ETIOPATHOGENESIS AND TREATMENT OF LOCALIZED PROVOKED VULVODYNIA

Päivi Tommola

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki in the Seth Wichmann Auditorium of the Department of Obstetrics and Gynecology, Helsinki University Hospital Haartmaninkatu 2, Helsinki, Finland

on June 9th 2017 at noon
Supervised by: Professor Jorma Paavonen
Department of Obstetrics and Gynecology
University of Helsinki and Helsinki University Hospital

and

Adjunct Professor Leila Unkila-Kallio
Obstetrics and Gynecology
University of Helsinki and Helsinki University Hospital

Reviewed by: Adjunct Professor Maija Haanpää
Department of Neurology
University of Helsinki

and

Adjunct Professor Satu Suhonen
Department of Obstetrics and Gynecology
University of Helsinki

Official opponent: Professor Juha Mäkinen
Department of Obstetrics and Gynecology
University of Turku and Turku University Hospital

Cover image by Päivi Tommola


Unigrafia
Helsinki 2017
To my daughter ...
# TABLE OF CONTENTS

## ABSTRACT

## ABBREVIATIONS

## LIST OF ORIGINAL PUBLICATIONS

## 1 INTRODUCTION

## 2 REVIEW OF THE LITERATURE

### 2.1 THE VULVAR VESTIBULE

- 2.1.1 Anatomy of the vulvar vestibule
- 2.1.2 Innervation and vascular supply of the vestibule
- 2.1.3 Physiology of the vestibule

### 2.2 LOCALIZED PROVOKED VULVDYNIA

- 2.2.1 Definition and classification
- 2.2.2 Diagnosis and clinical characteristics
- 2.2.3 Epidemiology and prevalence
- 2.2.4 Etiopathogenesis of LPV
  - Inflammatory factors
    - Immune cells
    - Inflammatory tissue milieu
  - Genetic predisposition to inflammation
  - Immuno-inflammatory characteristics of the vestibule
  - Factors related to pain perception
    - Peripheral pain mechanism and pain transmission
    - Vestibular mucosal neural tissue
    - Increased tactile and thermal sensitivity
    - Central modulation of pain
    - Co-morbidity with other pain conditions
    - Genetic factors
  - Musculoskeletal factors
  - Hormonal factors
  - Psychosocial factors
- 2.2.5. Evaluation of LPV in clinical practice and in research
  - Pain measurement
    - Dysspareunia
    - Vestibular tenderness
    - Assessment of sexual well-being
    - Mental and physical functioning
- 2.2.6 Treatment of LPV
  - Conservative treatment of LPV
  - Psychological interventions
  - Pelvic floor physical therapy
  - Medical treatment
  - Alternative treatment
  - Surgical treatment
    - Surgical techniques
    - Complications of surgery
    - Long-term outcome of surgical treatment


3 AIMS OF THE STUDY

4 PARTICIPANTS

4.1 Ethics

4.2 Subjects

4.2.1. Study groups

5. TREATMENT PROTOCOLS

5.1 Conservative management

5.2 Modified posterior vestibulectomy

6 MAIN OUTCOME MEASURES

7 METHODS

7.1 Hospital chart review

7.2 Face-to-face interview

7.3 Gynecological examination

7.4 Evaluation of the pelvic floor muscle function

7.5 Health questionnaires

7.6 Immunohistochemistry

7.6.1 Specimen selection

7.6.2 Staining procedures

7.6.3 Analyses

7.7 Statistics

8 RESULTS

8.1 LPV TREATMENT OUTCOME (Studies I and II)

8.1.1 Patient characteristics

8.1.2 Short-term well-being after surgery

8.1.3 Long-term well-being after surgery or conservative management

Patient satisfaction

Dyspareunia

Vestibular tenderness

Long-term complications of surgery

Pelvic floor muscles

Sexual well-being

Mood, quality of life and co-morbid conditions

8.2 VESTIBULAR MUCOSAL CHARACTERISTICS (Studies III, IV)

8.2.1 Characteristics of the immune system in the vestibular mucosa

Immune cells

Vestibule-associated lymphoid tissue, VALT

8.2.2 Neural tissue and nerve growth factor in the vestibular mucosa
Intraepithelial nerve fibers (IENF) 67
IENFs in relation to immune activation 68
Submucosal neural tissue 68
Nerve growth factor (NGF) 69

9 DISCUSSION 70

9.1 METHODOLOGICAL CONSIDERATIONS 70

9.1.1 Study groups 70
9.1.2 Evaluation of treatment outcomes 71
    VAS as an outcome measure 72
9.1.3 Histological samples 72
    Issues related to immunohistochemistry analyses 73

9.2 FROM ETIOPATHOGENESIS TO TREATMENT OUTCOME 74
9.2.1 Etiopathogenesis of LPV 74
    Vestibule-associated lymphoid tissue, VALT 74
    Immune activation 75
    Neoproliferation 75
9.2.2 Treatment of LPV 77
    Safety of posterior vestibulectomy 78
    Long-term well-being after vestibulectomy and conservative treatment 78
        Effectiveness of posterior vestibulectomy 79
        Sexual well-being 79
        Patient satisfaction 80
    Choice of treatment 81

10 CONCLUSION 83

11 FUTURE PROSPECTS 85

12 ACKNOWLEDGEMENTS 86

13 REFERENCES 90
ABSTRACT

Localized provoked vulvodynia (LPV), a subset of vulvodynia, is associated with pain induced by touch to the vulvar vestibular mucosa in the absence of any other recognizable disease. This results in severe dyspareunia. LPV mostly affects young fertile-aged women, with a peak incidence of 8–15% between the ages of 20–30 years. LPV severely impairs a patient’s quality of life, deteriorates a couple’s sexual health and can even result in infertility since women with the most serious symptoms have to abstain from sexual activity.

Details of the etiopathogenesis of LPV have remained unknown. Knowledge of particular patient characteristics that associate with or predispose one to LPV has increased in recent years, but the exact pain mechanism is still unknown. Studies on individual medical treatment options show that some patients, though not all, benefit from conservative management. Surgery has shown to be effective in refractory patients. Only a few studies report on true long-term results and complication analyses of the surgery are also rare.

The objective of this study was to investigate short-term and long-term results of posterior vestibulectomy and to compare the long-term well-being of LPV patients treated either conservatively or by surgery. The second aim was to characterize immune and neural details of the diseased vestibular mucosal tissue in order to explain the pain mechanism.

The study material consisted of 97 consenting patients with severe LPV who had been treated according to a multidisciplinary treatment algorithm at Helsinki University Hospital Vulva Clinic between years 1995–2007. Seventy of the patients were refractory to conservative treatment and ended up in surgery, a posterior vestibulectomy procedure; twenty-seven had responded favorably to conservative treatment and did not need surgery. We analyzed short-term well-being and complications of surgery in the 70 patients, and long-term well-being in 57
surgically treated and in 27 conservatively treated patients. Dyspareunia was evaluated by the visual analogue scale (VAS) and vestibular tenderness by gynecological examination including cotton swab testing. Sexual well-being, other health-related issues, and overall patient satisfaction were evaluated by validated instruments and face-to-face interviews. To investigate the etiopathogenesis of LPV, we analyzed the removed vestibular mucosal tissues of 27 surgically treated patients by immunohistochemical methods. As comparison we had vestibular mucosal tissue specimens from 15 healthy controls.

Posterior vestibulectomy was a day surgery procedure in 80% of the cases. Short-term complications occurred in 21.4% of the patients. Mild wound infection or inflammation represented two-thirds of the complications. The multidisciplinary treatment algorithm turned out to be effective. Around 90% of the patients in both groups reported long-term satisfaction with the treatment outcome. Surgery by posterior vestibulectomy was shown to be a safe and effective treatment option in patients refractory to conservative treatment.

In the immunohistochemical tissue analyses, we demonstrated vestibule-associated lymphoid tissue (VALT) in the vestibular mucosa and showed that VALT had become activated in LPV. This immune activation was further shown to be associated with neuroproliferation, which may partly explain the pain sensitization in LPV.

In conclusion, our results suggest that immunoinflammatory pathways play an essential role in the pain pathogenesis of LPV. An algorithm-based multidisciplinary treatment including selected conservative treatment modalities provides good long-term well-being in a number of patients. Surgery by posterior vestibulectomy is a safe and effective treatment option for refractory patients.
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAI</td>
<td>B cell activation index</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CBT</td>
<td>Cognitive behavioral therapy</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene-related peptide</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COC</td>
<td>Combined oral contraceptives</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
</tr>
<tr>
<td>DNIC</td>
<td>Diffuse noxious inhibitory control</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>EQ5D</td>
<td>EuroQOL5D (5-dimensional)</td>
</tr>
<tr>
<td>FSFI</td>
<td>Female Sexual Function Index</td>
</tr>
<tr>
<td>GCBT</td>
<td>Group cognitive behavioral therapy</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptophan (Serotonin)</td>
</tr>
<tr>
<td>HPF</td>
<td>High power field</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IENF</td>
<td>Intraepithelial nerve fiber</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>ISSVD</td>
<td>International Society for the Study of Vulvovaginal Disease</td>
</tr>
<tr>
<td>LPV</td>
<td>Localized provoked vulvodynia or localized provoked vestibulodynia</td>
</tr>
<tr>
<td>MALT</td>
<td>Mucosa-associated lymphoid tissue</td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose binding lectin</td>
</tr>
<tr>
<td>MOS</td>
<td>Medical Outcome Study</td>
</tr>
<tr>
<td>NF</td>
<td>Neurofilament</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>NRS</td>
<td>Numerical rating scale</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PGP9.5</td>
<td>Protein gene product 9.5</td>
</tr>
<tr>
<td>PI</td>
<td>Problem Index (McCoy)</td>
</tr>
<tr>
<td>p.o.</td>
<td>Per oral</td>
</tr>
<tr>
<td>QOL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>QST</td>
<td>Quantitative sensory testing</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>RVVC</td>
<td>Recurrent vulvovaginal candidiasis</td>
</tr>
<tr>
<td>SF-36</td>
<td>Medical Outcome Study 36-item Short Form general health survey</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>s.m.</td>
<td>Submucosal</td>
</tr>
<tr>
<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic antidepressant</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Transient receptor potential vanilloid -1</td>
</tr>
<tr>
<td>VALT</td>
<td>Vestibule-associated lymphoid tissue</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VVS</td>
<td>Vulvar vestibulitis syndrome</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals:


The original publications and Figures 8,9,10, and 11 are reproduced with permission of the copyright holders.
1 INTRODUCTION

Localized provoked vulvodynia (LPV), a subset of vulvodynia, is associated with pain induced by touching the vulvar vestibular mucosa in the absence of any other recognizable disease. This results in severe dyspareunia. (1,2). The pain is provoked by only a light touch, a phenomenon defined as allostynia.

LPV is a devastating health problem for young fertile-aged women around 20 to 30 years of age, and their partners. The peak incidence of 8-15% of the condition occurs at the time when the women are supposed to engage in intimate relationships and have children (3-5). Such intensive chronic pain greatly impairs quality of life. This particular sexual pain deteriorates the sexual health of the women and their partners and can even result in infertility, since women with the most serious symptoms have to abstain from sexual activity.

Despite substantial research efforts, details of the LPV etiopathogenesis have remained unknown. However, knowledge on particular risk factors and patient characteristics that associate with or predispose women to LPV has increased in recent years. Different vulvovaginal infections, especially recurrent candidiasis, and urinary tract infections are known risk factors for LPV (6-9). Animal models and in vitro studies have further produced data on the significance of Candida albicans infections as a pain generator in the vulvar vestibule (10,11). Additionally, LPV patients have been shown to carry a genetic predisposition to exaggerated immune-inflammatory responses (12-16). Studies conducted in cell culture have shown that fibroblasts originating from the vestibular mucosa of LPV patients are more responsive to fungal antigens than those from healthy controls (11). At the tissue level, chronic lymphocytic inflammation and an increased density of neural tissue are characteristic of LPV (17-20). Thus, immune-inflammatory pathways can be involved in the development of the altered pain sensation, but the exact mechanism is still unknown.
Currently available therapeutic approaches only manage the symptoms and not the underlying origin of pain. Studies on different medical treatment options show that some patients, but not all, benefit from conservative management, such as physical therapy, cognitive behavioral therapy, or topical or systemic medical treatment. However, often the improvement is not much greater than that achieved by placebo. Surgery has been shown to be effective in refractory cases and typically results in satisfaction rates of 70-90% (21,22). Reports on long-term satisfaction rates after any treatment modality, however, are sparse (22). Because the pain mechanism of LPV is not yet understood, and because there are multiple associated factors, a multidisciplinary treatment approach with individualized steps is recommended. Also, patients themselves prefer on-site multidisciplinary care (23).

LPV is not a generally known condition, not even among healthcare professionals (24). Thus, difficulties in identifying the condition may result in unnecessary prolongation of the patient’s misery with devastating symptoms. In addition, uncertainty of the choice of treatment options and the current poor evidence of various treatment modalities may cause unnecessary delay. Also, patients’ ignorance of the condition or reluctance to speak about sexual symptoms may delay management (25).

The first aim of this study was to investigate the long-term well-being of LPV patients treated either conservatively or by surgery. The second aim was to characterize immune and neural details of the diseased vestibular mucosal tissue in order to explain the pain mechanism. Last but not least, our purpose was to increase knowledge of this devastating pain problem among healthcare professionals, as well as in the general population.
2 REVIEW OF THE LITERATURE

2.1 THE VULVAR VESTIBULE

2.1.1 Anatomy of the vulvar vestibule

The vulva is the part of the female external genitals reaching from the mons pubis in the front to the anal orifice in the rear. It includes the perineum, labia majora, labia minora, clitoris and vestibule (Figure 1). The vulvar vestibule contains the urethral meatus and vaginal introitus, delineated by the hymeneal ring, and major and minor vestibular glands. The vestibule extends laterally from the hymeneal ring to a line of more keratinized skin on the labia minora, the Hart’s line. Anteriorly, the vestibule reaches upward to the frenulum of the clitoris in the anterior commissure and posteriorly downward to the posterior fourchette.

The vestibular mucosa is covered by a non-keratinized or thinly keratinized stratified squamous epithelium. The dermis is densely innervated, highly vascular and rich in elastic fibers and erectile tissue. Major vestibular glands are the anteriorly-located Skene’s glands with openings adjacent to the urethral meatus, and the posteriorly-located Bartholin’s glands with openings adjacent to the hymen. On the vulvar map, these gland openings are located at the 10 and 2 o’clock and at 5 and 7 o’clock positions, respectively (26). The major glands consist of tubuloalveolar mucus-secreting gland acini delineated by columnar epithelium. Minor vestibular glands are simple tubular mucus-secreting glands that enter directly onto the mucosal surface. These are scattered all-around the vestibular mucosa but are most common in the posterior region (27). (Figure 1)
2.1.2 Innervation and vascular supply of the vestibule

The dense dermal innervation of the vestibular mucosa consists of unmyelinated and myelinated nerve fibers originating from the perineal branch of the pudendal nerve and the dorsal nerve of the clitoris. These are the two main branches of the pudendal nerve arising from sacral roots S2-S4 (29,30). Extensions from this dermal neural plexus reach the epithelial layer in the vestibular area (19). In general, the external components of the female reproductive system, including the labia majora and minora, the vulva with the clitoris and vestibule, and the urethral meatus have a somatic innervation with sensations similar to those evoked in skin (i.e. sensations of touch, pain and temperature) (31). The vagina, being visceral tissue by definition, mainly has a visceral innervation from the inferior hypogastric plexus (30), but a somatic innervation is also included in the lower parts (31).
However, unlike in the vestibular mucosa, no epithelial extensions of the nerves have been shown in the vaginal mucosa (29).

Small branches of the pudendal arteries that originate pairwise from the internal iliac arteries deliver the main vascular supply of the vulva, with additional influx from branches of the femoral arteries. A dense network of arterioles are in charge of the vestibular mucosal vascular perfusion. Along with the innervation of the vestibule, the vascular supply and its regulation is partly similar to that of skin (32). Additionally, medial parts of the labia minora include non-erectile sexually-responsive vascular tissue that respond to sexual arousal with increased blood flow (33). Venous drainage passes through corresponding veins (32,34).

2.1.3 Physiology of the vestibule

A healthy vestibular and vaginal mucosal surface is covered by a thin layer of protective fluid, produced in the vestibule by the vestibular glands. This "just moist" condition is not sufficient to allow painless penile penetration (33). During sexual arousal, vaginal and vestibular blood flow is increased by arteriolar dilatation and vasocongestion, which results in enhanced plasma transudate. The increased lubrication needed for successful vaginal penetration is thus produced by the increase of blood flow, with vestibular glands playing a minor, if at all, role in that (35). Based on its special location, interposing embryologically unique endoderm between the external vulva (embryologic ectoderm) and vagina (embryologic mesoderm), the vestibule has special immunologic challenges (34,36). The vestibule is naturally subjected to trauma and contact with foreign proteins and must have the ability to produce both protective and adaptive immune responses (37,38).
2.2 LOCALIZED PROVOKED VULVODYNIA

2.2.1 Definition and classification

The first descriptions of a disorder very similar to what we nowadays define as vulvodynia can already be found in the literature in the late 19th century (39). Since then, the classification and terminology have undergone remarkable changes starting from the consensus of the International Society for the Study of Vulvovaginal Disease (ISSVD) in 1976 to introduce the term ‘burning vulva syndrome’ (1). The most recent update occurred in April 2015 at a consensus conference of ISSVD and two other societies, the International Pelvic Pain Society (IPPS) and the International Society for the Study of Women’s Sexual Heath (ISSWSH). This 2015 classification defines vulvodynia as vulvar pain of at least 3 months duration, without clear identifiable cause, which may have potential associated factors. Further, vulvodynia is characterized as either localized (affecting only a part of the vulvar area, e.g., the vestibule) or generalized (present in the whole vulvar area). Vulvodynia may be provoked (e.g., insertional, contact), spontaneous, or mixed, and the onset can be primary (present at the first attempted introital penetration) or secondary (appearing later in life after a period of painless intercourse). Vestibulodynia refers to the pain in the vulvar vestibule (i.e., the area surrounding the vaginal opening). The term vestibulodynia was recommended for the first time in 2003 by the ISSVD when it replaced the previous term “vulvar vestibulitis syndrome” (VVS). The terms localized provoked vulvodynia (LPV) and localized provoked vestibulodynia (LPV) are both widely used, and the condition may also be called as provoked vestibulodynia (PVD). All of these terms comply with the current recommended terminology. The latest classification includes a temporal pattern. Vulvodynia can be persistent, constant, or intermittent, as well as immediate or delayed (2). The 2015 classification, although implying idiopathic pain, includes a list of potential associated factors for vulvodynia (Table 1).
Table 1. Factors associated with vulvodynia (2)

<table>
<thead>
<tr>
<th>Co-morbidities and other pain syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Painful bladder, fibromyalgia, irritable bowel syndrome,</td>
</tr>
<tr>
<td>temporo-mandibular disorder</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genetic factors</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Hormonal factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacologically induced</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inflammation</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Musculoskeletal factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic muscle dysfunction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurologic mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central: spine, brain</td>
</tr>
<tr>
<td>Peripheral: neuroproliferation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Psychosocial factors</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Structural defects</th>
</tr>
</thead>
</table>

### 2.2.2 Diagnosis and clinical characteristics

LPV is characterized by pain upon light touch on the seemingly healthy vestibular mucosa (allodynia) (2,40). The diagnosis is based on the typical history of pain (i.e., long-standing (minimum of 3 months) severe pain on vestibular touch or on attempted vaginal entry and on the finding of allodynia in the vestibular mucosa verified with a cotton-swab palpation (also called cotton swab test or Q-tip test). Infections and other painful gynecological and dermatological conditions must be ruled out. Vulvar erythema to varying degrees may be present, but is not requisite for the diagnosis (2,40-42). Biopsy is not needed, except to rule out dermatological
conditions if necessary. The sensitized pain perception results in pain upon penetration of the vagina and causes introital, superficial dyspareunia. In the most severe cases, intercourse is impossible due to the severe pain. The most typical painful spots are the ductal openings of the major vestibular glands, the posteriorly-located Bartholin's glands and the anteriorly-located glands of Skene just beside the urethral meatus (26) (Figure1). Typically, the pain intensity varies over time. Remissions with or without relapses over 1 to 12 years time have been reported. Remission rates are greater in patients with less severe symptoms (24,43,44). Patients with an affected anterior vestibule often have symptoms related to the urinary tract (e.g., dysuria and difficulties in voiding) (45). Patients with involvement of both areas commonly report more severe pain than patients with involvement of only the posterior vestibule (45). Embryologically, the vestibule differentiates from the urogenital sinus and is thus of endodermal origin like the urethra and bladder (36). This may explain the coexistence of vulvodynia and pain symptoms in the urethra and bladder, and the umbilical hypersensitivity in some women with primary LPV (46-48).

2.2.3 Epidemiology and prevalence

LPV affects mainly fertile-aged women between the ages of 18-40 and is the most common cause of dyspareunia in this population. In several population-based and clinic-based studies prevalence rates from 8-18% for vulvodynia have been estimated (3,5,24,49,50). These prevalence studies do not always specify whether the provoked, unprovoked, or mixed type of vulvodynia is in question. The provoked subtype is more prevalent among young premenopausal women, representing two-thirds of all cases of premenopausal vulvodynia in one study (49). For LPV, an overall incidence rate of 4.2/100 person-years and an incidence rate for 20-year-old women of 7.6/100 person-years have been reported (4). Vulvodynia is more prevalent in white than black women, with a predominance
among Hispanic women (3,4). Assessing the exact prevalence of LPV is difficult since not all affected women seek medical attention, mainly because of a fear of being stigmatized (perceived stereotyping) as neurotic or a hypochondriac (3,25). Furthermore, among physicians, LPV is still largely misdiagnosed or ignored (3,50). In a population-based study, it was found that only 50% of symptomatic women sought for help, 30% of them had to visit 3 or more doctors and LPV still remained undiagnosed in 40% of the cases (49). In another study, 48.6% had sought for help and only 1.4% were diagnosed correctly (24). A recent population-based study evaluated rates of remission, relapse and persistence of vulvodynia. Up to a 50% rate of remission without relapse over 6 to 30 months was found, and 38.6% of the patients relapsed after remission. Greater odds for persistence or relapse were found in patients with provoked pain, higher pain ratings and long histories of pain (44).

2.2.4 Etiopathogenesis of LPV

The etiology of LPV is multifactorial. The list of potential associated factors that have been suggested to play a role in the development and maintenance of LPV are listed in the 2015 classification (2) (Table 1). These factors are likely to act interdependently, possibly, within a cyclical model, and LPV represents an endpoint of different factors that differ from woman to woman.

Inflammatory factors

Immune cells

Traditionally, the microscopic presence of inflammatory cell infiltrates has been equated with “inflammation” in the context of vulvodynia research. Many studies comparing LPV and healthy controls have reported elevated chronic lymphocytic
inflammation in LPV (17,18,51) but the presence of inflammatory cells has also been demonstrated in samples from healthy controls (52,53). A majority of the studies on immune cells report increased mast cell infiltrates in LPV (17,18,20,54), but disagreeing reports also exist (52,54,55). In one study, increased vestibular mucosal CD4-positive helper T cell densities in LPV were found (51).

**Inflammatory tissue milieu**

An inflammatory background for the pathogenesis of LPV pain has been supported by many, but not all studies (56,57). Already in 1987 Foster and Hasday showed elevated tissue levels of interleukin 1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in LPV (58). Bohm-Starke and colleagues found significantly higher levels of vascular perfusion in the posterior vestibule by Doppler in LPV than in controls (19). This result was later confirmed in another study (59). Furthermore, the abundance of mast cells in the vestibular mucosa in LPV is suggestive of a neuro-inflammatory process (17,18,20,54). Also, the detection of the calcitonin gene related peptide (CGRP) and transient receptor potential vanilloid-1 (TRPV1) receptor by immunohistochemistry in the abundant intraepithelial nerve fibers suggests that neuroinflammatory pathways are involved in LPV (60,61).

**Genetic predisposition to inflammation**

Different vulvovaginal infections, especially recurrent candidiasis, and urinary tract infections are known risk factors for LPV (6-9). Additionally, animal models have produced data suggestive of the significance of Candida albicans infection as a pain generator in the vulvar vestibule (10). There is evidence of a special genetic characteristics associated with an increased risk of recurrent vulvovaginal candidiasis (RVVC) in women with LPV (12,62). LPV patients, more often than healthy controls, carry a specific loss-of-function allele of the mannose binding lectin (MBL)-gene, the MBL*5B-allele. This results in reduced serum levels of MBL and possibly predisposes these women to fungal and other vulvo-vaginal infections (62). This MBL variant has also been associated with a reduced capacity for TNF-α production, another factor which could predispose one to exaggerated
inflammation (63). LPV patients also have a tendency to carry pro-inflammatory allele variants of the interleukin-1 receptor antagonist (IL-1RA) and the melanocortin-1 receptor (MC1R) genes (15,16). LPV patients also respond to immune stimulants with higher levels of IL-1β (14). All of these conditions result in an inability to terminate inflammatory reactions and, thus, may result in prolonged inflammation. Moreover, patients with LPV have been reported to have co-morbid inflammatory bowel disease at a rate higher than expected, as well as a family history of autoimmune disease (e.g., diabetes mellitus, rheumatoid arthritis) in a greater than expected percentage of relatives (8).

**Immunoinflammatory characteristics of the vestibule**

Foster et al. have suggested that in every woman the vulvar vestibule possesses unique inflammatory/immunologic responsiveness. Thus, LPV pain may reflect an extreme but natural phenomenon (38). This group has shown that vestibular fibroblasts from LPV patients produce higher levels of pro-inflammatory cytokines IL-6, IL-8, and IL-1β than fibroblasts from external vulva or fibroblasts from healthy controls when stimulated with *Candida albicans* and alpha melanocyte-stimulating hormone (α–MSH) (38). Recently, in an *in vitro* model, the group showed that fibroblasts from vestibular areas with the lowest thresholds for pain produced the highest levels of IL-6 and prostaglandin E2 (PG-E2) when challenged with fungal antigens (11). The fibroblasts were shown to express elevated levels of the Dectin-1 receptor, which is responsible for recognizing fungal antigens. Furthermore, the vestibular fibroblasts were able to recognize minimal amounts of fungal antigens, while external vulvar reference fibroblasts and fibroblasts from healthy controls remained nonresponsive (64).
Factors related to pain perception

Peripheral pain mechanism and pain transmission

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. In the peripheral tissue, free nerve endings called nociceptors respond to the potentially damaging stimuli. The nociceptors have two types of afferent nerve fibers, A-delta-fibers and C-fibers, that conduct the stimulus from the peripheral tissue to the spinal cord. A-delta fibers are thinly-myelinated fibers, from 1-5 μm in diameter, with a conductivity of 5-30 m/s that transmit the fast localized sharp pain stimulus. C-fibers are the thinnest unmyelinated fibers of less than 1 μm in diameter, with a conduction velocity of less than 1 m/s. Their stimulation gives rise to the secondary aching or burning pain sensations. The primary nociceptive neurons can regulate their chemical environment through mediators synthesized in the cell body. Secretion of inflammatory chemicals, such as substance-P (SP) and CGRP, after injury augments inflammation and causes neurogenic inflammation. Also, repeated noxious stimuli may cause activation of previously nonresponsive nearby nociceptors by this axon reflex. Primary afferent neurons terminate in the dorsal horn of the spinal cord where they connect with secondary afferent neurons and other primary afferent neurons via synaptic connections. In the dorsal horn, the transmission of the stimulus in the afferent neurons and in the synaptic connections is exposed to modulation by the dorsal horn inter-neurons. This modulation may be excitatory or inhibitory. In the dorsal horn, persistent firing from the C-fibers may also induce central sensitization. Inflammatory mediators produced and secreted at the tissue level, such as PGs, leukotrienes (LT), serotonin (5-HT), and nerve growth factor (NGF), have modulating effects on the afferent neurons, which may become more sensitive to noxious stimulation by changing their phenotype. Additionally, the nociceptive stimulus is modulated by supraspinal structures, the thalamus, the hypothalamus, and finally the cerebral cortex. The final perception of pain is a product of the brain’s abstraction and elaboration of sensory input (65,66) (Figure 2).
Vestibular mucosal neural tissue

First reports on neural tissue changes in LPV come from immunohistochemistry studies in 1998 by Bohm-Starke (19) and in 2007 by Bornstein (20). These studies analyzed vestibular mucosal neural tissue semi-quantitatively and reported more intraepithelial nerve fibers (IENF) in LPV than controls. Later, Bohm-Starke showed that these fibers were CGRP-positive, which confirms the nociceptive nature of the fibers (60). Tympanidis showed an increased density of nerves expressing TRPV1 in LPV (61). These receptors, activated by capsaicin, evoke a burning sensation and the release of neuroinflammatory peptides, and may evoke neurogenic inflammation (67). Afterward, additional studies have shown neural
hyperplasia in the vestibular mucosa in LPV, but no data on the density of epithelial nerve fibers exist (17,52,68).

**Increased tactile and thermal sensitivity**

Quantitative sensory testing (QST) is used to detect and determine pain thresholds for different qualities of sensation, namely mechanical (touch, vibration, and pressure) and thermal (cold and warm) sensations (69). In QST studies, patients with LPV invariably report lower detection and pain thresholds for the sensation parameters in the vestibular area (26,70). Some additional studies have shown similar increased sensitivity in the vestibule and in other parts of the body (71-73).

**Central modulation of pain**

Diffuse noxious inhibitory control, DNIC (today preferably called conditioned pain modulation, CPM) is a central nervous system endogenous pain inhibitor system that involves supraspinal structures (74,75). Pain at one body site inhibits pain at another body site through the inhibition of specific nociceptive and wide dynamic range neurons (nonspecific nociceptive neurons) in the dorsal horn (76,77). DNIC effects have been shown to be diminished or absent in some chronic pain conditions with a female predominance, such as fibromyalgia and chronic tension-type headaches (78,79). DNIC function in LPV has been investigated in two studies, both showing intact function (80,81).

**Co-morbidity with other pain conditions**

In a population-based study Reed, et al. screened 1,980 women for vulvodynia, fibromyalgia, irritable bowel syndrome, and interstitial cystitis, and found that women positive for any of the other pain conditions were at a 2.3-3.3 fold increased risk for having vulvodynia. If all the three of the other conditions screened positive, the odds for having vulvodynia was more than 5-fold increased (82). Co-morbidity with other pain conditions has also been shown to increase morbidity in general health measures and to predict unfavorable treatment
outcomes when compared with patients who report only vulvodynia symptoms (83,84). The co-morbidity with several other pain conditions in LPV patients supports the role of the central mechanisms' involvement in the maintenance of LPV pain (85).

**Genetic factors**

Differences in the opioid and serotoninergic systems affecting endogenous pain modulation might contribute to both pain hypersensitivity and psychosexual characteristics in LPV. Some genes involved in the opioid and serotoninergic systems have been studied in LPV patients. Heddini et al. showed that women carrying the 1438G and T102C alleles of the 5-HT receptor gene, 5-HT-2A, had an odds ratio (OR) of 2.9 for LPV. Women with the G-/C- genotypes also reported more frequent pain problems from body areas other than the vestibule. This polymorphism has been associated with an altered function of the serotonin receptor and has also been associated with fibromyalgia, temporo-mandibular dysfunction and chronic widespread pain (72). In Heddini et al.’s other study, women with LPV were shown to carry the 118A-allele of the µ-opioid receptor (OPRM1) gene more often than the controls. This 118A allele has been associated with higher pain sensitivity in females than the 118 G-allele (86). Specific single nucleotide polymorphisms in the guanosine triphosphatasecyclohydrolase (GCH1) gene are associated with reduced pain sensitivity in humans (87). However, the pain protective combinations of alleles of the GCH1-gene were not found to be less frequent in LPV patients than controls (88).

**Musculoskeletal factors**

Surface electromyography (EMG) in women with LPV has shown instability of the pelvic floor muscles, elevated resting tone of the muscles, and poor muscle recovery after contraction (89,90). Also, morphological changes of the pelvic floor musculature in women with LPV have been found with ultrasonography (91,92). In a recent study, tenderness of pelvic floor muscles was found to correlate, even
better than tenderness of the mucosal surface, with the reported intensity of intercourse pain. In this study, muscle tenderness was assessed by determining the pressure pain thresholds (PPT) of three muscle groups (the perineal muscle complex at the six o’clock position and the puborectalis muscles laterally on both sides). Mucosal tenderness was assessed by cotton swab testing and dyspareunia was assessed by using the Gracely pain scale (93). Muscle instability has been thought to be a consequence of a fear of penetration pain rather than a reason for the pain. However, since physical therapy aiming at relaxation of the pelvic floor muscles has proven to be effective in relieving pain, a causative or at least amplifying effect of the muscle instability behind the pain is likely (94,95). Several unrelated disorders can cause pelvic floor muscle dysfunction. In inflammatory conditions, such as inflammatory bowel disorders and pelvic endometriosis, visceral somatic reflexes can affect nociceptive and visceral afferent neurons and cause hypertonicity and shortening and contraction of the pelvic floor muscles (96). Hypertonicity, in turn, causes alterations in neurodynamics, impairs blood flow and causes tissue hypoxia, and can result in sensations of tingling, burning, and shooting pain (97). Overactivity of the pubococcygeus and puborectalis muscles that insert at the posterior vulva can cause allodynia of the posterior vestibule (96).

**Hormonal factors**

Several epidemiologic studies have investigated a possible etiological correlation between combined oral contraceptives (COC) and LPV. In a clinic-based study from 2002, the relative risk of LPV for ever-users of COC, as compared with non-users, was 6.6 (95% CI 2.5-17.4), confirming earlier findings (98). If COCs were initiated before the age of 16, the relative risk reached 9.3, and was higher for pills with low, compared to high, estrogenic potency (99). COC initiation at a young age and low estrogenic pill potency have been proven to be risk factors of LPV in further studies as well (100,101). However, a population-based study of 1,083 women did not show an increased risk of LPV in COC users at any age less than 50 (102). Serum levels of sex hormone binding globulin (SHBG) are higher in women with
than without COC. Consequently, circulating levels of free androgens are lower in women on COCs (103). Carriers of a specific polymorphism of the gene coding for androgen receptors (AR) (more CAG repeats in the gene activity-determining domain of the AR gene) show lower activity of the ARs. These women are possibly more susceptible to reacting unfavorably to low circulating levels of free androgens. This polymorphism has been suggested to be an explanation for the tendency of some women, but not all, to develop LPV while on COCs (104).

Furthermore, COCs have been shown to alter the steroid receptor distribution of vestibular mucosa in healthy women (105). Also, the vulvar vestibular mucosa of women using COCs displays more shallow and sparse dermal papillae than the mucosa of women in the follicular phase of a normal cycle (106). QST has shown a reduced mechanical pain threshold in the vestibule of women on COCs compared with women not using COCs (107). The expressions of steroid receptors in the vestibular mucosa of LPV patients and healthy controls have been analyzed in several studies with contradictory results. One study showed increased estrogen receptor (ER) α in LPV (108), and another study a total lack of ER α in a subgroup of women with LPV (109). Neither of these results has been confirmed by further studies, however (17,110).

Psychosocial factors

Several controlled studies have shown that women with LPV report higher scores of depression (4,98,111) and anxiety (112) than healthy controls. In one study, women with LPV reported increased anxiety and also showed signs of pain catastrophizing (i.e., rumination, magnification, and helplessness) in response to experimentally-induced non-genital pain (113). LPV patients also show blunted morning cortisol responses consistent with higher levels of stress, not found in healthy controls (114). It is not clear whether these psychological factors have developed as a consequence of coital pain or might also play a role in the etiopathogenesis (115-117). High levels of stress, anxiety, and depression are suggested to act as vulnerabilities to the development and maintenance of LPV.
pain (116,117). It is suggested that chronic stress activates the neuroendocrine system of the skin and may evoke neurogenic inflammation, which results in the sensitizing and sprouting of peripheral nociceptors (118). Since the nerve supply of the vestibular mucosa resembles that of skin, it is possible that such a mechanism is involved in LPV (31). A history of childhood abuse, physical or sexual, has also been found to associate with a greater risk of developing LPV, with more unsatisfactory LPV treatment outcomes, and with poorer sexual functioning (119-121).

2.2.5. Evaluation of LPV in clinical practice and in research

Pain measurement

Dyspareunia

Patients with LPV typically report knife-like sharp or burning pain that is provoked by vaginal penetration and localized to the vaginal opening. The ability to adequately measure pain in LPV, both in clinical practice and in a research context, is crucial. The McGill Pain Questionnaire (MPQ) is the most widely used method for measuring dyspareunia in LPV research. It provides a single value pain rating index (PRI) including sensory and affective modalities of pain (122,123). The visual analogue scale (VAS) and numerical rating scale (NRS) are instruments with which pain intensity is described with a single value ranging from 0 to 10, useful for both research and clinical purposes (124). A standardized tampon insertion and removal test, the Tampon Test, provides an alternative to measure introital pain for those women who are not sexually active (125). Recently, the Brief Pain Inventory (BPI), originally designed to measure cancer-related pain, has also been used in LPV research (126). The Brief Pain Inventory Interference Scale (BPIIS) reveals the extent to which pain interferes with various components of functioning (e.g., human relations) (127). The Patient Global Impression Change Scale (PGICS) measures the extent of improvement achieved by the treatment in question (127).
**Vestibular tenderness**

Increased sensitivity of the vestibular mucosa to cotton swab palpation is a prerequisite for the diagnosis of LPV (2). In clinical practice, vestibular tenderness measured by a cotton swab test is also used in treatment follow-up. Patients are asked to report the intensity of pain either with a VAS score, an NRS score from 0 to 10, or by verbal expression, in which evaluations range from no pain through moderate to severe pain. However, since cotton swab testing can be performed in several ways (varying in the number or order of tested sites, using moist or dry cotton swabs with varying degrees of pressure, etc.), its use in LPV trials has been criticized. Therefore, the traditional von Frey filaments have been used (128), and more sophisticated devices, vulvar algesiometers in particular, for more accurate testing of touch and pain thresholds have been developed (26,129,130).

**Assessment of sexual well-being**

A variety of instruments to measure sexual functioning are available. Some separately measure specific domains of sexual functioning, such as sexual satisfaction, partnership satisfaction, relationship adjustment, sexual problems, and sexual distress (131,132). Quite widely used, and also validated in women with vulvodynia, is the Female Sexual Function Index (FSFI) which includes 19 questions measuring 6 different domains of sexual functioning (desire, arousal, lubrication, orgasm, satisfaction, and pain), and gives separate scores for individual domains and a single value full-scale index (127,133,134). The original McCoy instrument for sexual functioning comprises roughly the same domains with 8 questions and gives separate indices for sexual satisfaction, partnership satisfaction, and sexual problems (135). Modifications of the original McCoy instrument have been used in Nordic studies on sexuality-related issues (136,137).
Mental and physical functioning

The Beck Depression Inventory-II (BDI), including 21 questions is the most used validated instrument to measure depressive symptoms (138). In LPV research, the 13-item short version of BDI is commonly used for depression evaluation (131,139). Anxiety may be measured separately with the most commonly used Spielberger's State/Trait Anxiety Inventory (STAI) (140) or cost-effectively with a combined questionnaire measuring anxiety and depression, the Hospital Anxiety and Depression Scale (HADS) (84,127,141). A validated instrument for quality of life (QOL) is the 36-item short-form health survey (SF-36), originally developed in 1992 for the Medical Outcomes Study (MOS). It produces eight scale scores for eight domains of health status including bodily pain, general health and social functioning, among others (142). Euro-QOL5D (EQ5D) is a five dimensional questionnaire for health-related QOL. It includes a VAS from 0 to 100 for subjective evaluation of the current general health of the patient, 100 representing the best possible health status (143). From the MOS collection, a specific option for measuring social support, the MOS Social Support Survey, is also derived (144). In addition to the validated instruments and questionnaires, many studies include simple questions on the patients’ overall satisfaction after a given treatment and their willingness to choose the treatment again (145-148).

2.2.6 Treatment of LPV

The quality of the research published on vulvodynia management varies, but progressively more randomized studies are included. In recent years, “Grading for Recommendation” and “Level of Evidence” evaluations are comprehensively included in the reviews of vulvodynia management (22,149). Also, multicenter randomized trials are currently ongoing (127). In the following text, the studies that are referred to are evaluated according to the Grading of Recommendations
Assessment, Development and Evaluation (GRADE) system Level of Evidence recommendations (1=high, 2=moderate, 3=low, 4=very low) (150,151). The level of evidence is expressed either after a single evaluated study or after the whole paragraph when it expresses the body of evidence for all above evaluated studies.

**Conservative treatment of LPV**

Because of the multifactorial, not yet totally understood background of LPV, a multidisciplinary treatment approach with individualized steps is recommended (42,152,153). The treatment usually starts with non-invasive options (psychological approach, physical therapy), is continued with medical treatments, either topical or systemic, followed by nonsurgical interventions when appropriate, and only in non-responding patients progresses to surgical interventions (154-157).

**Psychological interventions**

In addition to reducing pain, psychological interventions, such as cognitive behavioral therapy (CBT) or psychosexual or sexual counseling, target skills to better cope with pain (pain self-efficacy) and to restore sexual functioning (158-160). Several studies have shown that CBT, delivered either in individual, couple, or group formats (GCBT), is successful in both pain reduction in women and in the psychological and sexual functioning of both partners, with a long-term positive response (treatment gains being maintained up to 2.5 year afterward (161-164). In randomized studies, CBT has proven more effective in LPV treatment than a traditional talk therapy or topical steroid treatment (165,166). In recent years, mindfulness-based CBT has gained popularity in the treatment of chronic pain conditions, including LPV (117,158). Mindfulness emphasizes acceptance of the present moment as such without judgment. It increases self-efficacy, reduces chronic stress, and aims at accepting the pain and uncoupling the emotional and physical sensations of pain, allowing the woman to reconsider her response to vulvar pain (117). (Level of Evidence 2–4).
Pelvic floor physical therapy

Pelvic floor muscle instability is an inevitable consequence of the penetration pain in LPV, but it also plays a causative role (96). Biofeedback therapy to reduce hypertonicity and restore normal voluntary relaxation of the muscles has been shown to effectively reduce intercourse pain in LPV (94,95,161,167). Manual connective tissue manipulation and myofascial triggerpoint release techniques are used to reduce tissue restrictions and improve circulation (168). Vaginal dilators are used to normalize the muscle tone and provide systematic pain desensitization, which diminishes the fear of penetration pain (159,169). Compared to CBT, physical therapy proved less effective in improving sexual functioning, while both treatments were equally effective in reducing intercourse pain (164). Combined programs utilizing varied physical therapy techniques and psychosexual counseling have proven effective. However, most studies suffer from a lack of comparison groups and the reporting of non-standardized treatment methods and non-validated outcomes (94,95,170). (Level of Evidence 3-4)

Medical treatment

Topical and intralessional medical treatment

Research evidence on the efficacy of most of the topical medications in LPV is weak. However, these are commonly used in the management of LPV (Table 2). Sensitization of the peripheral nerves is a suspected mechanism for LPV pain, which rationalizes the use of topical anesthetics (171). Randomized controlled trials have not, however, shown any benefit from topical lidocaine (172,173). Studies with lower levels of evidence have shown varying effects from other medicines used either topically or intralessionally (Table 2). Although LPV was originally considered purely an inflammatory condition, topical corticosteroids did not seem to alleviate pain at all, and no early studies on topical corticosteroid therapy actually exist. Recently, one double-blind randomized cross-over study did not find any difference between the efficacy of low-potent and high-potent
corticosteroids in topical use. This study included only 15 patients instead of the planned 110, due to pharmaceutical problems. However, high-potent corticosteroids are not recommended because of the potentially severe side effects (174). A recent study showed that both low-potent corticosteroid cream and GCBT reduced intercourse pain and had a positive impact on sexual functioning, but GCBT yielded a more positive impact on both (166). The current concept of the role of neurogenic inflammation in LPV pain calls for a treatment option that addresses both antinociceptive and anti-inflammatory effects. Research targeting the possible mechanisms for controlling neurogenic inflammation is ongoing. Recently, a case report on the combined use of baclofen and palmitoylethanolamid in a topical cream to treat LPV and proctodynia with a successful outcome has been published (175).

Systemic medical treatment

Antidepressants, mostly tricyclics (TCA), have traditionally been used to treat generalized unprovoked vulvodynia, but a positive effect on LPV pain has also been found. The common co-morbidity with depression and anxiety in LPV rationalizes the use of antidepressants. Reed et al. found that, in 60% of vulvodynia patients, pain was diminished by more than 50% by TCA treatment, irrespective of the subtype of vulvodynia. Amitriptyline was the most effective medicine of the medications included in the study (amitriptyline, desipramine and other TCAs, paroxetine, and gabapentin). The dosages of amitriptyline were titrated on the basis of pain relief and tolerance, up to 225 mg nightly, but no data on the used dosages are reported (176) (Level of Evidence 3). In a randomized placebo controlled trial, Foster et al. found no difference in the efficacy of 150 mg daily desipramine, a TCA with secondary amine, in diminishing introital pain when compared with topical lidocaine or placebo (173) (Level of Evidence 1). Also, some reports on successful anticonvulsant therapy, mostly on gabapentin, in LPV treatment exist. However, recent reviews have concluded that current evidence is not solid enough to support antidepressant or anticonvulsant therapy in the treatment of LPV (149,177,178). However, in specific therapy-resistant cases
anticonvulsants may be considered, based on the less harmful side effects of anticonvulsants than antidepressants (149). Currently, a multi-center randomized controlled trial (RCT) on gabapentin treatment in LPV is ongoing (127).

Since RVVC is a known risk factor for LPV, long-lasting anti-candida medications have been regarded as successful and are commonly used. However, evidence from the only fluconazole intervention study does not support this. Treatment protocols with once a week oral (p.o.) fluconazole 150 mg treatment together with dietary restriction of oxalate for 6 months or the dietary restriction alone resulted in similarly satisfactory outcomes. Noteworthy is that in this study the number of study patients was only 40, including only 7 patients with a history of RVVC (179). No further research evidence either on the dietary restriction of oxalate or favoring a low-carbohydrate diet in LPV exists.

Discontinuation of COCs has been shown to significantly alleviate or totally abolish the vestibular tenderness in 15 % of LPV patients (105). A possible mechanism explaining this might be the normalization of serum levels of SHBG and free-circulating androgens with normal cycles (103,104).

**Alternative treatment**

Acupuncture has shown success in LPV treatment in two studies, one of them randomizing half of the group to acupuncture and the other half on a waiting list of any therapy (180,181). Involving practically no side effects, acupuncture seems a promising management option (Level of Evidence 3).
<table>
<thead>
<tr>
<th><strong>Intervention</strong></th>
<th><strong>Study Type N</strong></th>
<th><strong>Comparison</strong></th>
<th><strong>Main outcome measures</strong></th>
<th><strong>F/U time</strong></th>
<th><strong>Effect</strong></th>
<th><strong>Evidence Quality</strong></th>
<th><strong>Year of Publication / Ref</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical lidocaine cream 5% for 12 w</td>
<td>DB, RPCT 133 (33+33+33+34)</td>
<td>p.o.desipramine 150mg + combination of both + matching PBO</td>
<td>Tampon test (NRS)</td>
<td>12 w + 40 w</td>
<td>Pain reduction: lidocaine 20%, desipramine 24%, combination 36%, PBO 33%</td>
<td>1^6</td>
<td>2010 (173)</td>
</tr>
<tr>
<td>Topical lidocaine cream 2-5% for 4 m</td>
<td>RCT 37 (18+19)</td>
<td>EMG for 4 m</td>
<td>PPT, VAS 1</td>
<td>12 m</td>
<td>Similar positive effect in both groups</td>
<td>2^6</td>
<td>2006 (172)</td>
</tr>
<tr>
<td>Topical lidocaine cream 5% overnight for 7 w</td>
<td>Case series, Prospective 61</td>
<td>-</td>
<td>VAS 2</td>
<td>6 m</td>
<td>In 57% ≥ 50% reduction of dyspareunia</td>
<td>3^7</td>
<td>2003 (171)</td>
</tr>
<tr>
<td>Topical capsaicin cream 0.025% for 12 w</td>
<td>Case series, retrospective 47</td>
<td>-</td>
<td>Marinoff dyspareunia scale</td>
<td>6 w</td>
<td>Improvement</td>
<td>4^7</td>
<td>2005 (182)</td>
</tr>
<tr>
<td>Botulinum toxin A injection (20 U)</td>
<td>DB, RPCT 64 (32+32)</td>
<td>Saline injections</td>
<td>VAS 2, FSFI, SF-36, DLQI</td>
<td>6 m</td>
<td>No difference between groups</td>
<td>1^6</td>
<td>2009 (183)</td>
</tr>
<tr>
<td>Botulinum toxin A injection (100 U)</td>
<td>Case series, prospective 20</td>
<td>-</td>
<td>VAS 2, FSFI, DLQI</td>
<td>6 m</td>
<td>VAS 2 decrease from 8.3 to 2.7</td>
<td>3^7</td>
<td>2011 (184)</td>
</tr>
<tr>
<td>Botulinum toxin A injection (100 U)</td>
<td>Case series, retrospective 19</td>
<td>-</td>
<td>VAS 2, FSFI, DLQI</td>
<td>24 m</td>
<td>VAS 2 decrease from 8.7 to 3.1 37% CR</td>
<td>4^7</td>
<td>2016 (185)</td>
</tr>
<tr>
<td>Topical gabapentin cream 2% or 6% for 8 w</td>
<td>Case series, retrospective 32</td>
<td>-</td>
<td>VAS 2</td>
<td>8 w</td>
<td>VAS 2 decrease from 7.3 to 2.5</td>
<td>4^7</td>
<td>2008 (186)</td>
</tr>
<tr>
<td>Topical amitriptyline cream 2% for 3 m</td>
<td>Case series, Prospective 150</td>
<td>-</td>
<td>Symptoms and signs</td>
<td>3 m</td>
<td>10% CR, 46% PR, 44% NR</td>
<td>3^7</td>
<td>2012 (187)</td>
</tr>
<tr>
<td>Sodium chromoglycate cream 4% for 12 w</td>
<td>DB, RPCT 26 (13+13)</td>
<td>PBO</td>
<td>Dyspareunia</td>
<td>3 m</td>
<td>No difference between groups</td>
<td>1^6</td>
<td>2001 (188)</td>
</tr>
<tr>
<td>Intervention</td>
<td>Study Type</td>
<td>Comparison</td>
<td>Main Outcome measures</td>
<td>F/U time</td>
<td>Effect</td>
<td>Evidence Quality</td>
<td>Year of Publication / Ref</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>--------------</td>
<td>-------------------</td>
<td>-----------------------</td>
<td>----------</td>
<td>---------------------------------------------</td>
<td>------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Potent steroid cream (clobetasol 0.05%) for 4 w Fibroblast cutaneous lysate skin cream for 12 w</td>
<td>DB,RCT 7 + 8, DB,RPCT, Crossover 26</td>
<td>Mild steroid cream, HC 0.5% PBO cream</td>
<td>Pain, vestibular tenderness Dyspareunia Vestibular sensitivity Dyspareunia</td>
<td>6 w 12 w</td>
<td>No difference between groups Greater reduction of dyspareunia in active group, no difference in sensitivity Improvement</td>
<td>3 7</td>
<td>2004 (174) 2012 (128)</td>
</tr>
<tr>
<td>Topical badofen 5% + palmitoylethanolamid cream for 12 w</td>
<td>Case report 1</td>
<td>-</td>
<td>Dyspareunia CST</td>
<td>N/A</td>
<td>No difference between groups</td>
<td>4 7</td>
<td>2014 (175)</td>
</tr>
<tr>
<td>S.c. enoxaparin 40 mg injections for 90 days</td>
<td>DB,RPCT 40 (20+20)</td>
<td>Saline injections</td>
<td>Dyspareunia CST</td>
<td>6 m 24 m</td>
<td>Reduction of dyspareunia 29% vs. 4%; Decrease in sensitivity 30% vs. 11%</td>
<td>1 7</td>
<td>2012 (189)</td>
</tr>
<tr>
<td>Intralesional lidocaine + methylprednisolone injections weekly for 3 w for 13 w</td>
<td>Case series, prospective 19</td>
<td>-</td>
<td>Dyspareunia CST</td>
<td>6 – 24 m</td>
<td>32% CR, 36% PR, 32% NR</td>
<td>3 7</td>
<td>2001 (190)</td>
</tr>
<tr>
<td>Topical steroid (1% HC) for 13 w</td>
<td>RCT 97 (45+52)</td>
<td>GCBT</td>
<td>NRS ³, McGill, FSFI</td>
<td>6 m</td>
<td>Significant reduction in pain in both groups</td>
<td>2 7</td>
<td>2016 (166)</td>
</tr>
<tr>
<td>Intralesional interferon alpha</td>
<td>Case series, prospective</td>
<td>-</td>
<td>Dyspareunia (verbal)</td>
<td>6 m</td>
<td>49% partial improvement</td>
<td>3 7</td>
<td>1993 (191)</td>
</tr>
<tr>
<td>Topical nifedipine cream 2% or 4% for 6 w</td>
<td>RPCT 30(10+10+10)</td>
<td>PBO cream</td>
<td>VAS ²</td>
<td>3 m</td>
<td>All groups improved, No difference</td>
<td>1 6</td>
<td>2010 (192)</td>
</tr>
<tr>
<td>Topical estradiol 0.03% + testosterone 0.01% cream for 20 w</td>
<td>Case series, prospective 50</td>
<td>-</td>
<td>Vestibular pain score</td>
<td>-</td>
<td>73% decrease</td>
<td>3 7</td>
<td>2013 (103)</td>
</tr>
</tbody>
</table>

w=weeks, m = months, s.c. = sub-cutaneously, HC = Hydrocortisone, DB = double-blinded, RCT = randomized controlled trial, RPCT = randomized placebo controlled trial, PBO = placebo, EMG = electromyographic biofeedback, GCBT = group cognitive behavioral therapy, NRS = numerical rating scale, VAS = visual analogue scale, PPT = pressure pain threshold, SF-36 = Short Form general health survey, QOL=Quality of Life (VAS 0-100), FSFI = Female Sexual Function Index, DLQI = Dermatology Life Quality Index, CST = cotton swab test, F/U=follow-up, N/A=not available, CR=complete response, PR=partial response, NR=no response, Ref = reference.

¹ VAS for pain in PPT testing, ² VAS for dyspareunia (0-10), ³ NRS for dyspareunia, ⁴ Open label phase, ⁵ Level of Evidence: 1 = high, 2 = moderate, 3 = low, 4 = very low (Oxford Center of Evidence-Based Medicine – Levels of Evidence), ⁶ Evidence of no efficacy, ⁷ Evidence of efficacy, ⁸ Study interrupted
Surgical treatment

Surgical treatment of LPV aims at ablation of the painful vestibular mucosa. To reduce pain, surgery is by far the most effective treatment modality (145,193,194). Traditionally, surgery is recommended only for patients refractory to conservative management (22,131,157). A few early reports on laser ablation exist, all with lower success rates than achieved by cold knife surgery (34,195,196). In 1981, Woodruff reported the first case-series on 42 women with dyspareunia treated by a perineoplasty operation (197,198). Since then, Woodruff’s original perineoplasty technique has been modified, eventually resulting in techniques with less tissue removal but better coverage of the painful area of the mucosa (21).

Surgical techniques

Woodruff’s original perineoplasty

In the early modification of perineoplasty, a triangular area of skin, with its base at the outer margin of the vaginal outlet and its apex near the anal orifice, was excised. Three to four centimeters of vaginal mucosa were undermined and the liberated vaginal mucosa was approximated to the skin edges with absorbable stitches (198). Later, the technique was developed and tailored to cover the whole vestibule, including the hymeneal ring and adjacent 5 mm of vaginal tissue and the openings of the major vestibular glands, as well. The excision was 2–5 mm deep (197,199). Later, slightly modified perineoplasty, with the posterior extension not reaching down to the anal orifice but kept half way down the perineal skin, was introduced (200).

Vestibulectomy

In a vestibulectomy operation, the excision is limited to the vestibule only, not including the perineal skin beyond the Hart’s line but including all tender parts
extending to the anterior vestibule (145,201,202), or limited to the posterior part of the vestibule from 2 to 10 o'clock (203). Often, the procedure is begun with submucosal infiltration of a local anesthetic with epinephrine for intraoperative hemostasis and postoperative pain management (202). In a simplified version, only tender parts of the mucosa are excised (193).

Complications of surgery

Short-term complications

Based on the rich vascularization of the vulvar and perineal area, hemorrhage and hematoma formation are the most common short-term complications of vestibulectomy, reported with a frequency of 1–6.5% (195,202). Wound infections are also reported, but rarely with exact rates (204,205). One study on simplified vestibulectomy reported wound dehiscence or the need for re-suturing in 16% of patients (193). In conclusion, the risk of immediate postoperative complications seems low, but is poorly reported on.

Long-term complications

The ducts of the Bartholin's glands are usually transected in the vestibulectomy operations causing a risk for cyst formation from duct occlusion. Goetsch found a 9% risk for Bartholin's duct cysts in her dataset of 155 simplified vestibulectomies. The cysts were noted between 1.5 months and 2 years after the operation (206). A review found a 2-6% risk for Bartholin's duct cysts (21). In techniques excising more tissue, excessive scarring, a dissatisfying cosmetic appearance, and reduced lubrication are possible long-term complications. Foster et al. reported a dissatisfying appearance of the perineum in 7% and anal sphincter weakness in 1% of patients after more than four years post-surgery (207). Traas reported reduced lubrication in 24% of the patients (148) and Friedrich in 8% of the patients post-surgery (41,148). Rates of recurrence after a primarily successful operation vary from 1-13% (146,201,208).
Long-term outcome of surgical treatment

Most surgical treatment studies of LPV are retrospective case series and many of them lack proper evaluation of complications and data on long-term satisfaction with multiple outcome measures (21,22) (Level of Evidence 3 – 4). One RCT comparing surgery with GCBT and physical therapy exists, but this study suffers from a 24% loss of participants from the original surgery group (Level of Evidence 2b)(161). A majority of the studies report pain reduction as the only outcome measure (21). One group with two consecutive studies on the same LPV population (the latter with a continued follow-up period), evaluated both pain reduction and sexual functioning improvement as outcome measures. They reported complete responses in 68% of the patients (161,162). The reported overall satisfaction percentages vary from 42% to 100%, and the surgical technique seems not to have any major impact on the success rate (21). Instead, paying attention to the indications for surgery is important. Bornstein found an OR of 5.83 for operative failure among women with spontaneous pain in addition to dyspareunia at baseline (209). Predictors of a favorable surgical outcome are younger age of the patient (148) and secondary LPV (209). On the other hand, recurrences were more common in women with secondary than primary LPV (208). Since LPV patients, in addition to painful intercourse, suffer from poor sexual well-being and poor QOL, additional treatment modalities are often needed. A patient's willingness to attend psychological evaluation before surgery predicts a favorable outcome. (210). Also, combining physical therapy with surgery in one study increased the percentage of fully recovered patients from 60% to 90% (208).
3 AIMS OF THE STUDY

The first purpose of the study was to assess the safety and effectiveness of surgical treatment in LPV. The second purpose was to characterize the immune cells and neural tissue of the vestibular mucosa in LPV.

Specific aims of studies I – IV

**Study I:** To assess short-term complications and long-term well-being after posterior vestibulectomy for severe LPV

**Study II:** To compare long-term well-being of patients with severe LPV treated either conservatively or surgically, and to identify factors that predict the treatment outcome

**Study III:** To characterize the immune cells and immune response in the vestibular mucosa in LPV

**Study IV:** To identify associations between immune activation and intraepithelial nerve fibers in the vestibular mucosa in LPV
4 PARTICIPANTS

4.1 Ethics

The studies were approved by the local Ethical committee. All participants received oral and written information about the study they participated in and provided informed consent.

4.2 Subjects

4.2.1. Study groups

Women with severe LPV were identified in the patient registry of Helsinki University Hospital, Department of Obstetrics and Gynecology, Vulva Clinic. An invitation letter to attend a long-term follow-up study was sent to all 120 identified women. Of these women, 70 had undergone posterior vestibulectomy between 1995-2007 after failed conservative management. They formed the study population of the vestibulectomy follow-up study (Study I). The remaining 50 patients experienced a favorable response to conservative management during the convergent time period and did not need surgery. They formed the conservative treatment group of the comparative LPV treatment study (Study II). The participants of the comparative study were selected by matching patient pairs from both treatment groups. The patients were matched on age, timing of the treatment, severity of symptoms at baseline, and selection of applied treatments. The matching resulted in the exclusion of 18 surgically treated patients from the original cohort (Figure 3). Drop-out analysis did not show any clinically meaningful differences between the selected and excluded patients.

The inclusion criteria for all studies required that all patients had had more than a 12 month history of dyspareunia prohibiting or severely impairing intercourse
before treatment. Also, all had been managed according to a predefined algorithm (Figure 4). At the first admission to the Vulva Clinic, the diagnosis of LPV was based on the Friedrich's classical criteria: pain on vestibular touch or attempted vaginal entry causing dyspareunia, and a positive cotton swab test of the vestibular gland openings, with or without erythema of the involved mucosa (41). Dermatological conditions were ruled out by histopathologic examination of punch biopsies, when appropriate, and vaginal infections by bedside wet-mount microscopy. Dyspareunia was recorded by the VAS from 0 (no pain) to 10 (worst possible pain) (124). Reporting constant or intermittent spontaneous pain was not an exclusion criterion if provoked pain had been the major complaint of the patient.

Figure 3. Study protocol and study groups
LPV = localized provoked vulvodynia, F/U = follow-up
From the original group of 70 surgically treated patients, 27 patients with eligible representative archival vestibulectomy specimens and sufficient clinical data formed the study group of the etiopathogenetic studies (Studies III and IV). For Studies III and IV, we recruited 15 healthy control women with no vulvar complaints undergoing benign gynecological surgery. A 4 mm biopsy from the posterior vestibule at the 5 o’clock position was taken to obtain healthy vestibular tissue for comparison.

5. TREATMENT PROTOCOLS

5.1 Conservative management

All LPV patients at the Vulva Clinic were managed according to a predefined algorithm (Figure 4) in line with the international vulvodynia treatment guidelines (211,212).

After the diagnosis of LPV, patients were counseled on the treatment plan, with individualization when appropriate. Gentle care of the vulva, including recommendations to avoid vulvar irritants, was advised. COC withdrawal was recommended, if possible. Physical therapy, offered to all patients, included biofeedback therapy of the pelvic floor muscle dysfunction, sexual counseling, and education. Antifungals were prescribed for patients with a history of confirmed or suspected recurrent yeast infections. Corticosteroids were used as submucosal injections and TCAs prescribed when appropriate. Patients who did not respond to conservative management were offered surgery, a modified posterior vestibulectomy.
Figure 4. Treatment algorithm of localized provoked vulvodynia
COC = Combined oral contraceptives, VAS = Visual analogue scale, 
F/U = follow-up, p.o. = per oral, s.m. = submucosal

5.2 Modified posterior vestibulectomy

Vestibulectomy was performed under general anesthesia using the modified posterior vestibulectomy technique (Figure 5). Briefly, two parallel incisions with cutting electrocautery were made in the posterior vestibule reaching from 2 to 10 o’clock after infiltrating the submucosal layer with a lidocaine – epinephrine 0.05% solution. The inner incision line was made just inside the hymeneal ring and the peripheral incision to the Hart’s line. The posterior vestibular mucosa was then excised by superficial skinning vestibulectomy using cutting electrocautery. Three to four centimeters of vaginal mucosa was undermined, leaving tissue bridges for adequate blood supply. The denuded area was covered with the undermined
vaginal mucosa. The wound was closed with interrupted stitches using 2-0 absorbable suture material. A single dose of intravenous metronidazol (500 mg), followed by 400 mg three times daily p.o. for three days was used. Postoperative pain was managed with ibuprofen and paracetamol tablets according to the set treatment policy. Routine follow-up visits took place at one and two months. At the first visit, the women were encouraged to perform daily dilatation therapy with a vaginal probe and resuming intercourse was permitted.

Figure 5. Modified posterior vestibulectomy
Modified from Tommola et al. (213)

6 MAIN OUTCOME MEASURES

The outcome measures in Studies I and II were rates of short-term and long-term surgical complications, long-term overall patient satisfaction, improvement of dyspareunia, and vestibular tenderness and sexual functioning over the long-term. In Studies III and IV, the outcomes were characterization of immune cells and neural tissue in the vestibular mucosa.
7 METHODS

Methods used in the studies are shown in Table 3.

<table>
<thead>
<tr>
<th>Method</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital chart review</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Face-to-face interview</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Dyspareunia scoring (VAS)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Gynecological examination</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>including swab-touch test</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMG evaluation of the pelvic floor muscle function</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health questionnaires</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQ5D-VAS</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCoy Measure of sexual well-being, comprises three subscales:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sexual satisfaction (5 items), sexual problems (2 items), and</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>partnership satisfaction (2 items); higher scores indicate more</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sexual satisfaction, more problems, and more satisfaction with partner</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI short form</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>MOS Social Support Scale</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

1 Performed by one gynecologist who had not been involved in the treatment process
2 Does not apply to controls in Studies III and IV
3 EMG evaluation, Pelvimed 932 (Enraf Nonius B.V., Rotterdam, the Netherlands), Periform vaginal probe (Patterson Medical Ltd., Nottinghamshire, UK)
4 EQ5D-VAS, range 0 – 100, score of 100 represents the best possible health state (214)
5 McCoy Measure of sexual well-being, comprises three subscales: sexual satisfaction (5 items), sexual problems (2 items), and partnership satisfaction (2 items); higher scores indicate more sexual satisfaction, more problems, and more satisfaction with partner (135,136)
6 BDI, short form includes 13 items, range 0 – 39, greater value represents more depressive status (139), 7 MOS Social Support Scale, includes 9 items, scale 9 – 45

VAS = Visual Analogue Scale, EMG = Electromyography, EQ5D = Euro Quality Of Life 5-dimensional
BDI = Beck Depression Inventory, MOS = Medical Outcomes Studies
7.1 Hospital chart review

Hospital charts were reviewed and data appropriate for each study were collected as follows: preoperative symptoms (I - IV), preoperative clinical status (I - II), data on short-term recovery and post-operative complications (I), and details of the conservative treatment modalities used before opting for surgery (II - IV).

7.2 Face-to-face interview

At the long-term follow-up visit, all patients were interviewed following a semi-structured face-to-face interview. Any missing chart review data were also completed at this time. The interview included information on the patient’s intimate relationship status, menstrual cycle, parity, urogenital infections, pain symptoms (urogenital, gastrointestinal, and musculoskeletal), and information on other general health issues. Patients reported their current VAS score for dyspareunia, the effect of vestibulectomy on their symptoms (i.e., their personal judgment of the treatment outcome (complete recovery – partial recovery – no response)), their satisfaction with the treatment process as a whole, and their preference for choosing the operation again.

7.3 Gynecological examination

Gynecological examination was carried out at the long-term follow-up visit. It included inspection, identifying possible surgical scars and chronic vulvar fissures, evaluation of vestibular tenderness, and palpation of pelvic floor muscles and inner pelvic organs. Vestibular tenderness was evaluated with a cotton swab test separately in the anterior (from 11 to 1 o’clock) and posterior region (from 2 to 10 o’clock) with (equal) light touch administered each time. The result was recorded by using the categories “significant” (verbal expression of intense pain or
expression of pain by sudden movement), “mild” (verbal expression of mild pain), or “none” (no pain).

### 7.4 Evaluation of the pelvic floor muscle function

Pelvic floor muscle function was evaluated with EMG at the physical therapist appointment (Pelvimed 932, Periform vaginal probe). A resting tone value 10 µV or less was defined as good ability in voluntary relaxation.

### 7.5 Health questionnaires

Questionnaires were sent by mail before the follow-up visit to all consenting patients to be returned at the time of the visit. Questionnaires included basic sociodemographic data, information on urogenital symptoms, and information on possible additional treatments for dyspareunia after surgery. Validated instruments used were as follows (details are shown in Table 3): Subjective general health was measured with the EQ5D-VAS (214) and sexual well-being by the McCoy Sex Scale modified by Wiklund (135,136) with additional questions included on the intensity of the dyspareunia (from 1 to 7, higher number indicates more pain) and on the frequency of sexual acts during the previous month. Depression was measured by the 13-item version of BDI (139), and availability of social support was assessed by 9 items from the validated Finnish version of the MOS Social Support Scale with additional selected questions on social and emotional functioning (144,215). Questionnaires also included the validated Finnish version of Spielberger’s 20-item STAI, not analyzed in this study (215).
7.6 Immunohistochemistry

7.6.1 Specimen selection

Haematoxylin-Eosin (HE) stained 5μm sections from the 42 available paraffin blocks (27 patients, 15 controls) were analyzed by one experienced pathologist to confirm the quality of samples, to exclude dermatological diseases, and to grade lymphocytic inflammation.

7.6.2 Staining procedures

For the immunohistochemistry, further consecutive sections were stained to reveal specific immune cells and neural tissue elements according to the manufacturers' instructions. Details of the applied antibodies and the staining procedures are shown in Table 4. All sections were counterstained with hematoxylin.

7.6.3 Analyses

Immunohistochemical scoring was performed under light microscopy (Nikon Eclipse E800). The entire material was analyzed blinded to clinical data of the patients. All antigen stainings were analyzed for localization (stromal – epithelial – both) and densities of individual immune cell types and nerve fiber types in the vestibular mucosa (Table 4). Germinal centers were identified from the CD20 (cluster of differentiation) and CD3 stainings and the number counted from each section. The scoring of each section was based on the consensus of two investigators, disagreements were resolved by a joint review. A particular B cell activation index (BAI) was used to describe the overall level of B cell infiltration of each sample. BAI is the calculated sum (0 to 12) of three different parameters
analyzed from each sample: (1) overall density of B cells in the epithelium (score from 0 to 4), (2) overall density of B cells in the stroma (score from 0 to 4), and (3) absence (score 0) or presence (score 4) of germinal centers. Densities of nerve growth factor (NGF)-positive immune cells were analyzed from four different areas of the mucosa representing areas with or without B cells and with or without IENFs (Table 4).
<table>
<thead>
<tr>
<th>Antibody/Antigen</th>
<th>Clone, Catalog number, Manufacturer</th>
<th>Pre-treatment buffer (pH), Catalog number, Manufacturer</th>
<th>Dilution, Incubation time/°C</th>
<th>Detection system, Catalog number, Manufacturer</th>
<th>Staining Instrument</th>
<th>Evaluation/Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 T lymphocyte</td>
<td>2GV6, 790-4341, Roche Diagnostics Ltd, Switzerland</td>
<td>CC1 (pH 8.0), 950-124, Roche Diagnostics Ltd, Switzerland</td>
<td>RTU 32 min/RT</td>
<td>UltraView Universal DAB Detection Kit, 760-500, Roche Diagnostics Ltd, Switzerland</td>
<td>Ventana Benchmark XT</td>
<td>A single number score of the overall cell density for each section</td>
</tr>
<tr>
<td>CD20 B lymphocyte</td>
<td>L26, 760-2531, Roche Diagnostics Ltd, Switzerland</td>
<td>CC1 (pH 8.0), Roche Diagnostics Ltd, Switzerland</td>
<td>RTU 4 min/RT</td>
<td>UltraView Universal DAB Detection Kit, 760-500, Roche Diagnostics Ltd, Switzerland</td>
<td>Ventana Benchmark XT</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td>IgA Plasma cell</td>
<td>Polyclonal, A0262, Agilent Technologies, USA</td>
<td>Trypsin 0.5%, 37°C/25 min, Dicfo 250, 215310, BD Bioscience, USA</td>
<td>1:2000 30min/RT</td>
<td>EnVision Detection Systems Peroxidase/DAB, K5007, Agilent Technologies, USA</td>
<td>LabVision</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td>CD163 Dendritic cell</td>
<td>NCL-CD163,10D6, Leica Biosystems Inc. USA</td>
<td>Tris-EDTA (pH 9.0), S2367, Agilent Technologies, USA</td>
<td>1:100 40 min/RT</td>
<td>EnVision Detection Systems Peroxidase/DAB, K5007, Agilent Technologies, USA</td>
<td>LabVision</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td>CD68 Macrophage</td>
<td>M0876, PG-M1, Agilent Technologies, USA</td>
<td>CC1 (pH 8.0), Roche Diagnostics Ltd., Switzerland</td>
<td>1:1000 24min/RT</td>
<td>UltraView Universal DAB Detection Kit, 760-500, Roche Diagnostics Ltd., Switzerland</td>
<td>Ventana Benchmark XT</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td>CD117 Mast cell</td>
<td>Polyclonal, A4502, Agilent Technologies, USA</td>
<td>CC1 (pH 8.0), Roche Diagnostics Ltd., Switzerland</td>
<td>1:400 32 min/RT</td>
<td>UltraView Universal DAB Detection Kit, 760-500, Roche Diagnostics Ltd., Switzerland</td>
<td>Ventana Benchmark XT</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td>Antibody/ Antigen</td>
<td>Clone, Catalog number, Manufacturer</td>
<td>Pre-treatment buffer (pH), Catalog number, Manufacturer</td>
<td>Dilution, Incubation time/°C</td>
<td>Detection system, Catalog number, Manufacturer</td>
<td>Staining Instrument</td>
<td>Evaluation/ Analysis</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>CD117 Mast cell</strong></td>
<td>Polyclonal, A4502, Agilent Technologies, USA</td>
<td>CC1 (pH 8.0), Roche Diagnostics Ltd., Switzerland</td>
<td>1:400 32 min/RT</td>
<td>UltraView Universal DAB Detection Kit, 760-500, Roche Diagnostics Ltd., Switzerland</td>
<td>Ventana Benchmark XT</td>
<td>Mean number of staining positive cells</td>
</tr>
<tr>
<td><strong>PGP9.5 Small nerve fiber (C fiber)</strong></td>
<td>Polyclonal, RA95101, Ultra Clone Ltd., UK</td>
<td>No pre-treatment</td>
<td>1:1000 30min/RT</td>
<td>EnVision Detection Systems Peroxidase/DAB, K5007, Agilent Technologies, USA</td>
<td>LabVision</td>
<td>Linear density of IENFs, overall stromal density of nerve bundles</td>
</tr>
<tr>
<td><strong>NF2F11 Light chain neurofilament</strong></td>
<td>2F11, M0762 Agilent Technologies, USA</td>
<td>Tris-EDTA (pH 9.0), S2367, Agilent Technologies, USA</td>
<td>1:200 30min/RT</td>
<td>EnVision Detection Systems Peroxidase/DAB, K5007, Agilent Technologies, USA</td>
<td>LabVision</td>
<td>+ / -; overall stromal density of nerve bundles</td>
</tr>
<tr>
<td><strong>NGF Nerve growth factor</strong></td>
<td>Polyclonal, SC-548, Santa Cruz Biotechnology Inc., USA</td>
<td>EnVision FLEX Target Retrieval Solution (pH 6.1) K8005, Agilent Technologies, USA</td>
<td>0.5 mg/ml ON/4°C</td>
<td>MACH 4™ Universal AP Polymer Kit, M4U536, Biocare Medical Inc., USA</td>
<td>Manual</td>
<td>Mean number of staining positive cells</td>
</tr>
</tbody>
</table>

1 A single number score of the overall cell density both in the epithelium and stroma for each section (from one to three, 1 = low density, less than 50 cells/high power field (HPF x40 objective); 2 = moderate density, 50 – 100 cells/HPF; 3 = high density, more than 100 cells/HPF).  
2 The mean number of identified positive cells per visual field (HPF x40 objective) calculated from 2 – 4 HPFs.  
3 Number of PGP9.5-positive fibers / mm of epithelial outer surface,  
4 + = presence of NF2F11-positive fibers, - = absence of NF2F11-positive fibers.  
5 Positive immune cells per visual field (HPF x20 objective) were counted from 2-4 HPFs, each representing one of the 4 different types of areas (1. areas with increased B cell infiltration without intraepithelial nerve fibers (IENF), 2. areas without increased B cell infiltration with IENFs present, 3. areas with both increased B cell infiltration and IENFs, and 4. areas lacking both B cell infiltration and IENFs)  
CD = Cluster of Differentiation, PGP = Protein Gene Product, NF = neurofilament, CC = cell conditioning, RTU = ready to use, RT = room temperature, ON = overnight
7.7 Statistics

The data were analyzed using Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL, USA and SPSS versions 20 and 22; IBM corporation, Armonk, NY, USA).

Comparisons were within-group comparisons in Study I. In Study II, comparisons were made between surgically treated and conservatively treated patients. Power analysis of the difference in VAS for dyspareunia at follow-up indicated that a minimum of 24 women was needed in both groups to detect a clinically relevant 20% difference between the groups ($\alpha = 0.05$ and power $(1- \beta) = 0.8$). Studies III and IV included comparisons between patients and controls and within-group comparisons. Since the data did not follow normal distributions, we used non-parametric tests for comparing continuous data (Mann-Whitney U-test or Kruskall-Wallis test and Wilcoxon signed rank test). Results for continuous variables were reported as medians with minimums and maximums, except the VAS scores, which were reported as medians with interquartile ranges (IQR 25 %–75 %). Categorical data were compared with $\chi^2$ analysis or Fischer’s exact test. ORs with 95% CIs were calculated when appropriate. For correlations, Pearson’s and Spearman’s correlation tests were used. A two-tailed $p$-value < 0.05 was considered significant in all analyses.
8 RESULTS

8.1 LPV TREATMENT OUTCOME (Studies I and II)

8.1.1 Patient characteristics

At baseline, the median age of surgically treated patients was 24.5 years (range 16-48) and conservatively treated patients 23.5 years (range 18-32) \( p = 0.35 \). Most of the patients in both groups were nulliparous. The percentage of primary or secondary LPV was similar in both groups. Patients in the surgery group reported slightly higher baseline VAS for dyspareunia than patients in the conservative treatment group \( 9.0 \ [8.0 - 10.0] \) vs. \( 8.0 \ [8.0 - 9.0] \), \( p = 0.046 \). Otherwise the groups were identical. In addition to provoked pain, 26.3\% of patients in the surgery group reported spontaneous vulvar pain at baseline (no data for the conservative treatment group existed). During the conservative treatment period, the algorithm-based treatment modalities were equally commonly used in both groups (Table 5). No clinically significant differences between the consenting patients and the dropout patients or between the consenting patients and the selected patients in any of the studies were found. The median age of the patients in the immunohistochemistry studies was 27 years (range 18 - 48) and that of the controls 30 years (range 24 - 44) \( p = 0.017 \).
Table 5. Conservative treatment modalities used by study group

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Vestibulectomy N=39</th>
<th>Conservative treatment, N=27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discontinuation of COCs</td>
<td>9 / 25 (36.0)</td>
<td>12 / 17 (70.5)</td>
<td>0.094</td>
</tr>
<tr>
<td>Physical therapy</td>
<td>10 / 30 (33.3)</td>
<td>15 / 6 (57.7)</td>
<td>0.067</td>
</tr>
<tr>
<td>Long-term antifungal treatment</td>
<td>17 / 35 (48.6)</td>
<td>11 / 26 (42.3)</td>
<td>0.627</td>
</tr>
<tr>
<td>Submucosal or topical corticosteroids</td>
<td>18 / 31 (58.1)</td>
<td>9 / 26 (34.6)</td>
<td>0.077</td>
</tr>
<tr>
<td>Topical podophyllotoxin</td>
<td>19 / 31 (61.3)</td>
<td>19 / 26 (73.1)</td>
<td>0.347</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>13 / 31 (41.9)</td>
<td>7 / 21 (33.3)</td>
<td>0.532</td>
</tr>
</tbody>
</table>

Duration of the conservative treatment period before opting for surgery

<table>
<thead>
<tr>
<th></th>
<th>Vestibulectomy N=39</th>
<th>Conservative treatment, N=27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.0 (2–55) / 27</td>
<td>16.0 (3–129) / 22</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Values are medians (minimum - maximum) / number of data available for continuous variables, medians (IQR 25%–75%) for VAS values, and number of cases / data available (percentage) for categorical variables

1 Application of podophyllotoxin 5mg/ml (Wartec®, Glaxo Smith Kline) on tender points of vestibular mucosa following 5% acetic acid application, repeated three times every four weeks,
2 Amitriptyline 10 mg–50 mg, dose titrated on the basis of pain relief and tolerance
COC = combined oral contraceptive

8.1.2 Short-term well-being after surgery

Vestibulectomy was performed as day surgery in 80% of the cases. Actual short-term or immediate complications occurring less than one month after surgery were found in 21.4% of the cases (Table 6). Surgery was required to manage 5.7% of the post-operative bleedings. The results of the consenting and dropout patients did not differ significantly. However, Bartholin's duct cysts, occurring at 4, 16, and 24 months post-surgery, were more common in the dropouts. All Bartholin's duct cysts were managed by day surgery with no long-term sequelae.
Table 6. Short-term well-being after vestibulectomy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N = 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of operation, minutes</td>
<td>45 (20–90) / 58</td>
</tr>
<tr>
<td>Short-term complications</td>
<td>15 / 69 (21.4)</td>
</tr>
<tr>
<td>Postoperative hematoma</td>
<td>6 (8.6)</td>
</tr>
<tr>
<td>Wound pain or infection</td>
<td>11 (15.7)</td>
</tr>
<tr>
<td>Bartholin’s duct cyst</td>
<td>4/70 (5.7)</td>
</tr>
<tr>
<td>Absence from work, days</td>
<td>10.5 (3–24) / 60</td>
</tr>
<tr>
<td>Duration of wound pain, days</td>
<td>14 (0–90) / 49</td>
</tr>
<tr>
<td>Time to full recovery, weeks</td>
<td>5 (1–25) / 50</td>
</tr>
</tbody>
</table>

Values are medians (minimum - maximum)/ number of data available for continuous variables, medians (IQR 25–75%) for VAS for dyspareunia, and number of cases / data available (percentage) for categorical variables

1 Two patients had both hematoma and infection,
2 At 4, 16, and 24 months post-surgery
3 Data from questionnaire

8.1.3 Long-term well-being after surgery or conservative management

Among surgically treated patients, 53 underwent a face-to-face interview and gynecological examination and 55 returned questionnaires. Of the conservatively treated patients, 27 attended the interview and returned the questionnaires, and 24 were examined. EMG of the pelvic floor was carried out in 27 of the surgically treated patients.

The median follow-up period of the surgically treated patients was 36 (range 5–158) months in Study I and 47 (range 11–114) months in Study II, and 77 (range 34–131) months in the conservative treatment group in Study II.
Patient satisfaction

Overall long-term patient satisfaction was high, both after surgery and after conservative treatment. In both groups, approximately 90% of the patients reported complete or partial response. Complete response was achieved in 13 (36.1%) of the surgery patients and in 7 (25.9%) of the conservatively managed patients. No response was achieved by surgery in 4 (11%) and by conservative treatment in 2 (7.4%) of the patients. No patient in the surgery group and only one patient in the conservative treatment group reported a worse situation than at baseline. Among surgically treated patients, 84.9% were satisfied with the treatment process as whole, compared to 52.4% in the conservative treatment group ($p=0.08$). Up to 94.4% of all surgically treated patients would choose the operation again. Presence of spontaneous pain at baseline was not associated with an unsatisfactory treatment outcome by surgery (“P.T. et al., unpublished data”).

Dyspareunia

The VAS score for dyspareunia decreased 66.7% by surgery, from 9.0 (IQR 8.0–10.0) to 3.0 (IQR 0.6–4.9), $p<0.001$. Conservative treatment decreased dyspareunia by 78.1%, from 8.0(IQR8.0–9.0) to 2.0(IQR0.0–3.0), $p<0.001$ during follow-up. The VAS score immediately after the conservative treatment period was 5.0 (IQR 2.5–7.25) (N=12). VAS decline was similar in both treatment groups in the comparative study, $p=0.407$ (Figure 6).
Figure 6. VAS score for dyspareunia in the two LPV treatment groups at baseline and follow-up

Vestibular tenderness

Among 53 patients from Study I, surgery totally cured the posterior vestibular tenderness in 34 (64.2%) of the patients. Mild tenderness was present in 11 (20.8%) and significant tenderness in 8 (15.1%) of the patients. Anterior tenderness was present in 38 (71.7%) of the patients. This is not surprising since the anterior part of the vestibule is left intact in this procedure. In 12 patients,
anterior tenderness was significant and 3 of them reported no response to surgery, one having significant residual tenderness also in the posterior vestibule. Of the patients with a complete response, 52.9% had anterior swab test tenderness, and in 23.5% it was significant. None of the patients in the complete response group had significant posterior tenderness.

Swab-test results of the comparative study are shown in Figure 7. Noteworthy is the high percentage (62.5%) of patients with significant posterior tenderness after conservative treatment. However, these patients reported VAS scores of 2.0 (IQR 1.0–4.0), while those with significant residual posterior tenderness in the surgery group reported VAS scores of 5.3 (IQR 2.8–7.8), p=0.23.

![Swab-test results graph]

**Figure 7.** Cotton swab test tenderness at follow-up of patients with LPV treated by posterior vestibulectomy (surgery group) or by conservative management (conservative treatment group).

u = urethra  - - - = hymen, LPV = localized provoked vulvodynia

60
A detailed analysis regarding the reasons for non-optimal outcome (defined by the presence of one or more of the following: no response by subjective estimation, follow-up VAS score ≥7, significant tenderness present in the posterior vestibule, or reporting persistent dyspareunia) was performed among the patients in Study I. Only 6 (11.5%) of all patients reported the residual anterior tenderness as the main reason for their non-optimal outcome. In 4 (7.7%) patients, insufficient tissue removal and residual posterior tenderness was the most likely reason for the unsatisfactory outcome by surgery.

**Long-term complications of surgery**

Gynecological examination at follow-up revealed no actual long-term complications of surgery. A Bartholin's duct cyst in one patient had been cured by surgery with no long-term sequelae. Four patients had persistent or intermittent vulvar fissure, but three of the four had had the fissure already at baseline. None of the patients reported problems with surgical scars or with anal continence.

**Pelvic floor muscles**

A physical therapist evaluated the pelvic floor muscles in 27 surgically treated patients. Normal function (resting tone ≤ 10 μV) was found in 14 (51.8%) patients, with equal frequencies across all response groups. No difference in VAS scores or in the subjective long-term response evaluation was found between those who had attended physical therapy sessions during conservative treatment and those who had not.

**Sexual well-being**

More than two-thirds of the patients were sexually active during follow-up. The problem index (PI) score of 8 in Study I correlated well with the reported VAS
score, as well as with the reported intensity score (from 1 to 7) of dyspareunia in the McCoy questionnaire (Pearson's correlation coefficient 0.71, \( p<0.001 \) and 0.77, \( p=0.001 \), respectively). Long-term sexual well-being showed no differences between surgically treated and conservatively treated patients (Table 7). Patients with complete response reported a PI score of 5.0 (range 2.0–8.0) after surgery and 4.0 (range 2.0–7.0) after conservative treatment, \( p=0.304 \).

Table 7. Long-term sexual well-being of patients with localized provoked vulvodynia by study group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vestibulectomy N=39</th>
<th>Conservative treatment N=27</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexually active</td>
<td>30 / 37 (81.1)</td>
<td>18 / 25 (72)</td>
<td>0.402</td>
</tr>
<tr>
<td>Same partner as at baseline</td>
<td>22 / 36 (61.1)</td>
<td>9 / 26 (34.6)</td>
<td>0.106</td>
</tr>
<tr>
<td>Frequency of sexual acts during preceding month</td>
<td>2 (0-20) / 35</td>
<td>4 (0-15) / 24</td>
<td>0.135</td>
</tr>
<tr>
<td>Sexual satisfaction (^1)</td>
<td>22.5 (11-31) / 34</td>
<td>25 (9-29) / 25</td>
<td>0.718</td>
</tr>
<tr>
<td>Partnership satisfaction (^1)</td>
<td>12 (8-14) / 29</td>
<td>12 (7-14) / 18</td>
<td>0.250</td>
</tr>
<tr>
<td>Sexual problems(^1)</td>
<td>7 (2-14) / 34</td>
<td>7 (2-12) / 23</td>
<td>0.432</td>
</tr>
<tr>
<td>Pain problems (^2)</td>
<td>15 / 35 (42.9)</td>
<td>8/24 (33.3)</td>
<td>0.461</td>
</tr>
<tr>
<td>Dryness problems (^2)</td>
<td>19 / 34 (55.9)</td>
<td>11 / 23 (47.8)</td>
<td>0.550</td>
</tr>
<tr>
<td>Arousal problems (^2)</td>
<td>7 / 34 (20.6)</td>
<td>6 / 24 (25.0)</td>
<td>0.692</td>
</tr>
</tbody>
</table>

Values are medians (minimum - maximum) / number of data available for continuous variables and number of cases / data available (percentage) for categorical variables

\(^1\) Index in McCoy instrument

\(^2\) Pain, dryness, or arousal difficulty interfering with more than half of the sexual acts or occasions of intercourse
Mood, quality of life and co-morbid conditions

General health was quite satisfactory in both groups. EQ5D-VAS scores were 80 (48–100) after vestibulectomy and 82.5 (40–100) after conservative treatment, \( p=0.26 \). BDI scores for depression were low and the availability of social support similarly sufficient in both groups. No differences were found between the groups regarding other co-morbid pain conditions. The only difference found was that patients who benefitted from conservative treatment reported atopic skin problems more often than patients who needed surgery (46.2% vs. 14.3%, respectively, \( p=0.009 \); OR 0.2; 95% CI 0.1–0.7). Parity during follow-up was similar in both groups, 40.5% in the surgery group and 42.3% in the conservative treatment group (\( p=0.09 \)). Only one of the patients had delivered by cesarean section after surgical or conservative treatment during follow-up, all others had delivered vaginally.

8.2 VESTIBULAR MUCOSAL CHARACTERISTICS (Studies III, IV)

8.2.1 Characteristics of the immune system in the vestibular mucosa

Immune cells

A thorough analysis of different immune cell types in the vestibular mucosa revealed that all immune cells needed to initiate a cell-mediated immune response were present (Table 8). Dendritic cells (DC) are antigen-presenting cells and have an important role in initiating the immune response. DCs capture foreign antigens with distinct receptors on their surfaces, especially on their dendrites, and process them for presentation to T cells. DCs were found in both the stromal and epithelial layers of the mucosa, and were occasionally found to extend their dendrites through the epithelial surface (Figure 8).
T cell staining with the CD3 antibody collectively visualizes both helper (CD4+) and killer (CD8+) T cells. These were expressed in both the stromal and epithelial layers of the mucosa with no significant difference in densities between patients and controls ($p=0.280$).

CD20 antigen is expressed on B cells at most stages of development. CD20-positive B cells were present mainly in the stromal layer of the mucosa. Small amounts were also found in the epithelium in the most populated areas. B cells were more numerous in patients than in controls ($p<0.01$).

### Table 8. Immune cells in the vestibular mucosa

<table>
<thead>
<tr>
<th>Cell type</th>
<th>LPV (n=27) mean (95% CI)</th>
<th>Controls (n=15) mean (95% CI)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic cells</td>
<td>98 (86–110) $^2$</td>
<td>129 (101–157) $^3$</td>
<td>0.053</td>
</tr>
<tr>
<td>Macrophages</td>
<td>23 (17–29) $^2$</td>
<td>19 (15–24) $^3$</td>
<td>0.780</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>121 (97–145)</td>
<td>38 (15–60)</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>41 (31–52) $^2$</td>
<td>11 (8–15) $^4$</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Mast cells</td>
<td>35 (31–40)</td>
<td>33 (26–39) $^4$</td>
<td>0.512</td>
</tr>
<tr>
<td>Germinal centers $^5$</td>
<td>14 (51.9)</td>
<td>0 (0)</td>
<td>$&lt; 0.01$</td>
</tr>
</tbody>
</table>

$^1$ Values are mean cell counts per microscopy field; analyzed from 2–4 fields (x40 objective), $^2$ N = 24–26, $^3$ N = 8–10, $^4$ N = 12–14, $^5$ Number of samples (%) with one or more germinal center.

LPV = Localized provoked vulvodynia, CI = confidence interval.
Figure 8. Dendritic cells in the vestibular mucosa in LPV (CD163 staining). The inset shows a dendritic cell extending its projection through an intact epithelial surface.
Vestibule-associated lymphoid tissue, VALT

In areas with the highest lymphocyte densities, T and B cells formed germinal centers which represent secondary lymphoid tissue. Germinal centers were visualized by both the CD3 (Figure 9a) and CD20 (Figure 9b) antibodies showing the typical zone-wise arrangement of the individual cell types. Thus, we demonstrated that the vestibular mucosa has its own organized immune system that can be called vestibule-associated lymphoid tissue, VALT. The presence of germinal centers and increased densities of B cells, including mature plasma cells (Figure 10), were the major differences between LPV and control samples. This indicates activation of the VALT and implies immune activation in LPV.

Figure 9. Germinal center in the vestibular mucosa in LPV by a, CD3 (T cells) and by b, CD20 (B cells) stainings
8.2.2 Neural tissue and nerve growth factor in the vestibular mucosa

Intraepithelial nerve fibers (IENF)

Protein Gene Product (PGP) 9.5 detects the so-called small nerve fibers, the thinnest unmyelinated sensory C-fibers (less than 1 μm in thickness) which are easily identified by their typical appearance. The linear density of these intraepithelial C-fibers was higher in LPV (6.3/mm; 0.0–15.8; IQR 4.4–9.2) than controls (2.0/mm; 0.0–12.0; IQR 0.0–4.3, \( p=0.006 \)) but did not differ between primary and secondary LPV cases (Figure 11).

Neurofilament (NF) 2F11 detects neuronal axons by labeling the NFs of the cytoskeleton. NF proteins are exclusively expressed in neurons and are essential for myelination of the axons. Thus, nerve fibers visualized by NF2F11 can be interpreted as A-delta fibers. Intraepithelial A-delta fibers were found in 17 (63%) of the 27 LPV cases and in none of the controls (\( p<0.001 \)) with no difference between primary and secondary LPV (\( p=0.627 \)) (Figure 11).
**IENFs in relation to immune activation**

The density of intraepithelial C-fibers in patient samples was greater in the areas with increased B cell infiltration (5.3/mm; 0.0–23.3; IQR 3.0–9.4) than in the areas with no B cells (4.0/mm; 0.0–11.3; IQR 1.0–8.0, \( p=0.057 \)). The density of intraepithelial C-fibers was higher in samples with germinal centers (6.1/mm; 4.3–15.8; IQR 5.0–9.4) than in samples without germinal centers (3.0/mm; 0.0–13.4; IQR 0.0–8.4, \( p=0.020 \)). A positive correlation between the density of intraepithelial C-fibers and the BAI score was found (Spearman’s Rho 0.400, \( p=0.004 \); \( R^2 = 0.128 \)). Glandular epithelium also showed epithelial C-fibers and A-delta fibers with higher densities in glands surrounded by B lymphocyte infiltrates than in glands not surrounded by B lymphocytes. The density of T cells did not show any effect on neuroproliferation.

**Submucosal neural tissue**

In addition to solitary nerve fibers, PGP9.5 or NF2F11 stainings also visualized nerve bundles located in the subepithelial layer of the mucosa. The densities of

---

Figure 11. IENFs (white arrows) in vestibular mucosa in LPV a, PGP9.5-positive C-fibers and b, NF2F11-positive A-delta fibers.
these neural fasciculi did not differ between LPV and controls \((p= 0.110\) and \(p=0.498\)) or between primary and secondary LPV. No association between submucosal neural tissue density and signs of immune activation was found (data not shown).

**Nerve growth factor (NGF)**

NGF has important roles both in acute nociception and chronic pain (216). In samples from LPV patients, the number of NGF-positive immune cells was higher in areas with B cell infiltrates and IENFs (20.0; 1.0–102.0) than in areas lacking both B cell infiltrates and IENFs (4.0; 0.0–24.0), \(p<0.001\). The number of these cells did not differ between LPV and control samples (20.0; 1.0–102.0 vs. 17.5; 0.0–68.0, respectively, \(p=0.906\)). T cell density did not show any effect on the number of NGF positive immune cells (25.0; 1.0–102.0 for the low-density samples and 17.0; 0.0–60.0 for the high density samples, \(p=0.300\)).
9 DISCUSSION

9.1 METHODOLOGICAL CONSIDERATIONS

9.1.1 Study groups

We analyzed a patient series with confirmed diagnoses of LPV. Thorough clinical data of the patients were available, including precise details of the provided treatments. All patients had been managed at the same clinic during a 12 year period from 1995 to 2007. All had been managed according to a uniform treatment algorithm by experienced gynecologists with special knowledge on vulvar diseases. The follow-up evaluation was unbiased, performed by one gynecologist who had not been involved in the treatment process, which further ensures that the findings were objective.

The comparison group (conservative treatment group) to the surgically treated patients was matched by age, details of the individual disease histories, and timing of the treatment. Thus, the groups were identical except that the baseline VAS in the surgery group was slightly higher than in the conservative treatment group. The follow-up evaluation being almost 6 years after treatment among the conservatively treated patients may partly explain this group's fairly low participation (54%) in this study. Typically, LPV patients are young women with frequently changing life circumstances and therefore possibly not easy to reach. The dropout analyses on any of the study groups did not reveal any clinically significant differences between the follow-up attendees and non-attendees.

All of our studies were retrospective and we did not report comprehensive baseline data for outcome measures other than VAS for dyspareunia. For VAS, our baseline data included data from 52 (91.2%) surgically treated and 25 (92.6%)
conservatively treated patients. However, all included patients reported pre-treatment VAS for dyspareunia as high as or higher than 7. It is therefore obvious that their sexual well-being was severely compromised. A quarter of the patients also reported spontaneous vulvar pain of varying degrees before surgery. This was not considered to be of significance since the main complaint among them was the provoked pain.

9.1.2 Evaluation of treatment outcomes

Clinical trials of chronic pain, including LPV research, are recommended to assess outcomes representing six core domains: pain, physical functioning, emotional functioning, improvement and satisfaction with treatment, possible adverse events related to treatment, and participants’ adherence to the treatment (Initiative on Methods, Measurement, and Pain assessment in Clinical Trial, IMPACT) (217,218). We covered all of these requisites in our studies. For pain assessment, we used VAS to define pain intensity during sexual acts, the cotton swab test to evaluate pain in the vestibular mucosa, and evaluation of the frequency of pain was included in the McCoy questionnaire. All cotton swab tests were performed by only one examiner who always used the same technique, which improves the reliability of the comparisons (161). Physical functioning and emotional functioning were measured with validated instruments (EQ5D-VAS, BDI, MOS Social Support Scale, and McCoy) with available referral data from the general Finnish female population. We preferred the McCoy questionnaire for the evaluation of sexual well-being instead of the more widely used FSFI since the McCoy is better adjusted to measure the physical parameters of sexual functioning, which we considered essential in evaluating results of surgery. Improvement of dyspareunia was measured by VAS. The overall patient and treatment satisfactions and patients’ adherence to treatment were assessed with direct questions. Complication analyses served as reports on adverse events.
VAS as an outcome measure

The VAS (or NRS) is a simple, reproducible, and valid indicator of pain severity. The results of Study I further confirmed the validity of VAS in dyspareunia evaluation. VAS scores for dyspareunia after surgery were well in line with our findings of vestibular swab test tenderness and of the overall satisfaction of patients, and correlated well with dyspareunia reports in the McCoy questionnaire. Thus, the VAS score and its change can be recommended as one of the main outcome measures in sexual pain research, which has subsequently also been supported by other authors (42,156,218). A majority of the previous studies on surgical treatment report improvement of dyspareunia as the only long-term outcome and most studies do not define the improvement with an exact measure (21,22). Thus, reporting VAS for dyspareunia and improvement of dyspareunia with an exact VAS difference are notable strengths of our study.

9.1.3 Histological samples

Because of the thorough collection of the patient clinical data, we also could confirm that no treatments that could possibly interfere with the local immune tissue had been administered 6 months before surgery. The control vestibular tissue material from 15 healthy women is the largest amount of control tissue included in any study on vestibular mucosal details. An average 3-year age difference between patients and controls was unlikely to have any effect on the results since all were premenopausal. However, as in many previous studies on vestibular tissue, a challenge was the inevitable size difference between the patient and control specimens. In order to secure appropriate comparisons and to minimize the specimen size bias, the control biopsies were taken exactly from the five o’clock position of the posterior vestibule. This site has proven to be the most representative area of pain in LPV from studies on vulvar pain mapping (26). Also, using the linear density evaluation of the IENFs (numbers per length unit, mm) in Study IV diluted the significance of the size bias. On the other hand, since all of the
substantially large vestibulectomy specimens were viewed in their entirety, no
data were lost. This enabled, for instance, identification of areas with glandular
tissue and evaluation of the glandular epithelial nerve fibers, on which no previous
data existed. Since we used consecutive sections of each tissue block, we were able
to identify the same anatomical structures in all specimens. Thus, a comparison of
findings and combining the data was possible.

**Issues related to immunohistochemistry analyses**

All immunostainings were performed by experienced technicians according to the
manufacturers’ instructions. All antibodies were selected to perform the highest
specificity for each selected antigen to ensure correct identification of the immune
cells and neural elements.

PGP9.5 was selected since it is a well-established neuron-specific marker detecting
the thinnest unmyelinated sensory C fibers with typical appearance (219). PGP9.5
is the gold standard biomarker for IENFs, and is widely used in the diagnostics of
small fiber neuropathies (220). Since S-100 is not specific, we did not use it. In
addition to neural tissue, S-100 also stains dendritic cell extensions (221).

NF2F11 is a highly specific marker for NFs, and the monoclonal 2F11 antibody
detects the light molecular weight (NFL) phosphorylated form of the subunit, the
NFL subunit (70kDa), known to be crucial in the myelination process of the axons
(222). No previous reports on the NF2F11 expression in the vestibular mucosa
exist.

BAI was created to provide a single number index to illustrate the overall B cell
inflammation of each specimen and to serve as a continuous variable in statistical
processing.
9.2 FROM ETIOPATHOGENESIS TO TREATMENT OUTCOME

9.2.1 Etiopathogenesis of LPV

Vestibule-associated lymphoid tissue, VALT

Mucosal surfaces in the human body contain secondary lymphoid tissue, the mucosa-associated lymphoid tissue, MALT. In activated MALT, germinal centers, formed mainly by T and B lymphocytes, play a central role in initiating an immune response against foreign antigens and threats (37). Surprisingly, no previous reports on MALT in the vestibular mucosa exist. Since the vestibule represents one of the body orifices, it is assumed to have an important role in immune defense. Thus, our finding of the VALT can be considered of major significance, and in line with it are the earlier findings that the vulvar vestibular fibroblasts may also have an important role in the immunoinflammatory responses (11,38,64).

Also, our thorough analysis of the vestibular mucosal immune cells is the first ever presentation of the vestibular immunological microenvironment. Our results show that the distribution of immune cells in the vestibular mucosa differs from that of the vaginal mucosa. This difference implies a distinctive role for the vestibule in the immune defense and needs to be studied further (223).

Unlike others (17,18,20,54), we did not find an increased amount of mast cells in LPV. This may be because all patients in our study had a long disease history and mast cells typically appear in the early phase of inflammation (224). Others have previously shown an increased amount of lymphocytes in LPV, but no specific data on elevated B lymphocytes, plasma cells, or germinal centers as signs of immune activation exist. No previous reports on dendritic cells or macrophages exist, either (17,18). In one study, elevated levels of vestibular mucosal CD4-positive helper T cells in LPV were found (51). We could not confirm this, because we used the pan T cell marker CD3 and did not differentiate between the T cell types. Our finding of T cells in similar densities in patients and controls does not, however, exclude the
essential role of T cells in the immune response in the vestibular mucosa and the roles of specific T cell types in VALT are of further interest.

**Immune activation**

The presence of germinal centers is consistent with activation of the immune tissue locally. By nature, the vestibule is continuously and inevitably exposed to foreign antigens during sexual and reproductive life. Thus, both protective and adaptive characteristics of the immune response are required in the vestibule. The immune and neural systems are closely interrelated. While protecting against local infection, the activation of VALT could, in specific situations, result in an exaggerated inflammatory reaction and contribute to the sensitized pain perception in the vestibular mucosa. In LPV, this is possible since women with LPV have a tendency to carry a specific genetic predisposition to disproportionately intense immuno-inflammatory reactions (11,38). The demonstration of activated VALT can therefore be considered as one of the key findings in LPV etiopathogenesis.

**Neuroproliferation**

Previous studies have shown an overall abundance of neural tissue in the vestibular mucosa of LPV patients (225), but only semi-quantitative data on the IENFs have been reported (19). By calculating the linear density of IENFs, we were able to demonstrate an actual increase in the IENFs in LPV. The use of consecutive sections enabled us to re-identify anatomical details in each staining. Thus, precisely locating the areas of increased IENF densities in relation to immune cells was possible.

We found that epithelial neuroproliferation takes place around the areas of B cell infiltration and is associated with VALT. These findings also regarded the innervation of the glandular epithelium. An association between NGF-positive
immune cells, local B cell infiltrations, and neuproliferation was found. This suggests a possible role for NGF as a modulator between immune activation and neuproliferation. NGF has important roles in both acute nociception and chronic pain. NGF is known to induce nerve sprouting and to contribute to the generation of inflammatory hypersensitivity and alldynia (226-228). Inflammatory and immune cells are major sources of NGF (216). However, no difference in the density of NGF-positive immune cells between LPV and controls was found. It may be the difference in the amount of NGF produced by involved immune cells, not the number of cells producing it, that matters. But this needs to be studied further. Furthermore, we showed that NF2F11-positive IENFs, possibly representing nociceptors of myelinated A-delta fibers, were present in LPV but not in controls. No previous reports on A-delta fibers in LPV exist. The significance of this finding remains to be studied further.

The density of submucosal neural tissue did not differ between patients and controls. Similarly, the immune activation did not show any effect on it. Others have previously found higher densities of submucosal neural tissue in LPV than controls (17,110,225). These studies have reported use of the S-100 antibody, which is not specific to neural tissue and also stains dendritic cell extensions. As we showed, dendritic cells are numerous in the stroma and epithelium of the vestibular mucosa. So, the density of submucosal neural tissue may not play an important role in the sensitized pain perception in LPV, but IENFs seem to be of importance.

Our findings further disagree with previous reports suggesting a predominance of the overall neural tissue in primary over secondary LPV and higher densities of lymphocytes in secondary compared to primary LPV (17,110). We did not find any differences in the IENF density or in the density of subepithelial neural tissue between primary and secondary LPV, or any differences in the densities of immune cells between these groups.

Our finding of the increased IENF density in LPV and its association with the activated VALT suggest that immune activation explains the epithelial
neuroproliferation and may contribute to the LPV pain. Furthermore, the increased density of the glandular epithelial nociceptive nerve fibers and the presence of A-delta nociceptors may be of significance. Also, the prolonged inflammation may address a sensitizing effect on the IENFs’ signaling, possibly mediated via NGF (226,227). Possibly, chronic stress and its influence on the neuroendocrine system of the skin may also contribute to both the immune activation and the neuroproliferation. This could result in maintenance of the pain by peripheral and central sensitization (117). Overall, the finding of increased nociceptors in the sensitized mucosa and in the glands of the vestibule justifies surgical removal of the area and partly explains the effectiveness of surgery in the pain control.

9.2.2 Treatment of LPV

We studied the safety and effectiveness of posterior vestibulektomy to treat severe LPV. The reasonably long follow-up period after surgery provides data on the real long-term wellbeing of these patients. In addition, our study population enabled comparison of two LPV patient groups with similar histories regarding disease symptoms and applied conservative treatment modalities, but with different end results, one group ending up in surgical treatment. Several previous studies have shown that single conservative treatment modalities may provide some improvement (171,185,189,190). On the contrary, placebo-controlled studies have shown that the treatment effects are rarely any greater than that achieved by placebo (173,183). However, as long as a treatment benefits a patient without causing any harm, there is no absolute reason to avoid its use. So far, previous studies on the conservative treatment of LPV have been one-dimensional, investigating the efficacy of one treatment modality at a time. The results of the conservative treatment group in our study represent true long-term results of exclusively conservative management with a multidisciplinary approach (Figure 4). Thus, this is the first study with a genuinely multidisciplinary approach including only conservative treatment options with long-term follow-up (229).
Safety of posterior vestibulectomy

The sparse reporting in the literature on the short-term recovery and complications of surgical treatment for LPV emphasizes the importance of our vestibulectomy follow-up study. This certainly is important information to be delivered to patients who consider surgery as their treatment option.

Our results confirm that vestibulectomy mostly succeeds as a day surgery procedure with a rather rapid recovery. However, a one-and-a-half week absence from work was needed. A total short-term complication rate of 21% may be considered high, but most of the complications were minor complications. With surgery in this specific area, the risks of post-operative hemorrhage and wound infection are high because of the rich blood supply to the area and the immediate proximity of the richly-colonized rectum and perineal skin. The rate of 5.7% for post-operative hemorrhage requiring re-surgery of and that of wound infections (15.7%) are acceptable. Furthermore, some cases of wound inflammations may have been overdiagnosed as infections since all were non-febrile and mild. The rate of Bartholin's duct cyst formation (5.7%) is well in line with those reported in other studies (21). Noteworthy is that no complications of surgery with long-term sequelae occurred.

Long-term well-being after vestibulectomy and conservative treatment

The duration of follow-up in our studies surpasses most previous LPV treatment studies (154,156). Although a long follow-up may cause some recall bias, it certainly provides valuable data on the long-term well-being. In a previous study (162) and in our material, neither follow-up nor the degree of patient satisfaction were dependent on the duration of follow-up. Therefore, the 30 months difference in the duration of follow-up between the groups, due to practical reasons, was not
considered significant weakness. Since we used also other outcome measures, in addition to dyspareunia improvement, we provide important additional data.

Effectiveness of posterior vestibulectomy

Posterior vestibulectomy was effective in treating pain. The increased density of nociceptors in the vestibular mucosa rationalizes surgical removal of the area to reduce pain. The posterior vestibule has been proven to be the most painful area in LPV in previous as well as more recent studies (26,203). As with all surgery, the procedure should be extensive enough to cure the problem, but avoid unnecessary risks. Our results prove that the posterior vestibulectomy technique fulfills these criteria. Moreover, previous studies with other techniques, with either wider or more minimal tissue excision, do not report results superior to ours (145,147,148).

The residual anterior tenderness, inevitable in this technique, seems not to play a significant role in the long term. Only one-tenth of the patients reported the residual anterior tenderness as the main reason for their non-optimal outcome. In four patients, insufficient tissue removal and residual posterior tenderness was the most likely reason for the unsatisfactory outcome by surgery. Although the surgeon aims to cover the whole posterior vestibule in this surgery, it is possible that in some cases the peripheral extension is misjudged, resulting in residual tenderness in the posterior vestibule.

Sexual well-being

Surgical removal of mucosal tissue from the vestibule has been criticized for causing problems during sexual acts, beyond the original pain. An unsatisfying appearance of the perineum after surgery, sensitive scar tissue, and reduced lubrication are the most common complaints (148,207).
Our results show that dryness during sexual acts was not a problem more often after surgical treatment than after conservative treatment. The McCoy PI of 7 at follow-up found in both treatment groups suggests that surgery does not cause any additional harm in terms of painful scar tissue or excessive dryness. Also, a PI of 7 is only slightly higher than the PI score of 4.5 in a group of women with menorrhagia in an other Finnish study (137), a population with presumably less sexual problems than LPV patients. Moreover, the most important part of the arousal-related moisturizing derives from the increased blood flow of the vulvar and vaginal mucosa and not from the vestibular glands (35), which is supported by our results.

Both treatment groups also report equally good sexual satisfaction and partnership satisfaction evaluations in the long term, very similar to those reported by women with menorrhagia (137). In fact, sexual satisfaction evaluations of our patients are unusually high, since LPV is typically associated with reduced sexual satisfaction but not with reduced partnership satisfaction among patients and their partners (132,230). Noteworthy is the high percentage of parous women in both treatment groups.

**Patient satisfaction**

Overall patient satisfaction is an important outcome measure of treatment. A patient’s subjective evaluation of the outcome is most likely a combination of several separate outcome measures, including at least their experience of dyspareunia and quality of sexual life. Around 90% of surgically treated patients reported overall satisfaction with the treatment result (complete or partial response), in line with previous reports on surgical treatment (194,203,207). Interestingly, in our study the long-term satisfaction rates after conservative treatment were equally good. This result supports the current comprehension of the multidisciplinary care to be recommended in LPV management (152,153). A very recent study on LPV patients showed that what patients want is on-site multidisciplinary care (23).
Of interest is the slight difference between the groups in satisfaction with the treatment process as whole. The reason for this remains unclear. It is possible that the treatment result at short-term after conservative treatment was not satisfying enough (VAS 5.0) and improved only after a longer follow-up. The required duration of the conservative treatment period to show efficacy was slightly longer among those who chose only conservative treatment than those who chose surgery. The dissatisfaction with the treatment process might arise from the elongated duration of pain. However, since the long-term well-being from several measures was similar after both treatment policies, our results suggest a recommendation of 16 months for the conservative treatment to be continued before counseling the patient on surgery.

**Choice of treatment**

Previous studies have reported higher rates of poor outcome by surgery in patients with primary rather than secondary LPV and in patients who, in addition to provoked pain, have spontaneous vulvar pain at baseline (204,209,231). Our surgery results did not confirm these findings. Similarly, we did not confirm an earlier finding of inferior treatment response in patients who reported several other pain conditions (84). Thus, primary LPV or spontaneous vulvar pain and other concomitant pain conditions cannot be considered as contraindications for surgery.

In order to identify factors that could predict response to conservative treatment, we analyzed whether clinical differences existed between the two patient groups showing differing responses. Interestingly, atopic skin problems predicted a favorable response to conservative treatment. This might be explained by specific immunological etiopathogenetic background factors of LPV in these patients, but this requires further study. It is also possible that some patients – those refractory to conservative treatment modalities – may represent a specific subtype of LPV.
and would benefit from early surgery. With a better understanding of the LPV pain mechanism, individualization of the treatment may be possible.

Noteworthy is that our results of the patients in the surgery group do not exclusively represent results of the surgery, since conservative treatment had been initially administered to all patients. In some patients with severe symptoms, intense pain may, however, prohibit the application of other treatment modalities before surgery. In our study, this might explain that a lower percentage of patients in the surgery group than in the conservative treatment group had attended physical therapy sessions. These patients may be counseled immediately for vestibulectomy, without a period of conservative treatment attempted. In these cases a need for any further treatment, such as physical therapy or psychological and sexual counseling, should be evaluated after surgery.

Previous findings suggest that pelvic floor muscle dysfunction is the main reason for residual dyspareunia after surgery (208). In our study, all patients in the surgery group, irrespective of the outcome, had a similar ability regarding voluntary pelvic floor muscle relaxation after surgery. Although physical therapy did not show direct effect on the outcome, the whole process of physical therapy, including counseling and psychological support, augments the healing process, and is an essential part of multidisciplinary management.

A recent committee opinion on vulvodynia still recommends surgery only after nonsurgical treatment options have failed (42). Our results do not disagree, but emphasize that conservative treatment should follow a multidisciplinary algorithm.
10 CONCLUSION

Activated VALT and its association with the increased IENF density in LPV suggest that immune activation explains the epithelial neuroproliferation and may play an essential role in the altered pain sensation in LPV. Our findings thus further support the idea that inflammatory pathways initially have an essential role in the generation of LPV pain and that other associated factors may have an effect on the maintenance of pain. The idea that inherent factors associated with the vulvar vestibule have an impact in the disease is further supported by the striking effect of surgery.

We showed that there are patients who benefit from algorithm-based multidisciplinary conservative management. We also showed that patients refractory to conservative treatment benefit from posterior vestibulectomy, and that patients after both treatment policies report similar long-term satisfaction. Recently, evidence on the efficacy and desirability of the on-site multidisciplinary LPV management programs has increased. Hence, we conclude that multidisciplinary conservative management should be initially offered to all LPV patients, and that posterior vestibulectomy is rational, safe and effective in patients refractory to conservative treatment. The results of this study have enabled us to further develop our management algorithm (Figure 12).
Localized provoked vulvodynia (LPV)

Patient counseling
COC withdrawal
Vulvar care measures
Physical therapy: Bio-feedback, sexual counseling

6–12 months

Prolonged therapy with antifungals

VAS ≥ 7

Neuromodulatory pharmacotherapy

6 months

VAS < 7

Counsel for vestibulectomy

VAS ≥ 7

VAS < 7

F/U

1 p.o. fluconazol 150mg 1-2x/week for 2-6 months
2 topical lidocain 5% 4-5 x/day for 8 weeks or overnight, p.o. amitriptyline 10-150 mg, s.m. corticosteroid-anesthetic (Celeston® 2ml +bupivacain 3 ml) 3 - 4 x every 4 weeks topical gabapentin 4-6% 3 x /day for 8 weeks, topical podophillotoxin 5mg/ml 3 x every four weeks

Figure 12. Updated treatment algorithm of localized provoked vulvodynia
COC = Combined oral contraceptives, VAS = Visual analogue scale, F/U = follow-up, p.o. = per oral, s.m. = submucosal
11 FUTURE PROSPECTS

Our finding of VALT activation in LPV challenges future research of LPV pain mechanisms to focus on the interplay between the immune and neural systems. Also, details of the functions of individual immune cells in VALT are of interest. Further research is needed to find triggers of pain development other than fungal antigens. Additionally, other factors that might modulate the interaction between activated immune cells and pain biomodulators should be identified. Estrogen, a known biomodulator of inflammation, is one potential player in this pain pathway. Matrix metalloproteinase enzymes, in addition to being modulators of inflammation, might also play a role in neuroproliferation. The influence of anxiety and chronic stress on the immune response of the vestibular mucosa and on LPV pain maintenance is also of interest.

There is still a need for randomized trials on individual medical treatment options. The emphasis should be on agents that utilize the interaction of nociceptors and immunocompetent cells. Since multidisciplinary on-site management programs have proven effective, cost-effectiveness should also be evaluated. Furthermore, ongoing vulvodynia research is also important for the education of healthcare professionals as well as the general population.
12 ACKNOWLEDGEMENTS

This study was conducted at the Department of Obstetrics and Gynecology in Helsinki University Hospital between 2006 and 2016. I wish to express my gratitude to the former and present academic Heads of the Department, Professors Jorma Paavonen, Oskari Heikinheimo, and Juha Tapanainen, as well as the former and present administrative Heads of the Department, Professor Maija Haukkamaa, Adjunct professor Jari Sjöberg and Professor Seppo Heinonen for providing excellent working environments.

I owe my deepest and most sincere gratitude to my supervisors, Professor Jorma Paavonen and Adjunct professor Leila Unkila-Kallio. In addition to the scientific inspiration they have provided me with, I am sincerely grateful for the feeling of a special friendship that has developed between us over these years of working together.

I am grateful that Jorma originally evoked my interest in this issue. He has been a supervisor with the great wisdom to not press me too hard during any phases of the project. He has an exceptional talent in always seeing the essential elements of an issue, and his skills as a text editor are unique. I have always admired his endless enthusiasm and energy. He has also relentlessly supported me and encouraged me forward into the international network of vulva-related research, which has also been a great benefit for this study. Leila’s skills as a supervisor are indeed exceptional. In addition to her outstanding knowledge in biostatistics and her great experience with clinical issues, her supply of enthusiasm and the ability to keep up the most supportive attitude are neverending. Despite the major struggles she had in her personal life during some years of this project, she never got tired of my endless questions, and I could always rely on her support. Without her contribution, this study would never have been completed.
The official reviewers Adjunct professor Maija Haanpää and Adjunct professor Satu Suhonen are sincerely thanked for their professional and careful review of the thesis and for their invaluable comments that greatly improved this study. I particularly enjoyed the conversations with them. Satu Suhonen and Professor Aila Tiitinen are thanked for being the follow-up group for my doctoral studies.

I am sincerely grateful for my other co-authors, Professor Seppo Meri, Professor Eija Kalso, Adjunct professor Ralf Bützow, and Adjunct professor Anders Paetau. They all were, without hesitation, always ready to share their exceptional expertise and valuable time with me. Without their contribution the world of immunology, pain signaling and histopathology would have remained encrypted for me and this work would never have been possible.

I also want to thank Professor Timo Sorsa, who from outside the field of gynecological science, recognized the possibilities of the etiopathogenetic studies and encouraged me forward into the world of immunohistochemistry. I am grateful for our frequent and inspiring conversations, often together with Adjunct professors Jaana Hagström and Taina Tervahartiala, that convinced me of the rationale for many future prospects. Their expertise and continuing collaboration will be essential for my future research plans to take shape. Also Maarit Mentula MD,PhD and Medical student Emmiina Nevalainen, are warmly thanked for taking the responsibility of some of the future projects.

Colleagues, Päivi Härkki, Arto Leminen, Tomi Mikkola, Pontus Molander, Päivi Pakarinen, Juhani Toivonen and Juhani Vartiainen are warmly thanked for their support and their co-operation in helping to organize the collecting of the patient and control samples. The staffs at the operative theatre and the ward for surgical patients in the Women’s Hospital and Jorvi Hospital deserve also my appreciation for their co-operation.

I also thank Mia Kero, Head of the Immunohistochemistry Department of HUSLAB, and Kristiina Nokelainen, laboratory technician at Biomedicum research laboratory, for their excellent expertise and help with immunohistochemistry
issues. Physical therapists Vuokko Jernfors and Soile Rekonen are also thanked for contributing to this study. I owe special thanks to our devoted research nurses Pirkko Timonen, Minna Kaiponen and Teija Karkkulainen for their invaluable contribution in organizing the study patient visits and keeping the patient registries up-to-date, and secretaries Pia Nevalainen, Nina Nyholm and Maaria Puupponen who always kindly helped me with many kinds of issues related to publishing and employment. Alyce Whipp, MPH is warmly thanked for her careful and rapid linguistic review of the thesis text.

I want to thank all fellow researchers for their friendship, support and inspiration. Especially Mervi Halttunen-Niemen, Pekka Nieminen, Minna Tikkanen, Pauliina Tuomikoski, Päivi Galambosi, Hanna Hautamäki, Marja Kaijomaa, Kirsi Palva, and Leena Rahkonen. I owe special thanks to Pia Villa who, during this work, became a very dear friend of mine, hopefully for life. Also, I warmly thank Maija Jakobsson having been the best possible travel companion when attending many joint international congresses.

I owe my deepest gratitude to all colleagues whom I had the honor and pleasure to work with during my specialization in obstetrics and gynecology in Jorvi Hospital and in the Women's Hospital in the 1990s, especially Professor Markku Seppälä for providing such an inspiring atmosphere and Adjunct professor Pauli Kajanoja, my dear tutor. My sincere thanks also go to Maarit Angervo, Hille Erola, Päivi Härkki, and Riitta Meri, colleagues who have been my dear friends ever since the time of our specialization. You have always been there for me and brought support and joy to my life.

I want to thank my dear friends from many years back, Leena-Maija Aaltonen, Eeva Furman, Marjo Hakka-Kemppinen, and Johanna Mattson, who are also deeply involved in the academic world and hence are extremely occupied themselves, and also all other friends of mine for their support and friendship during these hectic years of struggling with my research.
I thank all of the patients who devoted their time to participate in this study and made this study possible. I also thank those other patients who, year after year, come to see me at my private practice, rely on me as their doctor regarding their most intimate issues and let me help them, hence making my work meaningful.

Finally, I wish to express my gratitude to my family. My late father provided me with the greatest persistence and relentlessness; my mother has always tried to teach me patience and tolerance. I thank my sister for just being my sister. I thank my partner, Veli, for his love and patience, his priceless help with computer issues, and his always endlessly supportive attitude. Last but far from least, I want to thank my daughter Nea. I thank her for being such a great daughter to love and to be proud of, and for being such a good friend of mine during the many years it was just the two of us. I hope that I have, with this thesis project, been able to inspire her in her future life perspectives.

This study was financially supported by Helsinki University Hospital (Research Grants TYH7249, TYH2011128, and TYH2013308), Helsinki University (Dissertation Completion Grant, Chancellor’s Travel Grants), the Finnish Medical Foundation, and The Finnish Society of Pediatric and Adolescent Gynecology.

Helsinki, April 20th 2017

Päivi Tommola
13 REFERENCES


(39) Skene AJC. Treatise on the disease of women. 1888.


(214) Sculpher MJ, Dwyer N, Byford S, Stirrat GM. Randomised trial comparing hysterectomy and transcervical endometrial resection: effect on health related


Long-term follow up of posterior vestibulectomy for treating vulvar vestibulitis

PÄIVI TOMMOLA, LEILA UNKILA-KALLIO & JORMA PAAVONEN

Department of Obstetrics and Gynecology, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland

Key words
Dyspareunia, surgical treatment, sexual problems, vestibulitis, vestibulectomy, vulvar pain

Correspondence
Päivi Tommola, MD, Department of Obstetrics and Gynecology, University Hospital Helsinki, PO Box 140, 00290 Helsinki, Finland. E-mail: päivi.tommola@kolumbus.fi

Conflicts of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Abstract
Objective. To evaluate the safety and the effectiveness of posterior vestibulectomy in the treatment of vulvar vestibulitis syndrome. Design. A retrospective cohort study. Setting. University Hospital, tertiary referral center. Population. Seventy women treated by posterior vestibulectomy for severe vulvar vestibulitis syndrome during 1995–2007 at the Department of Obstetrics and Gynecology, University Hospital, Helsinki. Methods. All operated women were invited to a long-term follow-up study. Patient characteristics, baseline visual analog scale (VAS) for dyspareunia and data from the postoperative period were collected. Of the 70 women, 57 attended the follow-up visit including face-to-face interview, gynecological examination with swab-touch test for vestibular tenderness, current VAS score for dyspareunia and McCoy questionnaire for sexual problems. Main Outcome Measures. Short-term and long-term complication rates, dyspareunia by VAS score, vestibular tenderness, sexual problem index and overall patient satisfaction. Results. Ninety-one per cent were satisfied with the outcome. The VAS for dyspareunia decreased from a median of 9 to 3 (66.7% decrease; p < 0.001). Posterior vestibular tenderness was absent in 34 patients (64.2%). Six (8.6%) patients developed postoperative bleeding and 11 (15.7%) mild wound infection. Bartholin’s cysts occurred in four (5.7%) patients. Conclusions. Posterior vestibulectomy is effective and safe in the treatment of severe vulvar vestibulitis syndrome and provides long-term patient satisfaction.

Abbreviations: IQR, interquartile range; ISSVD, International Society for the Study of Vulvovaginal Disease; SPSS, Statistical Package for Social Sciences; VAS, visual analog scale; VVS, vulvar vestibulitis syndrome

Introduction
Vulvar vestibulitis syndrome (VVS) is a subset of vulvodynia. It causes vulvar pain and dyspareunia, ruining the sexual life of young premenopausal women (1,2). Vulvar vestibulitis syndrome is characterized by severe pain in the vulvar vestibule provoked by touch or attempted vaginal entry. Prevalence rate up to 15% in general gynecologic practice population has been reported (3). The International Society for the Study of Vulvovaginal Disease (ISSVD) defines VVS as localized, provoked vulvodynia (4).

Treatment of VVS is challenging because the etiology is unknown. Treatment guidelines are mostly based on anecdotal clinical observations or uncontrolled data from case series (5,6). Surgery as a treatment option for severe VVS was first documented by Woodruff et al. in 1981 (7). Modifications of this original operation have been developed (8). However, studies on surgical treatment have been criticized for being non-randomized, having poor outcome definitions and lack of data of complications. In most studies, success is defined only by patients’ self report, with data collected by postal or telephone interviews. Here we report systematic short-term and long-term outcome of surgical treatment of severe VVS.

Material and methods
The study group consisted of 70 women who had undergone vestibulectomy during 1995–2007 in Helsinki University Central Hospital, Department of Obstetrics and Gynecology, Vulva Clinic. Data on preoperative symptoms, clinical status, short-term recovery and complications were collected from the hospital charts. All women were invited to a
follow-up visit in the same clinic. The local Ethical Committee had approved the study.

The Vulva Clinic is a tertiary referral center for patients with vulvar conditions, including vulvar vestibulitis. All study patients were managed according to a predefined algorithm, which is in line with the current vulvodynia treatment guidelines (5). The diagnosis of VVS was based on Friedrich’s classical criteria: pain on vestibular touch or attempted vaginal entry causing dyspareunia and positive swab-touch test of the vestibular gland openings with or without erythema of the involved mucosa (9). Swab-touch test result was recorded by using categories ‘significant’ (verbal expression of intense pain or expression of pain by sudden move), ‘mild’ (verbal expression of mild pain) or ‘none’ (no pain). Dyspareunia was recorded by visual analog scale (VAS) from 0 (no pain) to 10 (worst possible pain; 10). Dermatological conditions were ruled out by histopathological examination of punch biopsies when appropriate, and vaginal infections were ruled out by bedside wet-mount microscopy. Patients were also referred to a physical therapist. These visits included both electrostimulation to activate pelvic floor muscles and to relieve pain, and biofeedback therapy for better control and voluntary relaxation of pelvic floor muscles. Education was provided to relieve penetration pain and fear of intercourse. Patients who did not respond to the conservative management but continued to report VAS score 7 or more were counseled for vestibulectomy.

Vestibulectomies were performed under general anesthesia by three senior gynecological surgeons. A modified posterior vestibulectomy technique was used (Figure 1). Briefly, two parallel incisions with cutting electrocautery were made in the posterior vestibule reaching from 2 to 10 o’clock after infiltrating the submucous layer with lidocaine–adrenaline 0.05% solution. The inner incision line was made just inside the hymenal ring and the peripheral incision to the Hart’s line. The posterior vestibular mucosa was then excised by superficial skinning vestibulectomy using cutting electrocautery. The vaginal mucosa was undermined, leaving tissue bridges for adequate blood supply. The denuded area was covered with the undermined vaginal mucosa. The wound was closed with interrupted stitches using 2–0 absorbable Dexon®. A single dose of intravenous metronidazol (500 mg) followed by 400 mg three times daily for three days was given. Postoperative pain was managed with non-steroidal anti-inflammatory drugs (ibuprofen 400–600 mg or paracetamol 1g three times daily). Showering of the wound frequently was advised. The patients were told to avoid physical distress for three weeks. Sick leave was recommended for 7–21 days on an individual basis. Routine follow-up visits took place at one and two months. At the first visit the women were encouraged to perform daily dilatation therapy with a vaginal probe, and resumption of intercourse was permitted.

All women were invited by letter to participate in a long-term follow-up study. The follow-up visits were conducted during September 2005 and May 2008 by one senior gynecologist (P.T.), who had not been involved in the preoperative management or operations. At the follow-up visit the women were interviewed face to face using a structured questionnaire. They estimated dyspareunia by VAS score. Their current general health and the effect of vestibulectomy on symptoms, as well as their preference for choosing the same operation again were reviewed. Thereafter, a thorough gynecological examination was carried out. Vestibular tenderness was evaluated with a swab-touch test separately in the anterior (from 11 and to 1 o’clock) and posterior region (from 10 to 2 o’clock) with (equal) light touch administered each time. The test result was documented as described above. A follow-up visit to a physical therapist for evaluation of pelvic floor muscle function (Pelvimed 932, Enraf Nonius B.V., Rotterdam, the Netherlands; Periform vaginal probe, Patterson Medical Ltd., Nottinghamshire, UK) was offered. Resting tone value 10μV or less was defined as good ability in voluntary relaxation. The McCoy questionnaire for sexual well-being (11) was completed. To assess sexual problems...

Figure 1. (A) Modified posterior vestibulectomy technique. (B) Postoperative appearance, immediately (left) and after two months (right).
we used a sexual problem index, a calculated sum of reported frequency of dryness during sexual acts and frequency of dyspareunia (scale from 1 to 7 representing never to always; 12). Frequency of sexual acts during the preceding one month was recorded. The data were analyzed by Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL, USA). We report medians with interquartile range (IQR 25–75%) for visual analog scale (VAS) values, and numbers of cases/data available (percentage) for categorical variables.

Table 1. Baseline characteristics and short-term recovery after vestibulectomy (n=70).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at operation (years)</td>
<td>25.5 (18–50/70)</td>
<td></td>
</tr>
<tr>
<td>Birth control pill use at onset of symptoms</td>
<td>48/67 (68.6)</td>
<td></td>
</tr>
<tr>
<td>Parous</td>
<td>7/69 (10.1)</td>
<td></td>
</tr>
<tr>
<td>History of psychiatric problems</td>
<td>7/67 (10.4)</td>
<td></td>
</tr>
<tr>
<td>History of dyspareunia (years)</td>
<td>4.0 (1–18)</td>
<td></td>
</tr>
<tr>
<td>Baseline VAS score</td>
<td>9.0 (8.0–10.0/64)</td>
<td></td>
</tr>
<tr>
<td>Median operation time (minutes)</td>
<td>45 (20–90/46)</td>
<td></td>
</tr>
<tr>
<td>Short-term complications</td>
<td>15/70 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Postoperative hematoma</td>
<td>6 (8.6)</td>
<td></td>
</tr>
<tr>
<td>Wound pain or infection</td>
<td>11 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Bartholin's cyst</td>
<td>4/70 (5.7)</td>
<td></td>
</tr>
<tr>
<td>Primary sick leave (days)</td>
<td>10.5 (3–24/62)</td>
<td></td>
</tr>
<tr>
<td>Duration of wound pain (days)</td>
<td>14 (0–90/49)</td>
<td></td>
</tr>
<tr>
<td>Time to full recovery (weeks)</td>
<td>5 (1–25/50)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are medians (minimum–maximum)/number of data available for continuous variables, median (interquartile range 25–75%) for visual analog scale (VAS) values, and numbers of cases/data available (percentage) for categorical variables.

* Two patients had both hematoma and infection.

Results

Clinical characteristics of the study population are given in Table 1. Most women were relatively young nulliparas with a long history of severe VVS.

Short-term recovery and complication analysis

Data on short-term recovery and complications are shown in Table 1. Vestibulectomy was performed as day surgery, but 14 (20%) women needed an overnight stay because of pain, nausea, bleeding or difficulty in voiding. Half of the operations were performed in 45 minutes or less. Postoperative bleeding occurred in six women, of whom four (5.7%) required surgery. Eleven (15.7%) patients visited the outpatient clinic for postoperative pain or symptoms consistent with non-febrile wound infection or inflammation, two with hematomas and received a combination of oral cephalosporin and metronidazole for one week. Thus, any adverse effect with revisit occurred in 15 (21.4%) patients. Unilateral Bartholin’s cyst was detected in four (5.7%) women four, 16 and 24 months after surgery. All four were managed by day surgery.

Long-term follow up

Of the 70 women invited to attend the long-term follow up, 57 (81.4%) consented. The only difference between the 57 attendees and 13 non-attendees was history of psychiatric problems (5.4 vs. 36.4%, p<0.005). Fifty-three (93.0%) women were interviewed and examined, and 35 returned the questionnaire. Two of those examined did not return the questionnaire. Four patients returned the questionnaire only, and two of them participated in a structured telephone interview. The follow-up visit took place after a median of 36 months (range 5–158 months), at the age of 29 years (range 20–57 years). Of the women, 39 (68.4%) were married or cohabiting and 35 (63.6%) had the same partner as before. An additional five women had a regular sexual relationship and 11 (20%) were single. Women living in a relationship had a median of two (range 0–20) sexual acts during the preceding month. Eight (14.5%) women had delivered vaginally after vestibulectomy.

Topical anesthetic for dyspareunia was used by seven (13%) of 51 women. Fourteen (25.9%) women reported some degree of constant pain without touch. This did not depend on the duration of symptoms before treatment (p=0.573). Two (3.7%) women used oral amitriptyline alone or in combination with pregabalin. A second vestibulectomy operation had been performed on two women, both after four and a half years, one in the anterior vestibule with a Bartholin’s cyst extirpation and the other in the posterior vestibule.

The median VAS score for dyspareunia at follow up was 3.0 (IQR 0.6–4.9), significantly lower than the baseline score of 9.0 (IQR 8.0–10.0; p<0.001; Figure 2). The decrease in VAS score was 50% or more in 36 (69.2%) women and less than 15% in three (5.8%) women. The VAS score remained unchanged in two (3.8%) women, but no woman reported an increase in VAS score. Follow-up VAS was not associated with the patient’s age, length of history prior to operation or time after operation, previous history of constant pain, occurrence of a postoperative complication, vaginal delivery after the operation or current use of contraceptive pills (data not shown).

Tenderness in the posterior vestibule was absent in 34 (64.2%) women, mild in 11 (20.8%) and significant in eight (15.1%) women. The corresponding rates in the anterior vestibule, i.e. the area not included in the operation, were 15 (28.3%), 14 (26.4%) and 24 (45.3%). Significant tenderness in both regions was present in six (11.3%) women. Gynecological examination revealed no scarring. Four women had...
persistent vulvar fissure; three of the four had fissure already at baseline. Women with significant tenderness in the swab-touch test at follow up reported higher VAS scores for dyspareunia (4.0; IQR 2.0–5.0) than those with a non-tender test (0.75; IQR 0.0–3.75; \( p = 0.035 \)).

Resting tone of the pelvic muscles was measured in 27 (51%) women. Fourteen (51.8%) showed adequate voluntary relaxation (resting tone \( \leq 10 \mu V \) or less). They reported a median VAS score of 3.0 (IQR 0.8–6.3), while the score for those with resting tone more than \( 10 \mu V \) was 4.5 (IQR 0.8–5.5; \( p = 0.837 \)). Thirty-four (92%) of 37 women considered their current voluntary pelvic floor muscle relaxation as satisfactory.

Forty-four (80%) women reported a regular sexual relationship, 50 estimated current dyspareunia and 52 completed the McCoy questionnaire. Seven (14%) women reported no dyspareunia and 29 (58%) reported at least half of the intercourses as painless. Dyspareunia was persistent in 10 (20%) women. Median problem index score was 8.0 (range 2–14). Women with tenderness in the anterior vestibule reported higher problem index scores (median 8.0; range 2–14) than those with a non-tender anterior vestibule (median 6.0; range 2–13; \( p = 0.046 \)). The current VAS score and the problem index score were significantly correlated (Pearson’s correlation coefficient 0.71; \( p < 0.001 \)). Also, the VAS score correlated with the intensity of dyspareunia in the McCoy questionnaire (Pearson’s correlation coefficient 0.77; \( p < 0.001 \)).

The women were asked whether they had been cured by the operation (complete response), still had some complaints (partial response) or were the same or worse than before operation (no response). Of 54 women, 19 (35.2%) reported complete response, 30 (55.6%) partial response and five (9.3%) no response. The response did not depend on duration of the follow up (\( p = 0.401 \)), short-term complications (\( p = 0.320 \)) or the surgeon in charge (\( p = 0.970 \)). Table 2 shows other outcome measures based on subjective experience. Of the 54 women, 49 (90.7%) would have chosen the operation again, four (7.4%) were uncertain and one (1.9%) would have declined. This patient developed a postoperative hematoma and wound infection followed by refractory pain syndrome. All four women who were uncertain were in the partial response group, with significant tenderness in the anterior vestibule.

**Cases with non-optimal outcome**

Overall, 18 women had a non-optimal outcome (defined as no response by subjective estimation, follow-up VAS score \( \geq 7 \), significant tenderness present in the posterior vestibule, or reporting persistent dyspareunia: (Table S1). Such an outcome did not depend on duration of the disease before treatment (\( p = 0.343 \)) or duration of postoperative follow up (\( p = 0.213 \)). Of the eight patients with significant remaining posterior tenderness, four reported VAS scores \( \geq 4 \), three reported VAS scores <4 and one was not sexually active because of fear of intercourse. This patient and four other patients reported no response by surgery. Two of these had significant anterior tenderness with dyesthesitic pain, one reported dyesthesitic pain and one had a posterior vulvar fissure. Five other patients reported persistent dyspareunia, four of whom had anterior tenderness and one had a vulvar fissure. One patient reported a VAS score of 7 without tenderness. Thus, only four patients were considered as pure surgical failures.

**Discussion**

Posterior vestibulectomy resulted in 91% long-term satisfaction. Significant improvement of posterior vestibular tenderness was achieved in 85% of the patients. Earlier studies on surgical treatment have reported success rates up to 89% of cases (13). However, the outcome has been poorly defined in most previous studies, based on patients’ self-reports with no objective measures, and many previous studies lack data on complications, sexual functioning, residual tenderness and short-term recovery (8); however, such information is important when counseling patients for surgery.

We aimed to evaluate the short- and long-term well-being of 70 women who underwent modified posterior vestibulectomy. In our study, the inclusion criteria were similar to other studies (Friedrich’s criteria) and surgery was performed as the last treatment option after conservative treatment modalities had failed. This cohort study provides unique new data on the safety and effectiveness of vestibulectomy. The thorough gynecological examination and the interview at the follow-up visit were both conducted by a senior gynecologist who had
Table 2. Outcome measures and patient satisfaction of 54 patients with vestibulectomy.

<table>
<thead>
<tr>
<th></th>
<th>Complete response</th>
<th>Partial response</th>
<th>No response</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VAS score</strong> (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median VAS score</td>
<td>0.0 (0.0–1.0)</td>
<td>4.0 (2.5–5.3)</td>
<td>10 (7.0–10.0)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Change of VAS score of &gt;50%</td>
<td>19 (100)</td>
<td>16 (53.3)</td>
<td>1 (33.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Swab touch-test results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10 (58.8)</td>
<td>26 (86.7)</td>
<td>3 (60)</td>
<td>0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>7 (41.2)</td>
<td>4 (13.3)</td>
<td>2 (40.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Anterior vestibule tenderness</td>
<td></td>
<td></td>
<td></td>
<td>0.155</td>
</tr>
<tr>
<td>Significant</td>
<td>4 (23.5)</td>
<td>17 (56.6)</td>
<td>3 (60)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>5 (29.4)</td>
<td>8 (26.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (47.1)</td>
<td>5 (16.7)</td>
<td>2 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Posterior vestibule tenderness</td>
<td></td>
<td></td>
<td></td>
<td>0.086</td>
</tr>
<tr>
<td>Significant</td>
<td>0</td>
<td>7 (23.3)</td>
<td>1 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>1 (5.9)</td>
<td>8 (26.7)</td>
<td>2 (40.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16 (94.1)</td>
<td>15 (50.0)</td>
<td>2 (40.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Pelvic floor muscles</strong> (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting tone (μV)</td>
<td>11 (5.7–28)</td>
<td>10 (2.9–24)</td>
<td>7 (5–15)</td>
<td>0.699</td>
</tr>
<tr>
<td>Resting tone ≥10μV</td>
<td>3 (42.8)</td>
<td>9 (52.9)</td>
<td>2 (66.7)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Problem index score</strong> (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>28</td>
<td>1</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Note: Values are medians (minimum–maximum)/number of data available for continuous variables, median (interquartile range 25–75%) for visual analog scale (VAS) values, and numbers of cases/data available (percentage) for categorical variables.

* Scale 2–14 (less problems–more problems).

not been involved in the treatment of the patients, further increasing validity of the study.

Owing to the retrospective design, there are some limitations. Our study lacks detailed baseline data on sexual problems; however, all women entering operative treatment reported high VAS scores, indicating problems which severely interfered or totally prohibited sexual intercourse. A long interval between the operation and the follow-up visit may have biased some subjective statements.

The rate of immediate postoperative adverse effects was relatively high (21.7%). In this type of operation, postoperative hemorrhage or mild wound infections are not unexpected (14). Also, these patients may be anxious about their symptoms, leading to a low threshold for attending the outpatient clinic. It is noteworthy that all wound infections were non-febrile and mild. Some cases of wound inflammation may have been overdiagnosed as infections. Our rate of 5.7% of postoperative Bartholin’s cysts is in line with previous reports (15).

A VAS score was used to evaluate the severity of dyspareunia (10), enabling objective comparison of preoperative and postoperative conditions. The VAS score was obtained both at baseline and at follow up, and showed a 66.7% reduction. The VAS is a simple, reproducible and valid indicator of pain severity. In our hands, a VAS score ≥7 reflected no response to conservative management followed by counseling for vestibulectomy. A low VAS score at follow up correlated well with other measures of satisfactory response, i.e. lack of vestibular tenderness and low problem index score. Thus, the VAS score and its change can be recommended as a main outcome measure in sexual pain research.

Surgery did not totally correct posterior tenderness in eight (15%) of the patients as evaluated by the swab test. Although posterior vestibulectomy aims to cover the whole posterior vestibule from 2 to 10 o’clock, it is possible that the surgeon in some cases misjudges the peripheral extension, which may result in residual tenderness in the posterior vestibule. The high rate of residual anterior tenderness (45%) is not surprising, because the anterior vestibule is not included in this operation. It is worth considering whether total vestibulectomy might be a better choice. Some previous studies have used techniques other than posterior vestibulectomy without demonstrating superior results. Our choice of the posterior vestibulectomy technique was supported by this being the region where most patients locate the symptoms (16) and by an assumption that posterior tenderness has more impact on dyspareunia than anterior tenderness.

We disagree with previous studies claiming that pelvic floor muscle dysfunction is the main reason for residual dyspareunia (17). This is because our patients in all response groups had similar voluntary pelvic floor muscle relaxation. However, undoubtedly the whole process of physical therapy, including counseling, caring and psychological support, augments the healing process.
Overall patient satisfaction is an important outcome measure of surgery. Women’s subjective evaluation of the outcome most likely reflects the experience of pain and quality of sexual life, expressed by the VAS and problem index score. In our material, a VAS score of 0.5 and problem index score of 5.0 in the complete response group predicted high patient satisfaction. The problem index score was only slightly higher than the score after hysterectomy (4.1) performed for menorrhagia (12) in a population which presumably has less sexual problems than our VVS patients. However, the higher VAS score and problem index in the partial response group suggest that other treatment modalities beyond surgery are needed. Eight patients had residual posterior tenderness, but only four of them were considered surgical failures. Although surgery decreased tenderness, other co-existing problems explained poor outcome in other cases.

It is generally known that substantial relief of symptoms can be obtained by conservative treatment modalities as well. In some women, symptoms may even resolve spontaneously (6,18,19). This suggests that not all patients need surgery. In addition, a relatively large placebo effect is involved in all treatments of VVS, as demonstrated in a recent randomized placebo-controlled trial of oral desipramine and topical lidocaine (20). So far, surgery has been offered as the last resort of several ineffective treatment attempts. In the open label phase randomized trials because they are already frustrated with conservative treatment modalities may ultimately represent the most severe cases. In fact, women not responding to conservative treatment modalities may ultimately represent a specific subtype of severe VVS. Randomization of poor responders to surgery, to continue conservative treatment or to observation only might best reveal whether surgery is superior. However, these patients may be difficult to recruit into randomized trials because they are already frustrated with several ineffective treatment attempts. In the open label phase of the recent randomized controlled trial (20), patients opting for vestibulectomy instead of continuing medical therapy reported greatest improvement. Likewise, our patients opted for surgery after a long conservative treatment period with poor response. This may have eliminated the placebo effect, at least to some extent. Posterior vestibulectomy seems a safe, effective and well-tolerated treatment option for severe VVS when conservative treatment modalities have failed.

Funding

The study was supported by grants from the Helsinki University Hospital Research Funds.

References


Supporting information
The following supporting materials are available for this article:

Table S1. Details of 18 patients with non-optimal outcome (defined as ‘no response’, VAS ≥7 at follow up, significant residual tenderness in the posterior vestibule or persistent dyspareunia).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author.
Long-term well-being after surgical or conservative treatment of severe vulvar vestibulitis

PAIVI TOMMOLA, LEILA UNKILA-KALLIO & JORMA PAAVONEN

Department of Obstetrics and Gynecology, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland

Abstract

Objective. To compare long-term well-being of women who needed surgery or did not need surgery in the treatment of severe vulvar vestibulitis syndrome. We also attempted to identify factors explaining differences in the treatment response.

Design. An observational case-control study.

Setting. University Hospital Vulva clinic.

Population. Sixty-six women diagnosed with severe vulvar vestibulitis and treated initially by conservative management during 1994–2005. Thirty-nine women did not respond and underwent posterior vestibulectomy and 27 were managed without surgery.

Methods. Baseline patient characteristics, degree of dyspareunia, and details of management were collected from hospital charts. At the follow-up visit current dyspareunia, sexual well-being, somatic and mental health, and social support were analyzed. Vestibular tenderness was measured by swab-touch test. Main outcome measures. Visual analogue scale for dyspareunia, sexual well-being, vestibular tenderness, and overall patient satisfaction at follow-up.

Results. Dyspareunia decreased significantly in both groups. The visual analogue scale decreased 66.7% in the surgery group and 78.1% in the conservative treatment group (p = 0.407). Posterior swab-touch test was negative more frequently after vestibulectomy. Long-term sexual well-being did not differ between the two groups. Overall, 89% of the women in both groups were satisfied with the treatment. Women with atopic skin problems were less likely to need surgery (odds ratio 0.2; 95% confidence interval 0.1–0.7).

Conclusion. Women with severe vulvar vestibulitis syndrome who do not respond to conservative management achieve good long-term well-being and a decrease of dyspareunia by posterior vestibulectomy. The response is comparable to that achieved by conservative management among women who do not need surgery.

Abbreviations: VAS, visual analogue scale; VVS, vulvar vestibulitis syndrome.

Introduction

In vulvar vestibulitis syndrome (VVS), also called localized provoked vulvodynia, pain by touch in the vulvar vestibule causes dyspareunia and ruins the sexual life of many young women (1,2). The etiopathogenesis of the disease is unknown and treatment is challenging. The main symptom, dyspareunia, has been managed by topical anesthetics (3), corticosteroids (4), topical or systemic neuropathic pain medications (5,6) based on current guidelines of VVS treatment. However, the effectiveness and safety of most non-surgical interventions to treat VVS are poorly verified, with only short follow-up (7), and there are few randomized trials (8,9). Surgery, i.e. vestibulectomy (10,11), is usually offered to

Key message

Women with severe vulvar vestibulitis syndrome who do not respond to conservative management can achieve good long-term well-being and a decrease of dyspareunia by posterior vestibulectomy. The response is comparable to conservative management among women who do not need surgery.
women refractory to conservative management with good success; the proportion of women satisfied after surgery ranges from 78 to 91% (12,13). Most previous studies of surgical treatment place an emphasis only on the decrease of dyspareunia and few studies report sexual well-being or quality of life in the long-term (14). Few studies compare conservative treatment and surgery (9,15,16). To our knowledge, no study has compared the results of conservative management and surgery in women with severe symptoms. Patient characteristics predicting response to conservative management are unknown. To further evaluate the role of surgical treatment in severe VVS we report this case-control study on long-term well-being of severe VVS women managed by surgery or without surgery.

**Material and methods**

The study population consisted of 66 women diagnosed with severe vulvar vestibulitis syndrome (VVS) at the Vulva clinic, Department of Obstetrics and Gynecology, University Hospital, Helsinki, Finland during 1994–2005. The Vulva clinic is a tertiary referral center for women with vulvar conditions. Originally, we identified from hospital records 52 women treated by posterior vestibulectomy for severe VVS after failed conservative management. As controls we searched for 50 women with the same inclusion criteria (severe VVS symptoms with visual analogue scale (VAS) score for dyspareunia ≥7, duration of symptoms at least 12 months, absence of vulvar dermatoses, and equal timing of treatment period) who had not undergone vestibulectomy. Thirty-nine (75%) of 52 vestibulectomy women (surgery group) and 27 (54%) of 50 conservatively treated women (conservative treatment group) consented and were enrolled in a long-term follow-up study (Figure 1). The local Ethical Committee approved the study.

![Figure 1. Study design. VAS, visual analogue scale.](image)

All VVS women at the Vulva clinic are managed according to a predefined algorithm (Supporting Information Appendix S1) in line with international vulvodynia treatment guidelines (17). The diagnosis of VVS was based on the Friedrich’s classical criteria (18). Vestibular tenderness was evaluated with swab-touch test and intensity was recorded as 'none' (no pain), 'mild' (verbal expression of pain) or 'significant' (expression of pain by sudden move). Dyspareunia was recorded by VAS from zero (no pain) to 10 (worst possible pain). Vaginal infections were ruled out by bedside wet-mountain microscopy. Dermatological diseases were excluded by obtaining a punch biopsy when clinically indicated. After the diagnosis of VVS was confirmed, women were counseled about the treatment options available. All women were advised about general vulvar care measures. Oral contraceptives were withdrawn if possible. Long-lasting antifungal therapy with fluconazole was prescribed for women with history of recurrent yeast infections. When appropriate, local corticosteroid (injected into the submucosa in a mixture with local anesthetic or as a topical cream) was used, or amitriptyline orally was prescribed. In some women, mucosal neuromodulation with topical podophyllotoxin 5 mg/mL (Watlec®) application was used. Women were also referred to a physical therapist for bio-feedback therapy of the pelvic floor muscle dysfunction, sexual counseling, and education. Women who did not respond to conservative management were offered surgery, a modified posterior vestibulectomy (13).

Details of the conservative treatment modalities used during the initial treatment period (before opting for surgery or at dismissal after satisfactory conservative treatment) in both groups were collected from the hospital charts. The long-term follow-up visit of the surgery group took place in October 2005 and of the conservative treatment group in September 2009. Both visits were conducted by one senior gynecologist (P.T.) who had not been involved previously in the patient management. The difference in follow-up period between the groups was due to practical and funding limitations. At follow-up the women were interviewed face-to-face using a structured questionnaire. The effect of individual conservative treatments on symptoms was reviewed and the reason for opting for or refusing surgery was asked. Also, women reported possible additional treatments or medications used to treat symptoms after the Vulva clinic visits. The degree of dyspareunia was estimated by VAS score. Vestibular tenderness was evaluated with swab-touch test separately in the anterior (from 11 and to 1 o’clock) and posterior region (from 10 to 2 o’clock) with a light touch administered each time.

Somatic and mental health issues and psychosocial characteristics were evaluated with questionnaires. Subjective general health was assessed with the EuroQol-5-Dimension-visual analogue scale (EQ5D-VAS) with VAS scores
ranging from 0 to 100, a score of 100 representing the best possible health state (19). Depression was measured by the 13-item version of the Beck depression inventory (20) and availability of social support by nine items from the validated Finnish version (21) of the Medical Outcome Study (MOS) Support Scale (22). Sexual well-being was assessed by a modification of McCoy questionnaire (23,24) consisting of three indexes: sexual satisfaction index (five items on a scale from 1 to 7 representing the worst to the best situation), partnership satisfaction index (two items, similar scale), and index for sexual problems (a sum of reported frequency of dryness during sexual acts and frequency of dyspareunia; scale of 1 to 7 representing never to always). The modification of the McCoy questionnaire for sexual well-being is presented as Supporting Information in Appendix S2. The individual overall response at long-term was reviewed by asking whether the patient had been cured (complete response), still had some complaints (partial response) or experienced no improvement (no response or worse).

Power analysis of VAS difference for dyspareunia at follow-up indicated that 24 women were needed in both groups to detect a clinically relevant 20% difference between the groups ($\alpha = 0.05$ and power $(1 - \beta) = 0.8$). Based on previous results of vulvar vestibulitis treatment studies we estimated that decrease in VAS for dyspareunia could be 66% with surgery and 44% with conservative management (12,25). The data were analyzed by the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL, USA). We report medians with interquartile range of 25–75% for VAS values and medians with minimum and maximum values for other continuous data. For comparisons we used the Mann–Whitney U-test or Kruskal–Wallis test and Wilcoxon signed rank test for continuous data and chi-squared analysis or Fischer’s exact test for categorical data. Odds ratios with 95% confidence interval were calculated when appropriate. A two-tailed $p$-value of <0.05 was considered significant. For correlations, Pearson’s correlation coefficient was used.

**Results**

All 39 surgical women had opted for surgery because of insufficient relief of symptoms by conservative management. Of the 27 conservatively treated women, 23 had satisfactory resolution of symptoms, one patient did not report her response, and three had unsatisfactory treatment result but refused surgery – one because of fear and two reported no reason. The drop-out women in either group did not differ from the other women on any aspect at baseline. No data on their long-term outcome are available. Baseline clinical characteristics of the study groups are shown in Table 1.

**Table 1.** Baseline characteristics and conservative treatment modalities used by vulvar vestibulitis syndrome women by study group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vestibulectomy</th>
<th>Conservative treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>$39$</td>
<td>$27$</td>
</tr>
<tr>
<td>Age at first visit, years</td>
<td>24.5 (16–48)/30$^1$</td>
<td>23.5 (18–32)/24</td>
</tr>
<tr>
<td>Nulliparous at baseline</td>
<td>32/39 (82.1)</td>
<td>25/26 (96.1)</td>
</tr>
<tr>
<td>On OC at onset of symptoms</td>
<td>24/37 (61.5)</td>
<td>19/25 (76.9)</td>
</tr>
<tr>
<td>History of dyspareunia, yrs</td>
<td>5.0 (1–18)/35</td>
<td>4.0 (1–16)/23</td>
</tr>
<tr>
<td>Primary vestibulitis$^4$</td>
<td>7/27 (25.9)</td>
<td>8/26 (30.8)</td>
</tr>
<tr>
<td>VAS for dyspareunia at baseline</td>
<td>9.0 (8.0–10.0)/35</td>
<td>8.0 (8.0–9.0)/25</td>
</tr>
<tr>
<td>Discontinuation of OC</td>
<td>9/25 (36)</td>
<td>12/17 (70.5)</td>
</tr>
<tr>
<td>Physical therapy</td>
<td>10/30 (33.3)</td>
<td>15/26 (57.7)</td>
</tr>
<tr>
<td>Long-term anti-fungal treatment</td>
<td>17/35 (48.6)</td>
<td>11/26 (42.3)</td>
</tr>
<tr>
<td>Neuromodulation</td>
<td>18/31 (58.1)</td>
<td>9/26 (34.6)</td>
</tr>
<tr>
<td>Submucous or topical corticosteroids</td>
<td>19/31 (61.3)</td>
<td>19/26 (73.1)</td>
</tr>
<tr>
<td>Topical podophyllotoxin$^5$</td>
<td>13/31 (41.9)</td>
<td>7/21 (33.3)</td>
</tr>
</tbody>
</table>

$^1$Values are medians (minimum – maximum)/number of data available for continuous variables, median (interquartile range 25–75%) for VAS values, and numbers of cases/data available (percentage) for categorical variables.

$^2$Mann–Whitney U-test.

$^3$Chi-squared test.

$^4$Pain from first attempt of vaginal entry

$^5$Application of podophyllotoxin 5 mg/mL (Wartec®) on tender points of vestibular mucosa following 5% acetic acid application, repeated three times every four weeks (Appendix S1).

No significant differences were found between the groups. However, the VAS score for dyspareunia at baseline tended to be higher in the surgery group than in the conservative treatment group (9.0 vs. 8.0, $p = 0.046$). All women had long history of dyspareunia, most of them were nulliparous and under 25 years of age. The number of cases with primary vestibulitis (symptoms from first attempt of vaginal entry) was similar in both groups. The duration of the conservative treatment period was 18.5 (4–55) months in the surgery group and 16.0 (3–129) months in the conservative treatment group ($p = 0.596$). The time from treatment initiation to choosing operation was 9.0 (2–55) months and the median age of the women was 26.0 years (range 17–48, mean 27.9) when making the decision. The operation was performed at a median age of 27.0 years, (range 18–50, mean 28.6). The different treatment modalities used during the conservative treatment period were similar in both groups (Table 1).
The duration of follow-up was 47 (11–114) months in the surgery group and 77 (34–131) months in the conservative treatment group ($p < 0.001$). There was no difference between the two groups in the use of systemic medication or topical anesthetics to control dyspareunia during follow-up and none had tried any other treatment options (data not shown).

Gynecological examination with swab-touch test was performed in 35 vestibulectomy women and 24 conservatively treated women. Seven women refused examination for practical reasons but returned questionnaires and attended to the interview part of the study. No other vulvar diseases were found, except that three (7.7%) women in the surgery group had a fissure in the posterior fourcette (one recent and two recurrent fissures). Anterior tenderness did not differ between the groups but significant posterior tenderness was clearly less common in the surgery than in the conservative treatment group ($p = 0.001$) (Figure 2).

VAS scores for dyspareunia decreased significantly in both groups from baseline to follow-up visit; from 9.0 (8.0–10.0) to 3.0 (0.5–4.8), ($p < 0.001$) in the surgery group and from 8.0 (8.0–9.0) to 2.0 (0.00–3.0), ($p < 0.001$) in the conservative treatment group. The median changes in both groups were similar: 6.0 (3.8–8.0) (66.7%) and 6.3 (5.3–8.0) (78.1%), respectively ($p = 0.407$). Change of VAS did not depend on the duration of follow-up ($R^2 = 0.007$). In a subgroup analysis, women with significant posterior tenderness reported VAS scores of 5.25 (2.75–7.75) in the surgery group and 2.0 (1.0–4.0) in the conservative treatment group ($p = 0.229$).

Somatic or mental health, social, or partnership characteristics did not differ between the surgery and the conservative treatment groups except that atopic skin problems were more common among women in the conservative treatment group (Table 2). Need for surgery was less likely in women with atopic skin problems (odds ratio 0.2; 95% confidence interval 0.1–0.7). Pain related to urinary tract, symptoms suggestive of irritable bowel syndrome, and frequent musculoskeletal pains were equally common in both groups, as was the number of women with symptoms of two or more other pain disorders than vestibulodynia. Susceptibility to urogenital infections (candidiasis, bacterial vaginosis, other vaginitis, or urinary tract infection) did not differ between the groups (data not shown). In both groups around 75% of women were sexually active. Results of the McCoy instrument for sexual well-being were similar in both groups (Table 3). Conservatively treated women reported use of coital lubricants more often than surgically treated women (43.5% vs. 14.7%, respectively) ($p = 0.030$) (Table 3). Ten (52.6%) of 19 women in the conservative treatment group had changed partner during the follow-up period. Change of partner tended to be more common in the conservative treatment group than in the surgery group ($p = 0.065$) but was not associated with recovery.

The treatment response was reported as complete by 13 of 36 (36.1%) women in the surgery group and seven of 27 (25.9%) women in the conservative treatment group, as partial by 19 (52.8%) and 17 (63.0%), and as no response by four (11.1%) and two (7.4%), respectively, ($p = 0.567$). No woman in the surgery group was worse than at baseline, whereas one (3.7%) in the conservative treatment group reported a worse situation. The response did not depend on the duration of follow-up ($p = 0.441$). Four of the seven (57.1%) women with primary vestibulitis in the surgery group and one of the eight (12.5%) women in the conservative treatment group reported complete response.

At follow-up, less than optimal outcome (defined as no response by subjective estimation, follow-up VAS score ≥ 7, or presence of constant dyspareunia) was found in nine (23.1%) women in the surgery group and four (14.8%) women in the conservative treatment group, ($p = 0.534$). Three of these four control women were those who had had unsatisfactory result from the initial conservative treatment.

**Discussion**

This study showed that in women with severe VVS who do not respond to conservative management, posterior vestibulectomy can provide long-term satisfaction and an enjoyable sexual life, comparable with the well-being of those initially responding to conservative management who do not need surgery. This supports the prevailing opinion that surgery be offered to the most severe VVS cases when conservative management has failed.

To our knowledge, this is the first study comparing long-term results of surgery and conservative treatment of women with uniform definition of equally severe VVS at baseline. Also this is the first study reporting the very long-term well-being of these women and reveals that satisfactory treatment results are preserved for at least up to six years.

One advantage of the study was the use of a pragmatic treatment algorithm in the management of all study women as well as the use of several and versatile outcome measures. Women’s well-being and health determinants were broadly surveyed, including somatic and mental health, social characteristics and sexual well-being. The thorough interview and gynecological examination conducted by an expert was crucial in obtaining comparable clinical data. Further, as the great majority of the women were sexually active in both groups we also obtained relevant data on sexual well-being.

A major limitation of the study is probably the relatively small number of women. However, keeping to strict inclusion criteria enabled the comparison of women with as similar a
Vulvar vestibulitis treatment study

P. Tommola et al.

Figure 2. Long-term (4–6 years) results of swab-touch test tenderness of 59 women treated for severe vulvar vestibulitis syndrome by posterior vestibulectomy (surgery group) or by conservative management (conservative treatment group). U, uretra; C, clitoris, V, vagina; –– hymen.

Disease history and treatment history as possible and this may actually be viewed as an advantage. According to the power analysis the sample size was large enough to detect a 20% difference between the two groups’ current VAS scores for dyspareunia, the main symptom of VVS. The similar decrease of dyspareunia with both managements was striking. Of concern may be the relatively large proportion (46%) of women in the conservative treatment group who did not agree to participate. This may have introduced a drop-out bias as many unsatisfied women may have been reluctant to participate.

It is also worth considering whether the long duration of follow-up of the conservatively treated women had some impact on the results. It might, for example, have increased the proportion of women whose symptoms resolved spontaneously. Spontaneous recovery from VVS has been demonstrated recently (26). However, extending the duration of follow-up from six months to two and half years did not affect the results in another study (16). This is also supported by our findings as a change of VAS during follow-up was not time-dependent.

Significant posterior vestibular tenderness was more frequent in the conservative treatment group. Surprisingly, these women still reported quite low VAS scores. The long follow-up time of this group may have had a role in making a better adjustment to pain. Also, the ability to cope depends on special personality traits. Women with better self-efficacy tend to have higher pain tolerance than women with lower self-efficacy (27). Unfortunately, evaluation of personality traits was not included in our study.

A majority of the women were living in a relationship but reported low monthly frequency of sexual acts. Still, they experienced reasonably good sexual satisfaction. Possibly, women or couples with a long history of dyspareunia have adapted to be satisfied with having intercourse less frequently than healthy couples. Our finding of relatively high sexual and partnership satisfaction is consistent with results of previous studies on vulvodynia women and partners (28). The problem index scores in both groups were quite high, revealing residual pain and dryness. However, dryness during the sexual act did not constitute a problem more often after vestibulectomy than after conservative treatment. The sensation of dryness may be a fundamental symptom of VVS rather than a complication of surgery and may be managed with adequate lubricant use. The less frequent need of lubricants in the surgery group may be explained by less pain consistent with a favorable outcome.

Atopic skin problems predicted the response to conservative treatment. We were unable to identify any other explanatory factors. Conservative treatments were carried out rather similarly, and the women did not show any other differences regarding somatic or mental health, or social characteristics. Previous reports of primary vestibulitis suggest poor response to surgery (14,29). This was not supported by our results. More than half of the women with primary disease reported complete response to surgery.

© 2012 The Authors
Symptoms consistent with at least four pain syndromes other than vulvodynia predicted a poor response to conservative treatment in a recent study (30). In our study population such tendency was not obvious. Also, a history of sexual or physical abuse which was not surveyed in our women predicted a poor response to medical treatment in another recent study (31). It is possible that different subgroups of VVS exist with specific histopathologic features that determine responsiveness or influence the natural course of the disease. Our finding that atopic skin problems were more common among women who responded to conservative treatment suggests a specific immunological background in the pathophysiology of VVS, or a different immunological response of atopic skin type, or merely better tolerance to pain. This finding deserves further study. Change of partner had an impact on recovery in one study (14) but in our study, change of partner was not associated with outcome. Since it is difficult to predict the response to conservative management an initial conservative treatment period should be planned for all women at baseline. In this study, women refractory to conservative management were ready to choose surgery after median of nine months of failed conservative treatment attempts. Women with good response needed a treatment period of 16 months to render vestibulectomy unnecessary. Randomization of severe VVS women in the beginning might thus not be appropriate. Rather, randomization after a systematic conservative treatment period of poor responders either to surgery or to continue conservative treatment might be reasonable, but even this may be ethically problematic. Women with long history of severe VVS may be difficult to recruit into randomized trials. Furthermore, in our study, three of four conservatively treated women with non-optimal outcome in the long-term had already reported an unsatisfactory response after initial conservative management. This may mean that women refractory to conservative management possibly represent a specific subtype of VVS and should be encouraged to undergo vestibulectomy. In favor of the safety of surgery

### Table 2. Long-term somatic and mental health, social, and partnership characteristics of vulvar vestibulitis syndrome women by study group

<table>
<thead>
<tr>
<th></th>
<th>Vestibulectomy n = 39</th>
<th>Conservative treatment n = 27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at follow-up, years</td>
<td>31 (22–57)/39</td>
<td>32 (24–43)/27</td>
<td>0.561²</td>
</tr>
<tr>
<td>VAS for dyspareunia at follow-up</td>
<td>3.0 (0.5–4.8)/35</td>
<td>2.0 (0.0–3.0)/25</td>
<td>0.174²</td>
</tr>
<tr>
<td>Pain related to urinary tract</td>
<td>16/34 (47.1)</td>
<td>8/26 (33.3)</td>
<td>0.202²</td>
</tr>
<tr>
<td>Symptoms suggestive of IBS</td>
<td>19/35 (54.3)</td>
<td>10/26 (38.5)</td>
<td>0.221¹</td>
</tr>
<tr>
<td>Frequent joint pains</td>
<td>3/36 (8.3)</td>
<td>3/26 (11.5)</td>
<td>1.000²</td>
</tr>
<tr>
<td>Two or more other pain disorders</td>
<td>11/36 (30.6)</td>
<td>6/26 (23.1)</td>
<td>0.515³</td>
</tr>
<tr>
<td>Atopic skin problems</td>
<td>5/35 (14.3)</td>
<td>12/26 (46.2)</td>
<td>0.009³</td>
</tr>
<tr>
<td>EQ5D-VAS</td>
<td>80 (48–100)/35</td>
<td>82.5 (40–100)/26</td>
<td>0.264⁴</td>
</tr>
<tr>
<td>Depression (Beck)</td>
<td>3.0 (0–15)/37</td>
<td>3.0 (0–15)/26</td>
<td>0.994⁴</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>1/36 (2.8)</td>
<td>4/26 (15.4)</td>
<td>0.149³</td>
</tr>
<tr>
<td>Social support (MOS)</td>
<td>36.5 (21–45)/36</td>
<td>36.5 (28–45)/26</td>
<td>0.954³</td>
</tr>
<tr>
<td>Need for more social support</td>
<td>9/37 (24.3)</td>
<td>5/26 (19.2)</td>
<td>1.000²</td>
</tr>
<tr>
<td>Married or cohabiting</td>
<td>27/39 (69.2)</td>
<td>18/27 (66.7)</td>
<td>1.000²</td>
</tr>
<tr>
<td>Same sexual partner than before</td>
<td>22/36 (61.1)</td>
<td>9/26 (34.6)</td>
<td>0.106³</td>
</tr>
<tr>
<td>Single</td>
<td>7/37 (18.9)</td>
<td>7/25 (28)</td>
<td>0.402³</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>22/37 (59.5)</td>
<td>15/26 (57.7)</td>
<td>0.613³</td>
</tr>
<tr>
<td>Vaginal delivery during follow-up</td>
<td>7/37 (18.9)</td>
<td>11/27 (40.7)</td>
<td>0.090³</td>
</tr>
</tbody>
</table>

¹Values are medians (minimum − maximum)/number of data available for continuous variables, median (interquartile range 25%–75%) for VAS for dyspareunia, and numbers of cases/data available (percentage) for categorical variables
²Mann-Whitney U-test
³χ²-test
⁴Mann-Whitney U-test

### Table 3. Sexual health of vulvar vestibulitis syndrome women by study group

<table>
<thead>
<tr>
<th></th>
<th>Vestibulectomy n = 39</th>
<th>Conservative treatment n = 27</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexually active</td>
<td>30/37 (81.1)</td>
<td>18/25 (72)</td>
<td>0.402³</td>
</tr>
<tr>
<td>Use of coital lubricant</td>
<td>5/34 (14.7)</td>
<td>10/23 (43.5)</td>
<td>0.030³</td>
</tr>
<tr>
<td>Use of topical anesthetic</td>
<td>6/36 (16.7)</td>
<td>1/24 (4.2)</td>
<td>0.225³</td>
</tr>
<tr>
<td>Use of other ointment or cream</td>
<td>12/36 (33.3)</td>
<td>7/23 (30.4)</td>
<td>1.000²</td>
</tr>
<tr>
<td>Frequency of sexual acts during preceding month</td>
<td>2 (0–20)/35</td>
<td>4 (0–15)/24</td>
<td>0.135²</td>
</tr>
<tr>
<td>Sexual satisfaction³</td>
<td>22.5 (11–31)/34</td>
<td>25 (9–29)/25</td>
<td>0.718²</td>
</tr>
<tr>
<td>Partnership satisfaction⁴</td>
<td>12 (8–14)/29</td>
<td>12 (7–14)/18</td>
<td>0.250²</td>
</tr>
<tr>
<td>Sexual problems⁵</td>
<td>7 (2–14)/34</td>
<td>7 (2–12)/23</td>
<td>0.432²</td>
</tr>
<tr>
<td>Pain problem⁵</td>
<td>15/35 (42.9)</td>
<td>8/24 (33.3)</td>
<td>0.461³</td>
</tr>
<tr>
<td>Dryness problem⁵</td>
<td>19/34 (55.9)</td>
<td>11/23 (47.8)</td>
<td>0.550³</td>
</tr>
<tr>
<td>Arousal problem⁵</td>
<td>7/34 (20.6)</td>
<td>6/24 (25.0)</td>
<td>0.692³</td>
</tr>
</tbody>
</table>

¹Values are medians (minimum − maximum)/number of data available for continuous variables and numbers of cases/data available (percentage) for categorical variables
²Mann-Whitney U-test
³χ²-test
⁴Index in McCoy instrument
⁵Pain, dryness, or arousal difficulty interfering more than half of the intercourse
stands our recent study on posterior vestibulectomy reporting the rarity of long-term adverse outcome and the fact that over 90% of women would consent to vestibulectomy again (13).

In conclusion, posterior vestibulectomy is a good option for those severe VVS women who do not respond to conservative treatment. Both successful conservative management and surgery among refractory women result in an equally good long-term outcome.

Acknowledgments

We thank RN Pirkko Timonen for technical support and Nina Hedkrok for secretarial assistance.

Funding

The study was supported by grants from the Helsinki University Hospital Research Funds.

References


Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Algorithm for treatment of women with severe vulvar vestibulitis syndrome at Helsinki University Hospital Vulva Clinic. Algorithm for treatment of women with severe vulvar vestibulitis syndrome, Helsinki University Hospital Vulva Clinic. *Podophyllotoxin 5 mg/mL (Wartec®) is applied to tender points of vestibular mucosa following 5% acetic acid application, treated area is covered with cream and women are advised to wash the area after 24 hours. Application is repeated three times every four weeks.

Appendix S2. Modified McCoy questionnaire for sexual well-being.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.
Activation of vestibule-associated lymphoid tissue in localized provoked vulvodynia

Päivi Tommola, MD; Ralf Bützow, MD, PhD; Leila Unkila-Kallio, MD, PhD; Jorma Paavonen, MD, PhD; Seppo Meri, MD, PhD

OBJECTIVE: Localized provoked vulvodynia (LPV) may have inflammatory etiology. We wanted to find out whether the cell-mediated immune system becomes activated in the vestibular mucosa in LPV.

STUDY DESIGN: This was a controlled cross-sectional study. Vestibular mucosal specimens were obtained from 27 patients with severe LPV and 15 controls. Detailed clinical history of the patients was obtained. For immunohistochemistry, antibodies against CD3 (T cells), CD20 (B cells), IgA (mucosal plasma cells), CD163 (dendritic cells [DCs]), CD68 (macrophages), and CD117 (mast cells) were employed. Mann-Whitney U test and χ² test were used for statistical analyses.

RESULTS: More B lymphocytes and mature mucosal IgA-plasma cells were found in patients than in controls (P < .001 and P < .001, respectively). In LPV samples, B and T cells were arranged into germinal centers representing local immune activation. Germinal centers were not seen in controls. Antigen-presenting DCs and macrophages were found both in patients and controls with similar densities. DCs were found to extend their dendrites into the luminal space through an intact epithelium. Similar amounts of mast cells were found evenly scattered throughout the stroma of vestibular mucosa of both patients and controls.

CONCLUSION: We demonstrate here local organized vestibule-associated lymphoid tissue analogous to mucosa-associated lymphoid tissue. Vestibule-associated lymphoid tissue may emerge as a response to local infection or inflammation in LPV.

Key words: immune activation, inflammation, vestibulodynia, vulvar pain, vulvar vestibulitis


Localized provoked vulvodynia (LPV) is a pain syndrome with a suspected inflammatory background but the detailed characteristics of the inflammatory response are unknown. In particular, the role of specific immune system cells has not been defined. In

From the Department of Obstetrics and Gynecology, Helsinki University Central Hospital (Drs Tommola, Unkila-Kallio, and Paavonen); Department of Pathology (Dr Bützow); Research Programs Unit, Program of Immunobiology (Dr Meri); and Department of Bacteriology and Immunology, Haartman Institute (Dr Meri), University of Helsinki, Helsinki, Finland.

Received June 5, 2014; revised Aug. 9, 2014; accepted Oct. 27, 2014.

This study was supported by grants from the Finnish Society of Pediatric and Adolescent Gynecology and Helsinki University Hospital Research Funds (grant nos. TYH2012237, TYH2013340, TYH2011128, and TYH2013308).

The authors report no conflict of interest.

Preliminary results were presented at the VIII European Conference of the European Society for Infectious Diseases in Obstetrics and Gynecology, London, United Kingdom, Oct. 25-27, 2013, and at the XXII World Congress of the International Society for the Study of Vulvovaginal Disease, Rome, Italy, Oct. 6-13, 2013.

Corresponding author: Päivi Tommola, MD. paivi.tommola@kolumbus.fi

0002-9378/$36.00 © 2015 Elsevier Inc. All rights reserved. http://dx.doi.org/10.1016/j.ajog.2014.10.1098
this study we performed a thorough immunohistochemical analysis of the vestibular mucosal tissue focusing on differences in the characteristics of the local immune system between LPV patients and controls.

**MATERIALS AND METHODS**

**Study subjects**

We recruited 27 women with representative archival vestibulectomy specimens after surgically treated LPV during a period of 13 years from 1995 through 2007. The women were identified in the Helsinki University Central Hospital patient registry by matching the diagnosis (vulvar vestibulitis, vulvodynia, and dyspareunia) and the surgical procedure (posterior vestibulectomy). The total number of the original cohort was 70. It was not possible to reach 13 women, or they denied participating. Of the remaining 57 women we chose 27 with sufficient clinical data to form the patient population of this study. All the included patients were diagnosed as having localized provoked vestibulodynia with long disease history (mean, 5.3 years; 95% confidence interval [CI], 3.8–6.8; range, 2–18). The diagnoses in 8 patients were classified as primary (symptoms already at the first vaginal entry), in 15 patients as secondary (symptoms appearing later after an interval of painless intercourses), and in 4 patients the classification was unknown. Clinical data of these 27 LPV patients were collected from the patient records and by a face-to-face interview. Data on age, premenopausal status, general health, comorbidity, medications, and detailed disease history including duration and severity of symptoms, treatments before surgery, and response to surgery were systematically collected.\(^{16,19}\) As controls we recruited 15 premenopausal volunteers with no vulvar symptoms undergoing benign gynecological surgery under general anesthesia. No clinical data were collected. We obtained 4-mm punch biopsies from the posterior vestibule at 5 o’clock position from the controls. The study was approved by the local ethical committee. All participants provided informed consent.

**Tissues**

All vestibular tissues were routinely embedded in paraffin after a maximum of 24 hours fixation in 10% buffered formalin. We first stained 5-μm sections with hematoxylin–eosin for evaluation to exclude dermatological diseases, to confirm the quality of samples, and to grade lymphocytic inflammation. Immunohistochemistry on consecutive sections (5 μm in thickness; 10 μm for dendritic cells [DCs] and macrophages) was performed in the Helsinki University Central Hospital–Huslab tissue laboratory with routine staining procedures according to the manufacturers’ instructions. We employed antibodies against the following antigens: CD3 (clone 2GV6, RTU; Roche Diagnostics Ltd, Rotkreuz, Switzerland) for T lymphocytes, CD20 (clone L26, RTU; Roche Diagnostics Ltd) for B lymphocytes, CD68 (clone PG-M1, 1:1000; Dako, Glostrup, Denmark) for macrophages, CD117 (polyclonal, 1:1000; Dako) for mast cells, IgA (polyclonal, 1:2000; Dako) for mucosal plasma cells, CD163 (clone 10D6, 1:100; Leica Biosystems, Nussloch, Germany) for dendritic cells. For the first 4 antibodies above, we used a biotin-free polymer-based detection kit Ultraview (Roche Diagnostics Ltd). For the last 2 antibodies, we used a polymer-based detection kit Envision (Dako). All sections were counterstained with hematoxylin.

**Tissue analyses**

The entire material was analyzed blinded to clinical data. Histopathological

---

**FIGURE 1**

Antigen-presenting cells (DCs and macrophages) in vulvar vestibular mucosa

CD163 immunostaining for stromal and epithelial DCs in A, localized provoked vulvodynia patient and B, control sample. Inserts show DCs extending their projections through intact epithelial surface in greater magnification. CD68 immunostaining for macrophages in C, patient and D, control sample. Histological sections were counterstained with hematoxylin. Objectives A and B, ×40; C and D, ×20 (Nikon Eclipse E800).

DCs, dendritic cells.

inflammation was analyzed in hematoxylin–eosin sections and graded as none, mild, or moderate (R.B.) based on the amount of mononuclear infiltrates in stromal tissue beneath the vestibular epithelium at low-power magnification (×10).

Immunohistochemical scoring of the representative sections was performed under light microscopy (Nikon Eclipse E800). The stainings of all antigens were analyzed for localization (stromal, epithelial, both) and for densities of individual cell types in the vestibular mucosa. The scoring of each section was based on a consensus of 2 investigators (P.T. and J.P. or L.U-K. or S.M.) and disagreements were resolved by a joint review.

All immune cells, except T lymphocytes, were quantified from the most representative areas of inflammation by calculating the mean number of identified positive cells per field from 2-4 high-power fields (hpf) (×40 objective). T lymphocytes were scored by the overall cell density both in the epithelium and stroma. A single number score was given from 1-3 (1 = low density, <50 cells/hpf; 2 = moderate density, 50-100 cells/hpf; 3 = high density, >100 cells/hpf). Germinal centers were identified and counted from each section. For IgA, the overall staining intensity was additionally scored as 1 (mild intensity), 2 (moderate intensity), or 3 (strong intensity).

For statistical purposes and to reflect the overall level of B-cell infiltration we created a B-cell activation index (BAI) as follows. The most typical density of B cells in each section scored points from 0 (no B cells) to 4 (high amount of B cells), separately in the epithelium and in the stroma. The existence of at least 1 germinal center scored 4 points and absence scored 0 points. The sum of these 3 values gave a BAI from 0-12.

Statistical analyses of the data were performed with software (SPSS, version 20; IBM Corp, Armonk, NY). For comparisons between patients and controls, between patients with primary and secondary LPV, and between patients with different clinical characteristics, we used Mann-Whitney U test or Kruskal-Wallis test for continuous data and χ² analysis or Fisher exact test for categorical data when appropriate. For correlations Spearman rho test for nonparametric data was used. A P value of < .05 was considered significant in a 2-tailed analysis.

### TABLE 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>LPV (n = 27) Mean (95% CI)a</th>
<th>Controls (n = 15) Mean (95% CI)a</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic cells</td>
<td>96 (86–110)c</td>
<td>129 (101–157)d</td>
<td>.053</td>
</tr>
<tr>
<td>Macrophages</td>
<td>23 (17–29)e</td>
<td>19 (15–24)f</td>
<td>.780</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>121 (97–145)</td>
<td>38 (15–60)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Germinal centersc</td>
<td>14 (51.9)</td>
<td>0 (0)</td>
<td>.001</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>41 (31–52)g</td>
<td>11 (8–15)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Mast cells</td>
<td>35 (31–40)h</td>
<td>33 (26–39)</td>
<td>.512</td>
</tr>
</tbody>
</table>

CI, confidence interval; LPV, localized provoked vulvodynia.

a Values are mean cell counts per microscopy field, analyzed from 2-4 fields (×40 objective); b Mann-Whitney U test; c N = 24-26; d N = 9-10; e No. of samples (%) with ≥1 germinal center; f N = 12-14.


### FIGURE 2

T cells and B cells in vulvar vestibular mucosa

High density (score 3) of CD3⁺ T cells in A, localized provoked vulvodynia patient and B, control sample. CD20 staining for B cells shows greater density in C, patients than in D, controls. Histological sections were counterstained with hematoxylin and photomicrographed using ×20 objective.

RESULTS

Antigen-presenting cells

DCs can capture foreign antigens and process them for presentation to CD4⁺ helper T cells. CD163 immunostain revealed the typical morphology of DCs with the antigen-capturing dendrites. DCs were occasionally found to extend their dendrites through the epithelial surface to the luminal space (Figure 1, A and B). DCs were located both in the epithelium and stroma and tended to be more numerous in controls than in patients (Table 1). CD163 immunostaining for B cells. Histological sections were counterstained with hematoxylin and photomicrographed using ×10 objective.

Staining for macrophages demonstrated the chubby shape of the cell body and revealed the typical intracellular cytoplasmic granules (Figure 1, C and D). Macrophages were evenly scattered throughout the vestibular stroma with similar densities in patients and controls (Table 1).

T and B lymphocytes and plasma cells

T-cell staining (using the cytoplasmic pan T-cell marker CD3) visualized collectively both helper (CD4⁺) and killer (CD8⁺) T cells. These were expressed both in the stromal and epithelial layers of the vestibular mucosa with no significant difference in densities between patients and controls (Figure 2, A and B). T-cell density was high in 26.9% (7/26) of patients vs 21.4% (3/14) of controls, moderate in 30.8% (8/26) vs 57.1% (8/14), and low in 42.3% (11/26) vs 21.4% (3/14) of patients and controls, respectively (P = .280). T-cell densities were similar in primary and secondary LPV (P = .512; data not shown).

CD20 antigen is expressed on B cells at all stages of development, except the early pro-B cells and mature plasma cells capable of secreting antibodies. Anti-CD20 antibody showed that B lymphocytes were present mainly in the stromal layer of the mucosa. Small amounts were also found in the epithelium in the most populated areas (Figure 2, C and D). B lymphocytes were more numerous in patients than in controls (Table 1).

In areas with highest lymphocyte densities, T cells and B cells clearly formed lymphoid follicle-like structures. These were identified as germinal centers representing secondary lymphoid tissue. These germinal centers were visualized by both the CD3 (Figure 3, A through C) and CD20 (Figure 3, D through F) antibodies showing the typical zonewise arrangement of the individual cell types. Germinal centers were found only in LPV patients, but not in controls. The number of germinal centers in patients varied from 1-4 centers in 14 sections (52%), but was 0 in 13 sections. BAI was significantly higher in patients than in controls (median 5.0, range 1.0–9.0 and median 1.0, range 0–4.0, respectively; P = .001).

Mature mucosal plasma cells were stained on the basis of their ability to produce IgA-class antibodies. Compared to B cells, the plasma cells were bigger, had oval shape, showed high nucleus-to-cytoplasm ratio, and their nuclei had typical round cartwheel appearance. IgA-plasma cells were found in the subepithelial layer of the stroma (Figure 4). These were more numerous in patients than in controls (Table 1). In addition to the plasma cells’ cytoplasm, IgA was
also seen diffusely in the epithelial surfaces and stromas of mucosal samples. In a semiquantitative analysis no difference in the densities of IgA between patients and controls was found (data not shown).

**Mast cells**

Mast cells are tissue granulocytes. Typically they are prominent near the boundaries between the outside and inside environments, such as underneath mucosal surfaces, and adjacent to blood vessels. CD117+ mast cells were located in the stroma mostly as evenly distributed single cells, often adjacent to small capillaries (Figure 5) showing similar densities in patients and controls (Table 1).

**Clinical correlates of immunological parameters**

Histopathological inflammation was more pronounced in LPV than in controls and tended to be more severe in secondary than in primary LPV (Table 2). The grade did not associate with severity (visual analog scale score for preoperative dyspareunia) \( (P = .550) \) or duration of symptoms \( (P = .650) \).

The presence of germinal centers in patients was not associated with the histological grade of inflammation. Germinal centers were found in 35.7% (5/14) of patients with mild inflammation and in 69.2% (9/13) of patients with moderate inflammation \( (P = .082) \). The occurrence of germinal centers was not associated with the severity of dyspareunia or duration of symptoms \( (P = .550 \text{ and } P = .402, \text{ respectively}) \).

BAI scores were higher in patients with moderate than mild inflammation \( (5.9; 95\% \text{ CI}, 4.5–7.2 \text{ vs } 3.3; 95\% \text{ CI}, 1.9–4.6, \text{ respectively}; P = .011) \). No correlation was found between BAI and severity of dyspareunia (Spearman rho \(-0.172, P = .392\) ) or duration of symptoms (Spearman rho \(0.103, P = .610\) ).

Associations of selected patient characteristics and immunological parameters are presented in Table 3. Germinal centers were equally common in cases with primary or secondary LPV. BAI scores did not differ between these patient categories. History of recurrent genitourinary infection, history of autoimmune or inflammatory disease, or clinical response to surgery was not associated with the presence of germinal centers or with the BAI scores (Table 3).

**COMMENT**

In the present study we conducted a thorough analysis of different immune cell types in the vestibular mucosa of LPV patients and healthy controls. We were able to demonstrate the presence of key immune players and found evidence of immune activation in LPV. The vestibular area thus appears to have a localized immune system, which we call the vestibule-associated lymphoid tissue (VALT).

All patients had a clinically and histopathologically confirmed diagnosis of
severe LPV with accurate clinical data and good-quality archival tissue samples. The control group consisted of 15 healthy age-matched women. This is a major strength of our study since many previous studies on the histopathology of LPV reported very few or no controls. Control biopsies were taken exactly from the 5 o’clock position of the vestibule to secure appropriate comparison. This posterior site is the most representative vestibular area in LPV, as supported by a study on vulvar pain mapping and by the studies on the effect of surgical treatment of LPV.

Mucosal surfaces of the human body are known to contain secondary lymphoid tissue, the mucosa-associated lymphoid tissue (MALT). In MALT, B and T lymphocytes organize to form germinal centers, which play a central role in initiating an immune response against foreign antigens such as those from viruses and bacteria. The crucial point in the initiation of an immune response is the presentation of foreign antigens to T lymphocytes. Macrophages, DCs, and B cells are called professional antigen-presenting cells. By the antigen presentation, the T cells become activated. The activated T cells, in turn, are capable of activating a whole range of immune cells to mount different types of responses depending on the nature of the initial insult. This part of the activation of an adaptive immune response takes place in germinal centers. A proportion of the activated cells are B cells developing into antibody-producing mature plasma cells. In mucosal areas, B cells switch into plasma cells producing dimeric IgA-class antibodies that will subsequently be transported across the epithelium to the mucosal surfaces.

DCs capture different antigens with distinct receptors on their surfaces, especially on their dendrites. As shown in Figure 1, A and B, DCs can extend their dendrites into the luminal space through the epithelium in an intact mucosa to survey the mucosal surface and to capture potential antigens. Antigen-primed DCs in turn transport the antigen for presentation to T cells in the secondary lymphoid tissue. Our finding of a tendency towards fewer DCs in patients than in controls may be caused by the migration of these transporter cells to germinal centers. Similarly, in another study, loss of DCs was found in the vaginal and cervical epithelia of patients with an ongoing infection or inflammation compared to those without signs of inflammation.

In a recent study dense lymphocytic infiltrates were seen in hematoxylin-eosin-stained sections of vestibulodynia specimens. The authors speculated...
the possibility of MALT in the vestibular mucosa. Our immunohistochemical analysis reveals that the lymphocytic infiltrates in LPV indeed represent MALT, containing cells and structures needed to initiate a local immune response. Thus, the vestibular mucosa seems to have its own organized immune system that could be called VALT. Furthermore, our findings of germinal centers and large numbers of B cells and plasma cells support immune activation in LPV. In contrast, other types of immune cells, like macrophages and mast cells, were present in equal densities both in the patient tissues and healthy mucosal tissues.

Acute inflammation is associated with the release of cytokines, activation of complement, polymorphonuclear leukocytes, and mast cells. Acute inflammation is needed to create a milieu for activation of the adaptive immune response. Manifest LPV, however, represents a chronic condition with slow development and the acute phase of inflammation has undoubtedly already faded away. Our patients all had a long history of disease. This may partly explain why we did not find any excess of mast cells in contrast to some previous studies.8,26 In other tissues, eg, in the mucosa of gastrointestinal tract and in coronary arteries, a close association of mast cells with small vessels and nerve endings has been observed.27,28 In our material, mast cells were observed in close association with blood vessels (Figure 5). This suggests a link between the mast cell activation and vascular hyperemic response and possibly the interplay with neurons as well.

Although the immune activation in LPV was evident we found no association with the patients’ clinical characteristics, eg, severity of symptoms or history of infectious or autoimmune diseases. Similarly, there was no association between immune activation and response to surgical treatment. In the above-mentioned recent study histology did not predict response to surgery, either.25 However, 27 study subjects may not be enough for the subgroup analyses although they serve well for immunohistochemical evaluation of the cellular details of inflammation. Also, since the clinical data were collected retrospectively, recall bias may affect these results. Several studies and clinical observations suggest a susceptibility of LPV patients to vulvovaginal infections. Specifically, recurrent *Candida albicans* infection has been considered as a risk factor for LPV.24,25,29 By nature, the vestibule is extensively exposed to foreign antigens during sexual and reproductive life. Thus, both protective and adaptive characteristics of the immune response are required. While protecting against local infection an activated VALT could cause an exaggerated inflammatory reaction and contribute to the sensitized pain perception in the vestibular mucosa. Increasing interest in the role of immune response in pain context is emerging. The immune and neuronal systems are often interrelated and this may predispose to the development of LPV with disturbed pain perception. In this study we demonstrated a locally organized VALT, which may emerge as a response to local infection or inflammation. Although these results do not definitely prove that inflammatory response is related to pain, VALT may play an essential role in the development of LPV. Potential interplay between activated immune cells and biomodulators of the pain sensation poses a challenge to future research on the mechanisms of neurogenic inflammation. Better understanding of the link between immune system and pain may also provide new ways to treat LPV, eg, by immunomodulatory therapy.

### Table 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients positive (%)</th>
<th>GC + N (%)</th>
<th>P value for existence of GC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BAI score&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P value for BAI&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary LPV</td>
<td>8/23 (34.8)</td>
<td>4 (50)</td>
<td>4.0 (1–8)</td>
<td>5.0 (1–7)</td>
<td>.636</td>
</tr>
<tr>
<td>Secondary LPV</td>
<td>15/23 (65.2)</td>
<td>7 (47)</td>
<td>1.000&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.0 (1–9) vs 2.5 (1–7)</td>
<td>.289</td>
</tr>
<tr>
<td>History of recurrent genital infection</td>
<td>21/27 (77.8)</td>
<td>12 (57)</td>
<td>.089</td>
<td>5.0 (1–9) vs 5.5 (1–8)</td>
<td>.391</td>
</tr>
<tr>
<td>History of autoimmune or inflammatory disease&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16/26 (61.5)</td>
<td>7 (44)</td>
<td>.686</td>
<td>5.0 (1–9) vs 5.5 (1–9)</td>
<td>.111</td>
</tr>
<tr>
<td>Complete response to surgery</td>
<td>11/27 (40.7)</td>
<td>7 (64)</td>
<td>.252</td>
<td>4.0 (1–8) vs 5.5 (1–9)</td>
<td>.111</td>
</tr>
</tbody>
</table>

BAI, B-cell activation index; GC, germinal center; LPV, localized provoked vulvodynia.

* a $\chi^2$ analysis. * b Comparative BAI scores of patients with and without characteristic, median (range). * c Mann-Whitney U test. * d Fisher exact test. * e Thyroid disease, rheumatic disease, irritable bowel syndrome, bronchial asthma, atopic skin disease.


Immune activation enhances epithelial nerve growth in provoked vestibulodynia

Päivi Tommola, MD; Leila Unkila-Kallio, MD, PhD; Anders Paetau, MD, PhD; Seppo Meri, MD, PhD; Eija Kalso, MD, PhD; Jorma Paavonen, MD, PhD

BACKGROUND: Provoked vestibulodynia manifests as allodynia of the vulvar vestibular mucosa. The exact mechanisms that result in altered pain sensation are unknown. Recently, we demonstrated the presence of secondary lymphoid tissue, which is the vestibule-associated lymphoid tissue in the vestibular mucosa, and showed that this tissue becomes activated in provoked vestibulodynia.

OBJECTIVE: The purpose of this study was to examine whether expression of intraepithelial nerve fibers and nerve growth factor are related to immune activation in provoked vestibulodynia.

STUDY DESIGN: Vestibular mucosal specimens were obtained from 27 patients with severe provoked vestibulodynia that was treated by vestibulectomy and from 15 control subjects. We used antibodies against the protein gene product 9.5, the neuron specific neurofilament, and nerve growth factor for immunohistochemistry to detect intraepithelial nerve fibers and nerve growth factor expressing immune cells in the vestibular mucosa. For intraepithelial nerve fibers, we determined their linear density (fiber counts per millimeter of the outer epithelial surface), protein gene product 9.5 or presence (neuron specific neurofilament). Nerve growth factor was analyzed by counting the staining-positive immune cells. Antibodies against CD20 (B lymphocytes) and CD3 (T lymphocytes) were used to identify and locate mucosal areas with increased density of lymphocytes and the presence of germinal centers (ie, signs of immune activation). B-cell activation index was used to describe the overall intensity of B-cell infiltration.

RESULTS: We found more protein gene product 9.5—positive intraepithelial fibers in vestibulodynia than in the control samples (6.3/mm [range, 0.0—15.8] vs 2.0/mm [range, 0.0—12.0]; P=.006). Neuron specific neurofilament—positive intraepithelial fibers were found in 17 of 27 vestibulodynia cases (63.0%) and in none of the control cases. Protein gene product 9.5—positive intraepithelial fibers were more common in samples with more pronounced immune activation. The density of these fibers was higher in samples with than without germinal centers (6.1/mm [range, 4.3—15.8] vs 3.0/mm [range, 0.0—13.4]; P=.020). A positive correlation between the fiber density and B-cell activation index score of the sample was found (Spearman’s Rho, 0.400; P=.004; R²=0.128). No significant difference, however, was found in the density or presence of nerve fibers between samples with high and low T-cell densities. We identified areas of minor and major vestibular glands in 16 of the patient samples and in 1 control sample. Protein gene product 9.5—positive nerve fibers were found more often in glandular epithelium surrounded by B-cell infiltration than in glands without B cells (P=.013). Also, the presence of neuron specific neurofilament—positive fibers in glandular epithelium was associated with B-cell infiltrates (P=.053). Nerve growth factor—positive immune cells were more common in mucosal areas with than without B-cell infiltration and intraepithelial nerve fibers.

CONCLUSION: Excessive epithelial nerve growth in provoked vestibulodynia is associated with increased B-cell infiltration and the presence of germinal centers. This supports the fundamental role of immune activation in provoked vestibulodynia.

Key words: germinal center, immune activation, immunohistochemistry, inflammation, nerve fibers, NGF, NF2F11, PGP9.5, vestibulodynia, vulvar pain, vulvar vestibulitis, vulvodynia

Recently, we demonstrated the presence of secondary lymphoid tissue, which is the vestibule-associated lymphoid tissue (VALT) in the vestibular mucosa, and showed that VALT becomes activated in PVD. We showed higher numbers of B cells in PVD than in control samples but found no difference in the density of T cells between the groups. An exaggerated immunoinflammatory response and dysregulation of inflammation seem to be present in PVD. The close relation between immune and neuronal systems can activate neuroinflammatory processes and lead to sensitization of nerve fibers. Immune cells produce nerve growth factor (NGF), which may induce nerve sprouting and enhanced signaling of the nociceptive nerve endings. Thus, it is important to study the interrelation between immune activation and nerves in PVD. Previous studies have shown increased density of nerves in the vestibular mucosa in PVD and increased expression of transient receptor potential V1 (TRPV1) channels in these nerves, but no specific data on the density of intraepithelial nerve fibers (IENF) or expression of NGF exist.

We wanted to find out whether the density and localization of IENFs and the expression of NGF are related to immune activation in the vestibular mucosal tissue in PVD. In addition to the standard neural marker, the protein gene product 9.5 (PGP9.5), we used a specific marker for neurofilaments. To define the sites of immune activation, we used 2
standard markers, CD20 (B cells) and CD3 (T cells). We explored the differences in the expression of IENFs and NGF between PVD and control samples and in relation to different B-cell and T-cell densities.

Material and Methods

Study subjects

The study material consisted of 27 archival vestibulectomy specimens from posterior vestibulectomy operations. The patients were identified in the Helsinki University Hospital patient registry by matching the diagnosis (vulvar vestibulitis, vestibulodynia, and vulvodynia) and the surgical procedure (posterior vestibulectomy). Details of patient recruitment and data collection have been described previously. A good quality paraffin block of the tissue specimen was required. All the included patients had a long disease history (4.0 years; range, 2–18 years) of PVD. The diagnoses for 8 patients were classified as primary (symptoms already at the first vaginal entry); the diagnoses for 15 patients were classified as secondary (symptoms appearing later after an interval of painless intercourses), and the classification for 4 patients was unknown. All patients had been refractory to conservative treatments. The time from the last attempted medical management was >6 months. As control subjects, we recruited 15 healthy volunteers with no vulvar complaints who underwent benign gynecologic surgery. All participants were premenopausal. The median age of the patients with PVD was 27 years (range, 18–48 years); the median age of the control subjects was 30 years (range, 24–44 years; P = .017). A 4-mm punch biopsy specimen from the posterior vestibule at 5 o’clock position was obtained from the control subjects. Both patients and control subjects had provided informed consent. The local Ethical Committee approved the study.

Tissues

All vestibular tissues were embedded routinely in paraffin after a maximum of 24 hours fixation in 10% buffered formalin. Five-micrometer sections were first stained with hematoxylin-eosin to exclude dermatologic diseases and to confirm the quality of the samples. Immunohistochemistry for nerve fibers (10-μm sections) and B and T lymphocytes (5-μm sections) was performed at the Helsinki and Uusimaa Hospital District Laboratory Services tissue laboratory. Routine staining procedures according to the manufacturers’ instructions were followed (Table). Immunostaining for NGF (5-μm sections) was performed at the Department of Clinical Chemistry, University of Helsinki (manual staining procedure); Table).

Tissue analyses

Immunohistochemical scoring was performed under light microscopy (Nikon Eclipse E800; Nikon Instruments Inc, Melville, NY) at >200 magnification. The scoring of each section was based on a consensus of 2 investigators (P.T., A.P., or S.M.) who were blinded to clinical data of the patients. The number of PGP9.5-positive IENFs was counted to calculate the linear density of IENFs (number of nerve fibers /millimeters of epithelial outer surface). For identification of individual fibers, we used the criteria that had been validated for the diagnostics of small fiber neuropathies. Briefly, the fibers were considered to be separate if there were clearly 2 individual parallel fibers and if the distance between 2 different perpendicular sections of a stained axon exceeded 5 times the diameter of an axon. Only fibers clearly penetrating into the epithelium through the basal membrane were counted as IENFs. For neuron specific neurofilament (NF2F11)—positive IENFs only the presence or absence was documented. The overall density of neural fasciculi in the neural plexus region in the subepithelial stroma up to the depth of 1.25 mm (diameter of the ×20 high-power field) was scored semi-quantitatively for both neural markers. A single number score from 1–3 (1=low density, 2=moderate density, 3=high density) was given.

Evaluation of the vestibular glands was also limited to the depth of 1.25 mm. The glands were identified on the basis of typical morphologic condition. All comparisons were made between PVD samples and control samples. In PVD samples, densities of epithelial nerve fibers were also compared between areas with or without increased B-cell infiltration. The representative areas of B-cell infiltration in each sample were located with the use of CD20 staining. To reflect the overall level of B-cell infiltration of each sample, we used the B-cell activation index (BAI). BAI is the calculated sum (0–12) of 3 different parameters that were analyzed from each sample: (1) overall density of B cells in the epithelium (score, 0–4), (2) overall density of B cells in the stroma (score, 0–4), and (3) absence (score, 0) or presence (score, 4) of germinal centers. T-cell density was divided in the CD3-stained samples into 2 categories: “low density” (<50 cells/×20 high-power field) and “high density” (>50 cells/×20 high-power field). Germinal centers were visualized by CD20 and CD3 stainings.

For NGF quantification, 4 types of areas from the PVD samples were identified: (1) areas with increased B-cell infiltration without IENFs, (2) areas without increased B-cell infiltration with IENFs present, (3) areas with both increased B-cell infiltration and IENFs, and (4) areas lacking both B-cell infiltration and IENFs. The number of NGF-positive immune cells per visual field (×20 high-power field) in 3 of each type of areas in each sample was counted, and the mean number of positive cells was calculated. In the control samples, NGF-positive cells were evaluated only in the areas with IENFs present because no areas with increased B-cell infiltration were found.

The data were analyzed by Statistical Package for Social Sciences software (version 22; IBM Corporation, Armonk, NY). We report medians with minimum and maximum and interquartile range (IQR, 25–75%) when appropriate for continuous data. For comparisons, we used the Mann-Whitney U-test and Wilcoxon signed rank tests for continuous data and chi-square analysis or Fisher’s exact test for categoric data. For correlations, the Spearman’s correlation test was used. A 2-tailed probability value of <.05 was considered significant.
Results
Intraepithelial nerve fibers
Because PVD may be related to increased pain sensitivity, we looked at individual nerve fibers in the vestibular epithelium. Both in PVD samples and control samples, PGP9.5-positive IENFs were expressed typically in clusters. Thus, such areas were studied for PGP9.5-positive IENFs. PGP9.5-positive IENFs were found in 24 of the 27 PVD samples (88.9%) and in 8 of the 15 control samples (56.2%). The density of PGP9.5-positive IENFs was significantly higher in PVD (6.3/mm [range, 0.0–15.8]; IQR, 4.4–9.2) than in control samples (2.0/mm [range, 0.0–12.0]; IQR, 0.0–4.3; P = .006; Figure 1, A and B). No significant difference was found in the density of IENFs between 8 primary PVD cases (7.5/mm [range, 3.3–15.8]; IQR, 5.3–8.3) and 15 secondary PVD cases (5.0/mm [range, 0.0–12.4]; IQR, 2.5–9.2; P = .332).

NF2F11-positive IENFs typically occurred as solitary fibers or in clusters of 3–8 fibers (Figure 1, C) and were found in 17 of the 27 PVD cases (63%) and in none of the control cases (P < .001). NF2F11-positive IENFs were as common in primary and secondary PVD (P = .627).

IENFs in relation to B-cell infiltrates
Our previous study showed increased density of B lymphocytes in the vestibular

<table>
<thead>
<tr>
<th>Immunohistochemistry procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table</strong></td>
</tr>
<tr>
<td>Antibody</td>
</tr>
<tr>
<td>Protein gene product 9.5 (PGP9.5)</td>
</tr>
<tr>
<td>RA95101</td>
</tr>
<tr>
<td>Ultra Clone Ltd, Wellow, Isle of Wight, England</td>
</tr>
<tr>
<td>Neuron specific neurofilament (NF2F11)</td>
</tr>
<tr>
<td>M0762</td>
</tr>
<tr>
<td>Agilent Technologies Inc</td>
</tr>
<tr>
<td>CD20</td>
</tr>
<tr>
<td>760-2531</td>
</tr>
<tr>
<td>Roche Diagnostics Ltd, Rotkreuz, Switzerland</td>
</tr>
<tr>
<td>CD3</td>
</tr>
<tr>
<td>790-4341</td>
</tr>
<tr>
<td>Roche Diagnostics Ltd</td>
</tr>
<tr>
<td>Nerve growth factor (NGF)</td>
</tr>
<tr>
<td>sc-548</td>
</tr>
<tr>
<td>Santa Cruz Biotechnology Inc, Santa Cruz, CA</td>
</tr>
</tbody>
</table>

mucosa of patients with PVD. The density of PGP9.5-positive IENFs was greater in the areas with increased B-cell infiltration (5.3/mm; range, 0.0–23.3; IQR, 3.0–9.4) than in the areas with no B cells (4.0/mm; range, 0.0–11.3; IQR, 1.0–8.0; P = .057; Figure 1, D). In control samples, no areas with increased B-cell infiltration were found.

**IENFs in relation to signs of immune activation**

Germinal centers indicate immune activation and are the key component of the local lymphoid tissue, which we have termed as VALT. Infiltrating lymphocytes were primarily B cells that were stained by the CD20 marker. T cells were distributed more evenly across the samples and did not form clusters. The density of PGP9.5-positive IENFs was significantly higher in samples with germinal centers (6.1/mm; range, 4.3–15.8; IQR, 5.0–9.4) than in samples without germinal centers (3.0/mm; range, 0.0–13.4; IQR, 0.0–8.4; P = .020). A positive correlation between the density of PGP9.5-positive IENFs and the BAI score was found (Spearman’s Rho, 0.400; P = .004; R² = 0.128). NF2F11-positive IENFs were not associated with the presence or absence of germinal centers. However, the BAI scores of the samples with NF2F11-positive IENFs were higher (5.0 [range, 1.0–9.0]; IQR, 4.0–6.0) than the BAI scores of the samples without fibers (2.0 [range, 1.0–9.0]; IQR, 1.0–3.0; P = .055). No differences were found in the densities of PGP9.5-positive fibers between the low-density and high-density T-cell groups (6.8/mm [range, 0.0–15.8] and 4.6/mm [range, 0.0–12.4], respectively; P = 1.12) or in the presence of NF2F11-positive fibers (P = .080).

**Glandular epithelium and nerve fibers in relation to B lymphocytes**

Vestibular glands typically occur in the subepithelial layer of the vulvar vestibular mucosa. In the PGP9.5-stained PVD samples, 14 regions with glandular epithelium were identified. Of these, 9 were in areas with increased lymphocytic infiltration that showed a glandular epithelial nerve fiber density of 25.0/mm (range, 3.1–48.0; IQR, 11.3–32.0; Figure 2, A). Five regions were in areas without increased numbers of lymphocytes. Here the glands showed significantly lower epithelial nerve fiber density of 2.0/mm (range, 0.0–17.0; IQR, 0.0–16.5; P = .013). In the control samples, only 1 area of glandular epithelium was identified with an epithelial nerve fiber density of 2.0/mm, which was located in an area with no lymphocytic infiltration. In the NF2F11-stained PVD samples, 16 glandular regions were identified, 12 in areas with lymphocytes and 4 in areas without. Again, epithelial nerve fibers were present more commonly in glands that were surrounded by lymphocytic infiltrates (11/12) than in glands not surrounded by lymphocytic infiltrates (2/4; P = .053, Fisher’s exact test).

**Neural fasciculi in the subepithelial stroma in relation to signs of immune activation**

The densities of the neural fasciculi in the subepithelial stroma by PGP9.5 or
NF2F11 staining did not differ between PVD and control samples ($P=1.10$ and .498, respectively) or between primary and secondary PVD ($P=.289$ and .657, respectively). No association between neural tissue density and signs of immune activation was found (data not shown).

**NGF-positive immune cells in relation to lymphocyte infiltrates and IENFs**

NGF is essential in the development of peripheral nervous system by promoting growth and survival of neuronal cells. In mature tissue, it has important roles both in acute nociception and chronic pain. In the PGP9.5-stained PVD samples, the number of NGF-positive immune cells was higher in the areas with B-cell infiltrates and IENFs (20.0; range, 1.0–102.0) than in the areas lacking both B-cell infiltrates and IENFs (4.0; range, 0.0–24.0; $P<.001$). Because of wide variation, there was no significant difference between primary and secondary PVD in the density of NGF-positive cells (40.0 [range, 1.0–47.0] and 20.0 [range, 3.0–102.0], respectively; $P=.256$). Similarly, densities of these cells in areas with IENFs did not differ between PVD and control samples (20.0 [range, 1.0–102.0] vs 17.5 [range, 0.0–68.0], respectively; $P=.906$; Figure 3). The numbers of NGF-positive immune cells did not differ in relation to T-cell density either (25.0 [range, 1.0–102.0] for the low-density samples and 17.0 [range, 0.0–60.0] for the high-density samples; $P=.300$).

**Comment**

We conducted a thorough analysis of nerve fibers in the vestibular mucosa of patients with PVD and healthy control subjects by using 2 different neural markers, PGP9.5 and NF2F11. We showed that the density of IENFs in the vestibular mucosa was greater in PVD than in control subjects. Recently, we demonstrated the existence of the VALT and showed evidence of immune activation in PVD by identifying germinal centers and higher densities of B cells in PVD than in control subjects. We now show that the epithelial neuroproliferation is associated with VALT and takes place around the areas with B-cell infiltration. Our results suggest that immune activation explains the neuroproliferation and may contribute to the altered pain sensation in PVD.

Germinal centers emerge in the mucosa as a sign of immune activation. Although germinal centers mostly consist of B cells, T cells are also present in the follicular area and have important functional roles as helper cells that stimulate the immune responses. Density of T cells did not show effect on neuroproliferation. This is in line with our earlier study in which we, unlike others, found no difference in T-cell density between patients and control subjects. Further studies are needed to dissect the different T-cell subgroups in relation to nerve fibers and development of germinal centers.

PGP9.5 is a well-established neuron-specific marker that detects the thinnest unmyelinated sensory C fibers, which are <1 μm in thickness. PGP9.5 is the gold standard biomarker for IENFs. However, it is a pan-neuronal marker and, besides the small fibers, also stains subepithelial nerve bundles. Previous studies on PVD have shown an overall abundance of neural tissue in the vestibular mucosa of patients with PVD with no quantification of the IENF density. Thus, by calculation of the linear density of IENFs, the validated method of evaluating IENFs, we were able to demonstrate an actual increase in the IENFs in PVD. Furthermore, we found that only the expressions of IENFs differed between PVD and control samples and between inflammatory and noninflammatory areas; the overall density of neural tissue in the stroma (detected by PGP9.5 and NF2F11) was comparable. This finding disagreed with a previous study that showed neural hyperplasia by PGP9.5 in PVD.
FIGURE 3
Nerve growth factor positive immune cells in the vestibular mucosa

Immunostaining for nerve growth factor—positive immune cells (red) in A, provoked vestibulodynia and B, control. Histologic sections were counterstained with hematoxylin and photomicrographed with a ×20 objective.


compared with control samples and with studies that used S-100 staining. However, the S-100 antigen is not specific to neural tissue but may also stain structures of dendritic cells. Dendritic cells are numerous in the stroma and epithelium of vestibular mucosa, which may cause misinterpretation. We did not find any difference in the IENF density or in the density of subepithelial neural tissue between primary and secondary PVD, contrary to previous reports that have suggested predominance of the overall neural tissue in primary PVD.

NF2F11 detects neuronal axons by labeling the neurofilaments of the cytoskeleton. Neurofilament proteins are expressed exclusively in neurons and are essential for radial growth, axonal caliber maintenance, and myelination of the axons. In mature neurons, the neurofilaments are composed of 3 subunits with different molecular weights. Phosphorylated forms of the subunits are expressed by axons; the non-phosphorylated forms of the subunits are expressed by non-neuronal cells. Our study is the first to show NF2F11 expression in the vestibular mucosal tissue. We used the monoclonal 2F11 antibody, which detects the light molecular weight phosphorylated form of the subunit (the NFL subunit, 70 kDa), that is known to be crucial in the myelination process of the axons. Thus, it is possible that these nerve fibers that are detected by the NF2F11 antibody might be myelinated A-delta fibers that signal a faster mode of sharp pain. It is important to note that no NF2F11-positive epithelial nerve fibers were found in the control samples.

The unmyelinated C-fibers and thinly myelinated A-delta fibers together constitute the small caliber nerve fibers. These fibers transmit cold, warm, and mechanical nociceptive stimuli. Many neuropathic pain states (such as diabetic neuropathy, drug-induced neuropathies, and the burning mouth syndrome) are characterized by decreased density of these small nerve fibers. Decreased density of these IENFs in a skin biopsy is the hallmark of the diagnosis of small fiber neuropathies. Our current finding of a high density of intraepithelial small fibers in PVD suggests that PVD is not a small fiber neuropathy. To our knowledge, the only 2 other examples with increased pain sensitivity and increased density of IENFs that have been documented in the literature are rectal hypersensitivity disorder and dry eye disorder. Little is known about the distribution of nerve fibers in the genital mucosal tissue of healthy women. We, like others, demonstrated the histopathologic characteristic of increased lymphocytic inflammation, especially around the vestibular glands in PVD. We also demonstrated nerve fibers in the glandular epithelium by both PGP9.5 and NF2F11 stainings. In agreement with our finding of IENFs, the density of nerve fibers was higher in the glandular epithelium of glands with surrounding B-cell infiltrates than in area without B cells. These are the first data on the nerve supply of the vestibular glands. The glandular innervation may also be a relevant factor in the sensitized pain perception in PVD.

Recent research has revealed that neuropathic pain because of different diseases indicates different somatosensory profiles. By quantitative sensory testing, it is possible to distinguish between disorders with irritable nociceptors that show positive (ie, sensory gain) sensory signs and disorders with nonirritable nociceptors that show negative (ie, sensory loss) sensory signs. Many studies that have compared patients with PVD with pain-free control subjects have shown lower tactile, pain, and thermal thresholds and higher suprathreshold magnitude estimations for heat in the vulvar vestibule in PVD. Only findings that have suggested sensory gain have been reported. This might well be a result of increased density of irritable nociceptors.

Increased density and sensitivity of IENFs in PVD might be due to the upregulation of NGF because of a previous inflammatory process and immune activation. In addition to our finding of epithelial neuroproliferation and its association with immune activation, others have shown increased density of the TRPV1 in PVD. NGF induces nerve sprouting and contributes to the generation of inflammatory hypersensitivity and allodynia. Inflammatory and immune cells are major sources of NGF. In a recent study on experimental chronic prostatitis in mice, Schwartz et al showed elevated levels of NGF in the prostate tissue and upregulation of TRPV1 in the dorsal root ganglion neurons. We found an increased number of NGF-positive immune cells in the mucosal areas with B-cell infiltration and IENFs in PVD but could not show any difference in the number of NGF-positive cells between PVD and control samples. However, IENFs were present in only some of the
control samples, which may have caused bias. Furthermore, without analyzing the actual tissue level differences of NGF, no conclusions about the role of NGF in PVD pain generation can be drawn. It may be a question of the amount of NGF produced and not the number of cells producing it. Also, NGF might have been up-regulated already in an earlier phase of the disease and could no longer be verified in these samples that represent a late phase of the condition. NGF recently has been linked to many other syndromes that indicate chronic and neuropathic pain; research that targets blocking NGF is ongoing. Encouraging results on the novel protein kinase inhibitor dilmappimod in reducing cyto-kine production of the immune cells have been reported. Thus, as 1 potential drug candidate, the protein kinase inhibitor could inhibit neurogenic inflammation in PVD.

Our study is the first to report the linear density of IENFs in PVD and is the first to show the expressions of NF2F11-positive, possibly A-delta fibers, and innervation of the vestibular glands. A further strength is the higher number of proven cases and control cases than in most previous tissue studies on PVD. The 3-year age difference between cases and control cases, all being premenopausal, is unlikely to play a role. In line with many previous studies on vestibular tissue, our challenge was the size difference between the PVD and control samples. To minimize the bias and to secure appropriate comparison, the control biopsy samples were taken exactly from the 5 o’clock position of the posterior vestibule. This is the most representative area of pain in PVD that has been indicated by studies on vulvar pain mapping and studies on the effect of surgical treatment of PVD. In addition, the depth of all analyses was limited to 1.25 mm according to the depth of control biopsy samples to minimize the sample size bias. However, the size difference may have biased the evaluation of the focally distributed NF2F11 fibers. The lack of gland tissue in the control samples probably was due to the small size of the biopsy samples. Most importantly, however, the linear density evaluation of the C-fibers (numbers per length unit, millimeters) was not compromised because of the sample size difference. Dual staining to reveal the TRPV1 channels in the C-fibers would have been useful. The detection of myelin structures in the NF2F11-positive fibers could have confirmed the A-delta nature of the fibers. We provide new information to the question of the key mechanisms of alldynia in PVD. NGF may be up-regulated because of the activation of VALT and in turn induces sprouting and sensitization of the nociceptive nerve fibers, which suggests a key role to the interplay between immune and neural systems. Future research that will focus on mechanisms in PVD might lead ultimately to the development of new therapeutic interventions.

Acknowledgments

We thank Kristina Nokelainen and senior scientist Kati Räisänen from the Medicum, Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland, and Mia Kero from the Department of Pathology, University of Helsinki and Helsinki University Hospital, Finland, for their expertise in the immunohistochemistry procedures.

References


Kandel ER, Schwartz JH, Jessell TM. Prin-

28.

26.

dendritic cells in human atherosclerotic lesions:

et al. Immunohistochemical characterisation of

25.

24.

91-104.

9.5 using rabbit polyclonal and mouse mono-

clonal antibodies. Br J Exp Pathol 1988;69:

9.1-104.

23.

Bornstein J, Goldschmid N, Sabo E. Hyper-

innervation and mast cell activation may be

used as histopathologic diagnostic criteria for


24.

22.

21.

20.

19.

18.

17.

16.

15.

14.

13.

12.

11.

10.

9.

8.

7.

6.

5.

4.

3.

2.

1.

DECEMBER 2016 American Journal of Obstetrics & Gynecology 768.e8

Author and article information

From the Departments of Obstetrics and Gynecology (Dr Tommola, Unkila-Kallo, and Paavonen), Pathology (Dr Paetau), Bacteriology and Immunology, Haartman Institute (Dr Meri), and the Pain Clinic, Anesthesiology, Intensive Care, and Pain Medicine (Dr Kalso), University of Helsinki and Helsinki University Hospital, and the Research Programs Unit, Program of Immunobiology (Dr Meri), University of Helsinki, Helsinki, Finland.

Received April 22, 2016; revised June 19, 2016; accepted July 11, 2016.

Supported by grants from the Finnish Medical Foundation and Helsinki University Hospital Research Funds (grant nos. TYH20130308 and TYH2013340).

The authors report no conflict of interest.


Corresponding author: Päivi Tommola, MD, paivi.
tommola@kolumbus.fi

9.5 using rabbit polyclonal and mouse mono-

clonal antibodies. Br J Exp Pathol 1988;69:

9.1-104.

23.

Bornstein J, Goldschmid N, Sabo E. Hyper-

innervation and mast cell activation may be

used as histopathologic diagnostic criteria for


24.

22.

21.

20.

19.

18.

17.

16.

15.

14.

13.

12.

11.

10.

9.

8.

7.

6.

5.

4.

3.

2.

1.