SYMPTOM-DERIVED SUBGROUPING TO ELUCIDATE HETEROGENEITY IN PELVIC PAIN AND ENDOMETRIOSIS

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ABSTRACT

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Cyclic and non-menstrual pain in the pelvis and lower abdomen is associated with significant morbidity among women worldwide. The heterogeneity observed in the clinical presentation of pelvic pain and its associated disorders is not well understood. Endometriosis is among the most common disorders diagnosed in those presenting with pelvic pain. At the population level, there is great uncertainty regarding the true prevalence and incidence of endometriosis. At the individual level, pelvic endometriosis staging based on lesion type, location, or volume does not correlate well with pelvic pain symptoms. The overarching purpose of this dissertation was to describe and delineate the ways in which populations with pelvic pain – with and without endometriosis can be meaningfully partitioned.

The first aim was to characterize the population-level heterogeneity in the global distribution of endometriosis. We summarized and critically assessed studies of endometriosis frequency, distribution, and stage estimates between 1989-2019. We identified 69 studies describing the prevalence and/or incidence of endometriosis and examined stratification by population type and by indication for diagnosis, finding endometriosis prevalence ranging from 15.4% to 71.4% among women presenting with chronic pelvic pain. We did not find a change in frequency or distribution of endometriosis across the 30-year period.

The second aim was to identify subgroups of pelvic pain symptoms based on clustering patterns and investigate their association with comorbidities related to inflammation and chronic pain. Cross-sectional baseline data were analyzed from 1255 participants in the Women's Health

Study: from Adolescence to Adulthood (A2A) cohort, an ongoing longitudinal cohort study oversampled for adolescents and individuals with surgically confirmed endometriosis. We derived subgroups using latent class analysis (LCA) consisting of six variables: menstruation associated (cyclic; dysmenorrhea) and non-menstruation associated (acyclic) pelvic pain severity, frequency, and impact on daily activities. We identified five subgroups defined by pelvic pain characteristics and found distinct associations of comorbidity patterns, including endometriosis, differentially associated with these subgroups.

The third aim was to, again, utilize these identified five subgroups defined by pelvic pain characteristics and investigate their association with seven plasma-based inflammatory biomarkers selected for their previously observed associations with pelvic pain. We performed a three-step approach to examine these associations, accounting for non-linearity and classification uncertainty in the relationship between biomarkers and subgroup assignment, as well as adjusting for confounding by age and body mass index. We found that the most significant associations of pro-inflammatory cytokines were with the subgroup defined by those reporting both severe cyclic and severe acyclic pelvic pain compared to the subgroup inclusive of those reporting no pelvic pain. This severe multifactorial pelvic pain subgroup was the same group found in our second aim to be associated with the highest number of inflammatory and pain comorbidities.

Overall, this work contributes to understanding of the heterogeneity and patterning of symptomology in pelvic pain, with a lens on endometriosis. Considering the strengths and limitations of each study, subgroup associations with comorbid inflammatory and pain conditions and inflammatory biomarkers may suggest both distinct biologic pathways and shared underlying etiologies that warrant investigation in future research.

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KEY TO ABBREVIATIONS

A2A	Adolescence to Adulthood (Women's Health Study Cohort)
aBIC	Adjusted Bayesian Information Criterion
aOR	Adjusted Odds Ratio
BCH	Boston Children's Hospital
BIC	Bayesian Information Criterion
BLRT	Bootstrapped Likelihood Ratio Test
BMI	Body mass index
BWH	Boston Women's Hospital
CCL13	C-C Motif Chemokine Ligand 13, also known as MCP-4
CCL17	C-C Motif Chemokine Ligand 13, also known as TARC
CCR	Receptor for MCP-4/CCL13
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CV	Coefficient of variation (%)
CXCL10	C-X-C Motif chemokine 10, also known as IP-10
CXCR3	Receptor for IP-10/CXCL-10
FIML	Full information maximum likelihood
ICC	Intraclass correlation
IL-16	Interleukin 16
IL-8	Interleukin 8
IP-10	Interferon γ-induced protein 10 kDa, also known as CXCL-10
LCA	Latent Class Analysis
MAR	Missing at random

MCP-1	Monocyte chemoattractant protein 4, also known as CCL2
MCP-4	Monocyte chemoattractant protein 4, also known as CCL13
MNAR	Missing not at random
N	Number
NRS	Numerical rating scale
OR	Odds ratio
PCOS	Polycystic ovary syndrome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
R3STEP	The three-step procedure for antecedents in Mplus
rAFS	Revised American Fertility Society score
rASRM	Revised American Society for Reproductive Medicine score
RCS	Restricted cubic spline
SD	Standard deviation
TARC	Thymus and activation-regulated chemokine, also known as CCL17
TNF-α	Tumor Necrosis Factor alpha
VEGF	Vascular endothelial growth factor
VLMR	Vuong-Lo-Mendell-Rubin likelihood ratio test
WERF EPHect EPQ	World Endometriosis Research Foundation Endometriosis Endometriosis Phenome and Biobanking Harmonisation Project Patient Questionnaire
WHO	World Health Organization

CHAPTER 1 BACKGROUND AND OBJECTIVES

Definitions and epidemiology

Chronic pelvic pain, that is pain localized to the pelvis for 6 months or more, is a common and burdensome condition that impacts women worldwide. This pain can be constant or intermittent, of varying qualities and severity. The definition of chronic pelvic pain in women has varied over time including differences in time cutoffs and variation in the pain types included.[1] Most commonly the definition includes pain in the pelvis lasting more than 6 months.[2] However, the requirement for this cut-off if various impairments characteristic of central pain sensitization is reported.[3] Three types of pain commonly classified as a part of pelvic pain are cyclic pelvic pain, acyclic pelvic pain, and dyspareunia.[2] Cyclic pelvic pain, also called dysmenorrhea, is pain associated with menstruation. Acyclic pelvic pain is defined as pain not associated with menstruation, and in common definitions also not associated with acute events such as infections or pregnancy. Other types of pain include dyspareunia (pain associated with intercourse), considered a separate entity from acyclic pelvic pain.[2]

A World Health Organization (WHO) systematic review of 178 global studies from 1928 to 2004 reported prevalence estimates for chronic pelvic pain ranging from 2.1% to 24% for acyclic pelvic pain, and 16.8% to 81% for cyclic pelvic pain.[1] A follow-up review which used the more narrowly-defined definition of only acyclic pain lasting 6 months found 5.7% to 26.6%.[4] The uncertainty seen in prevalence estimates of chronic pelvic pain may be in part attributed to the variation in the condition, but also the absence of large population-level studies across the world.[4] Nevertheless, pelvic pain has significant economic impact. Between 1990-2021, the annual direct and indirect health care costs of chronic pelvic pain among women in the United States was an estimated $2.8 billion.[5] The condition has severe implications for the health

and well-being of those affected, as it has been estimated that more than 40% of laparoscopies and 10% to 15% of hysterectomies performed are for pelvic pain.[6] Chronic pelvic pain is multifactorial, with a range of gynecologic, gastrointestinal, urologic, neuromusculoskeletal and psychosocial conditions associated with it.[2] In nearly half of cases of chronic pelvic pain, there is at least one or more plausible causal factor associated such as endometriosis, irritable bowel syndrome, interstitial cystitis/painful bladder syndrome.[7] Among these endometriosis affects 15.4% to 71.4% of those being investigated for chronic pelvic pain, representing a substantial burden of disease.[8]

Endometriosis is a disease characterized by the presence of endometrial-like tissues in ectopic sites outside the uterus, most commonly in the pelvic area.[9] The etiology of endometriosis has historically been attributed to retrograde flow of menstrual discharge and deposition and establishment of endometrial cells in extra-uterine sites, as described by Sampson (1927).[10] In recent years, there has been increasing evidence to support other theories, including but not limited to coelomic metaplasia for retropelvic endometriosis, lymphatic and vascular metastasis for extra-pelvic endometriosis, and neonatal uterine bleeding.[11] A number of risk factors have been identified for the disease, catalogued in detail by Farland et al. (2017)[12] and Shafrir et al. (2018).[13] These factors range from those impacting the in-utero environment, menstrual and reproductive factors (such as parity), to physical characteristics (such as body mass index) among others.[12,13] Endometriosis is also associated with increased risk of clinical sequalae such as cardiovascular disease[14] and cancers such as ovarian cancer[15] and cutaneous melanoma[16].

The most commonly cited prevalence estimate for endometriosis is 5% to 10% among reproductive aged women between 15-49 years, with an estimated 2% affected by the most

severe categories of the disease.[11] Rarely, endometriosis is also seen in premenarcheal adolescents[17] and it is estimated to impact some 2% to 5% of post-menopausal women.[18] The highest incidence rate of endometriosis is reported in women between 25-29 years.[19] However, this group is also more likely to have access to diagnostics for indications such as infertility, than for example younger groups. In fact, in a study of 1000 women, the most common reasons for diagnosis with endometriosis included pelvic pain (80%), infertility (25%), and ovarian masses (20%). There were also some incidental diagnoses that occurred during surgery and imaging for other indications.[20] Furthermore, there is a large diagnostic delay associated with endometriosis that impacts measures of disease incidence. Across ten countries, the surgical diagnosis of endometriosis was found to take on average 6.7 years from the onset of symptoms.[21] A range of factors lead to this delay from misdiagnosis to the use of pain medications and contraception. This time delay is variable across countries, with a mean estimate of 4.4 years in the United States.[22] Additionally, the time to diagnosis has been found to vary based on the indication for clinical visit and symptom presentation. For example, in a cohort of Brazilian women it was found that those presenting with infertility were diagnosed a median of 4 years after the onset of symptoms. Yet, for adolescents presenting with chronic pelvic pain the wait was over a decade.[23]

Mechanisms underlying chronic pelvic pain and endometriosis

Chronic pelvic pain has been conceptualized as a regional pain syndrome resulting from interactions between multiple systems, or as described the symptom of an underlying etiology.[7,24] There are often overlaps between different types of pelvic pain. As well there are often overlaps between entities identified as distinct functional pain disorders such as irritable bowel syndrome, interstitial cystitis/painful bladder syndrome. Pain has historically been considered a protective evolutionary adaptation leading to organized response against noxious stimuli, though the

trajectory of its development across species is still not well-understood.[25] While hypotheses regarding the adaptive and maladaptive evolutionary mechanisms that may have led to sensitization and chronic pain including chronic pelvic pain have been proposed, evidence for these mechanisms is lacking.[26–28] Multiple models for the initiation of chronic pain have been proposed. This includes gate control theory, which was the first model integrating physiologic and psychologic factors into the experience of pain as proposed by Melzack and Casey (1965).[29] Later theories such as the diathesis-stress model proposed by Turk and Monarch (2012) expanded on this by examining how psychosocial factors may predispose some to higher risk of chronic pain than others.[30] Indeed, in addition to physiologic causes, chronic pelvic pain has been linked to psychologic and psychiatric disorders, as well as history of psychosocial trauma. In a study of 713 women with pelvic pain, 46.8% were found to have a history of physical or sexual abuse, and 31.3% screened positive for post-traumatic stress disorder.[31]

Pain has classically been described as generated via two mechanisms: nociceptive—due to activation of the nervous system due to non-neuronal tissue damage which is a normal physiologic function, and neuropathic—due to neuronal tissue damage.[32] Both mechanisms require evidence of damage, however many chronic pain conditions do not fall neatly into this dichotomy as they are caused by a dysfunction of the system, not gross pathologic damage. More recently, a third descriptor, termed ‘nociplastic’ pain has been conceptualized for where there is evidence of altered nociceptive function and processing in absence of direct damage.[33] It’s thought that chronic pelvic pain and other disorders such as fibromyalgia have a mix of nociceptive, neuropathic and nociplastic components. In this framework, these disorders emerge in a milieu of adverse physiologic and biopsychosocial factors.[34] Chronic pelvic pain then, can

be considered a synergistic entity emerging from a number of factors that compound each other's impact and enhance sensory dysfunction and overall central pain sensitization.[35]

Schliep et al. (2015) was among the first studies that characterized the typology of pain across the body in women who were undergoing laparoscopy or laparotomy. They found that women diagnosed with endometriosis, the majority with stage I peritoneum only disease, reported more cyclic pain (49.5% and 44.2%) compared to women diagnosed with other gynecologic disorders (31.0%) or no pelvic disorders (33.1%). They also reported more chronic pain (44.2%) compared to those with no pelvic disorders (30.2%).[36] Understanding the underlying pathologic processes and mechanisms directs approaches taken in the clinical treatment of pelvic pain. For example, in clinical treatment algorithms, pelvic pain with cyclical components is often treated with hormone therapy, and pelvic pain with a neuropathic component is treated with a combination of pain medications and anti-depressants.[7] However, the relationship between etiology, pathophysiology and symptomology in chronic pelvic pain is not fully clear or consistent, particularly as will be discussed in the context of endometriosis.

Endometriosis is a systemic disease involving aberrations in the hormonal, inflammatory and neurologic systems with an extremely heterogenous presentation.[9] The heterogeneity has been attributed to a number of factors: the diversity of patients, variations in the locations and histologic subtypes of the disease, and variations in the presentation of symptoms. The pelvis is the most common anatomic site of endometriosis presentation, followed by less common extra-pelvic sites. In the pelvis, the most common pathologic manifestations of the disease include superficial peritoneal lesions, endometrioma (ovarian lesions) and deeply infiltrating endometriosis.[37] In the pelvis, the most common pain characteristics include cyclic pelvic pain (dysmenorrhea), acyclic pelvic pain, and dyspareunia. Other symptoms associated with the

disease including abnormal uterine bleeding, infertility and subfertility, bladder dysfunction and pain, gastrointestinal dysfunction and pain and lower back pain.[9] Increase in the number of these symptoms is associated with increased likelihood of endometriosis.[38]

Multiple mechanisms have been proposed to explain how endometriotic lesions lead to pain symptoms such as dysmenorrhea. One proposed mechanisms is recurrent micro-bleeds in endometriotic lesions with associated inflammation.[39,40] It has also been hypothesized that the disconnect between disease staging and pain in endometriosis may be not due to the extent of the lesions but the way they interact with the neuronal system.[41] For example, via the irritation of nerves or direct invasion of nerves by endometriotic lesions.[42] Other mechanisms may be a result of mass effect, as for example in the case of infiltrating lesions on the uterosacral ligament which can place biomechanical pressure that causes dyspareunia during intercourse.[39] Conversely, in the case of ovarian endometriomas, larger diameters have been found to have an inverse relationship with cyclic and acyclic pelvic pain.[41] Overall, the symptomatic presentation of endometriosis has not been found to be associated with its broad pathophysiology.

A number of investigations have examined if symptom presentation and location correlate with surgical findings. A study of 96 women with endometriosis by Hsu et. al (2011) did not find associations between pain, pain location and lesion locations.[43] Similarly a study of 113 women by Ballard et al. (2010) found no associations between areas of pain and lesion locations, though women with endometriosis were more likely to experience dyschezia, pain with defecation, and describe specific pain sensations such as throbbing pain, compared to women with no endometriosis.[44] Schliep et al. (2015) also found no association between pain and location of lesions, though did find an association with deep lesions.[36] Renner et al. (2012) found that pain maps constructed pre-diagnostic laparoscopy for pelvic pain showed that those

eventually diagnosed with endometriosis had significantly different visual patterns of pelvic pain than those without endometriosis. They suggested that despite displacement from the anatomic location of lesions, the maps may still provide useful information.[45]

Measurement, diagnostics and classification of pelvic pain

Accurate and consistent measurement of pelvic pain represents a major challenge. While animal models have played an important role in understanding pathologies associated with pain, pain in itself is a highly complex and subjective internal state, not an external behavior, which makes it difficult to compare the full spectrum of pain states across species.[25] The same challenges are seen in human populations when comparing experiences of pain and there is little consensus regarding the best method to measure pain. Literature across a range of fields has focused on creating validated pain scales to objectively measure and compare pain in clinical settings and for research. Among the most commonly used validated, reliable scales in clinical settings are the Verbal Rating Scale (VRS), Visual Analogue Scale (VAS), and Numeric Rating Scale (NRS).[46] A systematic review of 258 studies of endometriosis between 1980 and 2012 found that VAS was the most commonly used scale, and recommended VAS and NRS for future use in endometriosis pain assessment.[47] The most comprehensive pain scales not only take into account pain localization and intensity and quality, but also impact on quality of life in various ways.[48] As an example, the World Endometriosis Research Foundation's Endometriosis Phenome and Biobanking Harmonization Project - Endometriosis Participant Questionnaire (WERF EPHect - EPQ) captures pain intensity on the NRS scale, but also uses additional questionnaires to capture cognitive and psychosocial components of pain such as pain catastrophizing.[49] Reliable widely-used contemporary tools that measure pain ultimately rely on self-reporting, hence the current focus in pain research is on biomarkers that can be used to tease

apart conscious self-reported experiences of pain and neurocognitive and physiologic experience of pain. Among the most promising developments is neuroimaging-based tools used to characterized evoked pain and chronic pain states in the brain.[50]

The gold standard diagnostic method for endometriosis is surgical visualization, often via laparotomy or laparoscopy.[51] However, it is an operator dependent and unreliable gold standard. The presence of a pathologic lesion may not be the cause of pain, and the lack of lesions on laparoscopic investigation does not preclude disease. A small study on the correlation between diagnosis and tissue samples found that only half of lesions considered clinically suspicious for endometriosis were microscopically proven to be endometriosis.[52] While the criteria for histopathologic diagnosis is the presence of two of three features in the lesion sampled: glands, stroma and hemosiderin-laden macrophages[53], there can be atypical or altered histopathologic presentation that causes diagnostic inaccuracy.[54] Classification of endometriosis is similarly characterized by challenges arising from the heterogeneity of the disease. The most commonly used staging system for endometriosis is the Revised American Society of Reproductive Medicine (rASRM) staging, historically called AFS and revised in 1996. It is based on surgical visualization of the location and depth, with four stages ranging from I-IV: minimal, mild, moderate, and severe.[51] However, it does not consider the presence of deep infiltrating endometriosis in certain sites. Also, rASRM has not been found to be associated with pain symptoms, nor treatment response, prognosis, fertility outcomes and sequalae.[36,55–57] Other classification systems include the ENZIAN system which considers the full spectrum of deep infiltrating endometriosis, and the endometriosis fertility index, which predicts fertility outcomes but neither correlates with pain symptoms.[57,58]

Pelvic pain and endometriosis in adolescents

Adolescent females (≤21 years of age) represent a population that is understudied for pelvic pain despite the fact that they commonly experience cyclic and acyclic pelvic pain.[59] Among adolescents, the prevalence of cyclic pelvic pain is an estimated 30% to 90%.[60] Compared to adults, adolescents can also have unique differential diagnoses for chronic pelvic pain that include congenital abnormalities and obstructive reproductive tract anomalies.[59] However the disorders commonly diagnosed among adults with chronic pelvic pain, such as endometriosis, are also of great relevance among adolescents. A meta-analysis found that among adolescents undergoing surgical investigation for chronic pelvic pain, 62% had visually confirmed endometriosis, with the estimate updated to 64% in an updated systematic review of literature between 2011 and 2019.[61,62] Though, these estimates may be higher than expected due to selection for individuals who have undergone surgery, they nevertheless indicate the severity of the problem in this group. Very few differences have been reported between adult versus adolescent symptom manifestations of endometriosis, one notable being that compared to adults, adolescents report more nausea and more superficial peritoneal endometriosis.[63] Additionally, one study of 57 adolescents also found higher recurrence of endometriosis in young women compared to older women after surgical treatment.[64]

While it is commonly thought that pelvic pain disorders such as endometriosis present less severely among adolescents, systematic reviews of adolescents with endometriosis reported that a notable minority, about a third, of the adolescents had moderate to severe endometriosis.[61,62] A study of 86 adolescents undergoing surgery for endometriosis found that among those with advanced stage endometriosis, ovarian endometriomas were the most common finding.[65] Nevertheless, a comparative cross-sectional study of 1,560 patients showed that severe

endometriosis is indeed more common in older ages.[66] This suggests that endometriosis is a progressive diseases that can worsen if left untreated from adolescence to adulthood, and associated with sequelae such as infertility and cancer. It is likely that endometriosis arises much earlier than thought in many adults. One study found that that among adults with endometriosis, one-third reported their symptoms started at menarche.[63] Another found that two-thirds of adults report their endometriosis symptoms commenced before 20.[67] Yet, adolescents with chronic pelvic pain continue to face diagnostic delays, which in a recent systematic review of 28 studies published between 2019 to 2021 was attributed to a lack of clinician awareness of updated diagnostic guidelines.[68] Chronic pelvic pain among adolescents is not as well-studied as adults, therefore, characterizing pelvic pain and its comorbidities in this group will provide novel insight and has the potential to aid in disease identification and development of early intervention strategies.

Heterogeneity: a challenge to unraveling biology

Despite difficulties with cross-species comparisons, studies across species using models of sensitivity states have revealed conserved biological mechanisms that contribute to adaptive and maladaptive pain states.[25] In recent years, there has been growing interest in characterizing unexplained genetic, pathological, and clinical heterogeneity as way to push precision medicine forward. Investigations of underlying biological heterogeneity and subtypes have been utilized across many disorders, and have proven particularly fruitful in cancer research with great implications for targeted therapies.[69] One of the earliest studies focusing on the heritability of chronic pelvic pain using twin pairs found a heritability of 0.43 (95% CI 0.25-0.56). The authors noted that almost all the genetic variance observed could be explained by underlying conditions, including endometriosis, dysmenorrhea, uterine fibroids and somatic distress (15%).[70] Hence, a

greater number of genetic studies have focused on the conditions that contribute pelvic pain, such as endometriosis. Familial and twin studies have established a genetic basis for the endometriosis.[71,72] Genome-wide studies to date have identified at least 14 genetic risk loci associated with the disease, including genes such as WNT4, which is involved in pathways of development of the female reproductive tract, and GREB1, which is involved in pathways of hormone dependent cell growth and proliferation.[73] Additionally, the genetic burden, that is greater number of risk loci in a given individual, has been associated with increased risk of severe endometriosis.[74] Genetic evidence of presentation heterogeneity including differences in somatic mutations between deep infiltrating lesions and other types of lesions have also been found, though their contribution to disease progression and symptomology is unclear.[75] However, to date, no studies have characterized the genetic basis for the heterogeneity in endometriosis pain symptomology.

To tackle the heterogeneity seen in endometriosis symptoms, and their overlap with other chronic pain associated disorders, research has turned to molecular biomarkers to understand etiology and for diagnostics.[76] These include serum, urine as well-as peritoneal-based biomarkers: glycoproteins, inflammatory cytokines, markers of oxidative stress, growth factors and peptides, angiogenesis molecules, autoantibodies, microRNAs, and a range of proteomic and metabolomic products.[76] However, the heterogeneity of endometriosis presents a challenge to biomarker identification. In a systematic review of 54 studies, 31 studies with 77 biomarkers for endometriosis could not distinguish between those with endometriosis from those without endometriosis.[77] Complex interactions between the immune system and the peripheral and central nervous system leads to the induction and maintenance of pathologic pain.[78] For this reason, inflammatory biomarkers in blood represent an area of particular interest as immune

dysregulation is thought to play an important role in the pathology and symptomatic presentation of endometriosis including pain.[79] Three studies have thus-far examined the association between serum-based biomarkers and pain symptomology in endometriosis.[79–81] These studies found elevated IL-16 in patients with endometriosis and chronic pelvic pain compared to those without pain,[80] inverse correlation between IL-19 and IL-22 levels and dyspareunia and acyclic pelvic pain intensity scores in patients with endometriomas,[81] and no association between IL-8 levels and pelvic pain in those with endometriomas.[82]

Basic science and clinical research in endometriosis group the diverse phenotypes seen in the disease into one disease entity. Attempts to categorize endometriosis into groups, often focus on single variables and not at patterns of symptomology.[83] The selection of appropriate controls has also been considered an issue in biomarker identification. As an example of such biases, a study focusing on diagnostic biomarkers for endometriosis found lower levels of inflammatory biomarkers IL-1β, IFN-γ, TNF- α and IL-6 in endometriosis cases compared to controls, contrary to their expectations. However, they speculated that the controls used in the study, selected from a clinical population, may have had non-endometriosis pelvic conditions such as adhesions that may increase the plasma concentrations of inflammatory cytokines.[84]

Subgrouping methods and applications

One way that researchers have tackled population heterogeneity across fields has been to focus on identifying unique disease presentation patterns based on specific criteria. For example, identification and partitioning of the heterogeneity of symptom patterns has also played a critical role in contemporary psychiatric research.[85] Clustering approaches have great potential in biomedical and clinical research, by allowing patients with similar patterns for specific characteristics to be identified as unique subgroups. They have been used to facilitate a range of

disorders with varying levels of success.[86] Subgroup identification can be conducted using various clustering approaches: heuristic, model-based, and density-based.[87] Model-based latent variable mixture modelling approaches have been widely adopted. These methods include latent class analysis (LCA), latent profile analysis (LPA), repeated measures latent class analysis (RMLCA) and latent transition analysis (LTA). LCA uses categorical variables called 'indicators' to identify cross-sectional patterns in the population, these emergent groups are called classes or subgroups. LPA is similar to LCA but uses continuous indicators. RMLCA and LTA are longitudinal approaches that assess changes in latent classes over time, but do not utilize the predetermined growth parameters seen in growth mixture models.[88]

LCA has many advantages when compared to other clustering techniques. As a probability-based technique it provides robust parameter estimates under the latent variable framework and considers measurement errors of indicators. LCA can also be expanded to use include other covariates, so called auxiliary variables, while accounting uncertainty in classification.[89,90] LCA has had a long history in fields such as psychology, but more recently has been adopted in clinical medicine as a tool to help identify hidden 'clinical phenotypes'.[91] Despite its strengths as a classification tool, a number of caveats must be addressed to properly utilize LCA. Individuals are assigned to classes based on probabilities; thus classification for each individual is associated with a degree of uncertainty. Using most probable class assignment as a variable in a regression analysis, therefore, can give inaccurate estimates. However, techniques such as the three-step method allow for conducting association analyses while accounting for classification uncertainty around class assignment.[88,89,92]

When deciding on optimal number of classes, there are many options as to what model fit statistic to use, which may impact the reliability of classes across different studies. As well,

researchers must choose specific labels for their classes, which may not accurately describe the complex nature of a given class.[93] Finally, the data-driven classes identified may reflect 'natural' subgroups with distinct etiologic and biologic underpinnings, or they may be descriptive statistical entities that simplify multidimensional data and classify complex patterns in a systematic and interpretable manner. Therefore, to understand and appropriately interpret emergent classes, it is essential that the validity and reliability is assessed. Validity can be examined by looking at the relationship of a given class with other external variables, and reliability by assessing classes in other populations or subpopulations.[94]

Systematic subgrouping of chronic pelvic pain and endometriosis in women has been limited, and the few studies that we identified based on a review of literature were highly variable in their objectives and approaches. Leserman et al. (2006) used assessment from one expert gynecologist to identify seven diagnostic subtypes based on symptomology and pain localization in a population drawn from a chronic pelvic pain clinic (N=289).[95] These diagnostic subtypes were labeled based on their predominant phenotype: diffuse abdominal/pelvic pain, vulvovaginal pain, cyclic pain, neuropathic pain, non-local pain, trigger points, and fibroid tumor pain. Important limitations of this study included the use of one individual to assign groups which limited any kind of interrater reliability assessment, as well limited power to detect differences between groups, given the small sample size with the large number of groups. Fenton et al. (2013) used exploratory factor analysis and latent profile analysis (LPA) to identify subgroups of pelvic pain in women seen at a pelvic pain referral center (N=476).[96] They identified high and low pain groups based on physical examination of pelvic regions using quantitative pressure threshold algometry and NRS pain ratings in 4 pelvic regions, consisting of 30 anatomic sites. An important limitation to this study was that all measurements were

conducted by a single clinician. Fenton et al. (2015) also used the same approach to identify classes of biopsychosocial dysfunction in patients with chronic pelvic pain.[97]

Chen et al. (2017) used latent class analysis (LCA) to identify symptom-based subtypes among women with dysmenorrhea (N=762), based on 14 somatic symptoms related to dysmenorrhea, ranging from abdominal cramps to nausea and constipation.[98] They identified three subgroups: a mild localized pain group, a severe localized pain group, and a severe multiple symptom group. An important limitation of this study included a convenient sample of internet users. Obbarius et al. (2019) used LCA to identify pain subgroup in a mixed group of men and women with chronic pelvic pain (N=411) from retrospective clinical data.[99] They used five categories of items to derive their subgroups: this included pain intensity, frequency and impairment using VAS rating, a pain perception scale, Patient Health Questionnaire 9-item (PHQ-9) which is used to identify depressive symptoms, Generalized Anxiety Disorder 7-item (GAD-7) which is used to identify anxiety symptoms, and the Short Form Health survey 8-Item (SF-8) which is used to assess general quality of life. They identified four classes labeled as low, moderate, high and extreme pain burden. Important limitations of this study included problems with detailed characterization of pain, including lack of information regarding pain location and timing. Urteaga et al. (2020) used self-tracking data from 4,368 women with endometriosis and based on a wide range of data including symptoms, quality of life, medication use.[100] They applied an unsupervised mixed membership modelling approach and identified four distinct groupings among patients with endometriosis that characterized the disease according to its severity and burden on life. One of the important limitations to this work was that the variables used to derive subgroups were so diverse and wide-ranging, from pain location and frequency to pain medication use, rendering interpretation of the four subgroups identified very challenging.

Major gaps in research

Upon review of literature we identified a number of gaps in research regarding chronic pelvic pain and endometriosis. Many studies have characterized the epidemiology of endometriosis in the past in different countries and subpopulations, leading to highly variable estimates cited across literature—including the commonly cited 10% prevalence.[11,13,101,102] A seminal review by Eskenazi and Warner (1997) characterized the frequency measures of endometriosis based on literature at that time.[102] Most recently, the Global Burden of Disease (2017) examined the frequency of endometriosis between 1990 and 2017, and found changes in age-standardized rates of the disease: including a 3.1% decrease from 1990-2007 and 3.0% decrease from 2007-2017. However, an assessment of the sources of these data, heterogeneity among them, or potential biases within the data were not considered.[103]

Second, there is increasing evidence that regardless of the range of underlying causes, chronic pelvic pain patients have common mechanisms driving their symptomology. These include but are not limited to alterations seen in nerve fibers and central gray matter volume.[104] Meanwhile, endometriosis has traditionally been treated in much of the literature as a homogenous entity. Hence, traditional statistical approaches used in investigating populations with the disease implicitly assume homogeneity and the lack of hidden subgrouping.[105] However recent associations of molecular differences, such as estrogen receptor-α immunoreactivity with symptom severity and recurrence, suggest that endometriosis may not be a single disease entity but one consisting of informative subtypes.[106,107] Given that chronic pelvic pain and endometriosis are associated with significant morbidity and their heterogeneity is not well-understood, there is a pressing need for studies that characterize the heterogeneity seen at the population level and at the individual level in these conditions. Specifically, approaches that

focus on the pattens of chronic pelvic pain symptoms and relate them to biology and pathophysiology of the multitude of diseases associated with pelvic pain may yield fruitful results beyond a single-pathology approach.

Overall objectives and specific aims

The overarching purpose of this dissertation is to describe and delineate the ways in which populations with pelvic pain – with and without endometriosis- can be meaningfully partitioned. The availability of the Women's Health Study: from Adolescence to Adulthood (A2A) cohort, an ongoing longitudinal cohort study oversampled for adolescents and for individuals with surgically confirmed endometriosis, provides a unique opportunity to conduct research that assesses the heterogeneity seen in pelvic pain and endometriosis in a large extensively phenotyped population. The aims of the current research are as follows:

Specific Aim 1: Characterize the population-level heterogeneity in endometriosis frequency, distribution, and stage estimates since 1989 globally and critically assess sampling and design when evaluating variations among these estimates.

Specific aim 1a: Compare endometriosis prevalence, incidence and severity by population source (general population versus clinic based) and geographic areas

Specific aim 1b: Evaluate reported epidemiologic measures by indication for diagnosis in clinical populations, including but not limited to pelvic pain

Specific aim 1c: Identify changes in epidemiologic measures across a 30-year period

Specific Aim 2: Identify symptom-based subgroups of chronic pelvic pain using data from a cohort with extensively phenotyped symptomology and examine the association between pelvic pain subgroups and comorbidities related to pain and inflammation.

Specific aim 2a: Use latent class analysis (LCA) to derive optimal pain symptom-based subgroups, and describe the distribution of socio-demographic and clinical variables across these subgroups

Specific aim 2b: Assess the comparative magnitude of relationships between pain and inflammation-related commodities and identified pelvic pain subgroups

Specific Aim 3: Utilize identified subgroups defined by pelvic pain characteristics and investigate the association between pelvic pain patterns and plasma-based inflammatory biomarkers in this population

Specific aim 3a: Assess the functional form of the relationship between biomarkers of interest and subgroups of pelvic pain

Specific aim 3b: Conduct an exploratory association analysis examining the direction of the relationship between inflammatory biomarkers and pelvic pain subgroups

CHAPTER 2 IS ENDOMETRIOSIS MORE COMMON AND MORE SEVERE THAN IT WAS 30 YEARS AGO?

ABSTRACT

Current estimates of endometriosis prevalence and incidence are highly variable, leading to uncertainty regarding true endometriosis frequency or validity of quantified changes over time. We present a comprehensive review of the prevalence, incidence and stage of endometriosis worldwide as reported over the past 30 years. We conducted a systematic search of observational studies utilizing PubMed, Web of Science, EMBASE, and CINAHL to identify research papers published in the English language between January 1989 and June 2019. Search terminologies were limited to titles containing: endometriosis and prevalence or incidence, or epidemiology, or frequency, or occurrence, or statistics. Two independent reviewers screened abstracts for study eligibility. Data from included studies were abstracted. Overall, 69 studies describing the prevalence and/or incidence of endometriosis met the inclusion criteria. Among these, 26 studies were from general population samples, 16 of which were from regional/national hospital or insurance claims systems. The other 43 studies were conducted in single clinic or hospital settings. Prevalence estimates for endometriosis varied widely from 0.2% to 71.4% depending on the population sampled. The prevalence reported from general population studies ranged from 0.7% to 8.6%, while among single clinic or hospital-based studies ranged from 0.2% to 71.4%. When defined by indications for diagnosis, endometriosis prevalence ranged from 15.4% to 71.4% among women with chronic pelvic pain, 9.0% to 68.0% among women presenting with infertility, and 3.7% to 43.3% among women undergoing tubal sterilization. A meta-regression was conducted with year as the predictor for prevalence. No trend across time was observed among ‘general population in country/region’ studies (β=0.04, p=0.12) nor among ’single

hospital or clinic' (β=-0.02, p=0.34) studies, however a decrease over time was observed among 'general population studies abstracted from health system/insurance systems' (β=-0.10, p=0.005). As with all human studies, population sampling and study design matter. Heterogeneity of inclusion and diagnostic criteria and selection bias overwhelmingly account for variability in endometriosis prevalence estimated across the literature. Thus, it is difficult to conclude if the lack of observed change in frequency and distributions of endometriosis over the past 30 years is valid.

INTRODUCTION

It is commonly stated that endometriosis affects approximately 10% of women of reproductive age worldwide, reaching up to 50% among infertile women.[13,101,108] Endometriosis has a heterogeneous clinical presentation with respect to symptoms, empiric treatment response and phenotype, which influence diagnosis sensitivity and specificity. Health disparities including access to experienced surgeons or imaging specialists impact the likelihood of being evaluated and diagnosed. While the gold standard for diagnosis remains surgically visualized lesions, skilled imaging can successfully identify ovarian endometrioma and deep endometriosis but not superficial peritoneal disease.[109]

Determining the frequency and distribution of chronic diseases such as endometriosis that can severely impact the quality of life is critical for public health and clinical care.[13,21,63,110,111] Prevalence quantifies the proportion of disease in a population at one point or period in time, while incidence is the rate of new disease occurrence or diagnosis in a population across a specified period.[112] Measuring true change in disease frequency over time requires stable definitions and likelihood of detection within the same or similar populations.

Quantifying changes in endometriosis frequency can provide insight into the etiology of the disease that may be attributed to correlated risk factors that also changed over the same time period. The Global Burden of Disease represents the broadest effort to quantify frequency measures for many diseases, tabulated using a mixture of nation-level morbidity, hospital discharge, and insurance data with the intention to allow countries to recognize their relative health challenges and change over time.[103] Endometriosis was included in 2017, with documentation given available data of a decrease in age-standardized rates of 3.1% (95% confidence interval = -6.3% to 0.5% change) from 1990-2007 and of -3.0% (-3.9% to -2.0% change) from 2007-2017. An assessment of the sources of these data, heterogeneity among them, or potential biases within the data are not considered. To the best of our knowledge no comprehensive systematic review has been conducted to examine changes in reported frequency measures in published literature over time.

The aim of this review was to apply a systematic approach to summarize endometriosis frequency, distribution, and stage estimates since 1989 from all areas of the world and to critically assess the studies' sampling and design when evaluating variations among those estimates.

METHODS

Protocol and registration

This study was developed in line with PRISMA guidelines and registered with the International Prospective Register of Systematic Reviews (PROSPERO).

Search strategy

A systematic computerized search was performed in four databases, including PubMed, Web of Science, EMBASE, and CINAHL for relevant manuscripts published from January 1,

1989 through June 30, 2019. Search terminologies were limited to titles containing: "endometriosis" AND "prevalence" or "incidence" or "epidemiology", or "frequency", or "occurrence", or "statistics." The searches were conducted independently by two authors (M.G., M.K.). Endnote X8 was used to retrieve full texts, organize and select studies.

Study selection, case definition and eligibility criteria

Studies were selected for eligibility based on the title and abstract and any disagreements were resolved by the third author (S.M.). Literature of interest included cross sectional and longitudinal studies in any population anywhere in the world, restricted to original research articles written in English that reported incidence and/or prevalence of endometriosis. Eligible studies meeting inclusion criteria underwent a full text review. A manual search of reference lists was also performed to identify other relevant publications. Studies with a case definition of diagnosed endometriosis based on clinical (e.g. physical exam, imaging) and/or surgical visualization with and without histologic confirmation, whether self-reported or abstracted, were included. Studies with a case definition of suspected or possible endometriosis based on symptoms without further imaging or surgical assessment were excluded. We excluded case series and case reports due to the lack of a comparison group and the absence of a denominator population. Manuscripts that did not contain empirical results, such as letters to editors, reviews, opinions/commentaries, expert committee reports, and conference abstracts were excluded. Since the research objective involved detection in humans, we excluded non-human studies.

Data extraction and quality evaluation

Relevant study characteristics and estimates abstracted included study design, publication date, study data date range, location and setting of study, population source, sample population or subpopulation, data source (e.g. insurance claims or medical records or self-report), sampling

method, age range, sample size, diagnostic or reporting criteria and method, response rate where relevant, prevalence and/or incidence when reported, and stratification by endometriosis stage when documented (Supplemental Table 2.1). Beyond stage, few studies reported data regarding symptom profile nor quantified symptom severity, and those that did were presented uniquely in form or detail that could not be harmonized nor compared and were therefore not incorporated into the systematic review. Prevalence and incidence estimates were reported with standard errors when reported. Population source was categorized as single hospital or clinic versus general population samples. General population samples were further sub-categorized as those that were drawn from nation/region-wide surveys or surveys in general public places (country/region) versus samples drawn from hospital systems and insurance claims. Study quality was assessed using a risk of bias tool is an adaptation of the GRADE criteria for prevalence studies by Hoy, et al. (2012) (Supplemental Table 2.2).[113]

Data analysis and synthesis

Studies were stratified by sampled population, and forest plots were generated to visualize the range of estimates. Studies were compared across time to examine any emerging trends and provide a narrative synthesis. All statistical analysis was conducted in R version 3.6.1[114] and meta-analyses were performed using the Metafor package for R.[115] To quantify interstudy heterogeneity, I2 was calculated and reported, with I2>75% representing high heterogeneity among studies. Estimates of pooled prevalence for various subgroups (by study setting, geographical location, and clinical indication) were reported both using fixed-effects and random-effects models. Under the fixed-effects model, study weighting was conducted using inverse variance with larger studies receiving more weight. Under the random effects model, a tau-squared measure for interstudy variation was applied to calculate the inverse variance. The

high heterogeneity observed among studies supports the preference for random-effects estimates. The Freeman-Turkey arcsine square root transformed proportions was used to normalize and stabilize the variances of prevalence estimates and calculate random effects summaries. Where applicable, pooled estimates were calculated after back-transformation using the DerSimonian and Laird procedure.[116] Meta-regression analyses were performed using mixed-effects models for proportions, examining the univariate association between prevalence and year of publication for all studies and then also stratified by general population versus single hospital/clinic population sources. A multivariable meta-regression model was applied that included year of publication (1989-2019), location of study (continent), and source population (general population versus single hospital/clinic). To examine directly if year of publication could explain part of the heterogeneity when stratified by source population, a meta-regression was conducted with year as the predictor for prevalence.

RESULTS

Study selection

A search of PubMed, Web of Science, EMBASE, and CINAHL with the above-mentioned search terms yielded 846 records (Figure 2.1). After excluding duplicates, 367 articles were screened. A total of 283 records were excluded based on relevance of the title and abstracts, with 84 full text manuscripts ultimately assessed for eligibility. After assessing the 84 full text articles, 34 articles were excluded. A search of the bibliographies of the remaining 50 articles yielded an additional 19 studies not captured in the initial search, for a total of 69 articles included in the systematic review.

Study characteristics

A total of 69 cross-sectional and cohort studies were included for qualitative synthesis in the 30-year period under review (1989-2019). Among the included studies, 16 studies reported the incidence of endometriosis [16-31] ,[19,117–131] 62 studies reported the prevalence (Table 2.1).[19,117–124,132–184] Among these 69 studies, 26 had a sampling frame from the general population, the remaining 43 were conducted in single hospital or clinic settings (Table 2.1). The largest proportion of studies was from Europe (38%) and the smallest from Australia (3%). Study sample sizes ranged from n=13 to a population of more than 14 million (Table 2.1 and Supplemental Table 2.1). 14 studies reported a population sample size ≥10,000 women. Prevalence estimates were higher in studies with smaller sample sizes (<10,000), while large studies typically reported prevalence less than 5%. However, these studies were more likely to include the adolescent population (lowest age limits ranged from 12-18) (Supplemental Table 2.1). 54 studies used a mix of case-ascertainment methods; most studies reported using laparoscopy, laparotomy, and other surgical procedure as the primary diagnostic tool or in association with ICD codes and self-reported questionnaires. Only 27 studies explicitly reported histologic verification. The remaining 15 studies reported relying on imaging findings only (e.g. ultrasound), use of diagnostic coding only, or self-reported questionnaires.

Figure 2.1 PRISMA flowchart of search strategy, screening and study selection process for systematic review.

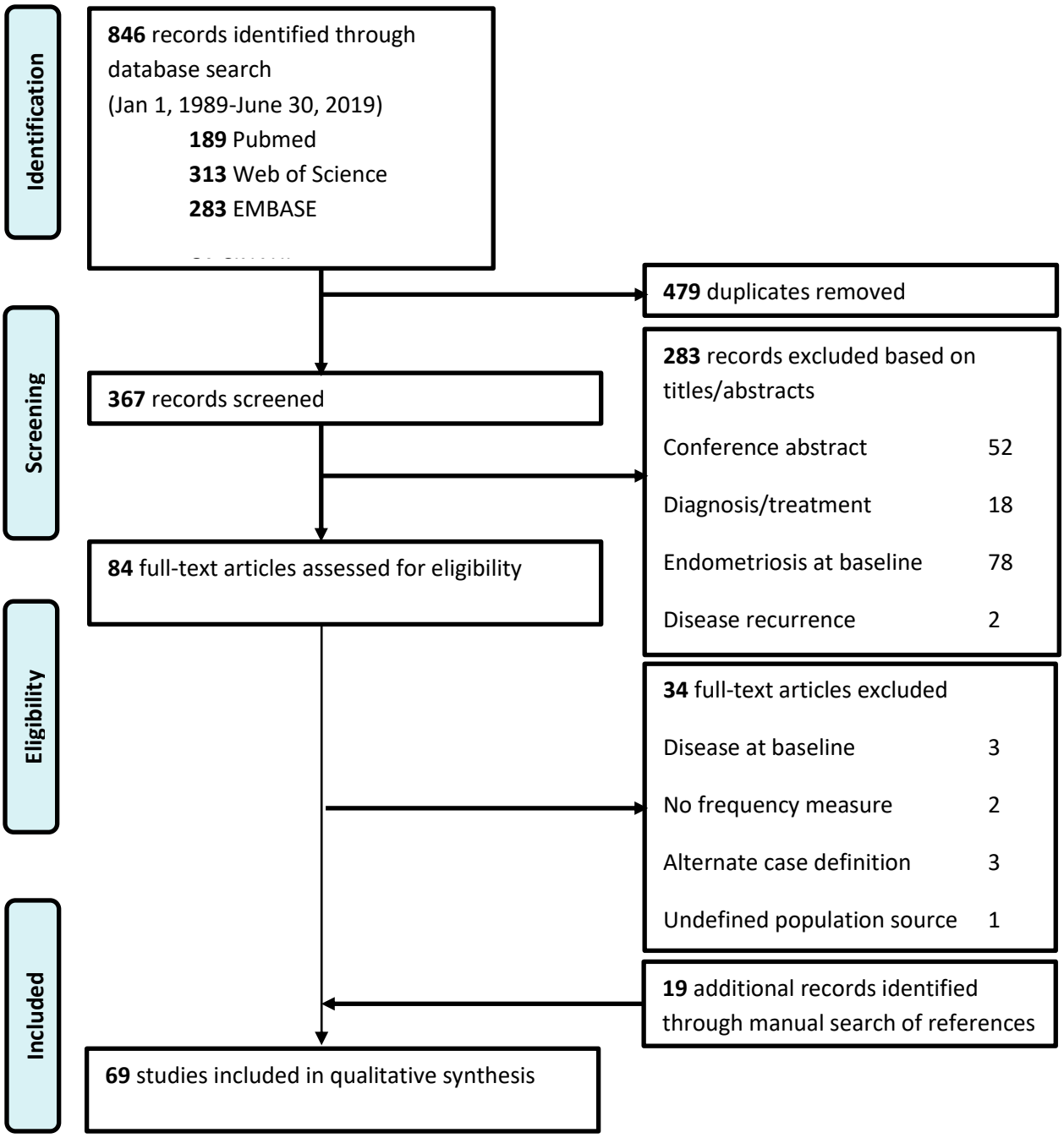


Table 2.1 Studies published from 1989-2019 reporting frequency and/or stage of endometriosis (N=69) by population source.

Population source	Number of studies (%) [Number reporting prevalence]	Range of prevalence estimates	Prevalence estimate fixed effect (95% CI)†	Prevalence estimate random effect (95% CI)†	Prevalence estimates I^2‡
General population	**26 (38%) [20]**		2.4% (2.4-2.4)	4.2% (2.2-7.0)	100.0 %
Country/region**	**9 (13%) [9]**	0.7-8.6%	4.3% (4.2-4.4)	3.4% (1.9-5.4)	99.7%
Study Population <10,000	6 [6]		2.4% (2.2-2.7)	3.3% (2.0-5.0)	94.4%
Study Population >=10,000	3 [3]		4.4% (4.4-4.5)	3.5% (1.0-7.3)	99.9%
Hospital system/insurance claims	**17 (25%) [11]**	0.8-23.2%	2.4% (2.4-2.4)	5.0% (2.1-9.1)	100.0%
Study Population <10,000	6 [4]		11.4% (11.1-11.8)	12.5% (6.0-21.0)	99.7%
Study Population >=10,000	11 [7]		2.4% (2.4-2.4)	2.2% (0.2-6.1)	100.0%
Single hospital or clinic	**43 (62%) [42]**	0.2-71.4%	15.9% (15.5-16.4)	22.9% (17.1-29.2)	99.1%
Study Population <100	6 [6]		38.0% (32.5-43.6)	42.4% (22.2-64.0)	92.6%
Study Population 100-<1000	31 [31]		24.5% (23.7-25.3)	22.9% (16.0-30.6)	98.8%
Study Population >=1000	6 [5]		8.1% (7.6-8.7)	8.0% (2.9-15.3)	99.2%
Geographic Region					
Africa (data from 1977-2017)	7 (10%) [7]	0.2-48.1%	11.0% (10.1-11.9)	10.6% (3.4-21.1)	99.0%
Americas (data from 1984-2014)*	15 (22%) [14]	0.7-69.6%	10.7% (10.7-10.8)	13.0% (9.6-16.8)	99.9%
Asia (data from 1970-2015)	19 (27%) [18]	1.0-71.4%	0.09% (0.09-0.09)	20.7% (12.1-31.0)	99.6%
Australia (data from 2012-2017)	2 (3%) [2]	3.4-3.7%	3.6% (3.2-4.1)	3.6% (3.2-4.1)	--
Europe (data from 1933-2018)	26 (38%) [21]	0.8-70.3%	7.7% (7.6-7.7)	11.5% (10.4-12.8)	99.9%

Abbreviations: CI = confidence interval

* N=14 North America, 1 South America

** Country/region: general population samples drawn from nation/region-wide surveys or surveys in general public places

† *Fixed effects model calculated using inverse variance with larger studies receiving more weight, random effects model calculated using a tau-squared measure for interstudy variation modified inverse variance (Metafor package for R 3.6.1)*

‡ I^2, measuring percent of variation among studies attributed to heterogeneity and not chance, calculated using Cochran's heterogeneity

Evaluating risk of bias within and among the 69 studies suggested moderate risk overall. Given the 10 criteria, four – likelihood of non-response bias, valid minimum prevalence time-period, acceptable case definition, and appropriate numerator and denominator for the parameter of interest – were required to be 100% present or absent by the inclusion or exclusion criteria. Among the other six risk of bias criteria, 92.8% of the 69 studies used the same mode of data collection for all participants, and the same high proportion (92.8%) of studies had a sampling

scheme that yielded a close representation of the target population. The problem then arises that that target population represented an unbiased selection of the general population in only 37.7% of studies, and some form of random selection was applied in only 29.0% of the studies.

Endometriosis Prevalence by study type and across 30 years

Overall, no clear time-trend for endometriosis prevalence across the past 30 years was observed (Figure 2.2). The meta-regression yielded that only the population source was a statistically significant predictor of prevalence (p-value <0.0001). No significant trend was observed among 'general population in country/region' studies ($\beta = 0.04$, p-value=0.12) nor among 'single hospital or clinic' ($\beta = -0.02$, p-value=0.34) studies. There was the suggestion of a decrease over time (i.e. a negative slope) when analyses were restricted to 'general population studies abstracted from health system/insurance systems' ($\beta = -0.10$, p-value=0.005).

Twenty-eight studies reported prevalence among a single gynecologic indication or subdivided a broader population by gynecologic indication, any of which may impact the likelihood of evaluation for the presence of endometriosis overall or at the specific study site (Table 2.2). Among these, 17 studies provided prevalence estimates among women with infertility or presenting for infertility treatment, yielding an overall prevalence of endometriosis of 27%, an estimate under fixed effects assumptions of 25%, and an estimate under random effects assumptions of 34%. Similarly, 11 studies provided prevalence estimates among women with chronic pelvic pain, among which there was an overall prevalence of endometriosis of 29%, an estimate under fixed effects assumptions, of 28%, and an estimate under random effects assumptions of 47%. Hysterectomy was an indication in the smallest number of studies (N=3) but included the largest number of women (N=9976), reporting a prevalence among all studies of 16%, an estimate under fixed effects assumptions of 16%, and an estimate under random effects

assumptions of 22% (Table 2.2). The overall prevalence by indication across the 30 years of publications did not suggest an increasing or decreasing trend (Figure 2.3).

Figure 2.2 Prevalence of endometriosis in studies published from 1989-2019 (N=62 studies), univariate meta-regression plot stratified by (a) prevalence in general population (b) prevalence in single clinic/hospital studies.

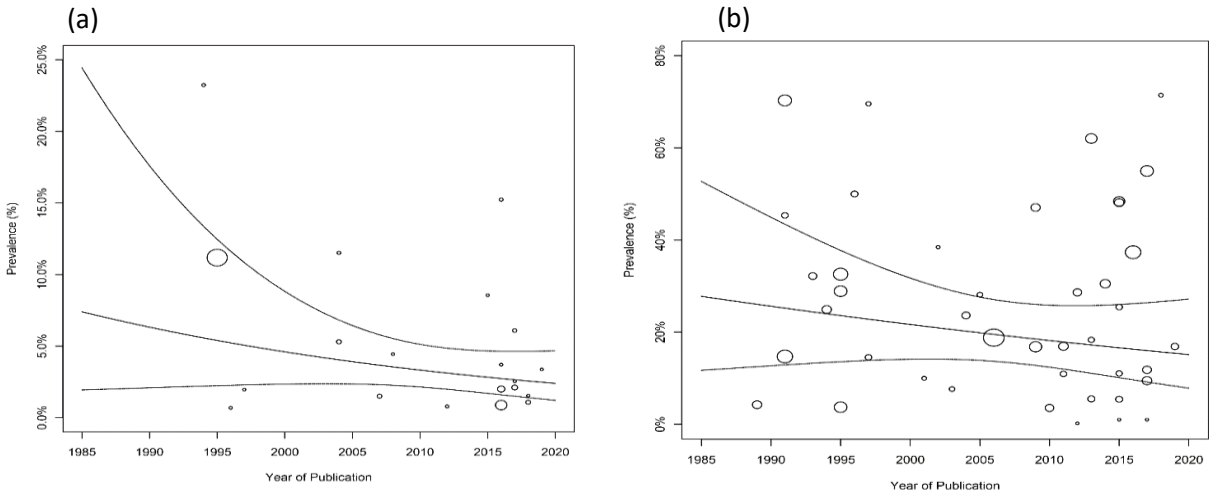


Figure 2.3 Prevalence of endometriosis in subpopulations define by gynecologic indications (infertility, chronic pelvic pain, tubal sterilization, hysterectomy, ovarian cancer) that underlie likelihood of evaluation for the presence of endometriosis (N=28 studies published 1989-2019), organized by year of publication.

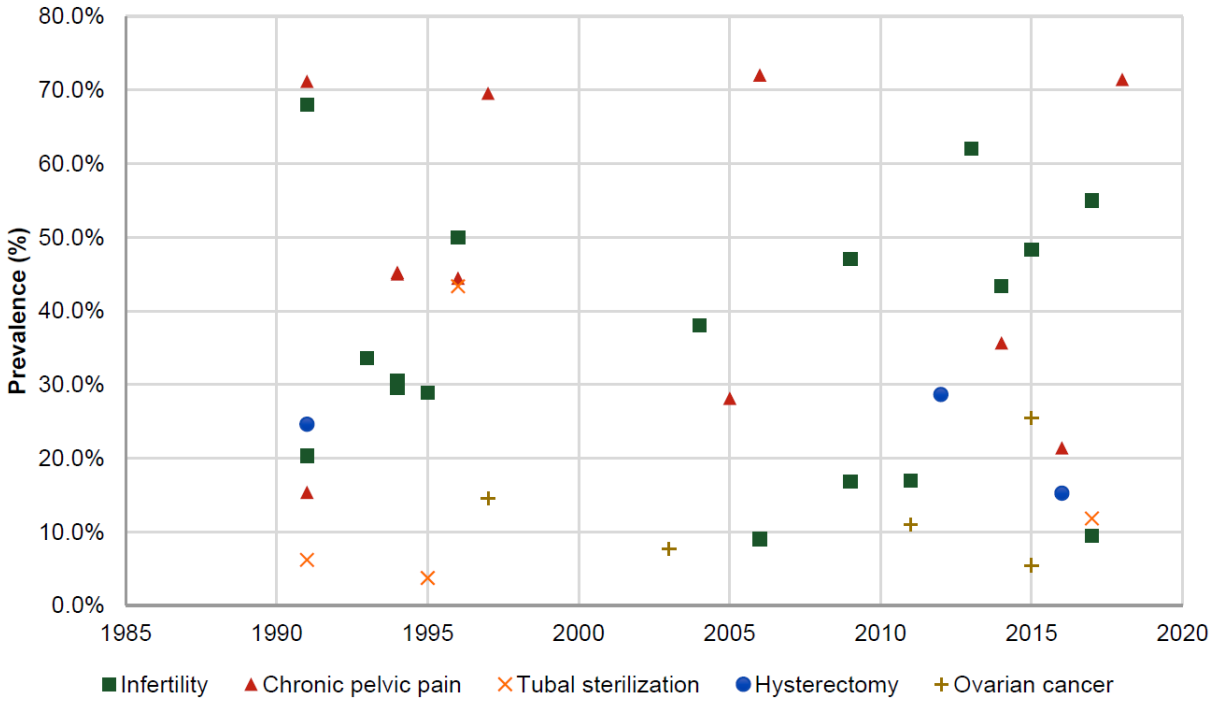


Table 2.2 Pooled prevalence estimates of endometriosis in subpopulations defined by gynecologic indications that underlie likelihood of evaluation for the presence of endometriosis, among studies published from 1989-2019.

Study characteristics	**Infertility**	**Chronic pelvic pain**	**Tubal sterilization**	**Hysterectomy**	**Ovarian cancer**
Number of studies	17	11	4	3	5
Total population	8172	5104	4477	9976	1171
Total endometriosis Cases	2193	1487	231	1561	117
Endometriosis proportion (overall)	26.80%	29.10%	5.20%	15.60%	10.00%
Range of prevalence estimates	9.0%-68.0%	15.4%-71.4%	3.7%-43.3%	15.2%-28.6%	5.4%-25.5%
Prevalence estimate: fixed effect (95% CI)†	24.8 (23.9-25.8)	28.1 (26.9-29.4)	4.4 (3.8-5.1)	15.5 (14.8-16.2)	9.3 (7.7-11.1)
Prevalence estimate: random effect (95% CI)†	34.4 (24.3-45.1)	46.6 (31.1-62.3)	10.6 (4.9-18.1)	22.2 (13.0-33.0)	11.7 (6.4-18.4)
Prevalence estimates I^2‡	98.90%	98.40%	96.00%	93.50%	89.50%

Abbreviations: CI = confidence interval

*References: Infertility [32,37-38,40,52,54,58-64,70,80,83-84], Chronic pelvic pain [32,35,37,40,54,56,58,65,70,79,80] , Tubal sterilization [37,58,76,81], Hysterectomy [58,65-66], Ovarian cancer [33,41,51,57,68]

† *Fixed effects model calculated using inverse variance with larger studies receiving more weight, random effects model calculated using a tau-squared measure for interstudy variation modified inverse variance (Metafor package for R 3.6.1)*

‡ I^2, measuring percent of variation among studies attributed to heterogeneity and not chance, calculated using Cochran's heterogeneity statistic (Q) and degrees of freedom (df): $I^2 = 100\% \times (Q - df)/Q$

Endometriosis Incidence

Among the 69 studies, 16 studies provided incidence estimates, with heterogeneous results. 7 studies provided information regarding incidence only, another 9 included additional information regarding prevalence at baseline or at a specified time-period during the study. Among these, six studies compared incidences within the sample population during different time periods.

Three studies reported no change in endometriosis incidence observed over time in populations based in Sweden,[128] Iceland[129] and the US.[120] One study based on healthcare services records for a large population in Israel reported an increase in incident endometriosis of 1.6% annually between 2000-2015.[119] Two studies reported a decrease in endometriosis incidence. One in a hospital-based population of orthodox Jewish women undergoing hysterectomies from 1970 through 1989 in Israel,[125] and the other inclusive of the Finnish Hospital Discharge

Register (FHPR) where the age-standardized incidence rate of surgically verified endometriosis decreased from 116 per 100,000 women in 1987 to 45 per 100,000 women in 2012.[130]

Two studies, Leibson et al. (2004)[120] in the US from 1987-1999 and Gylfason et al. (2010)[129] in Iceland from 1981-2000, both noted a marked increase in the use of laparoscopy over their study time-period. Leibson et al. (2004) further noted that while surgical diagnosis of endometriosis increased from 65% in 1970-1979 to 88% in 1987-1999, histologically verified diagnoses did not increase.[120,129]

Endometriosis Stage at Diagnosis

Endometriosis was staged during surgical visualization according to the revised American Fertility Society (rAFS)[185] or revised American Society for Reproductive Medicine (rASRM)[51] and reported in 26 studies (Supplemental Table 2.1). 19 studies provided staging details based on rAFS,[132,137,139,149,150,152,154,156,158–163,170,172,176,181,183] and 7 staged by the rASRM system.[126,129,134,138,140,164,167] 20 studies (77%) were either based in a single hospital or clinic or used data from a hospital or insurance system. Considerable heterogeneity among studies existed by geographic region and sample population, including age ranges, mean ages at time of staging, case definition for endometriosis, and indication for surgery (Supplemental Table 2.1). In addition, the rAFS and rASRM documented stage is reflective of one point in time and may vary over the natural history of endometriosis within each individual.

It is important to remind that endometriosis stage, although often conflated with "severity" terminology, is predictive of surgical complexity, but is not correlated with patients' symptom profile, symptom severity, nor treatment prognosis.[186] No data are available across the past 30 years to attempt to document change in presenting symptom severity, life impact, or short or long term prognosis among women with endometriosis.

DISCUSSION

As reported previously,[13,102] there continues to be large variation in prevalence estimates among studies - driven by heterogeneity in study populations, sampling scheme, endometriosis case definition, and indications for evaluation for the presence of endometriosis. In addition, all studies of endometriosis frequency document only those who successfully achieve an evaluation and diagnosis; the true frequency of undiagnosed endometriosis and its proportion among all women with endometriosis is unknown. General population studies yield an underestimate of the true prevalence of endometriosis due to diagnostics bias, while single hospital / clinic populations yield an overestimate due to selection bias.

Overall, 69 studies describing the prevalence and/or incidence of endometriosis met the inclusion criteria, of which 62 reported prevalence and 16 reported incidence or incidence rates, while 26 studies included details of endometriosis staging at the time of surgical diagnosis using the rAFS or rASRM criteria. There was no evidence for change in prevalence over time, among all women with endometriosis or when stratified by gynecologic indication for evaluation of the presence of endometriosis. Among six studies examining incidence of endometriosis across time in well-defined populations, data were highly inconsistent, with one study suggesting an increase in incidence,[119] two studies reporting a decrease,[125,130] and three studies reporting no change in incidence of endometriosis.[120,128,129]

This comprehensive review of the literature indicated that there have been very few high-quality, broadly representative cross-sectional or longitudinal studies for useful comparison among populations or across time periods. Fewer studies have examined the distribution of endometriosis by stage at surgical diagnosis. This critical limitation in research and publications

suggests that the individual or meta-analyzed estimates or plotted prevalence estimates across time cannot be used to rule in or rule out true changes in endometriosis prevalence or incidence. Beyond the limitations of the existing literature, there are fundamental issues with endometriosis diagnosis that must be overcome before a true population prevalence can be defined.[13] The lack of a non-invasive diagnostic creates insurmountable diagnostic biases driven by characteristics of those who can and those who cannot access definitive surgical or imaging diagnosis. Those with ovarian endometrioma or deep endometriosis can be diagnosed through imaging if they are geographically, economically, and socially able to achieve referral to and evaluation from an experienced imaging specialist.[187] For those with superficial peritoneal disease, definitive diagnosis via surgical evaluation is limited by severity of symptoms and response to empiric treatment, given the invasive nature and inherent risks of surgery. Even among those with adequately life impacting symptoms enough to warrant referral for a surgical evaluation, geographic and economic barriers to accessing endometriosis-focused surgeons remain. Beyond access to the appropriate skilled physician, the wide range of symptoms associated with endometriosis – many of which are stigmatized or normalized[21,63] – reduce the likelihood of referral and increase time to referral to the appropriate specialists.[13,21,188,189]

Social and cultural factors play a role in diagnostic bias as well. Black women within the US are found to have lower odds of being diagnosed with endometriosis compared to white women despite having the disease.[190] The bias in diagnosis itself may be influenced by variation in clinical symptoms among different populations not adequately captured or appreciated by standard clinical definitions, or may represent implicit bias in healthcare leading to alternate interpretation of the same symptoms affecting likelihood of diagnosis.[190] Moreover, there is considerable underrepresentation of studies from African and Asian countries compared to

European and North American populations (Table 2.1). High quality studies from these regions might alter existing global prevalence and incidence estimates, leading to more accurate overall estimates and improved public health focus. Additionally, diagnostic methods and definitions change over time, which will impact longitudinal measurements. The potential for detection bias must be considered given changing awareness of endometriosis, improved access to minimally invasive gynecologic surgery, and due to advances in imaging. It is extremely important to consider change in likelihood of diagnosis when attempting to determine true change in endometriosis incidence across time. Furthermore, it is important to consider the thoroughness of evaluation even among surgical populations. For example, endometriosis lesions may be missed during a surgery for tubal ligation that would have been observed and documented during a surgery for chronic pelvic pain. In general, studies estimating prevalence of endometriosis among highly selected populations, such as infertility centers or tertiary care hospitals, cannot be generalized more broadly. The estimates from these populations are an overestimate of the true proportion of women with endometriosis in the general population.

What are needed are studies that follow large numbers of diverse girls and women that collect data about demographic characteristics and gynecologic and other medical symptoms and experiences, including access to and interaction with the healthcare system. Several environmental and sociologic risk factors have been associated with endometriosis risk,[13] and change over time in exposure frequency and distribution could plausibly drive true changes in endometriosis incidence, as well as changes in symptom and phenotypic presentation. These may also underlie true differences among populations with respect to endometriosis prevalence. As increasing knowledge of and investment in endometriosis is made, it is essential to prioritize improved and unbiased quantification of endometriosis prevalence and incidence.

CHAPTER 3 IDENTIFYING SYMPTOM-BASED SUBGROUPS OF PELVIC PAIN USING LATENT CLASS ANALYSIS: ASSOCIATIONS WITH ENDOMETRIOSIS AND COMORBIDITIES

ABSTRACT

Chronic pelvic pain has a highly heterogenous symptom presentation, with variability that may be linked to clinically informative subgroups. We investigated subgroups of pelvic pain symptoms based on clustering patterns and their association with comorbidities related to inflammation and chronic pain. We included 1255 participants from the Women's Health Study: Adolescent to Adulthood (A2A) cohort, which oversampled for adolescents and surgically confirmed endometriosis cases. We conducted a latent class analysis (LCA) consisting of six indicators: menstruation associated (cyclic) pelvic pain severity, frequency, and impact on daily activities and non-menstruation associated (acyclic) pelvic pain severity, frequency, and impact on daily activities. The 3-step approach LCA was conducted to examine the associations between latent class membership, demographic, and clinical variables, and eighteen comorbidities, ten of which had prevalence of 10% or more in the population. We identified five subgroups (classes 1-5), consisting of a "no pelvic pain" subgroup, and four pelvic pain subgroups. Endometriosis cases appear, in varying proportions, ranging from 4% of in the "no pelvic pain subgroup" to 24%, 72%, 70%, and 94% respectively in the four pain subgroups, further evidence of its heterogeneity. Migraine headache was the only condition associated with greater odds of membership in all four pelvic pain subgroups relative to those with no pelvic pain (aOR=2.62, 95% CI=1.38,4.99 to aOR=7.78, 95% CI=4.82, 12.58). Subgroup associations with comorbid inflammatory and pain conditions suggest one condition can trigger another or shared underlying etiologies.

INTRODUCTION

Continuous or episodic chronic pain in the pelvis and lower abdomen lasting more than 6 months is associated with significant morbidity among women. Worldwide, 17% to 81% of reproductive aged women report pelvic pain associated with menstruation (cyclic), while 2% to 24% report pelvic pain that is not associated with menstruation, intercourse, or pregnancy (acyclic).[1] Among adolescents, the prevalence of cyclic pelvic pain ranges from 30 to 90%.[59,60]

Chronic pelvic pain is multifactorial, and caused by tissue injury, inflammation, ad neuropathic pain. Contributors to chronic pelvic pain include a range of inflammatory and pain conditions related to the reproductive system conditions, such as endometriosis, and non-gynecologic conditions, such as irritable bowel syndrome.[2] The variation in the frequency measures of chronic pelvic pain and associated disorders points to gaps in foundational knowledge regarding physiology and classification of heterogeneity. In endometriosis, for example, the clinical presentation of the disease is often different in adolescents than in adult women. For instance, adolescents more often have pelvic pain presenting with nausea than adults.[191] Furthermore, the degree of Reproductive Medicine (rASRM), which stage disease based on the presence and size of endometriosis lesions and adhesions in the abdominal cavity.[9,191,192] Similarly, histopathologic staging does not correlate well with symptoms including pain or comorbidities such as infertility, treatment response, or prognosis.[55–57] In other diseases such as breast cancer and ovarian cancer, defining distinct subgroups based upon clinical characteristics, histologic and genetic differences has led to novel insights into treatment options and risk factors.[193–196] Pelvic pain may similarly benefit from characterizing patterns of symptomology, or subgroups, and how they relate to underlying disease processes such as endometriosis.

The purpose of the current study was to identify symptom-based subgroups of chronic pelvic pain and uncover associations of the subgroups with eighteen comorbidities related to inflammatory and chronic pain. To achieve this goal, we used baseline data from a longitudinal cohort of adolescents and young adults with deeply-phenotyped pain symptomology. We identified groups of similar symptomatology with latent class analysis (LCA), a cross-sectional latent variable mixture modelling approach that clusters people with similar characteristics to uncover hidden clinical phenotypes.[91] While several studies have used LCA in the context of chronic pain, we identified only two prior studies that have used an LCA approach in pelvic pain, specifically for vulvar pain[197] and dysmenorrhea.[98]

METHODS

Study population

We used cross-sectional data completed at the point of enrollment into The Women's Health Study: From Adolescence to Adulthood (A2A) (N=1255), a prospective cohort study that enrolled females aged 7-55 years between November 2012 to June 2018 (A2A consist of N=1549 females, of which 81% met the inclusion criteria for this study). Participants were recruited from two tertiary care centers and surrounding communities in Boston, Massachusetts, USA using in-clinic eligible patient identification and hospital-catchment community advertising and word of mouth.[191] The population was oversampled for adolescents and individuals who had been diagnosed with endometriosis during a surgical procedure. Participants were excluded from the analysis did not complete the baseline questionnaire (N=239), had never menstruated (N=21), those whose menstrual status was unreported (N=6), and those missing all variables used to derive subgroups (N=28). Study participants completed a questionnaire upon enrollment on socio-demographic (e.g. age, race and ethnicity) and clinical characteristics (e.g. age at

menarche, weight, medication use) that was followed up with yearly questionnaires. Cyclic and acyclic pelvic pain were assessed using questions from an expanded form of the validated WERF EPHect Endometriosis Patient Questionnaire (EPQ).[49] The initial version of the baseline questionnaire assessed demographics, body mass index (BMI), physical activity, diet, smoking, alcohol consumption, reproductive factors, and other medical conditions as well as details on pain symptoms, treatment regimen, and medication use. In January 2014, an expanded version of the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonization Project (EPHect) [43] clinical questionnaire was adopted for use at baseline, although there was very little change in the questionnaire with the vast majority of the questions being the same.

Pelvic pain symptom indicators

Six pelvic pain variables from the questionnaire were used as indicators, defined as observed variables that are used to derive latent subgroups. Indicators for severity, frequency, and life impact were collected for both acyclic and cyclic pelvic pain. Acyclic pelvic pain was defined as worst pelvic/lower abdominal pain not caused by menstrual cramps, intercourse, surgery, pregnancy, or other injury and infections. Its severity and frequency measured over the last 3 months (survey from January 2014 onward) or last 12 months (survey prior to January 2014). Life impact was measured over the last 3 months. Cyclic pelvic pain was defined as dysmenorrhea or cramping, shooting, or stabbing pain that occurs during menses in the past 12 months. Continuous numerical rating scale (NRS) variables associated with severity for both acyclic and cyclic pelvic pain were converted to 3-level ordered categorical variables: 0-3 “none/mild pain,” 4-6 “moderate pain,” 7-10 “severe pain.”[47] Frequency for acyclic pelvic pain consisted of 3 categories: “no pain or <1 day/month or monthly but not weekly”, “weekly” and

"everyday". Frequency for cyclic pelvic pain consisted also of 3 categories: "never or occasionally", "often or usually" and "always". Measures of life impact, defined as the impact on carrying out daily activities such as work and school, were collapsed into a binary yes/no indicator for both acyclic and cyclic pelvic pain. A summary of pelvic pain indicators including analytic categories and data sources from which each variable was derived, is provided in Supplemental Table 3..

Demographics and clinical characteristics

Questionnaire items included age at completion of baseline survey (continuous), recruitment site (clinic-based, population-based), age at menarche (continuous), date of last menstrual period, self-reported weight and height was used to calculate body mass index categories (defined by WHO BMI categories for those ≥20 years, and CDC age and sex specific Z-score for <20 or less: underweight: BMI<18.5 kg/m^2 or Z-score≤-2, normal weight: BMI 18.5-24.9 kg/m^2 or Z-score is >-2 to <1, overweight: BMI 25-29.9 kg/m^2 or Z-score 1 to 2, obese: BMI≥30 or Z-score >2) and self-identified US-census socially-defined groups: race (Black, White and other racial categories consisting of Asian, American Indian/Alaska Native, Native Hawaiian or Pacific Islander, multiracial, other race and unknown/not reported) and ethnicity (Hispanic/Non-Hispanic).

Endometriosis and comorbidities

Participants were asked to self-report a range of comorbidities diagnosed by a physician, or self-report specific pain comorbidities.[198] Eighteen comorbid conditions related to inflammation and pain with greater than 10 cases included in the current analyses reported a diagnosis. These included surgically-diagnosed endometriosis (over-sampled at enrollment by cohort design with prevalence in the analytic sample of 48%), other gynecologic or genitourinary

conditions: fibrocystic or benign breast disease, painful bladder or interstitial cystitis, uterine fibroids, ovarian cysts, and polycystic ovarian syndrome; respiratory immune conditions: allergies (i.e. grasses, pollens, mold, food, latex, drugs, animals and other) and asthma; rheumatologic and neurological conditions: fibromyalgia, chronic fatigue syndrome, rheumatoid arthritis, migraine, lower back pain, muscle or joint pain unrelated to infections or sports injuries and leg pain; gastrointestinal or abdominal conditions: inflammatory bowel disease (Crohn's disease or ulcerative colitis), irritable bowel syndrome and non-pelvic abdominal pain. One version of the survey prior to 2014 (completed by N=276 participants) did not have questions assessing the presence of painful bladder/interstitial cystitis, and the short-form version of the WERF EPHect compliant survey, sent to participants who did not complete the survey after three follow up attempt (N=59), did not have questions assessing the presence of allergies, fibrocystic or benign breast disease, fibromyalgia, leg pain, lower back pain, muscle/joint pain, non-pelvic abdominal pain or rheumatoid arthritis. Participants who were missing information on a given comorbidity were dropped from the association analysis for that comorbidity. Of 18 conditions, 10 comorbid conditions had a prevalence of 10% or more in the population and were included in the final association analysis to minimize empty cells and unstable estimates and confidence intervals. Data cleaning and descriptive analysis, consisting of proportions for categorical variables and means and 95% confidence intervals or medians and 25% and 75% percentiles for continuous variables, was conducted in R (version 4.0.2).[199]

Missing data procedures

Strategies for dealing with missing indicators varied according to the underlying structure of missingness. Participants who had menstrual periods in the past year but did not provide answers to questions regarding acyclic or cyclic pelvic pain were considered missing at random

(MAR). However, a portion of the cohort was missing values for cyclic measures because they had not menstruated in the past 12 months – primarily due to hormonal ovarian suppression (see the flow chart in Supplemental Figure 3.1). This group was considered missing not at random (MNAR) as their missingness may have been conditioned on the severity of their dysmenorrhea or acyclic pain symptoms. For this group, missing cyclic pelvic pain variables were forward filled from historical age range data, where available (N=73 values for cyclic pain severity, 74 for cyclic pain frequency, and 203 for cyclic pain life impact) (Supplemental Table 3.2). To evaluate the impact of forward filling values, we conducted a sensitivity analysis with an LCA that excluded N=170 who had not menstruated in the past 12 months or whose date of last menstruation was unknown. The remaining missing indicators in the model were handled using the full information maximum likelihood (FIML) approach. FIML uses the information available for each individual to maximize the sample log-likelihood function for estimating parameters and standard errors, under the assumption that the indicators are missing at random.[200] The FIML procedure in LCA does not address missingness in non-indicator variables external to the latent class model. Therefore, in the association analyses between latent classes and other variables, participants who were missing demographic and clinical or comorbidity variables were excluded from 3-step approach LCA. Questions regarding history of pelvic pain across age ranges up to baseline were only available in the WERF EPHect compliant version, which consisted of 73.3% of surveys and consequently completed by 58.1% of those with endometriosis and 87.1% of those without.

Latent class analysis

Latent class analysis (LCA) was used to identify subgroups of women with similar pelvic pain characteristics based on six indicators of pelvic pain. LCA uses the EM (expectation-

maximization) algorithm to produce maximum likelihood estimates of model parameters, including identifying the typologies (i.e. subgroups of people who are alike) within a population.[88,201,202] We selected this method, rather than other distance-based clustering techniques (e.g. k-means clustering), as probability-based techniques provide robust parameter estimates under latent variable framework and take into consideration measurement errors of the indicators.[203,204] Latent class analysis was conducted in Mplus (version 8.6).[205]

We determined the optimal number of latent classes based on five criteria in step-wise order:[88] The first and primary criterion was statistical fit, measured via adjusted Bayesian information criterion (aBIC), followed by Bayesian information criterion (BIC), and Akaike information criterion (AIC) and likelihood ratio test methods: Bootstrapped Likelihood Ratio test (BLRT) and Vuong-Lo-Mendell-Rubin test (VLMRT).[206] Lower aBIC, BIC and AIC were preferred, and BLRT and VLMR-LRT p-values <0.05 (rejecting the null hypothesis that a model with one less class is sufficient compared to the current model). The second criterion was homogeneity, suggesting that all individuals in a latent class provide a similar response pattern on indicators. Conditional response probability showed the quality of indicators in measurement, which ideally should be 0 or 1, but is often not the case in empirical data. In our study, we used 0.5 as a threshold for conditional response probabilities. The third criterion, class separation, is measured by entropy, with values close to 1 indicating clear class separation.[207] We also looked at variable-specific entropy contribution, which does not have a cut-off criteria but was used to compare the indicators' ability to separate classes.[208] Our fourth criterion, local conditional independence, indicates that within a given class any correlation between two indicators is explained solely through the latent class structures, which may reflect underlying biology or other latent constructs in the sampled population. This was measured by calculating bivariate

residuals, where less than 1%-5% of residuals having significant values (>|1.96|) suggest a good model.[209] The fifth criterion was the qualitative interpretability of the emergent classes based on domain-specific knowledge.[202]

We tabulated proportions of the study population in each latent class-defined subgroup based on estimated posterior probabilities. We estimated pelvic pain item response probabilities, which are proportions endorsing each category of a variable conditional on class membership. Conditional response probabilities were examined to assign labels or pain type subgroups to facilitate interpretation. We conducted descriptive statistics of demographics and clinical covariates and comorbidities across classes. The estimates were based on most-likely latent class membership of each individual in each class, which does not consider uncertainty around the estimated posterior probabilities of latent class assignment for each individual .[88] We conducted a sensitivity analysis using the same methods but restricting the population to adolescents only, defined as those 12-24 years of age, to examine if the subgroups derived and associations would be different compared to full sample population.[210]

Association analyses

To examine the relationships between comorbidities and the subgroups of pelvic pain, a bias-adjusted three step-approach was used for LCA with covariates. This approach estimates the measurement model (i.e. LCA without covariates), assigns latent class membership to participants, and associates the class to an external variable accounting for classification uncertainty.[89,211,212] Demographic, clinical variables, surgically-confirmed endometriosis status, and other comorbidities were tested as predictors of class membership using multinomial logistic regression via the R3STEP procedure which incorporates the most likely latent class indicator variable and uncertainty rates associated with each (Mplus 8.6).[89] We estimated unadjusted and

age-adjusted odds ratios. We adjusted for age because age is associated with changes in pain perception and is a risk factor for endometriosis and a number of comorbidities included in the analysis.[213]

RESULTS

Sample characteristics

A total of 1255 participants were included in the analytic population. Overall, participants predominantly identified as White (81.0%) or Black (4.3%) (Table 3.1). Participants ranged from 12-55 years of age (median=23 years, 37.3% younger than 21 years). Approximately 47.6% had surgically diagnosed endometriosis (N=597). Those with surgically diagnosed endometriosis were younger (median age=19 years compared to 24 years among those who did not have an endometriosis diagnosis). Those with endometriosis also were more likely to have been recruited from clinics (96.7% versus 13.7%) and fewer menstruated in the last 12 months (78.1% versus 94.0%) compared to those not diagnosed with endometriosis.

The most prevalent comorbidities were lower back pain (69%), migraine (49%), non-pelvic abdominal pain (46%), muscle or joint pain unrelated to infections or sports injuries (39%), and allergies (33%). The prevalence of 17 of 18 comorbidities was higher among those with surgically diagnosed endometriosis; the one exception was PCOS with 2.5% prevalence among those with endometriosis compared to 5.2% in those without. The median number of comorbidities for participants was 3 (range 0-14). Those diagnosed with endometriosis had on average 5 comorbidities versus 2 for those without an endometriosis diagnosis.

Table 3.1 Baseline characteristics of all study participants (N=1255), and the cohort without endometriosis diagnosis (N=658) and with endometriosis (N=597)

	Total number of participants (N=1255)	No endometriosis diagnosis[1] (N=658)	Endometriosis diagnosis[1] (N=597)
Age at completion of baseline survey			
Mean (SD)	23.4 (7.2)	25.7 (6.7)	20.9 (6.8)
≤15 years	121 (9.6%)	11 (1.7%)	110 (18.4%)
16-20 years	348 (27.7%)	96 (14.6%)	252 (42.2%)
21-30 years	617 (49.2%)	438 (66.6%)	179 (30.0%)
31-40 years	125 (10.0%)	81 (12.3%)	44 (7.4%)
≥41 years	44 (3.5%)	32 (4.9%)	12 (2.0%)
Age at menarche[2]			
Mean (SD)	12.1 (1.5)	12.4 (1.4)	11.8 (1.4)
<10 years	53 (4.2%)	17 (2.6%)	36 (6.0%)
10-11 years	356 (28.4%)	150 (22.8%)	206 (34.5%)
12-13 years	649 (51.8%)	353 (53.7%)	296 (49.6%)
>13 years	196 (15.6%)	137 (20.9%)	59 (9.9%)
Body mass index[2,3]			
Underweight	34 (2.71%)	26 (3.96%)	8 (1.3%)
Normal weight	800 (63.8%)	433 (65.9%)	367 (61.5%)
Overweight	282 (22.5%)	134 (20.4%)	148 (24.8%)
Obese	138 (11.0%)	64 (9.7%)	74 (12.4%)
Race[4]			
Black	54 (4.30%)	39 (5.93%)	15 (2.5%)
White	1017 (81.0%)	475 (72.2%)	542 (90.8%)
Other and Unknown	184 (14.7%)	144 (21.9%)	40 (6.70%)
Hispanic ethnicity[4]			
Hispanic	97 (7.8%)	56 (8.6%)	41 (7.0%)
Non-Hispanic	1140 (92.1%)	596 (91.4%)	544 (92.8%)
Source of enrollment			
Clinic based	667 (53.1%)	90 (13.7%)	577 (96.7%)
Non-clinic based	588 (46.9%)	568 (86.3%)	20 (3.4%)
Hormone medication use[2,5]			
Never	238 (19.0%)	219 (33.3%)	19 (3.2%)
Ever	1016 (81.0%)	438 (66.7%)	578 (96.8%)
Ever used for birth control	440 (44.9%)	332 (56.2%)	108 (27.8%)
Ever used for pain	389 (39.7%)	100 (16.9%)	289 (74.5%)
Pain medication use[2,6]			
Never	732 (61.6%)	487 (76.2%)	245 (44.6%)
Less than 2 days per week	102 (8.6%)	48 (7.5%)	54 (9.8%)
2 or more days per week	354 (29.8%)	104 (16.3%)	250 (45.5%)

Table 3.1 (cont'd)

	Total number of participants (N=1255)	No endometriosis diagnosis[1] (N=658)	Endometriosis diagnosis[1] (N=597)
Date of last menstrual period (LMP)			
Within the last 3 months	910 (72.5%)	554 (84.2%)	356 (59.6%)
3-6 months ago	122 (9.7%)	47 (7.1%)	75 (12.6%)
6-12 months ago	53 (4.2%)	18 (2.7%)	35 (5.9%)
>12 months ago	164 (13.1%)	36 (5.5%)	128 (21.4%)
Not in last 3 months, LMP unknown	6 (0.5%)	3 (0.5%)	3 (0.5%)
Comorbid conditions[7,8]			
Gynecologic/genitourinary conditions			
Fibrocystic /benign breast disease	10 (0.8%)	4 (0.6%)	6 (1.1%)
Painful bladder/interstitial cystitis	10 (1.1%)	3 (0.5%)	7 (2.0%)
Uterine Fibroids	22 (1.8%)	10 (1.5%)	12 (2.02%)
Ovarian cysts	147 (11.8%)	20 (3.1%)	127 (21.5%)
Polycystic ovarian syndrome (PCOS)	49 (3.9%)	34 (5.2%)	15 (2.5%)
Respiratory/immune conditions			
Allergies	389 (32.7%)	171 (26.8%)	218 (39.7%)
Asthma	280 (22.5%)	115 (17.5%)	165 (28.0%)
Rheumatologic/neurologic conditions			
Fibromyalgia	16 (1.35%)	5 (0.8%)	11 (2.0%)
Chronic fatigue syndrome (CFS)	14 (1.1%)	2 (0.3%)	12 (2.0%)
Rheumatoid arthritis	12 (1.0%)	5 (0.8%)	7 (1.3%)
Migraine	610 (48.6%)	231 (35.1%)	379 (63.5%)
Lower back pain	811 (69.0%)	370 (58.6%)	441 (81.1%)
Muscle/joint pain[9]	448 (38.5%)	195 (31.0%)	253 (47.2%)
Leg pain	355 (30.6%)	146 (23.2%)	209 (39.4%)
Gastrointestinal/abdominal conditions			
Inflammatory bowel disease: Crohn's disease or ulcerative colitis	14 (1.1%)	5 (0.8%)	9 (1.5%)
Irritable bowel syndrome (IBS)	148 (11.8%)	47 (7.2%)	101 (17.0%)
Non-pelvic abdominal pain	543 (46.3%)	178 (28.2%)	365 (67.3%)
Number of comorbidities (18 total)			
Mean (SD)	3.57 (2.34)	2.34 (1.75)	4.91 (2.15)
Median [Min, Max]	3.00 [0, 14.0]	2.00 [0, 9.00]	5.00 [1.00, 14.0]

*Abbreviations SD: standard deviation

[1] 'No endometriosis diagnosis' (comparison) defined as female participants without a known diagnosis of endometriosis; 'Endometriosis diagnosis' (cases) defined as participants with surgically confirmed endometriosis

[2] Number of participants missing information for each characteristic variable: age at menarche (1 total: 1 comparison), body mass index (1 total: 1 comparison), hormonal medication use (1 total: 1 comparison), pain mediation use (67 total: 19 comparisons, 48 cases)

[3] Underweight for age <20 (z-score >-9 to <=-2), age ≥20 (>0 to < 18.5); normal weight for age <20 (z-score >-2 to <1), age ≥20 (>=18.5 to <25); overweight for age <20 (z-score >=1 to <=2), age ≥20 (>=25 to <30); obese for age <20 (z-score >2), age ≥20 (>=30)

[4] Black participants include Hispanic (N=10) and Non-Hispanic (N=42), White participants include Hispanic (N=50) and Non-Hispanic (N=954); Other and unknown category participants are Asian (93 total: 91 comparison, 2 cases), American Indian/Alaska Native (1 total: 1 case), Native Hawaiian or pacific islander (1 total: 1 comparison), multiracial (57 total: 36 comparison, 21 cases), other race (25 total: 11 comparison, 14 case), unknown (7 total: 5 comparison, 2 cases), including Hispanic (N=37) and Non-Hispanic (N=144), missing Hispanic ethnicity status (N=18)

Table 3.1 (cont'd)

[5] Lifetime use of hormone medication, including birth control pills, patches, rings, injections, implants, hormonal intrauterine device, for any reason including but not limited to acne, bad cramping, irregular periods, birth control, fertility treatments.
[6] Current regular use of pain medications including acetaminophen, non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, celecoxib, rofecoxib, naproxen, mefenamic acid, ketorolac), opioid analgesics (e.g. hydrocode-combination, oxycodone with acetaminophen, oxycodone HCL, acetaminophen with codeine, codeine, morphine)
[7] Four versions of the survey were administered over the enrollment of the cohort, with distributions of cases and comparison group varying over each version: survey prior to January 2014: version 1 (155 total: 9.0% comparison, 91.0% cases); survey prior to January 2014: version 2 (121 total: 43.8% comparison, 56.2% cases), survey from January 2014 onward: version 3 (920 total: 62.2% comparison, 37.7% cases), survey from January 2014 onward: version 4 short-form (59 total: 30.5% comparison, 69.5% cases) contained no questions regarding allergies, fibrocystic or benign breast disease, fibromyalgia, leg pain, lower back pain, muscle/joint pain, non-pelvic abdominal pain, rheumatoid arthritis
[8] Number of participants missing information for each comorbid condition is as follows: Allergies (67 total: 19 comparisons, 48 cases), Asthma (8 total: 1 comparisons, 7 cases), CFS (8 total: 1 comparisons, 7 cases), Crohn's or ulcerative colitis (5 total: 1 comparisons, 4 cases), Fibrocystic or benign breast disease (67 total: 19 comparisons, 48 cases), Fibromyalgia (67 total: 19 comparisons, 48 cases), IBS (5 total: 1 comparisons, 4 cases), Leg pain (94 total: 28 comparisons, 66 cases), Lower back pain (80 total: 27 comparisons, 53 cases), Muscle/joint pain (90 total: 29 comparisons, 61 cases), Non-pelvic abdominal pain (81 total: 26 comparisons, 55 cases), Ovarian cysts (9 total: 2 comparison, 7 cases), Painful bladder/interstitial cystitis (337 total: 85 comparison, 252 cases), PCOS (8 total: 1 comparisons, 7 cases), Rheumatoid arthritis (67 total: 19 comparisons, 48 cases), Uterine fibroids (5 total: 1 comparisons, 4 cases)
[9] Muscle/joint pain is defined as those unrelated to infections/sports injuries

Table 3.2 Indicator item response proportions conditional on class for 5-class model of pelvic pain symptomology

	Overall	Class 1 (22.6%)	Class 2 (27.7%)	Class 3 (20.3%)	Class 4 (8.5%)	Class 5 (21.0%)
Qualitative description of subgroups		**No pelvic pain**	**Moderate cyclic pain only**	**Severe cyclic pain only**	**Severe acyclic, moderate cyclic pain**	**Severe acyclic, severe cyclic pain**
Acyclic pelvic pain: Severity[1]						
None/mild	0.68	0.97	0.99	0.88	0.04	0.01
Moderate	0.09	0.02	0.02	0.10	0.44	0.10
Severe	0.23	0.01	0.00	0.02	0.53	0.89
Acyclic pelvic pain: Frequency[2]						
No pain	0.78	0.99	0.99	0.97	0.44	0.20
Weekly	0.14	0.01	0.01	0.02	0.46	0.43
Everyday	0.09	0.00	0.00	0.01	0.10	0.37
Acyclic pelvic pain: Life impact[3]						
No	0.75	0.97	1.00	0.98	0.35	0.08
Yes	0.25	0.01	0.00	0.04	0.65	0.92
Cyclic pelvic pain: Severity[4]						
None/mild	0.26	0.83	0.18	0.00	0.22	0.00
Moderate	0.25	0.17	0.56	0.07	0.38	0.04
Severe	0.49	0.00	0.26	0.93	0.41	0.96
Cyclic pelvic pain: Frequency[5]						
Never/occasionally	0.34	0.99	0.22	0.01	0.33	0.00
Often/usually	0.26	0.01	0.56	0.18	0.52	0.09
Always	0.40	0.00	0.22	0.81	0.16	0.91
Cyclic pelvic pain: Life impact[6]						
No	0.70	0.99	0.92	0.39	0.80	0.23
Yes	0.30	0.01	0.08	0.61	0.20	0.77

[1] Acyclic pelvic pain severity reported on 0-10 numeric rating scale: none/mild= 0-3; moderate=4-6; severe 7-10, in the past 3 months (survey from January 2014 onward) or past 12 months (survey prior to January 2014)

[2] Acyclic pelvic pain frequency in the last 3 months (survey from January 2014 onward) or 12 months (survey prior to January 2014): no pain='no pain in the past 3 months/12 months', <1 day/month', 'one day a month', 'two to three days a month', weekly='one day/week', '> one day/week'; everyday='every day')

[3] Acyclic pelvic pain life impact: yes= frequently interferes (survey prior to January 2014) or moderately-extremely interferes (survey from January 2014 onward) with normal social activities with 'work or school' or 'activities at home'

Table 3.2 (cont'd)

[4] Cyclic pelvic pain severity reported on 0-10 numeric rating scale: none/mild= 0-3; moderate=4-6; severe 7-10; severity of usual period pain (survey prior to January 2014), period pain in the past 12 months (survey from January 2014 onward), severity at last period in past 3 months if last 12 months missing (survey from January 2014 onward), for non-menstruating in past 12 months severity in current age range or if not available in the previous age range (age range defined as <15, 16-20, 21-30, 31,40, >41) (survey from January 2014 onward)

[5] Cyclic pelvic pain frequency: never/occasionally= 'never', 'occasionally (less than a quarter of my periods); moderate='often', 'usually'; always= 'always' (reported in survey from January 2014 onward only), for non-menstruating in the past 12 months, frequency in current age range or if not available in the previous age range (survey from January 2014 onward)

[6] Cyclic pelvic pain life impact: yes= 'pain that prevents from going to work or school or carrying out daily activities (even if taking pain-killers)' available only for the last period, if last period during the last 3 months (in survey from January 2014 onward only), for non-menstruating in the past 3 months, life impact at current age range, or if not available life impact at the previous age range (survey from January 2014 onward)

Latent subgroups

According to the five criteria consisting of information criteria and test statistics of 2-class to 9-class LCA, the five-class model was considered optimal (Supplemental Table 3.3). The class distributions (class 1 to 5) reflected an ordering of groups from 'no pelvic pain' (class 1) to 'severe acyclic, severe cyclic pelvic pain' (class 5) (Table 3.2). Class 1 ('no pelvic pain') was a subgroup with no acyclic pelvic pain experienced by 97% of class members and no cyclic pelvic pain experienced by 83% of class members. Class 2 ('moderate cyclic pelvic pain only') was a subgroup where 56% individuals experienced moderate cyclic pelvic pain associated with periods and 99% experienced no acyclic pelvic pain and 92% experienced no life impact because of this pain. Class 3 ('severe cyclic pelvic pain only') was a subgroup where, like class 2, members experienced only cyclic pelvic pain with 88% experiencing no acyclic pelvic pain. However, unlike class 2, 93% of class 3 experienced severe cyclic pelvic pain. Class 4 ('severe acyclic, moderate cyclic pelvic pain') was a subgroup that, similar to class 2, experienced cyclic pelvic pain with low life impact. However, in class 4, 53% were characterized by severe acyclic pelvic pain (53%), and life impacted by acyclic pelvic pain (65%). Finally, class 5 ('severe acyclic, severe cyclic pelvic pain') consisted of individuals experiencing the most severe categories for five of six pelvic pain indicators. The sociodemographic characteristics (age and race) of individuals assigned to the five classes were largely comparable. Some clinical characteristics were also comparable for age at menarche and body mass index. However, clinical characteristics like the date of last menstruation and medication use varied widely between groups (Supplemental Table 3.4).

A sensitivity analysis excluding those who had not menstruated in the past 12 months (remaining sample size N=1085) showed a 5-class model, very similar to the groups described

above, to be the best fitted model (Supplemental Table 3.5). A sensitivity analysis including only adolescents 12-24 years of age (N=828) showed a 3-class model to be the best fitted model in this subset (Supplemental Table 3.6). Examining how individuals were assigned in the main analysis versus the adolescent-only restricted group, 99% of participants assigned to labeled Class 'Adolescent A' were drawn from severe acyclic, moderate cyclic (class 4) and severe cyclic and acyclic pelvic pain group (class 5). Class 'Adolescent B' was 99% participants assigned in the full model to cyclic pain-only groups: moderate cyclic pain only (class 2) and severe cyclic pain only (class 3). 96% of Class 'Adolescent C' consisted of those previously assigned to the no pelvic pain class (class 1), and a portion of those with moderate cyclic pain only (class 2) (Supplemental Table 3.7 and Supplemental Table 3.8).

Unadjusted associations of pain-variable defined subgroups with covariates are described in Supplemental Table 3.9. Higher age at completion of the survey was associated with lower odds of being in the 'severe acyclic, severe cyclic pelvic pain' subgroup (class 5) (OR=0.88, 95% CI=0.83,0.94) compared to the reference no pelvic pain subgroup (class 1). Higher age at completion of survey also was associated with lower odds of being in the severe cyclic pain only subgroup (class 3) (OR=0.89, 95% CI=0.84,0.93) compared to the no pelvic pain subgroup (class 1). Similarly, higher age at menarche was associated with lower odds of being in class 5 (OR=0.67, 95% CI=0.58,0.77) and class 3 (OR=0.67, 95% CI=0.58,0.78) compared to class 1. Neither BMI nor Black/White racial categories was significantly associated with being in any of the classes.

Association between comorbidities and derived latent classes

The distribution of surgically diagnosed endometriosis ranged from 4.0% in class 1 to 94.1% in class 5 (Supplemental Table 3.4). Having surgically diagnosed endometriosis was

quantitively the strongest predictor of being in the three subgroups marked by severe pain (Table 3.3). Migraine was the only condition associated with probability of being in all four pain subgroups compared to the no pain group, ranging from the lowest magnitude of association with being in the 'moderate cyclic pain, severe acyclic pain' (OR=2.62, 95% CI=1.38,5.00) to highest in the 'severe acyclic, severe cyclic pelvic pain' class (OR=7.78, 95% CI=4.82,12.56). Higher number of comorbidities was also significantly associated with being in all the pelvic pain subgroups.

All ten comorbidities with ≥10% prevalence and one comorbidity with ≤10% prevalence were associated with the 'severe cyclic pain only' subgroup (class 3) compared to the 'no pelvic pain' subgroup (class 1) (Table 3.3 and Supplemental Table 3.10). No comorbidity was exclusively associated with this group. Allergies and asthma were only associated with the 'severe cyclic pain only' (class 3) and the 'severe acyclic, severe cyclic pelvic pain subgroup' (class 5) subgroups. Eight of ten comorbidities with ≥10% prevalence in the population were associated with 'severe acyclic, moderate cyclic pain' subgroup (class 4) relative to the 'no pelvic pain' subgroup (class 1). Finally, all ten comorbidities with ≥10% prevalence and 2 with ≤10% prevalence were associated with the 'severe acyclic, severe cyclic pelvic pain' subgroup (class 5) relative to the 'no pelvic pain' subgroup (class 1); the only morbidity that was solely associated with the 'severe acyclic, severe cyclic pelvic pain' subgroup (class 5) relative to the 'no pelvic pain' subgroup (class 1), was fibromyalgia (OR=62.75, 95% CI=1.98,1993.3). Fibromyalgia had a very low prevalence in this population, only affecting 1.35% of the total population and thus associated with wide confidence intervals. However, 11 of the 16 affected (67%) were in class 5.

Table 3.3 Age-adjusted odds ratios and 95% confidence intervals for associations between comorbid conditions with N≥100 and 5-class model of pelvic pain

Comorbid condition	**Class 1[1] [Ref]**	**Class 2 aOR (95% CI)[2]**	**Class 3 aOR (95% CI)[2]**	**Class 4 aOR (95% CI)[2]**	**Class 5 aOR (95% CI)[2]**
Qualitative description of subgroups	No pelvic pain	Moderate cyclic pain only	Severe cyclic pain only	Severe acyclic, moderate cyclic pain	Severe acyclic, severe cyclic pelvic pain
Gynecologic/genitourinary conditions					
Endometriosis	(1.00)	19.61 (0.68, 563.33)	407.23 (17.55, 9451.19)	184.20 (7.54, 4500.49)	2289.96 (87.7, 59810.11)
Ovarian cysts	(1.00)	2.45 (0.52, 11.53)	5.27 (1.49, 18.69)	15.57 (4.42, 54.84)	18.45 (5.68, 59.96)
Respiratory/immune conditions					
Allergies	(1.00)	1.19 (0.68, 2.06)	1.76 (1.08, 2.86)	1.86 (0.98, 3.54)	2.45 (1.54, 3.88)
Asthma	(1.00)	1.86 (0.98, 3.56)	1.88 (1.04, 3.42)	1.96 (0.90, 4.27)	3.38 (1.94, 5.86)
Rheumatologic/neurologic conditions					
Migraine	(1.00)	2.92 (1.72, 4.94)	5.68 (3.48, 9.27)	2.62 (1.38, 4.99)	7.78 (4.82, 12.56)
Lower back pain	(1.00)	1.48 (0.92, 2.38)	3.89 (2.35, 6.42)	2.94 (1.50, 5.76)	6.35 (3.80, 10.59)
Muscle/joint pain	(1.00)	1.51 (0.89, 2.57)	2.08 (1.30, 3.34)	3.03 (1.59, 5.77)	2.84 (1.78, 4.53)
Leg pain	(1.00)	1.49 (0.81, 2.75)	2.54 (1.50, 4.30)	2.08 (1.01, 4.27)	4.93 (2.99, 8.16)
Gastrointestinal/abdominal conditions					
Irritable bowel syndrome	(1.00)	2.56 (0.81, 8.10)	3.42 (1.21, 9.70)	9.54 (3.28, 27.77)	9.40 (3.55, 24.86)
Non-pelvic abdominal pain	(1.00)	1.41 (0.81, 2.44)	2.67 (1.67, 4.26)	6.878 (3.53, 13.41)	12.72 (7.56, 21.41)
Total number of comorbidities[3]	(1.00)	1.28 (1.10, 1.50)	1.98 (1.71, 2.28)	1.91 (1.61, 2.27)	2.58 (2.23, 3.00)

Abbreviations: adjusted odds ratio (aOR), confidence interval (CI)

[1] Reference latent class has an OR of 1.00

[2] Adjusted odds ratio (aOR) interpretation: tests of categorical latent variable using multivariable multinomial logistic regression using the 3-step procedure accounting for classification uncertainty, using 5-class model of pelvic pain, increase in odds of membership in each class relative to membership in reference class 1 (no pelvic pain) given one unit change in predictor (absence/presence of condition), controlling for age at time of enrollment in the study

[3] aOR interpretation: increase in odds of membership in each class, with one unit increase in the number of comorbidities (range 0 to 14)

DISCUSSION

Identification of latent subgroups

In a cohort of women oversampled for those with surgically confirmed endometriosis, we identified five subgroups of chronic pelvic pain, differentiated by cyclic and acyclic pelvic pain severity, frequency, and life impact. We observed that 13 of 18 comorbidities associated with inflammation and pain, including endometriosis, were heterogeneously distributed across subgroups. Relative to the subgroup with no pelvic pain, the severe pain subgroup was associated with the highest number of comorbidities. Migraine was associated with all four subgroups experiencing chronic pelvic pain.

At baseline this cohort was by design over-sampled for individuals with surgically diagnosed endometriosis (48%). Indications for laparoscopy to evaluate for endometriosis include pelvic pain, dyspareunia, and infertility. The distribution of those with endometriosis was the smallest in the no pelvic pain subgroup, and largest in the group reporting both severe acyclic, severe cyclic pelvic pain. Endometriosis was only significantly associated with odds of being in the three of four pain groups compared to the no pelvic pain subgroup, and not with the moderate cyclic pain only group. The co-clustering of women with and without endometriosis across multiple pain groups suggests there may be commonalities in underlying pain pathologies within groups.

Two studies have used LCA to examine dimensions of pain symptomology involving the pelvis, focusing on vulvodynia and dysmennorhea.[98,197] Other studies focused on chronic pelvic pain describe ‘subgroups’ of the syndrome with respect to the presence of specific symptoms or comorbidities (e.g., chronic pelvic pain with provoked vestibulodynia or with painful bladder symptoms).[214] We identified one cross-sectional study of 289 women which classified chronic

pelvic pain into seven diagnostic subtypes using expert opinion based on a range of reported symptoms and localization of pain.[95] The current study is the first to apply a systematic data driven approach to identify patterns of chronic pelvic pain in a large population with strong adolescent and endometriosis representation, and further explore the pelvic pain determined class associations with other comorbidities.

Compared to a class defined by no pelvic pain, our analysis revealed two subgroups that included those experiencing only cyclic pelvic pain – one marked by moderate cyclic pain and the other severe cyclic pain. The two other groups were both marked by the presence of severe acyclic pelvic pain – one group accompanied by moderate cyclic pain, and one group accompanied by severe cyclic pain. Interestingly, in a sensitivity analysis where we restricted the age of participants to 12-24, the data fit best into a three-class solution. Statistically, our sample size was reduced by one-third in this model. In LCA, smaller sample sizes can lead to insufficient power to detect low prevalence but substantively important classes.[202] Most adolescents experiencing cyclic pelvic pain are thought to have primary dysmenorrhea in absence of known pathology[215].. Nevertheless, it is biologically plausible that a portion of individuals experiencing moderate pain associated only with menstruation may be comparable to those experiencing no pain and may be appropriately co-clustered.

Association between comorbidities and derived latent classes

In this study, we observed that the subgroup reporting both severe cyclic and severe acyclic pelvic pain (class 5) had the largest number of comorbidities and was strongly associated with endometriosis. This group also had a stronger association with endometriosis than the severe acyclic pain, moderate cyclic pain subgroup (class 4), when both were compared to the no pelvic pain subgroup. It is possible that class 5 was one affected by central pain sensitization or

hypothalamic-pituitary-adrenal (HPA) dysregulation, both of which lead to widespread pain and increased comorbidity.[216] While the causal mechanisms of central pain sensitization are not well characterized, it manifests as pain hypersensitivity, whether inflammation and neural lesions are present or not.[217] Class 5 was associated with allergies, asthma, and fibromyalgia. Fibromyalgia is considered a prototypical central pain sensitization syndrome, with observed evidence of structural and function changes in the central nervous system.[218] Neuro-immune interaction is thought to play a critical role in pathologic pain, and both peripheral and central sensitization.[78] HPA axis dysregulation is associated with stress response and elevated levels of corticotropin-releasing factor (CRF) which increase cortisol, but also is responsible for mast cell activation and downstream activation of pain nociceptors.[216] Elevated basal levels of cortisol have been found in fibromyalgia.[219] In a recent study using data from the A2A cohort, participants with any autoimmune and/or inflammatory conditions had an increased odds of also having endometriosis; it is unclear if these findings are due to shared immune profiles or other biological mechanisms.[198]

Intriguingly, while most comorbidities, including endometriosis, were not equally distributed among the four pelvic pain symptom-defined groups, migraine was strongly associated with all four pelvic pain subgroups compared to the group with no pelvic pain. While is known women with endometriosis are more likely to have migraines[220], the lifetime prevalence of migraine headaches is an estimated 67% among women with chronic pelvic pain regardless of endometriosis status.[221] Recent literature has conceptualized a third type of chronic pain— nociplastic pain, that is chronic pain not characterized by direct nociceptive activation or neuropathy, which nevertheless involves altered nociceptive function.[33] Nociplastic pain features peripheral and central pain sensitization with increased processing or decreased inhibition of pain

stimuli.[34] The mechanisms for nociplastic pain are thought to be heterogenous, emerging and propagated within a complex biopsychosocial framework.[222] This conceptualization aligns well with the heterogeneous subgroups that we have observed in chronic pelvic pain.

Strengths and limitations

The strengths of this study include a large sample size of participants who provided detailed information via a validated collection tool on multiple dimensions of pelvic pain. This enabled us to use the best available indicators and appropriately forward fill missing data based on historical information. The experience of pain involves complex pathways involving physiologic and psychological mechanisms and feedback processes.[223] The included variables captured multidimensional aspects of perceived pain, detecting what could have been missed in univariable-oriented approaches. Through LCA we incorporated different dimensions of symptomology in our model. This person-centered approach accounts for patterns and complexity of symptomology in a highly heterogeneous condition.

The cohort is also relatively young compared to most other studies of endometriosis, allowing us to capture an earlier timepoint in the endometriosis journey. Given that endometriosis is associated with increased risk of infertility[224] and chronic diseases such as cardiovascular disease[14,225], autoimmune disorders[226,227], and cancer[228], one could treat co-clustering in a severe pelvic pain subgroup as a risk indicator for further causal investigations in these individuals. This cohort is overrepresented with individuals recruited from clinics, and those with a surgical diagnosis of endometriosis, providing deep phenotyping that does not exist in standard clinical care. In addition, the composition of this group allowed us to detect more extreme subgroups while reflecting a spectrum of pelvic pain presentations.

In clinical populations, one of the most common treatment modalities for cyclic pelvic pain is ovulatory suppression,[7,229] which leads to an inability to quantify severity or frequency of cyclic pelvic pain among those who receive this treatment. In contrast, acyclic pelvic pain status can still be assessed in those receiving ovulatory suppression treatment. For this reason, we quantified cyclic pain information using pre-survey historical data for the 13.5% of our population without known menses in the past year. We also conducted a sensitivity analysis excluding this group and found that our classification remained robust.

Limitations to this work include potential recall bias for the history of pelvic pain prior to study enrollment and completion of the baseline questionnaire. Those currently experiencing life-impacting pain may recall their past severity and frequency of pain differently from those who also experienced pain in the past but currently have some or complete remediation. It is important to note that this medical history at enrollment is wholly consistent with clinical settings where symptoms are self-reported, and rarely functionally tested. Further, most data used in this study were from reports of current experience (in the last 3 or the most recent 12 months) and did not utilize more distant recalled time periods.

Second, in assessing comorbidities there is a possibility of detection bias for those who have had greater contact with the medical system. Particularly, those within the cohort who had not been surgically diagnosed with endometriosis were largely enrolled from the communities within the hospitals' catchment area, without the same pattern of potential medicalization around their pelvic pain experience compared to those with endometriosis. This detection bias would drive associations between pain and comorbidities away from the null. However, while there were strong associations with some of the subgroups and endometriosis, endometriosis was not associated with all four pain subgroups. As well, pelvic pain is often experienced without

healthcare intervention. It is also possible that there are women in the non-pain group who have comorbidities not yet diagnosed. In the case of endometriosis, in the 5 to 10 years of follow-up in the cohort from which participants were drawn, only 3 without an endometriosis diagnosis at baseline have had a new diagnosis.

Third, as our intention was to establish classes defined by pelvic pain symptoms, we did not examine past and current medication use in these analyses beyond the impact on menstrual cyclicity, thus impeding quantification of dysmenorrhea severity. Our groups experiencing severe pelvic pain are a sum of those not using pain-modifying analgesics or hormonal medication and those whose pain is inadequately remediated by the pain-modifying medications that they are taking. Therefore, individuals may be misclassified into less severe pain groups than they would have been had they not been on interventions, which would drive the associations that we observed toward the null.

Fourth, the cohort by design is over-represented with individuals with surgically diagnosed endometriosis. While this has many strengths as described, these results may not generalize to a chronic pelvic pain population that includes a different proportion of those with endometriosis. Also, with the intentional prioritization for enrolling adolescents into this cohort, these classes may not have emerged as they have in a population oversampled for rASRM stage III/IV disease; although, rASRM stage correlates poorly with pain presentation or pain treatment prognosis.[55–57] Finally, while representative of the population diagnosed with endometriosis in the participating hospitals, the systemic biases impacting receipt of treatment for pelvic pain across the globe, but potentially maximized in tertiary care settings may have driven the high proportion of White participants relative to other race/ethnicities. Future discovery should target enrollment among historically underrepresented populations.[230]

Conclusion

This study identified five subgroups of pelvic pain in a population oversampled for adolescents and surgically diagnosed endometriosis. These subgroups covered a spectrum of experiences with different patterns of pelvic pain types, severity, frequency, and life impact. We then examined the association between these subgroups and comorbidities associated with pain and inflammation. Pelvic pain is described regional pain syndrome resulting from interactions between multiple systems, or as described the broad symptom of underlying pathologies such as endometriosis. This research allows for delineation of several unique patterns of pelvic pain and raises the question that if comorbidities cluster differently in association with these patterns, could the underlying mechanisms generate these specific patterns be biologically distinct. The methods described here can be used in future analyses, incorporating further indicators such as observed measures and biomarkers of pain. This may allow for classes to be further refined from descriptive to ones that reflect intrinsic subtypes reflecting ever more precise pathophysiologic pathways. Consequently, we may better understand the etiology, diagnosis, and prognosis of chronic pelvic pain and associated disorders.

CHAPTER 4 DIFFERENCES IN INFLAMMATION-ASSOCIATED BIOMARKERS BETWEEN PATIENT CLASSES DEFINED BY PELVIC PAIN

ABSTRACT

Pelvic pain in women is a multi-factorial and heterogenous condition that has been associated with increased inflammation. This study investigated the association between eight target serum-based inflammatory biomarkers and previously identified patient subgroups classes defined by pelvic pain characteristics. This cross-sectional study included a subset of participants in the Women's Health Study: from Adolescence to Adulthood (A2A; N=625), a deeply-phenotyped cohort oversampled for adolescents and those with surgically diagnosed endometriosis. Seven target inflammatory markers were included: interleukin (IL)-8, IL-16, interferon gamma-induced protein (IP)-10/CXCL10, monocyte chemotactic protein (MCP)-1, MCP-4, thymus- and activation-regulated chemokine (TARC)/CCL-17, and tumor necrosis factor (TNF)-α. Cubic regression spline functions were used to explore non-linearity in relationships between biomarkers and five subgroups of pelvic pain (classes 1-5) previously defined using latent class analysis (LCA). Multinomial logistic regression analysis was conducted using the LCA three-step approach to examine associations between biomarkers and subgroups, accounting for classification uncertainty. Five biomarkers showed non-linear associations and two biomarkers showed linear associations with the subgroup that included participants who reported both severe cyclic and acyclic pain (class 5) compared to the subgroup that included participants who reported no pelvic pain (class 1). Greater odds of being in this subgroup with both severe cyclic and acyclic pain was associated with the highest concentration quintile compared to the middle three concentration quintiles for three biomarkers: IL-16 (aOR=2.16, CI=1.03, 4.56), IP-10 (aOR=2.78, CI=1.32, 5.85), and MCP-4 (aOR=3.33, CI=1.43, 7.77). The unique patterns in

which inflammatory biomarkers are associated with symptom-based subgroups provides novel insight and important questions regarding the association between pain and pathology that merit further investigation.

INTRODUCTION

Pelvic pain represents a large burden on patients due to both its high prevalence worldwide and heterogeneity, characterized by multifactorial but poorly understood etiologies. Among women, the prevalence of cyclic pelvic pain (associated with menstruation) is 16.8% to 81.0%, and acyclic pelvic pain (not associated with menstruation, intercourse, pregnancy) is 2.1% to 24.0%.[1] Chronic pelvic pain with a duration lasting more than 6 months among women has been conceptualized as a syndrome with no known underlying etiology, associated with other functional pain syndromes such as irritable bowel syndrome, or caused by pathologic disease processes, such as endometriosis which has a prevalence of 15.4% to 71.4% among those being investigated for chronic pelvic pain.[8] The evaluation and treatment of chronic pelvic pain is based on symptomology if the cause is known, or disease-specific guidelines if there is a known disease process.[7] However, even in the case of a known disease process, determining the etiology or pathophysiology of pelvic pain is difficult as there are often multiple simultaneous comorbidities.

In recent years clinical and technological advances have led to the discovery of novel noninvasive serum-based biomarkers, which are indicators of biologic or pathologic processes in various diseases. Serum-based biomarkers can be classified as prognostic, predictive, or surrogate.[231] They can not only provide insight into disease progression and treatment response, but also in investigations of the natural history of a disease or process.[232] Inflammatory cytokines, secreted proteins that allow for communication between cells and are involved in the

coordination of immunologic response, have been implicated in many pathologic pain conditions.[233] Cytokines such as interleukin IL-6 and tumor necrosis factor (TNF)-α have been shown to promote neuro-immune pain processes.[233] Numerous studies have reported associations of individual cytokines with the presence of single disease processes such as endometriosis.[234–236]

Given that pelvic pain and associated disease entities are highly heterogenous, in symptom presentation and underlying pathophysiology,[9] symptom-based population clustering offers a way to identify patterns of multiple symptoms in a complex, multifactorial condition.[237] Recently, we conducted a symptom-based data-driven subgroup identification analysis driven by patient reported pelvic pain characteristics.[238] These subgroups were drawn from a cohort oversampled for adolescents with surgically diagnosed endometriosis. We identified five subgroups based on six dimensions of pain: ranging from those who experience no pelvic pain, to those who experience both severe acyclic, severe cyclic pelvic pain. We further found heterogeneity in the distribution of comorbidities across these subgroups. The objective of the current study was to examine the associations between eight plasma-based biomarkers and these previously defined subgroups of pelvic pain in the same cohort. We hypothesized that elevated levels of pro-inflammatory markers would be associated with assignment to the most severe pain classes.

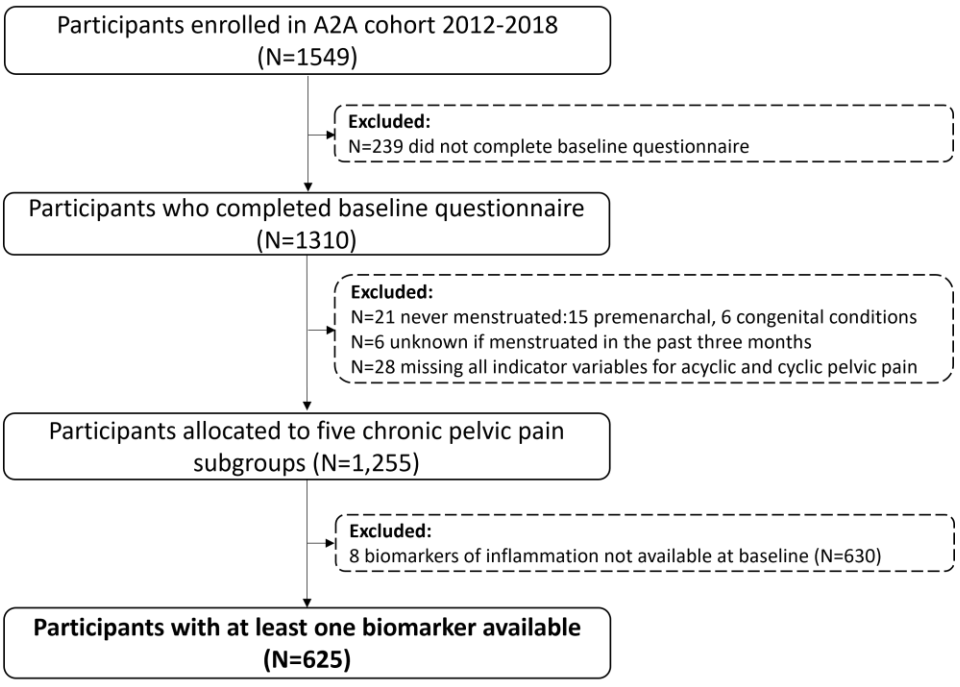
METHODS

Study population

This cross-sectional study used data from the baseline questionnaire and biologic sample collections from an ongoing cohort, The Women's Health Study: From Adolescence to Adulthood (A2A; N=625), described in detail previously.[191] In brief, this prospective cohort

study enrolled female participants age 7 to 55 years from the Boston Children's Hospital (BCH) and Brigham and Women's Hospital (BWH) and the general population in the hospitals' catchment area between November 2012 to June 2018. By design, the cohort oversampled for adolescents and for participants with surgically diagnosed endometriosis. 1549 participants were enrolled in the cohort, 1255 met our eligibility criteria (completed the baseline questionnaire and had a value for at least one of six pain variables for cyclic and acylic pelvic pain severity, frequency and life impact) and were thus included in a latent class analysis.[238] Of these, a subset provided blood samples, and had data for at least one of seven inflammatory biomarkers of interest that have previously been linked with endometriosis and other pelvic pain conditions (N=625; Figure 4.1).[76,82,84,239–241]

Figure 4.1 Flowchart of inflammatory biomarker cohort in the Adolescent to Adulthood (A2A) study



Biomarker measurement

Target biomarkers for this study were seven inflammatory cytokines that previously have been associated with chronic pelvic pain and associated conditions: Interleukin (IL)-8, IL-16, interferon gamma-induced protein (IP)-10/CXCL10, monocyte chemotactic protein (MCP)-1, MCP-4, thymus- and activation-regulated chemokine (TARC)/CCL-17, and tumor necrosis factor (TNF)-α. Per WERF EPHect guidelines on fluid biospecimen collection, blood samples were collected in participating clinical sites via venous blood draw from participants and were processed into plasma, serum and buffy coats and stored at ≤80°.[242] 77.9% of samples were collected within 30 days of survey completion (N=487; Supplemental Figure 4.1). Plasma samples were sent and assayed at a central laboratory (Martinez-Maza Laboratory, University of California, Los Angeles, CA).

Two Luminex Multiplex Assay panels were used to assess biomarker levels using a bead-based multiplex immunoassay approach (R&D Systems, Minneapolis, MN) and the BioPlex 200 Luminex array read (BioRad, Hercules, CA) and BioPlex Manager (version 4.1.1) were used to read and quantify levels. The lower limit of detection was defined as the lowest calculated value on the BioPlex Manager-generated standard curve. This standard curve was used to extrapolate and assign values to those below the limit of detection. Biomarkers selected for analysis were those whose extrapolation rates did not exceed 10% (Supplemental Table 4.2). Each value was adjusted by batch, the batch-adjustment process re-calibrated the biomarker levels to one of an "average" batch, which gave an adjusted value on the same scale as the original marker using methods described by Rosner et al. (2008).[243]

Biomarker concentrations were reported in pg/ml. Concentrations of the seven biomarkers were skewed and were therefore natural log transformed to approximate a normal

distribution for statistical testing. Outliers, which were determined using the generalized extreme studentized deviate many-outlier method[244], were removed (0 to 3% of values). Intra-assay coefficients of variation (CV) were reported, where CV<15% suggest low error due to biomarker variability between subjects. Five of seven biomarkers had CVs<15%: IL-16, IP-10, MCP-1, MCP-4, TARC. The sampling frame for the descriptive analysis consisted of those participants who had an assigned class and values above the limit of detection for at least one biomarker of interest (N=625), whereas the sampling frame for the association analysis was restricted to participants who had values above limit of detection for all biomarkers (N=590) (Supplemental Table 4.2). Biomarkers were categorized into quintiles based the distribution of a given biomarkers across the entire sampling frame (Supplemental Table 4.3)

Pelvic pain subgroups

Latent class analysis (LCA) was used to identify subgroups of women with similar patterns of pelvic pain using six indicators of pelvic pain in our full cohort. Briefly, information about acyclic and cyclic pelvic pain was collected using the validated EPHect Endometriosis Patient Questionnaire (EPQ).[49] Information collected included severity (using numerical rating scale NRS), frequency and life impact for both pain types. Acyclic pelvic pain was defined as worst pelvic/lower abdominal pain not caused by menstrual cramps, intercourse, surgery, pregnancy, or other injury and infections over the 12 months prior to questionnaire completion. Cyclic pelvic pain was defined as dysmenorrhea or cramping, shooting, or stabbing pain that occurred during menses over the 12 months prior to questionnaire completion (survey prior to January 2014) or 3 months (survey after January 2014). Participants for whom cyclic pelvic pain variables were not available because the participant was not menstruating in the year prior to the completion of the survey were treated as missing not at random and their cyclic pelvic pain data

was forward filled from historical age range data, where available. Cleaning and descriptive analysis of data was conducted in R version 4.0.2 and latent class analysis conducted in Mplus 8.6.[199,245] Full description of methods is provided in Ghiasi et al. (under review).[238]

Classes assigned to participants in the current study were those that were assigned in the previous study using the full cohort (N=1255). In that study, a 5-class model was identified as the best fitting model using fit criterion: statistical fit and interpretability. Each of the participants were assigned a most-likely latent class membership, and the uncertainty around the estimated posterior probabilities of latent class assignment was recorded. We conducted a sensitivity LCA using the same methods above but restricting the population to participants in the current study only (N=625), recognizing reduction sample size and population composition could impact the latent classes formed. We found that despite the restricted sample, a 5-class solution provided once again the best statistical fit with the lowest aBIC and lowest entropy as well (Supplemental Table 4.4). The five classes in this smaller model were qualitatively similar to the five classes in the model including all eligible participants (N=1255). For each individual participant, we compared the most likely class assignment in the full model versus the subset model and found high agreement between the subgroups to which participants were assigned (Cohen's Kappa=0.85, $p<0.01$).

Sociodemographic and clinical covariates

At enrollment, study participants completed a questionnaire on socio-demographic and clinical characteristics, pain symptoms and quality of life which expands on the validated WERF EPHect Endometriosis Patient Questionnaire (EPQ).[49] Self-reported information collected included age at completion of the baseline survey (continuous), age at menarche (continuous), US Census Bureau socially-defined groups[246]: race (Black, White, other self-identified groups

and unknown) and Hispanic ethnicity, date of last menstrual period, body mass index (kg/m^2, continuous), cigarette smoking history (never, former, current), parity (nulliparous, parous), health conditions and comorbidities (presence of endometriosis, count of other comorbidities), use of hormonal medication (never, ever, and if ever used indication for use), and use of analgesic medication (never, <2 days per week, ≥2 days per week), analgesic medication use within 48 hours prior to blood draw, recruitment site (clinic-based, population-based). Data cleaning and descriptive analysis, consisting of proportions for categorical variables and means and 95% confidence intervals or medians and 25% and 75% percentiles for continuous variables, was conducted in R (version 4.0.2).[199]

Evaluating correlations between and linearity assumption for biomarkers

Bivariate associations were assessed between biomarkers, using a correlation matrix for the seven biomarkers and age and body mass index. This was used to identify strong collinearity between biomarkers (defined as spearman rank correlation coefficient $r_s>0.70$, $p<0.05$) to facilitate interpretation and robustness of associations.[247] We corrected for multiple comparisons using Bonferroni correction (significance level $p=0.05/12=0.0042$) (Supplemental Figure 4.2).

Before conducting latent class regression analysis, we sought to understand the functional form of the relationship between biomarkers of interest and latent classes, and if they were best treated as linear or non-linear in our models.[248] Biomarkers were transformed using restricted cubic spline analysis.[249] A restricted cubic spline is a series of piecewise polynomials that meet at 'knots'. The restricted cubic spline is continuous and smooth providing a better fit over linear spline functions, but also imposes linearity on the two tails of the curve with reduced degrees of freedom which advantages it over unrestricted cubic spline functions.[250,251] In general 3-5 knots are recommended, and provide a good fit without overfiiting.[251,252]

We conducted multiple logistic regression analyses with assignment to pain subgroups (class 2 to class 5) compared to the reference no pelvic pain subgroup (class 1) as dependent variables, and each restricted cubic spline transformed continuous biomarker values and the covariates age at enrollment and body mass index (BMI) as independent variables. Age and BMI were selected as covariates because they have been both been identified as being associated with changes in biomarker levels for our biomarkers of interest.[253] For each biomarker, we tested unadjusted and adjusted linear, and restricted cubic spline transformed 3, 4, 5, and 6 knot models with pre-specified percentiles recommended by Harrel et al. (2015), which positions smaller percentile intervals at distribution tails.[251] Model fit was statistically assessed using Akaike's information criterion (AIC) to assess the value in increasing model complexity (Supplemental Table 4.5 and Supplemental Figure 4.3).

For each biomarker, twelve spline models across four subgroups, with various parameters were compared using batch corrected log-transformed values for biomarkers regressed on odds of being in the most severe pain class (class 5) compared to the no pain subgroup (class 1) (Supplemental Table 4.5). Broadly, age and BMI adjusted models showed the best fit. While linear models were best for most biomarkers across two subgroups, the odds of assignment to the severe acyclic and cyclic pelvic pain group (class 5) compared to the no pain group (class 1) was non-linear for most biomarkers. Therefore, in conducting bias-adjusted three step approach we provided both estimates for both linear and non-linear models and interpreted accordingly.

Bias-adjusted three step association analyses between biomarkers and latent classes

To explore the relationship between all previously derived latent classes and biomarkers, we used a bias adjusted three-step approach. A schematic of this approach is provided in Supplemental Figure 4.4. Inflammatory biomarkers were tested as predictors of latent class

membership using with the R3STEP procedure (Mplus 8.6).[89] Briefly, this approach estimates the measurement model, assigns participants to a latent class, and associates the class using multinomial logistic regression to an external variable while simultaneously accounting for classification uncertainty.[89,211,212] To assess linear relationships, biomarkers were input as a continuous predictor. To assess non-linear (u-shaped) relationships, biomarkers were categorized into tertiles, and the middle tertile selected as the reference group and compared to the top and bottom tertile. We presented unadjusted odds ratios (ORs) and age-BMI-adjusted ORs, with age and BMI parametrized as non-transformed continuous variables in years and kg/m^2 respectively. We also conducted a sensitivity analysis restricting to biomarker values which were obtained from blood samples within a 60 days window of the questionnaire (N=487).

RESULTS

Description

The characteristics of the sampling frame compared to the overall portion of the cohort who completed the baseline questionnaire is reported in Supplemental Table 4.1. Those who had at least one biomarker value available were on average younger (22.5 years) compared to those with no biomarkers available (24.4 years). They were more likely to have been recruited from clinics, with 56.8% of their enrollment source being clinic based versus those with no biomarkers available where 49.5% were recruited from clinics. 51.8% of those with biomarker data available have surgically diagnosed endometriosis, versus 43.3% of those without biomarker data. Those who had biomarkers available had higher levels of ever using hormonal medication (85.3%) compared those who did not (76.8%). However, their date of last menstrual period, pain medications used, and other characteristics were all highly comparable.

Latent classes of pelvic pain

The proportion of participants assigned to each pelvic pain-defined subgroup class was relatively similar apart from the severe acyclic pelvic pain, moderate cyclic pelvic pain class, (Class 4), which was considerably smaller: 22.1% (Class 1: 5.80% with endometriosis), 23.2% (Class 2: 22.1% with endometriosis), 22.6% (Class 3: 75.2% with endometriosis), 6.7% (Class 4: 66.7% with endometriosis), and 25.4% (Class 5: 94.3% with endometriosis) (Table 4.1). Some notable patterns of characteristic distributions among the classes included medication use. Nearly one quarter of those in the no pelvic pain subgroup who had never used hormonal medications, whereas only 3.1% of those in the severe cyclic and severe acyclic pain subgroup had never used hormone medication. While 14.5% of those in the no pelvic pain subgroup reported using pain medication 2 or more days a week, whereas half of those in the most severe pain group report regularly using pain medications. Examining the distribution of biomarker quintiles showed that some biomarkers were bimodally distributed. For example, in the severe acyclic and cyclic pain subgroup, MCP-4 was distributed in a bimodal manner, i.e. 61.5% of the class was in either the lowest or highest quintile (Supplemental Table 4.3).

Table 4.1 Description of the five latent classes of pelvic pain and their characteristics for participants with at least one inflammatory biomarker available (N=625)

Characteristic	No pelvic pain Class 1 (N=138)	Moderate cyclic pelvic pain only Class 2 (N=145)	Severe cyclic pelvic pain only Class 3 (N=141)	Severe acyclic, moderate cyclic pain Class 4 (N=42)	Severe cyclic and acyclic pain Class 5 (N=159)
Proportion in population	22.1%	23.2%	22.6%	6.7%	25.4%
Age at enrollment					
Mean (SD)	25.5 (6.88)	23.8 (6.91)	21.2 (6.61)	21.4 (7.24)	20.2 (5.78)
Median [Min, Max]	24.0 [14.0, 52.0]	23.0 [13.0, 51.0]	19.0 [12.0, 42.0]	19.5 [12.0, 50.0]	18.0 [13.0, 42.0]
≤15 years	3 (2.17%)	10 (6.90%)	27 (19.1%)	5 (11.9%)	28 (17.6%)
16-20 years	26 (18.8%)	31 (21.4%)	53 (37.6%)	18 (42.9%)	75 (47.2%)
21-30 years	83 (60.1%)	86 (59.3%)	47 (33.3%)	16 (38.1%)	46 (28.9%)
31-40 years	20 (14.5%)	12 (8.28%)	13 (9.22%)	2 (4.76%)	9 (5.66%)
≥41 years	6 (4.35%)	6 (4.14%)	1 (0.709%)	1 (2.38%)	1 (0.629%)
Age at menarche[1]					
Mean (SD)	12.5 (1.26)	12.0 (1.42)	11.7 (1.39)	11.9 (1.35)	11.8 (1.35)
Median [Min, Max]	12.5 [9.00, 15.0]	12.0 [9.00, 15.0]	12.0 [8.00, 15.0]	12.0 [9.00, 15.0]	12.0 [8.00, 15.0]
<10 years	1 (0.725%)	6 (4.17%)	11 (7.80%)	2 (4.76%)	6 (3.77%)
10-11 years	26 (18.8%)	46 (31.9%)	46 (32.6%)	15 (35.7%)	58 (36.5%)
12-13 years	81 (58.7%)	69 (47.9%)	74 (52.5%)	20 (47.6%)	83 (52.2%)
>13 years	30 (21.7%)	23 (16.0%)	10 (7.09%)	5 (11.9%)	12 (7.55%)
Body mass index at enrollment[1,2]					
Mean kg/m^2 (SD)	23.5 (4.35)	24.5 (5.10)	24.9 (6.09)	24.0 (4.60)	25.1 (6.19)
Median [Min, Max]	22.6 [17.0, 42.8]	23.1 [16.2, 46.2]	23.3 [15.6, 60.8]	22.9 [16.1, 38.4]	23.5 [17.6, 57.2]
Underweight	4 (2.90%)	5 (3.47%)	0 (0%)	0 (0%)	1 (0.629%)
Normal weight	97 (70.3%)	91 (63.2%)	88 (62.4%)	27 (64.3%)	98 (61.6%)
Overweight	26 (18.8%)	33 (22.9%)	36 (25.5%)	13 (31.0%)	40 (25.2%)
Obese	11 (7.97%)	15 (10.4%)	17 (12.1%)	2 (4.76%)	20 (12.6%)
Race[3]					
Black	9 (6.52%)	8 (5.52%)	4 (2.84%)	2 (4.76%)	11 (6.92%)
White	95 (68.8%)	113 (77.9%)	109 (77.3%)	36 (85.7%)	136 (85.5%)
Other identification	34 (24.6%)	24 (16.6%)	28 (19.9%)	4 (9.52%)	12 (7.55%)

Table 4.1 (cont'd)

Characteristic	No pelvic pain Class 1 (N=138)	Moderate cyclic pelvic pain only Class 2 (N=145)	Severe cyclic pelvic pain only Class 3 (N=141)	Severe acyclic, moderate cyclic pain Class 4 (N=42)	Severe cyclic and acyclic pain Class 5 (N=159)
Hispanic ethnicity[3]					
Hispanic	12 (8.7%)	18 (12.8%)	15 (10.9%)	4 (10.0%)	10 (6.29%)
Non-Hispanic	126 (91.3%)	123 (87.2%)	122 (89.1%)	36 (90.0%)	149 (93.7%)
Cigarette smoking status at enrollment[1]					
Never smoker	129 (93.5%)	131 (90.3%)	129 (91.5%)	38 (90.5%)	143 (89.9%)
Past smoker	7 (5.07%)	7 (4.83%)	10 (7.09%)	2 (4.76%)	7 (4.40%)
Current smoker	2 (1.45%)	3 (2.07%)	1 (0.709%)	1 (2.38%)	2 (1.26%)
Parity[1]					
None	118 (86.8%)	139 (96.5%)	132 (94.3%)	39 (92.9%)	150 (94.9%)
≥1 births	18 (13.2%)	5 (3.47%)	8 (5.71%)	3 (7.14%)	8 (5.06%)
Health conditions and co-morbidities[4]					
Endometriosis	8 (5.80%)	32 (22.1%)	106 (75.2%)	28 (66.7%)	150 (94.3%)
≥2 comorbidities	86 (62.4%)	106 (73.1%)	132 (93.6%)	40 (95.2%)	153 (96.2%)
Hormone medication use[5]					
Never used	34 (24.6%)	31 (21.4%)	16 (11.3%)	6 (14.3%)	5 (3.14%)
Ever used	104 (75.4%)	114 (78.6%)	125 (88.7%)	36 (85.7%)	154 (96.9%)
For birth control	60 (50.8%)	58 (51.8%)	30 (28.3%)	9 (33.3%)	23 (23.0%)
For pain control	12 (10.2%)	25 (22.3%)	71 (67.0%)	17 (63.0%)	71 (71.0%)
Regular pain medication use[1,6]					
Never	109 (79.0%)	104 (74.8%)	68 (49.3%)	24 (57.1%)	68 (44.4%)
< 2 days per week	9 (6.52%)	10 (7.19%)	14 (10.1%)	4 (9.52%)	10 (6.54%)
≥2 days per week	20 (14.5%)	25 (18.0%)	56 (40.6%)	14 (33.3%)	75 (49.0%)
Within 48 hours prior to blood draw[7]	16 (15.8%)	23 (24.5%)	23 (29.1%)	4 (19.0%)	22 (32.8%)
Time since last menstrual period (LMP) to enrollment					
In last 3 months	124 (89.9%)	117 (80.7%)	110 (78.0%)	30 (71.4%)	93 (58.5%)
3-6 months ago	4 (2.90%)	7 (4.83%)	17 (12.1%)	4 (9.52%)	22 (13.8%)
6-12 months ago	4 (2.90%)	4 (2.76%)	3 (2.13%)	2 (4.76%)	8 (5.03%)

Table 4.1 (cont'd)

Characteristic	No pelvic pain Class 1 (N=138)	Moderate cyclic pelvic pain only Class 2 (N=145)	Severe cyclic pelvic pain only Class 3 (N=141)	Severe acyclic, moderate cyclic pain Class 4 (N=42)	Severe cyclic and acyclic pain Class 5 (N=159)
>12 months ago	5 (3.62%)	17 (11.7%)	11 (7.80%)	6 (14.3%)	33 (20.8%)
Not in last 3 months, LMP unknown	1 (0.725%)	0 (0%)	0 (0%)	0 (0%)	3 (1.89%)
Source of enrollment					
Clinic based	22 (15.9%)	46 (31.7%)	108 (76.6%)	29 (69.0%)	150 (94.3%)
Non-clinic based	116 (84.1%)	99 (68.3%)	33 (23.4%)	13 (31.0%)	9 (5.66%)
Mean days between enrollment and blood draw (SD)	6.8 (21.2)	7.9 (27.5)	9.8 (87.2)	2.6 days (32.1)	7.79 days (63.5)

Abbreviations: SD=standard deviation

[1] Number of participants missing information for each characteristic variable: age at menarche (N=1), body mass index (N=1), cigarette smoking status at enrollment (N=13), parity (N=5), regular pain medication use (N=15)

[2] Underweight for age <20 (z-score >-9 to <=-2), age ≥20 (>0 to < 18.5); normal weight for age <20 (z-score >-2 to <1), age ≥20 (>=18.5 to <25); overweight for age <20 (z-score >=1 to <=2), age ≥20 (>=25 to <30); obese for age <20 (z-score >2), age ≥20 (>=30)

[3] Black participants include Hispanic (N=6) and Non-Hispanic (N=26), White participants include Hispanic (N=30) and Non-Hispanic (N=454); Other and unknown category participants are Asian (N=47), American Indian/Alaska Native (N=1), multiracial (N=16), other race (N=34), unknown (N=4), including Hispanic (N=23) and Non-Hispanic (N=76)

[4] Comorbidities include gynecologic or genitourinary conditions: surgically-diagnosed endometriosis, fibrocystic or benign breast disease, painful bladder or interstitial cystitis, uterine fibroids, ovarian cysts, and polycystic ovarian syndrome (PCOS); respiratory immune conditions: allergies and asthma; rheumatologic and neurological conditions: fibromyalgia, chronic fatigue syndrome, rheumatic arthritis, migraine, lower back pain, muscle or joint pain unrelated to infections or sports injuries and leg pain; gastrointestinal or abdominal conditions: inflammatory bowel disease, irritable bowel syndrome and non-pelvic abdominal pain

[5] Lifetime use of hormone medication, including birth control pills, patches, rings, injections, implants, hormonal intrauterine device, for any reason including but not limited to acne, bad cramping, irregular periods, birth control, fertility treatments.

[6] Current regular use of pain medications including acetaminophen, non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, celecoxib, rofecoxib, naproxen, mefenamic acid, ketorolac), opioid analgesics (e.g. hydrocode-combination, oxycodone with acetaminophen, oxycodone HCL, acetaminophen with codeine, codeine, morphine)

[7] Pain medication used within 48 hours prior to blood draw for plasma biomarker sampling

Table 4.2 Age and body mass index-adjusted odds ratios associating biomarkers to latent classes of pelvic pain (pg/ml unit increase)

		No pelvic pain Class 1[1] (N=138)	Moderate cyclic pelvic pain only Class 2[2] (N=145)	Severe cyclic pelvic pain only Class 3[2] (N=141)	Severe acyclic, moderate cyclic pain Class 4[2] (N=42)	Severe acyclic, severe cyclic pain Class 5[2] (N=159)
Linear[3]						
IL-8	Q5 vs. (ref Q1)[5]	(1.00)	0.41 (0.1,1.64)	0.86 (0.28,2.64)	0.55 (0.13,2.28)	1.05 (0.34,3.19)
	Continuous		0.71 (0.32,1.56)	0.78 (0.38,1.58)	0.61 (0.13,2.87)	1.11 (0.55,2.24)
TARC	Q5 vs. (ref Q1)[5]	(1.00)	0.87 (0.27,2.8)	0.65 (0.22,1.95)	2.41 (0.23,25.54)	0.61 (0.24,1.57)
	Continuous		0.81 (0.47,1.4)	0.75 (0.48,1.17)	1.06 (0.54,2.1)	0.7 (0.46,1.06)
Non-Linear[4]						
IL-16	Q1 (ref Q2-4)[5]	(1.00)	1.38 (0.51,3.74)	1.48 (0.68,3.22)	0.43 (0.06,2.87)	1.59 (0.76,3.31)
	Q5 (ref Q2-4)[5]	(1.00)	1.81 (0.72,4.56)	1.59 (0.75,3.41)	0.31 (0.02,5.8)	2.16 (1.03,4.56)*
IP-10	Q1 (ref Q2-4)[5]	(1.00)	1.69 (0.66,4.32)	1.71 (0.77,3.79)	0.68 (0.05,9.11)	2.11 (0.98,4.56)
	Q5 (ref Q2-4)[5]	(1.00)	1.97 (0.77,5.01)	1.74 (0.81,3.72)	3.08 (1.03,9.2)*	2.78 (1.32,5.85)*
MCP-1	Q1 (ref Q2-4)[5]	(1.00)	1.55 (0.6,4.02)	0.82 (0.35,1.96)	1.81 (0.53,6.19)	2.1 (0.99,4.43)
	Q5 (ref Q2-4)[5]	(1.00)	1.69 (0.66,4.32)	1.01 (0.48,2.12)	1.41 (0.38,5.2)	1.92 (0.92,3.97)
MCP-4	Q1 (ref Q2-4)[5]	(1.00)	0.60 (0.2,1.81)	0.73 (0.36,1.48)	1.11 (0.28,4.4)	1.44 (0.72,2.88)
	Q5 (ref Q2-4)[5]	(1.00)	3.40 (1.25,9.23)*	1.4 (0.59,3.3)	1.79 (0.47,6.87)	3.33 (1.43,7.77)*
TNF-a	Q1 (ref Q2-4)[5]	(1.00)	1.37 (0.47,3.94)	2.07 (0.91,4.71)	1.65 (0.35,7.75)	1.83 (0.79,4.26)
	Q5 (ref Q2-4)[5]	(1.00)	0.94 (0.38,2.32)	1.22 (0.60,2.48)	1.96 (0.63,6.06)	1.35 (0.68,2.70)

Notes: Significant associations observed denoted (*)

[1] Reference latent class is those with no pelvic pain, class 1, with an OR=1.0.

[2] Odds of membership in this class given one unit change in biomarker value (continuous) or quintile (binary)

[3]Linear multinomial regression model assumption for two biomarkers: IL-8, TARC

[4]Non-linear multinomial regression model assumption for five biomarkers: IL-16, IP-10, MCP-1, MCP-4, TNF-a

[5]Reference quintile(s): concentration quintile 1 (lowest) in binary linear models, concentration quintiles 2, 3 and 4 (middle) in non-linear models

Association between biomarkers of inflammation and latent classes of pelvic pain

There was, as expected, moderate correlation between inflammatory biomarkers. Most of the correlations were in the positive direction (Supplemental Figure 4.2), but the correlation was moderate enough that it does not impact the interpretation. The highest positive correlations were seen between MCP-1 and MCP-4 (Spearman rank correlation $\rho=0.60$, $p\leq0.001$) and MCP-4 and TARC ($\rho=0.40$, $p\leq0.001$). Regression splines with parameters based on best fitting AIC values were plotted with 95% confidence interval bands, adjusted to age and body mass index (Supplemental Figure 4.3). Spline plots revealed a non-linear relationship between many of the biomarkers and probability of being in class 5 compared to class 1. Five biomarkers: IL-16, IP-10, MCP-1, MCP-4 and TNF-α showed strong non-linear relationships with the probability of being in the severe cyclic and acyclic pain subgroup (class 5) versus the no pelvic pain subgroup (class 1).

We found the severe acyclic, severe cyclic pelvic pain subgroup (class 5) was associated with highest and lowest quintiles of concentration, depending on the inflammatory biomarker, compared to the middle three quintiles of concentration used as the reference group (adjusted: Table 4.2 and unadjusted: Supplemental Table 4.5). In the model adjusted for age and BMI the highest concentration quintile of IL-16 (aOR=2.16 95% CI=1.03,4.56), IP-10 (aOR=2.78 95% CI 1.32, 5.85) and MCP-4 (aOR=3.33 95% CI 1.43,7.77) had increased odds of being assigned to the severe acyclic, severe cyclic pelvic pain subgroup (class 5) compared to class 1. The highest concentration quintile, when compared to the middle three concentration quintiles, was also associated with increased odds of being in the severe acyclic, moderate cyclic pelvic pain subgroup (class 4) compared to class 1 for IP-10 (aOR=3.08 95% CI=1.03,9.2) and the moderate cyclic pain only subgroup (class 2) compared to class 1 for MCP-4 (aOR=3.40 95%

CI=1.25,9.23). The only association that was no longer significant after adjustment was between MCP-1 and the severe acyclic, severe cyclic pelvic pain subgroup (Class 5).

DISCUSSION

In this investigation of five subgroups of women defined by pelvic pain characteristics, we identified six biomarkers associated with one or more subgroups. The inflammatory cytokines were most strongly associated with the class that included participants who reported both severe acyclic and sever cyclic pelvic pain compared to the subgroup who reported no pelvic pain, independent of age and BMI. That various pain conditions are associated with inflammatory biomarkers is well-established. Many populations with persistent pain have elevated levels of pro-inflammatory cytokines: this includes those with fibromyalgia[254], complex regional pain syndrome[255] among others. Similarly, we expected the severe acyclic, severe cyclic pelvic pain subgroup identified in this population to have elevated levels of inflammation-associated cytokines; in our previous findings this subgroup was marked by high levels of inflammatory and pain comorbidities compared to others. We found that as expected high levels of some inflammatory markers were associated with increased odds of being in this subgroup, however, somewhat unexpectedly low levels of other inflammatory biomarkers also increased odds of being in this group. This may in part be related to the unique ways in which specific inflammatory biomarkers are linked to pathology and pain.

One of the strongly associated biomarkers with physiologic actions beyond inflammation is interferon γ-induced protein 10 kDa (IP-10) also known as CXCL10. Our study showed an association between the highest quintiles of IP-10 and the two pelvic pain subgroups marked by severe acyclic pelvic pain. This cytokine is released in response to interferon-gamma (IFNγ) that binds the CXCR3 receptor.[256] In addition to its pro-inflammatory properties it modulates

angiogenesis.[257,258] In particular, IP-10 has been associated with being in an anti-angiogenic state.[259] Vascular endothelial growth factor (VEGF) is thought to be a critical factor in progression of endometriosis, marked by vascularization and angiogensis.[260] The expression of the IP-10 receptor CXCR3 leads to the blockage of VEGF and thus reduces angiogenesis.[261] Overall, increased levels of IP-10 has been associated with hyperalgesia and allodynia in neuropathic pain in murine models, and its nociceptive effects are reduced after inhibiting its receptor CXCR3.[262–265] Murine models have suggested that IP-10 induces neuropathic pain via increased permeability of blood-spinal cord barrier.[266] Disruption and changes in permeability in this barrier are broadly thought to lead to chronic pain sensitization.[267]

Several studies have examined levels of IP-10 in women with chronic pelvic pain. A study of endometriosis patients (N=120) found decreased levels of IP-10 in peritoneal fluid of those with advanced endometriosis.[268] Another study (N=147) found low levels of serum IP-10 in women with endometriosis compared to women without endometriosis, as well as low levels in those with advanced endometriosis compared to early endometriosis.[269] The latter study proposed that the absence of the anti-angiogenic properties of IP-10 may be allowing for the development of endometriosis lesions, and explain their paradoxically lower levels in advanced disease. In in our descriptive analysis, we found that IP-10 were positively correlated with pro-inflammatory cytokines IL-16 and MCP-4. We also found that the top tertile of IP-10, with the middle tertile as the reference group, was associated with increased odds of being in the most severe cyclic and acyclic pain subgroup (class 5) and the severe acyclic, moderate cyclic pain group (class 4). Our subgroups are derived solely from pain symptomology among women with various comorbidities including endometriosis, and not on histopathologic presence of any disease. For instance, the three severe pain groups have respective proportions of participants

surgically diagnosed with endometriosis as follows 75.2% (class 3: severe cyclic pelvic pain only), 66.7% (class 4: severe acyclic, moderate cyclic pelvic pain), and 94.3% (class 5: severe acyclic and cyclic pelvic pain), but IP-10 is associated at high concentrations only with the latter two. In summary, IP-10 levels may positively correlate with severe pain symptomology but simultaneously have an inverse relationship with pathological presentation in diseases such as endometriosis. We did not see high levels of IP-10 significantly associated with the two cyclic pain only subgroups (class 2 and class 3).

The highest quintile of the inflammatory biomarkers IL-16 and MCP-4 were uniquely associated with increased odds of being in the most severe acyclic, severe cyclic pelvic pain subgroup (class 5) relative to the subgroup with no pain (class 2). IL-16 is produced by a variety of cell types and has a wide range of pro-inflammatory actions.[270] It has been implicated in inflammatory disease such as atopic dermatitis, inflammatory bowel disease, a range of autoimmune diseases, infections and airway disorders including asthma.[271] With respect to pain, and particularly pelvic pain, genetic variation leading to aberrant expression of IL-16 have been investigated in multiple studies, however, there are mixed findings regarding the clinical implications of increased IL-16 in pelvic pain and associated diseases. In a study of a Greek population, consisting of women with endometriosis (N=159) and without (N=146), an IL-16 gene polymorphism (rs11556218) was associated with increased risk of the disease.[272] Similar findings were found among Iranian women for two IL-16 polymorphisms (rs11556218 and rs4072111), the latter of which had not been found to be significant in the Greek population.[273] A study in a Chinese population, consisting of women with endometriosis (N=230) and without (N=203), an IL-16 gene polymorphism (rs4778889) was associated not only with endometriosis, but also with the subgroup that experienced pain symptoms compared to those without any pain

symptoms.[274] While the pathway between the polymorphisms and pain is not clear, in the latter study, it was suggested based on a previous study with this specific polymorphism in asthma,[275] that individuals with the aberrant polymorphism may produce higher levels of IL-16.

Monocyte chemoattractant protein-4 (MCP-4) also known as CCL13 is involved in both allergic and non-allergic inflammation via CCR3 signalling.[276] MCP-4 plays a critical role in the chemotaxis of eosinophils, which are forefront effectors in allergic diseases such as atopic dermatitis. It has also been linked to a number of chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease (IBS).[277] A study comparing children with and without atopic dermatitis (N=152) which examined the levels of 44 plasma analytes including cytokines, it was found that levels of IL-16, MCP-4 and TARC were significantly higher in those with atopic dermatitis, while other cytokines levels were not associated with increased risk of atopic dermatitis.[278] While IP-10, IL-16 and MCP-4 are all increased in the most severe cyclic and acyclic pain group, IP-10 and IL-16 act via T helper I effector cells while MCP-4 acts via T helper II effector cells. The T helper I dominated inflammatory profile is generally considered to be the prototypic pro-inflammatory profile, yet in patients with chronic pain conditions some studies have observed surprising shifts to a T helper II profile.[279] Autoimmune disease, allergies and asthma have been found to be more common in women with endometriosis.[227] In our previous study we found a significantly increased risk of allergies and asthma in the most severe cyclic and acyclic pain group (class 5). There is some evidence that hypersensitivities may actually impact pain levels experienced, for example a study in men with chronic pelvic pain found that episodes were triggered by elevated pollen levels.[280] Overall our findings are consistent with previous findings for IL-16 and MCP-4, and suggest that these two

cytokines should be further investigated in contexts of the association between their dual role in mediating allergies and hypersensitivity and pelvic pain disorders.

Strengths and limitations

The current study has several important strengths and limitations. First, the major strength of this study is a unique and large cohort oversampled with adolescents and with individuals with severe pelvic pain compared to the general population. Secondly, despite the cross-sectional design of the study, there is some temporal ordering, as the symptoms used to derive latent classes were generally described as symptoms over the previous year when the survey was completed, whereas most blood samples were collected within the month when the baseline survey was completed. Third, we examined non-linearity in associations between biomarkers and latent classes and accounted for it in our models. A major limitation in this study is that we use a single measurement of inflammatory biomarkers in association with chronic inflammatory status, and many cytokines which have short half-lives in blood and can be sensitive to acute events.[281] However, studies examining intraclass correlations (ICCs) of cytokine measurements over time in healthy populations have reported that single measurements of our biomarkers of interest are, to varying degrees, representative of average levels over time.[253] Second, the different subgroups identified through our LCA approach may not fully represent different biological underpinnings that would be linked to variations in levels of inflammatory biomarkers. We nevertheless conducted sensitivity analyses that demonstrate that the pain characteristic defined classes remained stable across multiple subsets of the population. Third, the biomarkers in the study had moderate correlation, and it is possible that some associations could be attenuated if these correlations are adjusted for. As well, the population in the current study is primarily White of European descent, in addition to being overrepresented

with adolescents and those with endometriosis. This limits the generalizability of findings from this study to other populations. However, to our knowledge this cohort is also one with the closest measurement of inflammatory biomarkers to symptom onset, while many historical studies have only measured biomarker levels in adults many years after symptom onset and treatment at the time of blood collection.

Conclusion

We found significant associations between inflammatory biomarkers and subgroups of chronic pelvic pain, though the strength of these associations varied depending on the biomarker of interest. Most differences remained significant after adjustment for age and body mass index. This exploratory study raised two important questions regarding pain subgroups and their associations with underlying pathologies that merit further investigation. First, do specific cytokines such as IP-10 have different or even paradoxical associations with histopathologic phenotypes versus pain phenotypes that may lead to discrepancies seen between pathology and pain, for example in the case of endometriosis. Second, are there true non-monotonic associations between cytokines in the context of complex pelvic pain disorders. The approach used to group pelvic pain in the current study focused exclusively on symptoms; future studies may incorporate other clustering variables including biomarkers and genotypes within the clustering algorithm and offer additional biological-based insights into the heterogeneity of pelvic pain.

CHAPTER 5 GENERAL DISCUSSION

Overview

Worldwide an estimated 2.1% to 24% of women experience acyclic pelvic pain, 16.8-81% cyclic pelvic pain, and an estimated 10% are affected by endometriosis, one of the important causes of pelvic pain.[1,9] Chronic pelvic pain is multifactorial, and the underlying pathologies associated with it, are highly heterogenous in their symptomatic presentation, treatment response and prognosis. Improved characterization of the heterogeneity seen in chronic pelvic pain and endometriosis paves the way for better understanding of the underlying mechanisms driving pain and lays the foundation for improved treatments. The purpose of this dissertation was to characterize the population level heterogeneity of chronic pelvic pain and endometriosis via three aims. First, we aimed to understand the temporal and geographic distribution and heterogeneity of endometriosis at the global level. We conducted a systematic search of literature over a 30-year period and identified 69 studies which were used to identify endometriosis incidence and prevalence in the general population and in clinical populations, as well, endometriosis distribution by indications for diagnosis, such as pelvic pain symptoms. We then proceeded to focus on pelvic pain and its comorbidities, with a lens on endometriosis. Thus, as part of our second aim, we assessed pain symptoms clustering in a large extensively phenotyped cohort oversampled for adolescents and those with surgically diagnosed endometriosis. To identify these subgroups, we conducted latent class analysis based on six indicators of pelvic pain. We identified five subgroups and found the highest aggregation of comorbidities among those classified in the severe acyclic and severe cyclic pelvic pain subgroup. Then, as a part of our third aim, we proceeded to examine the associations between seven plasma-based inflammatory biomarkers and subgroups of pelvic pain. We found that being

in the severe acyclic and severe cyclic pelvic pain subgroup was associated with the highest concentration quintile for three inflammatory biomarkers. Overall, our results from the two latter aims demonstrated that subgroups of pelvic pain can be identified using a data-driven approach. While a substantial portion of the subgroups with severe acyclic or cyclic pelvic pain consisted of those with surgically diagnosed endometriosis, participants with many other comorbidities also had increased odds of being classified into these subgroups. The direct and indirect effects of multiple comorbidities and associated inflammatory cytokines may contribute to the generation of unique patterns of symptomatic phenotypes.

Summary of major findings

In aim 1, we investigated population-level heterogeneity in endometriosis. We found there was large variation in estimates of prevalence between studies examining endometriosis incidence and prevalence globally. A systematic search of literature yielded 846 records, and among these, we identified 69 studies that provided epidemiologic measurements over a 30-year period between 1989 and 2019. Of these, all provided prevalence estimates and 16 provided incidence estimates. Just above one-third of the studies we examined had a sampling frame from the general population, and 14% had sample sizes ≥10,000 women. Studies based on Europe represented the majority of data (38%), while there were very few studies from Australia and Africa. We did not identify a clear change in trends across time for endometriosis incidence or prevalence, only observing a slight decrease in prevalence (β =-0.10, $p<0.05$) in our analysis of general population data abstracted from health system/insurance systems. Overall, we identified prevalences ranging from 0.7% to 8.6% in the general population, and 0.2% to 71.4% in clinical populations. Our overall prevalence for the general population estimated via a random effects model was 4.2% (95% CI 2.2,7.0) which is lower than the often-reported estimate of 10%.

Meanwhile the random effects estimate of prevalence for single clinic and hospitals yielded a prevalence of 22.9% (95% CI 17.1-29.2), which is much higher than 10%. We stratified clinical populations to examine the prevalence of endometriosis diagnosis by indication. The top two indications characterized in studies were infertility described in 17 studies, and chronic pelvic pain described in 11 studies. The prevalence range of endometriosis among those with infertility was 9.0% to 68.0%, with an overall prevalence estimated using a random effects model of 34.4% (95% CI 23.9, 45.1). The prevalence range of endometriosis among those with chronic pelvic pain was 15.4% to 71.4%, with the overall prevalence estimated via a random effects model of 46.6% (95% CI 31.1, 62.3). To assess if there have been changes in endometriosis severity, we examined reported prevalence of surgical staging. We found extremely heterogenous results contingent on a variety of sampling frame and study design factors. We also found no data that would allow us to assess objective changes in endometriosis symptomatic severity, life impact or prognosis over time.

In aim 2, we identified subgroups of women with similar patterns of pelvic pain using a data-driven clustering technique in a cohort drawn from clinics and the general population. Our sampling frame of the Women's Health Study: Adolescence to Adulthood cohort consisted of 1255 participants, of which 37.3% were younger than 21 years, and the majority of whom identified as white (81.0%) or black (4.3%). Participants had a median of 3 (range=0 to 14) comorbid conditions, including eighteen gynecologic/genitourinary, respiratory/immune, rheumatologic/neurologic, and gastrointestinal/abdominal. The most prevalent condition was migraine, impacting 69.0% of participants. As well, 47.6% of participants had surgically diagnosed endometriosis. Using latent class analysis we identified five subgroups, which were qualitatively described as 'no pelvic pain' (class 1), 'moderate cyclic pelvic pain only' (class 2),

‘severe cyclic pelvic pain only’ (class 3), ‘moderate cyclic and severe acyclic pelvic pain’ (class 4) and ‘severe acyclic and severe cyclic pelvic pain’ (class 5). In association analyses, the highest number of comorbidities (12) were associated with class 5 relative to class 1. Migraine was the only comorbidity associated with all pelvic pain subgroups, with the strongest magnitude as a predictor of being assigned to class 5 relative to class 1 (OR=7.78, 95% CI 4.82, 12.56). The distribution of surgically diagnosed endometriosis in these subgroups ranged from 4.0% in class 1 to 94.1% in class 5, and it was the strongest predictor of being in the three most severe pain subgroups compared to class 1. In terms of conditions exclusively associated with subgroups, allergies and asthma were associated only with class 3 and class 5, while chronic fatigue syndrome was only associated with class 4, and fibromyalgia only with class 5, though the latter two had very wide confidence intervals due to small number of cases overall.

In aim 3, we used subgroups of pelvic pain identified in the second aim to examine differences in inflammation-associated plasma-based biomarkers based on pain symptom patterns. We found moderate correlations between levels of inflammatory biomarkers and identified non-linear relationships between many biomarkers and probability of being assigned to a given pelvic pain subgroup. Restricted cubic spline models revealed that five inflammatory biomarkers: IL-16, IP-10, MCP-1, MCP-4, TNF-a had strong non-linear relationships with the probability of being in the severe cyclic and acyclic pain subgroup (class 5) versus the no pelvic pain subgroup (class 1). The highest concentration quintiles of IL-16, IP-10 and MCP-4 were associated with higher odds of being in the severe acyclic, severe cyclic pelvic pain subgroup (class 5) compared to the no pelvic pain subgroup (class 1). We also found that the highest concentration quintile of IP-10 was associated with higher odds of being in the moderate cyclic and severe acyclic pelvic pain subgroup (class 4), and the highest concentration quintile of MCP-

4 with moderate cyclic pelvic pain only subgroup (class 2), both relative to the no pelvic pain subgroup (class 1). Overall, the severe acyclic, severe cyclic pelvic pain subgroup (class 5) had the strongest associations with pro-inflammatory biomarkers, suggesting systemic immunomodulatory aberrations in this subgroup.

Public health implications and future directions

Overall, the results from the three studies included in this dissertation provide a basis for a range of questions and methods in future research concerning chronic pelvic pain and endometriosis. In our comprehensive review of the literature on endometriosis, we found that there have been few high-qualities studies that measure the prevalence of the disease across diverse populations around the world. There is, in particular, a dearth of studies from Asia and Africa. We also found few large-scale cross-sectional or longitudinal studies that allow for meaningful comparisons across time. Conducting higher quality studies across populations and across time will allow for the derivation of more accurate estimates of the true prevalence and incidence of the disease. Epidemiologic measures of endometriosis are also severely limited by diagnostic limitations of the disease. Endometriosis is challenging to diagnose, and currently the standard for diagnosis for pelvic endometriosis remains laparoscopic visualization. However, surgical referral requires patients to meet a threshold of symptoms and have appropriate access to specialist care.[9] We found low population prevalence of diagnosed endometriosis, but very high prevalence among clinical populations with indications such as pelvic pain. Given the high prevalence of pelvic pain globally and the challenges with diagnosing endometriosis, many population measures are likely underestimating the true prevalence of the disease. The development of non-invasive diagnostics at the point-of-care will have a meaningful impact not just on research but also providing a pathway to relief to millions suffering from endometriosis

and its sequalae. In our two studies focused on subgroups of pelvic pain, we found that disparate patient symptom profiles in a cohort of women could be meaningfully aggregated into clusters and understood as patterns of symptomology. Studies with larger and more diverse populations may provide further evidence for unique patterns of pelvic pain, their association with specific comorbidities and biomarkers, and provide a stratum for research in understanding the underlying pathways to different types of pain.

Strengths and limitations

Broadly, there are several important limitations that should be considered in examining the results from these studies. First are biases associated with study populations under consideration. In terms of case definitions, our first study relies on estimates of diagnosed endometriosis, whether self-reported or abstracted from records. Our second and third study use the gold standard diagnostic criteria (surgically-confirmed diagnosis) for endometriosis but rely on self-report for other comorbid conditions. Diagnosis of endometriosis and many other chronic disease comorbidities may be inaccurate for reasons ranging from limited diagnostic tools to poor access to care and so on. The A2A cohort follow-up over an eight-year period has shown that extremely few individuals not diagnosed with endometriosis were later diagnosed, reducing risk of misclassification bias for endometriosis in this specific cohort. However, we have not assessed if this holds true for other comorbidities reported, and if fewer individuals are diagnosed with a given comorbidity than there is true disease, then any comparisons of estimates may be pulled toward the null. Second there are limitations associated with symptom-based latent classes. Two of our studies rely on latent class analysis which is a data-driven approach, meaning that any classes identified could be statistical phenomenon, lacking true validity and reliability.[94] While we have addressed the validity of the classes by examining their associations

with other external variables, our examination of reliability was limited to a subset of the sample and not a different population. Additionally, as we established latent classes of pelvic pain, we did not incorporate past and current pain-modifying and hormonal medication use in our models. For instance, our most severe subgroups are individuals that are not using pain-modifying medication or are using these medications without adequate pain control. On the other hand, it is possible there are individuals in none or moderate subgroups who, without the intervention of pain-modifying medications, would have been classified into severe pain subgroups. Third, the cohort in this analysis is by design is over-represented with adolescents and individuals with surgically diagnosed endometriosis and thus these results may not generalize to a chronic pelvic pain population with a different composition.

Conclusion

While chronic pelvic pain and endometriosis represent a large disease burden globally, their heterogenous distribution and presentation is not well-understood. As a part of this dissertation we identified the variable range in epidemiologic measures pertaining to endometriosis, one of the great contributors to chronic pelvic pain in women. In doing so we highlighted the need for higher quality longitudinal studies particularly at the population level, examining not just diagnosis of endometriosis but also the symptomology including severity, frequency and life impact of pain associated with the disease over populations and time. As efforts are underway across disciplines to better understand the underlying mechanisms that lead to the pathology and symptomology observed in among patients with endometriosis and pelvic pain, it is essential to explore underlying causes of the considerable heterogeneity in clinical presentation. The current dissertation contributes to the field as the first to focus on a latent class analysis approach to derive pelvic pain symptom-based subgroups and examine how

inflammation and pain-associated comorbidities and inflammatory biomarkers relate to these subgroups. In doing so this dissertation draws attention to the importance of moving beyond a single-symptom or single-disease paradigm in assessing complex multifactorial conditions such as pelvic pain. Moving forward, studies examining pelvic pain and endometriosis may benefit from person-centered approaches that incorporate multiple dimensions and allow for assessment of patterns of symptomology in assessment of risk factors, treatment progression, and prognosis of pelvic pain and associated disorders.

APPENDICES

APPENDIX A: CHAPTER 2 SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 2.1 Summary of abstracted data from studies published from 1989-2019 included in the systematic review (N=69) detailing location, sample size, sampled population, and frequency estimates - ordered by first author.

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Abbas (2012, Germany, N=62,323)	2004-2008	General population of women covered by insurance groups specified	Statutory Health Insurance (SHI) Sample AOK Hesse/KV Hesse (ICD-10: N80 diagnostic codes)	General population[1]: Hospital system and/or claims records	15-54 32, median age for symptomatic patients	0.8% (in 2007)	Incidence = 3.5 per 1,000 women (95% CI 3.0-4.0), age-standardized	--
Ajossa (1994, Italy, N=305)	1991-1993	Premenopausal patients undergoing laparoscopy for infertility, chronic pelvic pain, non-malignant ovarian cyst, or uterine myoma	Medical records from a single hospital (Laparoscopy, laparotomy and histology)	Single hospital or clinic	15-57	24.9%	Not calculated	rAFS[2] staging overall: I=37% II=10% III=32% IV=21% Proportion with each stage presented by indication for diagnosis. Among infertile: stage I=56%, II=11%, III=22%, IV=11%; chronic pelvic pain: I=50%, II=17%, III=17%, IV=17%; benign ovarian cyst: I=18%, III=46%, IV=36%; uterine myoma: I=33%, II=25%, III=33%, IV=8.4%
Akbarzadeh-Jahromi (2015, Iran, N=110)	2008-2013	Patients with epithelial ovarian cancer	Medical records from two gynecologic centers (Histology)	Single hospital or clinic	24-83 49.93 ±9, mean age for diagnosed patients	25.5%	Not calculated	--
Al-Jefout (2017, Jordan, N=1772)	2015	General population recruited in public locations in three cities	Self-administered Questionnaires (Self-reported questionnaires)	General population[1]: country or region	15-55	2.5%	Not calculated	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Al-Jefout (2018, UAE, N=3572)	2016	Women recruited via email working or studying at a single university	Self-administered Questionnaire (Self-reported questionnaires)	General population[1]: country or region	18-55	1.5%	Not calculated	--
Al-Jefout (2018, Jordan, N=28)	2010-2014	Patients with chronic pelvic pain refractory to conventional therapy	Medical records from two hospitals (Laparoscopy)	Single hospital or clinic	15-21 18.4 ±2, mean age at diagnosis	71.4%	Not calculated	rASRM[3] Stage at laparoscopy: I=45% II=40% III=10% IV=5%
Balasch (1996, Spain, N=100)	Not stated	Patients undergoing laparoscopy for infertility, chronic pelvic pain, or sterilization	Medical records from a single hospital (Laparoscopy and/or histology)	Single hospital or clinic	33.3 ± 4, mean age of sample	50.0%	Not calculated	Proportion with each stage presented by indication for diagnosis. rAFS[2] stage among infertile: I=81%, II=15%, III=4%; pelvic pain: I=88%,II=13%; fertile: I=100%
Bocker (1994, Israel, N=1434)	1970-1989	Orthodox Jewish women undergoing hysterectomy	Medical records from a single medical center (Histology)	Single hospital or clinic	49.1 mean age of sample (1970-1979), 51.2 mean age of sample (1980-1989)	Not calculated	Presented as proportions per decade and not as rates. 1.52% (1970-1979); 0.70% (1980-1989); 1.12% (1970-1989)	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Buck Louis (2011, USA, N=495 operative cohort, 131 population cohort)	2007-2009	Endometriosis: Natural History, Diagnosis, and Outcomes (ENDO) Study -- Case/control study of women undergoing laparotomy or laparoscopy for any clinical indication plus general population presumed controls	Assembled cases and controls in 5 clinical centers in Utah and 9 in California; presumed controls underwent MRI (Surgically visualized, histology, MRI)	General population[1]: Hospital system and/or claims records	18-44 33 ± 7, mean age of sample	Not calculated	Presented as proportions and not as rates. Operative cohort: 41% for any surgical visualization, 0.7% for histology only no visualization, 7% MRI only; population cohort: 11% MRI only	rASRM[3] Stage at laparoscopy for operative cohort: I=50% II=21% III=18% IV=11%
Camilleri (2011, Malta, N=437)	2003-2008	Patients of reproductive age with infertility undergoing laparoscopy	Medical records from two hospitals (Laparoscopy)	Single hospital or clinic	31±4, mean age of sample	16.9%	Not calculated	rASRM[3] Stage at laparoscopy: I=51% II=8% III=8% IV=5% Unspecified=35%
Cea Soriano (2017, UK, N=866, 295)	2000-2010	General UK population, medical records from THIN/HES database	The Health Improvement Network (THIN) and Hospital Episode Statistics (HES) (ICD-10 diagnostic code and review of free-text comments/validated questionnaires)	General population[1]: Hospital system and/or claims records	12-54	2.1% (in 2010)	IR=1.46 per 1,000 person years (95% CI 1.43-1.50)	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Chu (1995, Taiwan, N=752)	1993	Patients undergoing laparoscopy for indications unrelated to endometriosis	Medical records from a single medical center (Laparoscopy and histology)	Single hospital or clinic	>18	32.6%	Not calculated	rAFS[2] stage among asymptomatic 65%(I) 32% (2/3) 3%(IV)
Darwish (2006, Egypt, N=2493)	1998-2005	Patients undergoing laparoscopy for infertility, chronic pelvic pain and other indications	Medical records from a single hospital (Laparoscopy)	Single hospital or clinic	26.8±5, mean age of sample	18.8%	Not calculated	rASRM[3] Stage at laparoscopy: I=34.9% II=39.6% III=10.3% IV=15.2%
Dzatic-Smiljkovic (2011, Serbia, N=210)	2000-2004	Patients with epithelial ovarian cancer	Medical records from a single hospital (Surgery and histology)	Single hospital or clinic	Not stated	11.0%	Not calculated	--
Eggert (2008, Sweden, N=1, 081, 058)	1990-2004	Swedish-born patients living in Sweden first hospitalized between 1990-2004 (data for foreign born also available)	Swedish total population, National hospital discharge, and national cause of death registries (ICD-9: 617, ICD-10: N80 diagnostic codes)	General population[1]: Hospital system and/or claims records	20-41	Not calculated	IR=1.019 per 1,000 person-years (age-standardized, for Sweden-born)	--
Eisenberg (2018, Israel, N=570, 781)	2000-2015	General population of women covered by healthcare provider covering 1/4 of the country	Computerized databases of Maccabi Healthcare Services (MHS) (ICD-9: 617 diagnostic codes)	General population[1]: Hospital system and/or claims records	15-55 40.4 ± 8, mean age at diagnosis	1.1%	Incidence= 0.72 per 1,000 women (95% CI 0.65-0.80)	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
El-Sayed (2017, Egypt, N=100)	2015-2017	Patients with Polycystic ovary syndrome (PCOS) undergoing laparoscopic ovarian drilling	Medical records from a single university hospital (Laparoscopy)	Single hospital or clinic	25-35	1.0%	Not calculated	--
Esselen (2016, USA, N=838)	1998-2013	Patients with endosalpingiosis	Medical records from a single university hospital (ICD-9: 617 diagnostic code)	Single hospital or clinic	17-89 52, median age of sample	37.4%	Not calculated	--
Fawole (2015, Nigeria, N=239)	2008-2010	Patients undergoing first diagnostic laparoscopy for a gynecologic indication	Self-administered Questionnaire and medical records from a single hospital (Laparoscopy)	Single hospital or clinic	18-45	48.1%	Not calculated	--
Ferrero (2010, Italy, N=1291)	2007-2009	Premenopausal patients visiting general practitioners for non-gynecologic concerns	Self-administered Questionnaires and prospective medical records from a single hospital (Self-reported questionnaire for pain, MRI and surgery for subset)	Single hospital or clinic	30-39, age range of 52% of those diagnosed with endometriosis	3.6%	Not calculated	--
Fisher (2016, Australia, N=7427)	2012	Cohort of women in Australian Longitudinal Study on Women's Health (ALSWH) randomly selected from national Medicare database	National Australian Longitudinal Study of Women's Health (ALSWH) (Self-reported questionnaire)	General population[1]: Hospital system and/or claims records	34-39	3.7%	Not calculated	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Flores (2008, Puerto Rico, N=1285)	Not stated	General population recruited at public locations (health fairs, shopping centers, universities, businesses, etc.)	Self-administered Questionnaires (Self-reported questionnaire on presumptive and surgical diagnosis with endometriosis, no validation reported)	General population[1]: country or region	27.3 mean age recruited, 33.3 mean age of diagnosed	4.4% Overall 4.0% Self-reported surgically confirmed	Not calculated	--
Fuldeore (2017, US, N=48, 020)	2012	General US population	Three national response panels, assembled by survey companies (Self-reported questionnaire on presumptive and surgical diagnosis with endometriosis, no validation reported)	General population[1]: country or region	18-49	6.1%	Not calculated	--
Gao (2019, Sweden, N=3476)	1933-1972	Cohort of women born in Sweden between 1933-1972	National birth records and patient registries (ICD-8 : 625.3; ICD-9: 617, ICD-10: N80 diagnostic codes)	General population[1]: Hospital system and/or claims records	15-50	Not calculated	IR = 1.08 per 1,000 p-y	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Gylfason (2010, Iceland, N=231000)	1981-2000	National sample of women discharged from hospitals	Nationwide hospital databases, medical records, and pathology registries (ICD-8 : 625.3; ICD-9: 617, ICD-10: N80 diagnostic codes, medical and surgical notes)	General population[1]: Hospital system and/or claims records	15-69	Not calculated	*Presented as proportions and not as rates.* Incidence (crude)= 0.1% for visually confirmed, 0.06% for histologically confirmed; Incidence (age standardized) = 0.1% for visually confirmed, 0.05% for histologically confirmed	rASRM[3] Stage at laparoscopy: I/II=37% III/IV=63%
Hager (2019, Austria, N=225 (152 infertile))	2008-2018	Patients with Polycystic ovary syndrome (PCOS) undergoing laparoscopic ovarian drilling for clomiphene citrate resistance	Medical records from a single hospital (Laparoscopy)	Single hospital or clinic	28.3 ±5, mean age of sample	16.9%	Not calculated	rAFS[2] Stage at laparoscopy: I=86.8% II=13.2% III=0% IV=0%
Heinig (2002, Germany, N=13)	1995-2001	Patients with endo-salpingiosis	Medical records from a single university hospital (Surgery, histology)	Single hospital or clinic	24-82 43, mean age of sample	38.5%	Not calculated	rAFS[2] stage: I=0% II=20% III=80% IV=0%
Jimbo (1997, Japan, N=172)	1980-1995	Patients with epithelial ovarian cancer	Medical records from a single medical center (Surgery and histology)	Single hospital or clinic	15-78	14.5%	Not calculated	

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Khawaja (2009, Pakistan, N=796)	1999-2005	Patients undergoing evaluation for primary or secondary infertility	Medical records from a single hospital (Laparoscopy and histology)	Single hospital or clinic	16-47 29 ±5, mean age of sample	16.8%	Not calculated	rAFS[2] Stage at laparoscopy: I=40.1% II=33.7% III=16.9% IV=9.3%
Kjerulff (1996, USA, N=31617)	1984-1992	Women participating in nationally representative survey	National Health Interview Survey (Self-reported questionnaire about clinical diagnosis, no validation reported)	General population[1]: country or region	18-50	0.7%	Not calculated	--
Koninckx (1991, Belgium, N=643)	1987-1990	Patients Undergoing laparoscopy for infertility and/or chronic pelvic pain	Medical records from a single university hospital (Laparoscopy)	Single hospital or clinic		70.3%	Not calculated	Proportion with each stage presented by indication for diagnosis. rAFS[2] Stage at laparoscopy among infertility: I=46%, II=23%, III=23%, IV=9%; pain: I=42%, II=36%, III=28%, IV=3%; infertility and pain: I=19%, II=33%, III=33%, IV=14%
Kriplani (2001, India, N=70)	Not stated	Patients with Polycystic ovary syndrome (PCOS) undergoing laparoscopic ovarian drilling	Medical records from a single university hospital (Laparoscopy)	Single hospital or clinic	20-38 26.4, mean age of sample	10.0%	Not calculated	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Laufer (1997, USA, N=46)	1990-1994	Adolescent patients with chronic pelvic pain greater than 3 months not responsive to therapy	Medical records from two clinical centers (Laparoscopy plus histology)	Single hospital or clinic	13-21 16, mean age of sample	69.6%	Not calculated	rAFS[2] Stage at laparoscopy: I=79.4% II=22.6% III=0% IV=0%
Leibson (2004, USA, N=8229)	1987-1999	General population in a Minnesota county with history of 1 or more surgeries	Medical records from Mayo Clinic and affiliated hospitals (ICD-9: 617 diagnostic code, clinical and surgical diagnosis stratified and reported)	General population[1]: Hospital system and/or claims records	>15	11.5% (annual likelihood of surgical diagnosis)	IR=1.87 per 1,000 person-years(p-y) (95% confidence interval (CI)=1.76- 1.99); (IR=2.46 /1000p-y when definition standardized to 1970-1979	--
Machado-Linde (2015, Spain, N=496)	1971-2010	Patients with epithelial ovarian cancer	Medical records from a single hospital (Surgery, histology)	Single hospital or clinic	51.4 ± 12, mean age of sample	5.4%	Not calculated	--
Mahmood (1991, UK, N=1542)	Not stated	Premenopausal Caucasian patients undergoing laparoscopy for infertility, sterilization, chronic abdominal or pelvic pain, or total abdominal hysterectomy for dysfunctional uterine bleeding	Medical records from a single hospital (Laparoscopy, hysterectomy, histology)	Single hospital or clinic	34 ± 7, mean for diagnosed; 33.5 ± 6 mean for not diagnosed	14.7%	Not calculated	Proportion with each stage presented by indication for diagnosis. rAFS[2] Stage at laparoscopy among infertile: I/II=65%, III=25%, IV=10%; sterilization: I/II=81%, III=19%, IV=0%; abdominal pain: I/II=88%, III=12%, IV=0%; hysterectomy: I/II=75%, III=21%, IV=3%

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Matorras (1995, Spain, N=602)	1985-1991	Patients with a history of infertility greater than 2 years undergoing laparoscopy	Medical records from a single medical center (Laparoscopy, histology)	Single hospital or clinic	29.5 ± 3.4, mean age of sample	28.9%	Not calculated	rAFS[2] Stage at laparoscopy: I=50.6% II=23.0% III=14.4% IV=12.1%
Mazlouman (2004, Iran, N=220)	Not stated	Patients with infertility and without infertility	Medical records from a single hospital (Laparoscopy, histology)	Single hospital or clinic	25-40	23.6%	Not calculated	Proportion with each stage presented by indication for diagnosis. rAFS[2] Stage at laparoscopy for fertile: I=50%, II=36%, III=14%, IV=0%; infertile: I=8%, II=24%, III=39%, IV=29%
Meuleman (2009, Belgium, N=221)	2003-not stated	Patients with 1 year or greater history of infertility with a regular cycle whose partner had a normal semen analysis	Medical records from a single medical center (Laparoscopy and histology)	Single hospital or clinic	19-42 31.4±3 mean age for diagnosed with stage I/II; 3.4±4 for diagnosed with stage III/IV	47.1%	Not calculated	rAFS[2] Stage at laparoscopy: I/II=63.5% III/IV=36.5%
Mishra (2015, India, N=372)	2012-2013	Patients with primary or secondary infertility undergoing ultrasound, hystero-laparoscopy and chromopertubation test	Medical records from a single medical center (Ultrasound and laparoscopy, histology)	Single hospital or clinic	19-40 29±4 mean age of sample	48.4%	Not calculated	rAFS[2] Stage at laparoscopy: I=66% II=22% III=6% IV=6%

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Mishra (2017, India, N=502)	2014-2015	Patients with primary or secondary infertility undergoing ultrasound, hystero-laparoscopy and chromopertubation test	Medical records from a single medical center (Ultrasound and laparoscopy, histology)	Single hospital or clinic	19-44 28.6±4 mean age of sample	55.0%	Not calculated	rAFS[2] Stage at laparoscopy: I=66% II=18% III=8% IV=8%
Missmer (2004, USA, N=90, 065)	1989-1999	Nurses' Health Study II cohort	Self-administered Questionnaires (Self-reported clinical diagnoses with laparoscopy-confirmation)	General population[1]: country or region	25-42	5.3% (at baseline in 1989)	IR=13.8 per 1000p-y (women with past infertility); IR=2.37 per 1000p-y (women with no past infertility)	--
Moen (1997, Norway, N=4034)	1990-1992	General population of women attending cardiovascular disease screening program	Self-administered questionnaires (Self-reported questionnaire regarding diagnosis, operation and treatment, no validation reported)	General population[1]: country or region	40-42	2.0%	*Presented as proportions and not as rates.* 0.3% annual incidence	--
Moini (2013, Iran, N=403)	2009-2010	Patients with infertility referred for laparoscopy	Medical records from two clinics (Laparoscopy)	Single hospital or clinic	30.85±5, mean age for diagnosed with stage I/II; 31.4±5 mean age for diagnosed with stage III/IV	62.0%	Not calculated	rASRM[3] Stage at laparoscopy: I=21% II=28% III=33% IV=18%

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Morassutto (2016, Italy, N=1674573)	2011-2013	Patients living in areas covered by regional data linkage system in the Northeast Italy region	Linked hospital discharge records (ICD-9: 617, supported by laparoscopy or surgery)	General population[1]: Hospital system and/or claims records	15-83	2.0%	*Presented as proportions and not as rates.* Incidence= 0.06% in the region (for histologically verified), 0.14% (all diagnoses with or without histologic verification)	--
Mowers (2016, USA, N=9622)	2013-2014	Patients undergoing laparoscopy or abdominal hysterectomy for non-malignant indications	Michigan Surgical Quality Collaborative (N=52 hospitals representing 30% of all MI hospitals) (Laparoscopy or surgery)	General population[1]: Hospital system and/or claims records	<45: 61% overall, 73% of cases and 58% of non-cases	15.2%	Not calculated	--
Naphatthalung (2012, Thailand, N=220)	2011-2012	Patients undergoing hysterectomy with indication of adenomyosis or uterine myoma	Medical records from a single university hospital (Laparotomy and histology)	Single hospital or clinic	40-50 45.6±3, mean age for sample	28.6%	Not calculated	--
Nomelini (2013, Brazil, N=507)	1985-2007	Patients with leiomyomas, gynecologic premalignant or malignant neoplasms	Medical records from a single hospital (Surgeries including hysterectomies and laparotomies, histology)	Single hospital or clinic	13-79 39, mean pre-menopausal 59, mean post-menopausal	5.5% 6.9% among pre-menopausal; 1.52% among post-menopausal	Not calculated	rASRM[3] Stage at laparoscopy: I=42% II=27% III=12% IV=4% Other sites= 15%

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Oral (2003, Turkey, N=183)	1995-2001	Patients with epithelial ovarian cancer	Medical records from a single university hospital (Surgery, histology)	Single hospital or clinic	26-70, age range for diagnosed only	7.7%	Not calculated	--
Osefo (1989, Nigeria, N=1385)	1977-1987	Patients from Igbos subpopulation undergoing pelvic operations	Medical records from a single university hospital (Surgeries including hysterectomies and laparotomies, histology)	Single hospital or clinic	19-60	4.3%	Not calculated	--
Parazzini (1994, Italy, N=3684)	1991-1992	Patients with sterilization, chronic pelvic pain, fibroids, non-malignant ovarian cyst	Multicenter (N=23) ob-gyn departments in Italy (Laparoscopy, laparotomy and histology)	General population[1]: Hospital system and/or claims records	15-54	23.2%	Not calculated	Proportion with each stage presented by indication for diagnosis. rAFS[2] Stage at laparoscopy among chronic pelvic pain: stage I=37%, II=24%, III=30%, IV=10%; Fibroids: stage I=36%, II=11%, III=45%, IV=8%; Sterilization: stage I=51%, II=22%, III=20%, IV=7%; Ovarian cysts: stage I=13%, II=5%, III=62%, IV=20%
Ragab (2015, Egypt, N=654)	2012-2014	Adolescent patients with severe dysmenorrhea in rural and urban areas	Self-administered Questionnaire and medical record follow-up (Mainly ultra-sound, MRI, laparoscopy and histology)	General population[1]: country or region	15.2 ±4, mean age for sample	8.6% US suspicion of endometriosis 6.7%% by MRI or laparoscopy and histology	Not calculated	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Rawson (1991, USA, N=86)	Not stated	Patients undergoing laparoscopy for pelvic evaluation or for hysterectomy	Medical records from a single fertility center and hospital (Laparoscopy)	Single hospital or clinic	32.6, mean age for diagnosed; 35.2 mean age without endometriosis	45.3%	Not calculated	rAFS[2] Stage at laparoscopy: I=72% II=20% III=3% IV=5%
Reid (2019, Australia, N=652)	2017	General population of women with membership with Qualtrics marketing research company	Self-administered Questionnaires (Self-reported questionnaire for diagnosis of endometriosis over the last 3 years, no validation reported)	General population[1]: country or region	18-50	3.4%	Not calculated	--
Rouzi (2015, Saudi Arabia, N=190)	2008-2013	Patients undergoing laparoscopy for infertility, chronic pelvic pain, ectopic pregnancy, pelvic mass, or IUD removal	Medical records from a single hospital (Laparoscopy)	Single hospital or clinic	33.8±9, mean age for sample	11.1%	Not calculated	--
Saavalainen (2018, Finland, N=49,956 (nationwide cohort of diagnosed))	1987-2012	Patients discharged from private and public hospitals receiving a surgical diagnosis of endometriosis	Finnish Hospital Discharge Register (FHPR) (ICD-9: 617, ICD-10: N80 diagnostic codes, laparoscopy, laparotomy and other surgical procedures)	General population[1]: Hospital system and/or claims records	12-85 38.8, median age of diagnosed in 1987-1990; 33.3 median age of diagnosed in 2006-2010	Not calculated	IR=116 (CI=1.12–1.21) in 1987 to 45 (42–48) in 2012 per 100,000 women (age-standardized)	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Salah (2013, Egypt, N=120)	Not stated	Patients with Polycystic ovary syndrome (PCOS) undergoing laparoscopic ovarian drilling	Medical records from a single university hospital (Laparoscopy)	Single hospital or clinic	21-45	18.3%	Not calculated	--
Sangih-aghpeykar (1995, USA, N=3384)	1987-1993	Multiparous patients undergoing sterilization	Medical records from a single hospital (Laparoscopy)	Single hospital or clinic	<25 20% of cases, 23% of non-cases; ≥36 21% of cases, 13.5% of non-cases	3.7%	Not calculated	rAFS[2] Stage at laparoscopy: I=91.3% II=4.8% III=4% IV=0%
Seaman (2007, UK, N=369, 000)	1992-2001	Patients registered with clinical practices contributing data (private and temporary patients excluded)	UK General Practice Research Database (UK General Practice Research Database (GPRD) diagnostic code (laparoscopy, endoscopy, laparotomy), medications used to treat endometriosis)	General population[1]: Hospital system and/or claims records	15-55 35.1, mean age of diagnosed	1.5% (all cases) 1.2% (restricted to definite and probable)	IR=0.97 per 1000p-y (all cases); IR=0.77 per 1000p-y (restricted to definite and probable cases)	--
Somigliana (2012, Uganda, N=528)	2009-2010	Patients undergoing gynecologic consultations in a single hospital	Medical records from a single hospital (Clinical finding, US)	Single hospital or clinic	≤24 31% of sample; ≥35 32% of sample	0.2%	Not calculated	--
Sorouri (2015, Iran, N=100)	2011-2012	Patients with Polycystic ovary syndrome (PCOS) undergoing laparoscopic ovarian drilling	Medical records from a single university hospital (Laparoscopy)	Single hospital or clinic	27.6-28.0, mean for procedure subgroups	1.0%	Not calculated	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
Stanford (2005, Canada, N=64)	Not stated	Patients undergoing laparoscopy for chronic pelvic pain	Medical records from a single gynecologic private practice (referral center) (Laparoscopy and histology)	Single hospital or clinic	15-58 29.1, mean age for sample	28.1%	Not calculated	--
Tanma-hasamut (2014, Thailand, N=331)	2011-2012	Patients undergoing surgery for non-malignant gynecologic disease	Medical records pre and post-surgery (Laparoscopy, laparoscopy and histology)	Single hospital or clinic	39.4 ±17, mean age for sample	30.5%	Not calculated	--
Tissot (2017, France, N=465)	1989-2009	Patients undergoing sterilization	Medical records from a single university hospital (Histology)	Single hospital or clinic	15-49 40.7, mean age for sample	11.8%	Not calculated	rAFS[2] Stage at laparoscopy: I=70% II=13% III=15% IV=4%
Velebil (1995, USA, N=5067500)	1988-1990	Patients hospitalized for any gynecologic disorder	National hospitalization discharge survey (ICD-9: 617 diagnostic codes)	General population[1]: Hospital system and/or claims records	15-44	11.2% (1989-1990 total discharges)	Incidence: 3.24 per 1,000 women average annual rate	--
Vessey (1992, UK, N=17, 032)	1968/74-1990	General population visiting 17 family planning clinics in England and Scotland	Surveys conducted at the clinics (Laparoscopy or laparotomy abstracted from discharge summaries)	General population[1]: Hospital system and/or claims records	25-39, age range at study entry	Not calculated	Incidence: 18.4 cases per 1,000 women (1968-1990). Person-years reported only for sub-groups.	--

Supplemental Table 2.1 (cont'd)

Author (Year, Country)	Data Dates	Sampled Population description	Data Source (Diagnostic Method)	Population Source	Age Range	Prevalence	Incidence	Staging Details
von Theobald (2016, France, N=14, 239, 197)	2008-2012	Patients hospitalized at least once for endometriosis across the country	French hospital discharge database and program of medicalization of information system (PMSI) (ICD-10: N80 diagnostic code)	General population[1]: Hospital system and/or claims records	15-49	0.9%	Not calculated	--
Waller (1993, UK, N=174)	1990-1992	Patients with complaint of infertility, stratified by fertility status of partners	Physical examination and medical records (Laparoscopy)	Single hospital or clinic	21-45 30.8, mean age for sample with fertile partners; 30.6 mean age for sample with infertile partners	32.2% overall; 27.5% among women with infertile partners; 33.6% among women with fertile partners	Not calculated	rAFS[2] Stage at laparoscopy: I=71% II=23% III=5% IV=0%
Yamamoto (2017, USA, N=717)	2008-2009	Patients with history of infertility undergoing first IVF cycle	Medical records from a single infertility clinic (Clinical symptoms, subset with laparoscopic evidence)	Single hospital or clinic	35.8±4, mean age for sample	9.5%	Not calculated	--

[1] General population includes country, region, hospital system, insurance claims records

[2] American Society for Reproductive Medicine. The American Fertility Society Revised American Fertility Society classification of endometriosis. *Fertility and Sterility.* 1985;43:351-354.

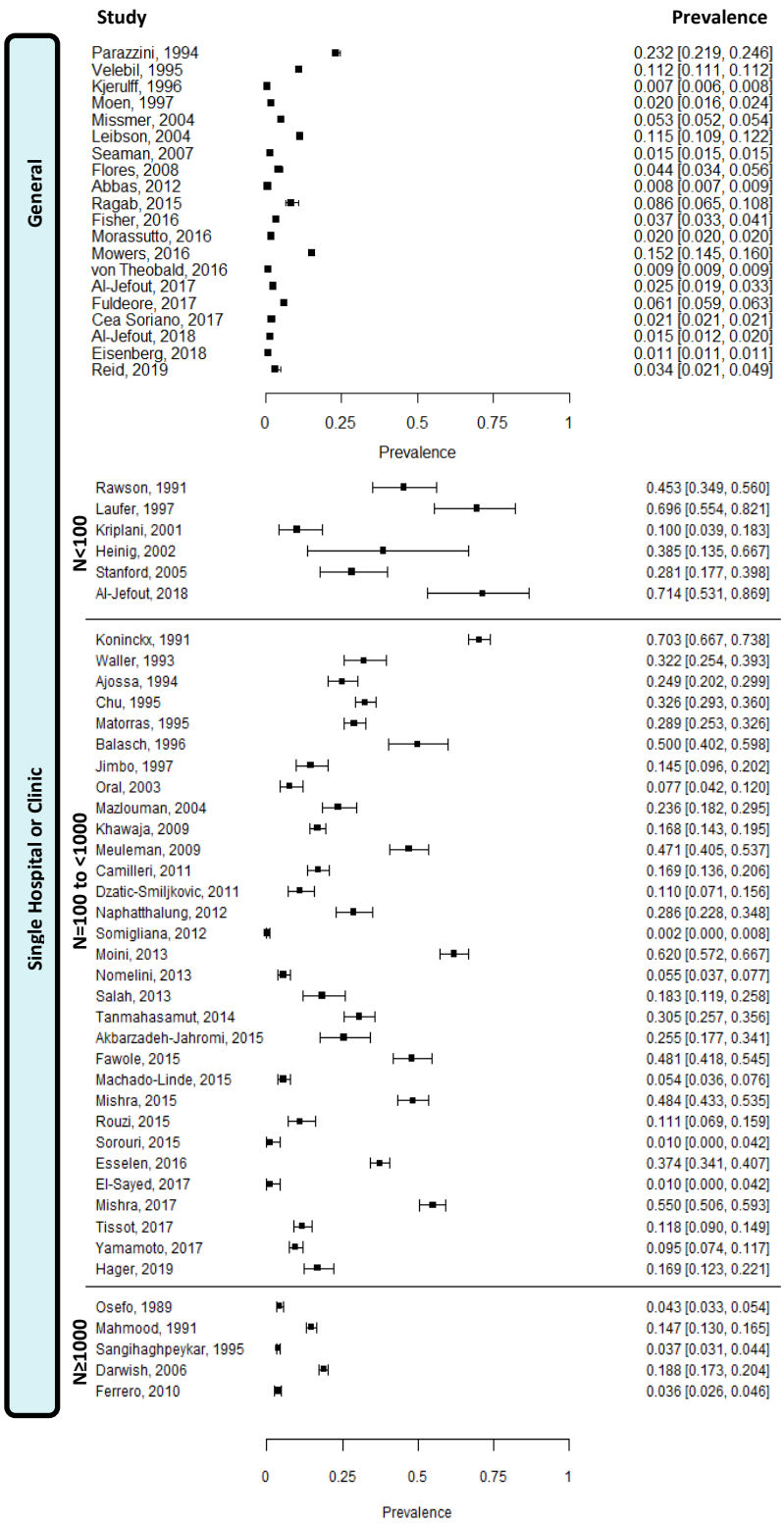
[3] Canis M, Donnez JG, Guzick DS, et al. Revised American society for reproductive medicine classification of endometriosis: 1996. *Fertility and Sterility.* 1997;67(5):817-821.

Supplemental Table 2.2 Adapted risk of bias tool for prevalence study quality assessment.

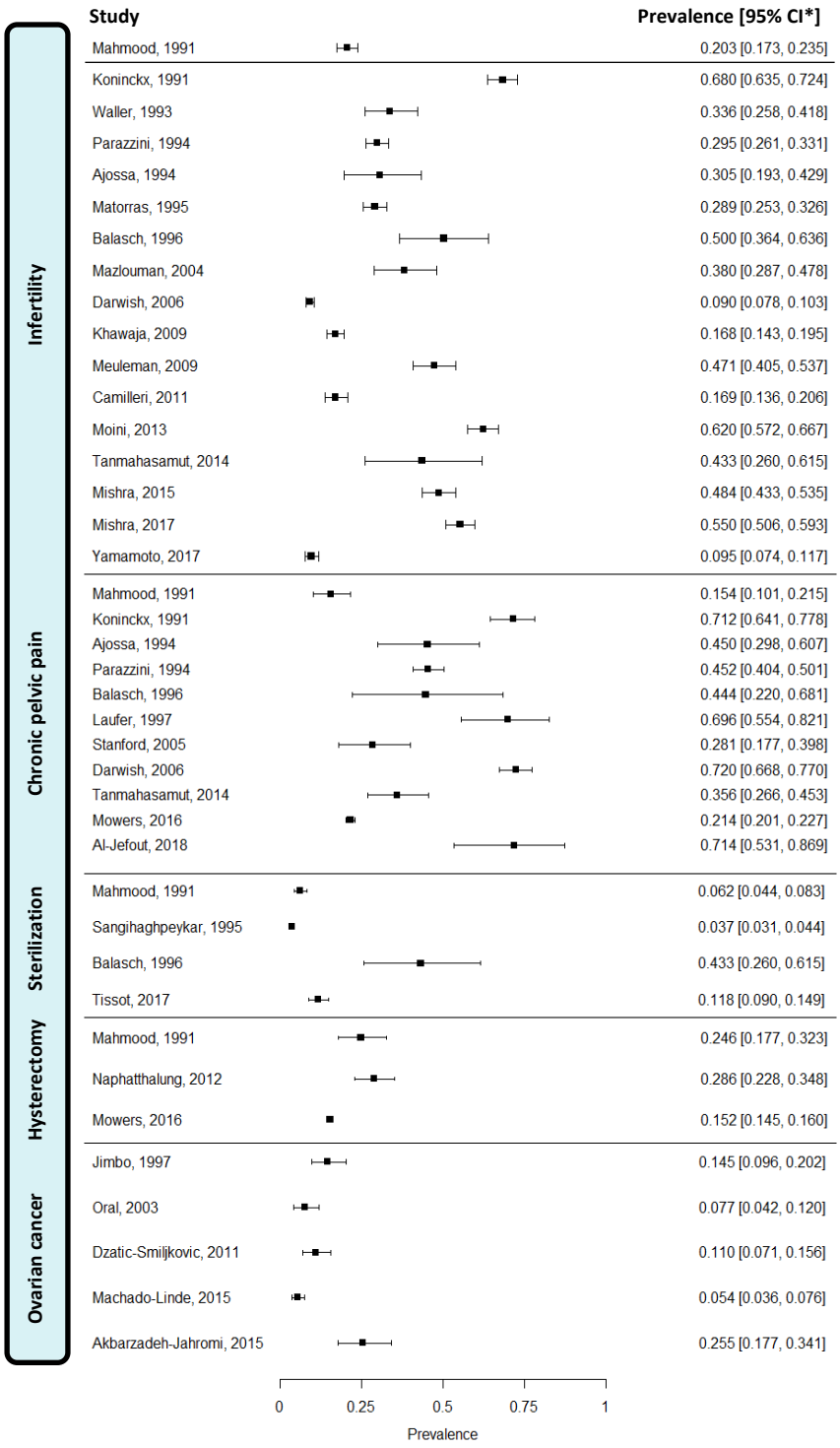
Risk of bias item	Answer criteria	Score assigned
1. Was the study's target population a close representation of the national population in relation to relevant variables, e.g. age, sex, occupation?	**Yes (LOW RISK):** The study's target population was a close representation of the national population.	0
	No (HIGH RISK): The study's target population was clearly NOT representative of the national population.	1
2. Was the sampling frame a true or close representation of the target population?	**Yes (LOW RISK):** The sampling frame was a **true or close** representation of the target population.	0
	No (HIGH RISK): The sampling frame was NOT a **true or close** representation of the target population.	1
3. Was some form of random selection used to select the sample, OR, was a census undertaken?	**Yes (LOW RISK):** A census was undertaken, OR, some form of random selection was used to select the sample (e.g. simple random sampling, stratified random sampling, cluster sampling, systematic sampling).	0
	No (HIGH RISK): A census was NOT undertaken, AND some form of random selection was NOT used to select the sample.	1
4. Was the likelihood of non-response bias minimal?	**Yes (LOW RISK):** The response rate for the study was >/=75%, OR, an analysis was performed that showed no significant difference in relevant demographic characteristics between responders and non-responders	0
	No (HIGH RISK): The response rate was <75%, and if any analysis comparing responders and non-responders was done, it showed a significant difference in relevant demographic characteristics between responders and non-responders.	1
5. Were data collected directly from the subjects (as opposed to a proxy)?	**Yes (LOW RISK):** All data were collected directly from the subjects.	0
	No (HIGH RISK): In some instances, data were collected from a proxy.	1
6. Was an acceptable case definition used in the study?	**Yes (LOW RISK):** An acceptable case definition was used.	0
	No (HIGH RISK): An acceptable case definition was NOT used.	1
7. Was the study instrument that measured the parameter of interest (e.g. prevalence of low back pain) shown to have reliability and validity (if necessary)?	**Yes (LOW RISK):** The study instrument had been shown to have reliability and validity (if this was necessary), e.g. test-retest, piloting, validation in a previous study, etc.	0
	No (HIGH RISK): The study instrument had NOT been shown to have reliability or validity (if this was necessary).	1
8. Was the same mode of data collection used for all subjects?	**Yes (LOW RISK):** The same mode of data collection was used for all subjects.	0
	No (HIGH RISK): The same mode of data collection was NOT used for all subjects.	1
9. Was the length of the shortest prevalence period for the parameter of interest appropriate?	**Yes (LOW RISK):** The shortest prevalence period for the parameter of interest was appropriate (e.g. point prevalence, one-week prevalence, one-year prevalence).	0
	No (HIGH RISK): The shortest prevalence period for the parameter of interest was not appropriate (e.g. lifetime prevalence)	1
10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	**Yes (LOW RISK):** The paper presented appropriate numerator(s) AND denominator(s) for the parameter of interest (e.g. the prevalence of low back pain).	0
	No (HIGH RISK): The paper did present numerator(s) AND denominator(s) for the parameter of interest but one or more of these were inappropriate.	1

Note: This instrument was dapted from Hoy et al. (2012)[113]

Supplemental Figure 2.1 Distribution of endometriosis prevalence by population source ordered by year of publication. *95% confidence interval around prevalence estimate calculated using



Supplemental Figure 2.2 Distribution of endometriosis prevalence by gynecologic indication ordered by year of publication. *95% confidence interval around prevalence estimate



APPENDIX B: CHAPTER 3 SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 3.1 Summary of pelvic pain indicator variables, including analytic category levels and description, data sources from each version of the survey and indicator quality in 5-class latent class analysis

	Variable description	Analytic category levels	Data sources from survey versions and sections			
			Survey prior to January 2014: Status at baseline	**Survey from January 2014 onward: Status at baseline**	**Survey from January 2014 onward (Version 3 only) Current and previous age range**	**Variable-specific entropy[2]**
Acyclic pelvic pain	Severity	• 0 None/mild (NRS 0-3) • 1 Moderate (NRS 4-6) • 2 Severe (NRS 7-10)	✓ 'Worst pain' / last 12 months	✓ 'Worst pain' / last 3 months		0.36
	Frequency	• 0 No pain, <1 day/month, monthly but not weekly • 1 Weekly (1-6 days per week) • 2 Every day	✓ 'How often' in last 12 months	✓ 'How long' in last 3 months		0.23
	Life impact[1]	• 0 No • 1 Yes	✓ Last 12 months	✓ Last 3 months		0.29
	Severity	• 0 None/mild (NRS 0-3) • 1 Moderate (NRS 4-6) • 2 Severe (NRS 7-10)	✓ Severity of usual period pain, for menstruating in past 12 months	✓ Period pain severity at worst in the past 12 months, or worst pain at last period in past 3 months if last 12 months missing	✓ For non-menstruating in past 12 months: Worst pain in current age range or if not available in the previous age range	0.31
Cyclic pelvic pain	Frequency	• 0 Never/occasionally • 1 Often/usually • 2 Always		✓ Period pain frequency in the past 12 months	✓ For non-menstruating in the past 12 months, frequency in current age range or if not available in the previous age range	0.29
	Life impact[1]	• 0 No • 1 Yes		✓ Life impact at last period in the past 3 months	✓ For non-menstruating in the past 3 months, life impact at current age range, or if not available life impact at the previous age range	0.15

*Abbreviations NRS: Numerical rating scale, with 0 as no pain and 10 worst pain imaginable

[1] Life impact is defined as pain that interfered with work or school, or daily activities at home

[2] Variable-specific entropy contribution for 5-class model, allows for quality comparison between indicators in how they identify latent classes; does not have a recommended threshold or provide statistical significance testing

Supplemental Table 3.2 Summary of pelvic pain indicator variables missingness

	Acyclic pelvic pain			Cyclic pelvic pain		
	Severity	**Frequency**	**Life impact**	**Severity**	**Frequency**	**Life impact**
Total available	1223	1232	1230	1049 0=272 (25.9%) 1=264 (25.2%) 2=513 (48.9%)	859 0=291 (33.9%) 1=227 (26.4%) 2=341 (39.7%)	723 0 = 564 (78.0%) 1 = 159 (22.0%)
Missing in original sample	60	51	53	234	424	560
due to no menstruation or unknown	N/A	N/A	N/A	174 = no menstruation >12 months 6 =unknown	174 = no menstruation >12 months 6=unknown	356 (no menstruation >3 months) 6=unknown
Added using data from current age range and previous age range (V3/4 only)1	N/A	N/A	N/A	+73 0=20 (27.4%) 1=16 (21.9%) 2=37 (50.7%)	+74 0=22 (29.7%) 1=16 (21.6%) 2=36 (48.6%)	+203 0=83 (40.9%) 1=120 (59.1%)
Remain missing on all variables, dropped from sample	28	28	28	28	28	28
Available	1223	1232	1230	1122	933	926
Missing	32	23	25	133[1]	322[2]	329[2]
Total sample size				1255		
Overall distribution	0=833 (68.1%) 1=106 (8.67%) 2=284 (23.2%)	0=958 (77.8%) 1=168 (13.6%) 2=106 (8.60%)	0=918 (74.6%) 1=312 (25.4%)	0=292 (26.0%) 1=280 (25.0%) 2=550 (49.0%)	0=313 (33.5%) 1=243 (26.0%) 2=377 (40.4%)	0=647 (69.9%) 1=279 (30.1%)
Distribution for no endometriosis	0=588 (91.0%) 1=27 (4.18%) 2=31 (4.80%)	0=624 (95.7%) 1=24 (3.68%) 2=4 (0.613%)	0=618 (95.1%) 1=32 (4.92%)	0=269 (42.6%) 1=224 (35.5%) 2=138 (21.9%)	0=294 (51.3%) 1=175 (30.5%) 2=104 (18.2%)	0=520 (90.9%) 1=52 (9.09%) 2=86 (13.1%)
Distribution for diagnosed with endometriosis	0=245 (42.5%) 1=79 (13.7%) 2=253 (43.8%)	0=334 (57.6%) 1=144 (24.8%) 2=102 (17.6%)	0=300 (51.7%) 1=280 (48.3%)	0=23 (4.68%) 1=56 (11.4%) 2=412 (83.9%)	0=19 (5.28%) 1=68 (18.9%) 2=273 (75.8%)	0=127 (35.9%) 1=227 (64.1%) 2=243 (40.7%)

[1] Remaining missing: consisted of participants who had periods in the past 12 months but reported no severity for past 12 months or 3 months, or who had not menstruated in the past 12 months but had not reported historical data or did not have historical data available because they completed the WERF EPHect compliant survey administered starting in January 2014.
[2] Remaining missing for frequency of pain with periods and life impact of pain with periods: consisted of those who had not menstruated and who had not reported current age range or historical data

Supplemental Table 3.3 Determination of class structure by comparison of indicators of fit for latent class models comprising of 1-9 classes

Number of classes	Parameters	Log-likelihood	AIC	BIC	aBIC	BLRT p-Value	VLMR-LRT p-Value	Entropy
1	10.00	-5277.02	10574.03	10625.38	10593.61	.	.	.
2	21.00	-4250.79	8543.59	8651.42	8584.72	0.00	0.00	0.93
3	32.00	-4004.85	8073.71	8238.02	8136.38	0.00	0.00	0.82
4	43.00	-3940.23	7966.47	8187.27	8050.68	0.00	0.00	0.82
5[1]	54.00	-3900.32	7908.64	8185.93	8014.40	0.00	0.00	0.76
6	65.00	-3879.33	7888.65	8222.42	8015.95	0.00	0.06	0.77
7	76.00	-3868.42	7888.85	8279.10	8037.69	0.08	1.00	0.77
8	87.00	-3859.18	7892.36	8339.10	8062.75	0.24	1.00	0.78
9	98.00	-3852.16	7900.31	8403.53	8092.24	1.00	0.73	0.80

*Abbreviations AIC: Akaike information criterion, BIC: Bayesian Information Criterion, aBIC: sample-adjusted, Bayesian Information Criterion, BLRT: bootstrap likelihood ratio test, VLMRT-LRT: Vuong-Lo-Mendell-Rubin likelihood ratio test
**Notes: Model fit was evaluated by using fit statistics (AIC, BIC, aBIC), likelihood ratio tests (BLRT, VLMR-LRT), entropy and interpretability
[1] Selected as final model based on low aBIC, followed by BIC values, VLMR-LRT p value <0.05 for the goodness of fit of model of 5-classes relative to a model of 4 classes, overall entropy, and interpretability

Supplemental Table 3.4 Descriptive characteristics of the five most-likely latent classes identified

	Total (N=1255)	Class 1 (N=300)	Class 2 (N=332)	Class 3 (N=256)	Class 4 (N=98)	Class 5 (N=269)
		No pelvic pain	Moderate cyclic pain only	Severe cyclic pain only	Severe acyclic, moderate cyclic pain	Severe acyclic, severe cyclic pelvic pain
Age at completion of baseline survey						
Mean (SD)	23.4 (7.16)	26.1 (7.08)	24.3 (6.79)	21.7 (6.63)	22.8 (7.36)	21.3 (7.03)
≤15 years	121 (9.6%)	5 (1.7%)	20 (6.02%)	44 (17.2%)	11 (11.2%)	41 (15.2%)
16-20 years	348 (27.7%)	43 (14.3%)	68 (20.5%)	88 (34.4%)	34 (34.7%)	115 (42.8%)
21-30 years	617 (49.2%)	194 (64.7%)	198 (59.6%)	97 (37.9%)	40 (40.8%)	88 (32.7%)
31-40 years	125 (10.0%)	41 (13.7%)	32 (9.64%)	24 (9.38%)	11 (11.2%)	17 (6.32%)
≥41 years	44 (3.5%)	17 (5.7%)	14 (4.22%)	3 (1.17%)	2 (2.04%)	8 (2.97%)
Age at menarche[1]						
Mean (SD)	12.1 (1.5)	12.5 (1.4)	12.2 (1.44)	11.8 (1.47)	12.2 (1.41)	11.7 (1.42)
<10 years	53 (4.2%)	5 (1.7%)	11 (3.32%)	19 (7.42%)	4 (4.08%)	14 (5.20%)
10-11 years	356 (28.4%)	64 (21.3%)	89 (26.9%)	77 (30.1%)	27 (27.6%)	99 (36.8%)
12-13 years	649 (51.8%)	163 (54.3%)	167 (50.5%)	136 (53.1%)	51 (52.0%)	132 (49.1%)
>13 years	196 (15.6%)	68 (22.7%)	64 (19.3%)	24 (9.38%)	16 (16.3%)	24 (8.92%)
Body mass index[1,2]						
Underweight	34 (2.71%)	15 (5.00%)	10 (3.02%)	4 (1.56%)	0 (0%)	5 (1.86%)
Normal weight	800 (63.8%)	198 (66.0%)	219 (66.2%)	159 (62.1%)	64 (65.3%)	160 (59.5%)
Overweight	282 (22.5%)	59 (19.7%)	72 (21.8%)	60 (23.4%)	25 (25.5%)	66 (24.5%)
Obese	138 (11.0%)	28 (9.3%)	30 (9.06%)	33 (12.9%)	9 (9.18%)	38 (14.1%)
Race[3]						
Black	54 (4.3%)	16 (5.3%)	15 (4.52%)	6 (2.34%)	4 (4.08%)	13 (4.83%)
White	1017 (81.0%)	216 (72.0%)	267 (80.4%)	211 (82.4%)	86 (87.8%)	237 (88.1%)
Other / Unknown	184 (14.7%)	68 (22.7%)	50 (15.1%)	39 (15.2%)	8 (8.16%)	19 (7.06%)
Hispanic ethnicity[3]						
Hispanic	97 (7.8%)	24 (8.0%)	26 (8.0%)	22 (8.7%)	5 (5.3%)	20 (7.5%)
Non-Hispanic	1140 (92.1%)	276 (92.0%)	297 (91.7%)	230 (91.3%)	89 (94.7%)	248 (92.5%)
Source of enrollment						
Clinic	667 (53.1%)	39 (13.0%)	111 (33.4%)	191 (74.6%)	71 (72.4%)	255 (94.8%)
Non-clinic	588 (46.9%)	261 (87.0%)	221 (66.6%)	65 (25.4%)	27 (27.6%)	14 (5.20%)
Hormone medication use[1,4]						
Never	238 (19.0%)	85 (28.4%)	92 (27.7%)	34 (13.3%)	13 (13.3%)	14 (5.20%)
Ever	1016 (81.0%)	214 (71.6%)	240 (72.3%)	222 (86.7%)	85 (86.7%)	255 (94.8%)
Ever used for birth control	440 (44.9%)	148 (55.6%)	148 (55.0%)	63 (32.0%)	26 (41.3%)	55 (29.9%)
Ever used for pain	389 (39.7%)	31 (11.7%)	65 (24.2%)	124 (62.9%)	36 (57.1%)	133 (72.3%)

Supplemental Table 3.4 (cont'd)

	Total (N=1255)	Class 1 (N=300)	Class 2 (N=332)	Class 3 (N=256)	Class 4 (N=98)	Class 5 (N=269)
Pain medication use[1,5]						
Never	732 (61.6%)	237 (80.9%)	223 (71.9%)	123 (50.4%)	45 (49.5%)	104 (41.6%)
Less than 2 days per week	102 (8.6%)	18 (6.1%)	29 (9.4%)	23 (9.4%)	14 (15.4%)	18 (7.2%)
2 or more days per week	354 (29.8%)	38 (13.0%)	58 (18.7%)	98 (40.2%)	32 (35.2%)	128 (51.2%)
Date of last menstrual period (LMP)						
Within the last 3 months	910 (72.5%)	252 (84.0%)	247 (74.4%)	186 (72.7%)	65 (66.3%)	160 (59.5%)
3-6 months ago	122 (9.7%)	24 (8.0%)	18 (5.42%)	32 (12.5%)	8 (8.16%)	40 (14.9%)
6-12 months ago	53 (4.2%)	6 (2.0%)	15 (4.52%)	13 (5.08%)	6 (6.12%)	13 (4.83%)
>12 months ago	164 (13.1%)	16 (5.3%)	51 (15.4%)	25 (9.77%)	19 (19.4%)	53 (19.7%)
Not in last 3 months, LMP unknown	6 (0.48%)	2 (0.7%)	1 (0.301%)	0 (0%)	0 (0%)	3 (1.12%)
Comorbid conditions[6,7]						
Gynecologic/genitourinary conditions						
Endometriosis	597 (47.6%)	12 (4.0%)	79 (23.8%)	185 (72.3%)	68 (69.4%)	253 (94.1%)
Fibrocystic or other benign breast disease	10 (0.8%)	3 (1.0%)	2 (0.645%)	2 (0.820%)	0 (0%)	3 (1.20%)
Painful bladder/interstitial cystitis	10 (1.1%)	1 (0.4%)	1 (0.40%)	4 (2.17%)	0 (0%)	4 (2.44%)
Uterine Fibroids	22 (1.8%)	3 (1.00%)	3 (0.909%)	4 (1.56%)	3 (3.13%)	9 (3.35%)
Ovarian cysts	147 (11.8%)	11 (3.69%)	20 (6.08%)	23 (8.98%)	22 (23.4%)	71 (26.4%)
Polycystic ovarian syndrome (PCOS)	49 (3.9%)	17 (5.69%)	11 (3.34%)	7 (2.76%)	3 (3.13%)	11 (4.09%)
Respiratory / immune conditions						
Allergies	389 (32.7%)	76 (25.9%)	87 (28.1%)	85 (34.8%)	34 (37.4%)	107 (42.8%)
Asthma	280 (22.5%)	44 (14.7%)	69 (21.0%)	56 (22.0%)	23 (24.0%)	88 (32.7%)
Rheumatologic/ neurologic conditions						
Fibromyalgia	16 (1.4%)	1 (0.341%)	2 (0.645%)	2 (0.820%)	0 (0%)	11 (4.40%)
Chronic fatigue syndrome	14 (1.1%)	1 (0.334%)	3 (0.912%)	2 (0.787%)	3 (3.13%)	5 (1.86%)
Rheumatoid arthritis	12 (1.01%)	3 (1.02%)	1 (0.323%)	4 (1.64%)	0 (0%)	4 (1.60%)
Migraine	610 (48.6%)	78 (26.0%)	151 (45.5%)	152 (59.4%)	47 (48.0%)	182 (67.7%)
Lower back pain	811 (69.0%)	154 (53.1%)	191 (62.0%)	189 (77.1%)	67 (76.1%)	210 (86.1%)
Muscle/joint pain[8]	448 (38.5%)	81 (28.0%)	107 (34.9%)	98 (40.5%)	43 (48.9%)	119 (49.8%)
Leg pain	355 (30.6%)	57 (19.8%)	78 (25.4%)	76 (31.8%)	28 (31.5%)	116 (48.7%)
Gastrointestinal/abdominal conditions						
Inflammatory bowel disease: Crohn's disease or ulcerative colitis	14 (1.1%)	1 (0.334%)	2 (0.606%)	2 (0.781%)	3 (3.13%)	6 (2.23%)
Irritable bowel syndrome (IBS)	148 (11.8%)	14 (4.68%)	27 (8.18%)	25 (9.77%)	22 (22.9%)	60 (22.3%)
Non-pelvic abdominal pain	543 (46.3%)	72 (24.7%)	100 (32.5%)	114 (46.9%)	61 (69.3%)	196 (80.3%)
Number of comorbidities						
Mean (SD)	3.57 (2.34)	2.10 (1.65)	2.81 (1.98)	4.02 (2.04)	4.36 (2.20)	5.41 (2.23)
Median [Min, Max]	3.00 [0, 14.0]	2.00 [0, 8.00]	3.00 [0, 9.00]	4.00 [0, 9.00]	4.00 [0, 10.0]	5.00 [1.00, 14.0]

Supplemental Table 3.4 (cont'd)

*Abbreviations SD: standard deviation
[1] Number of participants missing information for each characteristic variable: age at menarche (1 total) body mass index (1 total), hormonal medication use (1 total), pain mediation use (67 total)
[2] Underweight for age <20 (z-score >-9 to <=-2), age ≥20 (>0 to < 18.5); normal weight for age <20 (z-score >-2 to <1), age ≥20 (>=18.5 to <25); overweight for age <20 (z-score >=1 to <=2), age ≥20 (>=25 to <30); obese for age <20 (z-score >2), age ≥20 (>=30)
[3] Black participants include Hispanic and Non-Hispanic, White participants include Hispanic and Non-Hispanic; Other and unknown category participants are Asian (93 total), American Indian/Alaska Native (1 total), Native Hawaiian or pacific islander (1 total), multiracial (57 total), other race (25 total), unknown (7 total), all including Hispanic and Non-Hispanic
[4] Lifetime use of hormone medication, including birth control pills, patches, rings, injections, implants, hormonal intrauterine device, for any reason including but not limited to acne, bad cramping, irregular periods, birth control, fertility treatments.
[5] Current regular use of pain medications including acetaminophen, non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, celecoxib, rofecoxib, naproxen, mefenamic acid, ketorolac), opioid analgesics (e.g. hydrocode-combination, oxycodone with acetaminophen, oxycodone HCL, acetaminophen with codeine, codeine, morphine)
[6] Four versions of the survey were administered over the enrollment of the cohort, with distributions of cases and comparison group varying over each version: survey prior to January 2014: version 1 (155 total); survey prior to January 2014: version 2 (121 total), survey from January 2014 onward: version 3 (920 total), survey from January 2014 onward: version 4 short-form (59 total) contained no questions regarding allergies, fibrocystic or benign breast disease, fibromyalgia, leg pain, lower back pain, muscle/joint pain, non-pelvic abdominal pain, rheumatoid arthritis
[7] Number of participants missing information for each comorbid condition is as follows: Allergies (67 total), Asthma (8 total), CFS (8 total), Crohn's or ulcerative colitis (5 total), Fibrocystic or benign breast disease (67 total), Fibromyalgia (67 total), IBS (5 total), Leg pain (94 total), Lower back pain (80 total), Muscle/joint pain (90 total), Non-pelvic abdominal pain (81 total), Ovarian cysts (9 total), Painful bladder/interstitial cystitis (337 total), PCOS (8 total), rheumatoid arthritis (67 total), Uterine fibroids (5 total)
[8] Muscle/joint pain is defined as those unrelated to infections/sports injuries

Supplemental Table 3.5 Latent class analysis limited to sample with periods in the past year only (N=1085), determination of class structure by comparison of indicators of fit for latent class models comprising of 1-9 classes

Number of classes	Parameters	Log-likelihood	AIC	BIC	aBIC	BLRT p-Value	VLMR-LRT p-Value	Entropy
1	10.00	-4597.63	9215.26	9265.16	9233.39	.	.	.
2	21.00	-3708.72	7459.43	7564.21	7497.51	0.00	0.00	0.94
3	32.00	-3483.32	7030.64	7190.30	7088.66	0.00	0.00	0.84
4	43.00	-3426.85	6939.71	7154.25	7017.67	0.00	0.00	0.84
5[1]	54.00	-3392.85	6893.70	7163.12	6991.61	0.00	0.12	0.77
6	65.00	-3379.32	6888.64	7212.95	7006.49	0.00	0.17	0.78
7	76.00	-3369.02	6890.04	7269.23	7027.84	0.00	1.00	0.80
8	87.00	-3363.75	6901.51	7335.58	7059.25	0.00	0.65	0.82
9	98.00	-3355.10	6906.19	7395.15	7083.88	0.00	0.97	0.85

*Abbreviations AIC: Akaike information criterion, BIC: Bayesian Information Criterion, aBIC: sample-adjusted Bayesian Information Criterion, BLRT: bootstrap likelihood ratio test, VLMRT-LRT: Vuong-Lo-Mendell-Rubin likelihood ratio test
**Notes: model fit was evaluated by examining aBIC
[1] Selected as final model based on low aBIC, low BLRT p-value, low VLMRT p-value overall entropy, and interpretability

Supplemental Table 3.6 Latent class analysis restricted to adolescent participants aged 12-24 (N=828) determination of class structure by comparison of indicators of fit for latent class models comprising of 1-9 classes, model fit was evaluated by examining aBIC

Number of classes	Parameters	Log-likelihood	AIC	BIC	aBIC	BLRT p-Value	VLMR-LRT p-Value	Entropy
1	10.00	-3457.22	6934.44	6981.63	6949.87	.	.	.
2	21.00	-2763.30	5568.59	5667.69	5601.00	0.00	0.00	0.94
3[1]	32.00	-2609.44	5282.89	5433.90	5332.27	0.00	0.00	0.82
4	43.00	-2567.46	5220.92	5423.84	5287.28	0.00	0.88	0.82
5	54.00	-2541.18	5190.36	5445.18	5273.70	0.00	0.22	0.76
6	65.00	-2523.60	5177.20	5483.93	5277.52	0.00	0.00	0.78
7	76.00	-2515.31	5182.63	5541.27	5299.92	0.38	1.00	0.80
8	87.00	-2509.31	5192.62	5603.17	5326.89	0.67	1.00	0.80
9	98.00	-2503.18	5202.36	5664.82	5353.61	0.67	0.84	0.81

*Abbreviations AIC: Akaike information criterion, BIC: Bayesian Information Criterion, aBIC: sample-adjusted Bayesian Information Criterion, BLRT: bootstrap likelihood ratio test, VLMRT-LRT: Vuong-Lo-Mendell-Rubin likelihood ratio test
[1] Selected as final model based on low aBIC, low BLRT p-value, low VLMRT p-value overall entropy, and interpretability

Supplemental Table 3.7 Indicator item response proportions conditional on class for 3-class model of pelvic pain symptomology restricted to adolescent individuals aged 12-24 (N=828)

	Overall	Adolescent A (31.2%)	Adolescent B (30.3%)	Adolescent C (38.5%)
Acyclic pelvic pain: Severity[1]				
None/mild	0.65	0.02	0.92	0.94
Moderate	0.08	0.15	0.06	0.04
Severe	0.27	0.83	0.02	0.02
Acyclic pelvic pain: Frequency[2]				
No pain	0.76	0.23	0.98	0.99
Weekly	0.15	0.45	0.02	0.00
Everyday	0.10	0.32	0.00	0.01
Acyclic pelvic pain: Life impact[3]				
No	0.72	0.12	0.98	0.98
Yes	0.28	0.88	0.02	0.02
Cyclic pelvic pain: Severity[4]				
None/mild	0.21	0.04	0.00	0.57
Moderate	0.25	0.11	0.26	0.36
Severe	0.54	0.86	0.74	0.07
Cyclic pelvic pain: Frequency[5]				
Never/occasionally	0.29	0.04	0.01	0.75
Often/usually	0.26	0.19	0.35	0.21
Always	0.45	0.77	0.65	0.04
Cyclic pelvic pain: Life impact[6]				
No	0.65	0.33	0.56	0.97
Yes	0.35	0.67	0.45	0.03

[1] Acyclic pelvic pain severity reported on 0-10 numeric rating scale: none/mild= 0-3; moderate=4-6; severe 7-10, in the past 3 months (survey from January 2014 onward) or past 12 months (survey prior to January 2014)

[2] Acyclic pelvic pain frequency in the last 3 months (survey from January 2014 onward) or 12 months (survey prior to January 2014): no pain='no pain in the past 3 months/12 months', <1 day/month', 'one day a month', 'two to three days a month', weekly='one day/week', '> one day/week'; everyday='every day')

[3] Acyclic pelvic pain life impact: yes= frequently interferes (survey prior to January 2014) or moderately-extremely interferes (survey from January 2014 onward) with normal social activities with 'work or school' or 'activities at home'

[4] Cyclic pelvic pain severity reported on 0-10 numeric rating scale: none/mild= 0-3; moderate=4-6; severe 7-10; severity of usual period pain (survey prior to January 2014), period pain in the past 12 months (survey from January 2014 onward), severity at last period in past 3 months if last 12 months missing (survey from January 2014 onward), for non-menstruating in past 12 months severity in current age range or if not available in the previous age range (age range defined as <15, 16-20, 21-30, 31,40, >41) (survey from January 2014 onward)

Supplemental Table 3.7 (cont'd)

[5] Cyclic pelvic pain frequency: never/occasionally= ‘never’, ‘occasionally (less than a quarter of my periods); moderate=’often’, ‘usually’; always= ‘always’ (reported in survey from January 2014 onward only), for non-menstruating in the past 12 months, frequency in current age range or if not available in the previous age range (survey from January 2014 onward)
[6] Cyclic pelvic pain life impact: yes= ‘pain that prevents from going to work or school or carrying out daily activities (even if taking pain-killers)’ available only for the last period, if last period during the last 3 months (in survey from January 2014 onward only), for non-menstruating in the past 3 months, life impact at current age range, or if not available life impact at the previous age range (survey from January 2014 onward)

Supplemental Table 3.8 Individual class assignment using the full model versus age restricted model (N=828)

Individual assigned class in age-restricted model (N=828)	Adolscent classes	Individual assigned class in full model (N=828 of 1255)				
		Class 1 No pelvic pain	Class 2 Moderate cyclic pain only	Class 3 Severe cyclic pain only	Class 4 Severe acyclic, moderate cyclic pain	Class 5 Severe acyclic, severe cyclic pain
	Adol A[1]			3	54	201
	Adol B[2]		73	175	3	
	Adol C[3]	165	141	3	10	

Supplemental Table 3.9 Unadjusted odds ratios for tests of categorical latent variable using univariable multinomial logistic regression with the 3-step procedure accounting for classification uncertainty

Predictors[1]	**Class 1[2] [Ref]**	**Class 2 OR (95% CI)[3]**	**Class 3 OR (95% CI)[3]**	**Class 4 OR (95% CI)[3]**	**Class 5 OR (95% CI)[3]**
	No pelvic pain	Moderate cyclic pain only	Severe cyclic pain only	Severe acyclic, moderate cyclic pain	Severe acyclic, severe cyclic pelvic pain
Demographic and clinical characteristics					
Age at completion of survey	(1.00)	0.98 (0.95, 0.10)	0.89 (0.85, 0.93)	0.96 (0.90, 1.03)	0.88 (0.83, 0.94)
Age at menarche	(1.00)	0.88 (0.75, 1.05)	0.67 (0.58, 0.78)	0.88 (0.72, 1.08)	0.67 (0.58, 0.77)
Body mass index [1]					
Underweight	(1.00)	0.55 (0.17, 1.80)	0.23 (0.043, 1.17)	0.00 (0.00, 0.00)	0.34 (0.12, 1.00)
Normal weight	(1.00)	1.05 (0.64, 1.72)	0.82 (0.54, 1.25)	1.029 (0.56, 1.90)	0.746 (0.50, 1.11)
Overweight	(1.00)	1.17 (0.65, 2.08)	1.31 (0.80, 2.15)	1.468 (0.75, 2.88)	1.361 (0.85, 2.19)
Obese	(1.00)	0.87 (0.38, 1.99)	1.52 (0.81, 2.85)	0.842 (0.29, 2.47)	1.618 (0.88, 2.96)
Race (Black and White)[2]	(1.00)	0.76 (0.27, 2.14)	0.28 (0.07, 1.20)	0.582 (0.14, 2.44)	0.722 (0.30, 1.72)
Ethnicity (Hispanic and Non-Hispanic)	(1.00)	0.99 (0.48,2.35)	1.13 (0.55,2.33)	0.57 (0.14,2.26)	0.944 (0.46,1.93)
Comorbid conditions					
Gynecologic conditions					
Endometriosis	(1.00)	27.34 (0.42, 1794.71)	598.85 (11.25, 31883.03)	247.97 (4.48, 13737.93)	3265.11 (55.77, ***)
Fibrocystic or other benign breast disease	(1.00)	0.50 (0.03, 8.74)	0.77 (0.1, 5.97)	0.00 (0.00, 0.00)	1.11 (0.20, 6.21)
Painful bladder/interstitial cystitis	(1.00)	0.24 (0.00, 30413.19)	6.21 (0.61, 62.54)	0.00 (0.00, 0.00)	5.95 (0.55, 63.87)
Uterine Fibroids	(1.00)	0.74 (0.05, 10.15)	1.607 (0.28, 9.38)	3.175 (0.43, 23.42)	3.37 (0.71, 16.06)
Ovarian cysts		1.98 (0.54, 6.73)	3.07 (1.12, 8.41)	9.72 (3.28, 28.81)	11.41 (4.45, 29.22)
Polycystic ovarian syndrome (PCOS)	(1.00)	0.49 (0.15, 1.57)	0.41 (0.13, 1.25)	0.46 (0.09, 2.37)	0.66 (0.28, 1.57)
Respiratory immune conditions					
Allergies	(1.00)	1.08 (0.63, 1.86)	1.64 (1.05, 2.56)	1.68 (0.9, 3.17)	2.22 (1.45, 3.41)
Asthma	(1.00)	1.80 (0.95, 3.42)	1.84 (1.05, 3.21)	1.90 (0.89, 4.06)	3.30 (1.96, 5.54)

Supplemental Table 3.9 (cont'd)

Predictors[1]	Class 1[2] [Ref]	Class 2 OR (95% CI)[3]	Class 3 OR (95% CI)[3]	Class 4 OR (95% CI)[3]	Class 5 OR (95% CI)[3]
Rheumatologic/neurologic conditions					
Fibromyalgia	(1.00)	2.39 (0.04, 126.94)	2.86 (0.1, 78.11)	0.00 (0.00, 0.00)	16.85 (0.81, 351.91)
Chronic fatigue syndrome (CFS)	(1.00)	5.49 (0.03, 1077.65)	3.75 (0.03, 473.81)	19.80 (0.16, 2443.95)	9.80 (0.09, 1085.37)
Rheumatoid arthritis	(1.00)	0.00 (0.00, 0.00)	1.61 (0.35, 7.44)	0.00 (0.00, 0.00)	1.35 (0.30, 6.13)
Migraine	(1.0)	3.00 (1.76, 5.12)	5.87 (3.65, 9.46)	2.81 (1.50, 5.27)	8.03 (5.02, 12.85)
Lower back pain	(1.00)	1.44 (0.89, 2.32)	3.79 (2.31, 6.21)	2.78 (1.43, 5.39)	6.27 (3.74, 10.49)
Muscle/joint pain	(1.00)	1.47 (0.87, 2.48)	1.94 (1.24, 3.02)	2.70 (1.45, 5.03)	2.75 (1.78, 4.25)
Leg pain	(1.00)	1.45 (0.81, 2.62)	2.13 (1.3, 3.47)	1.74 (0.86, 3.52)	4.43 (2.77, 7.1)
Gastrointestinal/abdominal conditions					
Inflammatory bowel disease: Crohn's disease or ulcerative colitis	(1.00)	2.29 (0.03, 159.96)	2.86 (0.08, 100.42)	13.68 (0.42, 450.44)	8.53 (0.31, 235.88)
Irritable bowel syndrome (IBS)	(1.00)	2.27 (0.74, 6.92)	2.66 (1.04, 6.83)	7.90 (2.86, 21.86)	7.26 (3.01, 17.52)
Non-pelvic abdominal pain	(1.00)	1.46 (0.84, 2.54)	3.16 (2.01, 4.97)	7.42 (3.80, 14.51)	14.78 (8.89, 24.59)
Number of comorbidities	(1.00)	1.30 (1.11, 1.53)	1.95 (1.70, 2.234)	1.94 (1.64, 2.29)	2.55 (2.21, 2.94)

[1] Body mass index categorized as indicator variables of underweight for age <20 (z-score >-9 to <=-2), age ≥20 (>0 to < 18.5); normal weight for age <20 (z-score >-2 to <1), age ≥20 (>=18.5 to <25); overweight for age <20 (z-score >=1 to <=2), age ≥20 (>=25 to <30); obese for age <20 (z-score >2), age ≥20 (>=30)

[2] Binary variable, comparison was made between Black (Hispanic and non-Hispanic) and White (Hispanic and non-Hispanic) as reference group

Supplemental Table 3.10 Age-adjusted odds ratios and 95% confidence intervals for associations between eight comorbidity conditions and 5-class model of pelvic pain

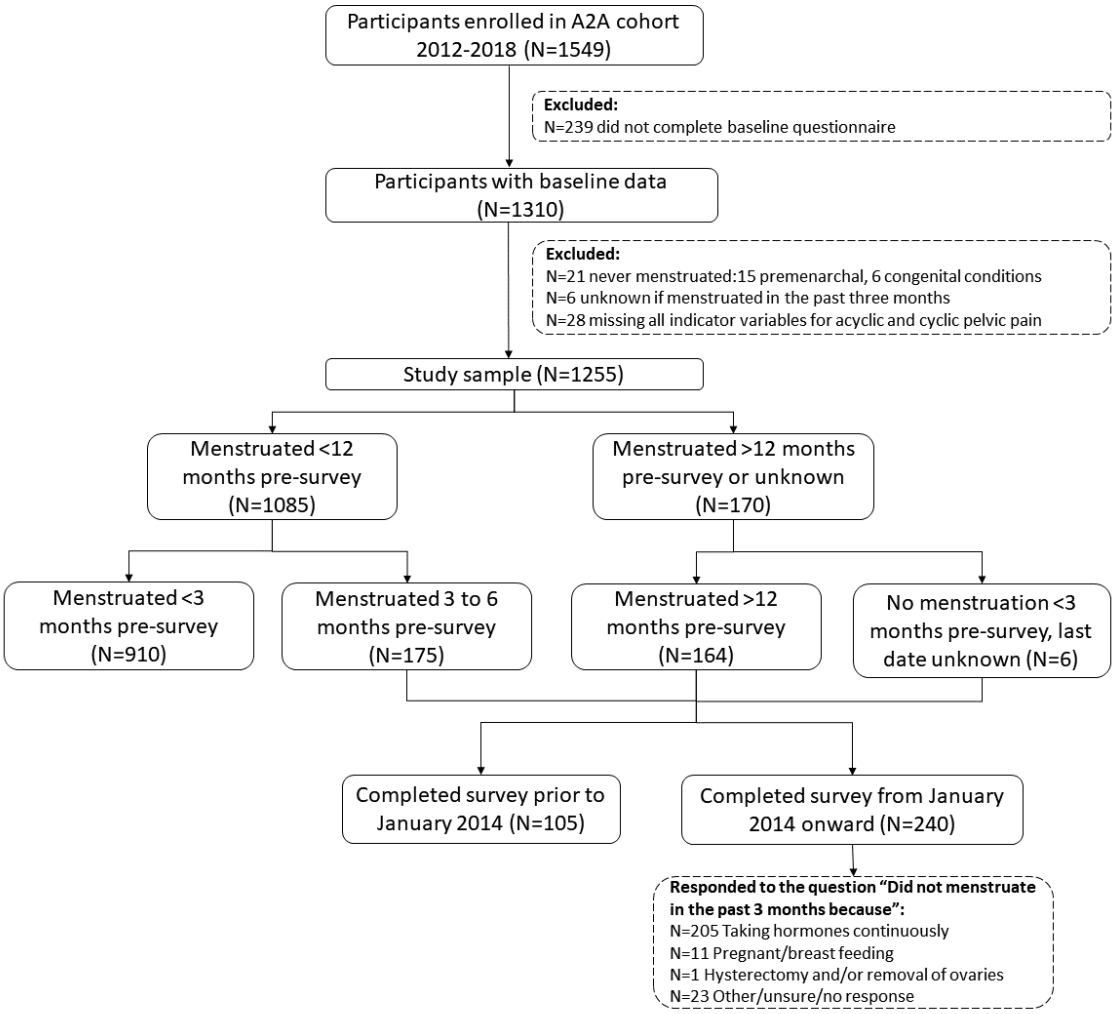
Comorbid condition	Class 1[1] [Ref]	Class 2 aOR (95% CI)[2]	Class 3 aOR (95% CI)[2]	Class 4 aOR (95% CI)[2]	Class 5 aOR (95% CI)[2]
Qualitative description of subgroups	No pelvic pain	Moderate cyclic pain only	Severe cyclic pain only	Severe acyclic, moderate cyclic pain	Severe acyclic, severe cyclic pelvic pain
Gynecologic/genitourinary conditions					
Fibrocystic or other benign breast disease	(1.00)	0.53 (0.02, 17.62)	2.08 (0.17, 26.13)	0.00 (0.00, 0.00)	2.87 (0.36, 22.58)
Painful bladder/interstitial cystitis	(1.00)	1.26 (0.02, 79.97)	41.52 (0.38, 4555.4)	0.00 (0.00, 0.00)	59.00 (0.50, 6952.66)
Uterine Fibroids	(1.00)	1.49 (0.12, 18.87)	11.84 (1.20, 116.92)	7.56 (0.68, 84.64)	34.76 (3.69, 327.8)
Polycystic ovarian syndrome (PCOS)	(1.00)	0.50 (0.16, 1.58)	0.47 (0.139, 1.56)	0.44 (0.07, 2.78)	0.78 (0.31, 1.93)
Rheumatologic/neurologic conditions					
Fibromyalgia	(1.00)	2.51 (0.01, 1344.36)	12.47 (0.20, 798.82)	0.00 (0.00, 0.00)	62.75 (1.98, 1993.29)
Chronic fatigue syndrome	(1.00)	3.58 (0.11, 114.76)	5.26 (0.22, 123.89)	22.081 (1.12, 437.26)	8.84 (0.31, 252.14)
Rheumatoid arthritis	(1.00)	0.00 (0.00, 0.00)	1.59 (0.37, 6.81)	0.00 (0.00, 0.00)	1.29 (0.27, 6.16)
Gastrointestinal/abdominal conditions					
Inflammatory bowel disease: Crohn's disease or ulcerative colitis	(1.0)	2.24 (0.06, 79.87)	5.38 (0.28, 103.75)	16.84 (0.84, 337.63)	14.85 (0.86, 256.19)
Number of comorbidities	(1.0)	1.28 (1.10, 1.50)	1.98 (1.71, 2.28)	1.91 (1.61, 2.27)	2.58 (2.23, 3.00)

Abbreviations: adjusted odds ratio (aOR), confidence interval (CI)

[1] Reference latent class has an OR of 1.00

[2] Adjusted odds ratio (aOR) interpretation: tests of categorical latent variable using multivariable multinomial logistic regression using the 3-step procedure accounting for classification uncertainty, using 5-class model of pelvic pain, increase in odds of membership in each class relative to membership in reference class 1 (no pelvic pain) given one unit change in predictor (absence/presence of condition), controlling for age at time of enrollment in the study

Supplemental Figure 3.1 Description of participants in the full A2A cohort (N=1549), study sample (N=1255), and sensitivity analysis limited to participants who had periods in <12 months pre-survey (N=1085)



APPENDIX C: CHAPTER 4 SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 4.1 Characteristics of study participants in the study with at least one of seven biomarkers available (N=625) compared to the overall cohort who had baseline information available (N=1255) and compared to those with no biomarkers available (N=630)

Characteristic	A2A cohort with baseline data (N=1255)	No biomarkers available (N=630)	Biomarker available[1] (N=625)
Age at enrollment			
Mean (SD)	23.4 (7.16)	24.4 (7.31)	22.5 (6.89)
Median [Min, Max]	23.0 [12.0, 55.0]	23.0 [12.0, 55.0]	22.0 [12.0, 52.0]
≤15 years	121 (9.64%)	48 (7.62%)	73 (11.7%)
16-20 years	348 (27.7%)	145 (23.0%)	203 (32.5%)
21-30 years	617 (49.2%)	339 (53.8%)	278 (44.5%)
31-40 years	125 (9.96%)	69 (11.0%)	56 (8.96%)
≥41 years	44 (3.51%)	29 (4.60%)	15 (2.40%)
Age at menarche[2]			
Mean (SD)	12.1 (1.45)	12.2 (1.51)	12.0 (1.39)
Median [Min, Max]	12.0 [7.00, 15.0]	12.0 [7.00, 15.0]	12.0 [8.00, 15.0]
<10 years	53 (4.23%)	27 (4.29%)	26 (4.17%)
10-11 years	356 (28.4%)	165 (26.2%)	191 (30.6%)
12-13 years	649 (51.8%)	322 (51.1%)	327 (52.4%)
>13 years	196 (15.6%)	116 (18.4%)	80 (12.8%)
Body mass index at enrollment[2,3]			
Mean kg/m^2 (SD)	24.4 (5.57)	24.4 (5.66)	24.5 (5.47)
Median [Min, Max]	23.0 [15.6, 65.8]	22.9 [16.0, 65.8]	23.0 [15.6, 60.8]
Underweight	34 (2.71%)	24 (3.81%)	10 (1.60%)
Normal weight	800 (63.8%)	399 (63.3%)	401 (64.3%)
Overweight	282 (22.5%)	134 (21.3%)	148 (23.7%)
Obese	138 (11.0%)	73 (11.6%)	65 (10.4%)
Race[4]			
Black	54 (4.30%)	20 (3.17%)	34 (5.44%)
White	1017 (81.0%)	528 (83.8%)	489 (78.2%)
Other identification	184 (14.7%)	82 (13.0%)	102 (16.3%)

Supplemental Table 4.1 (cont'd)

Characteristic	A2A cohort with baseline data (N=1255)	No biomarkers available (N=630)	Biomarker available[1] (N=625)
Hispanic ethnicity			
Hispanic	97 (7.84%)	38 (6.1%)	59 (9.59%)
Non-Hispanic	1140 (92.2%)	584 (93.9%)	556 (90.4%)
Cigarette smoking status at enrollment[2]			
Never smoker	1142 (91.0%)	572 (90.8%)	570 (91.2%)
Past smoker	56 (4.46%)	23 (3.65%)	33 (5.28%)
Current smoker	19 (1.51%)	10 (1.59%)	9 (1.44%)
Parity[2]			
None	1149 (92.3%)	571 (91.4%)	578 (93.2%)
1 or more births	96 (7.71%)	54 (8.64%)	42 (6.77%)
Health conditions and co-morbidities[5]			
Surgically diagnosed endometriosis	597 (47.6%)	273 (43.3%)	324 (51.8%)
Two or more comorbidities	989 (78.9%)	472 (75.0%)	517 (82.8%)
Hormone medication use[6]			
Never	238 (19.0%)	146 (23.2%)	92 (14.7%)
Ever	1016 (81.0%)	483 (76.8%)	533 (85.3%)
Ever used for birth control	440 (44.9%)	260 (50.4%)	180 (38.9%)
Ever used for pain	389 (39.7%)	193 (37.4%)	196 (42.3%)
Regular pain medication use[2,7]			
Never	732 (61.6%)	359 (62.1%)	373 (61.1%)
Less than 2 days per week	102 (8.59%)	55 (9.52%)	47 (7.70%)
2 days or more per week	354 (29.8%)	164 (28.4%)	190 (31.1%)

Supplemental Table 4.1 (cont'd)

Characteristic	A2A cohort with baseline data (N=1255)	No biomarkers available (N=630)	Biomarker available[1] (N=625)
Date of last menstrual period (LMP)			
In last 3 months	910 (72.5%)	436 (69.2%)	474 (75.8%)
3-6 months ago	122 (9.72%)	68 (10.8%)	54 (8.64%)
6-12 months ago	53 (4.22%)	32 (5.08%)	21 (3.36%)
>12 months ago	164 (13.1%)	92 (14.6%)	72 (11.5%)
Not in last 3 months, LMP unknown	6 (0.478%)	2 (0.317%)	4 (0.640%)
Source of enrollment			
Clinic based	667 (53.1%)	312 (49.5%)	355 (56.8%)
Non-clinic based	588 (46.9%)	318 (50.5%)	270 (43.2%)

Abbreviations: SD=standard deviation

[1] At least one or more of 8 select biomarkers (IL-6, IL-8, IL-16, IP-10, MCP-1, MCP-4, TARC, TNF-a) available

[2] Number of participants missing information for each characteristic variable: age at menarche (N=1), body mass index (N=1), cigarette smoking status at enrollment (N=38), parity (N=10), regular pain medication use (N=67)

[3] Underweight for age <20 (z-score >-9 to <=-2), age ≥20 (>0 to < 18.5); normal weight for age <20 (z-score >-2 to <1), age ≥20 (>=18.5 to <25); overweight for age <20 (z-score >=1 to <=2), age ≥20 (>=25 to <30); obese for age <20 (z-score >2), age ≥20 (>=30)

[4] Black participants include Hispanic (N=6) and Non-Hispanic (N=26), White participants include Hispanic (N=30) and Non-Hispanic (N=454); Other and unknown category participants are Asian (N=47), American Indian/Alaska Native (N=1), multiracial (N=16), other race (N=34), unknown (N=4), including Hispanic (N=23) and Non-Hispanic (N=76)

[5] Comorbidities include gynecologic or genitourinary conditions: surgically-diagnosed endometriosis, fibrocystic or benign breast disease, painful bladder or interstitial cystitis, uterine fibroids, ovarian cysts, and polycystic ovarian syndrome (PCOS); respiratory immune conditions: allergies and asthma; rheumatologic and neurological conditions: fibromyalgia, chronic fatigue syndrome, rheumatic arthritis, migraine, lower back pain, muscle or joint pain unrelated to infections or sports injuries and leg pain; gastrointestinal or abdominal conditions: inflammatory bowel disease, irritable bowel syndrome and non-pelvic abdominal pain

[6] Lifetime use of hormone medication, including birth control pills, patches, rings, injections, implants, hormonal intrauterine device, for any reason including but not limited to acne, bad cramping, irregular periods, birth control, fertility treatments.

[7] Current regular use of pain medications including acetaminophen, non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, celecoxib, rofecoxib, naproxen, mefenamic acid, ketorolac), opioid analgesics (e.g. hydrocode-combination, oxycodone with acetaminophen, oxycodone HCL, acetaminophen with codeine, codeine, morphine)

Supplemental Table 4.2 Coefficients of variation (CV%) for seven biomarkers of inflammation (N=625).

Inflammatory marker	Inter-assay coefficient of variation (%)[1]	Overall extrapolation rate (%)[2]	Outliers removed (N)[3]	Values available after outlier removal (N)[3]	Sampling frame (N) for analysis[4]
IL-8	16.70%	3.1%	0 (0%)	625	590
IL-16	13.10%	0.0%	5 (1%)	620	590
IP-10/CXCL10	9.20%	0.2%	5 (1%)	620	590
MCP-1/CCL2	9.90%	0.0%	1 (0%)	624	590
MCP-4/CCL13	10.60%	0.0%	0 (0%)	625	590
TARC/CCL17	14.30%	7.2%	16 (3%)	609	590
TNF-α	17.60%	0.8%	10 (2%)	615	590

Note: Biomarker levels were measured in plasma samples using multiplex microarray immunoassays.

[1] CVs were calculated from blinded quality control samples embedded in each batch.

[2] Levels were automatically calculated from the standard curve using BioPlex Manager (v 4.1.1) software, and for each, values that were too low on the standard curve were extrapolated and reported in pg/ml.

[3] Outliers were determined using the generalized extreme studentized deviate many-outlier method.

[4] Sampling frame consisted of those participants who had baseline data available and data for at least one biomarkers of interest (N=625), then for regression samples were restricted to participants those who had values above the limit of detection for all biomarkers (N=590).

Supplemental Table 4.3 Distribution of biomarker quintiles across five pre-assigned latent classes of pelvic pain.

Biomarker concentration quintile (Q)[1]	No pelvic pain Class 1 (N=138)	Moderate cyclic pelvic pain only Class 2 (N=145)	Severe cyclic pelvic pain only Class 3 (N=141)	Severe acyclic, moderate cyclic pain Class 4 (N=42)	Severe acyclic and cyclic pain Class 5 (N=159)
IL-8					
Q1	21 (15.7%)	24 (17.6%)	18 (13.4%)	9 (23.7%)	19 (12.8%)
Q2	22 (16.4%)	31 (22.8%)	26 (19.4%)	4 (10.5%)	21 (14.2%)
Q3	35 (26.1%)	23 (16.9%)	35 (26.1%)	8 (21.1%)	33 (22.3%)
Q4	25 (18.7%)	28 (20.6%)	23 (17.2%)	5 (13.2%)	23 (15.5%)
Q5	31 (23.1%)	30 (22.1%)	32 (23.9%)	12 (31.6%)	52 (35.1%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)
IL-16					
Q1	27 (20.1%)	29 (21.3%)	30 (22.4%)	5 (13.2%)	32 (21.6%)
Q2	31 (23.1%)	21 (15.4%)	22 (16.4%)	6 (15.8%)	25 (16.9%)
Q3	25 (18.7%)	19 (14.0%)	24 (17.9%)	12 (31.6%)	23 (15.5%)
Q4	26 (19.4%)	30 (22.1%)	24 (17.9%)	9 (23.7%)	20 (13.5%)
Q5	25 (18.7%)	37 (27.2%)	34 (25.4%)	6 (15.8%)	48 (32.4%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)
MCP-1					
Q1	23 (17.2%)	26 (19.1%)	20 (14.9%)	9 (23.7%)	35 (23.6%)
Q2	27 (20.1%)	28 (20.6%)	29 (21.6%)	6 (15.8%)	16 (10.8%)
Q3	28 (20.9%)	21 (15.4%)	28 (20.9%)	10 (26.3%)	21 (14.2%)
Q4	30 (22.4%)	26 (19.1%)	24 (17.9%)	3 (7.89%)	27 (18.2%)
Q5	26 (19.4%)	35 (25.7%)	33 (24.6%)	10 (26.3%)	49 (33.1%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)
MCP-4					
Q1	33 (24.6%)	22 (16.2%)	25 (18.7%)	9 (23.7%)	38 (25.7%)
Q2	23 (17.2%)	22 (16.2%)	29 (21.6%)	7 (18.4%)	15 (10.1%)
Q3	28 (20.9%)	24 (17.6%)	28 (20.9%)	5 (13.2%)	25 (16.9%)
Q4	28 (20.9%)	24 (17.6%)	21 (15.7%)	7 (18.4%)	17 (11.5%)
Q5	22 (16.4%)	44 (32.4%)	31 (23.1%)	10 (26.3%)	53 (35.8%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)

Supplemental Table 4.3 (cont'd)

Biomarker concentration quintile (Q)[1]	No pelvic pain Class 1 (N=138)	Moderate cyclic pelvic pain only Class 2 (N=145)	Severe cyclic pelvic pain only Class 3 (N=141)	Severe acyclic, moderate cyclic pain Class 4 (N=42)	Severe acyclic and cyclic pain Class 5 (N=159)
IP-10					
Q1	25 (18.7%)	29 (21.3%)	29 (21.6%)	5 (13.2%)	32 (21.6%)
Q2	33 (24.6%)	21 (15.4%)	23 (17.2%)	7 (18.4%)	28 (18.9%)
Q3	27 (20.1%)	24 (17.6%)	22 (16.4%)	3 (7.89%)	17 (11.5%)
Q4	24 (17.9%)	26 (19.1%)	24 (17.9%)	8 (21.1%)	18 (12.2%)
Q5	25 (18.7%)	36 (26.5%)	36 (26.9%)	15 (39.5%)	53 (35.8%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)
TARC					
Q1	26 (19.4%)	31 (22.8%)	29 (21.6%)	4 (10.5%)	34 (23.0%)
Q2	27 (20.1%)	30 (22.1%)	28 (20.9%)	10 (26.3%)	28 (18.9%)
Q3	23 (17.2%)	28 (20.6%)	32 (23.9%)	4 (10.5%)	29 (19.6%)
Q4	32 (23.9%)	20 (14.7%)	21 (15.7%)	12 (31.6%)	31 (20.9%)
Q5	26 (19.4%)	27 (19.9%)	24 (17.9%)	8 (21.1%)	26 (17.6%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)
TNF-a					
Q1	20 (14.9%)	23 (16.9%)	26 (19.4%)	6 (15.8%)	22 (14.9%)
Q2	27 (20.1%)	26 (19.1%)	13 (9.70%)	5 (13.2%)	26 (17.6%)
Q3	31 (23.1%)	26 (19.1%)	22 (16.4%)	7 (18.4%)	17 (11.5%)
Q4	27 (20.1%)	30 (22.1%)	39 (29.1%)	7 (18.4%)	37 (25.0%)
Q5	29 (21.6%)	31 (22.8%)	34 (25.4%)	13 (34.2%)	46 (31.1%)
Missing	4 (2.9%)	9 (6.2%)	7 (5.0%)	4 (9.5%)	11 (6.9%)

[1] Biomarker levels were measured in plasma samples using multiplex microarray immunoassays. Levels were automatically calculated from the standard curve using BioPlex Manager (v 4.1.1) software, and for each, values that were too low on the standard curve were extrapolated and reported in pg/ml.

Supplemental Table 4.4 Latent class solution and fit for latent class sensitivity analysis including only participant subset with biomarkers available (N=625)

Number of classes	Parameters	LL	AIC	BIC	aBIC	BLRT P-Value	VLMR-LRT P-Value	Entropy
1	10	-2654.26	5328.51	5372.89	5341.14	.	.	.
2	21	-2098.2	4238.4	4331.59	4264.92	0.00	0.00	0.94
3	32	-1957.41	3978.82	4120.83	4019.24	0.00	0.00	0.87
4	43	-1928.72	3943.44	4134.27	3997.75	0.00	0.05	0.83
5[1]	54	-1904.38	3916.76	4156.40	3984.96	0.00	0.00	0.80
6	65	-1893.19	3916.39	4204.84	3998.47	0.00	0.54	0.81
7	76	-1885.87	3923.74	4261.01	4019.72	0.50	0.71	0.82
8	87	-1879.18	3932.36	4318.45	4042.24	1.00	0.62	0.84
9	98	-1876.62	3949.23	4384.13	4072.99	1.00	1.00	0.85

*Abbreviations AIC: Akaike information criterion, BIC: Bayesian Information Criterion, aBIC: sample-adjusted Bayesian Information Criterion, BLRT: bootstrap likelihood ratio test, VLMRT-LRT: Vuong-Lo-Mendell-Rubin likelihood ratio test

[1] Selected as final model based on low aBIC, overall lowest entropy, and interpretability

Supplemental Table 4.5 Comparison of model fit using Akaike Information Criterion (AIC) for twelve models consisting of linear and restricted cubic splines

ID	Model[1]	Knots (K)	Knot location (percentile)[2]	Covariates	IL-8 AIC	IL-16 AIC	IP-10 AIC	MCP-1 AIC	MCP-4 AIC	TARC AIC	TNF-α AIC
1	Linear				392.80	392.21	391.35	393.61	389.77	391.93	391.34
2	RCS	3	0.1, 0.5, 0.9		394.55	386.99	387.84	391.21	386.82	393.53	392.55
3	RCS	4	0.05, 0.35,0.65,0.95		395.92	386.78	388.10	387.82	387.72	391.39	394.59
4	RCS	5	0.05,0.275,0.5,0.725,0.95		397.42	388.47	386.87	385.68	389.02	393.29	396.05
5	RCS	6	0.05,0.23,0.41,0.59,0.77,0.95		399.36	386.57	388.39	386.02	389.14	394.97	396.35
6	RCS	4	0.20,0.40,0.60,0.80		396.11	388.19	388.43	389.96	387.92	392.52	394.41
7	Linear			Age, BMI	327.19	326.64	327.35	328.12	327.55	324.27	327.82
8	RCS	3	0.1, 0.5, 0.9	Age, BMI	328.02	318.75	326.22	326.78	326.37	326.26	327.36
9	RCS	4	0.05, 0.35,0.65,0.95	Age, BMI	329.44	319.29	324.69	323.93	328.30	325.22	329.22
10	RCS	5	0.05,0.275,0.5,0.725,0.95	Age, BMI	329.35	321.02	325.47	323.69	330.10	327.08	330.47
11	RCS	6	0.05,0.23,0.41,0.59,0.77,0.95	Age, BMI	331.14	317.25	326.53	324.85	331.03	328.98	328.02
12	RCS	4	0.20,0.40,0.60,0.80	Age, BMI	329.58	320.44	326.26	326.00	328.14	326.04	328.62
				Model number selected	7	11	9	10	8	7	8
				Model features	Linear	RCS K=6	RCS K=4	RCS K=5	RCS K=3	Linear	RCS K=3

Abbreviations: AIC= Akaike Information Criterion, RCS=restricted cubic spline

[1] Biomarker is transformed with restricted cubic spline (RCS) and regressed on the log odds of odds of being in class 5 (N=159) compared to referent class 1 (N=138)

[2] Knot location is based on percentile threshold among individuals in the cohort with biomarkers available who did not have surgically diagnosed endometriosis (N=301)

Supplemental Table 4.6 Unadjusted pairwise odds ratios using multinomial logistic regression logistic associating biomarkers to latent classes of chronic pelvic pain

		No pelvic pain Class 1[1] (N=138)	Moderate cyclic pelvic pain only Class 2[2] (N=145)	Severe cyclic pelvic pain only Class 3[2] (N=141)	Severe acyclic, moderate cyclic pain Class 4[2] (N=42)	Severe acyclic, severe cyclic pain Class 5[2] (N=159)
Linear[3]						
IL-8	Q5 vs. (ref Q1)[5]	(1.00)	0.73 (0.23,2.35)	1.23 (0.47,3.23)	0.73 (0.2,2.72)	1.88 (0.76,4.65)
	Continuous		0.83 (0.37,1.85)	0.89 (0.47,1.68)	0.66 (0.16,2.68)	1.45 (0.75,2.8)
TARC	Q5 vs. (ref Q1)[5]	(1.00)	0.84 (0.27,2.59)	0.79 (0.32,1.98)	3.6 (0.26,50.23)	0.71 (0.3,1.71)
	Continuous		0.79 (0.46,1.35)	0.79 (0.52,1.2)	1.06 (0.54,2.06)	0.74 (0.5,1.09)
Non-Linear[4]						
IL-16	Q1 (ref Q2-4)[5]	(1.00)	1.36 (0.53,3.48)	1.37 (0.67,2.79)	0.4 (0.07,2.28)	1.57 (0.78,3.14)
	Q5 (ref Q2-4)[5]	(1.00)	2.19 (0.87,5.52)	1.78 (0.85,3.73)	0.48 (0.07,3.1)	2.8 (1.38,5.68)*
IP-10	Q1 (ref Q2-4)[5]	(1.00)	1.53 (0.59,3.94)	1.5 (0.72,3.12)	0.74 (0.12,4.5)	1.87 (0.91,3.81)
	Q5 (ref Q2-4)[5]	(1.00)	2.07 (0.81,5.24)	1.95 (0.94,4.06)	3.29 (1.11,9.76)*	3.21 (1.59,6.5)*
MCP-1	Q1 (ref Q2-4)[5]	(1.00)	1.53 (0.58,4.02)	0.89 (0.39,2.01)	1.88 (0.56,6.36)	2.21 (1.06,4.55)*
	Q5 (ref Q2-4)[5]	(1.00)	1.86 (0.74,4.64)	1.41 (0.69,2.9)	1.74 (0.5,6.02)	2.85 (1.43,5.68)*
MCP-4	Q1 (ref Q2-4)[5]	(1.00)	0.67 (0.24,1.87)	0.73 (0.37,1.45)	1.03 (0.31,3.37)	1.59 (0.84,3.04)
	Q5 (ref Q2-4)[5]	(1.00)	3.59 (1.34,9.63)*	1.66 (0.71,3.86)	2.12 (0.56,7.97)	4.56 (2.03,10.24)*
TNF-a	Q1 (ref Q2-4)[5]		1.19 (0.42,3.34)	1.59 (0.73,3.46)	1.44 (0.35,5.93)	1.18 (0.54,2.61)
	Q5 (ref Q2-4)[5]		1.09 (0.44,2.69)	1.40 (0.70,2.78)	2.23 (0.74,6.67)	1.70 (0.89,3.25)

Notes: Significant associations observed denoted (*)

[1] Reference latent class is those with no pelvic pain, class 1, with an OR=1.0.

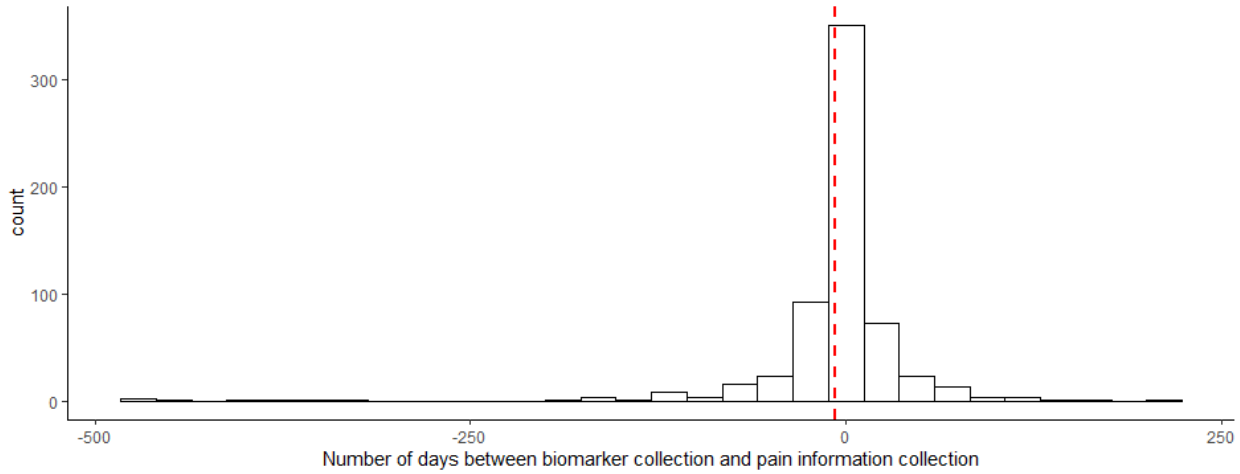
[2] Odds of membership in this class given one unit change in biomarker value (continuous) or quintile (binary)

[3]Linear multinomial regression model assumption for four biomarkers: IL-8, TARC

[4]Non-linear multinomial regression model assumption for five biomarkers: IL-16, IP-10, MCP-1, MCP-4, TNF-a

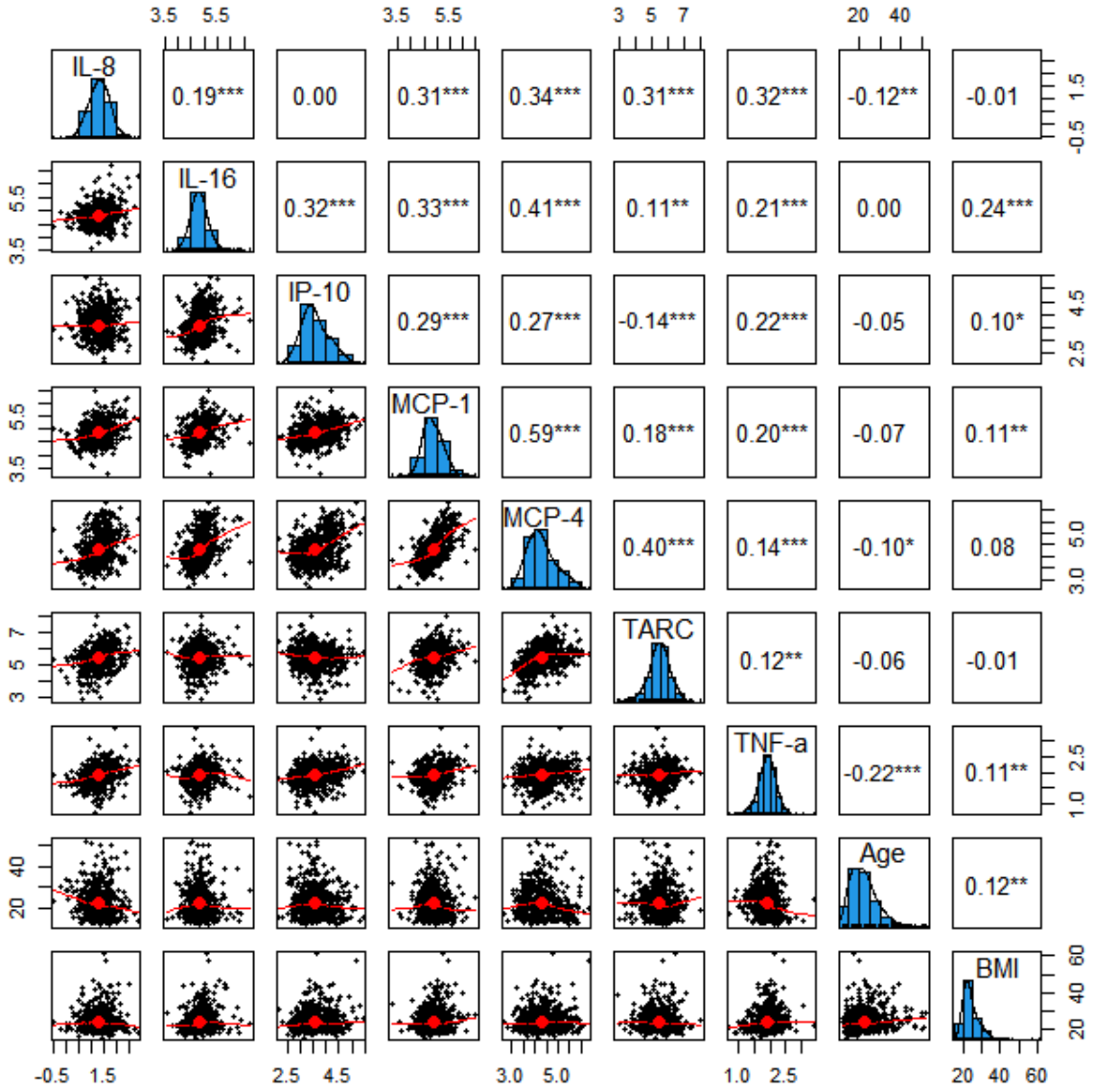
[5]Reference quintile(s): concentration quintile 1 (lowest) in binary linear models, concentration quintiles 2, 3 and 4 (middle) in non-linear models

Supplemental Figure 4.1 Biomarker collection (days) from collection of information about symptomology.



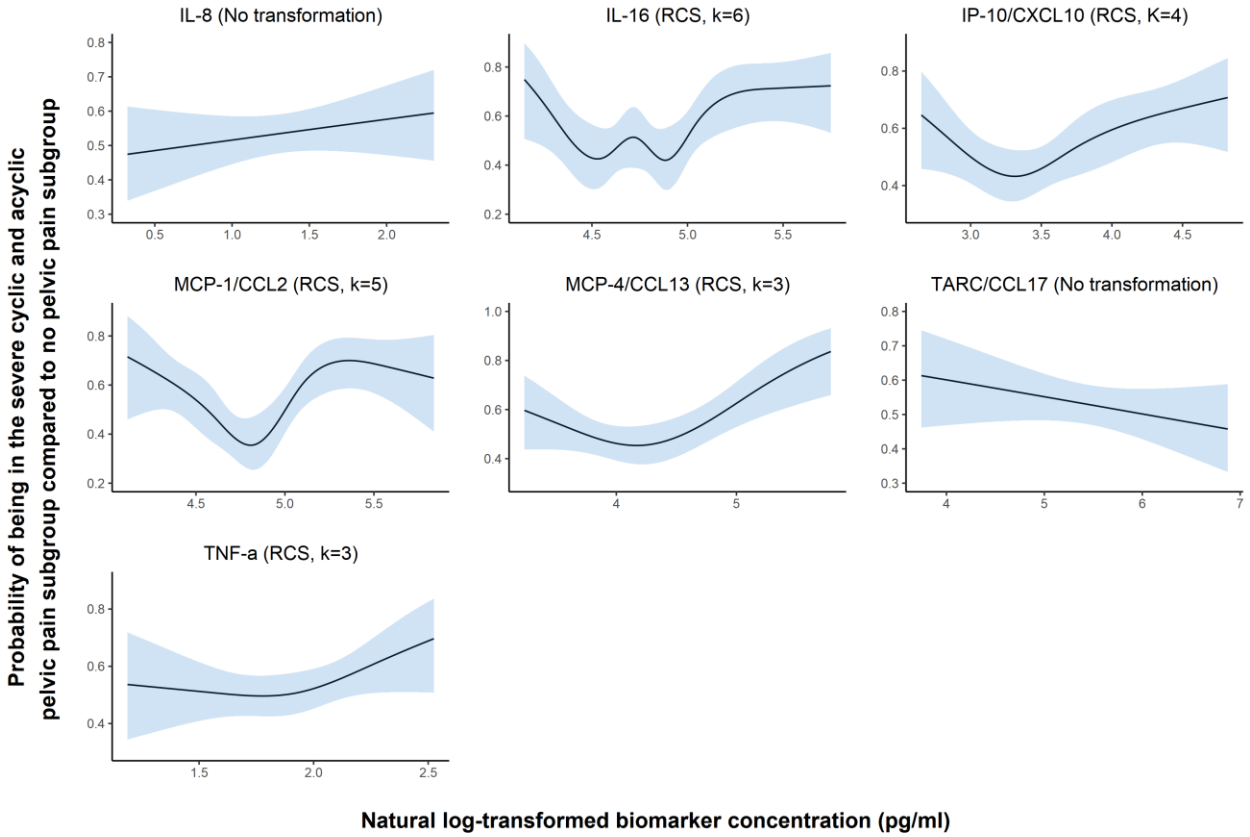
Notes: Negative values mean blood was drawn before survey was completed, positive values mean blood was drawn after survey was completed. Bin represents a 30-day period.

Supplemental Figure 4.2 Correlation plots displaying associations between inflammatory biomarkers in study participants (N=625)



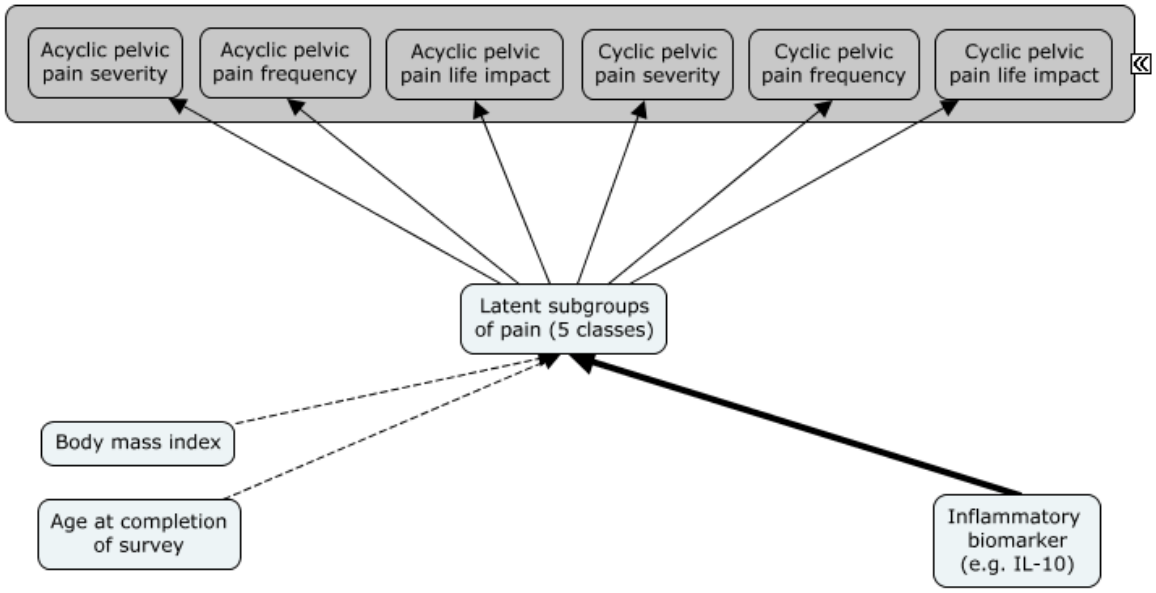
Notes: Histogram for log-transformed biomarkers (diagonal), scatter plots of correlations (below the diagonal), spearman rank correlation coefficient (above diagonal). Stars indicate statistical significance, p-values P ≤ 0.05*, ≤0.01**, ≤0.001***. Bonferroni corrected significance level is p=0.05/12=0.0042

Supplemental Figure 4.3 Restricted Cubic regression spline figures of biomarkers versus probability transformed log odds of being assigned to the most severe pain subgroup (class 5, N=159) versus no pain subgroup (class 1, N=138), age and BMI-adjusted.



Note: Logistic regression models for odds of being in the severe acyclic, severe cyclic pelvic pain subgroup (class 5, N=159) compared to referent no pelvic pain subgroup (class 1, N=138) using restricted cubic splines. The number of knots for each model is reported above individual graphs. Pointwise 95% confidence bands are shown. All y-axes have the same scale (logit scale converted to probability scale ranging from 0-100%), x-axes are natural log-transformed concentrations (pg/ml).

Supplemental Figure 4.4 Schematic diagram of regression analysis



Notes: Grey boxes represent observed indicators used to estimate underlying latent class subgroups of chronic pelvic pain. Thick black line represents the unadjusted association analyses in each model. Dashed black line represents covariate included in the adjusted model. Dashed grey line represented the moderating effect of classes on the relationship between covariate and outcome of interest. (Multinomial logistic regression using the R3STEP approach to estimate the odds ratio (OR) of being in each group compared to reference group as the levels of an inflammatory biomarker increases, adjusted for age and body mass index

APPENDIX D: IRB DETERMINATION

MICHIGAN STATE
UNIVERSITY

Modification / Update APPROVAL
Revised Common Rule

February 14, 2022

To: Stacey Ann Missmer

Re: **MSU Study ID:** STUDY00001042
IRB: Biomedical and Health Inst. Review Board (BIRB)
Principal Investigator: Stacey Ann Missmer
Category: Expedited 5, 7
Submission: Modification / Update MOD00004644
Submission Approval Date: 2/14/2022
Effective Date: 2/14/2022
Study Expiration Date: **None; however modification and closure submissions are required (see below).**

Title: What is Endometriosis (WISE)? Deep Phenotyping to Advance Diagnosis and Treatment
Funding Title: Menstrual health during the Covid-19 pandemic: A longitudinal study among young people with and without endometriosis
Funding Source: NIH
Funding Status: Funded

Office of Regulatory Affairs
Human Research Protection Program

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This submission has been approved by the Michigan State University (MSU) Biomedical and Health Inst. Review Board (BIRB). The submission was reviewed by the Institutional Review Board (IRB) through the Non-Committee Review procedure. The IRB has found that this study protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., 2018 45 CFR 46, 21 CFR 50, 56, other applicable regulations).

This letter notes that this modification changes the determination made on 1/28/2019 that this activity does not involve human subjects to human subjects research and was approved as Expedited 5, 7 on 2/14/2022.

How to Access Final Documents
To access the study's final materials, including those approved by the IRB such as consent forms, recruitment materials, and the approved protocol, if applicable, please log into the Click™ Research Compliance System, open the study's workspace, and view the "Documents" tab. To obtain consent form(s) stamped with the IRB watermark, select the "Final" PDF version of your consent form(s) as applicable in the "Documents" tab. Please note that the consent form(s) stamped with the IRB watermark must typically be used.

Expiration of IRB Approval: The IRB approval for this study does not have an expiration date. Therefore, continuing review submissions to extend an approval

period for this study are not required. **Modification and closure submissions are still required (see below).**

Modifications: Any proposed change or modification with certain limited exceptions discussed below must be reviewed and approved by the IRB prior to implementation of the change. Please submit a Modification request to have the changes reviewed.

New Funding: If new external funding is obtained to support this study, a Modification request must be submitted for IRB review and approval before new funds can be spent on human research activities, as the new funding source may have additional or different requirements.

Immediate Change to Eliminate a Hazard: When an immediate change in a research protocol is necessary to eliminate a hazard to subjects, the proposed change need not be reviewed by the IRB prior to its implementation. In such situations, however, investigators must report the change in protocol to the IRB immediately thereafter.

Reportable Events: Certain events require reporting to the IRB. These include:

- Potential unanticipated problems that may involve risks to subjects or others
- Potential noncompliance
- Subject complaints
- Protocol deviations or violations
- Unapproved change in protocol to eliminate a hazard to subjects
- Premature suspension or termination of research
- Audit or inspection by a federal or state agency
- New potential conflict of interest of a study team member
- Written reports of study monitors
- Emergency use of investigational drugs or devices
- Any activities or circumstances that affect the rights and welfare of research subjects
- Any information that could increase the risk to subjects

Please report new information through the study's workspace and contact the IRB office with any urgent events. Please visit the Human Research Protection Program (HRPP) website to obtain more information, including reporting timelines.

Personnel Changes: Key study personnel must be listed on the MSU IRB application for expedited and full board studies and any changes to key study personnel must to be submitted as modifications. Although only key study personnel need to be listed on a non-exempt application, all other individuals engaged in human subject research activities must receive and maintain current human subject training, must disclose conflict of interest, and are subject to MSU HRPP requirements. It is the responsibility of the Principal Investigator (PI) to maintain oversight over all study personnel and to assure and to maintain appropriate tracking that these requirements are met (e.g. documentation of training completion, conflict of interest). When non-MSU personnel are engaged in human

research, there are additional requirements. See HRPP Manual Section 4-10, Designation as Key Project Personnel on Non-Exempt IRB Projects for more information.

Prisoner Research: If a human subject involved in ongoing research becomes a prisoner during the course of the study and the relevant research proposal was not reviewed and approved by the IRB in accordance with the requirements for research involving prisoners under subpart C of 45 CFR part 46, the investigator must promptly notify the IRB.

Site Visits: The MSU HRPP Compliance office conducts post approval site visits for certain IRB approved studies. If the study is selected for a site visit, you will be contacted by the HRPP Compliance office to schedule the site visit.

For Studies that Involve Consent, Parental Permission, or Assent Form(s):

Use of IRB Approved Form: Investigators must use the form(s) approved by the IRB and must typically use the form with the IRB watermark.

Copy Provided to Subjects: A copy of the form(s) must be provided to the individual signing the form. In some instances, that individual must be provided with a copy of the signed form (e.g. studies following ICH-GCP E6 requirements). Assent forms should be provided as required by the IRB.

Record Retention: All records relating to the research must be appropriately managed and retained. This includes records under the investigator's control, such as the informed consent document. Investigators must retain copies of signed forms or oral consent records (e.g., logs). Investigators must retain all pages of the form, not just the signature page. Investigators may not attempt to de-identify the form; it must be retained with all original information. The PI must maintain these records for a minimum of three years after the IRB has closed the research and a longer retention period may be required by law, contract, funding agency, university requirement or other requirements for certain studies, such as those that are sponsored or FDA regulated research. See HRPP Manual Section 4-7-A, Recordkeeping for Investigators, for more information.

Closure: If the research activities no longer involve human subjects, please submit a Continuing Review request, through which study closure may be requested. Closure indicates that research activities with human subjects are no longer ongoing, have stopped, and are complete. Human research activities are complete when investigators are no longer obtaining information or biospecimens about a living person through interaction or intervention with the individual, obtaining identifiable private information or identifiable biospecimens about a living person, and/or using, studying, analyzing, or generating identifiable private information or identifiable biospecimens about a living person.

For More Information: See the HRPP Manual (available at hrpp.msu.edu).

Contact Information: If we can be of further assistance or if you have questions, please contact us at 517-355-2180 or via email at IRB@msu.edu. Please visit hrpp.msu.edu to access the HRPP Manual, templates, etc.

Expedited Category. Please see the appropriate research category below for the full regulatory text.

Expedited 1. Clinical studies of drugs and medical devices only when condition (a) or (b) is met.
(a) Research on drugs for which an investigational new drug application (21 CFR Part 312) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.)
(b) Research on medical devices for which (i) an investigational device exemption application (21 CFR Part 812) is not required; or (ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.

Expedited 2. Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows:
(a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or
(b) from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Expedited 3. Prospective collection of biological specimens for research purposes by noninvasive means.
Examples: (a) hair and nail clippings in a nondisfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax or by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor; (h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Expedited 4. Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for

expedited review, including studies of cleared medical devices for new indications.) Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

Expedited 5. Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)

Expedited 6. Collection of data from voice, video, digital, or image recordings made for research purposes.

Expedited 7. Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.)

Expedited 8. Continuing review of research previously approved by the convened IRB as follows:
(a) where (i) the research is permanently closed to the enrollment of new subjects; (ii) all subjects have completed all research-related interventions; and (iii) the research remains active only for long-term follow-up of subjects; or
(b) where no subjects have been enrolled and no additional risks have been identified; or
(c) where the remaining research activities are limited to data analysis.

Expedited 9. Continuing review of research, not conducted under an investigational new drug application or investigational device exemption where categories two (2) through eight (8) do not apply but the IRB has determined and documented at a convened meeting that the research involves no greater than minimal risk and no additional risks have been identified.

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