Review

Local and Systemic Inflammation in Localized, Provoked Vestibulodynia

A Systematic Review

K. Jane Chalmers, B Phty (Hons), Victoria J. Madden, PhD, Mark R. Hutchinson, PhD, and G. Lorimer Moseley, PhD

OBJECTIVE: To synthesize and critically evaluate all available evidence investigating whether localized, provoked vestibulodynia is associated with a specific inflammatory profile at both a local and a systemic level.

DATA SOURCES: Comprehensive electronic searches were performed in MEDLINE, EMBASE, Scopus, PubMed, Web of Science, Cumulative Index to Nursing and Allied Health Literature, the Cochrane Collaboration databases, and ClinicalTrials.gov. The search strategy was developed using MeSH terms related to localized, provoked vestibulodynia, and inflammatory markers.

METHODS OF STUDY SELECTION: Two independent investigators screened titles and abstracts and performed data extraction and risk of bias assessments. Studies were included if they reported at least one baseline inflammatory marker in women with localized, provoked vestibulodynia and compared them with healthy women. Reference lists from published reviews on localized, provoked vestibulodynia were screened for additional studies.

TABULATION, INTEGRATION, AND RESULTS: There were 1,619 studies identified. Eighteen studies met the inclusion criteria, including 400 women with localized, provoked vestibulodynia and 212 healthy women in a control group. Risk of bias assessment revealed that the methodologic quality was generally low. Fifteen studies investigated local inflammation and three studies investigated systemic inflammation. On a local level, the number of mast cells expressed in vestibular tissues was greater in women with localized, provoked vestibulodynia expressed than in women in the control group. Several studies reported undefined inflammatory infiltrate in vestibular tissues to a greater level in women with localized, provoked vestibulodynia than in women in the control group. Systemically, levels of natural killer cells were lower in women with localized, provoked vestibulodynia than in women in the control group. There were no systemic differences in systemic interferon-α and interferon-ϒ levels between groups.

CONCLUSION: There is limited and contradictory evidence regarding the characteristics of local and systemic inflammation in women with localized, provoked vestibulodynia.

(Obstet Gynecol 2016;0:1–11)
DOI: 10.1097/AOG.0000000000001510

From the Sansom Institute for Health Research, University of South Australia, and the Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, University of Adelaide, Adelaide, South Australia, Australia.

K. Jane Chalmers is supported by a Research Grant from Pelvic Pain Foundation and the Gould Experimental Science Grant from the University of South Australia. Victoria J. Madden is supported by the Oppenheimer Memorial Trust (South Africa): and an Innovation Postdoctoral Fellowship from the National Research Foundation of South Africa. Mark R. Hutchinson is supported by an Australian Research Council Research Fellowship ID DP110100297. G. Lorimer Moseley is supported by a Principal Research Fellowship from the National Health & Medical Research Council of Australia ID 1061279.

Presented as a poster at the International Association for the Study of Pain 15th World Congress on Pain, October 6–11, 2014, Buenos Aires, Argentina, and at PainAdelaide, March 30, 2015, Adelaide, Australia.

Corresponding author: G. Lorimer Moseley, PhD, Sansom Institute for Health Research, University of South Australia, GPO Box 2471, Adelaide, SA 5001; e-mail: lorimer.moseley@gmail.com.

Financial Disclosure
Dr. Moseley has received royalties from NOI Group Publishing for Explain Pain; Explain Pain Handbook: Protectometer; Painful Yarns: Metaphors and Stories to Help Understand the Biology of Pain; *and* The Graded Motor Imagery Handbook. *He receives speaker fees for lectures on pain and its treatment. He has been a consultant for Pfizer, Kaiser Permanente, and Providence Health Care. The other authors did not report any potential conflicts of interest.*

ISSN: 0029-7844/16

Localized, provoked vestibulodynia is a condition characterized by cutting pain, burning pain, or a combination of both at the entrance to the vagina when the area is provoked by pressure.[1] Despite being a very common condition, affecting approximately 12% of women over their lifetimes,[2] very little is known about localized, provoked vestibulodynia.

There have been several reviews published that explore the possible etiology of localized, provoked vestibulodynia[3–6]; however, a consensus on what causes and perpetuates the condition has not been reached. Localized, provoked vestibulodynia is commonly referred to as a condition that is driven by inflammation.[6–10] However, data have emerged that cast doubt over this view (eg, the failure of treatments aimed at reducing inflammation in localized, provoked vestibulodynia[11,12]).

There have been many investigations and several narrative reviews into the local and systemic inflammatory profile in women with and without localized, provoked vestibulodynia. Remarkably, however, there seems to have been no attempt to critically appraise this literature or obtain an overall impression of the state of the evidence. We contend that this is a critical gap and that it is important to understand the mechanisms behind localized, provoked vestibulodynia to be confident in assessing and treating it and to augment the search for more effective interventions.

We aimed to synthesize and critically evaluate all available evidence concerning the local and systemic inflammatory profile in women with and without localized, provoked vestibulodynia and to obtain a picture of the current state of the evidence in this field.

SOURCES

A protocol for the review was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. We performed an electronic search of published studies up to November 2015 included in the following databases: MEDLINE (through OvidSP), EMBASE (through OvidSP), Scopus, PubMed, Web of Science, Cumulative Index to Nursing and Allied Health Literature, the Cochrane Collaboration, and ClinicalTrials.gov. The search strategy was developed using MeSH terms and free terms and was devised with the assistance of a research librarian at the University of South Australia (Appendix 1, available online at http://links.lww.com/AOG/A827). Any related reviews, books, and book chapters were also hand-searched for additional studies.

Any studies that measured biomarkers of inflammation in human participants with localized, provoked vestibulodynia and in women in a control group were included if they were published or in press at the time of the search. No restrictions were placed on the duration of localized, provoked vestibulodynia or the age of participants, although participants had to be premenopausal. Vulvar pain is common postmenopause as a result of hormonal changes such as atrophic vaginitis. Often these symptoms are incorrectly labeled as localized, provoked vestibulodynia[13]; therefore, we excluded menopausal and postmenopausal women to avoid these potentially incorrectly diagnosed women. There were no restrictions on the language or publication date of articles.

STUDY SELECTION

Two reviewers (K.J.C. and V.J.M.) independently screened the titles and abstracts of all studies. After this screening, the two reviewers then independently assessed the eligibility of full-text articles. Studies were retained if they met the following criteria: 1) participants were premenopausal women; 2) measured at least one unstimulated biomarker of inflammation in both women with localized, provoked vestibulodynia and women in the control group, excluding mRNA and other genetic biomarkers; and 3) localized, provoked vestibulodynia was diagnosed according to recognized criteria (ie, not self-diagnosed). Any discrepancies were resolved through discussion between the two reviewers. If discrepancies were unresolved through discussion, they were discussed by the wider research team until a resolution was obtained.

The same two reviewers also completed the quality assessment using an adapted version of the STrengthening the Reporting of OBservational studies in Epidemiology statement,[14] as has previously been used in a similar systematic review.[15] In the "other" column of the tool, reviewers were particularly concerned with the reliability and validity of sampling and assessment methods and the standardization of the tests in terms of anatomic location and time of day. These considerations were important because both aspects may have systematic effects on inflammatory profiles: levels of inflammation in separate vestibular anatomic locations can differ, even in women with no pain (eg, Foster and Hasday[16] and Bornstein et al[17]), and there are demonstrated diurnal variations in the production of cytokines in healthy humans.[18] Each criterion was scored as "yes," "no," or "unclear" based on the published information available. Any discrepancies were again resolved through discussion.

A customized and previously piloted data extraction form was then used by the same two reviewers to extract information relating to our objective from each of the included studies. Data extracted included participant characteristics such as age, pain score, symptom duration, parity, and whether any forms of hormonal contraception were currently in use; and information on the biomarker of inflammation studied such as the nature and location of the sample, sample

handling and analysis procedures as well as the levels of the biomarker present in the sample.

Where the article did not contain the necessary information, three attempts were made to contact the authors by e-mail to request the additional information. Authors were given 3 weeks to provide the information and received two reminder e-mails if they missed the deadline or had not responded.

We sought to pool inflammation data from studies where adequate and appropriate data were available. We planned, a priori, to pool data from studies that had assessed the same cytokine using identical methods and control groups.

RESULTS

Figure 1 outlines the process of the literature search and selection of studies for inclusion in this review. The initial search yielded 1,619 studies with an additional seven found through searching the reference lists of relevant articles. After removing duplicates, the titles and abstracts of 1,158 articles were screened for eligibility. Seventy-four articles were identified as being relevant, so the full texts of these articles were accessed. Fifty-six articles were excluded in the full-text review, leaving 18 studies eligible for inclusion in this review according to our a priori criteria. There were four discrepancies between reviewers that were resolved through initial discussion.

Key data from these 18 studies are summarized in Tables 1 and 2. Eleven studies reported their results quantitatively[16,19–27]; four studies reported their results semiquantitatively using a level of inflammation descriptor (eg, "none," "mild," "moderate," and "severe").[28–31] Three studies[17,32,33] reported both quantitative and semiquantitative results.

The overall risk of bias of included studies was high (Table 3). The study with the lowest risk of bias was that by Masterson et al,[34] which scored a low risk on all but three categories, in which it scored unclear (blinding, attrition bias, and "other bias") as a result of a lack of depth in reporting their methodology. None of the 18 included studies scored a low risk of bias in the attrition bias or "other bias" categories. These categories were scored either unclear or high risk of bias for two main reasons: 1) failure to report how participants were recruited and how many potential participants refused to take part in the study, and 2) failure to discuss the reliability and validity of the methodology that was used. Bornstein et al[17] and Eva et al[20] closely followed Masterson et al[34] with low risk of bias.

The authors of 17 articles were contacted for further information required for the review; of these, eight responses were received. However, only half (four) were able to provide the necessary data.

Table 4 outlines the results from the 18 studies. Although systematic reviews routinely include a risk of bias assessment, that assessment is often not taken into account during the extrapolation of results. More confidence can be placed in the findings of studies with lower risk of bias because their findings are more likely to represent the true effect, not that of chance or

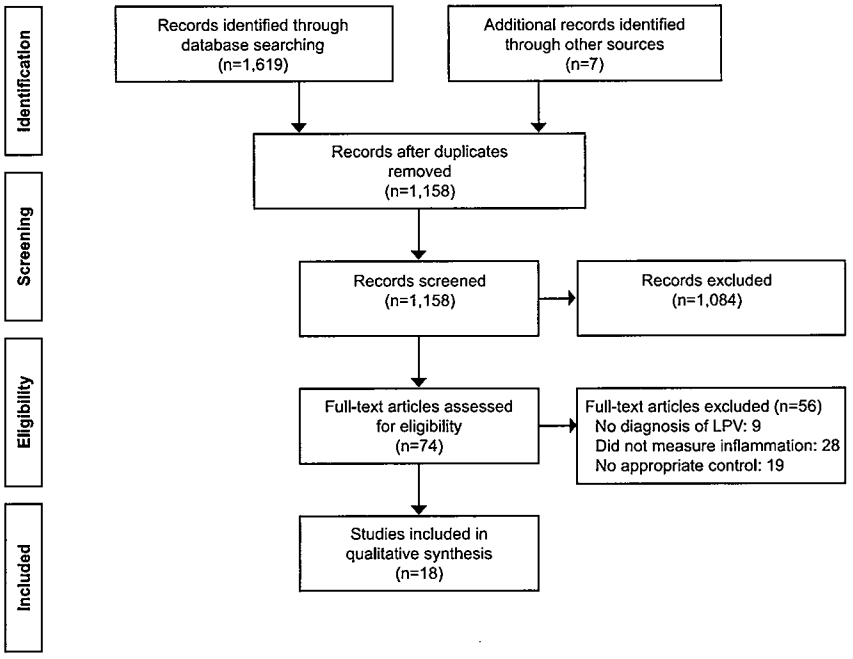

Fig. 1. Literature search and selection of studies per PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; www.prisma-statement.org). LPV, localized, provoked vestibulodynia.

Chalmers et al. Inflammation in Localized, Provoked Vestibulodynia. Obstet Gynecol 2016.

Table 1. Key Participant Characteristics From Studies Investigating Local Inflammation

Study	LPV Criteria	Sample Type	Control Participant Characteristics (n=212)			
			n	Age (y)	Pain Score	Other
Chaim et al, 1996[19]	Vulvar burning, focal tenderness, introital dyspareunia	Biopsy	—	—	—	Undergoing posterior repair for pelvic relaxation
Foster and Hasday, 1997[16]	Greater than 1 y vulval burning and dyspareunia, vulval allodynia, no other pathologies, 3- to 6-mo trial of conservative therapy failure	Biopsy	10	48 (median)	All 0/10 VAS CTT	Normal neurologic examination; undergoing rectocele; 6/10 Caucasian; parity median 3
Lundqvist et al, 1997[28]	Friedrich's criteria	Biopsy	11 (11 sections)	18–25 (range)	—	All HPV-negative
Chadha et al, 1998[29]	Dyspareunia greater than 6 mo, burning painful sensation at introitus	Biopsy	12	—	—	Undergoing laparoscopic sterilization
Slone et al, 1999[30]	Friedrich's criteria	Biopsy	5 (6 sections)	—	—	Archived tissues
Bornstein et al, 2004[17],*	Friedrich's criteria	Biopsy	7	18–48 (range)	—	Undergoing corrective or cosmetic surgery
Halperin et al, 2005[33],*	Friedrich's criteria	Biopsy	16	27.5 (range 20–45)	—	Undergoing reconstructive surgery
Eva et al, 2007[20]	Friedrich's criteria	Biopsy	16	—	—	Women undergoing nonpainful gynecologic surgery; 1 postmenopausal; all Caucasian
Foster et al, 2007[22]	As according to Bergeron et al, 2001	Biopsy	5	43.4±6.2	—	Negative yeast cultures
Korcheva and Morgan, 2008[31]	—	Biopsy	9	—	—	Archived tissues
Goetsch et al, 2010[32],*†	Greater than 1 y dyspareunia, extreme tenderness on light touch	Biopsy	4	40.3±3.7 (range 36–45)	—	Undergoing surgery
Leclair et al, 2014[25],‡	ISSVD criteria	Biopsy§	4	40.3±3.7 (range 36–45)	—	Undergoing surgery
		Biopsy‖	2	36 and 33	—	—
Seckin-Alac et al, 2014[26]	Friedrich's criteria	Biopsy	13	—	—	Women undergoing rectocele repair
Foster et al, 2015[21],¶	Friedrich's criteria	Biopsy	4 (8 samples)	33.5	All 0/10 NRS on CTT	All Caucasian
Tommola et al, 2015[27]	—	Biopsy	15	30 (range 24–44)	—	Women undergoing benign gynecologic surgery

LPV, localized, provoked vestibulodynia; VAS, visual analog scale; CTT, cotton tip test; HPV, human papillomavirus; ISSVD, International Society for the Study for Vulvovaginal Disease; NRS, numeric rating scale.
Age and LPV duration presented as mean or mean±standard deviation and (range), unless otherwise stated.
* Reported both quantitative and semiquantitative data.
† Appear to have used the same cohort as Leclair et al, 2014.[25]
‡ Appear to have used the same cohort as Goetsch et al, 2010.[32]
§ Group samples assessed using immunohistochemistry.
‖ Group samples assessed using flow cytometry.
¶ Appear to have used the same cohort as a study not included in this review (Falsetta ML, Foster DC, Woeller CF, Pollock SJ, Bonham AD, Haidaris CG, et al. Identification of novel mechanisms involved in generating localized vulvodynia pain. Am J Obstet Gynecol 2015;213:38.e1–12).

LPV Participant Characteristics (n=400)				
n	Age (y)	Pain Score	LPV Duration	Other
16	27.5±5.2 (20–36)	—	6 mo–12 y	15 Caucasian; 11 nulliparous; undergoing perineoplasty
12	33 (median)	8/10 VAS CTT (median); all greater than 5/10 VAS CTT	Greater than 1 y, (range 2–9 y)	All Caucasian; parity median 1
20 (24 sections)	20–29 (range)	—	—	All HPV negative
12	—	—	23 mo	2 positive for candida, 1 positive for chlamydia
14 (15 sections)	43±11.7 (range 23–61)	—	6 mo–8 y (range)	Archived tissues
40	—	—	—	Undergoing perineoplasty
24	23.8 (range 19–35)	—	6 mo–10 y (range)	All primary LPV
35	—	—	—	All Caucasian
5	43.0±4.1	—	104±70 mo	Negative yeast cultures
18	—	—	—	—
10	28.4±7.1 (range 20–46)	—	6.5 y (median) (range 2–25 y)	Primary LPV
10	30.8±7.5 (range 21–40)	—	4.5 y (median) (range 1–20 y)	Secondary LPV
10	28.4±7.1 (range 20–46)	—	6.5 y (median) (range 2–25 y)	Primary LPV
10	30.8±7.5 (range 21–40)	—	4.5 y (median) (range 1–20 y)	Secondary LPV
4	26, 33, 28, and 23	—	—	All primary LPV
15	—	—	—	—
4 (8 samples)	33.5	8/10 NRS CTT (median) (range 7–9/10)	72±26 mo	All 0/10 NRS CTT for external to vulva; all Caucasian
27	27 (median) (range 18–44)	—	—	8 primary LPV, 15 secondary LPV, 4 unsure

the involvement of confounding factors. We aimed to integrate the results of the constituent articles with the risk of bias assessment results to provide a more confident explanation of the findings within studies. The full study results can be found in tabulated form in Appendices 2–4, available online at http://links.lww.com/AOG/A827.

Levels of interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α), and "inflammation" as well as numbers of B cells and mast cells were assessed by multiple studies. However, the studies used various sample analysis techniques or assessed heterogeneous sites, precluding the possibility of pooling data or undertaking a meta-analysis.

Table 2. Key Participant Characteristics From Studies Investigating Systemic Inflammation

Study	LPV Criteria	Sample Type	Control Participant Characteristics				LPV Participant Characteristics				
			n	Age (y)	Pain Score	Other	n	Age (y)	Pain Score	LPV Duration	Other
Masterson et al, 1996[34]	ISSVD	Blood	17	—	—	HPV-negative	22	—	—	—	—
Gerber et al, 2002[23],*	Friedrich's	Blood	47	—	—	Mostly Caucasian	62	—	—	—	Mostly Caucasian
Jayaram et al, 2014[24]	Friedrich's	Vaginal swab	15	32.6±8.1	—	Normal body mass	30	30.8±5.7	—	28 mo (median) (2–264 y)	6 primary LPV, 24 secondary LPV; normal body mass

LPV, localized, provoked vestibulodynia; ISSVD, International Society for the Study for Vulvovaginal Disease; HPV, human papillomavirus.
* Appear to have used the same cohort as a study not included in this review (Gerber S, Bongiovanni AM, Ledger WJ, Witkin SS. Defective regulation of the proinflammatory immune response in women with vulvar vestibulitis syndrome. Am J Obstet Gynecol 2002;186:696–700).

Four studies[17,29,31,32] reported a greater amount of nondescriptive inflammatory infiltrate in women with localized, provoked vestibulodynia than in women in the control group[12,24,26,27]; however, two studies[30,33] reported no differences between the groups,[25,28] and one study reported a greater level of inflammatory infiltrate in the control group than in the localized, provoked vestibulodynia group.[28]

Four studies reported higher numbers of mast cells present in the vestibular tissues of women with localized, provoked vestibulodynia than in those of women in the control group.[12,20,26,27] Furthermore, Goetsch et al[32] reported higher numbers of mast cells existing only in tender areas, suggesting a site-specific difference in mast cell count. Two studies reported similar mast cell counts in women with localized, provoked vestibulodynia and healthy women in the control group.[27,33]

Local levels of vestibular TNF-α were investigated in three studies[16,20,22]; of these, two found no

Table 3. Risk of Bias Results and Overall Bias Score of the 18 Included Studies

Study	Bias Category							Overall Score (0–7)
	Statistical Methods	Selection Bias	Blinding	Attrition Bias	Detection Bias	Reporting Bias	Other Bias	
Chaim et al, 1996[19]	?	?	●	?	○	●	?	6
Masterson et al, 1996[34]	○	○	?	?	○	○	?	3
Foster and Hasday, 1997[16]	●	●	●	?	○	○	?	5
Lundqvist et al, 1997[28]	?	?	?	?	○	●	?	6
Chadha et al, 1998[29]	?	?	●	?	?	●	?	7
Slone et al, 1999[30]	?	?	?	?	●	○	?	6
Gerber et al, 2002[23]	○	?	?	?	?	○	?	5
Bornstein et al, 2004[17]	●	?	●	?	○	●	?	6
Halperin et al, 2005[33]	?	●	●	?	?	○	?	6
Eva et al, 2007[20]	○	?	●	?	○	●	?	5
Foster et al, 2007[22]	●	?	●	?	●	●	?	7
Korcheva et al, 2008[31]	?	?	?	?	?	○	?	6
Goetsch et al, 2010[32]	●	?	●	?	?	○	?	6
Jayaram et al, 2014[24]	●	●	?	?	?	○	?	6
Leclair et al, 2014[25]	●	?	?	?	?	○	?	6
Seckin-Alac et al, 2014[26]	?	?	?	?	?	○	?	6
Foster et al, 2015[21]	●	●	?	?	?	○	?	6
Tommola et al, 2015[27]	?	?	?	?	?	●	?	7

● =high risk; ○ =low risk; ?=unclear of risk (not stated in article or in discussions with author[s]).
Overall score calculated as UNCLEAR/HIGH=1, LOW=0; the highest possible score is 7; a higher number indicates an increased risk of bias.

Table 4. The Results of the Included Studies Investigating Local Inflammation and Systemic Inflammation Weighted According to Risk of Bias

Marker	Study	Sample Type	No. of Participants		Result
			Controls	LPV	
Local inflammation					
B cells	Tommola et al, 2015[27]	Biopsy	15	27	
	Leclair et al, 2014[25]	Biopsy	4	20	*
GM-CSF	Foster et al, 2007[22]	Biopsy	5	5	
IL-1α	Eva et al, 2007[20]	Biopsy	16	35	†
IL-1β	Foster and Hasday, 1997[16]	Biopsy	10	12	†
	Eva et al, 2007[20]	Biopsy	16	35	†
	Foster et al, 2007[22]	Biopsy	5	5	
IL-2	Foster et al, 2007[22]	Biopsy	5	5	
IL-4	Foster et al, 2007[22]	Biopsy	5	5	
IL-6	Foster et al, 2007[22]	Biopsy	5	5	
	Foster et al, 2015[21]	Biopsy	4	4	*
IL-8	Foster et al, 2007[22]	Biopsy	5	5	
IL-10	Foster et al, 2007[22]	Biopsy	5	5	
IL-12	Foster et al, 2007[22]	Biopsy	5	5	
IFN-γ	Foster et al, 2007[22]	Biopsy	5	5	
Inflammation	Chadha et al, 1998[29]	Biopsy	12	12	*
	Bornstein et al, 2004[17]	Biopsy	7	40	†
	Korcheva et al, 2008[31]	Biopsy	9	18	
	Goetsch et al, 2010[32]	Biopsy	4	10	†
	Slone et al, 1999[30]	Biopsy	5	14	
	Halperin et al, 2005[33]	Biopsy	16	24	
	Lundqvist et al, 1997[28]	Biopsy	11	24	*
Macrophages	Tommola et al, 2015[27]	Biopsy	15	27	
Mast cells	Chaim et al, 1996[19]	Biopsy	NS	16	
	Bornstein et al, 2004[17]	Biopsy	7	40	†
	Korcheva et al, 2008[31]	Biopsy	9	18	
	Goetsch et al, 2010[32]	Biopsy	4	10	†
	Halperin et al, 2005[33]	Biopsy	16	24	†
	Tommola et al, 2015[27]	Biopsy	15	27	
PGE_2	Foster et al, 2015[21]	Biopsy	4	4	*
T cells	Tommola et al, 2015[27]	Biopsy	15	27	

(*continued*)

Table 4. The Results of the Included Studies Investigating Local Inflammation and Systemic Inflammation Weighted According to Risk of Bias (*continued*)

Marker	Study	Sample Type	No. of Participants		Result
			Controls	LPV	
CD3+ T cells	Leclair et al, 2014[25]	Biopsy	4	20	—●—*
CD4+ T cells	Leclair et al, 2014[25]	Biopsy	4	20	↑*†
CD8+ T cells	Leclair et al, 2014[25]	Biopsy	4	20	↓*
CD4+:CD8+ T cells	Leclair et al, 2014[25]	Biopsy	4	20	↑*
IL-1β+ T cells	Seckin-Alac et al, 2014[26]	Biopsy	13	15	—●—
TNF-α+ T cells	Seckin-Alac et al, 2014[26]	Biopsy	13	15	↑
TNF-α	Foster and Hasday, 1997[16]	Biopsy	10	12	—●—†
	Foster et al, 2007[22]	Biopsy	5	5	—●—
	Eva et al, 2007[20]	Biopsy	16	35	↓†
Systemic inflammation					
IL-1β	Jayaram et al, 2014[24]	Vaginal swab	15	30	—●—
IFN-α	Gerber et al, 2002[23]	Blood	47	62	—●—
IFN-γ	Gerber et al, 2002[23]	Blood	47	62	—●—
NK Cells	Masterson et al, 1996[34]	Blood	17	22	↓

LPV, localized, provoked vestibulodynia; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α; NK, natural killer; NS, not stated.

↑=LPV>women in the control group; ↓=Women in the control group>LPV; —●—=No difference between control and LPV groups.

The results within this table are weighted according to the risk of bias results from included studies. That is, studies with low risk of bias are represented by thicker lines, indicating more confidence in the results, and vice versa for studies with higher risk of bias.

* Results collated from multiple sites or analysis methods in the one study.

† Results based on statistical analysis performed by the systematic review author team.

difference in the levels of TNF-α,[16,22] and a separate study reported lower levels of TNF-α in women with localized, provoked vestibulodynia than in healthy women in the control group.[20]

The number of B cells present in vestibular tissues was assessed by Leclair et al,[25] who reported no difference between groups. However, Tommola et al[27] also investigated levels of B cells, but found a greater number in the vestibular tissues of women with localized, provoked vestibulodynia than healthy women in the control group.

Four studies reported no difference in local levels of IL-1β in women with localized, provoked vestibulodynia and in healthy women in the control group.[16,20,22,24] Similarly, two studies reported no difference in local levels of IL-6 between groups.[21,22]

Several studies investigated different aspects of T cells. Tommola et al[27] reported similar numbers of T cells in women with localized, provoked vestibulodynia and in healthy women in the control group. When specific staining of T cells was assessed, greater numbers of TNF-α-positive[26] and CD4-positive[25] T cells were found in women with localized, provoked vestibulodynia than in healthy women in the control group. Conversely, women with localized, provoked vestibulodynia displayed lesser numbers of CD8-positive T cells than healthy women in the control group displayed.[25] No differences in the number of CD3-positive[25] and IL-1β-positive[26]-stained T cells were observed between groups.

Many markers of inflammation were investigated by only a single study, in which they were not

significantly different between groups: granulocyte macrophage colony-stimulating factor,[22] IL-1α,[20] IL-2,[22] IL-4,[22] IL-8,[22] IL-10,[22] IL-12,[22] interferon-α,[22] interferon-ϒ,[22] prostaglandin E_2,[21] and macrophages.[27]

No systemic markers of inflammation were investigated by more than one study.

Masterson et al[34] reported lesser numbers of natural killer cells in the blood of women with localized, provoked vestibulodynia than healthy women in the control group. Levels of systemic IFN-α and IFN-ϒ were investigated by Gerber et al,[23] who reported no differences between the localized, provoked vestibulodynia and healthy groups.

DISCUSSION

This systematic review highlights the lack of a consistent inflammatory profile in women with localized, provoked vestibulodynia. The methodologic quality of the 18 included studies was poor, casting further doubt over our current understanding of inflammation in localized, provoked vestibulodynia.

Women with localized, provoked vestibulodynia express higher than normal numbers of mast cells in vestibular tissues, which is not altogether surprising given the presentation of the condition. Activated mast cells produce nerve growth factor and proinflammatory cytokines,[35] both of which can stimulate and sensitize peripheral nociceptive fibers,[36] and cause hyperplasia of these nerves.[37] This sensitization and hyperplasia may lower the sensory threshold of the vestibular tissues, leading to allodynia, one of the clinical features of localized, provoked vestibulodynia. Interestingly, however, there was no observed increase in the number of proinflammatory cytokines in women with localized, provoked vestibulodynia.

Many studies reported the presence of undefined "inflammation" in the vestibular tissues of women with localized, provoked vestibulodynia, but also healthy women. This is not altogether surprising: the environmental conditions of the vulva cause bacteria and fungi to flourish,[38] evoking normal inflammatory processes as part of the ongoing immune response. The results from this review suggest that a low level of vestibular inflammation is normal in healthy women.

Natural killer cell counts are significantly lower in the blood of women with localized, provoked vestibulodynia than they are in healthy women.[34] Natural killer cells play a large role in the body's defense against fungal *Candida* infections of the vulva and vagina, and a deficiency in these cells may lead to repeated infections. Because women with localized, provoked vestibulodynia are deficient in natural killer cells, it is not surprising that 80% of women with localized, provoked vestibulodynia (as contrasted with 21% of healthy women) report a history of recurrent candidiasis.[39] It is plausible to suggest that a deficiency in natural killer cells may lead to recurrent infections of candidiasis in the vulva, which may eventually manifest as localized, provoked vestibulodynia. That recurrent candidiasis may lead to localized, provoked vestibulodynia is not a new proposition[40,41]; however, just how recurrent candidiasis might trigger the condition remains to be investigated.

Besides the high risk of bias described previously, interpretation of the current result should consider other pertinent issues. First, this review did not subdivide localized, provoked vestibulodynia groups into primary localized, provoked vestibulodynia, where pain is present from the first attempt of vaginal penetration, or secondary localized, provoked vestibulodynia, when pain appears after a period of pain-free vaginal penetration. There is some evidence to suggest that these two subdivisions may present with different etiologies.[25,32] Second, the methods of investigating inflammation varied greatly across studies. In many cases a lack of reporting led to confusion over the anatomic location from which the samples were taken. There was also great variation in the specific laboratory techniques used to investigate levels of inflammatory markers; these techniques have different sensitivity thresholds, which may explain some of the variation observed in the results. Finally, we only included studies that investigated baseline inflammatory markers. Studies that used methods of stimulation or provocation were excluded from this review but may have added valuable data. There is evidence to suggest that women with localized, provoked vestibulodynia express more proinflammatory cytokines (eg, IL-1β[42] and IL-6[22]) and fewer anti-inflammatory control mechanisms (eg, IL-1 receptor antagonist[42]) in response to stimulation in vitro. Recent research has investigated the so-called "priming" of immune cells to assess immune cells responses to repeated immune challenges. Normal immune cells return to a dormant state after provocation, but others remain in a constant state of readiness to respond to the next immune challenge with a heightened proinflammatory response.[43] Priming of immune cells can occur through activation of Toll-like receptor 4, a receptor on the surface of immune cells that recognizes the presence of damage or danger. *Candida* infection is known to activate Toll-like receptor 4,[44] and so it is plausible that the

recurrent candidiasis infections commonly reported by women with localized, provoked vestibulodynia led to the priming of immune cells within the vestibule. This may explain why there are limited reports of baseline vestibular inflammation in women with localized, provoked vestibulodynia, as evidenced by this review, yet there is some evidence of a heightened proinflammatory response to vestibular stimulation in these women. This mechanistic hypothesis remains to be explored.

We propose that localized, provoked vestibulodynia reflects vestibular tissues in a submaximal-immune state or primed state. In this state, immune processes are clearly occurring but are insufficient to produce a proinflammatory response. As such, and notably, there seems to be no indication for the use of anti-inflammatory therapies to treat localized, provoked vestibulodynia.

REFERENCES

1. Haefner HK. Report of the International Society for the Study of Vulvovaginal Disease terminology and classification of vulvodynia. J Low Genit Tract Dis 2007;11:48–9.
2. Harlow BL, Stewart EG. A population-based assessment of chronic unexplained vulvar pain: have we underestimated the prevalence of vulvodynia? J Am Med Womens Assoc 2003;58:82–8.
3. Bergeron S, Binik YM, Khalifé S, Pagidas K. Vulvar vestibulitis syndrome: a critical review. Clin J Pain 1997;13:27–42.
4. Akopians AL, Rapkin AJ. Vulvodynia: the role of inflammation in the etiology of localized provoked pain of the vulvar vestibule (vestibulodynia). Semin Reprod Med 2015;33:239–45.
5. Davis GD, Hutchison CV. Clinical management of vulvodynia. Clin Obstet Gynecol 1999;42:221–33.
6. Zolnoun D, Hartmann K, Lamvu G, As-Sanie S, Maixner W, Steege J. A conceptual model for the pathophysiology of vulvar vestibulitis syndrome. Obstet Gynecol Surv 2006;61:395–401.
7. Farage MA, Galask RP. Vulvar vestibulitis syndrome: a review. Eur J Obstet Gynecol Reprod Biol 2005;123:9–16.
8. Gaunt G, Good A, Stanhope CR. Vestibulectomy for vulvar vestibulitis. J Reprod Med 2003;48:591–5.
9. Gerber S, Witkin SS, Stucki D. Immunological and genetic characterization of women with vulvodynia. J Med Life 2008;1:432–8.
10. Pukall CF, Smith KB, Chamberlain SM. Provoked vestibulodynia. Womens Health (Lond Engl) 2007;3:583–92.
11. Friedrich E Jr. Vulvar vestibulitis syndrome. J Reprod Med 1987;32:110–4.
12. Landry T, Bergeron S, Dupuis MJ, Desrochers G. The treatment of provoked vestibulodynia: a critical review. Clin J Pain 2008;24:155–71.
13. Arnold LD, Bachmann GA, Kelly S, Rosen R, Rhoads GG. Vulvodynia: characteristics and associations with co-morbidities and quality of life. Obstet Gynecol 2006;107:617–24.
14. Gallo V, Egger M, McCormack V, Reid WM, Fong AC, Crow J, et al. STrengthening the Reporting of OBservational studies in Epidemiology–Molecular Epidemiology (STROBE-ME): an extension of the STROBE statement. Eur J Clin Invest 2012;42:1–16.
15. Parkitny L, McAuley JH, Di Pietro F, Stanton TR, O'Connell NE, Marinus J, et al. Inflammation in complex regional pain syndrome: a systematic review and meta-analysis. Neurology 2013;80:106–17.
16. Foster DC, Hasday JD. Elevated tissue levels of interleukin-1β and tumor necrosis factor-α in vulvar vestibulitis. Obstet Gynecol 1997;89:291–6.
17. Bornstein J, Goldschmid N, Sabo E. Hyperinnervation and mast cell activation may be used as histopathologic diagnostic criteria for vulvar vestibulitis. Gynecol Obstet Invest 2004;58:171–8.
18. Petrovsky N, Harrison LC. The chronobiology of human cytokine production. Int Rev Immunol 1998;16:635–49.
19. Chaim W, Meriwether C, Gonik B, Qureshi F, Sobel JD. Vulvar vestibulitis subjects undergoing surgical intervention: a descriptive analysis and histopathological correlates. Eur J Obstet Gynecol Reprod Biol 1996;68:165–8.
20. Eva LJ, Rolfe KJ, MacLean AB, Reid WM, Fong AC, Crow J, et al. Is localized, provoked vulvodynia an inflammatory condition? J Reprod Med 2007;52:379–84.
21. Foster DC, Falsetta ML, Woeller CF, Pollock SJ, Song K, Bonham A, et al. Site-specific mesenchymal control of inflammatory pain to yeast challenge in vulvodynia afflicted and pain-free women. Pain 2015;156:386–96.
22. Foster DC, Piekarz KH, Murant TI, LaPoint R, Haidaris CG, Phipps RP. Enhanced synthesis of proinflammatory cytokines by vulvar vestibular fibroblasts: implications for vulvar vestibulitis. Am J Obstet Gynecol 2007;196:346.e1–8.
23. Gerber S, Bongiovanni AM, Ledger WJ, Witkin SS. A deficiency in interferon-α production in women with vulvar vestibulitis. Am J Obstet Gynecol 2002;186:361–4.
24. Jayaram A, Witkin SS, Zhou X, Brown CJ, Rey GE, Linhares IM, et al. The bacterial microbiome in paired vaginal and vestibular samples from women with vulvar vestibulitis syndrome. Pathog Dis 2014;72:161–6.
25. Leclair CM, Leeborg NJ, Jacobson-Dunlop E, Goetsch MF, Morgan TK. CD4-positive T-cell recruitment in primary-provoked localized vulvodynia: potential insights into disease triggers. J Low Genit Tract Dis 2014;18:195–201.
26. Seckin-Alac E, Akhant S, Bastu E, Tuzlalik S, Yavuz E. Elevated tissue levels of tumor necrosis factor-α in vulvar vestibulitis syndrome. Clin Exp Obstet Gynecol 2014;41:691–3.
27. Tommola P, Bützow R, Unkila-Kallio L, Paavonen J, Meri S. Activation of vestibule-associated lymphoid tissue in localized provoked vulvodynia. Am J Obstet Gynecol 2015;212:476.e1–8.
28. Lundqvist EN, Hofer P, Olofsson J, Sjöberg I. Is vulvar vestibulitis an inflammatory condition? A comparison of histological findings in affected and healthy women. Acta Derm Venereol 1997;77:319–22.
29. Chadha SM, Gianotten WL, Drogendijk AC, Weijmar Schultz WC, Blindeman LA, van der Meijden WI. Histopathologic features of vulvar vestibulitis. Int J Gynecol Pathol 1998;17:7–11.
30. Slone S, Reynolds L, Gall S, Peiper S, Martin A, Ackermann D, et al. Localization of chromogranin, synaptophysin, serotonin, and CXCR2 in neuroendocrine cells of the minor vestibular glands: an immunohistochemical study. Int J Gynecol Pathol 1999;18:360–5.

31. Korcheva V, Morgan TK. Chronic vestibulitis in young and postmenopausal women: significance of inflammation, mast cells, and neural proliferation. Lab Invest 2008;88: 210A.
32. Goetsch MF, Morgan TK, Korcheva VB, Li H, Peters D, Leclair CM. Histologic and receptor analysis of primary and secondary vestibulodynia and controls: a prospective study. Am J Obstet Gynecol 2010;202:614.e1–8.
33. Halperin R, Zehavi S, Vaknin Z, Ben-Ami I, Pansky M, Schneider D. The major histopathologic characteristics in the vulvar vestibulitis syndrome. Gynecol Obstet Invest 2005;59: 75–9.
34. Masterson BJ, Galask RP, Ballas ZK. Natural killer cell function in women with vestibulitis. J Reprod Med 1996; 41:562–8.
35. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol 2005;6:135–42.
36. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 1999;353: 1959–64.
37. Weström LV, Willén R. Vestibular nerve fiber proliferation in vulvar vestibulitis syndrome. Obstet Gynecol 1998;91: 572–6.
38. Young GL, Jewell D. Topical treatment for vaginal candidiasis (thrush) in pregnancy. The Cochrane Database of Systematic Reviews 2001, Issue 4. Art. No.: CD000225. DOI: 10.1002/ 14651858.CD000225.
39. Mann MS, Kaufman RH, Brown D Jr, Adam E. Vulvar vestibulitis: significant clinical variables and treatment outcome. Obstet Gynecol 1992;79:122–5.
40. Furlonge CB, Thin RN, Evans BE, McKee PH. Vulvar vestibulitis syndrome: a clinico-pathological study. BJOG 1991;98:703–6.
41. Farmer MA, Taylor AM, Bailey AL, Tuttle AH, MacIntyre LC, Milagrosa ZE, et al. Repeated vulvovaginal fungal infections cause persistent pain in a mouse model of vulvodynia. Sci Transl Med 2011;3:101ra191.
42. Gerber S, Bongiovanni AM, Ledger WJ, Witkin SS. Defective regulation of the proinflammatory immune response in women with vulvar vestibulitis syndrome. Am J Obstet Gynecol 2002; 186:696–700.
43. Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31.
44. Gil M, Gozalbo D. Role of Toll-like receptors in systemic Candida albicans infections. Front Biosci (Landmark Ed) 2008;14: 570–82.