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PROVOKED VESTIBULODYNIA – EVALUATION OF A TREATMENT MODALITY AND EARLY LIFE HEALTH

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Provoked Vestibulodynia – Evaluation of a Treatment Modality and Early Life Health
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my parents.

Mom, look! I am an author now.

Dad, its okey that you don't read this thesis. I haven´t read yours.

I miss you both.

POPULAR SCIENCE SUMMARY OF THE THESIS

Background

Provoked vestibulodynia, PVD, is a pain condition that affects many women, especially in younger years. The pain is located around the lower opening of the vagina, and differs in intensity from person to person. This burning sensation is triggered by touch or stretch to this area, e.g. when trying to insert a tampon or engaging in vaginal intercourse. Some experience a burning sensation after intercourse, whilst others are so sensitive that even tight-fitting clothes can be extremely uncomfortable.

Several different forms of this condition exist with the most common referred to as primary and secondary PVD. Those afflicted by primary PVD describe having always had this pain, even from the first time they tried to insert a tampon or engage in intercourse. Those with secondary PVD have had a pain free period before developing this disease.

A closely related condition to PVD is vaginismus, which is an involuntary muscle spasm of the pelvic floor muscles. It is thought that this is a protective response to the vaginal pain, and therefore the muscles contract and close the vagina. This reflex then becomes permanent and the muscles remains contracted, which further adds to the problem of pain sensitivity. This muscle spasm can be difficult to treat and requires special care focusing on pelvic floor function.

The treatment options today are based on limited scientific evidence taken from clinical experience which include recommendations for physiotherapy, de-sensitizing treatments of the vestibular mucosa, pharmacotherapy and psychotherapy to name a few.

Why some people develop PVD and others not, is not entirely clear, especially for those who have primary PVD. The research group have theorized that events during fetal development and the first year of life may trigger the development of this disease. Other studies have seen a link between being born prematurely and develop pain conditions as an adult, and we wanted to explore this specifically for PVD.

What did we try to do and how?

This thesis has evaluated how botulinum toxin A, BTA, can be used as a treatment for PVD. This drug has a passing paralytic effect on the muscle in which it is injected, which could help break the muscle spasm and hopefully reduce the pain sensitivity.

In the studies exploring the effect of BTA, a small dose was injected in the bulbocavernosus muscles on patients with PVD and compared it with placebo. The treated muscles are located around the vaginal opening. Several different parameters were evaluated, particularly if pain during intercourse or tampon insertion was reduced. The participants also answered questionnaires pertaining to sexual and mental health throughout the studies. The pressure in

the vagina was measured with a manometer, to try and see if we could get an objective muscle effect of BTA.

The manometer had never been used for this purpose, so one of the papers evaluated the device, by testing it three groups of women; PVD women, women who had never given birth and women who had given birth three months prior to the measurement. These three groups were chosen based on the differing status of their pelvic floor muscles (PFM).

To explore if birth related events could have a link to developing PVD later in life data from several different medical registries in Sweden was used. Among the theories explored was whether repeated painful experiences during birth and during the first year of life could be a risk factor. Such painful experiences included surgery, repeated blood sampling and certain diseases etc. We also looked at prematurity, birth weight and some maternal characteristics.

What did we discover?

In the studies evaluating the effect in BTA, no reduction in pain during intercourse was observed compared with placebo. However over time the BTA group experiences and overall reduction in pain. The group receiving BTA did try to have sex more often, and had a muscular effect with lower vaginal pressures during the time when the drug was active, which was seen as a positive effect of the treatment.

The tested manometer could detect differences in pressures between some of the evaluated groups. It also measured very consistently, with minor variations between the measurements. It did have some drawbacks, and optimally, the measuring balloon should be a bit larger for easier handling and possibly more accurate measurements.

In the registry studies we could see that being born prematurely and small for gestational age was a risk factor for developing PVD later in life. Painful experiences during the first year of life as mentioned earlier, were not associated to be afflicted by PVD later in life.

ABSTRACT

Background:

Provoked vestibulodynia (PVD) is a common cause of dyspareunia and have severe negative impact on the quality of life and sexual health in those afflicted. PVD can affect women at a young age without a clear cause and a theory is that birth-related events could contribute to the development of this condition. One important clinical finding is increased tension of the pelvic floor muscles (PFM). Various physiotherapeutic interventions are recommended as first line treatment, but these are not always effective and more options are needed.

Objective

The main aim of this thesis was to evaluate the effect of BTA-injections in the bulbocavernosus muscles as a treatment for PVD. Another aim was to investigate if birth-related events could be a contributing factor to the etiology of PVD.

Material and Methods

We conducted a randomized controlled trial (RCT) were we evaluated the effect of bilateral injections of 50 U BTA in the bulbocavernosus muscles in women diagnosed with PVD, compared with placebo. Injections were given twice with three months' interval. Forty-four women were randomized to BTA and 44 to placebo (in total 88 women). Our primary outcome measure was reduction in dyspareunia or pain at tampon use during the last month. Secondary outcomes were reduction in vaginal pressure as a measure of PFM function, pain at weekly tampon insertion, adverse events and sexual function and distress six months after the first treatment (Study I). A long-term follow-up of the RCT with additional pelvic floor exercises and psychometric evaluation, was conducted at 12 months (Study IV). In an effort to objectively investigate the PFM function in women with PVD, we evaluated a vaginal manometer measuring various vaginal pressures as a proxy for muscle function in women with PVD (n=60), nulliparous women (n=34) and primiparous women (n=34) (Study III).

In Study II we conducted a register study were all women born in Sweden between 1973 and 2001 were categorized into those with and without a PVD/vaginismus diagnosis between 2001 and 2016. Here we estimated the association between health during infancy and onset of PVD/vaginismus later in life.

Results

In Study I and IV twice repeated injections of 50 U BTA in women with PVD did not reduce dyspareunia or pain at tampon use. Secondary results showed a significant decrease in pain at weekly tampon insertion. The vaginal manometer detected a transient lower maximum contraction and 10-second endurance contraction in the BTA group compared with the placebo group during the time period when the drug was active. No severe adverse events were reported. The group receiving BTA also had an increased number of intercourse attempts compared with placebo at both 6- and 12-months' follow-up.

In study III, all pressure variables showed a similar pattern when comparing the groups. The vaginal resting pressure was lower in primiparous women compared to nulliparous women but not the PVD group. The maximum contraction and endurance pressure was similar for PVD and nulliparous women. Primiparous women exhibited lower pressure in both these variables compared with the other groups. Intra-rater reliability for the VRP variable was moderate and good to excellent intra-rater reliability for MCP and EP. The inter-rater reliability showed excellent reliability for all variables.

In Study II adverse health at birth, such as preterm delivery, low birth weight and small for gestational age was found to be associated with developing PVD/vaginismus later in life. In contrast to our hypothesis, we found no evidence to suggest that pain exposure early in life is positively associated with developing PVD/vaginismus later on in life.

Conclusions

BTA does have a detectable muscular effect on women with PVD, but the effect on dyspareunia and the optimal dose to reduce this still remains to be determined. After 12 months, increased sexual function and significant increase in number of intercourse attempts were observed in the BTA group. The vaginal manometer was deemed a functional tool to detect differences in vaginal pressures during different states of PFM activity and varying parity with good intra- and inter-rater reliability. Adverse events at birth such preterm delivery, SGA and low birth weight could be contributing factors for developing PVD/vaginismus later in life.

LIST OF SCIENTIFIC PAPERS

I. **Philip Haraldson**, Hanna Mühlrad, Ulrika Heddini, Kent Nilsson, Nina Bohm-Starke
Botulinum Toxin A as a Treatment for Provoked Vestibulodynia
Obstetrics and Gynecology, 2020, 2020;3:524-532

II. Hanna Mühlrad, **Philip Haraldson**, Bernard L Harlow, Marie Anell Olofsson, Nina Bohm-Starke
Early Life Health in Women with Provoked Vestibulodynia and/or Vaginismus
Journal of Women's Health, 2021;6:799-806

III. **Philip Haraldson**, Nina Bohm-Starke, Matilda Jakobsson, Kent Nilsson, Ulrika Heddini
An Evaluation of a Vaginal Manometer Designed for Women with Provoked Vestibulodynia
In manuscript, submitted.

IV. **Philip Haraldson,** Hanna Mühlrad, Ulrika Heddini, Kent Nilsson, Nina Bohm-Starke
Botulinum Toxin A for Provoked Vestibulodynia: 12 months' follow-up of a Randomized Controlled Trial
In manuscript, submitted.

CONTENTS

LIST OF ABBREVIATIONS

BTA	Botulinum Toxin A
CBT	Cognitive Behavioral Therapy
CI	Confidence Interval
EMG	Electromyography
EP	Endurance Pressure
FSFI	Female Sexual Function Index
FSDS	Female Sexual Distress Scale
IBS	Irritable Bowel Syndrome
ICC	Intra Class Correlation Coefficient
ICD	The International Classification of Diseases
ISSVD	The International Society for the Study of Vulvovaginal Diseases
KKÅ	Klassifikation av kirurgiska åtgärder
KVÅ	Klassifikation av vårdåtgärder
LISA	Longitudinal integrated database for health insurance and labour market studies
NPR	National Patient Registry
MBR	Medical Birth Registry
MCP	Maximum Contraction Pressure
MRI	Magnetic Resonance Imaging
aOR	Adjusted Odds Ratio
OR	Odds Ratio
PFM	Pelvic Floor Muscles
PSS	Percieved Stress Scale
PVD	Provoked Vestibulodynia
RCT	Randomized Controlled Trial
SBU	The Swedish Agency for Health Technology Assessment and Assessment for Social Services
SCAS	Spencer Children Anxiety Scale

SNQ	Swedish Neonatal Quality Registry
TLR	Toll-Like Receptors
U	Units
VAS	Visual Analogue Scale
VRP	Vaginal Resting Pressure

1 INTRODUCTION

1.1 HISTORY

Some sources believe that the first recorded reference to some form of vulvodynia could date as far back as the first century CE. Soranus, a medical doctor in ancient Greece, makes a mention of "satyriasis in females" which some have interpreted as vulvodynia (1). In more modern times Skene described in the late 19th century a condition of excessive sensitivity of the vulva. Thirty years later Kelly also makes a mention of very sensitive red spots in the hymenal mucosa, as a source of dyspareunia. In 1970 the International Society for the Study of Vulvovaginal Diseases (ISSVD) was founded and in 1976 they recognized idiopathic vulvar pain as its own condition and named it "the burning vulva syndrome" (2). The research on vulvar pain conditions has since then been growing and terminology and description expanded. In the 1980´s Friedrich coins the term "vulvar vestibulitis syndrome", after which the disorder is more widely recognized (1). During the 1990´s, the term vestibulodynia was introduced, and is what is used today to describe the pain condition in focus of this thesis. At the end of the 1990s the ISSVD takes charge of the terminology and classification of vulvar pain disorders and has since then released several reports and updates, the one currently being used released in 2015 (3).

1.1.1 Vulvodynia

Vulvodynia is characterized by vulvar pain that can occur either spontaneous or upon touch, pressure or stretch of the vulvar tissue. The 2015 Consensus Terminology and Classification of persistent Vulvar Pain and Vulvodynia defines vulvodynia as vulvar pain persistent ≥ 3 months without an identifiable cause, which may have associated co-morbidities. Vulvodynia is then divided into two main categories: generalized and localized. These two categories are then further subdivided into: provoked, unprovoked and mixed types. Generalized vulvodynia is pain in a large part of the vulvar region, whereas localized vulvodynia is pain in a specific area e.g. vestibulodynia or clitorodynia. Provoked refers to pain upon contact with vulvar tissue, and unprovoked is pain without an external stimuli (3). The most common subtype of vulvodynia is localized Provoked Vestibulodynia (PVD) where the pain is located around the vaginal opening, most often in the posterior part (4).

1.1.2 Epidemiology

No global epidemiological studies assessing prevalence of PVD has been performed, but there are studies conducted in individual countries and regions. One occurring problem with these studies is the mix of terminology, and several vulvodynia subtypes are grouped together which makes it difficult to assess the incidence and prevalence of PVD alone. One study from the US estimated that up to 16% of women experience vulvodynia during their lifetime (5). Another survey in the same country reported that around 8% of women may develop vulvar pain consistent with vulvodynia by the age of forty (6). A web-based study from Spain reported a lifetime prevalence of vestibulodynia of 13% (7) and another web-based

Portuguese study found a lifetime prevalence of 16% (8). Incidence has been estimated to 4.2 cases per 100-person years (9).

A Swedish study published in 2003 found a prevalence of severe dyspareunia of 9.3% in women aged 20-60, with the highest prevalence (13%) seen in ages 20-29. The study did not differentiate between different types of dyspareunia (10). A prospective study among university students in Sweden found that up to 29% reported coital pain during intercourse at the time of the study (11). Another Swedish questionnaire study aimed at adolescents seeking care at a youth clinic reported that 50% had coital pain during the last month, and half of them had significant problems (12). In a large Swedish population survey in 2017, 19% of women aged 16-29 reported pain during or after intercourse. The report does not however, specify what type of pain the respondents were suffering from (13).

A survey on vestibulitis from 2018, published by the Swedish National Board of Health and Welfare, show a steady increase in patients seeking care for vestibulitis, vaginismus and dyspareunia between 2001 and 2016 (14). It is unclear if the increase reflects a rising incidence in the population, or an enhanced awareness in the society and among professionals. The report does stress that the condition is underreported, which could be attributable to several factors. One is that visits youth centers and primary care units do not register diagnoses or ICD codes to the national patient registry, and these appointments are therefore not included in national registers for specialized care. Another factor is that terminology and classification is not used coherently nationwide (14). Previous studies have also shown that at large portion of women with dyspareunia do not seek care at all for their problems (15).

2 LITERATURE REVIEW

2.1 PATHOPHYSIOLOGY

The mechanisms underlying vulvodynia and PVD are to a large extent unknown but are considered multifactorial, both regarding development and maintenance of the disease, including both biomedical and psychosocial causes (16, 17). The neuropathophysiology of vulvodynia is complex and changes in both peripheral and central sensory systems have been described (16, 17). Vulvodynia is characterized by mechanical allodynia and hyperalgesia in the vulvo-vaginal region. An increased number of nociceptive nerve endings in the vestibular mucosa has been seen in patients with PVD (18, 19). This increased nociceptive innervation has been shown to have negative consequences for patients with this condition (20). MRI scans show higher activity in cerebral pain regulatory centers in PVD patients, similar to other chronic pain conditions (21). Other MRI brain studies have shown increased gray matter volume in pain modulatory and stress related areas, which correlate to clinical symptoms of PVD, compared with healthy controls (22, 23).

Inflammation, both local and systemic, has been hypothesized as a pathophysiological pathway for vulvodynia (17). Several studies have looked into this possible link but the results have been inconsistent. A systematic review from 2016 evaluated the evidence of inflammation in patients with PVD (24). The studies reviewed had looked at cytokine levels, prostaglandin E2, T cells, mast cells, natural killer cells and macrophages in women with PVD. The authors of the review noted that the methodologic quality of the included studies was generally low and the methods investigating inflammation varied greatly. The conclusion was that the evidence of an altered inflammatory response in women with PVD compared with controls were limited and contradictory (24). However, there were findings of an increased number of mast cells in the vestibular tissue in several studies (25-27). Mast cells can stimulate and sensitize peripheral nerve endings (28) and together with a hyperplasia of the nerve endings lead to allodynia in the vestibular mucosa (18).

As a consequence, there is currently no indication for the use of anti-inflammatory therapies for PVD (24). A more recent published study looked at the expression of toll-like receptors (TLR) in women with PVD. TLR receptors are involved in innate immune responses to microbial assaults, and the result of the study showed a higher expression in patients with PVD. It was proposed that suppression of this response may be a new therapeutic option for vulvodynia patients (29). Based on clinical experience and scientific results recurrent vulvovaginal candida infections is probably the single most important factor for the onset and development of vulvodynia, adding to the theory that inflammation is part of the mechanism behind the condition (30, 31). Numerous women with secondary PVD are telling the same story of vulvo-vaginal candidiasis initiating pain and discomfort, which is further supported by epidemiological as well as experimental data (14, 32, 33).

2.1.1 Primary and Secondary PVD

In addition to the several subtypes of vulvodynia described by the ISSVD terminology (3), two other forms are described in the literature and referred to as primary and secondary PVD (34). Primary PVD is characterized by vulvar pain since the first attempt at vaginal penetration, with e.g. tampon or sexual activities. Secondary PVD patients have experienced pain-free penetrative activities before developing PVD (35). The literature researching the prevalence of these two types is scarce, but one clinical paper estimated the groups to be equally large (36). It has been theorized that these two subtypes may have different etiologic origins and in 2016 Pukall reviewed the literature on this matter (35). Those with primary PVD report higher pain intensity, more genetic susceptibility, more evidence of inflammation, less successful treatment outcomes and different neural activation patterns (35). The literature was however not conclusive, and more research is deemed necessary.

2.1.2 Hormonal factors

It is known that female sexual hormones affect the pelvic floor and vaginal tissue. Estrogen influences the physiology, mucosal composition and thickness of the vagina and increases vaginal blood flow (37). The effect of progesterone on vaginal tissues are less studied, and therefore not as well understood. The role of hormonal contraceptive use and the development of PVD have been investigated in several studies. Some clinically based studies have reported a possible link between use of oral contraceptives and an increase in risk of developing vulvodynia (38, 39). This finding was however, not reported in more recent population-based studies (40, 41). A systematic review, conducted in 2016, looked at the effect of hormonal contraceptives on pelvic floor function, bladder, vaginal and sexual symptoms and found that oral contraceptives could increase the risk of both interstitial cystitis and vulvar vestibulitis (42). The review also concluded that more animal/molecular studies were needed to study the effect of estrogen and progesterone on bladder, bowel and vaginal tissues in women in reproductive age to understand the mechanism of action better in this group (42).

2.1.3 Muscle dysfunction

A condition that often is associated with PVD is various degrees of vaginismus, i.e. an involuntary spasm or hypertension of the PFM. Patients with PVD are also found to have a heightened general pelvic floor tonus, poorer muscle control and weaker muscle contractility compared with healthy controls (43, 44). Evidence suggests that this hyperactivity in the pelvic floor muscles (PFM) is chronic and contributes to maintaining and exacerbating the pain in affected individuals. If this muscle dysfunction is a cause of vulvar pain or a result of it remains unanswered (16, 17).

Several methods to evaluate the pelvic floor function in women with PVD exist. Digital palpation is one of the most common methods (45), and has the advantage of being easily accessible and quick, but is highly subjective and lack precision (46).

4D trans perineal ultrasound has been used, which has the advantage of a pain free assessment of the pelvic floor (44). The methodology is however expensive and requires a trained user to be reliable.

Electromyography (EMG) has also been used in PVD patients to measure muscular contractile activity with conflicting results. Some studies have found higher resting activity in vulvodynia patients compared with controls (47, 48) a finding not confirmed by others (49). This might be due to different methodologies (surface, intravaginal, or needle) used, each with individual limitations (50).

2.1.4 Genetic factors

A familiarity analysis done on PVD patients who underwent vestibulectomy has shown a familial clustering of the condition. Using population-based genealogy, the relative risk for relatives to patients who underwent this surgery could be calculated and the conclusion that there is a possible genetic predisposition for PVD was drawn (51). Several PVD related polymorphisms have been described in pain regulatory genes for both serotonergic and opioid systems (52, 53), immune modulatory genes which affect inflammatory response (54-56) and an androgen receptor gene modulating lubrication in women taking combined oral contraceptives (57).

2.1.5 Psychosocial factors

PVD has been linked to a substantial negative impact on quality of life, severe impact on sexual function, and increased psychological distress (16, 31). Other studies looked at severe emotional- and physical abuse during childhood, and found that women with vulvodynia were more likely to have experienced childhood maltreatment (58, 59). A Canadian survey on adolescent women also reported an association between coital pain and a history of sexual abuse (60). Other population based studies have shown associations between anxiety and depression disorders and vulvodynia (61). A review of psychosexual factors and etiology of PVD found that anxiety, fear of pain, hypervigilance, catastrophizing, and depression are more frequently reported by women with PVD than healthy women of the same age (62).

2.1.6 Early life health

Previous research on early life health and PVD is scarce, but suggests that preterm exposure to pain stimuli has long term consequences in terms of sensory stimuli perception, neuroendocrine stress and anxiety (63-66). Moreover, earlier studies also suggest a link between chronic pain and mental illness, but much of these relationships remain unclear (67). In addition to human suffering, chronic pain disorders can have a negative impact on labor market outcomes. Young women with mental illness have a 31% greater risk for sick leave than men of the same age in Sweden (68). Improved knowledge of chronic pain disorders in general, and pain disorders for young women in particular, can therefore be of great value for both the affected individuals and society at large.

2.2 DIAGNOSIS, SCREENING AND PREVENTION

The diagnosis of vulvodynia is mainly based on medical history, vulvo-vaginal examination and laboratory assessment. The medical history should include onset and characteristics of symptoms, previous gynecological history and other co-morbidities. Psychological symptoms and relationship factors should be assessed as well (3). The gynecological examination is done to support the diagnosis and differentiate vulvodynia from other vulvar pain disorders. The external appearance is usually normal, but erythema can sometimes be seen in the vulvar vestibule, particularly around Skene's and Bartholin's glands, and in the posterior fourchette (69). The cotton swab test is commonly used to assess vulvar pain, and consist of applying a light pressure with a cotton swab around the vulvar vestibulum perpendicular to the tissue and the patient assess the pain verbally, often using a numeric scale (70, 71).

During the pelvic examination an assessment of the pelvic floor muscle tension and tenderness is included, since dysfunction in the PFM is associated with pain and negative psychosexual consequences (72). Several methods to evaluate PFM tension has been described, but there is no clear consensus or standardized way to do this. One study developed a standardized protocol for assessing pelvic pain starting with an external examination of the sacroiliac joints, anterior superior iliac spine and the pubic symphysis. Thereafter, the levator anii and obturators internus muscles were examined bilaterally (73). In vulvodynia patients it is also important to examine the bulbocavernosus, transverse perineal and the ischicavernosus muscles. The patient is asked to contract and relax the pelvic floor muscles, to reveal the level of muscle tension and control.

Wet mount microscopy should be performed to rule out candida infections, bacterial vaginosis or trichomoniasis. In some cases, cultures are needed. Hypoestrogenic states, which can occur with some oral contraceptives, during breast feeding or in postmenopausal women should be also be excluded (74).

Differential diagnosis to be considered during examination are infections, inflammatory, neoplastic and neurologic diseases (3). Candida vulvovaginitis is heavily overrepresented in women with vulvodynia and often occur concomitantly and requires adequate parallel treatment.

2.3 MANAGEMENT

The management of vulvodynia today has no international consensus. Evaluation of conducted RCTs has resulted in very low certainty of evidence, which means that it is not possible to conclude the effects of most interventions (75, 76).Treatment guidelines are usually based on experts' opinion and it is proposed that treatment should be tailored individually (3). A multidisciplinary team consisting of a gynecologist, midwife/ physiotherapist and a psychologist/counselor is recommended. This multidisciplinary treatment approach is aimed at covering psychological interventions, pelvic floor physical therapy, psychosexual support, medical treatment and surgery (77).

A first step in the treatment is vulvar care measures which includes using cotton underwear, avoiding vulvar irritants, cessation of soap use in the vulvar region in favor of oil and water. Use of daily emollients in the vulva is recommended, preferably after shower (78). Medical treatment then focuses on desensitizing the vulvar mucosa and daily applications of topical lidocaine is often tried. Women with intensive mucosal pain may benefit from this treatment (78). Other topical remedies tested include estrogen, fibroblast lysates, muscle relaxers, capsaicin, tricyclic antidepressants and anticonvulsants but they all have questionable efficacy (79).

The next step in medical treatment is oral medication which fall into two categories: antidepressants and anticonvulsants. Tricyclic antidepressants; amitriptyline, nortriptyline and desipramine all have a proven neuropathic pain-relieving effect. All medications have been used primarily for the treatment of generalized and unprovoked vulvodynia and amitriptyline and nortriptyline show the greatest effect (79, 80). For PVD, desipramine was not superior compared with topical lidocaine or placebo in one double-blinded RCT evaluating pain during intercourse (81). Gabapentin, an anticonvulsant, has also been used and tested for vulvodynia patients but the results are inconclusive. One RCT did not see any difference between the drug and placebo regarding pain outcomes, but an improved sexual function was found in the intervention group (82).

Alongside local desensitization, physical therapy is considered first line treatment with the aim to restore a normal pelvic floor tonus and the patient´s awareness of their pelvic floor, especially in patients with concomitant vaginismus. Most trials evaluating this modality lack a comparison group, and the effectiveness of the treatment should therefore be interpreted with caution. Nevertheless, in one RCT studying the effect of multimodal physiotherapeutic interventions compared with topical lidocaine, a significantly better results for pain-reduction during intercourse and sexual function was seen in the intervention group. The results were seen at the end of treatment, and also at six months' follow-up (83). Furthermore, a systematic review of physical therapy for women did show a consistent effectiveness of physical therapy for women with PVD with a significant pain reduction in 71-80% of the women (84).

Cognitive-behavioral therapy (CBT) for reducing pain, fear of pain and increasing sexual function is also recommended. The psychological distress is considered secondary to vulvodynia and CBT does not treat the underlying causes, but literature does support psychological therapy as part vulvodynia treatment (85, 86).

Vestibulectomy is usually considered the last option for women with PVD. It is used when little or no benefit has been observed with conservative treatment. Surgery is also the most studied treatment for PVD, and the exact surgical technique seem to be of minor importance for the result (87). Most published papers report a high success rate with significant pain relief in 78.5-88.8% of the patients, where many have had a complete response. The surgical procedure is deemed a safe option with few adverse events for this patient group (87). There is currently only one published RCT comparing surgery to EMG biofeedback and group CBT

for three months (85). The results are in favor for surgery, showing less pain during intercourse at 6 months' and two and a half years follow-up (86).

2.4 NEUROTOXIC AGENTS

Botulinum toxin A has been used for patients with PVD, and to some extent been evaluated scientifically. It is most commonly used in patients who do not respond to first line treatments, especially physiotherapy. It is most applicable on patients with PVD and vaginismus, as the injection sites are limited to the vulvar vestibule, and in generalized vulvodynia it is therefore not clinically useful. BTA is a neurotoxin that has a transient paralytic effect on muscle tissue, and also inhibits the release of substance P, a neurotransmitter marking inflammation and pain (88). It is this paretic effect, causing muscle relaxation that is theorized to cause a pain-relieving effect.

There are a limited number of previous RCTs on BTA for PVD. Petersen et al, 2009, found a significant pain reduction in both BTA (20 Allergan units) and placebo groups after a single treatment, with no difference between the groups (89). The main outcome was reported at six months' follow-up when a possible effect of BTA would theoretically have passed (90). In a small RCT by Diomande et al (91), no effect was shown after a single injection of either 50 or 100 U of BTA. In this study the injections were given subcutaneously in the dorsal vestibulum of the vagina, and a spread to underlying muscles might have occurred. Interestingly, still symptomatic patients were offered an additional 100 U injection after three months which resulted in a significant pain reduction. However, the second part of the study was performed open label. Both RCTs also concluded that the treatment seems to be a safe treatment option (89, 91).

Another uncontrolled, open label study on PVD patients reported a significant effect of 100 U of BTA injected in the bulbocavernosus muscles bilaterally, up to 2 years after treatment (92). In a Danish open label study, 100 U of BTA was injected in the puborectalis muscles bilaterally, and showed significant improvement in dyspareunia, cotton swab test and quality of life (93).

One thing to note is the lack of discussion on BTAs role as a paralytic agent in the injected muscle. One of the rationales of using BTA is to lower pelvic floor tonus and thus decrease the pain. None of the studies discussed above have had heightened pelvic floor tonus as an inclusion criterion, and only one discussed its role briefly as such in the discussion (92).

2.5 PROGNOSIS

The treatment for PVD falls in to three major categories; non-pharmacological, pharmacological and surgical. The evaluation of each treatment in studies is commonly done separately, but clinically a combined approach is often used and also recommended in international consensus guidelines (77). Generally, psychological interventions, pelvic floor rehabilitation and vestibulectomy has shown the most promising results in studies (94). In an observational case-control study, long-term follow up for conservative treatment (median 77

months follow-up) of PVD was compared with surgery (median 47 months follow-up). In both groups a similar long-term outcome was observed for pain and sexual function, where coital pain reported using a VAS decreased 67% in the surgery group and 78% in the conservative group. In both groups 89% where satisfied with their treatment. The high success rate of conservative treatment strengthens the conclusion that this is preferable as a first-line option (95). Another study found better treatment outcomes for women with secondary PVD compared with primary PVD. The number of concomitant pain conditions was also seen to correlate with treatment outcome, in that women with fewer other pain conditions had a higher likelihood of a successful treatment outcome (96).

Regardless of treatment option, symptoms may be reduced but still persist to some extent, and symptoms may re-occur following any treatment. Therefore, more treatment options are needed for this group. A complete reduction of pain can be an unrealistic goal, and the ability to develop coping strategies to deal with lasting symptoms are usually helpful to improve quality of life (94).

2.6 PREVENTION

The literature evaluating preventive measures and their efficacy is scarce, but there are some parts that is worth highlighting. Several studies report the importance of a general, basic good skin care using emollients and avoiding irritants in the vulvar region (97, 98), and this should apply also to people without a pain condition. The advice to use cotton underwear can also be stressed here, since it maintains a good environment for the vulvar skin and mucosa.

Education about female genital anatomy, general sexual health and what type of care that is available in the society for issues related to these topics, should also be included in preventive measures. There is no evidence to show that this affects incidence and prevalence on vulvodynia as such, but education about such measures are known to improve health conversation and lower stigma around these problems (99). It is also known that many women with vulvar pain have not been in contact with healthcare, and the condition is underreported (6, 15). Continuous education of healthcare professionals is also an important factor in order to recognize and correctly treat PVD (100). There is also clinical experience that earlier detection and treatment of PVD, makes for a more successful treatment outcome.

3 RESEARCH AIMS

This thesis had two major aims. The first one was to evaluate botulinum toxin A's effect on pain, pelvic floor function and psychosexual health in women with provoked vestibulodynia PVD. The second aim was to evaluate if events in early life correlate to the development of provoked vestibulodynia PVD later in life.

The specific aims of the studies were:

3.1 STUDY I

- The primary aim was to evaluate reduction of pain during intercourse or tampon insertion in PVD-patients 6 months after two injections of 50 units botulinum toxin A compared with placebo.
- Secondary aims were evaluation of pain at weekly tampon insertion, reduction in pelvic floor pressure and evaluation of psychosexual health.

3.2 STUDY II

- To explore the association between birth-related events and the risk of developing PVD/vaginismus during adulthood.

3.3 STUDY III

- To evaluate a vaginal manometer for measurements of intra-vaginal pressure as a correlate of pelvic floor muscle function in women with PVD. The manometer was evaluted in PVD patients and two healthy control groups with varying pelvic floor morphology.
- Intra- and inter- rater reliability were also assessed.

3.4 STUDY IV

- The primary aim was to evaluate reduction of pain during intercourse or tampon insertion in PVD-patients 12 months after two injections of 50 units botulinum toxin A with added pelvic floor exercises compared with placebo.
- Psychosexual health was evaluated as a secondary outcome.

4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

Ethical permit: Dnr 2016/390-31 The study has been reviewed and ethically approved by the Regional Ethical Review Board in Stockholm.

Study I, III and IV will be considered as one here, since they are all part of the same RCT and therefore fall under the same ethical permit. The main focus of the RCT is to evaluate neurotoxin injections, as a treatment for women with PVD. The neurotoxin was injected in two muscles of the pelvic floor corresponding to the location of the vestibular pain. The aim of the treatments is to lower muscle tension in these muscles and thus decrease local pain. The study was performed in accordance to good clinical practice and followed the Helsinki declaration (101).

Botulinum toxin A is an already approved drug for several medical indications, but not, as of yet, for vulvodynia. Therefore, before treatment started a thorough inquiry about contraindication for BTA was performed and possible side effects were disclosed to the participants. In previous studies, using similar BTA doses as in our study, the risk for complications was shown to be very low. Most often, transient flu-like symptoms were reported in a minority of cases. During our trial, participants were regularly asked to report side effects throughout the study period with the option to report them via a weekly online log as well as directly to the investigators.

The treatment itself was perceived as painful as the injections were administered in the painful genital region. The injection site is difficult to anesthetize with topical anesthetics and injected anesthetics was not an option. To assure that the drug was injected into the correct muscles, we use EMG needles connected to a device registering electrical muscular activity.

The possible gain for the patients receiving active treatment is less dyspareunia, improved sexual function and quality of life. Those receiving placebo will probably not have the same gain, even though we believe a certain placebo effect will occur. An ethical concern is that the placebo group will be denied active treatment for 12 months (study period). All participants were therefore offer self-care instructions for PVD between month 6 – 12 of the trial. The placebo group were offered the BTA treatment after the completion of the study.

Under the course of the study the patients filled out in depth questionnaires about their sexual health and sexual lives, which was paramount for us to evaluate treatment effect. To ensure that integrity was not violated and to maintain anonymity, the participants were given a personal study identification number used for all personal responses. The questionnaires were filled in and stored safely online, and could only be accessed by the persons involved in the study. The access was granted through a personal login and the code for the study numbers were safely locked in and stored separately from all collected data in the research department. The answers of the questionnaires will only be published in an aggregated anonymized way to avoid identification of any study participants.

EPN number: 2017/299-31. The study has been reviewed and ethically approved by the Regional Ethical Review Board in Stockholm.

Study II bears the common ethical dilemmas for registry studies. The data is collected in an anonymous form from Statistics Sweden and The Swedish National Board of Health and Welfare. Information and results are presented in median values for an entire group and never on individual level. The data material is anonymized and accessed via the MONA system, an online service from Statistics Sweden for delivery of data. Eligible researchers acquire a personal login. This ensures that unauthorized persons do not get access to the data.

Research on register data can be defended by a positive risk-benefit relationship, as the benefit of increased knowledge for the population outweighs the potential harm for the individual.

4.2 STUDY SUBJECTS

4.2.1 Study I and IV.

For the two studies we assessed 124 women with PVD and out of those 88 were included in the study and randomized to injections with either botulinum toxin A (n=44) or placebo (n=44). The study was conducted between May 2016 and June 2018. Inclusion criteria were: nulliparous women age 18 – 40 years, pain during intercourse or tampon insertion reported as ≥ 60 mm on a visual analogue scale (VAS) 0-100 mm, where 0 represents no pain and 100 worse possible pain, with a minimum duration of ≥ 3 months, and heightened tonicity in the bulbocavernosus muscles on digital palpation. The diagnostic criteria for PVD are in accordance to International Society for the Study of Vulvovaginal Diseases (ISSVD) 2015 Consensus terminology of persistent vulvar pain (3).

Exclusion criteria were: local infection, dermatologic disease or other causes for coital pain, severe psychiatric or somatic disease, contraindication to botulinum toxin A (peripheral motor neuron disease such as myasthenia gravis, amyotrophic lateral sclerosis, Lambert-Eaton syndrome and diabetes), daily use of pain medication, pregnancy and urinary or flatulence incontinence.

4.2.2 Study II

Here we identified all women born in Sweden between 1973 and 2000 through the Medical birth registry, in total a number of 1 418 317 women. These individuals were matched to the Death Registry (1973-2016) and Longitudinal Integrated database for health insurance and labor market studies (LISA). Mothers of female offspring without a valid identification number were excluded (n=11643). Women dying younger than 15 years (n=10304) or not living until year 2001 (n=817) were also excluded. Moreover, women with missing information on certain key covariates (birth year, gestational age, birth weight, educational attainment, immigrant status) were excluded (n=36238). Using the National Patient Registry, women with and without diagnosed PVD/vaginismus during 2001-2016 were identified. A

number of 9237 women with diagnosed with PVD (n=6648) and vaginismus (n=3567) were identified.

4.2.3 Study III

In this study we included three groups of women. Women with PVD (n=60) that took part in the RCT on BTA. The inclusion and exclusion criteria for them was the same as in Study I and IV. The two other groups were nulliparous women (n=34) and primiparous women (n=34). Another 10 nulliparous women were also recruited to evaluate inter-rater reliability. Inclusion criteria for these two groups of women without any prior history of PVD were; 1) Nulliparous women, age 18-40. 2) Primiparous women age 18-40 with vaginal delivery without major obstetric tear (less than grade 3-4), 3 to 4 months prior to study inclusion. Exclusion criteria for both groups were: previous or ongoing history of vulvodynia or other chronic pain condition, vulvovaginal infections, pregnancy, urinary and/or flatulence incontinence, major psychiatric or medical disease.

4.3 SAMPLE SIZE CALCULATION

4.3.1 Study I and IV

For the BTA RCT the sample size was calculated to achieve a power of 0.80, two-sided test with $\alpha = 0.05$ to show a significant difference based on reduction of 20 units on the visual analogue scale (VAS) 0-100 with a mean coital pain measurement reduction of 76 to 56 and standard deviation of 31. Based on these values 38 participants are required in each group. To compensate for eventual withdrawals, we chose to include 44 women in each group.

4.3.2 Study II

For this study, it was not possible to calculate statistical power in advance which is determined by the characteristics of the analyzed data (effect size, variation, sample size). However, the study population consisting of all women born in Sweden 1973-2000 with and without diagnosed PVD during 2001-2016 is likely to render a large enough sample to be able to show sufficient power for the statistical analyses.

4.3.3 Study III

This study is regarded as a pilot study since no previous data on vaginal pressure measured by this device existed. However, our study design and method to measure vaginal pressure resembles a study by Naess et (102). We therefore included a similar number of participants in our control groups (n=34), but increased the number of participants with PVD (n=60).

4.4 METHOD

4.4.1 Study I and IV

The studies are a double-blind randomized placebo-controlled trial with 50 units of botulinum toxin A or placebo injected in the bulbocavernosus muscles, repeated twice with a three months' interval. Injections sites were in the lateral and medial portion of both sides of the bulbocavernosus muscle, part of the superficial PFM, and below the plane of the levator anii muscle, to achieve sufficient spread of the substance within the muscle.

Study I is the 6 months' follow-up of the BTA trial on PVD, whereas Study 4 is the 12 months' follow-up after the participants had been instructed to perform pelvic floor exercises during the 6 to 12 months' period. Study IV also expanded more on psychosexual evaluations.

4.4.2 VAS outcomes

Two different pain variables measured by VAS (0-100 mm) were used. The primary outcome for Study I and IV was reduction of self-reported dyspareunia or pain at tampon use during the previous month. Since a large proportion of PVD patients do not engage in vaginal intercourse, pain at tampon use was used as a proxy marker.

As a secondary outcome for Study I, all patients received standard tampons, and performed a weekly tampon insertion test, reporting the pain during the first six months of the trial. The results were reported in a weekly online log, aiming to detect and assess the onset and duration of a possible effect of the BTA-injections.

4.4.3 Questionnaires

As further secondary outcomes, two different validated questionnaires on sexual function were used. The Female Sexual Function Index (FSFI) assesses sexual function during the previous four weeks (103, 104) in women engaging in intercourse. The Female Sexual Distress Scale (FSDS) assesses sexually related personal distress in women during the previous 30 days (105). Both were used in Study I and IV.

At baseline, the patients were first screened for inclusion and exclusion criteria by filling out a questionnaire to map background data (ethnicity, education level, work status) and previous medical history of general and reproductive health.

In Study IV, the psychometric evaluation was expanded with two different questionnaires. One was the Perceived Stress Scale (PSS) which measures stress levels during the last month (106). The second one was the Spencer Children Anxiety Scale (SCAS) which was initially developed for children and adolescents (107) but has also been used on adults. It focuses on anxiety.

4.4.4 Intra-vaginal pressure

In addition, intra-vaginal pressure was measured as a secondary outcome, this as a proxy for PFM function. In study I and IV we performed the same measurements as in Study III. For more detail on the measurements, see method section on Study III. The measurements were performed before the intramuscular BTA injections, since a pain provocation could affect muscular tone and thus cloud the results.

4.4.5 Pelvic floor training

At six months from baseline all participants were introduced to a protocol of pelvic floor exercises, which they were instructed to perform three times a week until the end of the study period (Study IV). They were then categorized at the 12 months' follow-up according to the frequency of performed training sessions as high trainers (≥3 times per week), or low trainers (<3 times per week).

4.4.6 Statistics

Students t-test were used to analyze differences in means in the outcome variables.
For Study I, post-treatment period is defined as the average of all visits after Visit 1, i.e. Visits 2-5. Using a repeated measure design, we explored the impact of botulinum toxin A by comparing the botulinum toxin A (BTA) group to the placebo group across Visits 1-5. For this analysis we used ordinary least square regressions for continuous outcomes and logistic regressions for binary outcomes. For capturing the difference in differences in the outcome across each visit, we included interaction terms between each visit and the BTA group (108). The statistical analysis was conducted using STATA 15.1.

In Study IV we report means and standard deviations of all outcome variables at baseline, 6 months' follow-up and 12 months' follow-up. The panel structure of our data facilitated an analysis of differences in means between the BTA and placebo group across visits. We computed differences in means between BTA and placebo over time using ordinary least square regressions, in which we included interaction terms between each visit and the BTA group (108). Standard errors were clustered at the level of each participant to allow for serial correlation in the error terms across each individual (108). The statistical analysis was conducted using STATA 16.1.

4.5 STUDY II.

4.5.1 Data collection

Data from several different registries were accessed and combined for the period 1973-2016. This is possible in Sweden, since all residents have a unique personal identification number. All data was handled in an anonymous form on an aggregated level. The registries used in our study were from:

- National Board of Health and Welfare
    - National Patient Registry (NPR). Provides information on all in patient visits from 1987, and specialized outpatient clinics since 2001.
    - Medical Birth Registry. Contains data on conditions during pregnancy, delivery and portpartum for pregnancies beyond gestational week 22, availible since 1973.
    - Cause of Death Registry. To ensure that the study population is alive.
- Statistics Sweden
    - Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA). This registry holds information on labor market outcomes, highest educational attainment etc. The registry started in 1990.
    - Population registry – contains data on birth, death, citizenship, marital status and migration.
    - Multi-generation registry. Is used to identify the link between mothers and their chdilren.
- Swedish Neonatal Quality Registry (SNQ). This registry started in 2001 and contains information on perinatal diagnoses.

4.5.2 Classification and terminology

The NPR contains diagnoses set by a physician. For inpatient care, diagnoses are set according to the International Classification of Diseases Eighth Revision (ICD-8) through 1986, ICD-9 from 1987-1996, and ICD-10 from 1997 to the present. For outpatient care, diagnoses are set according to ICD-10 as of 2001. In Sweden, PVD and vaginismus are diagnosed according to the ICD-10 system where N76.3 includes provoked vestibulodynia defined as "*Subacute and chronic vulvitis"* and vaginismus is diagnosed with codes N94.2 or F52.5.

We defined pain exposure during infancy as having at least one medical condition that is associated with painful stimuli and/or procedures during delivery up until one year postnatally. Pain exposure due to medical conditions were coded according to the ICD-8-9-10 system in the Medical Birth Registry and according to the ICD-9 and -10 in the National (in-) Patient Registry. Exposure to pain due to medical procedures and treatments are coded according to the classification of care measure and classification of surgical procedures (KVÅ "*Klassifikation av vårdåtgärder*" and KKÅ "*Klassifikation av kirurgiska åtgärder*") for 1973-1996 and 1996-2005.

4.5.3 Statistics

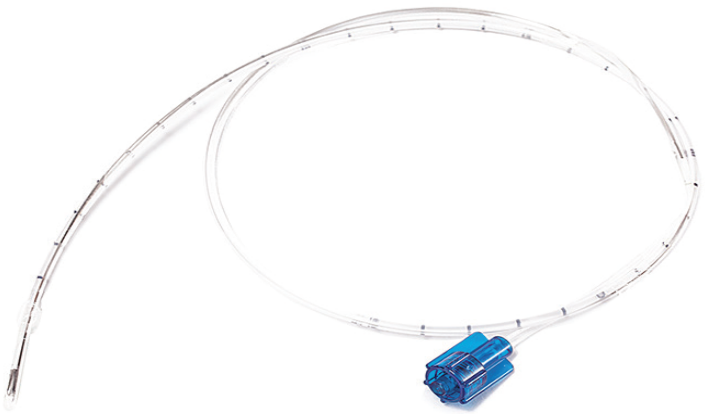
The difference between patient and maternal characteristics for women with and without PVD/vaginismus was examined using Kruskal-Wallis test. Furthermore, the link between patient and maternal characteristics and PVD/vaginismus was examined by Cramer's V coefficient. We adjusted for birth year, maternal age, highest educational attainment, and immigrant status of the mother. These covariates were chosen because their distribution differed among mothers of women with and without diagnosed PVD/vaginismus.

The probability of occurrence of events, i.e., pain exposure and health at birth, is modeled using an event probability regression, from which the odds ratio (OR) and adjusted OR of PVD/vaginismus is calculated. The same procedure is used when analyzing how pregnancy and delivery outcomes for mothers to female offspring with and without PVD/vaginismus are associated with diagnosed PVD/vaginismus in female offspring.

4.6 STUDY III

In this study the "T-DOC Air charged, Laborie Medical Technologies" catheter was evaluated and its properties of measuring intra-vaginal pressure was in focus. It was chosen since it can be placed intravaginally without eliciting pain in women with PVD.

Figure 1. T-DOC catheter used in Study I, III and IV.



The measurements were performed with the participants in a lithotomy position with the pressure inducer placed in the lower part of the vagina, and the measuring balloon of the catheter was placed at the plane of the bulbocavernosus muscles. A practice session was conducted with each participant where they were instructed how to contract the PFM with minimal additional contractions from the hip-, glutei- and abdominal muscles.

Intra-vaginal pressure was recorded during three different states of PFM activity:

1. Vaginal Resting Pressure VRP was recorded for 20 s. Measured in mmHg.
2. Maximum Contraction Pressure, MCP. Three short maximum contractions. Measured in mmHg.
3. Endurance Pressure, EP. A 10 s prolonged, maximum contraction. Measured in mmHg/s.

The measurements were performed twice with a few minutes' rest in between, and mean values of the two measurements were used for statistical analyses. Nulliparous women performed a repeated test two weeks later to further test the reproducibility of the results.

To measure inter-rater reliability, the two examiners consecutively performed the measurements in 10 healthy nulliparous women at the same visit. These 10 women were recruited for this purpose only, and not part of the first group of 34 nulliparous women.

4.6.1 Statistics

Statistical analysis was performed using SPSS (IBM) version 27. PFM variables are summarized as mean values with standard deviations (±SD). All data were continuous with skewed distributions according to normality tests. Differences in VRP, MCP and EP between the three groups were analyzed using Kruskal-Wallis test, including a post hoc test to define which of the PFM values that differed significantly from each other. Intra-rater reliability in each group was analyzed using the non-parametric Wilcoxon signed-rank test, where measure 1 was compared with measure 2 from visit 1. To further test intra-rater reliability, Wilcoxon signed-rank test was used to analyze the re-test the nulliparous women did two weeks later where the first measurement from visit 1 was compared with the first measurement from visit 2. p-values< 0.05 were considered significant.

In addition, both intra- and inter-rater reliability was assessed by calculating Intra Class Correlation (ICC) (109). For both the intra- and interrater reliability an ICC two-way mixed effect model checking for absolute agreement was calculated. ICC was graded as values < 0.5 poor reliability, between 0.5-0.75 moderate reliability, values between 0.75-0.9 good reliability and values > 0.9 excellent reliability (110).

5 RESULTS

5.1 STUDY I AND IV

Here are results from Study I and IV, which are presented together since they are part of the same RCT with six (Study I) and twelve (Study IV) months follow-up. Eighty-eight women were included and randomized, all of whom received the allocated treatment. An overview of the randomization and follow up is presented below (Fig. 2). The mean duration of PVD symptoms for both groups was 6 years. The groups were similar with regards to background characteristics and previous treatment. Few were lost to follow-up. An overview of patients' background characteristics is presented below (Table 1).

Figure 2. Flowchart Study I and IV. Six in the BTA group and seven patients in the placebo group were lost to follow-up.

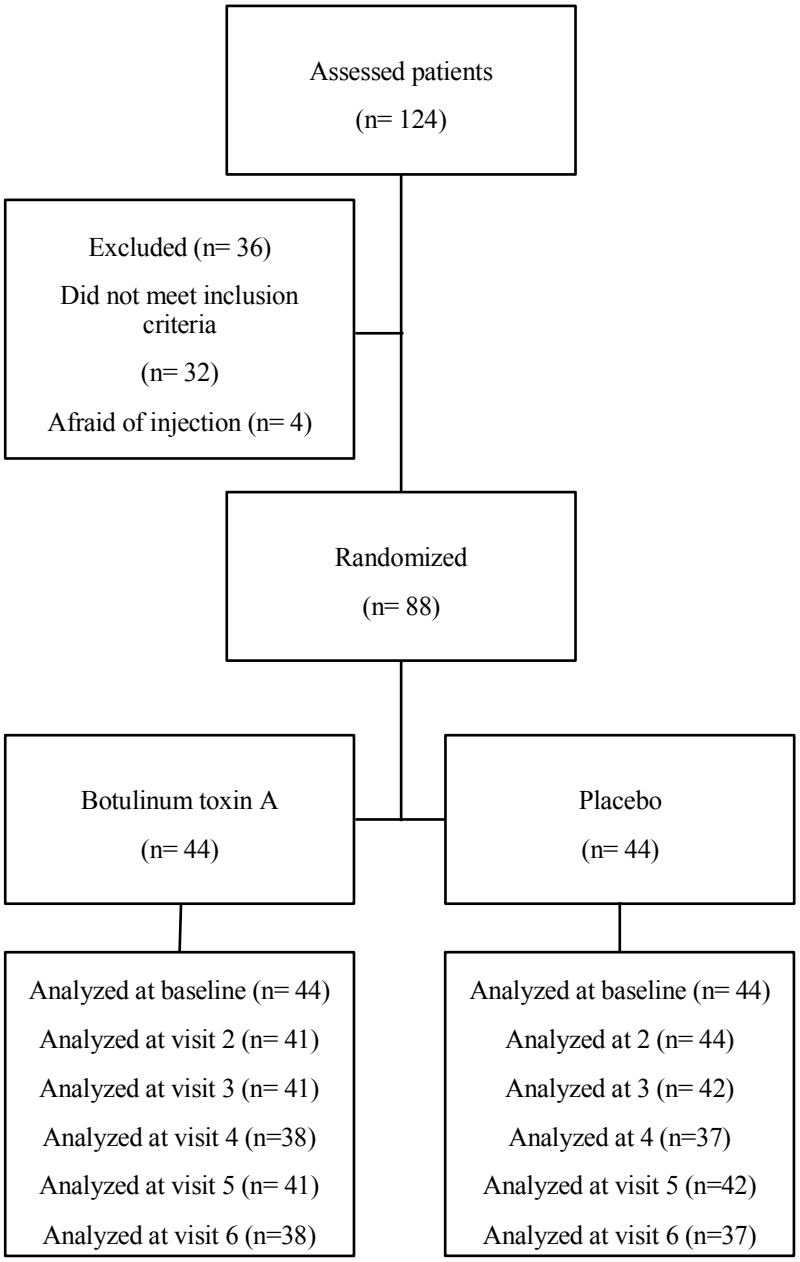


Table 1. Clinical background data for PVD patients in Study I and IV.

	Botox n=44	**Placebo** n=44
Age		
Age >25	21 (48)	20 (45)
Age 25-29	18 (41)	18 (41)
Age 30-34	2 (5)	6 (14)
Age >34	3 (7)	0 (0)
Socio-economic outcomes		
Study	12 (27)	8 (18)
Work	25 (57)	27 (61)
Work and study	5 (11)	9 (20)
Permanent partner	2 (5)	34 (77)
Immigrant	6 (14)	2 (5)
Other conditions		
Migraine	5 (11)	5 (11)
IBS	15 (34)	6 (14)
Back pain	20 (45)	25 (52)
Muscular pain	4 (9)	8 (18)
Mental disorder	8 (18)	10 (23)
Menstrual pain	31 (70)	30 (68)
Painful urination	6 (14)	7 (16)
History of infections		
Yeast infection	36 (82)	33 (75)
Herpes	2 (5)	5 (11)
Urinary Tract Infection	29 (66)	27 (61)
Bacterial Vaginosis	11 (25)	4 (9)
Primary or secondary provoked vestibulodynia *		
Primary	22 (50)	16 (36)
Secondary	24 (56)	27 (63)

*Primary provoked vestibulodynia = pain at first tampon insertion or intercourse attempt. Secondary provoked vestibulodynia = pain at tampon use or vaginal intercourse, with a prior history of a pain free period. Data are n (%).

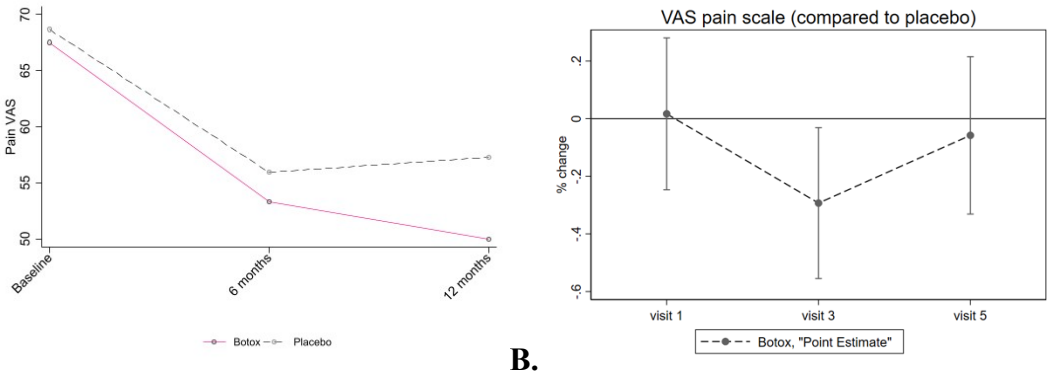
5.1.1 Primary outcome

For the primary outcome, there was no difference at baseline in VAS (0-100) for dyspareunia or pain at tampon use during the last month between the BTA (VAS mean 68±18) and the placebo group (VAS mean 67±25) (Fig. 2). In Study I at 3 months (visit 3), there was a significant difference between BTA (VAS mean 50±25) compared to placebo (VAS 62±24). At six months, there was a lower but non-significant pain rating in VAS by 7 units, 95% CI [-15.0, 0.4], in the BTA group compared with the placebo group (Fig 3b).

In Study IV, the primary outcome was evaluated up until 12 months after the first injection (visit 6). At the six month's follow-up, the participants were instructed how to perform weekly pelvic floor exercises during the following 6 months. At this point the groups reported

lower pain ratings with no statistically significant difference between the groups. However, the BTA group reported in total a mean reduction of 17.5 units on VAS and the placebo group 11.4 units, compared with their inclusion scores (Fig. 3a).

Figure 3. Primary outcome.



A. **B.**

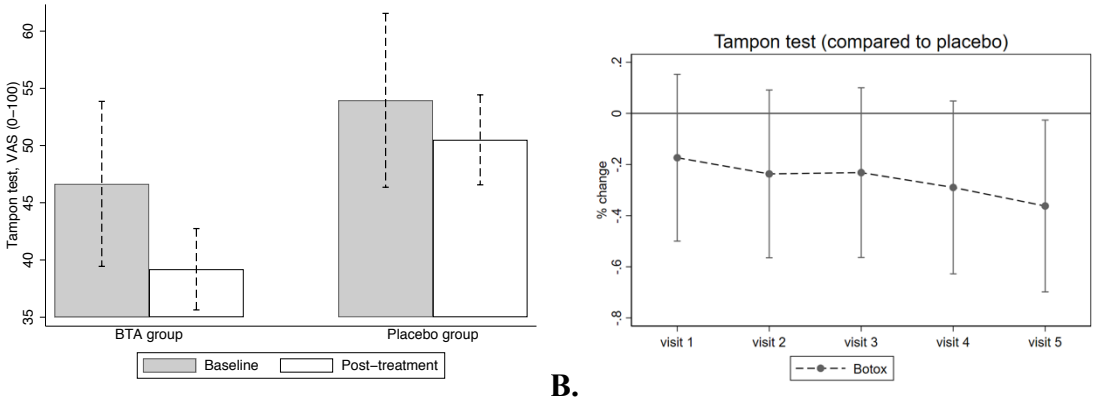
Fig. 3a. Overview of dyspareunia/pain at tampon use measured by VAS (0-100) at baseline, 6 and 12 months.

Fig. 3b. Percental change in VAS BTA compared to placebo, first 6 months. t-test with 95% CI.

5.1.2 Tampon test

In the weekly tampon test in Study I, which was only performed up until visit 5 (six months), pain rating at baseline was similar between the BTA (VAS mean 47±23) and placebo group (VAS mean 54±25). Between-group comparison post treatment showed a significant reduction of 11 VAS units lower in the BTA group compared with the placebo group, 95% CI [-16.59, -6.03], Fig 4a, Fig 4b.

Figure 4. Tampon Test.



A. **B.**

Fig. 4a. The bar charts show the means and CIs in pain rating, VAS (0-100), for the BTA and placebo group separately before (visit 1) and after treatment (visit 2-5).

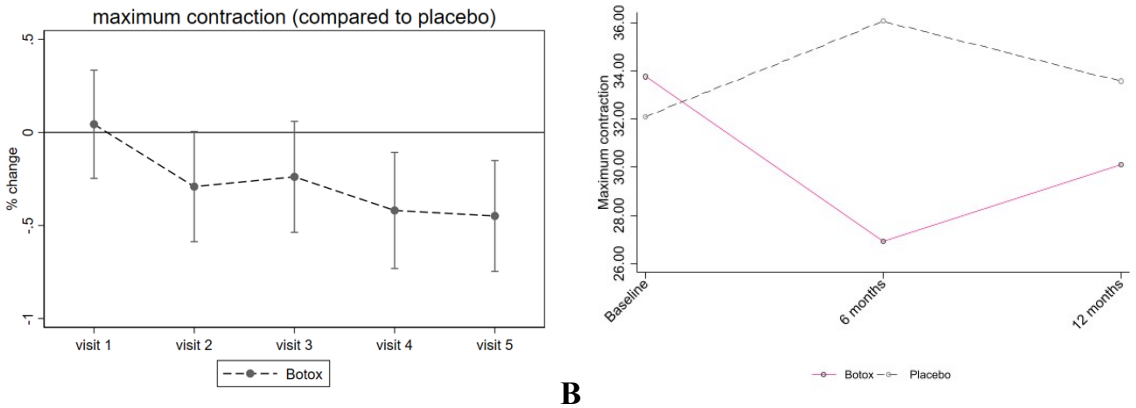
Fig. 4b. Tampon test visit 1-5. Percental change in VAS with 95% CI for the BTA and placebo group.

5.1.3 Vaginal pressure

During the course of Study I and IV, all pressure variables exhibit changes between the groups. At baseline VRP, differed significantly between the BTA group (mean 20 mmHg, ± 7) compared with the placebo group (mean 24 mmHg ±10), and should therefore be interpreted with caution. Both group exhibited slightly lower results a 6 months, BTA (mean 17.4mmHg ±5.6) and placebo (mean 23.0±8.9).

Neither the maximum contraction pressure (Fig. 5a & 5b) nor endurance pressure (Fig. 6a & 6b) showed any differences between the groups at baseline (Study I and IV). Both pressures were lower for the BTA group compared with placebo at 6 months (Study 1). At the end of Study IV all pressures were restored to baseline levels.

Figure 5. Maximum Contraction Pressure.

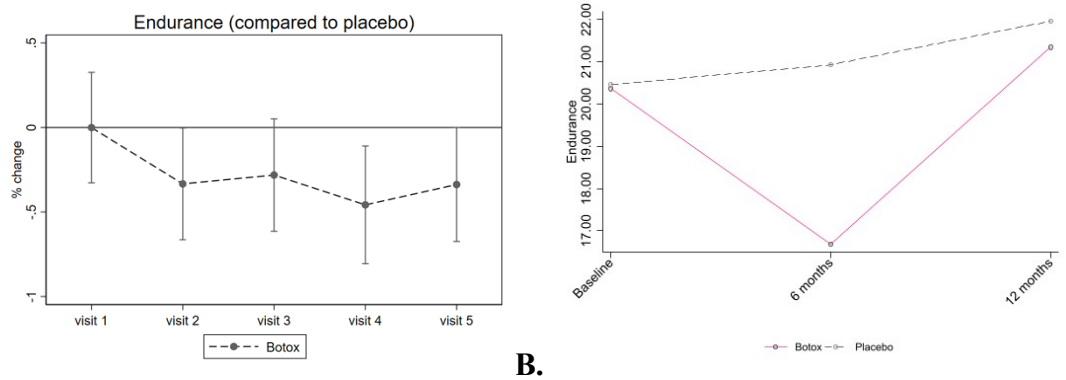


A **B**

Fig. 5a. Percental change in MCP with for the BTA and placebo group during the first six months. The between group comparison post-treatment showed a reduction of 7 mmHg in the BTA group compared with placebo 95% CI [-12.70, -2.38] (Study I).

Fig. 5b. Mean Maximum Contraction Pressure in mmHg at baseline, 6- and 12-months' follow-ups (Study IV).

Figure 6. Endurance Pressure.



A. **B.**

Fig. 6a. Percental change in EP for the BTA group compared to placebo. The EP variable also showed a significant decrease by 4 mmHg/10 s, 95% CI [-7.72, -1.16], for the BTA group compared to placebo (Study I).

Fig. 6b. Mean Endurance Pressure in mmHg at baseline, 6- and 12-months' follow-ups.

5.1.4 Psychosexual evaluation

5.1.4.1 FSFI

For all participants attempting intercourse (n=34 at baseline), the FSFI score at baseline for both the BTA group (mean 20±4) and the placebo group (mean 18±3) suggests sexual dysfunction in both groups. At the six months' follow-up there was no significant change in the scores between the groups (Study I, Fig 7).

At the twelve month's follow-up (Study IV) both groups had improved their scores somewhat, the BTA group to a mean of 23 (±5) and the placebo group to a mean of 20± (5.0), p=.048, but both groups were still displaying sexual dysfunction on group level (Fig. 7). Here we also performed an additional analysis including also those not engaging in intercourse which showed a similar result.

Figure 7. FSFI.

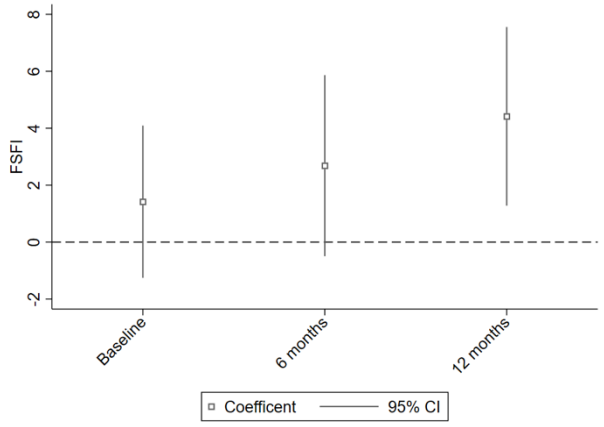


Fig. 7. FSFI scores for those attempting intercourse. BTA compared with placebo, t-test with 95% CI.

5.1.4.2 FSDS

At baseline, there was no significant difference in FSDS scores between the BTA group (mean 33±11) and the placebo group (mean 30±9). At the post-treatment follow-up at six and twelve months, there was no significant change in the scores between the groups. The pre- and post-treatment scores were all within the range of sexual distress, i.e. ≥ 15.

5.1.4.3 Intercourse attempts

The FSFI contains information on intercourse activity and at baseline there was no difference in that variable between the groups. In the BTA group, 43% were engaging in intercourse activity at baseline, as compared with 34% in the placebo group. Between-group comparison at visit 5 (Study I), showed a significantly higher increase in intercourse activity in the BTA group by 27% compared with the placebo group. That result still remained at the 12 months' follow-up, where 74% in the BTA group attempted intercourse compared to 43% in the placebo group, p=0.005 (Fig 8).

Figure 8. Intercourse Attempts.

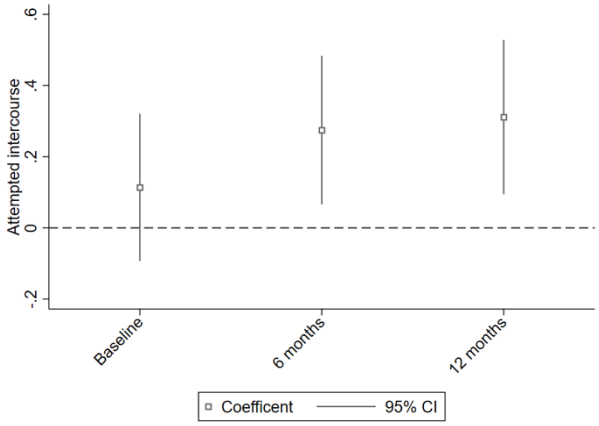


Fig. 8 . Attempted Intercourse, t-test with 95% CI. BTA compared with placebo.

5.1.4.4 Stress and anxiety

In Study IV PSS and SCAS was also evaluated. None of these psychometric scales differed between the groups at baseline or at any of the study visits.

5.1.4.5 Pelvic floor training.

Few women adhered to the pelvic floor training program during Study IV. Only 26/78 were classified as high trainers, 16 in the BTA group and 10 in the placebo group. Therefore, further analyzes of this variable was not possible.

5.1.4.6 Adverse Events

No severe side-effects were reported and there were no differences in side-effects between the BTA and placebo groups. The most common side-effect in both groups was rapidly resolving pain at the injection site.

5.2 STUDY II

5.2.1 Patient and maternal characteristics

The majority of women diagnosed with PVD/vaginismus received their diagnosis between ages 20-30 (Fig. 9). They were older and more educated compared to the non-PVD women.

Figure 9. Age distribution PVD.

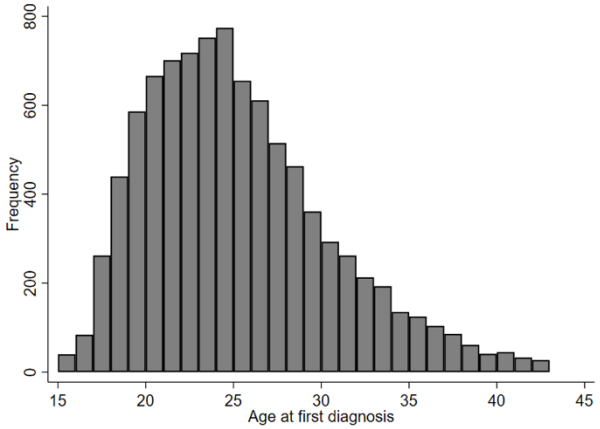


Fig. 9. Age at first PVD/vaginismus diagnosis. Frequency of age at first PVD/vaginismus diagnosis identified using the National Patient Registry (outpatient visits) during 2001–2016.

Mothers who had given birth to female offspring with PVD/vaginismus had a more advanced maternal age, higher educational attainment, and were less likely to be born outside of Sweden (Table 2). However, the Cramer's V coefficient showed a low correlation between maternal characteristics and PVD/vaginismus.

Table 2. Patient and Maternal Characteristics

	PVD/vaginismus *N=9247*		**Women without PVD/ vaginismus** *N=1 350 068*		**P-values from Kruskal-Wallis test**	**Cramer's V**
	No. of women	**%**	**No. of women**	**%**		
Patient Characteristics						
Age						
15-20	154	1.67	208118	15.42	***	-0.031
20-24	1259	13.62	258767	19.17	***	-0.012
25-29	2532	27.38	270156	20.01	***	0.015
30-34	2371	25.64	219233	16.24	***	0.021
35-39	1991	21.53	214005	15.85	***	0.013
>40	940	10.17	179789	13.32	***	-0.008
Education						
No schooling	1	0.01	1546	0.14		-0.003
Elementary	376	4.14	76952	6.87	***	-0.010
High school	3316	36.55	539546	48.15	***	-0.021
University	5380	59.30	502395	44.84	***	0.026

Maternal characteristics of female offspring with and without PVD/vaginismus.						
Age						
<25	2208	23.88	365152	27.05	***	-0.006
25-29	3575	38.66	504574	37.37	*	0.002
30-34	2433	26.31	343360	25.43		0.002
35-39	889	9.61	118283	8.76		0.002
>40	142	1.54	18699	1.39		0.001
Education						
No schooling	281	3.04	53897	3.99		-0.004
Elementary	807	8.73	134095	9.93	*	-0.003
High school	4400	47.58	668204	49.49	**	-0.003
University	3759	40.65	493872	36.58	***	0.007
Born in Sweden	8391	90.74	1180551	87.44	***	0.008

Notes: Differences in characteristics are calculated using Kruskal-Wallis test, statistically significant differences are indicated by p-values: * p<0.05; ** p<0.01; *** p<0.001

5.2.2 Health during infancy

Offspring born prematurely had an elevated risk of developing PVD/vaginismus later in life. The risk increase was 1.15 for preterm delivery <37 w, to a 1.18-fold increase for extremely preterm births before week 32 (Table 3). The preterm before w 32 were, however, very rare events with wider confidence intervals. A similar pattern was seen for women born with low birth weight compared with women with normal birth weight. Having a birth weight <2,500 g was associated with a 1.24-fold higher risk of developing PVD/vaginismus later in life, and a birth weight <1,500g was associated with a 1.41-fold risk. Furthermore, we observed an increased risk among SGA babies after multivariate adjustment (aOR: 1.20, 95% CI:1.08-1-34).

APGAR scores <7 and pain exposure during infancy were not associated with developing PVD/vaginismus.

Table 3. Birth and neonatal outcomes in women with and without PVD/vaginismus

	PVD/Vaginismus		**Women without PVD/ vaginismus**							
	N	***%***	***N***	***%***	***OR***	***CI***		***Adj. OR***	***CI***	
Preterm, 36-32 weeks	482	5,21%	62269	4,61%	1,15	(1,05-1,26)	**	1,15	(1,05-1,26)	**
Preterm, <32 weeks	58	0,63%	7747	0,57%	1,20	(0,93-1,56)		1,18	(0,91-1,53)	
Birthweight, 2500-1500 g	421	4,55%	50622	3,75%	1,22	(1,10-1,34)	***	1,24	(1,12-1,36)	***
Birthweight, <1500 g	62	0,67%	6811	0,50%	1,45	(1,13-1,86)	**	1,41	(1,10-1,82)	**
Small for gestational age	333	3,68%	40283	3,07%	1,13	(1,01-1,26)	*	1,20	(1,08-1,34)	**
APGAR at 5 minutes <7	70	0,83%	11244	0,90%	0,92	(0,72-1,16)		0,95	(0,75-1,20)	
Pain exposure	1200	12,98%	190186	14,09%	0,95	(0,89-1,00)		0,98	(0,92-1,04)	

Notes: Adjusted for birth year, maternal age, maternal immigrant status and highest maternal educational attainment. For all outcomes total N= 1359315 except for APGAR<7 (total N= 1254833) and SGA (total N= 1321744).

5.2.3 Pregnancy related complications

Gestational diabetes, hypertonia and preeclampsia in mothers with female offspring was not associated with PVD/vaginismus. Offspring delivered by cesarean section had an increased risk of developing PVD/vaginismus (aOR=1.12, 95% CI: 1.05-1.19).

5.3 STUDY III

The average age for patients with PVD was 25±4 years, for nulliparous women 25±3 years, whereas the primiparous were older with a mean age of 32±4 years ($p< 0.001$). An overview of the study participants can be seen in figure 10. The PVD patients reported a mean pain rating during intercourse or tampon insertion of VAS 69±21 at inclusion, and the mean duration of symptoms of PVD was 6±4 years.

Figure 10. Flow chart Study III.

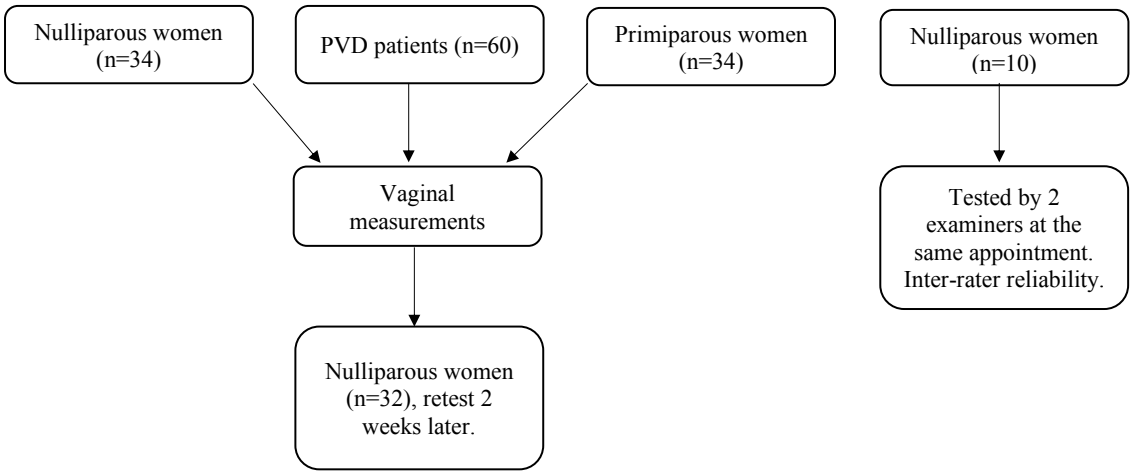


Fig. 10. Flow chart over the study participants. After inclusion in the study, the same measurements were conducted in all participants. A second group of nulliparous women (n=10) were recruited to test inter-rater reliability.

The pressure measurements show the same order in all parameters, where nulliparous women exhibit the highest pressure and primiparous women the lowest, whilst the PVD patients are somewhere in between. An overview of the vaginal pressure measurements is presented in Table 4.

Table 4. Vaginal pressure measurements

Vaginal pressure variables	1. PVD (n = 60)	2. Nulliparous women (n = 34)	3. Primiparous women (n = 34)	Comparison between groups, *p*-value
VRP (mmHg)	21.6 (± 7.8)	24.5 (± 9.8)	19.1 (± 4.2)	**.040** .094 (1 vs 2) .271 (1 vs 3) **.011 (2 vs 3)**
MCP (mmHg)	34.8 (± 21.2)	35.9 (± 19.1)	23.8 (± 13.8)	**.010** .694 (1 vs 2) **.009 (1 vs 3)** **.006 (2 vs 3)**
EP (mmHg/s)	217.1 (± 151.7)	267.6 (± 149.4)	145.7 (± 102.8)	**< .001** .060 (1 vs 2) **.007 (1 vs 3)** **<.001 (2 vs 3)**

Differences between groups were analyzed with Kruskal-Wallis test. Results are presented as means with standard deviations within parenthesis. Significant differences are presented in bold. PVD=provoked vestibulodynia. VRP=vaginal resting pressure, MCP=maximum contraction pressure, EP=endurance pressure.

5.3.1 Vaginal resting pressure

The nulliparous women had a 5.4 mmHg higher VRP compared with the primiparous women, (p=.011). No other differences were observed between the groups.

5.3.2 Maximum contraction pressure

PVD patients and nulliparous women had a 11.0 mmHg (p=.009) and 12.1 mmHg (p=.006) higher MCP respectively compared with the primiparous group. The small difference between PVD patients and nulliparous women was not significant.

5.3.3 Endurance pressure

The most pronounced pressure difference was seen regarding the pressure during a prolonged 10s contraction (EP). Here the nulliparous women had the highest pressure/s followed by the PVD patients whereas the primiparous women had a markedly lower pressure/s (121.9 mmHg/s lower than nulliparous (p<.001) and 71.4 mmHg/s lower than PVD (p=.007)). No difference between the PVD patients and nulliparous women were found.

5.3.4 Intra-rater reliability

The intra-rater reliability was tested by comparing measure 1 and 2 from the first visit for all three pressure variables within all three groups. There was a difference in VRP between the two measurements, where the second measurement yielded a higher pressure in all groups, see Table 2. For the MCP, the second measurement showed a 4.0 mmHg (p=.042) higher pressure in the PVD group and a 4.1 mmHg (p=.041) higher pressure in the nulliparous

group. No differences between measure 1 and 2 were found for the endurance variable in any of the groups, Table 5.

Table 5. Intra-rater reliability all groups

Vaginal pressure variables	PVD (n=60)	Nulliparous women (n=34)	Primiparous women (n=34)
VRP (mmHg)	Measure 1: 21.4 (±11.2) Measure 2: 21.7 (±7.6) ***p*=.010**	Measure 1: 22.5 (±9.3) Measure 2: 26.4 (±12.3) ***p*=.003**	Measure 1: 17.7 (±5.3) Measure 2: 20.6 (±3.9) ***p*=.001**
ICC VRP (CI)	.444 (.063-.666)	.727 (.451-.864)	.686 (.213-.860)
MCP (mmHg)	Measure 1: 33.0 (±21.6) Measure 2: 37.0 (±22.4) ***p*=.042**	Measure 1: 34.1 (±18.6) Measure 2: 38.2 (± 21.0) ***p*=.041**	Measure 1: 23.1 (±13.8) Measure 2: 24.6 (±15.5) *p*=.567
ICC MCP (CI)	.798 (.666-.881)	.927 (.841-.965)	.893 (.787-.946)
EP (mmHg/s)	Measure 1: 216.7 (±178.4) Measure 2: 217.6 (±149.2) *p*=.127	Measure 1: 274.0 (±169.7) Measure 2: 261.2(±143.6) *p*=.343	Measure 1: 146.0 (±106.4) Measure 2: 145.5 (±111.3) *p*=.761
ICC EP (CI)	.828 (.706-.896)	.894 (.788-.947)	.881 (.761-.941)

Comparison of measure 1 and 2 from the first visit, was analyzed using Wilcoxon signed-rank test and ICC. Values are presented as means with standard deviation in parenthesis. Significant differences are presented in bold. ICC= Intra Class Correlation values are presented with 95% CI within parenthesis. PFM=pelvic floor muscles. PVD=provoked vestibulodynia. VRP=Vaginal resting pressure, MCP= maximum contraction pressure, EP=endurance pressure.

To further test the reproducibility of the results, the nulliparous women were retested after two weeks. Results of VRP, MCP and EP from the first examination were compared with results from the second examination. No significant differences were found for any variable between the two occasions when tested with Wilcoxon signed rank test. ICC was also calculated to test intra-rater reliability. All three variables were examined between the groups. The VRP variable shows a moderate correlation (0.728), whilst both the MCP (0.882) and endurance (0.812) show a good correlation between the examinations.

5.3.5 Inter-rater reliability

To test the inter-rater reliability, an additional 10 healthy nulliparous women were tested consecutively by the two examiners at a single appointment. For the VRP and MCP variables, ICC shows an excellent correlation between the examiners, VRP (0.919), MCP (0.973) and for EP a moderate correlation (0.610).

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Three different study designs were part of this thesis and will be discussed below.

6.1.1 Randomized Controlled Trial

RCT is a type of experimental study where an exposure is studied in a defined population and specific outcomes are evaluated. It is used to assess the causal effect of a medical intervention, and is regarded the hallmark of evidence-based medicine. Certain criteria are set up for the study population, i.e. the inclusion and exclusion criteria. The exposure is predefined and the individuals are randomized to two or more groups. Classic randomization groups are active treatment or placebo. The randomization aims to minimize confounding, by making the two groups as homogenous as possible. There are some concerns with RCTs, since they usually are time consuming, resource demanding and can be affected by compliance problem especially with a long-follow up period.

Study I and IV used an RCT design to study the effect of BTA injections effect compared with placebo (exposure) on PVD. The study was performed double-blinded i.e., both participants and caretakers were blinded to the allocated treatment during the study period. This minimizes the risk of influencing the results.

Cochrane has described bias in randomized trials using the following terms which will be discussed below (111).

6.1.1.1 Selection bias

Selection bias refers to systematic differences in the baseline characteristics of the study groups.

In Study I and IV, the participants are randomized to active treatment or placebo, thereby minimizing this risk. Between the BTA and placebo group there were no differences in baseline characteristics, which confirms a successful randomization.

6.1.1.2 Performance bias

Performance bias is when the care provided to trial participants differs systematically between the experimental and control groups. In Study I and IV, the groups and caretakers were blinded to treatment allocation, which minimizes this risk. Since this is a superiority trial, intention-to-treat was analyzed, in order to further avoid bias.

6.1.1.3 Detection bias

Detection bias is a systematically different outcome assessment between the study groups.

In Study I and IV, the data was prospectively collected during the study period, and both the study participants and care takers were blinded to the treatment. This reduces the risk of this misclassification.

6.1.1.4 Attrition bias

Attrition bias is the systematic difference between groups in withdrawal from a study, i.e. a differential loss to follow up or other forms of exclusions from the analysis. In the RCT, six women were lost to follow up in the BTA group at twelve months and seven in the placebo group. This is considered few losses with no difference between the groups.

6.1.2 Cohort Studies

Cohort studies are one of the most common methods in epidemiological research. The association between an exposure and certain outcomes can be studied in observational cohort studies. This is an epidemiological analysis of how risk factors affect the incidence of disease. It is a type of longitudinal study where the subjects are followed over time and are grouped together depending on exposure, and are compared with the event of interest (outcome). In Study II, we used prospectively collected data for a retrospective cohort study. We combined several nationwide registries (defined above) to study the association between neonatal events (exposure) and PVD/vaginismus (outcome) later in life. Since the quality of registries used is generally high and covers the entire population a disease such as PVD can be studied.

The major limitation of a retrospective cohort study is the risk of bias, since the data collection is primarily not done with the specific study in mind. Data on exposures, outcomes and potential confounders are restricted to the collected data. This increases the risk of bias. Using a prospectively cohort design could be an option, but is very time consuming and expensive since the time from exposure to event could be very long.

Systematic errors are faults in the study not introduced by chance but by inaccuracy, i.e. they are non-random. Systematic error is also termed bias and can occur with the incorrect selection of the study population (selection bias), error in the exposure measurements (misclassification/information bias) and a distortion of exposure (confounding). Systematic errors are not affected by the size of the study. The terminology used for bias in cohort studies differs somewhat from randomized trial and will be discussed below.

6.1.2.1 Confounding

A confounder is a variable that can influence both the outcome and exposure of interest and lead to misinterpretation of the results. It is therefore important to identify potential confounders and adjust for them in the analysis. In the design of the study, the effect of confounding can be minimized in three ways: by randomization, restriction and/or matching.

In Study II we adjusted the sample for birth year, maternal age, highest educational attainment and immigrant status of the mother. These variables were selected due to the fact

that their distribution differed in mothers of women with and without diagnosed PVD/vaginismus.

6.1.2.2 Measurement bias

Measurement bias can either be non-differential (random) and thus lead to an underestimation of effect, or differential. A differential bias can lead to both under- and over- estimation of the effect studied.

As the data in Study II the data was also prospectively collected, the risk of recall bias or differential record of information is reduced. However, the main outcome of interest, PVD/vaginismus, was only captured through the NPR which contains diagnoses set in specialized care. It could lead to an underestimation of the effect, since those diagnosed and treated only at youth clinics only are not included in our sample. As always when investigating a diagnosis, all undiagnosed cases are not captured. This is known to be the case for many vulvodynia patients, since a large portion of them do not seek medical care for these kinds of problems. Patients with higher socioeconomic status are also more prone to seek specialized care and could therefore be overrepresented in our sample (112). Women who experienced medical issues as a child, might also be more likely to seek medical care as an adult. This fact might also explain the finding in our study that mothers of female offspring with PVD/vaginismus are older, more educated and less likely to be an immigrant.

6.1.2.3 Selection bias

In a retrospective cohort study selection bias can occur if the subjects are somehow related to the outcome of interest. Study II is a population-based cohort study, where the subjects were identified through the MBR. The MBR has an almost completed coverage of births in Sweden, so the risk of selection bias is low. The sample was restricted by excluding women with missing information on key covariates (birth year, gestational age, birth weight, educational attainment and immigrant status). If the excluded subjects are different from the included ones, that could lead to selection bias. The number excluded (n=36 238) was however small compared to the overall final sample (n=1,359,315). Further restriction was excluding women dying before the age of 15. Mothers of female offspring without a valid identification number were excluded as well.

6.1.3 Internal validity

Internal validity is the extent to which a study is free from bias and how the results among the study population can be applied to similar individuals outside the study. It can however, be affected by systematic error, which in clinical trials are selection, performance, detection and attrition bias. In a cohort study, as described above, selection bias, measurement bias and confounding influence the internal validity. In the setup of a study, it is therefore important to keep these concepts in mind, in order to avoid them as much as possible. In observational studies, such as the cohort study, these factors are always present to some extent which makes inference about causality impossible to do.

The internal validity of study I and IV is deemed high since we have a selected population, the exposure is assigned before outcome is assessed.

For Study II the internal validity is inherently low, since the observed population is diverse and the exposure is ascertained before the outcome.

6.1.4 External Validity

External validity is the generalizability of the results. One prerequisite for high external validity is also a high internal validity.

In Study I and IV the inclusion and exclusion criteria were based on ISSVD international consensus guidelines for PVD (3). The age inclusion criterion was set to 18-40 years, which is the age when most common age at which PVD is diagnosed. Heightened tonicity of the PFM palpation was also an inclusion criterion, but is unfortunately a subjective evaluation method, and could potentially exclude patients and thus cloud the generalizability. Nulliparity was also an inclusion criterion to keep the group homogenous and minimize the risk of other concomitant birth related pelvic pain conditions. Other exclusion criteria such as infection, dermatologic disease, contraindications to BTA etc., were conditions were BTA would never have been used.

Study II were population based and nation-wide, which strengthens its external validity. There are however some drawbacks, since there is some concern with information bias. A cohort study of this type, also have some residual confounding.

6.1.5 Random Error

Random errors are related to variations due to chance and can affect the reliability of the results. These cannot be eliminated but can be expressed quantitatively using p-values and confidence intervals. Traditionally in medical research p-values are set to < 0.05, which was used in Study I, III and IV. A confidence interval of 95% is commonly used, and was used in Study 2.

6.2 METHOD EVALUATION

In Study III we evaluated a vaginal manometer for the use in patients with PVD and compared the result with two healthy control groups: nulliparous and primiparous women. The aim was to analyze if reliable measurements of intra-vaginal pressure corresponding to pelvic floor muscle strength and endurance were achieved, including analyzes of the manometer's intra- and interrater reliability.

6.2.1 Evaluated Groups

Two different control groups were evaluated, along with the PVD group. They were chosen because of know differences in pelvic floor morphology, a difference that was theorized to be detectable with this method.

6.2.2 Other Methods

There are several other methods to assess the pelvic floor function in PVD women. However, several of them include comparatively large intravaginal devices, which in this patient group is unfitting, since it can provoke pain and thus cloud the results. Trans perineal ultrasound could have been an option for this group, since it is pain free however this method was not available at our department at the time of the study.

6.2.3 Parameters evaluated

Three different parameters were evaluated: vaginal resting pressure, maximum contraction pressure and endurance pressure. These parameters were chosen in partly since they represent different states of pelvic floor activity. A second reason was that we wanted to assess the same parameters as were evaluated by Næss et al, with a method similar to ours (102). Unfortunately, that specific method is not commercially available.

In order to ensure a correct evaluation of the parameters, the assessors performed a few test on patients together prior to study start. This was done in order to agree on position of patient, the exact position of the manometer and how to instruct the person to perform the different contractions during the pressure measurements.

One thing we noted during the course of the study was that some patients, had difficulties to find out how to correctly contract their muscles

The manometer was held in place by the assessor, aided by the markings of the catheter. The measuring cuff is small, only a few mm long and wide, which served the purpose of not provoking any pain in the PVD patients. However, the manometer was sensitive to movements so a small shift in place could alter the measurement. We therefore conclude that manometer with a longer cuff would be better suited for this type of evaluation.

The pressure measurements all showed the same order of the groups in the results. The highest resting and contraction pressures were seen in the nulliparous group, the lowest pressures in the nulliparous group with PVD in between. Our hypothesis was that the PVD group would elicit a significantly higher VRP than both control groups, since on clinical examination they had the highest muscle tonus. This was also the result in the study by Næss et al (102). The conflicting result might be a result of the pressure cuff we used being too small to detect small differences at this level.

6.2.4 Test-retest reliability

Intra-rater reliability is the test-retest consistency of measurements by a single rater. Here the ICC was moderate in the VRP variable and good to excellent in the others. Inter-rater reliability is the extent to which two or more raters agree. The ICC showed a moderate to excellent reliability in all the examined variables.

6.3 FINDINGS AND IMPLICATIONS

6.3.1 Botulinum toxin A effect on Provoked Vestibulodynia.

6.3.1.1 Pain evaluation

The primary outcome for both Study I and IV was the reduction of dyspareunia or pain at tampon use after BTA injections. In Study I it was evaluated up until six months and in Study IV until 12 months. In neither of these studies could we see that BTA was superior to placebo with regards to the primary outcome. The tendency was lower VAS scores for the BTA group over time compared with placebo, but the difference did not reach statistical significance.

In our secondary outcome in Study I, the participants performed a weekly tampon test, and here the groups differed, with a favorable pain reduction in the BTA group.

6.3.1.2 BTA as a drug

The profile of BTA is a transient paretic effect on muscles, and it might dampen neurogenic inflammation. These effects are theoretically very suitable to treat PVD. The effect on inflammation is still not clear, but future data might provide insight into this mechanism. We chose to inject the bulbocavernosus muscles because they are easily accessible, and have been injected before with few reported side effects. The area injected, however, is comparatively small, and more muscles injected might have yielded more promising results. Clinically the patients are usually also tense in a larger part of the pelvic floor than just the bulbocavernosus muscle.

At the time of planning the studies, few previous studies on BTA and PVD were published. In a study by Damsted's, they gave a single injection of 20 U of BTA (89). They did not see any difference in pain reduction between the intervention and the control group. We therefore chose to increase the dose to 50 U, repeated twice. More data has arrived since the start of our RCT, and even doses up to 100 U have been used in open label studies, without severe side effects and reduction in pain. As a next step, 100 U compared to placebo would be interesting to evaluate in a future trial.

If a larger part of the pelvic floor is to be injected with BTA as a treatment, i.e. increasing the number of injections, the question of anesthesia becomes relevant. In our study we performed 4 injections at on treatment, which is painful for the patient but tolerable. Much more than that might require sedation in order to be accepted.

6.3.1.3 Muscular effect

One cornerstone in the treatment of PVD, is to reduce the hypertonicity in the pelvic floor, often present in these patients. Physiotherapy is the first line treatment to restore pelvic floor function, but is not always sufficient. BTAs transient paretic effect is therefore alluring as a treatment for these patients, in an attempt to achieve pain reduction secondary to the diminished hypertonicity. Previous studies have primarily evaluated the pain component of PVD and discussion on reducing muscle spasms has been scarce.

In an attempt to analyze BTAs muscle effect, we measured intravaginal pressure as a proxy for muscular activity. At baseline the VRP differed between the two groups and should therefore be interpreted with caution. The other two variables, MCP and EP, were equal between the groups at baseline, and here the groups differed at 6 months' post-treatment (Study 1). Both MCP and EP were lower for the BTA group compared with the placebo group. This is in line with the theorized result, confirming a clear paretic effect. At the final follow-up at 12 months all remnants of BTA should have dissipated, and therefore no longer detectable. Concordant with this all pressure variables were restored back to baseline levels at the 12 months' follow-up, (Study IV).

Since physiotherapy is the first line treatment for pelvic floor dysfunction of this kind, all participants were introduced to and instructed to perform pelvic floor exercises between 6 and 12 months. This was also thought to add to the effect of BTA, and thus prolong the hopefully positive effect for these patients. However, few patients adhered to the protocol and analysis of the intervention is therefore hard to do. If more patients had completed the training as instructed it might have resulted in remaining lower vaginal pressures in the BTA group.

In hindsight, a closer follow-up of the training would have been preferable, as there were no appointments between 6 and 12 months. Optimally, the instructions and follow-ups should have been given by a physiotherapist or midwife experienced in handling these patients, in a similar way as in clinical practice.

6.3.1.4 Psychometric evaluation

As further secondary outcomes we evaluated several psychometric parameters. The FSFI and FSDS were used in both Study I and IV. Both these questionnaires are widely used in the entire field of sexual health research. At baseline both groups displayed sexual dysfunction according to the FSFI (Study I and IV), with no difference between the groups. At the 6 months' follow-up there was still no difference between the groups (Study I), but at 12 months' the BTA groups result differed favorably compared with placebo (Study IV). The difference was however small, and the clinical effect could be questioned.

One of the parameters in the FSFI is the evaluation of frequency of intercourse attempts. At baseline there was no difference between the groups (Study I and IV), but at 6 months there was a 33% increase for the BTA group compared to baseline whilst the placebo group did not increase their number of attempts (Study I). This result remained at the 12 months' follow-up (Study IV).

In Study IV, we expanded the psychometric evaluation, with two additional questionnaires that focused on stress and anxiety. Both groups were similar at baseline and the scores did not improve for any of the groups during the follow-up period. Since most other outcomes did not improve, or showed a rather slight clinical improvement, we did not expect this to differ either. The study also did not include any specific focus or therapy addressing stress and anxiety.

6.3.1.5 Clinical implication

The optimal dose and injection site is still to be determined for BTA treatment. It is also important to select the right patients. PVD has several clinical presentations; some women have a pronounced mucosal involvement with severe pain sensitivity. Others have a more defined muscular involvement. Given the nature of the drug those with a more problematic muscular involvement part are the ones likely to benefit from treatment with BTA.

We would also like to stress that in our opinion BTA treatment should be complementary to other physiotherapeutic interventions for PVD.

6.3.2 Early life health

In Study II we could see that there was an association between premature birth and being small for gestational age and developing PVD/vaginismus later in life. Both these conditions increases susceptibility for disease later in life

Since the time from exposure to event is very long (often 20+ years), there might very well be other factors during this time span that affects the development of PVD/vaginismus. However, the literature examining this period of life in this patient group is very scarce.

6.3.2.1 Pain exposure

Contrary to our belief that the development of PVD/vaginismus could be linked to a higher degree of pain exposure early in life, no such link was confirmed in this study. An explanation could be that we might have included too many diagnoses in our search, which diluted the sample.

6.3.2.2 Socioeconomic factors

We found that mothers of female offspring suffering from PVD were older, higher educated and less likely to be immigrants. This might be a selected sample since previous studies have found an association between high socioeconomic status and the consumption of specialized care (112).

6.3.2.3 Data on diagnosis

The diagnosis of PVD/vaginismus in our sample, is mainly registered in specialized care, which is not entirely representative for this diagnosis. Many of these patients are receiving care at youth centers, and diagnoses made at these centers are not collected in any official registry. If the youth center patients had been included the age at first diagnosis in the study group (Fig. 6) would most probably have been lower. . This problem have also been discussed in a report on vestibulitis by the Swedish National Board of Health and Welfare (14).

6.3.3 Vaginal manometer

The vaginal manometer was found to be able to detect differences in vaginal pressures in varying states of PFM activity. The intra- and inter- rater reliability is also deemed acceptable.

The device could distinguish between the groups to some extent. The primiparous women exhibited the lowest pressures consistently in all parameters, which was in line with our expectations. However it could not detect statistical differences between the PVD women and nulliparous women, which was surprising. On clinical examination the PVD women had a pronounced hypertension compared to the nulliparous, therefore we expected to see a difference in the VRP variable. In the study by Næss et al, they did detect a higher VRP for PVD women, but not for the other variables (102).

Since the probe is sensitive to where it is placed, we believe a slightly longer probe would more accurately measure pressure. In clinical practice it would be handy with a tool that can visualize improvements for the patients. The device is easy to use and easy to instruct others in how to use it. It is also possible that with regards to vaginal pressure it is better suited to have the patient as its own control, and not to compare to other groups.

7 CONCLUSIONS

The conclusions for the study specific findings are presented below

7.1 STUDY I

- Primary outcome: 50 U of BTA injected in the bulbocavernosus muscles did not reduce dyspareunia or pain at tampon use, during 6 months.
- Secondary outcomes: A favorable pain reduction in the weekly tampon test and a reduction of contraction pressures were seen after BTA injections. The group receiving BTA increased their number of intercourse attempts compared with placebo.

7.2 STUDY II

- Being born premature <37 weeks, low birth weight or SGA were associated with developing PVD/vaginismus later on in life.
- Painful exposures around birth and during first year of life was not seen as a risk factor for developing PVD/vaginimus.
- Mothers to patients with PVD/vaginismus were sligthly older, had higher educational attainment and were less liketly to have a positive immigrant status.

7.3 STUDY III

- The vaginal manometer could detect differences in vaginal contraction pressures between women with varying pelvic floor morphology.
- The manometer exhibited good intra- and inter- rater realiability.

7.4 STUDY IV

- Primary outcome: 50 U of BTA injected in the bulbocavernosus muscles did not reduce dyspareunia or pain at tampon use at 12 months' follow-up.
- Secondary outcomes: The reduced vaginal contraction pressures seen at six months after BTA injections were restored to baseline at twelve months. The pelvic floor training intervention could not be analyzed since few patients adhered to the program. The increased number of intercourse attempts in the BTA group remained at 12 months' follow-up.

8 POINTS OF PERSPECTIVE

We face several challenges regarding PVD and other vulvodynia conditions. As mentioned above, education and awareness of these conditions is important, for both the public and health professionals, since early diagnosis and adequate treatment help minimize the burden of disease (113). In Sweden sexual health education is mandatory in schools, and offers a natural platform where such issues could be addressed. Social media is another arena where these issues are being discussed. The involvement of professionals is desirable in these arenas to provide adequate information, to avoid misleading the public.

The etiology of PVD and other vulvodynia diagnoses remains to a large extent unclear. As presented above, there are several etiological theories that needs further investigation (114). Since vulvodynia has several subgroups, clinical presentations and potential etiological pathways, interdisciplinary collaboration in research and treatment is something to strive for. Longitudinal studies are needed to identify potential risk factors in the development of vulvodynia (16).

Epidemiological studies on PVD and other vulvar pain conditions have focused primarily on white women in Europe, North America and Australia, and studies outside these regions are few (115). To further understand the health burden these conditions cause world-wide, more epidemiological studies are needed.

On a more local perspective, the National Board of Health and Welfare in Sweden published a report on vestibulitis in 2018. The report mapped the health care offered Swedish women with vulvar conditions. It concluded that only 11 out of 21 regions had one or more specialized vulvar clinic, aiming to treat women with these kind of diseases. Where you live geographically thus impacts the care you may receive in Sweden. The report also highlights the need for further education within the professional as well as the public field (14).

The care available today is usually not always effective and time consuming both for patients and care givers. The need for more accessible and effective treatments is great, but remains a challenge. The Swedish Agency for Health Technology Assessment and Assessment for Social Services (SBU) recently published a report and evaluation on the available treatments today for PVD (76). The report concluded that generally the treatment studies are not of sufficient scientific quality, due to methodologically shortcomings, and therefore the treatment effects is not possible to assess.

The report also discuss the need for a core outcome set for PVD which has also been apparent during the work with our studies (76). A core outcome set is a minimum set of important outcomes for a certain condition that should be evaluated in trails for a specific condition. Such outcomes would be of great value for both patients and clinicians since it would make the comparison between studies much easier. The goals for treatment would also be clearer.

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