.
33. Lagana AS, Condemi I, Retto G, Muscatello MR, Bruno A, Zoccali RA, et al. Analysis of psychopathological comorbidity behind the common symptoms and signs of endometriosis. European Journal of Obstetrics, Gynecology, and Reproductive biology. 2015;194:30-3.
34. Longo LD. Classic pages in obstetrics and gynecology. Aberrant portions of the mullerian duct found in an ovary: William Wood Russell Johns Hopkins Hospital Bulletin, vol. 10, pp. 8--10, 1899. American Journal of Obstetrics and Gynecology. 1979;134(2):225-6.
35. Benagiano G, Brosens I, Lippi D. The history of endometriosis. Gynecologic and Obstetric Investigation. 2014;78(1):1-9.
36. Sampson JA. Perforating hemorrhagic (chocolate) cysts of the ovary: Their importance and especially their relation to pelvic adenomas of endometrial type ("adenomyoma" of the uterus, rectovaginal septum, sigmoid, etc.). Archives of Surgery. 1921;3(2):245-323.
37. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertility and Sterility. 2012;98(3):511-9.
38. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstetrics and Gynecology. 1984;64(2):151-4.
39. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and Immune Dysfunction in Endometriosis. BioMed Research International. 2015;2015:795976.
40. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. Fertility and Sterility. 1999;71(4):701-10.

41. Saha R, Pettersson HJ, Svedberg P, Olovsson M, Bergqvist A, Marions L, et al. Heritability of endometriosis. Fertility and Sterility. 2015;104(4):947-52.
42. Guo SW. Epigenetics of endometriosis. Molecular Human Reproduction. 2009;15(10):587-607.
43. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Annals of the New York Academy of Sciences. 2008;1127:106-15.
44. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nature Reviews Endocrinology. 2014;10(5):261-75.
45. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Molecular and Cellular Endocrinology. 2006;248(1-2):94-103.
46. Bruner-Tran KL, Rier SE, Eisenberg E, Osteen KG. The potential role of environmental toxins in the pathophysiology of endometriosis. Gynecologic and Obstetric Investigation. 1999;48 Suppl 1:45-56.
47. Porpora MG, Resta S, Fuggetta E, Storelli P, Megiorni F, Manganaro L, et al. Role of environmental organochlorinated pollutants in the development of endometriosis. Clinical and Experimental Obstetrics & Gynecology. 2013;40(4):565-7.
48. Borrelli GM, Carvalho KI, Kallas EG, Mechsner S, Baracat EC, Abrao MS. Chemokines in the pathogenesis of endometriosis and infertility. Journal of Reproductive Immunology. 2013;98(1-2):1-9.
49. Wu MY, Ho HN. The role of cytokines in endometriosis. American Journal of Reproductive Immunology (New York, NY : 1989). 2003;49(5):285-96.
50. Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. Fertility and Sterility. 1991;56(1):45-51.
51. Koninckx PR, Kennedy SH, Barlow DH. Endometriotic disease: the role of peritoneal fluid. Human Reproduction Update. 1998;4(5):741-51.
52. Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Frontiers in Immunology. 2013;4:9.
53. Mosbah A, Nabiel Y, Khashaba E. Interleukin-6, intracellular adhesion molecule-1, and glycodelin A levels in serum and peritoneal fluid as biomarkers for endometriosis. International Journal of Gynaecology and Obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2016;134(3):247-51.
54. Tuten A, Kucur M, Imamoglu M, Oncul M, Acikgoz AS, Sofiyeva N, et al. Serum YKL-40 levels are altered in endometriosis. Gynecological endocrinology : the Official Journal of the International Society of Gynecological Endocrinology. 2014;30(5):381-4.
55. Eisenberg VH, Zolti M, Soriano D. Is there an association between autoimmunity and endometriosis? Autoimmunity Reviews. 2012;11(11):806-14.
56. Nothnick WB. Treating endometriosis as an autoimmune disease. Fertility and Sterility. 2001;76(2):223-31.

57. Sinaii N, Cleary SD, Ballweg ML, Nieman LK, Stratton P. High rates of autoimmune and endocrine disorders, fibromyalgia, chronic fatigue syndrome and atopic diseases among women with endometriosis: a survey analysis. Human Reproduction (Oxford, England). 2002;17(10):2715-24.
58. Nielsen NM, Jorgensen KT, Pedersen BV, Rostgaard K, Frisch M. The co-occurrence of endometriosis with multiple sclerosis, systemic lupus erythematosus and Sjogren syndrome. Human Reproduction (Oxford, England). 2011;26(6):1555-9.
59. Laschke MW, Menger MD. The gut microbiota: a puppet master in the pathogenesis of endometriosis? American Journal of Obstetrics and Gynecology. 2016;215(1):68.e1-4.
60. Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. BJOG : an International Journal of Obstetrics and Gynaecology. 2019.
61. Chiquet-Ehrismann R, Chiquet M. Tenascins: regulation and putative functions during pathological stress. The Journal of Pathology. 2003;200(4):488-99.
62. Harrington DJ, Lessey BA, Rai V, Bergqvist A, Kennedy S, Manek S, et al. Tenascin is differentially expressed in endometrium and endometriosis. The Journal of Pathology. 1999;187(2):242-8.
63. Tan O, Ornek T, Seval Y, Sati L, Arici A. Tenascin is highly expressed in endometriosis and its expression is upregulated by estrogen. Fertility and Sterility. 2008;89(5):1082-9.
64. Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. Journal of Cell Science. 2016;129(23):4321-7.
65. Eyster KM, Hansen KA, Winterton E, Klinkova O, Drappeau D, Mark-Kappeler CJ. Reciprocal communication between endometrial stromal cells and macrophages. Reproductive Sciences. 2010;17(9):809-22.
66. Hood BL, Liu B, Alkhas A, Shoji Y, Challa R, Wang G, et al. Proteomics of the human endometrial glandular epithelium and stroma from the proliferative and secretory phases of the menstrual cycle. Biology of Reproduction. 2015;92(4):106.
67. Riedl S, Tandara A, Reinshagen M, Hinz U, Faissner A, Bodenmuller H, et al. Serum tenascin-C is an indicator of inflammatory bowel disease activity. International Journal of Colorectal Disease. 2001;16(5):285-91.
68. Chung HW, Wen Y, Chun SH, Nezhat C, Woo BH, Lake Polan M. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3 mRNA expression in ectopic and eutopic endometrium in women with endometriosis: a rationale for endometriotic invasiveness. Fertility and Sterility. 2001;75(1):152-9.
69. Liu H, Wang J, Wang H, Tang N, Li Y, Zhang Y, et al. The plasma and peritoneal fluid concentrations of matrix metalloproteinase-9 are elevated in patients with endometriosis. Annals of Clinical Biochemistry. 2016;53(Pt 5):599-605.
70. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annual Review of Cell and Developmental biology. 2004;20:781-810.
71. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Developmental Cell. 2009;17(1):9-26.

72. Shi J, Chi S, Xue J, Yang J, Li F, Liu X. Emerging Role and Therapeutic Implication of Wnt Signaling Pathways in Autoimmune Diseases. Journal of Immunology Research. 2016;2016:9392132.
73. Moparthi L, Koch S. Wnt signaling in intestinal inflammation. Differentiation; Research in Biological Diversity. 2019;108:24-32.
74. Silva-Garcia O, Valdez-Alarcon JJ, Baizabal-Aguirre VM. The Wnt/beta-catenin signaling pathway controls the inflammatory response in infections caused by pathogenic bacteria. Mediators of Inflammation. 2014;2014:310183.
75. Matsuzaki S, Botchorishvili R, Pouly JL, Canis M. Targeting the Wnt/beta-catenin pathway in endometriosis: a potentially effective approach for treatment and prevention. Molecular and Cellular Therapies. 2014;2:36.
76. Gaetje R, Holtrich U, Karn T, Cikrit E, Engels K, Rody A, et al. Characterization of WNT7A expression in human endometrium and endometriotic lesions. Fertility and Sterility. 2007;88(6):1534-40.
77. Matsuzaki S, Darcha C. Involvement of the Wnt/beta-catenin signaling pathway in the cellular and molecular mechanisms of fibrosis in endometriosis. PLoS One. 2013;8(10):e76808.
78. Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, et al. Wnt signaling through inhibition of beta-catenin degradation in an intact Axin1 complex. Cell. 2012;149(6):1245-56.
79. Canonical Wnt pathway with Wnt 2019 [Available from: https://commons.wikimedia.org/wiki/File:Canonical_Wnt_pathway_with_Wnt..jpg (accessed on October 10th 2019).
80. Xiong W, Zhang L, Yu L, Xie W, Man Y, Xiong Y, et al. Estradiol promotes cells invasion by activating beta-catenin signaling pathway in endometriosis. Reproduction (Cambridge, England). 2015;150(6):507-16.
81. Matsuzaki S, Darcha C. In vitro effects of a small-molecule antagonist of the Tcf/ss-catenin complex on endometrial and endometriotic cells of patients with endometriosis. PLoS One. 2013;8(4):e61690.
82. Kikuchi A. Roles of Axin in the Wnt signalling pathway. Cellular Signalling. 1999;11(11):777-88.
83. Shafrir AL, Farland LV, Shah DK, Harris HR, Kvaskoff M, Zondervan K, et al. Risk for and consequences of endometriosis: A critical epidemiologic review. Best Practice & Research Clinical Obstetrics & Gynaecology. 2018;51:1-15.
84. Parazzini F, Esposito G, Tozzi L, Noli S, Bianchi S. Epidemiology of endometriosis and its comorbidities. European Journal of Obstetrics, Gynecology, and Reproductive biology. 2017;209:3-7.
85. Parazzini F, Cipriani S, Bravi F, Pelucchi C, Chiaffarino F, Ricci E, et al. A metaanalysis on alcohol consumption and risk of endometriosis. American Journal of Obstetrics and Gynecology. 2013;209(2):106.e1-10.

86. Porpora MG, Scaramuzzino S, Sangiuliano C, Piacenti I, Bonanni V, Piccioni MG, et al. High prevalence of autoimmune diseases in women with endometriosis: a case-control study. Gynecological Endocrinology : the Official Journal of the International Society of Gynecological Endocrinology. 2019:1-4.
87. Stephansson O, Falconer H, Ludvigsson JF. Risk of endometriosis in 11,000 women with celiac disease. Human Reproduction (Oxford, England). 2011;26(10):2896-901.
88. Jess T, Frisch M, Jorgensen KT, Pedersen BV, Nielsen NM. Increased risk of inflammatory bowel disease in women with endometriosis: a nationwide Danish cohort study. Gut. 2012;61(9):1279-83.
89. Schink M, Konturek PC, Herbert SL, Renner SP, Burghaus S, Blum S, et al. Different nutrient intake and prevalence of gastrointestinal comorbidities in women with endometriosis. Journal of Physiology and Pharmacology : an Official Journal of the Polish Physiological Society. 2019;70(2).
90. Schomacker ML, Hansen KE, Ramlau-Hansen CH, Forman A. Is endometriosis associated with irritable bowel syndrome? A cross-sectional study. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2018;231:65-9.
91. Brinton LA, Gridley G, Persson I, Baron J, Bergqvist A. Cancer risk after a hospital discharge diagnosis of endometriosis. American Journal of Obstetrics and Gynecology. 1997;176(3):572-9.
92. Heidemann LN, Hartwell D, Heidemann CH, Jochumsen KM. The relation between endometriosis and ovarian cancer - a review. Acta Obstetricia et Gynecologica Scandinavica. 2014;93(1):20-31.
93. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. Journal of Assisted Reproduction and Genetics. 2010;27(8):441-7.
94. Luscombe GM, Markham R, Judio M, Grigoriu A, Fraser IS. Abdominal bloating: an under-recognized endometriosis symptom. Journal of Obstetrics and Gynaecology Canada : JOGC = Journal d'Obstetrique et Gynecologie du Canada : JOGC. 2009;31(12):1159-71.
95. Ballweg ML. Impact of endometriosis on women's health: comparative historical data show that the earlier the onset, the more severe the disease. Best Practice & Research Clinical Obstetrics & Gynaecology. 2004;18(2):201-18.
96. Wattiez A, Puga M, Albornoz J, Faller E. Surgical strategy in endometriosis. Best Practice & Research Clinical Obstetrics & Gynaecology. 2013;27(3):381-92.
97. Ferrero S, Camerini G, Ragni N, Remorgida V. Endometriosis and irritable bowel syndrome: co-morbidity or misdiagnosis? BJOG : an International Journal of Obstetrics and Gynaecology. 2009;116(1):129; author reply -30.
98. Lee CE, Yong PJ, Williams C, Allaire C. Factors Associated with Severity of Irritable Bowel Syndrome Symptoms in Patients with Endometriosis. Journal of Obstetrics and Gynaecology Canada : JOGC = Journal d'Obstetrique et Gynecologie du Canada : JOGC. 2018;40(2):158-64.
99. Moore JS, Gibson PR, Perry RE, Burgell RE. Endometriosis in patients with irritable bowel syndrome: Specific symptomatic and demographic profile, and response to the low FODMAP diet. The Australian & New Zealand Journal of Obstetrics & Gynaecology. 2017;57(2):201-5.

100. Hammar O, Roth B, Bengtsson M, Mandl T, Ohlsson B. Autoantibodies and gastrointestinal symptoms in infertile women in relation to in vitro fertilization. BMC Pregnancy and Childbirth. 2013;13:201.
101. Ohlsson B, Sjoberg K, Alm R, Fredrikson GN. Patients with irritable bowel syndrome and dysmotility express antibodies against gonadotropin-releasing hormone in serum. Neurogastroenterology and Motility : the Official Journal of the European Gastrointestinal Motility Society. 2011;23(11):1000-6, e459.
102. Muller-Lissner S, Bassotti G, Coffin B, Drewes AM, Breivik H, Eisenberg E, et al. Opioid-Induced Constipation and Bowel Dysfunction: A Clinical Guideline. Pain Medicine (Malden, Mass). 2017;18(10):1837-63.
103. Hirsch M, Begum MR, Paniz E, Barker C, Davis CJ, Duffy J. Diagnosis and management of endometriosis: a systematic review of international and national guidelines. BJOG : an International Journal of Obstetrics and Gynaecology. 2018;125(5):556-64.
104. Bedaiwy MA, Alfaraj S, Yong P, Casper R. New developments in the medical treatment of endometriosis. Fertility and Sterility. 2017;107(3):555-65.
105. Saridogan E, Becker CM, Feki A, Grimbizis GF, Hummelshoj L, Keckstein J, et al. Recommendations for the surgical treatment of endometriosis-part 1: ovarian endometrioma. Gynecological Surgery. 2017;14(1):27.
106. Saha L. Irritable bowel syndrome: Pathogenesis, diagnosis, treatment, and evidence-based medicine. World Journal of Gastroenterology : WJG. 2014;20(22):6759-73.
107. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clinical Gastroenterology and Hepatology : the Official Clinical Practice Journal of the American Gastroenterological Association. 2012;10(7):712-21.e4.
108. Lacy BE, Patel NK. Rome Criteria and a Diagnostic Approach to Irritable Bowel Syndrome. Journal of Clinical Medicine. 2017;6(11).
109. Drossman DA, Dumitrascu DL. Rome III: New standard for functional gastrointestinal disorders. Journal of Gastrointestinal and Liver Diseases : JGLD. 2006;15(3):237-41.
110. Ford AC, Lacy BE, Talley NJ. Irritable Bowel Syndrome. The New England Journal of Medicine. 2017;376(26):2566-78.
111. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. Jama. 2015;313(9):949-58.
112. Dai C, Jiang M. The incidence and risk factors of post-infectious irritable bowel syndrome: a meta-analysis. Hepato-gastroenterology. 2012;59(113):67-72.
113. Tap J, Derrien M, Tornblom H, Brazeilles R, Cools-Portier S, Dore J, et al. Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome. Gastroenterology. 2017;152(1):111-23.e8.
114. Sundin J, Ohman L, Simren M. Understanding the Gut Microbiota in Inflammatory and Functional Gastrointestinal Diseases. Psychosomatic Medicine. 2017;79(8):857-67.

115. Gibson PR, Barrett JS, Muir JG. Functional bowel symptoms and diet. Internal Medicine Journal. 2013;43(10):1067-74.
116. Henstrom M, Diekmann L, Bonfiglio F, Hadizadeh F, Kuech EM, von Kockritz-Blickwede M, et al. Functional variants in the sucrase-isomaltase gene associate with increased risk of irritable bowel syndrome. Gut. 2018;67(2):263-70.
117. Wall GC, Bryant GA, Bottenberg MM, Maki ED, Miesner AR. Irritable bowel syndrome: a concise review of current treatment concepts. World Journal of Gastroenterology. 2014;20(27):8796-806.
118. Johannesson E, Simren M, Strid H, Bajor A, Sadik R. Physical activity improves symptoms in irritable bowel syndrome: a randomized controlled trial. The American Journal of Gastroenterology. 2011;106(5):915-22.
119. Ianiro G, Eusebi LH, Black CJ, Gasbarrini A, Cammarota G, Ford AC. Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. Alimentary Pharmacology & Therapeutics. 2019;50(3):240-8.
120. Surrey ES, Soliman AM, Johnson SJ, Davis M, Castelli-Haley J, Snabes MC. Risk of Developing Comorbidities Among Women with Endometriosis: A Retrospective Matched Cohort Study. Journal of Women's Health (2002). 2018;27(9):1114-23.
121. Wu CY, Chang WP, Chang YH, Li CP, Chuang CM. The risk of irritable bowel syndrome in patients with endometriosis during a 5-year follow-up: a nationwide population-based cohort study. International Journal of Colorectal Disease. 2015;30(7):907-12.
122. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology. 2004;126(3):693-702.
123. Bellini M, Gambaccini D, Stasi C, Urbano MT, Marchi S, Usai-Satta P. Irritable bowel syndrome: a disease still searching for pathogenesis, diagnosis and therapy. World Journal of Gastroenterology. 2014;20(27):8807-20.
124. El-Salhy M. Irritable bowel syndrome: Diagnosis and pathogenesis. World Journal of Gastroenterology : WJG. 2012;18(37):5151-63.
125. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: A microbiome-gut-brain axis disorder? World Journal of Gastroenterology : WJG. 2014;20(39):14105-25.
126. Ohman L, Simren M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nature reviews Gastroenterology & Hepatology. 2010;7(3):163-73.
127. Anaf V, Chapron C, El Nakadi I, De Moor V, Simonart T, Noel JC. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. Fertility and Sterility. 2006;86(5):1336-43.
128. Fond G, Loundou A, Hamdani N, Boukouaci W, Dargel A, Oliveira J, et al. Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. European Archives of Psychiatry and Clinical Neuroscience. 2014;264(8):651-60.

129. Haug TT, Mykletun A, Dahl AA. Are anxiety and depression related to gastrointestinal symptoms in the general population? Scandinavian Journal of Gastroenterology. 2002;37(3):294-8.
130. Lee C, Doo E, Choi JM, Jang SH, Ryu HS, Lee JY, et al. The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis. Journal of Neurogastroenterology and Motility. 2017;23(3):349-62.
131. Chen LC, Hsu JW, Huang KL, Bai YM, Su TP, Li CT, et al. Risk of developing major depression and anxiety disorders among women with endometriosis: A longitudinal follow-up study. Journal of Affective Disorders. 2016;190:282-5.
132. Hansen KE, Kesmodel US, Baldursson EB, Kold M, Forman A. Visceral syndrome in endometriosis patients. European Journal of Obstetrics, Gynecology, and Reproductive biology. 2014;179:198-203.
133. Azizi Z, Javid Anbardan S, Ebrahimi Daryani N. A review of the clinical manifestations, pathophysiology and management of opioid bowel dysfunction and narcotic bowel syndrome. Middle East Journal of Digestive Diseases. 2014;6(1):5-12.
134. Moore J, Barlow D, Jewell D, Kennedy S. Do gastrointestinal symptoms vary with the menstrual cycle? British Journal of Obstetrics and Gynaecology. 1998;105(12):1322-5.
135. Boland K, Nguyen GC. Microscopic Colitis: A Review of Collagenous and Lymphocytic Colitis. Gastroenterology & Hepatology. 2017;13(11):671-7.
136. Park T, Cave D, Marshall C. Microscopic colitis: A review of etiology, treatment and refractory disease. World Journal of Gastroenterology : WJG. 2015;21(29):8804-10.
137. Pardi DS. Diagnosis and Management of Microscopic Colitis. The American Journal of Gastroenterology. 2017;112(1):78-85.
138. Roth B, Gustafsson RJ, Jeppsson B, Manjer J, Ohlsson B. Smoking- and alcohol habits in relation to the clinical picture of women with microscopic colitis compared to controls. BMC Womens Health. 2014;14:16.
139. Twist K, Ablett J, Wearden A, Paine P, Vasant D, Lal S, et al. Gastrointestinal dysmotility: A qualitative exploration of the journey from symptom onset to diagnosis. Neurogastroenterology and Motility : the Official Journal of the European Gastrointestinal Motility Society. 2018:e13339.
140. Paine P, McLaughlin J, Lal S. Review article: the assessment and management of chronic severe gastrointestinal dysmotility in adults. Alimentary Pharmacology & Therapeutics. 2013;38(10):1209-29.
141. Wingate D, Hongo M, Kellow J, Lindberg G, Smout A. Disorders of gastrointestinal motility: towards a new classification. Journal of Gastroenterology and Hepatology. 2002;17 Suppl:S1-14.
142. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. Journal of Internal Medicine. 1993;233(1):45-51.

143. Ohlsson B, Orho-Melander M, Nilsson PM. Higher Levels of Serum Zonulin May Rather Be Associated with Increased Risk of Obesity and Hyperlipidemia, Than with Gastrointestinal Symptoms or Disease Manifestations. International Journal of Molecular sciences. 2017;18(3).
144. Bengtsson M, Hammar O, Mandl T, Ohlsson B. Evaluation of gastrointestinal symptoms in different patient groups using the visual analogue scale for irritable bowel syndrome (VAS-IBS). BMC Gastroenterology. 2011;11:122.
145. Bengtsson M, Sjoberg K, Candamio M, Lerman A, Ohlsson B. Anxiety in close relationships is higher and self-esteem lower in patients with irritable bowel syndrome compared to patients with inflammatory bowel disease. European Journal of Internal Medicine. 2013;24(3):266-72.
146. Roth B, Bengtsson M, Ohlsson B. Diarrhoea is not the only symptom that needs to be treated in patients with microscopic colitis. European Journal of Internal Medicine. 2013;24(6):573-8.
147. Roth B, Ohlsson B. Gastrointestinal symptoms and psychological well-being in patients with microscopic colitis. Scandinavian Journal of Gastroenterology. 2013;48(1):27-34.
148. Roth B, Berntorp K, Ohlsson B. The Expression of Serum Antibodies Against Gonadotropin-releasing Hormone (GnRH1), Progonadoliberin-2, Luteinizing Hormone (LH), and Related Receptors in Patients with Gastrointestinal Dysfunction or Diabetes Mellitus. Drug Target Insights. 2014;8:45-50.
149. Roth B, Gustafsson RJ, Ohlsson B. Auto-antibodies and their association with clinical findings in women diagnosed with microscopic colitis. PLoS One. 2013;8(6):e66088.
150. Bengtsson M, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). BMC Gastroenterology. 2007;7:16.
151. Bengtsson M, Persson J, Sjolund K, Ohlsson B. Further validation of the visual analogue scale for irritable bowel syndrome after use in clinical practice. Gastroenterology Nursing : the Official Journal of the Society of Gastroenterology Nurses and Associates. 2013;36(3):188-98.
152. Yoo HY, Park B, Joo J, Kim JS, Lee Y, Lim MC, et al. Validation of the Korean version of visual analogue scale for irritable bowel syndrome questionnaire for assessment of defecation pattern changes. Annals of Surgical Treatment and Research. 2018;94(5):254-61.
153. Kamijo K, Ishikawa K, Tanaka M. Clinical evaluation of 3rd generation assay for thyrotropin receptor antibodies: the M22-biotin-based ELISA initiated by Smith. Endocrine Journal. 2005;52(5):525-9.
154. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid : Official Journal of the American Thyroid Association. 2003;13(1):3-126.

155. Laboratoriemedicin Skåne Analysportalen 2019 [Available from: http://www.analysportalen-labmedicin.skane.se/viewAnalys.asp?Nr=1389] (accessed on Jul 11, 2019).
156. Celiac Fusion / tTG DGP ELISA Wicklow, Ireland2016 [Available from: http://www.trinitybiotech.com/products/celiac-fusion-ttgdgp-elisa/] (accessed on June 20, 2018).
157. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One. 2014;9(4):e95192.
158. Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. Nucleic Acids Research. 2011;39(15):e102.
159. Olink Proteomics [Available from: https://www.olink.com/] (accessed on Sept 30. 2018).
160. Laboratoriemedicin Skåne Analysportalen. 2019 [Available from http://www.analysportalen-labmedicin.skane.se/] (accessed on Jul 11, 2019)
161. Bengtsson J. Anvisning, F-Kalprotektin Medicinsk Information. 2017.
162. Poulsen JL, Brock C, Olesen AE, Nilsson M, Drewes AM. Evolving paradigms in the treatment of opioid-induced bowel dysfunction. Therapeutic Advances in Gastroenterology. 2015;8(6):360-72.
163. Galleri L, Luisi S, Rotondi M, Romagnani P, Cobellis L, Serio M, et al. Low serum and peritoneal fluid concentration of interferon-gamma-induced protein-10 (CXCL10) in women with endometriosis. Fertility and Sterility. 2009;91(2):331-4.
164. Lee HH, Farber JM. Localization of the gene for the human MIG cytokine on chromosome 4q21 adjacent to INP10 reveals a chemokine "mini-cluster". Cytogenetics and Cell Genetics. 1996;74(4):255-8.
165. Van den Steen PE, Husson SJ, Proost P, Van Damme J, Opdenakker G. Carboxyterminal cleavage of the chemokines MIG and IP-10 by gelatinase B and neutrophil collagenase. Biochemical and Biophysical Research Communications. 2003;310(3):889-96.
166. Sikora J, Smycz-Kubanska M, Mielczarek-Palacz A, Kondera-Anasz Z. Abnormal peritoneal regulation of chemokine activation-The role of IL-8 in pathogenesis of endometriosis. American Journal of Reproductive Immunology (New York, NY : 1989). 2017;77(4).
167. Arend RC, Londono-Joshi AI, Straughn JM, Jr., Buchsbaum DJ. The Wnt/beta-catenin pathway in ovarian cancer: a review. Gynecologic Oncology. 2013;131(3):772-9.
168. Zhang L, Xiong W, Xiong Y, Liu H, Liu Y. 17 beta-Estradiol promotes vascular endothelial growth factor expression via the Wnt/beta-catenin pathway during the pathogenesis of endometriosis. Molecular Human Reproduction. 2016;22(7):526-35.
169. Dao DY, Yang X, Chen D, Zuscik M, O'Keefe RJ. Axin1 and Axin2 are regulated by TGF- and mediate cross-talk between TGF- and Wnt signaling pathways. Annals of the New York Academy of Sciences. 2007;1116:82-99.

170. Jenne CN, Kubes P. Platelets in inflammation and infection. Platelets. 2015;26(4):286-92.
171. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scandinavian Journal of Gastroenterology. 2015;50(1):74-80.
172. Fraenkel PG. Anemia of Inflammation: A Review. The Medical Clinics of North America. 2017;101(2):285-96.
173. Gamage NU, Tsvetanov S, Duggleby RG, McManus ME, Martin JL. The structure of human SULT1A1 crystallized with estradiol. An insight into active site plasticity and substrate inhibition with multi-ring substrates. The Journal of Biological Chemistry. 2005;280(50):41482-6.
174. Sak K, Everaus H. Sulfotransferase 1A1 as a Biomarker for Susceptibility to Carcinogenesis: From Molecular Genetics to the Role of Dietary Flavonoids. Current Drug Metabolism. 2016;17(6):528-41.
175. Hemmerich S. Carbohydrate sulfotransferases: novel therapeutic targets for inflammation, viral infection and cancer. Drug Discovery Today. 2001;6(1):27-35.
176. Rizner TL. The Important Roles of Steroid Sulfatase and Sulfotransferases in Gynecological Diseases. Frontiers in Pharmacology. 2016;7:30.
177. Gomez-Lechon MJ. Oncostatin M: signal transduction and biological activity. Life Sciences. 1999;65(20):2019-30.
178. Beigel F, Friedrich M, Probst C, Sotlar K, Goke B, Diegelmann J, et al. Oncostatin M mediates STAT3-dependent intestinal epithelial restitution via increased cell proliferation, decreased apoptosis and upregulation of SERPIN family members. PLoS One. 2014;9(4):e93498.
179. West NR, Hegazy AN, Owens BMJ, Bullers SJ, Linggi B, Buonocore S, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nature Medicine. 2017;23(5):579-89.
180. Burri E, Beglinger C. The use of fecal calprotectin as a biomarker in gastrointestinal disease. Expert Review of Gastroenterology & Hepatology. 2014;8(2):197-210.
181. Menees SB, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. The American Journal of Gastroenterology. 2015;110(3):444-54.
182. Manoharan B, Haider AS, Samira AM, Bharathan B, Parra-Blanco A. Isolated appendiceal endometriosis resulting in intussusception. Oxford Medical Case Reports. 2018;2018(10):omy099.
183. Lorencatto C, Petta CA, Navarro MJ, Bahamondes L, Matos A. Depression in women with endometriosis with and without chronic pelvic pain. Acta Obstetricia et Gynecologica Scandinavica. 2006;85(1):88-92.
184. Patten SB, Barbui C. Drug-induced depression: a systematic review to inform clinical practice. Psychotherapy and Psychosomatics. 2004;73(4):207-15.

185. Warnock JK, Bundren JC, Morris DW. Depressive symptoms associated with gonadotropin-releasing hormone agonists. Depression and Anxiety. 1998;7(4):171-7.
186. Friedl F, Riedl D, Fessler S, Wildt L, Walter M, Richter R, et al. Impact of endometriosis on quality of life, anxiety, and depression: an Austrian perspective. Archives of Gynecology and Obstetrics. 2015;292(6):1393-9.
187. Lovkvist L, Bostrom P, Edlund M, Olovsson M. Age-Related Differences in Quality of Life in Swedish Women with Endometriosis. Journal of Women's Health (2002). 2016;25(6):646-53.
188. De Palma G, Collins SM, Bercik P. The microbiota-gut-brain axis in functional gastrointestinal disorders. Gut Microbes. 2014;5(3):419-29.
189. Vork L, Weerts Z, Mujagic Z, Kruimel JW, Hesselink MAM, Muris JWM, et al. Rome III vs Rome IV criteria for irritable bowel syndrome: A comparison of clinical characteristics in a large cohort study. Neurogastroenterology and Motility : the Official Journal of the European Gastrointestinal Motility Society. 2018;30(2).
190. Miller LE. Study design considerations for irritable bowel syndrome clinical trials. Annals of Gastroenterology. 2014;27(4):338-45.
191. Yuk JS, Park EJ, Seo YS, Kim HJ, Kwon SY, Park WI. Graves Disease Is Associated With Endometriosis: A 3-Year Population-Based Cross-Sectional Study. Medicine. 2016;95(10):e2975.
192. Shigesi N, Kvaskoff M, Kirtley S, Feng Q, Fang H, Knight JC, et al. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. Human Reproduction Update. 2019;25(4):486-503.
193. Aghajanova L, Stavreus-Evers A, Lindeberg M, Landgren BM, Sparre LS, Hovatta O. Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology. Fertility and Sterility. 2011;95(1):230-7, 7.e1-2.
194. Detti L, Uhlmann RA, Fletcher NM, Diamond MP, Saed GM. Endometrial signaling pathways during ovarian stimulation for assisted reproduction technology. Fertility and Sterility. 2013;100(3):889-94.
195. Cordeddu L, Bergvall M, Sand E, Roth B, Papadaki E, Li L, et al. Severe gastrointestinal dysmotility developed after treatment with gonadotropin-releasing hormone analogs. Scandinavian Journal of Gastroenterology. 2015;50(3):291-9.
196. Aziz I, Tornblom H, Palsson OS, Whitehead WE, Simren M. How the Change in IBS Criteria From Rome III to Rome IV Impacts on Clinical Characteristics and Key Pathophysiological Factors. The American Journal of Gastroenterology. 2018;113(7):1017-25.