“Towards better clinicopathological diagnosis of lichen planus”

Tania Day MD (Columbia University) FRANZCOG FACOG

A thesis submitted in fulfilment of the requirements for the
degree of Doctor of Philosophy in Medicine

School of Medicine and Public Health
University of Newcastle
August 2018

This research was supported by an Australian Government Research Training
Program (RTP) Scholarship

Declarations

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am the first of more than two authors. I have included as a part of the thesis co-authorship declaration forms signed by all authors and endorsed by the Faculty Assistant Dean of Research Training, attesting to my contribution to the joint publications (attached in appendix).

Tania Day

Acknowledgements

Robert and I met on a boat from Greece to Turkey before I began medical school. He lived in Sydney, had a satisfying career, and knew it was impractical to begin a relationship with a young woman who was contractually required to spend the next 12 years studying, training, and working for the US government. Thankfully, he put pragmatism aside, immigrated to the USA, and never complained as we moved house four times in ten years. Our arrangement was that I would apply for my medical credentials to be recognized in Australia, and we would move there once my obligations were fulfilled in the USA. We spent the winter of 2011 in Sydney awaiting AHPRA registration, followed by a year of supervised practice at Maitland Hospital, before I was granted Fellowship with RANZCOG. After that, we traveled New South Wales looking for a town and a hospital to call home after so many years of itinerancy. Within a year of moving to Newcastle, we discussed the possibility of doing a PhD - a degree that might be described as supernumerary for a Senior Staff Specialist at a tertiary hospital. He encouraged me to do it if I thought it would be fulfilling or useful. For 20 years and counting, Robert's unwavering love, support, and optimism have been the bedrock of my life and career.

Lori Boardman ran the Colposcopy and Vulvar Clinics at Women and Infants' Hospital (WIH) of Rhode Island during the late 1990s and early 2000s, and was actively involved in research supported by Brown University and the National Institutes of Health. I am one of many WIH trainees whose careers would have been less rewarding had we not known Lori. During our rotation in her clinics, she provided us with a unique and important knowledge base in vulvovaginal and HPV-mediated disease, and inspired us to engage in research as an intellectual pursuit rather than a tick-box exercise. She guided me from planning to publication of two research projects during training, and provided a model for how to be an academic gynaecologist and compassionate leader. Her mentorship was transformative, and I am very grateful.

After training, I served four years in the US Navy, with tours in Okinawa, Japan, and Jacksonville, Florida. In both departments, I was the only gynaecologist with any formal training in vulvovaginal disease. Women referred with these issues were directed towards me and remained under my care while stationed at each site. It was rewarding to help women who often had been searching for a diagnosis and treatment plan for years. I remember that time fondly for the collegiality and interdepartmental collaboration that eased the transition from training to specialist practice.

The Maitland Hospital had a special clause in the full-time Staff Specialist position description to help attract and retain talent - one day a week could be spent pursuing a subspecialty interest of one's choice. Wanting to maximize this opportunity, I sought counsel from Felicity Park, who was working towards her Maternal-Fetal Medicine fellowship at the time. Felicity did her core training in Newcastle and is a visionary in her efforts to improve women's health in the Hunter region and beyond. Knowing my background and interests, she encouraged me to set up a vulval clinic at John Hunter Hospital. She took me on a tour of the facility and introduced me to departmental leadership. The clinic started by concentrating a trickle of referrals into a single session, but volume grew rapidly as local doctors heard about the service and saw positive results for their patients. Her encouragement and guidance were critical in transforming this idea into reality, and navigating the still unfamiliar bureaucracy of the Australian public health system.

Not long after the vulval clinic began, Jim Scurry called me to discuss a biopsy result. He asked if I had an interest in vulvovaginal disease, offered his help and mentorship, and gave me a copy of his book. We then ran some Clinicopathologic Correlation sessions and co-authored a case report on vulval pityriasis versicolour. He encouraged me to attend the 2013 International Society for the Study of Vulvovaginal Disease (ISSVD) meeting, where we discussed doing a research project so that I could qualify for ISSVD Fellowship. I soon recognised the incredible luck of this geographic circumstance - a world-renown vulval dermatopathologist was based in Newcastle because of family and lifestyle, and I had landed there for similar reasons with an

ambition to build an excellent clinical service in vulvovaginal disease. We started to meet once a week to review slides, discuss cases, and work on projects. Jim's tutelage has provided me with an understanding of vulvovaginal disease usually not accessible to gynaecologists, and his mentorship is foundational to this thesis and my career evolution.

Clinicopathologic research is only possible with the cooperation of clinicians who refer specimens to our Pathology department for expert assessment. The network of specialists who refer to Jim extends across New South Wales and Victoria, and most of these doctors are nationally or internationally recognized as experts in vulvovaginal disease. Their willingness to extract and share de-identified clinical data made this work possible. I am extremely thankful for their insights and editorial contributions, which have made the work richer and more applicable to the problems faced by busy clinicians. Likewise, our patients' willingness for their stories and photographs to be incorporated into research is commendable, as is their near-universal statement of hope that their experiences can improve the lives of other women.

While I was writing the manuscript on perianal disease, Felicity asked if I had considered doing a PhD by publication. Jim thought it an excellent idea and offered to be a supervisor. Pursuit of the degree while working full-time required the support of several departmental leaders. Professor Ian Symonds agreed to be the University supervisor, and has provided me with the practical advice and contacts so essential to progress through the degree requirements. Professor Henry Murray has consistently supported my academic pursuits, ensured I had administrative time, and always offered a smile as he signed off on multiple ethics applications. Carol Azzopardi, the Service Manager for Maternity and Gynaecology, has supported the development specialised clinics and my research efforts. Ken Jaaback and Geoff Otton, both Gynaecologic Oncologists, have also been invaluable to this work as referrers, educators, reliable sources of wisdom and encouragement, and co-authors. It has been a great privilege to work with so many inspiring people over the years, to be trusted by women to care for them, to know the generosity of genuine mentorship, and to hope to provide that gift to others.

List of publications included as part of the thesis

Day T, Holland S, Scurry J. Normal vulvar histology: variation by site. *Journal of Lower Genital Tract Disease* 2016;20(1):64-9.

Day T, Borbolla-Foster A, Phillips S, Pagano R, Dyall-Smith D, Scurry J, Garland SM. Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis? *Journal of Lower Genital Tract Disease* 2016;20(3):267-71.

Day T, Knight V, Dyall-Smith D, Dennerstein G, Pagano R, Tran H, Tan Y, Yap D, Weigner J, Scurry J. The interpretation of non-diagnostic vulvar biopsies. *Journal of Lower Genital Tract Disease* 2018;22(1):74-81.

Day T, Bohl T, Scurry J. Perianal lichen dermatoses: a review of 60 cases. *Australasian Journal of Dermatology* 2016;57(3):210-15.

Day T, Burston K, Dennerstein G, Pagano R, Scurry J. Vestibulovaginal sclerosis versus lichen sclerosus. *International Journal of Gynecological Pathology* 2018;37(4):356-63.

Day T, Moore S, Bohl TJ, Scurry J. Cormorbid lichen planus and lichen sclerosus. *Journal of Lower Genital Tract Disease* 2017;21(3):204-8.

Day T, Weigner J, Scurry J. Classic and hypertrophic vulvar lichen planus. *Journal of Lower Genital Tract Disease* 2018;22(4):387-95.

Day T, Bowden N, Jaaback K, Otton G, Scurry J. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *Journal of Lower Genital Tract Disease* 2016;20(2):174-179.

Day T, Otton G, Jaaback K, Weigner J, Scurry J. Is vulvovaginal lichen planus associated with squamous cell carcinoma? *Journal of Lower Genital Tract Disease* 2018;22(2):159-165.

I warrant that I have obtained, where necessary, permission from the copyright owners to use any of my own published work in which the copyright is help by another party.

Table of contents

1. Abbreviations 8

2. Abstract 9

3. Overview and literature review 10 - 39

- 3.1 Overview of lichen planus and lichen sclerosus 10
- 3.2 Epidemiology and quality of life 12
- 3.3 Anatomy and histology 14
- 3.4 Clinical diagnosis and treatment: studies of LS and LP 16
- 3.5 Clinical diagnosis and treatment: perianal disease 21
- 3.6 Association between lichenoid disorders and vulvar cancer 22
- 3.7 Histopathology of lichenoid disorders and clinicopathologic correlation 24
- 3.8 The problem of non-diagnostic biopsy in presumed lichen planus 30
- 3.9 Conditions that complicate clinicopathologic assessment of lichenoid disorders 32
- 3.10 Histopathology of dermatosis-associated neoplasia 36

4. Studies 40 - 108

- 4.1 Normal vulvar histology: variation by site 40
- 4.2 Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis? 47
- 4.3 The interpretation of non-diagnostic vulvar biopsies 53
- 4.4 Perianal lichen dermatoses: a review of 60 cases 62
- 4.5 Vestibulovaginal sclerosis versus lichen sclerosus 69
- 4.6 Cormorbid lichen planus and lichen sclerosus 78
- 4.7 Classic and hypertrophic vulvar lichen planus 84
- 4.8 Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia 94
- 4.9 Is vulvovaginal lichen planus associated with squamous cell carcinoma? 101

5. Ethics 109

6. Conclusion and recommendations 110

7. Bibliography 114

8. Appendix 128 - 146

- 8.1 Co-authorship declarations 129 - 137
- 8.2 Copyright permissions 138 - 146

1. Abbreviations

DEVIL	differentiated exophytic verruciform intraepithelial lesion
DIV	desquamative inflammatory vaginitis
DLQI	Dermatology Life Quality Index
DNA	deoxyribonucleic acid
dVIN	differentiated vulvar intraepithelial neoplasia
GVHD	graft-versus-host disease
H&E	hematoxylin and eosin
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
IHC	immunohistochemistry
-IN	intraepithelial neoplasia
ISSVD	International Society for the Study of Vulvovaginal Disease
LAST	Lower Anogenital Squamous Terminology
LP	lichen planus
LS	lichen sclerosus
LSC	lichen simplex chronicus
LSIL	low-grade intraepithelial neoplasia
MALT	mucosa-associated lymphoid tissue
MCJ	mucocutaneous junction
PAS	periodic acid-Schiff
PBI	patient benefit index
PCR	polymerase chain reaction
PCV	plasma cell vulvitis
PEH	pseudoepitheliomatous hyperplasia
QoL	quality of life
RCT	randomised controlled trial
SCC	squamous cell carcinoma
SIR	standardized incidence ratio
VAAD	vulvar acanthosis with altered differentiation
VAS	visual analog scale
VVC	vulvovaginal candidosis
VVS	vestibulovaginal sclerosis
uVIN	usual vulvar intraepithelial neoplasia

2. Abstract

Vulvovaginal lichen planus (LP) is a T-cell mediated inflammatory dermatosis characterised by quality of life impacts, irreversible anatomic changes, long-term treatment, and a reported increase in vulval cancer risk. Major knowledge gaps include that there are no consensus-based diagnostic criteria, no validated outcome measures, and little agreement on treatment strategies. The lack of diagnostic criteria produces a major limitation of clinical studies - not all participants have the disease of interest. Progress on diagnostic criteria is hindered by a lack of histopathologic research. The thesis aim was to address deficiencies in the clinicopathologic literature on vulvovaginal LP in order to lay the groundwork for international consensus guidelines on diagnosis.

Methodology for all studies was similar. The local pathology database was searched for diagnoses of interest. Slides were reviewed to select specimens meeting inclusion and exclusion criteria, then assessed for histopathologic features. Clinical notes and photographs were obtained from referring specialists. Clinical and histopathologic data were analysed together in an effort to describe patterns of presentation and diagnostic conundrums.

There are six key findings of the nine incorporated studies.

- Determination of anatomic site is fundamental to establishing a diagnosis.
- LP often presents with infectious and dermatologic comorbidities; identification requires liberal use of microbiology and biopsy at morphologically-distinct areas.
- There are two patterns of basal layer abnormality in erosive LP: the well-known degenerative pattern, and the newly described regenerative pattern.
- Non-recognition of the regenerative pattern contributes to the high non-diagnostic biopsy rate, along with clinician factors such as suboptimal biopsy timing or placement, and mistaking candidosis or vulvodynia for LP.
- Classic and hypertrophic LP have complex clinicopathologic appearances with multiple avenues for misdiagnosis.
- The evaluation of dermatosis-associated neoplasia requires an appreciation of all of the above components in order to avoid misattribution of vulvar cancers to LP.

3. Overview and literature review

3.1 Overview of lichen planus and lichen sclerosus

The evaluation and management of vulval dermatoses lies at the intersection of dermatology, gynaecology, anatomical pathology, and sexual health. Women present with an array of symptoms including sexual pain, vulvovaginal discomfort, pruritus, and skin changes. These symptoms result in reduced quality of life (QoL) and reduction or elimination of sexual activity. An accurate diagnosis is essential both for appropriate management and to guide ongoing surveillance, as some diagnoses are associated with an increased risk of neoplasia.

LP and lichen sclerosus (LS) are T-cell mediated chronic inflammatory disorders [1]. The pathophysiology involves epitopic alteration of the basal cells of the epithelium, which leads to lymphocytic attack yielding a cycle of cellular damage and repair [2]. The reason for this epitopic alteration is unknown, but a similar phenomenon occurs in graft-versus-host disease (GVHD) and lichenoid drug reactions [3,4]. This process manifests histopathologically as a lichenoid tissue reaction - a band of lymphocytes adjacent to damaged epithelium. In addition to their common mechanism, vulval LP and LS may coexist, have shared clinical features, and require a similar management approach. LS is more prevalent and dominates the existing literature, with study findings sometimes extrapolated to LP.

LS typically presents with pruritus and appears as pallor and textural change, often in a figure-of-8 distribution encompassing the vulva and perianus. The disease is often accompanied by evidence of rubbing and scratching seen as linear erosions, petechiae, ecchymoses, and increased skin markings. LS typically results a distinctive pattern of vulval architectural change, with flattening of the clitoral hood, resorption of the labial minora, and diminished elasticity of the posterior fourchette. The accepted wisdom is that LS is confined to keratinised skin. However, three case reports suggest it rarely occurs in the vagina and vestibule [5-7].

Three types of LP occur on the vulva: erosive, classic, and hypertrophic [8,9]. The traditional description of erosive LP is painful well-demarcated glazed erythema occurring on non-keratinised epithelium of vestibule and vagina. Apposed eroded surfaces may adhere resulting in fusion of labia minora or vaginal obliteration. This type also may affect the oral cavity, tracheo-oesophageal complex, conjunctiva, and lacrimal ducts, also accompanied by scarring and functional impairment. Classic LP appears on keratinised skin anywhere on the body with pale, red-purple, or brown papules and plaques that may spontaneously resolve [10]. Hypertrophic LP is described as pruritic violaceous plaques on keratinised skin. The perianus may be a site of predilection, but this has not been well documented [9,11,12]. The non-erosive subtypes receive scant attention in dermatopathology textbooks and cohort studies of vulval LP [3,4,13-15]. The association between LS, differentiated vulvar intraepithelial neoplasia (dVIN), and squamous cell carcinoma (SCC) is well established, while an increased risk of neoplasia in vulval LP is suggested but not substantiated [16-22].

Multiple cohort studies have documented a non-diagnostic biopsy rate of at least 30% in LP [13, 23-25]. LS is a usually a more straightforward clinicopathologic diagnosis, but atypical presentations and comorbid conditions may complicate matters. Although treatment of LP and LS is similar, inability to distinguish between the two has implications in the realms of clinical management, health care policy, and research. Vulvovaginal LP is usually more difficult to treat than LS, and thus is considered to be an indication for long-term subspecialist-led care [26,27]. In contrast, LS is a common disease with a wide severity spectrum that also carries a meaningful risk of cancer, so practice guidelines must consider the roles of general practitioners and gynaecologic oncologists in addition to vulval specialists [28-33].

An evidence-based management strategy for LP and LS should be based upon several concordant and methodologically-sound clinical trials. This has not yet been possible because two essential tools are lacking: uniform diagnostic criteria and a set of standardised outcome measures. The current knowledge base in lichenoid disorders is

inadequate to make real progress on their elaboration. This body of work is a step towards a better clinicopathologic diagnosis of LP.

3.2 Epidemiology and quality of life

The prevalence of vulval lichenoid disorders is difficult to determine. Limited epidemiologic literature documents LS in 0.1-0.3% of a general hospital population, 1.7% of women in a standard gynaecology practice, and 3% of female nursing home residents [34-36]. LP at any site is estimated to affect 1% of women, with the oral cavity most commonly involved [37,38]. Vulvovaginal disease was observed in 25-57% of women with oral LP, was found on biopsy in 3.7% of women attending a multidisciplinary vulval clinic, and was diagnosed in 6% of postmenopausal women with chronic vaginal complaints [39,40]. Multiple factors contribute to potential underestimation of disease prevalence: disease may be asymptomatic, women may defer care-seeking, and medical practitioners may fail to make the diagnosis [41,42].

A qualitative study identified QoL themes common across all vulval skin conditions [43]. Women reported a delay in accessing medical care due to fear, embarrassment, and a belief their disease could be malignant or sexually transmitted. The interval between symptoms and definitive diagnosis ranged from 18 months to ten years [43]. Women modified their daily activities, especially choice of clothing and approach to sitting, walking, and exercise. Sexual function was identified as a major area of concern, with women noting pain, shame about anatomic changes, fear of disappointing partners, diminished femininity, and withdrawl from intimate relationships. The majority of women reported potentially damaging self-treatment practices, including over-washing and application of products obtained from the chemist. Inadequate awareness of vulval disease resulted in inappropriate use of antimycotic medication, lack of self-examination, and non-recognition of anatomical changes that occurred over time. Reinforcing these findings, a study of women presenting to a specialty vaginitis clinic found that 65% had used ‘over the counter’ and alternative therapies for their vulvovaginal symptoms, and compared to non-users had higher levels of perceived stress and greater levels of interference with their social lives [44].

There are no validated QoL measures specific to vulvar lichenoid disorders. The Dermatology Life Quality Index (DLQI) is a widely-used measure of dermatology-specific QoL, and has been recommended for use in erosive LP patients [45-49]. It is self-administered by patients over age 16 and the average completion time is 126 seconds [49]. The ten questions address symptoms, embarrassment, interference with sport, work/study, daily tasks, and social activities, impact on choice of clothing, sexual and relationship difficulties, and treatment-related problems. Respondents tick one of five responses with associated scores of not at all (0), a little (1), a lot (2), very much (3), or not relevant (0). A validated five-band system that describes life impact may be applied to ease clinical interpretation: very large (11-20), moderate (6-10), small (2-5), or none (0-1). For general inflammatory skin conditions, the Minimal Clinically Important Difference is estimated at four points.

While the DLQI is well-studied in many dermatology populations, there is scant literature on its use in genital dermatoses [50,51]. A Dutch study administered the DLQI to 212 participants with vulval LS; the mean score was 11.92 indicating a substantial life impact. This result is similar to patients with generalised skin conditions such as psoriasis, hyperhidrosis, and dermatomyositis [52]. Analysis of responses suggested that the only DLQI domain relatively unaffected by vulval LS was work/study, and the most dramatic impact was in sexual difficulties. Survey of women with erosive LP indicates that the DLQI does not fully reflect their experiences, and does not directly address treatment outcomes such as improvement in pain, scarring, and sexual function [46]. A study that aimed to assess the validity of a new disease-specific questionnaire for VIN used the DLQI along with the Hospital Anxiety and Depression Scale, the Sabbatsberg Self-Rating Scale, and the Process Outcome Specific Measure [53]. This work demonstrated correlation between the total scores of the VIN questionnaire and DLQI ($r=0.69$), and internal consistency of the DLQI in VIN patients (0.93). The VIN questionnaire included items not addressed in the DLQI related to fertility, sexual frequency and enjoyment, repeated gynaecological examinations, and potential risk for developing cancer, all of which are applicable to women with LP. However, it is unclear how much

additional information is gained by administering more questionnaires, and if that outweighs the time and inconvenience of collecting the data.

A study of 96 women with LS used the two previously developed indexes, the Skindex-29 and the Patient Benefit Index (PBI), to assess health-related QoL after three months of treatment with clobetasol [54]. The authors reported that disease was verified by histopathology, but less than half of specimens showed diagnostic features. The Skindex-29 is intended for chronic skin diseases and categorises questions into 'Emotions', 'Symptoms', and 'Functioning'; it has been validated in psoriasis and eczema and used in two studies of vulvovaginal conditions. The PBI has 23 items each corresponding to 'Patient Needs' and 'Patient Benefit' and the result is a global benefit score; this tool was designed to assess the impact of treatment on QoL and had not been used previously for vulvovaginal disease. The questionnaires were mailed to subjects at a single time point, thus more than 6 months had elapsed since treatment in over 60% of women. The results of the Skindex-29 suggested that persistent symptoms had an ongoing impact on women's QoL. The PBI found benefit from clobetasol, but women's own goals for treatment were only achieved in 56-73%. The analysis was not stratified by time elapsed since treatment, or use of any ongoing therapy. The authors interpreted their results favourably, reporting that LS is worth treating because this yields a substantial impact on QoL, and that clobetasol has a "very high benefit score." An alternative view is that the time-limited treatment protocol was inadequate, as most women had ongoing symptoms and emotional impacts due to LS, and a third did not attain reasonable therapeutic goals. This study demonstrates that the collection of QoL data is a pointless exercise if study methodology neither guarantees that enrolled women have the disease of interest, nor assesses subjects at a similar point in the disease trajectory. It also reveals a tendency to persistently endorse traditional treatment protocols, despite data that suggest suboptimal outcomes.

3.3 Anatomy and histology

The vulva is a unique dermatologic site, most similar to the mouth, but with a wider array of functions and challenges. The mons, external surfaces of the labia majora, and

buttocks are covered in hair bearing skin replete with apocrine, eccrine, and sebaceous glands. At the clitoral hood, labia minora, and central perineum this transitions to hairless skin, which contains mammary-like glands and superficially-located sebaceous glands. Keratinised epidermis then changes to hormonally-sensitive non-keratinised squamous epithelium, also called squamous mucosa, in a circumferential zone delineated by the clitoral frenulum, inner labia minora, and fossa navicularis [55,56]. There are multiple mucinous glands beyond this transition zone, located periurethrally and at the base of the hymen [57]. The vagina is lined with squamous mucosa, until it transitions to columnar epithelium of the cervix. No mucinous or other glands are found in the vagina. At the anus, the distance over which these transitions occur is extremely short - hair bearing skin of the perianus becomes hairless skin of the anal verge, then non-keratinised epithelium of the anal canal, then columnar epithelium of the rectum. The squamocolumnar junction is well established as a site of vulnerability to human papillomavirus (HPV), but it is unclear if the mucocutaneous junction (MCJ) has an analogous susceptibility to infection or immunologically-mediated disease. This histopathologic complexity means that there is no single subspecialty with a monopoly on vulval specimens. Expertise in this area is built on a foundation of gynaecologic and dermatologic pathology, augmented by interest, experience, and interaction with clinicians who have likewise cultivated their knowledge and skills in vulval disorders.

Recognition of normal underpins the evaluation of abnormal, but this is lacking for vulval histopathology. Two autopsy studies published over three decades ago documented measurements of epithelial thickness at various sites on the vulva, but did not comment on a variety of other important features such as stratum corneum morphology, the granular cell layer, exocytosis, basal layer variation, and how these relate to biopsy site [58,59]. There is contention about the definition of normal with regards to the findings of parakeratosis, compact stratum corneum, and a moderate or dense lymphocytic infiltrate [59-64]. When physiologic or non-specific histologic findings are misinterpreted as disease, clinicians may persist in a dermatologic diagnosis when some other aetiology should instead be considered for the woman's symptoms.

A major barrier to understanding normal vulval anatomy is access to biopsy specimens from asymptomatic reproductive-age women. Several Scandinavian studies have recruited healthy women to provide biopsies from the base of the vestibule. Lundqvist and colleagues enrolled 20 women with chronic vestibular pain and 11 healthy volunteers aged 18 to 25 to undergo a punch biopsy at the opening of the Bartholin's gland [65]. Both groups showed glycogenated squamous mucosa of variable thickness and most specimens demonstrated mucinous glands (22/35) and ducts (21/35) labeled as Bartholin's glands and/or minor vestibular glands. The lymphocytic infiltrate was minimal in three specimens from each group, and abundant in four pain cases. A lymphoid follicle was detected in three controls and two cases, while a focal moderate infiltrate was found in one additional control and 12 cases, often near a gland duct. The authors were the first to identify that the vestibule is an immunologically active site containing lymphoid follicles and germinal centres, and cast doubt on the previously held belief that vulvovaginal pain is an inflammatory process. Finnish researchers recruited 15 asymptomatic premenopausal women undergoing benign gynaecologic surgery to have a 4mm punch biopsy from the 5 o'clock position of the posterior vestibule [66]. These biopsies showed similar numbers of T-cells, dendritic cells, macrophages, and mast cells when compared with posterior vestibulectomy specimens obtained from women with unexplained chronic vulvovaginal pain. Differences were found in the number of B cells, germinal centres, and plasma cells per microscopy field. The authors agreed with Lundqvist's conclusion that the vestibule demonstrates localised mucosa-associated lymphoid tissue (MALT), similar to the oral cavity and anal canal. However, this study did not comment on any other features of vestibular histopathology.

3.4 Clinical diagnosis and treatment: studies of LS and LP

The majority of the literature relating to LS and LP addresses clinical management. These studies are heterogeneous in their inclusion and exclusion criteria, selected interventions, and outcome assessment. In 2013, Simpson and colleagues published a systematic review of 28 randomised controlled trials (RCT) on vulval skin conditions and found 25 different types of outcome measures, with one to 13 different outcomes per study [67]. Only 21% of studies identified the primary outcome in the abstract or

methods. There were nine RCT on LS and one on LP. The latter randomised 34 Pakistani women with a clinical diagnosis of LP to aloe vera versus placebo gel and used 'Thongprasom criteria' to assess response; this is a 0 to 5 scale based on the size and appearance of oral LP lesions that has not been validated for vulval disease [68,69]. The intervention is not a standard component of current or historic LP treatment or vulval care practices. Eight of the LS studies reported clinician-rated improvement in skin appearance, each using novel grading systems. Two of these compared clobetasol propionate with topical pimecrolimus, and required biopsies before and after the intervention. The reported outcome in one was histopathology and the Female Sexual Distress Scale, and the other used histopathology, a 10-point visual analog scale (VAS) for pain and itch, and an Investigator Global Assessment scale of 0 to 3 for overall severity, lichenification, and fissuring [70,71]. Both studies found clobetasol to be superior. A crossover study of a topical antihistamine versus placebo recorded composite scores of severity and duration of symptoms, clinical appearance, and tolerability. Eligibility was based on clinical diagnosis; there was no difference between groups in LS appearance but itch was improved by the intervention [72]. A RCT on adjunctive use of silk versus cotton underwear used a scale of 0 to 3 to measure seven symptoms and ten signs [73]. Enrollment was primarily based on clinical diagnosis; modest improvement was noted with silk briefs. Five of the RCT contained in the systematic review assessed testosterone, which has subsequently been disregarded as a therapy for LS [74-78]. The authors concluded the overall quality of studies was poor, and some "lacked stringency of disease definition" [67]. Standardised core outcome measures for vulval skin conditions were recommended as an essential next step to improve quality of clinical trials and enhance comparison of results; these should be focused on patient- and clinician-rated assessments of severity, impact on daily function, and overall QoL, and be confirmed through international consensus.

In the intervening five years, one RCT has been published on the treatment of LP. It compared one session of photodynamic therapy to six weeks of clobetasol propionate for treatment of vulvovaginal LP [79]. Participants (n=37) had a clinical diagnosis of erosive LP, of whom 4 (11%) did not have a biopsy and 40% had a non-diagnostic report. The

outcome assessment at 6 and 24 weeks post-treatment was a novel 'GELP score' that incorporated a 10-point VAS and scores of 0 to 3 for four examination characteristics: size of area involved, intensity of erythema, striae, and number of erosions. The mean reduction in GELP score was similar between the two groups, as were reports of treatment-associated discomfort. An accompanying commentary pointed out the difficulty in recruitment to a RCT on LP management; of 90 women invited to enroll in the study, 28 declined, 22 had insufficient disease activity, and three dropped out after randomisation to clobetasol [80]. This foreshadowed the outcome of an attempt by Simpson and colleagues to complete a RCT on second-line treatment of vulvovaginal erosive LP [81,82]. They aimed to recruit 96 non-pregnant women with moderate or severe disease despite treatment with clobetasol, and randomise them into one of four arms: hydroxychloroquine, methotrexate, mycophenalate mofetil, and prednisolone. A biopsy excluding dVIN and SCC was required for study entry, and participants could not have comorbid LS or previous malignancy. After 14 months, the 12 study sites were able to recruit only 22 women [83]. Obstacles to recruitment included mild disease (n=50), refusal to take a tablet treatment (n=20), recent exposure to a study drug (n=15), comorbid LS (n=14), previous malignancy (n=9), and unwillingness to have a biopsy (n=6). They also found that many potential participants were not using topical steroids appropriately, and had clinical improvement once research staff advised them on proper use. Of the 14 subjects who took the study medications, four met the definition of treatment success - disease assessment of 'none' or mild' and improvement on review of clinical photographs. In a commentary explaining the decision to close the study, the authors identified several 'lessons learned' and advised that any future trial consider referral of potential participants to specific centres, involvement of patients in trial design, a simple protocol, a set of core outcome measures, and funding for research visits.

Since 2013, there have been several RCT on LS management, with continued heterogeneity in case ascertainment, interventions selected, and outcome measures. A Chinese study compared photodynamic therapy to clobetasol in women with biopsy-proven LS, using outcomes of lesion size and a 4-point VAS for symptoms and signs [84]. Both groups showed improvement at the 6-month follow-up. A German group

randomised 30 women with biopsy-proven LS to clobetasol versus UV-A1-phototherapy, with a 21-point 'total clinician's score' as the primary outcome measure, and found steroids were superior [85]. A RCT of clobetasol versus topical tacrolimus assessed change over time at five anatomic sites grading the overall appearance from 0 to 3 [86]. This study enrolled women and girls, most of whom had supportive histopathology, but hyperkeratotic disease was excluded. The results again suggested steroids are more effective. A RCT comparing clobetasol to mometasone furoate used a "Global Subjective Scale" and a "Global Objective Scale", the former obtained by an interviewer administering a 10-point VAS for the symptoms of itching and burning, and the latter based on examination of five findings graded on a 0 to 3 scale [87]. Supportive histopathology was not required for enrollment, and the two treatments showed similar efficacy. These authors published a continuation of the study comparing twice a week maintenance therapy with the two topical steroid preparations, and found similar benefit in both groups [88].

The largest prospective cohort study in LS to date was published in 2015. It followed 507 women with a clinical and histopathologic diagnosis for a mean of 4.5 years and graded disease by scoring clinical hyperkeratosis as 1+ to 4+ [89]. Women were prescribed a daily steroid ointment regimen titrated to disease severity and aimed at normalising the skin appearance. Outcome data included categorical assessment of symptom resolution, dyspareunia, progression of scarring/adhesions, requirement for division of adhesions, steroid dermatitis, and diagnosis of neoplasia. Women who self-reported compliance with the recommended treatment regimen had reduced risk of persistent symptoms, dyspareunia, and architectural change, and fewer cases of dVIN or SCC during follow-up.

An effort to use electronic-Delphi consensus methodology to produce a severity scale for adult LS represents the first step towards the standardised core outcome measures advocated by Simpson [90]. The authors reviewed 338 publications to generate a list of 103 items used to describe LS, "spanning the categories of symptoms, signs, histologic findings, immunohistochemical markers, QoL, and sexual function," but elected not to

include histopathologic criteria due to a concern this would suggest a biopsy was required at every visit. They administered a survey over three rounds to 66 members of the International Society for the Study of Vulvovaginal Disease (ISSVD) who regularly care for women with LS, and this produced 24 descriptors of symptoms, signs, and architectural changes. Many of the consensus items are words that have specific dermatopathologic definitions, but are often misused as synonyms: whiteness/sclerosis, ulceration/erosion, and lichenification/hyperkeratosis. Meanwhile, some of the items that did not reach consensus are used interchangeably with items that did, such as pallor/hypopigmentation versus whitening, and atrophy versus parchment-like skin. There was no consensus on a preferred method of measuring the items, and the majority of those surveyed did not wish to use previously validated scales for sexual function and QoL. This effort revealed that clinicians are not using the same vocabulary to describe disease, have disparate opinions about what findings are important and impacted by effective treatment, and are discordant on the measurement options of categorical responses, 5-point Likert scales, and validated questionnaires.

The remainder of the literature on LP management is comprised several single-centre retrospective cohorts and one UK-based multicenter audit of 172 cases of erosive LP. The latter did not describe criteria used by clinicians to make the diagnosis, and reported that vulval biopsy was performed in 77% of women and was non-diagnostic in 29% of these [45]. This audit was accompanied by a survey of British vulvologists that identified wide variation in treatment, with 75% of clinicians prescribing steroids as first-line management, while 17 different topical and systemic medications were used as second-line management. Of the several retrospective cohorts, only one specified the type and location of the LP [24]. When histopathology was obtained, non-diagnostic biopsy rates ranged from 22-56% [13-15,24]. One study of 100 consecutive women with clinical LP reported that all had a diagnostic biopsy, but failed to describe the criteria used and listed an array of inconsistent and irrelevant histopathologic features [91]. All the cohort studies on LP suffer the same major limitation - there are no consensus-based criteria for clinicopathologic diagnosis of LP that reliably distinguish it from LS and other dermatoses [92]. As a result, it is unclear what percentage of the subjects had the disease

of interest. Although requirement for a supportive biopsy might improve case ascertainment, this is hampered by the dearth of pathologists skilled in vulval disease and the likelihood that current histopathologic descriptions are inadequate.

3.5 Clinical diagnosis and treatment: perianal disease

The perianus is defined as the circumferential area within 5cm of the anal verge [93]. By definition, the perineum and perianus overlap in women, and vulval dermatologic conditions often are contiguously expressed across the perianus and natal cleft. These territories have traditionally been designated as the domain of colorectal surgeons and gastroenterology specialists. Unfortunately, their literature uses the descriptive term, 'pruritus ani,' to encompass the diverse dermatologic and infectious aetiologies of symptomatic abnormal perianal skin [94,95]. It is reported to be a male-predominant syndrome occurring in the fourth to sixth decades of life, but this fails to identify the referral bias of colorectal practices. 'Pruritus ani' is categorised as primary or secondary, but the explanations applied to these terms reveal a misunderstanding of dermatologic definitions. Primary disease is stated to be idiopathic, but is described as being related to allergens or irritants such as heat, moisture, friction, excrement, and exogenous topicals. This should instead be called lichen simplex chronicus (LSC) arising from atopy or from irritant and allergic contact dermatitis. Secondary disease is defined as pruritus due to some underlying diagnosis, which may be infectious, dermatologic, structural, or neoplastic. This should instead be identified as the causative disorder, which may have superimposed LSC.

This flawed conceptualisation of perianal skin disease unsurprisingly results in problematic research into diagnosis and treatment. Three studies of perianal pruritus, together containing more than 350 affected patients, reported no LP and only two cases of LS. Instead they attribute symptoms to 'intertrigo' (used interchangeably with candidosis in one study), haemorrhoids, erythrasma, acute and chronic contact dermatitis, streptococcal infection, psoriasis, and 'idiopathic' [96-98]. Biopsy was performed at the discretion of clinicians, and its frequency and results were not reported. It is likely that these authors misattributed cases to easily identifiable microbiologic results, such as

Candida albicans and *Streptococcus*, and failed to recognise underlying chronic dermatoses like LP, LS, or psoriasis. Rather than acknowledging the limitations of their own knowledge and investigative methods, several of these publications report that 'idiopathic' disease may be attributable to patients' behavioral and psychological problems [95,98].

3.6 Association between lichenoid disorders and vulvar cancer

There are two types of vulvar SCC - approximately 30% of cases are HPV-dependent and the other 70% are HPV-independent and usually associated with LS [99,100]. Multiple studies suggest that vulvar SCC occurs in 5% of women with LS, and a recent prospective cohort study suggests that effective treatment may mitigate this risk [89]. Vulvovaginal LP has also been described in association with vulval neoplasia in several case reports and series, and SCC is occasionally noted in women with erosive LP during long-term follow-up [101-103]. Limitations of this literature include lack of histopathologic confirmation of the LP diagnosis, inadequate follow-up, and failure to identify if neoplasia could be related to HPV or other carcinogens. The largest study to date reported 38 cases of LP-associated SCC and dVIN occurring at a single European center [104]. However, this study did not convincingly establish the anatomic or microscopic site of LP nor describe the diagnostic criteria used, and did not account for the possibility of comorbid LS. The clinical photographs accompanying the manuscript were similarly unconvincing, with at least half being consistent with severe LS.

A Finnish study of 7,616 women with LS demonstrated a markedly increased risk of vulvar SCC with a Standardised Incidence Ratio (SIR) of 40.3 [95% CI 36.3-46.7], with the risk remaining elevated over the whole period of follow-up [41]. This translated to 2.1% of women with LS being diagnosed with vulvar SCC during a mean of 8.8 years of follow-up. The vaginal cancer SIR was 3.69 [95% CI 1.01-9.44]. There was no change in cancers of the oral cavity or extragenital skin, while the risk of cervical cancer was decreased. These authors used similar methodology to evaluate LP at any site and cancer risk in a cohort of 13,100 women. In contrast to LS, the overall SIR was only 1.15 [95% CI 1.09-1.2]. The SIR was elevated for cancers of the tongue (12.4), oral cavity (7.97),

lip (5.17), larynx (3.47), vulva (1.99), and oesophagus (1.95), with no increased risk for skin or vaginal cancers [42]. In both studies, data were unavailable on tobacco, high-risk HPV results, immune compromise, or subtype of SCC or VIN, thus the authors were unable to elaborate on the HPV-dependent or independent status of these malignancies. There was no discussion about the possibility of cancer arising in comorbid LS. The authors identified that LP may be an incorrect diagnosis in some women, and an unknown fraction of LP cases are never diagnosed. Any of these limitations could falsely elevate the SIR.

A study of 201 consecutive incident vulvar SCC cases demonstrated that increased risk of recurrence was related to adjacent LS, with multivariate analysis showing a 3-fold increase in local recurrence [105]. In contrast, excision margin status did not impact on risk of local recurrence or second field tumours. The authors acknowledged that their analysis was limited by the need to divide women into three simplified groups: LS irrespective of VIN, any VIN but no LS, and no LS or VIN. However, they were able to report descriptive data on the various combinations of diagnoses in the 79 women with LS and SCC: 32% had dVIN, 24% had HSIL (usual VIN), 5% had both dVIN and HSIL, and 6% had undefinable VIN. Consistent with the types of precursors seen, a third of these cases were positive for high-risk HPV. This rate is similar to a 2006 study of 27 women with LS and any type of VIN, in which 31% were positive for HPV DNA and all but one was type 16 [106]. Approaching this issue from another angle, a study of vulvar cancers conclusively associated with HPV showed 6/281 (2%) had features of LS and/or dVIN [107]. Although the rate of transcriptional activity of HPV in normal vulval skin compared to skin affected by LS or LP is unknown, it is feasible that the combination of damaged skin and local immunosuppression increases HPV acquisition and persistence. It is also possible that HPV promotes in carcinogenesis in the dermatosis-dVIN pathway. Taken together, these studies suggest that close surveillance of women with lichenoid dermatoses may detect dVIN, HSIL, or both, and that treatment of the field of abnormal epithelium is a priority in the prevention of recurrent cancer.

3.7 Histopathology of lichenoid disorders and clinicopathologic correlation

The histopathologic interpretation of vulval specimens follows the general methods of dermatopathology. Assessment of a slide involves acknowledgement of the stain, usually hematoxylin and eosin (H&E) for general review or periodic acid-Schiff (PAS) to highlight mycosis, glycogen, and the basement membrane. Next, an attempt is made to establish the site: hair bearing skin, hairless skin, MCJ, or non-keratinised squamous epithelium. For inflammatory diseases, the steps are then (1) layer-by-layer identification of abnormalities, (2) aggregation of abnormalities into reaction patterns, (3) differential diagnoses, and (4) clinicopathological correlation [108]. The key reference point in the identification of abnormalities is comparison with normal.

The six main tissue reaction patterns in vulval dermatoses are lichenoid (interface), acanthotic (psoriasiform), spongiotic, vesiculobullous, granulomatous, and vasculopathic [109]. The initial three comprise most vulval skin conditions. The lichenoid reaction displays a closely-applied band-like lymphocytic infiltrate and evidence of basal layer damage. Basal layer damage manifests as apoptotic bodies, vacuolar change of basal cells, and/or squamatisation, the latter defined as a change in morphology of normal basilar keratinocytes to horizontally-disposed cells with a mature squamous appearance [3,110]. The acanthotic reaction shows thickened epithelium with elongated rete ridges. This is seen in psoriasis and LSC. The spongiotic reaction pattern shows intracellular edema. This is seen in acute contact dermatitis and infectious processes.

The differential diagnosis for a lichenoid tissue reaction on the vulva includes LS, LP, lichenoid drug reaction and fixed drug eruption, GVHD, cutaneous lupus erythematosus, and cutaneous T-cell lymphoma [111]. The latter three are rare and have other systemic manifestations that aid in diagnosis. GVHD requires previous bone marrow transplant, and often is multifocal. Drug reactions may be difficult to distinguish from LP as many common medications are implicated and the interval between exposure and rash may be days or weeks. Typically, drug reactions display an intermittent rather than chronic course, occur in non-typical age groups and anatomic locations, and biopsy sometimes shows excessive stromal eosinophils.

The traditional description of erosive LP in dermatopathology textbooks includes erosion, epithelial thinning, a band-like lymphocytic infiltrate in the lamina propria, evidence of basal layer degeneration, and absence of abnormal stromal collagen [4]. Features of classic LP include hyperkeratosis, wedge-shaped hypergranulosis, sawtooth or spiky acanthosis, basal layer damage, a band-like lymphocytic infiltrate, and absence of abnormal dermal collagen [3]. Hypertrophic LP demonstrates changes of classic LP with superimposed lichenification seen as marked hyperkeratosis and hypergranulosis, irregular elongated rete ridges, and papillary dermal fibrosis [3,4]. Some cases lack features specific to LS or LP, and may be labelled non-specific lichenoid reaction.

LS is distinguished from LP primarily by sclerosis - the presence of abnormal collagen in the papillary dermis or lamina propria. The collagen may be hyalinised, edematous, or fibrotic, or a combination of the three. The term 'homogenised' is used interchangeably with hyalinised. The abnormal collagen often occurs in an obvious subepithelial band, creating a tri-laminar appearance of epidermis, a pink layer of collagen, and a purple layer of lymphocytes. The reason that sclerosis occurs in LS but not LP is unknown. The precise mechanism for collagen change also remains unclear, but appears to begin as an edematous protein-rich exudate from blood vessels that becomes hyalinised through dehydration, deposition of type 5 collagen, loss of elastic fibers, and accumulation of decomposed fibrin [112-115]. In some cases, this may be further organised into fibrosis. The presence of abnormal collagen in the upper dermis occurs in a limited number of dermatologic diagnoses. These include dermal scar due to trauma or radiation, localised scleroderma, cutaneous T-cell lymphoma, and malignant atrophic papulosis [116-119]. Again, these display an array of other clinical features inconsistent with LS.

Fadare recently described another item on the list of differential diagnoses for sclerosis [120]. Three postmenopausal women presented with a chief complaint of dyspareunia and were found to have white papules and plaques in the distal vagina. Two had excisions and one had punch biopsies. Histopathology showed an atrophic epithelium, keratinisation in two, and a thick band of stromal sclerosis. The photomicrograph of one specimen suggested focal squamatisation, but no basal layer changes were reported. This

was proposed to be a distinct clinicopathologic entity, designated ‘vaginal stromal sclerosis.’ The hypothesised mechanism involves oestrogen-deficiency leading to focal injury that that results in florid sclerosis. It remains unclear if this entity has any relationship to LS, and if its incidence is higher than would be suggested by this case series.

A significant problem with the histopathologic literature on lichenoid disorders is the proposition of phenomena called ‘early LS’ and ‘late (burnt-out) LS’. Proponents argue that abnormal collagen may be absent in new disease, and that old disease does not display a lichenoid tissue reaction and instead shows isolated fibrosis. This hypothesis assumes there is correlation between duration of disease and histopathologic appearance, but this has not been established in the literature and is logistically difficult to study [121,122]. Nevertheless, several authors have attempted to describe clinicopathologic situations that represent ‘early’ or ‘late’ LS. For example, Regauer and colleagues stated that LS “begins periclitorally and spread to the interlabial sulcus, the labia minora and majora” - an uncited statement that is not expressed by other experienced clinician-researchers [27,109,123]. They followed with the assertion that “…pathognomonic LS is usually seen in older women with atrophic, lichenified, cigarette-paper thin skin, resorption of the labia minora…and stenosis of the vaginal introitus representing the end-stage of LS after decades of disease progression” - again an unreferenced statement with multiple conflicting adjectives and an erroneous implication that children and younger women are less likely to have typical disease manifestations [27]. Clinical photographs accompanying these statements demonstrated either mild LS, or an appearance more consistent with LP. The authors then reported that biopsies from women with ‘early LS’ lack sclerosis, but will show focal basement membrane thickening, exocytosis, ectatic blood vessels, and acanthotic change to appendegeal structures. However, all of these findings may occur in any lichenoid tissue reaction.

Fung and colleagues took a different approach to the study of purported ‘early LS’. They reviewed 68 vulval LS specimens to find nine cases that had a transition from a non-specific lichenoid pattern to an area of pathognomonic LS, and compared these to six

cases of penile LP [110]. They proposed that this transition zone is a proxy for 'early LS' implying that the disease spreads by lateral extension; however, there is no evidence for this concept. They reported that transitional LS more commonly had psoriasiform rete ridges and exocytosis, while LP cases more often had spiky rete ridges, wedge-shaped hypergranulosis, and apoptotic bodies. The authors acknowledged that only the most typical histopathologic pattern of LP can be distinguished from peripheral LS, thus advised clinicians to take biopsies from the worst or most representative area rather than sampling the edge of a lesion.

It is possible that the histopathology of paediatric LS is a better representation of 'early LS'. However, biopsy is uncommonly performed in children. An Australian series of 46 pre-pubertal girls noted a biopsy rate of 13%, while a Finnish study of 44 girls aged 2 to 18 years reported that 66% had tissue sampling [124,125]. The latter described findings of hyperkeratosis in 59%, lymphocytic infiltrate in 57%, and homogenised collagen in 52%; LS was stated to be confirmed in 26/29 (90%) but the authors did not define their diagnostic criteria. Disease in children cannot be assumed to be recent in onset. Delay in diagnosis is usually 1-2 years, with some girls waiting eight years for LS to be identified and treated [125,126]. Photomicrographs are few, limited to case reports, and demonstrate the usual tri-laminar appearance [127-129]. Although a clinicopathologic study on paediatric LS would be worthwhile, the evidence to date does not suggest it would elucidate the appearance of newly developed LS.

Weyers challenged the assumption that a lack of sclerosis signals 'early LS.' He performed a robust review of 73 specimens from 68 men and women with genital LS, and described multiple different histopathologic patterns, arguing against a simplistic time-linear categorisation of LS [121]. There were seven cases that showed an interesting constellation of features: absent or minimal sclerosis, parakeratosis, marked acanthosis, and foci of dyskeratotic cells above dermal papillae. All were from the vulva of elderly women, three had clinical photographs consistent with severe LS, and half had long-term clinical follow-up showing improvement of disease with treatment and no SCC. Eight other cases showed areas of both hyperplasia and atrophy in the same specimen, arguing

against the concept that epithelial atrophy is a 'late' finding. Eleven cases showed a saw-tooth rete ridge pattern usually associated with classic LP. Weyers hypothesised that the chronic course of LS permits the interaction and evolution of multiple pathogenic factors, leading to substantial variation in histopathologic appearance. His work validates previous studies that suggest LS is not characterised by a homogenous centre and a spreading margin, but instead affected skin undergoes a constant cycle of injury and repair in response to multiple variables, and this may be non-contiguous at the microscopic and gross level [115,122].

The evidence for 'late LS' appearing as isolated dermal fibrosis comes from a comparison of LS near SCC to LS without cancer [130]. Most women with HPV-independent SCC are elderly and have a long duration of under-treated disease, as the aetiology of these cancers likely involves gradual acquisition of carcinogenic mutations in scarred inflamed epithelium. Thus, the authors surmised that SCC usually arises out of long-standing LS. This study found that 57% of LS 1mm from SCC was fibrotic, compared to 0-3% elsewhere, and fibrotic cases had often lost other diagnostic features of LS. However, it is unknown how long the process of fibrosis takes, what impact cancer has on adjacent epithelium, and how to distinguish the fibrosis from dermal scar in this group of women who often had previous biopsies and excisions.

Several publications detail attempts to construct histopathologic scoring systems for LS that might differentiate it from other dermatoses [110,123]. A subsequent analysis applied two of these systems to 31 biopsies from women with a variety of clinical diagnoses including LS, LP, and LSC. This study found that LS could be excluded if dermal sclerosis was absent, but that neither system could distinguish LP from other dermatologic diagnoses [131]. The authors also noted that the classic features of LP on keratinised skin are often not observed on the vulva, and that oral LP occurred concurrently with both LS and LP and should not be regarded as evidence of the latter. Rather than attempt a scoring system, Weyers reported on novel subtle findings that help distinguish LS from LP. He evaluated 100 specimens from genital skin with solid clinicopathologic correlation for a diagnosis of LS, of which 29 lacked sclerosis. These

were compared to 15 cases each of the primary differential diagnoses: LP, LSC, and cutaneous T-cell lymphoma. Patterns of abnormal collagen consistent with LS included small foci of sclerosis at the tips of dermal papillae, pericapillary sclerosis, marked fibrosis expanding the papillary dermis, and thickened individual collagen fibres with lymphocytes aligned in horizontal rows between fibres. Other features reported as more common in LS than LP were: lack of apoptotic bodies in the papillary dermis, psoriasiform hyperplasia, exocytosis through the entire thickness of the epithelium, and alignment of lymphocytes in the basal layer. He argued that the histopathologic definition of LS should be expanded, and that pathologists should carefully review each section, performing step sections if needed, in order to detect small foci of homogenised collagen that permit a firm diagnosis of LS.

A recent international electronic-Delphi consensus exercise on the diagnostic criteria of erosive LP yielded a definition of disease less stringent than that in dermatopathology textbooks [132]. This study included 73 members of the ISSVD, of whom seven were pathologists. The exercise resulted in three histopathologic criteria for the diagnosis of erosive LP: 1) a well-defined inflammatory band in the superficial connective tissue at the dermo-epidermal junction, 2) the band consists predominantly of lymphocytes, and 3) signs of basal layer degeneration like vacuolar change, apoptotic bodies, and abnormal keratinocytes. There was a consensus that biopsy is required only for diagnostic doubt or suspicion of malignancy. The exercise also resulted in six clinical criteria: 1) well-demarcated erosions, 2) a hyperkeratotic white border, 3) pain or burning, 4) scarring or architectural change, 5) vaginal inflammation, and 6) involvement of other mucosal sites. The authors proposed that any three features - clinical or histopathologic - would suffice for diagnosis. Unfortunately, this strategy cannot reliably distinguish LP from other vulval dermatoses, particularly LS and plasma cell vulvitis (PCV). The items generated from this exercise do not consider the possibility of other abnormal basal layer patterns. These criteria were applied retrospectively to 72 women with a firm clinical diagnosis of erosive LP, of whom 45 had accessible biopsy reports [133]. Most (33/45, 73%) had a band-like lymphocytic infiltrate, while only 60% had basal layer degeneration, and 13% had non-specific findings. Yet, over 90% of women had erythematous erosions and

pain/burning respectively, and 88% had architectural change, resulting in 97% of patients having three or more of Simpson's criteria for diagnosis. The authors noted that the clinical symptoms and signs are non-specific, and often shared with LS, and stated that raising the number of required criteria may decrease false-positives.

3.8 The problem of non-diagnostic biopsy in presumed lichen planus

Multiple studies have documented that vulval skin disorders are underappreciated by the medical community, and often associated with a delay in diagnosis [134]. Some authors advocate a low threshold for biopsy, both to improve detection of dermatoses and ensure neoplasia is not missed [135,136]. Bowen and colleagues performed a retrospective study of women referred to an academic clinic with 'vulvodynia unresponsive to therapy', which was defined as chronic vulval pain without an identifiable cause that had not improved with treatments attempted in the private sector. These women underwent a thorough clinical evaluation and stepwise investigations and therapies based on the differential diagnosis. Ultimately, there were 90 specimens obtained and sent to dermatopathologists for assessment. A diagnosis was obtained in 61%, most commonly LS, dermatitis, and LP; 5 (6%) women were found to have neoplasia. Biopsies were described as non-specific in 39% of cases, without further detail on their appearance. The authors cautioned against the use of vulvodynia as "a wastebasket term for any patient complaining of vulvar pain" and advised instead that women who do not respond to initial empiric treatments should have a biopsy evaluated by a skilled pathologist. A similar Australian study of 525 women with dyspareunia or chronic vulval pain categorised women into two groups - 'normal skin' or 'abnormal skin' - after a comprehensive clinical assessment and vaginal swab [137]. They likewise found that 61% had a dermatologic or infectious aetiology of their pain, with candidosis, LP, LS, and psoriasis being most often identified. The authors did not report the number or results of biopsies performed, leaving open the possibility that some biopsies did not confirm the clinical diagnoses.

There are no studies addressing the reverse problem of non-diagnostic biopsy done for suspected dermatologic disease. This is a difficult area to study because there are

multiple variables that influence how clinicians integrate the results of histopathology into their final diagnosis. These may include the degree of suspicion based on history and examination findings, the perceived expertise of the pathologist, the clinical trajectory of the woman, practice pattern variation between individuals, and clinicians' beliefs around the causes of chronic vulval pain. The term 'vulvodynia' continues to generate controversy [138-140]. The debate centres on the extent to which persistent pain can be explained by a specific disorder, with critics of the term 'vulvodynia' arguing that its promotion discourages the pursuit of the underlying aetiologies of pain [137,138]. In a similar vein, some experts argue that neurologic and musculoskeletal pain should be considered causative diagnoses, and not 'associated factors' as labeled in the recent ISSVD consensus terminology statement [138,140]. Interpretation of women's symptoms and signs occurs through the prism of each clinician's belief structure around the aetiology and management of pain. Just as some may fail to appreciate a skin condition and instead misapply the term 'vulvodynia', others may over-diagnose dermatoses, especially LP, in an effort to provide answers to women suffering with chronic pain. Vulval erythema is a non-specific finding, especially when it manifests in the vestibule and is poorly-demarcated. Multiple authors have documented vulval erythema as a feature of vulvodynia, in studies that have reliably excluded the possibilities of dermatosis and infection [65,140,141]. If painful erythema is thought to represent LP but the biopsy is non-diagnostic, there may be reluctance to revise the original diagnosis. Further complicating the situation, the placebo-response rate for pain conditions has been documented as at least 30%, so a proportion of women will respond to topical steroids in absence of an inflammatory skin condition [141,142]. This may reinforce the clinician's belief that a diagnosis of LP is correct, despite a normal biopsy. If women have a suboptimal response to steroids, the clinician may provide a diagnosis of superimposed pain syndrome, rather than revisiting the accuracy of the original diagnosis.

It is also possible that the substantial non-diagnostic biopsy rate of LP in cohort studies is due in part to histopathologic patterns consistent with disease that have not yet been documented. The cycle of basal layer damage and repair would suggest that changes

consistent with regeneration would sometimes be apparent on histopathology of lichenoid disorders. This might include enlarged or hyperchromatic nuclei, mitoses, and maturational alterations; these findings have been suggested but not described in genital LP [92]. A regenerative pattern has been recognized in oral LP as consistent with either reactive change or emerging intraepithelial neoplasia, and is labeled 'atypical lichenoid stomatitis [2]. However, the mouth is a carcinogen-rich environment compared to the vulva. Compared to oral LP and LS, vulval LP has suffered a relative lack of academic attention, resulting in a significant knowledge gap around the potential spectrum of its histopathologic appearance.

3.9 Conditions that complicate clinicopathologic assessment of lichenoid disorders

Useful clinicopathologic diagnostic criteria for vulval LP must be based on an understanding of the disease itself as well as the common comorbid states and differential diagnoses. Lichenification is the clinical manifestation of the itch-scratch cycle, and histologically is called LSC. This may be caused by atopic, irritant, or allergic dermatitis affecting otherwise normal skin; scratching causes an acute insult to convert into a chronic dermatitis. LSC may also be superimposed on any pruritic dermatologic or infectious disease, such as LS, LP, psoriasis, candidosis, and dermatophytosis [135,143]. The skin appears thickened with increased skin markings, erosions, excoriations, and ecchymoses. Linear fissures in skin folds may occur and cause stinging pain. The colour varies from gray-pink to violaceous, depending on the underlying aetiology, and post-inflammatory hyperpigmentation can occur. Histopathology shows hyperkeratosis, hypergranulosis, acanthosis, papillary dermis fibrosis, and a degree of lymphocytic infiltrate. Epithelial loss due to scratching may limit the pathologist's ability to detect an underlying dermatosis.

Psoriasis is a common chronic proliferative skin condition that has diverse manifestations, including genital disease, and may affect multiple sites simultaneously. Vulval psoriasis presents with itch or pain, and the appearance depends on location. On hair bearing skin of mons, labia majora, and perianus, there are well-demarcated erythematous plaques with scale, often symmetric in distribution. On moist or hairless

skin like labia minora and natal cleft, there is erythema, oedema, and fissuring. Non-keratinised epithelium of vestibule and vagina is spared. Superinfection with *Candida albicans* or *Staphylococcus aureus* is common, occurring in up to 20% [144]. The aetiology of psoriasis includes abnormal activation of CD4 and CD8 T-cells, and anomalous proliferation and differentiation of keratinocytes [145]. Thus, it is associated with other autoimmune dermatologic diseases, including vitiligo, LS, and LP. Psoriasis was found in 4.5% of women with erosive LP, and 8% of women with LS [125, 146]. In addition to possible comorbidity, psoriasis is an important item on the list of differential diagnosis of LP, along with candidosis, dermatophytosis, extramammary Paget's disease, and HSIL. Histopathology is often non-specific; however, subcorneal pustules and psoriasiform rete ridge morphology are sometimes present and permit a more specific diagnosis [3]. The microscopic findings of lichenified psoriasis may be indistinguishable from LSC.

Candida albicans is both a commensal and a pathogen in the lower genital tract. Healthy asymptomatic non-pregnant women have a point prevalence of 20%, and over one year 70% of women have at least one positive culture [147]. The balance between colonisation and disease is mediated by multiple factors, including the vaginal microbiome, genetic polymorphisms in Toll-like receptors and mannose-binding lectin, and activity of inflammasomes, interleukin signaling, and interferon production [148]. These in turn are influenced by the woman's hormonal and medical situation, particularly oestrogen levels, diabetes, antibiotic use, local or systemic immunosuppressive medications, and dermatologic disease [147,148]. Several studies on LP and LS have mentioned the problem of superinfection. The RCT of photodynamic therapy versus clobetasol for genital LP reported 2/17 (12%) women allocated to steroids developed vulvovaginal candidosis (VVC) [79]. The RCT on silk underwear as an LS adjunct documented 3/42 (7%) women developed candidal vulvovaginitis and were treated with oral antimycotics [73]. Candidosis occurred in 4% of women with LP and 6% of LS managed with an individualised daily topical steroid regimen; all resolved with oral or topical antimycotics [13,146]. Review articles note that superinfection should be suspected when there is a sudden increase in pain, discharge, erythema, erosions, or non-

response to therapy in women with a lichenoid dermatosis [8,12]. Recurrent or chronic VVC is also an important differential diagnosis for women with pain accompanied by erythema and oedema of the labia minora and vestibule [149,150]. Prominent discharge is often lacking in chronic disease, and microbiology is plagued by false-negatives [151]. In contrast to LP, women with isolated VVC will not demonstrate architectural change, and will not have sustained improvement with topical steroids. The histopathology of VVC is not well described, but likely is non-specific or spongiotic, and shows organisms in the stratum corneum in less than 30% of cases [152].

Plasma cell vulvitis (PCV) and desquamative inflammatory vaginitis (DIV) are poorly understood phenomena that manifest as pain and punctate or confluent orange-red discoloration, with DIV also characterised by purulent vaginal discharge [40,153,154]. PCV occurs at the vestibule and DIV is primarily a vaginal disease. Abnormalities are restricted to non-keratinised squamous epithelium. Histopathology of PCV features a dense lymphoplasmacytic infiltrate, stromal capillaritis and hemosiderin, and thinned epithelium with a normal basal layer. Histopathology of DIV has not been well described. A cohort of 101 patients with a clinical diagnosis of DIV had 11 biopsies, three of which showed findings consistent with PCV, six showed spongiotic dermatitis, and two were within normal limits [156]. PCV is a leading differential diagnosis for erosive LP, and some have hypothesised that DIV is a variant of vaginal LP [157]. More recently, there is evidence to suggest that PCV and DIV are both immunologically-mediated reactive phenomena with historical triggers present in more than half of cases [40,156]. Documented triggers include pelvic surgery, severe medical illness, viral gastroenteritis, prolonged or high-dose antibiotics, genital infections, medications, and hormonal alterations [156,158]. Treatment of DIV is clindamycin topical cream, sometimes in combination with potent topical steroids [155,156]. Management of PCV follows a similar pattern, but there is scant evidence to support its efficacy [153,154]. It is unknown to what extent these diseases complicate LP and LS. In a retrospective cohort of 171 women with DIV, Sobel found 5 (3%) with LS and excluded them from further analysis.

Vitiligo is a chronic autoimmune disorder involving T-cell-mediated attack on melanocytes, leaving the affected skin depigmented. As a result, it is a leading differential diagnosis for LS, as both demonstrate well-demarcated pallor. However vitiligo is not pruritic and the skin texture is normal. Similar to LP and LS, it occurs in at least 1% of the population and is regarded as an autoimmune disease with a genetic predisposition. Theories about its pathogenesis include epitopic alteration due to sun exposure, topical immunomodulators, the Koebner phenomenon, and 'Wolf's isotopic response' that holds one skin disease may trigger a second unrelated condition [159]. A cohort of women with erosive LP found vitiligo in 2% [25]. Multiple case reports document simultaneous LS and vitiligo, including comorbid genital disease [160]. In dark-skinned children, several authors have described a 'vitiligoid variant' of LS that is markedly depigmented yet symptomatic - it is unknown if these cases represent LS in isolation or adjacent to vitiligo, or the two diseases superimposed [160,161]. Treatment options for vitiligo are limited and of variable success, thus management is directed primarily at any comorbid disorder.

Pseudoepitheliomatous hyperplasia (PEH) is a benign reactive proliferation of squamous cells seen as separated nests and tentacles extending into the dermis. This appearance is morphologically similar to SCC, except that PEH lacks the cellular atypia, abnormal mitoses, and desmoplastic reaction that accompany invasive cancer. PEH has been documented in fungal, syphilitic, and tuberculous infections, on the edges of burns and fistulae, and in nodular prurigo [162,163]. Several case reports have described PEH occurring in non-genital hyperplastic LP, and one documented PEH complicating vulval LP, in each case raising a concern for cancer [164-187]. PEH has been documented in 12.5% of lichenified LS cases, and these cases were more likely to show extravasated erythrocytes and fresh fibrin than cases of LS without PEH. Lee and colleagues hypothesized that increased tissue trauma may be a pathogenic factor for PEH, linking it to other conditions with dermal scarring. As the conditions seen with PEH are also associated with cancer, the pathologist must recognize that ancillary measures are of limited value in distinguishing between the two, and that a careful assessment of cytologic atypia is the best tool for accurate diagnosis. Genetic testing has been used in a

research setting to exclude malignancy when there is florid PEH, and may in future be an important mechanism for avoiding a misdiagnosis of cancer and unnecessary vulvectomy [163].

3.10 Histopathology of dermatosis-associated neoplasia

The World Health Organisation classification for vulvar tumours categorises SCC into five types: verrucous, keratinising, non-keratinising, warty, and basaloid [168]. The latter four are all types of conventional SCC, with the ability to metastasise. Keratinising and verrucous cancers are usually associated with chronic vulval dermatoses, while the other three types are typically HPV-dependent lesions with HSIL (usual VIN) as the immediate precursor. Verrucous SCC is a well-differentiated non-metastasising neoplasia with minimal nuclear atypia that spreads through an expansile blunt interface [169]. Previous histopathologic studies of both vulval and oral verrucous SCC specimens demonstrate foci of atypia or invasion consistent with conventional SCC in 20-35% [170-172] It is unclear if verrucous and keratinising SCC are two consecutive steps along the same carcinogenic pathway, or if they represent two different mechanisms of neoplastic transformation [173].

The terminology for vulvar squamous intraepithelial neoplasia (-IN) has changed at least 15 times in the past 100 years, with the most recent ISSVD consensus statement published in 2016. This was generated after release of the Lower Anogenital Squamous Terminology (LAST) project, which unified nomenclature for HPV-dependent lesions at every affected site: cervix, vulva, vaginal, perianus, and anus. It elaborated a two-tiered system: HSIL for pre-cancerous lesions previously labeled with the suffixes IN-2 and IN-3, and LSIL for non-neoplastic lesions that represent transient HPV infection and previously were called IN-1 [174]. The LAST project emphasised the role of immunohistochemistry (IHC) for p16 to aid in identification of HSIL when the differential diagnosis includes LSIL or other entities. As the LAST project's brief was HPV-dependent lesions, the document did not mention dVIN or specifically describe the challenges of distinguishing it from HSIL. The ISSVD argued that dVIN should be

identified in any discussion of neoplastic squamous lesions, and subsequently produced a revised consensus terminology for vulvar pre-invasive disease.

The concept of dVIN was first introduced by the ISSVD in 1986 and confirmed in 2004 [168]. The microscopic appearance was delineated in a landmark study published in 2000 by Yang and Hart [18]. They described 12 cases of dVIN encountered at least 1cm away from the margin of an SCC, performed IHC for p53, and did PCR for selected HPV types. Seven had a concurrent or subsequent keratinising SCC, four carried a diagnosis of LS, and ten were reported to have LSC. The typical clinical appearance was a grey-white plaque ranging from 0.5 to 3.5cm in size. Specimens typically had parakeratosis, acanthosis with elongated branched rete ridges, and a relatively orderly maturation pattern. Most of the suprabasilar squamous cells were abnormal, often enlarged with large vesicular hyperchromatic nuclei, macronucleoli, abundant eosinophilic cytoplasm consistent with premature differentiation, and prominent intracellular bridges. These were most obvious when they extended deeply into the rete ridges or abutted the dermis. Sometimes the differentiated cells formed whorls with keratin pearls. When compared to suprabasilar cells, the basal cells were smaller, their nuclei were more hyperchromatic, and they showed a variable degree of nuclear atypia with scattered mitotic figures. IHC for p53 showed strong diffuse basilar staining in 83% of cases, while 17% were aberrant negative. One dVIN case was positive for HPV 31/33/51 in low copy number, and all cancers were negative for HPV. The authors concluded that dVIN is the immediate precursor to keratinising SCC, and warned that it is easily mistaken for non-neoplastic conditions like LSC and LS. They also noted that dVIN appears to have shorter transition time to invasive cancer than HSIL, and this may explain why it is infrequently diagnosed. Only women undergoing close surveillance for vulval dermatoses are likely to have biopsies performed at the pre-invasive stage; most other women will progress to an invasive cancer which either overgrows the precursor lesion or is unreported by pathologists who focus attention on the cancer [Yang].

The next substantial advance in the histopathologic description of dVIN occurred in 2009, when Ordi and colleagues described a basaloid variant that shares epidemiologic,

clinical, and immunohistochemical characteristics with standard dVIN [175]. Of 110 HPV-negative SCC, there were 51 cases of dVIN, of which four showed diffuse replacement of the whole epidermis by a homogenous population of small keratinocytes with large atypical nuclei. The epidermis was thickened and parakeratotic, sometimes with coalescent rete ridges giving an appearance of flat acanthosis. All cases were positive for p53, negative for p16, and negative for HPV DNA. The adjacent skin showed LS in one and LSC in two. Clinically, lesions were described as red-brown plaques with a rough surface. The authors highlighted that these cases are similar in appearance to HSIL, and advised IHC for p16 and p53 to aid in correct classification. In a previous work, Ordi's group duplicated the 80/20% ratio for p53 positivity in dVIN initially described by Yang [176]. Keratinising SCC was p16 positive in 9% and p53 positive in 70%, while all three verrucous SCC were negative for both. Interestingly, this study also documented that extramammary Paget's disease was positive for p16 in 100% of cases, and for p53 in 83%, and basal cell carcinomas were positive for each in 60% of cases. This underscores the need for close attention to histopathological features to make the correct diagnosis, with use of IHC restricted to an adjunctive role.

Recent evidence suggests there should be an expansion in the definition of HPV-independent intraepithelial neoplasia. Vulvar acanthosis with altered differentiation (VAAD) is a descriptive term for an unusual epithelial appearance with marked verruciform hyperplasia, plaque-like parakeratosis, hypogranulosis, a layer of pale-staining squamous cells, premature maturation, and absence of basal atypia [169]. This was introduced as a possible precursor lesion for verrucous SCC, but has subsequently been identified with keratinising SCC. Since then, a variety of verruciform lesions with abnormal maturation patterns that do not meet criteria for VAAD have been described [173]. Watkins and colleagues elaborated the defining characteristics of these lesions: 1) exophytic with prominent acanthosis or verruciform architecture, 2) lacking histomorphic features of HPV-related lesions, and 3) lacking sufficient basilar atypia to warrant a diagnosis of dVIN [173]. The authors coined a provisional term 'differentiated exophytic vulvar intraepithelial lesion' (DEVIL) for cases meeting these criteria. They encountered 25 examples of DEVIL, of which 14 were accompanied by SCC, and did

IHC and molecular profiling. While there was variation across the lesions, most examples of DEVIL lacked copy number alterations and TP53 mutations, but showed activating PIK3CA mutations. The authors proposed that DEVIL may represent a third pathway to vulvar cancer, but acknowledged that a direct link between DEVIL and SCC has not yet been established. The variety of genetic changes encountered suggests that these lesions exist on a spectrum between reactive phenomena that may resolve with treatment of the underlying dermatologic condition, to neoplastic entities that require excision or immunotherapy.

4. Studies

4.1 Normal vulvar histology: variation by site

A major problem in vulvar histopathology is that normal is not well defined, and it cannot be assumed to be the same as non-genital skin. The vulvar milleu is unique - the skin must accommodate sexual, reproductive, and excretory functions, and their attendant antigenic stimuli. The vulva also endures more friction, heat, moisture, and pressure than other body sites, related to both ambulation and sitting. Moreover, within a small surface area there is a transition from the glandular epithelium of inner cervix, to the squamous mucosa of cervix and vagina, to hairless skin of labia minora and central perineum, to hair bearing skin of mons, labia majora, lateral perineum, and perianus. This study demonstrated that vulvar histology is different to non-genital skin, and varies depending on specific location and epithelium type. Specifically, the results suggest that there is a lateral to medial transition in stratum corneum morphology, epithelial thickness, and lymphocytic infiltrate. The work also established that the junction between keratinised and non-keratinised skin has specific characteristics that should be interpreted as normal when there is correlation between anatomic and histopathologic site. A complete description of the range of appearances of vestibular squamous mucosa was not possible, as only 17 of 118 specimens were obtained from this site.

Normal Vulvar Histology: Variation by Site

Tania Day, MD,[1,2] Seán M. Holland, MBBS,[3] and James Scurry, FRCPA[2,4]

Objective: The aim of the study was to assess the histology of normal vulvar skin with attention to anatomic location and epithelium type.

Materials and Methods: We performed a retrospective histologic review of 118 vulvar biopsies and excisions obtained between 2010 and 2014 with adjacent normal skin or mucosa. Exclusions included age younger than 18 years, vestibulectomy, labiaplasty, inflammatory dermatoses, and insufficient normal tissue for assessment. Stratum corneum morphology was assessed as basket weave, compact, or intermediate. Stratum granulosum cell layer number and epithelial thickness were recorded. Dermal lymphocytic infiltrate was described as nil, sparse, moderate, or dense. Fischer exact test, Pearson χ^2, and Student *t* test were used for statistical analysis.

Results: There were 7 cases from mons pubis, 11 from perineum, 83 from labia, and 17 from vestibule. In the skin, the stratum corneum morphology was basket weave in 31%, compact in 35%, and intermediate in 34%. Stratum corneum at the mons pubis was uniformly basket weave, whereas at perineum, it was either compact or intermediate (7/7 vs 0/11; $p < .001$); the labia demonstrated all 3 morphologies. Parakeratosis (PK) was identified at the specimen edge in 4 cases of hairless skin and 7 cases of squamous mucosa. Mean epithelial thickness and dermal lymphocytic infiltration were similar in specimens with and without PK.

Conclusions: Compact stratum corneum of vulvar skin and a zone of PK at the mucocutaneous junction may be normal histological findings. Pathologists need to be aware of site-related differences of the vulvar epithelium to avoid overdiagnosis of pathological conditions.

Key Words: vulva, histology, stratum corneum, parakeratosis, mucocutaneous junction

(*J Lower Gen Tract Dis* 2016;20: 64–69)

The histopathologic interpretation of vulvar specimens follows the general methods of dermatopathology. For inflammatory diseases, the steps are (1) layer-by-layer identification of abnormalities, (2) aggregation of abnormalities into reaction patterns, (3) differential diagnoses, and (4) clinicopathological correlation.[1] The key reference point in the identification of abnormalities is comparison with normal.

Distinct anatomic zones of the vulva are covered by hair-bearing skin, hairless skin, or squamous mucosa, but histological differences in the surface epithelium of these 3 zones are not well described.[2–4] Assumptions include that keratinized epithelium at different sites is similar in appearance, the stratum corneum is uniformly basket weave, and that parakeratosis (PK) is an abnormal finding outside of squamous mucosal membranes.[2,5–7]

The possibility of site-related differences in vulvar epithelium was raised in an autopsy study of 52 cases, which showed increasing keratin layer thickness and decreasing epithelial thickness from medial to lateral vulva; the morphology of the stratum corneum was not documented.[6] This study also found that stratum corneum was absent in 62% of specimens from inner labia minora. We have observed that clinically normal vulvar skin may show a compact stratum corneum and that the mucocutaneous junction between inner labia minora and vestibule shows PK. We hypothesize that the presence of compact stratum corneum on keratinized skin and PK at the mucocutaneous junction are normal phenomena and represent important site-related differences on the vulva.

To investigate histologic features of normal vulvar skin, we undertook a retrospective histological assessment of specimens obtained from mons pubis, labia, vestibule, and perineum with examples of hair-bearing skin, hairless skin, and squamous mucosa.

METHODS

The Pathology North, Hunter New England, histopathology database identified 1,032 cases labeled "vulva," "perineum," "mons," "labia," "labium," or "vestibule" between 2010 and 2014 inclusively. This retrospective observational histologic case series was assessed by Hunter New England Research Ethics and Governance unit and exempted from further review. Exclusion criteria included cases of vestibulectomy and labiaplasty, evidence of inflammatory dermatosis, age younger than 18 years, insufficient perilesional tissue to permit assessment, and sites not relevant to this study such as groin, perianus, or glans clitoris. The resultant 118 cases with examples of normal skin or mucosa were biopsies or excisions of lesions to include naevi, melanosis, condyloma acuminata, fibroepithelial polyps, vulvar high-grade squamous intraepithelial lesion (HSIL), basal cell carcinoma (BCC), and squamous cell carcinoma arising from vulvar HSIL. These cases were grouped by anatomical location as mons pubis, labia, vestibule, or perineum, according to clinical notes. Specimen labeling did not uniformly distinguish between labia majora and minora. All slides were stained with hematoxylin and eosin (H&E).

The 3 authors reviewed each case on a multihead microscope to document epithelium type, stratum corneum morphology, stratum granulosum cell layer number, epithelial thickness in millimeters (mm), presence or absence of papillary dermal fibrosis, and degree of dermal lymphocytic infiltration. Epithelium type was assessed as hair-bearing skin, hairless skin, or squamous mucosa. Hair-bearing skin occurs on mons pubis, labia majora, and lateral perineum, whereas hairless skin occurs at the interlabial sulcus, labia minora, and central perineum.[2,4] Site determination in keratinized skin is aided by variations in the presence and appearance of adnexal structures such as hair follicles and sebaceous glands.[5] The mucocutaneous junction is a zone of transition between hairless skin and squamous mucosa; on inner labia minora, this is called Hart's line. Hart's line is not uniformly visible on clinical examination but marks the lateral boundary of the vestibule; the medial boundary is the hymen. The intervening vestibular mucosa is nonkeratinized and easily distinguished from noneroded keratinized skin. Specimens containing evidence of the mucocutaneous junction were classified into either hairless skin or mucosa, according to the predominant epithelium type present.

The stratum corneum morphology was assessed as basket weave, compact, or intermediate. Basket weave was defined as an open weave of keratin with clear spaces between each layer of keratin. Compact was defined as a solid eosinophilic band of keratin where the individual keratin layers could not be seen.

[1]Maternity and Gynaecology, John Hunter Hospital, [2]University of Newcastle, Callaghan, New South Wales; [3]Obstetrics and Gynaecology, Gold Coast University Hospital, Gold Coast, Queensland; and [4]Anatomical Pathology, Pathology North, Hunter New England, Newcastle, New South Wales, Australia
Reprint requests to: Tania Day, MD, 2 Lookout Rd, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au
The authors have declared they have no conflicts of interest.
This project was assessed by the ethics board and exempted from review.

TABLE 1. Stratum Corneum Morphology of Normal Vulva, Stratified by Location

	All (n = 118)	Mons pubis (n = 7)	Perineum, hair bearing (n = 6)	Perineum, hairless (n = 5)	Labia, hair bearing (n = 40)	Labia, hairless (n = 43)	Squamous mucosa (n = 17)
Stratum corneum, n (%)							
Basket weave	30 (25.4)	7 (100)	0	0	20 (50)	3 (7)	NA
Compact	34 (28.8)	0	1	3	7 (17.5)	23 (54)	NA
Intermediate	33 (28)	0	5	2	13 (32.5)	13 (30)	NA
PK	11 (9.3)	0	0	0	0	4 (9)	7 (41)
None	10 (8.4)	NA	NA	NA	NA	NA	10 (59)

NA indicates not applicable.

Stratum corneum with features in between these types was recorded as intermediate. Parakeratosis is the persistence of nuclei in the keratinocytes of the stratum corneum. The epithelial thickness was measured from the top of the granular cell layer to the tip of the deepest rete ridge closest to the resection line. The number of cell layers in the stratum granulosum was counted at the site where the epithelial thickness was measured. Dermal fibrosis was defined as the presence of coarse collagen fibers perpendicular to the skin surface. The degree of lymphocytic infiltration was defined as nil, sparse, moderate, or dense. Sparse reflected occasional scattered lymphocytes, moderate was a clustering of lymphocytes, and dense was a nodular or band-like sheet of closely packed lymphocytes.

Descriptive statistics, distributions, means, and SDs were calculated. Categorical data were compared with Fischer exact test or Pearson χ^2 and means were compared with the Student t test.

RESULTS

The mean age of the cases was 50 years with a range of 18 to 86 years. There was no association between age and histologic findings. The anatomical distribution, epithelium type where relevant, and stratum corneum morphology are described in Table 1. In the skin, the stratum corneum appearance was basket weave in 31% (see Figure 1), compact in 35% (see Figure 2), and intermediate in 34% (see Figure 3). Stratum corneum at the mons pubis uniformly showed basket-weave morphology, whereas at perineum, it was either compact or intermediate (7/7 vs 0/11; $p < .001$); the labia demonstrated all 3 morphologies. Hair-bearing skin was more likely to demonstrate basket-weave morphology than hairless skin (27/53 [51%] vs 3/48 [6%]; $p < .001$). On comparison of hairless to hair-bearing skin, the former was associated with a greater epithelial thickness (0.19 vs 0.16 mm; $p = .02$) and mean granular cell layer number (3.1 vs 2.6; $p = .009$). No specimen demonstrated dense dermal lymphocytic infiltration or papillary dermal fibrosis. Of 118 cases, dermal lymphocytes were sparse in 89 (75%) and moderate in 14 (12%, see Figures 2, 3). Histologic features stratified by anatomic site, with labial specimens subdivided into hairless and hair-bearing skin, are recorded in Table 2.

Compact stratum corneum was associated with a greater mean epithelial thickness (0.2 vs 0.15 mm; $p = .005$) and mean granular cell layer number (3.1 vs 2.3; $p < .001$) than basket-weave stratum corneum (see Table 3). The differences in lymphocyte infiltration across the 3 stratum corneum morphology groups were statistically significant ($p = .03$).

Parakeratosis was identified at the edge of 4 specimens of hairless skin and seven of squamous mucosa (see Figures 4, 5). Parakeratosis was not observed in hair-bearing skin. Table 4 describes the histologic features of hairless skin and squamous mucosa with and without PK. Mean epithelial thickness in specimens with PK was similar to those without (0.18 vs 0.19; $p = .56$), as was dermal lymphocytic infiltration (nil or sparse lymphocytes 8/11 [73%] vs 47/54 [87%]; $p = .35$).

DISCUSSION

We assessed 118 cases of normal vulvar skin and mucosa and found site-specific differences in stratum corneum morphology and PK at the mucocutaneous junction. Specimens from the

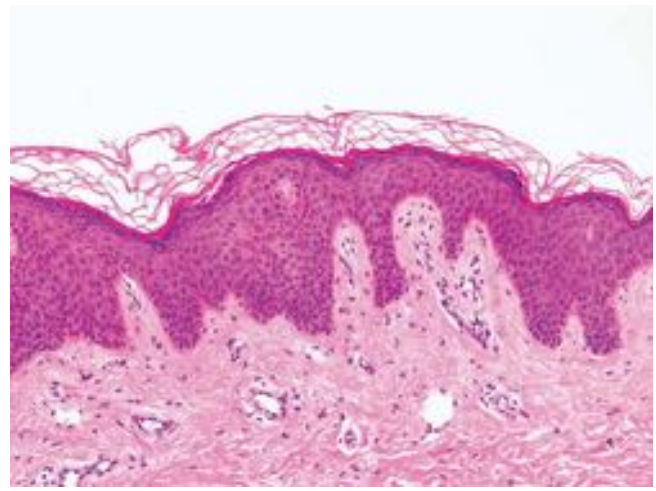

FIGURE 1. Hair-bearing skin with basket-weave morphology of the stratum corneum. H&E ×10.

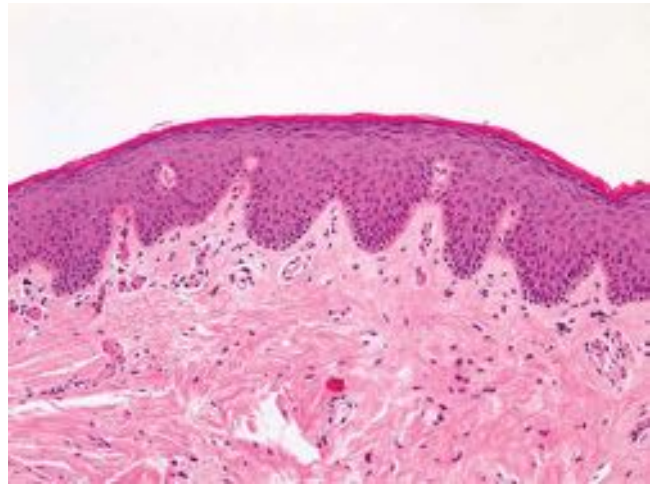

FIGURE 2. Hair-bearing skin with compact morphology of the stratum corneum and sparse dermal lymphocytic infiltration. H&E ×10.

mons pubis universally showed basket-weave stratum corneum, the labia could be compact, intermediate, or basket weave, whereas the perineum was intermediate or compact but not basket weave. The hairless epithelium of central perineum and labia minora was thicker with more granular cell layers. These site-related differences may represent an evolutionary functional adaptation related to pressure from sitting. Supporting the contention of site-specific adaptation, the variation in stratum corneum and epithelial thickness at different anatomic locations is well documented. Epidermal thickness varies from 0.05 mm at the eyelid to 0.6 mm at palms and soles, with most other locations between 0.07 and 0.18 mm, whereas mean stratum corneum thickness varies from 0.006 mm at the corner of the eye to 0.02 mm at outer forearm.[8–10] There is also evidence for functional differences across sites.[11]

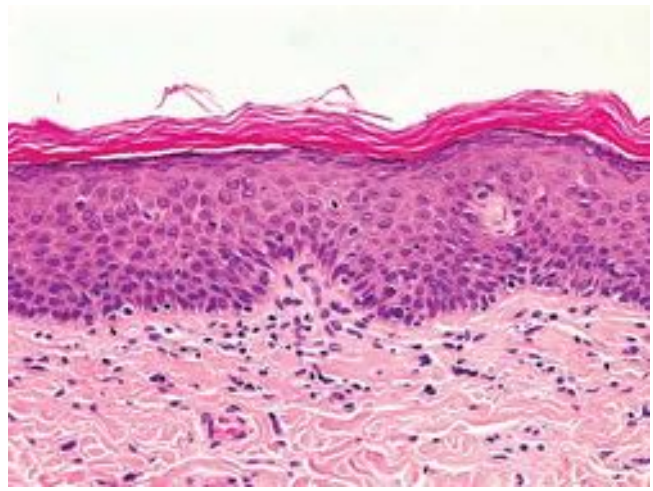

FIGURE 3. Hair-bearing skin with intermediate morphology of the stratum corneum and moderate dermal lymphocytic infiltration. H&E ×10.

TABLE 2. Histological Features of Skin From Mons, Perineum, and Labia

	Total (n = 97)	Mons (n = 7)	Perineum (n = 11)	Labia, hair bearing (n = 40)	Labia, hairless[a] (n = 39)
Granular cell layer number, mean (SD)	2.8 (0.94)	1.6 (0.72)	2.4 (0.77)	3 (0.86)	3 (0.87)
Epithelial thickness, mean (SD), mm	0.17 (0.07)	0.16 (0.03)	0.2 (0.06)	0.15 (0.06)	0.2 (0.12)
Lymphocytic infiltration, n (%)					
Nil	12 (12)	1 (14)	0	6 (15)	5 (13)
Sparse	74 (76)	6 (86)	8 (73)	30 (75)	30 (77)
Moderate	11 (11)	0	3 (27)	4 (10)	4 (10)

[a]Excludes 4 specimens with a parakeratotic stratum corneum.

TABLE 3. Histological Features Stratified by Stratum Corneum Morphology

	Basket weave (*n* = 30)	Intermediate (*n* = 33)	Compact (*n* = 34)
Granular cell layer number, mean (SD)	2.3 (0.89)	2.9 (0.92)	3.1 (0.8)
Epithelial thickness, mean (SD), mm	0.15 (0.05)	0.17 (0.07)	0.2 (0.08)
Lymphocytic infiltration, *n* (%)			
Nil	8 (27)	3 (9)	1 (3)
Sparse	21 (70)	26 (79)	27 (79)
Moderate	1 (3)	4 (12)	6 (17)

When compared with skin on the forearm, skin from labia majora has a higher transepidermal water loss, blood flow, and friction coefficient.[12] We were unable to assess for an anterior to posterior transition across the labia because information on exact location was not consistently documented in the clinical or requisition notes. Our findings of a relationship between skin type, stratum corneum morphology, and epithelial thickness are consistent with Jones' observation of a medial to lateral decrease in epidermal thickness.[6]

We considered an alternative explanation that compact stratum corneum is a manifestation of lichen simplex chronicus (LSC) so mild as to not be clinically evident. We rejected this hypothesis for several reasons. We excluded cases in which pruritus was recorded on requisition notes. No case had papillary dermal fibrosis, a key histologic finding of LSC. The dermal lymphocytic infiltrate was nil or sparse in 82% of specimens with compact stratum corneum. Lichen simplex chronicus is primarily a disease of hair-bearing skin, but we found compact stratum corneum more

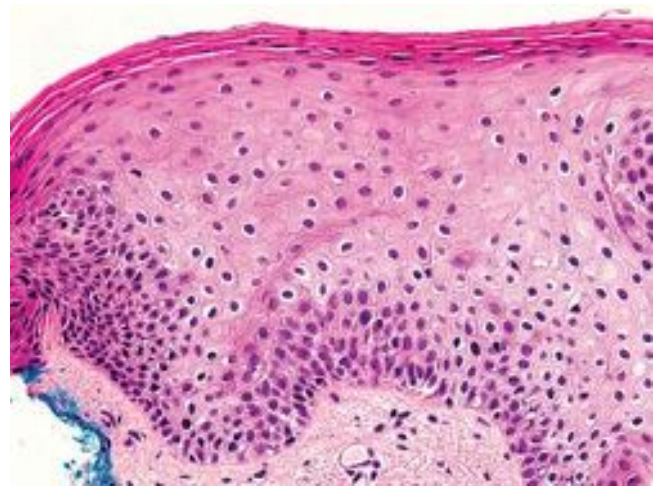

FIGURE 4. Parakeratosis at the left-hand side of an excision of a combined lesion of benign lentigo and stromal melanocytosis, which contains the mucocutaneous junction. H&E ×40.

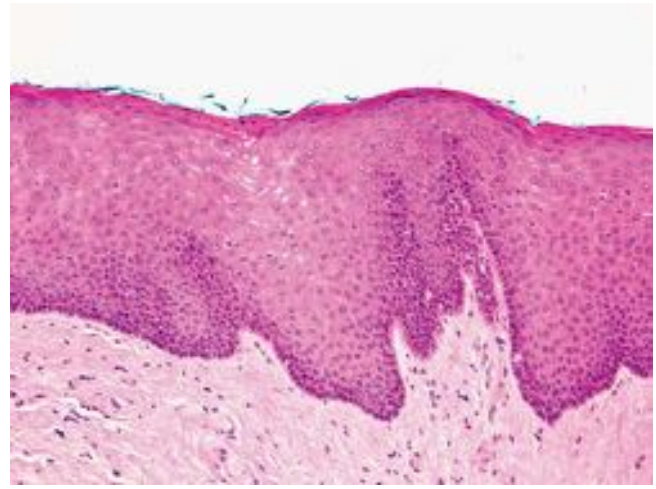

FIGURE 5. Hairless skin with PK at the mucocutaneous junction. H&E ×10.

TABLE 4. Hairless Skin and Squamous Mucosa Stratified by PK

	Hairless skin with PK (n = 4)	Hairless skin without PK (n = 44)	Squamous mucosa with PK (n = 7)	Squamous mucosa without PK (n = 10)
Epithelial thickness, mean (SD), mm	0.13 (0.02)	0.19 (0.07)	0.2 (0.08)	0.18 (0.07)
Lymphocytic infiltration, n (%)				
Nil	1 (25)	5 (11)	0	2 (20)
Sparse	3 (75)	32 (73)	4 (57)	8 (80)
Moderate	0	7 (16)	3 (43)	0

PK indicates parakeratosis.

often in hairless skin.[13,14] The mean epidermal thickness of specimens with compact stratum corneum was 0.2 mm, consistent with previous reports, which document a normal range in adults of 0.12 to 0.35 mm.[6,15] The implication is that an isolated finding of compact stratum corneum should not generate a diagnosis of mild LSC, particularly if the specimen is from the labia minora or perineum. Histopathologic findings that would support a diagnosis of LSC include significant hyperkeratosis, hypergranulosis, acanthosis, any degree of dermal fibrosis, and a moderate or dense lymphocytic infiltrate.[7]

Inclusion of vestibulectomy and labiaplasty samples would have provided more information on the mucocutaneous junction, but we elected not to include these given the unsettled debate as to the histologic normality of these specimens.[16–21] We document 11 cases in which PK occurred at the edge of a specimen of hairless skin or mucosa, supporting the concept of a zone of PK at the normal mucocutaneous junction. Studies of normal orolabial and inner eyelid mucocutaneous junctions likewise demonstrate PK.[22,23] Nauth and Schilke[24] previously reported PK in up to 50% of clinically normal vulvar skin specimens, particularly in women younger than 50 years, but other authors state that PK is a manifestation of inflammation.[5,6] We found no correlation between the presence of PK and other features of inflammation such as degree of lymphocytic infiltrate or increased epithelial thickness. Most specimens, regardless of anatomic site, had sparse dermal and lamina propria lymphocytes, in keeping with previous reports.[6,15,25] We interpreted these lymphocytes as normal skin and mucosa-associated lymphoid tissue. In the setting of scant to moderate lymphocytes and otherwise normal appearance, specimens with PK from a site consistent with the mucocutaneous junction should be evaluated as normal.

Access to biopsies and excisions from multiple facilities across Australia provides a case series sufficiently robust to compare subgroups. However, any retrospective histology study is limited by the presence of a clinical indication for biopsy or excision. We strove to exclude cases suggestive either clinically or histopathologically of an underlying inflammatory dermatosis but had limited access to some case histories. Some authors suggest that even grossly normal peritumoral skin adjacent to condyloma or BCC in fact has subtle morphological or immunohistochemical abnormalities.[26,27] Although our research question would be optimally addressed through prospective collection of vulvar biopsies from asymptomatic volunteers, there are ethical and logistic barriers to recruiting women across the age spectrum for clinically unnecessary genital procedures. Further studies of normal vulvar histology might additionally assess the distribution and size of lymphatics and sebaceous glands in labia minora, the presence of perisebaceous lymphocytic infiltrates, anterior to posterior variation in the labia, and stratum corneum thickness taking into account morphologic differences.

In summary, we have shown that compact stratum corneum of vulvar skin and a zone of PK at the mucocutaneous junction may be considered normal findings. Pathologists need to be aware of site-related differences of the vulvar epithelium to avoid overdiagnosis of pathological conditions.

REFERENCES

1. High W, Tomasini C, Argenziano G, et al. Basic principles of dermatology. In: Jorizzo J, Schaffer J, Bolognia J, et al., eds. *Dermatology*. 3rd ed. Philadelphia, PA: Elsevier Saunders; 2012:1–42.
2. Calonje E, Neill S, Bunker C, et al. Diseases of the anogenital skin. In: Brenn T, Lazar A, McKee P, et al., eds. *McKee's Pathology of the Skin*. 4th ed. Elsevier Saunders; 2012:437–519.
3. Neill S, Lewis F. Basics of vulval embryology, anatomy, and physiology. In: *Ridley's the Vulva*. 3rd ed. Hoboken, NJ: John Wiley and Sons; 2009:20–5.
4. van der Putte SCJ. External perineum. In: Lange R, ed. *The Development of the Perineum in the Human: A Comprehensive Histological Study With a Special Reference to the Role of Stromal Components*. Heidelberg, Germany: Springer; 2005:31.
5. Scurry J, Melville KR. Normal vulva and developmental anomalies. In: Heller DS, Wallach RC, eds. *Vulvar Disease: A Clinicopathological Approach*. Boca Raton, FL: Taylor and Francis Group LLC; 2007:18–22.
6. Jones IS. A histological assessment of normal vulval skin. *Clin Exp Dermatol* 1983;8:513–21.
7. Lazar AJF, Wang W. Skin. In: Kumar V, Abbas A, Aster J, eds. *Robbins Basic Pathology*. 9th ed. Philadelphia, PA: Elsevier Saunders; 2013: 851–70.
8. Sandby-Møller J, Poulsen T, Wulf HC. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. *Acta Derm Venereol* 2003;83:410–3.
9. Robertson K, Rees JL. Variation in epidermal morphology in human skin at different body sites as measured by reflectance confocal microscopy. *Acta Derm Venereol* 2010;90:368–73.
10. Lee Y, Hwang K. Skin thickness of Korean adults. *Surg Radiol Anat* 2002;24:183–9.
11. Tagami H. Location-related differences in structure and function of the stratum corneum with special emphasis on those of the facial skin. *Int J Cosmet Sci* 2008;30:413–34.
12. Farage M, Maibach HI. The vulvar epithelium differs from the skin: implications for cutaneous testing to address topical vulvar exposures. *Contact Dermatitis* 2004;51:201–9.
13. O'Keefe RJ, Scurry JP, Dennerstein G, et al. Audit of 114 non-neoplastic vulvar biopsies. *Br J Obstet Gynaecol* 1995;102:780–6.
14. Lynch PJ. Lichen simplex chronicus (atopic/neurodermatitis) of the anogenital region. *Dermatol Ther* 2004;17:8–19.

15. Harper WF, McNicol EM. A histological study of normal vulval skin from infancy to old age. *Br J Dermatol* 1977;96:249–53.

16. Scurry J, Marchitelli C, Moyal-Barracco M. Labioplasty specimens: when should pathological findings be considered as abnormal? *J Lower Gen Tract Dis* 2015;19:e49–50.

17. Barrett MM, Carlson JA. Reply to Scurry et al.: vulvar lymphangiectasis, an indicator of lymph stasis, is an authentic sign of abnormal histopathology in labiaplasty specimens. *J Lower Gen Tract Dis* 2015; 19:e50.

18. Brokenshire C, Pagano R, Scurry J. The value of histology in predicting the effectiveness of vulvar vestibulectomy in provoked vestibulodynia. *J Lower Gen Tract Dis* 2014;18:109–14.

19. Scurry J, Pagano R, Locker B, et al. Nerve and inflammatory cell density in localized provoked vestibulodynia. *J Lower Gen Tract Dis* 2009; 13:S23.

20. 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.

21. Halperin R, Zehavi S, Vaknin Z, et al. The major histopathologic considerations in the vulvar vestibulitis syndrome. *Gynecol Obstet Invest* 2005;59:75–9.

22. Barrett AW, Morgan M, Nwaeze G, et al. The differentiation profile of the epithelium of the human lip. *Arch Oral Biol* 2005;50:431–8.

23. Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat* 2011;218:449–61.

24. Nauth H, Schilke E. Cytology of the Exfoliative Layer in Normal and Diseased Vulvar Skin - Correlation with Histology. *Acta Cytol* 1982;26:269–83.

25. Sargeant P, Moate R, Harris J, et al. Ultrastructural study of the epithelium of the normal human vulva. *J Submicrosc Cytol Pathol* 1996;28:161–70.

26. Paul J, Carlson JA. Lymphangiectases are common underlying warts and in normal peritumoral skin: histologic evidence of decreased immune surveillance. *Am J Dermatopathol* 2011;33:152–60.

27. Monhian N, Jewett BS, Shan RB, et al. Matrix metalloproteinase expression in normal skin associated with basal cell carcinoma and in distal skin from the same patients. *Arch Facial Plast Surg* 2005;7:238–43.

4.2 Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis?

There is no previous study of the appearance and clinical course of cutaneous fungal and yeast infections seen on histopathology of affected vulval skin. Multiple publications suggest that candidal superinfection may complicate lichenoid dermatoses, but it is unknown how this impacts on histopathologic assessment. It is standard dermatopathology practice to inspect the PAS of an acanthotic tissue reaction for evidence of spores or hyphae, but it is unclear if the number and shape of these elements can distinguish between yeast and dermatophytes. It is not universal to apply the same step to evaluation of the lichenoid reaction. There is also no literature addressing treatment of mycosis in the setting of vulval lichenoid dermatoses. The working hypothesis was that yeast and dermatophytes have distinct histopathologic patterns that may be used to aid in definitive diagnosis. The study demonstrated the reverse - histopathology cannot reliably distinguish between candidosis and dermatophytosis, and neither can organism-specific polymerase chain reaction (PCR). This work also documented the low rates of correct provisional diagnosis, the complicated appearance of comorbid mycosis and lichenoid dermatoses, and the wide variation in treatment regimens employed by the cohort of referring vulval specialists.

Can Routine Histopathology Distinguish Between Vulvar Cutaneous Candidosis and Dermatophytosis?

Tania Day, MD,[1,2] Ailsa Borbolla Foster, MBBS,[1] Samuel Phillips, BSc(Hons),[3] Ross Pagano, FRANZCOG,[4] Delwyn Dyall-Smith, FACD,[5] James Scurry, FRCPA,[2,6] and Suzanne M. Garland, MD[3,7]

Objectives: This study aimed to determine if vulvar cutaneous candidosis and dermatophytosis can be distinguished by routine histopathology.

Materials and Methods: Twenty-four cases of periodic acid-Schiff–stained vulvar biopsies with a diagnosis of cutaneous mycosis were reviewed and histopathological characteristics on both periodic acid-Schiff and hematoxylin and eosin were recorded. Data were collected on age, clinical impression, microbiological results, and treatment, and all specimens underwent multiplex polymerase chain reaction analysis.

Results: The mean age was 60 years, and all but 3 women had at least 1 risk factor for mycosis including 15 (62.5%) with lichen sclerosus and/or planus managed with topical corticosteroids. A clinical suspicion of tinea or candidosis was documented in 12 (50%) of the cases. Vulvovaginal swabs showed *Candida* species in 9 women; one skin scraping was positive for *Trichophyton rubrum*. Microbiology was not obtained in 8 patients, 5 had a negative swab, and 1 had negative skin scrapings. No histopathological or morphological features distinguished *Candida* species from dermatophytes. Organisms appeared as basophilic structures in the stratum corneum in 15 (62.5%) hematoxylin and eosin–stained slides. Polymerase chain reaction results were positive for *Candida* species in 5 (21%) and for dermatophytes in 3 (13%), negative in 13, and unassessable in 3 cases.

Conclusions: Vulvar cutaneous candidosis and dermatophytosis cannot be reliably distinguished by routine histopathology or specific polymerase chain reaction. A high index of suspicion combined with adequate microbiological testing remains the best approach to differentiating between the 2, which impacts on counseling, treatment, and prognosis.

Key Words: vulva, histopathology, dermatophytosis, candidosis, lichenoid

(*J Lower Gen Tract Dis* 2016;20: 00–00)

Cutaneous mycoses are superficial infections of the skin, hair, or nails in which the organism is limited to the stratum corneum but provokes a host immune response. These are classified into dermatophytosis, usually caused by the fungi *Trichophyton*, *Microsporum*, and *Epidermophyton*, and candidosis, typically caused by *Candida albicans* and related yeast species. Genital dermatophytosis spares mucosa and is termed *tinea cruris*, whereas candidal infection of the female genital tract may affect mucosa and skin and is called *vulvovaginal candidosis* (VVC).[1] Both are common, yet clinical recognition and organism-specific diagnosis pose a challenge. Both infections may present as a pruritic erythematous rash, with a differential diagnosis that includes dermatitis, lichen simplex chronicus, lichenoid dermatoses, psoriasis, and neoplasia.[2] Microbiology of swabs or scrapings may yield the diagnosis, but its utility is limited by long culture times and sensitivities of 30% to 70% and 36% to 42% for nonacute *Candida* and dermatophytes, respectively.[3–5] Histopathology of skin biopsy can detect organisms in the stratum corneum, and the use of the periodic acid-Schiff (PAS) stain improves diagnostic yield.[6] However, the role of histopathology in differentiating between candidosis and dermatophytosis remains unclear, with conflicting reports regarding the relative significance of detection on hematoxylin and eosin (H&E) versus PAS.[7–9]

This study aimed to describe the clinical course, microbiological results, histopathological findings, and polymerase chain reaction (PCR) analysis of vulvar biopsies showing mycosis in an effort to determine if candidosis and dermatophytosis may be distinguished on routine histopathology.

MATERIALS AND METHODS

The Pathology North, Hunter New England database was searched between January 2012 and June 2014 for vulvar biopsies with a histopathological diagnosis of mycosis. The Hunter New England Research Ethics and Governance Unit approved this retrospective case series (HREC 14/09/10/5.04); signed written consent was obtained for the use of clinical photographs. Data collected included age, clinical impression, vulvar dermatological diagnoses, and presence of risk factors including diabetes mellitus, obesity, physiological or pharmaceutical estrogen, urinary incontinence, and use of topical corticosteroids. Concurrent and previous microbiological results and recommended treatment were also recorded. The final presumed diagnosis was based on the examination findings, results of investigations, and clinical assessments subsequent to the biopsy.

Slides stained with H&E and PAS were retrieved and reviewed; no additional histopathological studies were performed. The pathologist (J.S.) was blinded to the clinical history and microbiological results. The case was included if organisms were identified in the stratum corneum of PAS-stained slides; subsequently, the H&E preparations were assessed. Exclusion criteria were age of less than 18 years and unavailable clinical information. Biopsy location was recorded as hair-bearing skin or hairless skin; precise anatomical location was not uniformly documented. The quantity of visible organisms on both PAS and H&E was recorded as sparse, moderate, or profuse, with absent as an option for H&E-stained slides. We looked for the aleurioconidia (fruiting bodies) and true hyphae, which characterize dermatophytes versus the spores and nonseptated pseudohyphae of *Candida* species.[8] The dermal inflammatory infiltrate was assessed as absent, mild, or moderate to dense. Any additional diagnostic features were recorded.

All cases were sent as formalin-fixed paraffin-embedded (FFPE) tissue blocks for PCR analysis. Samples underwent deparaffinization via a histolene-based method. Digested tissue 200 μL was extracted using the MagNA Pure 96 automated system, then the total nucleic acids were eluted into a volume of 100 μL in

[1]Maternity and Gynaecology, John Hunter Hospital; and [2]Faculty of Health and Medicine, University of Newcastle, Newcastle, New South Wales; [3]Department of Microbiology and Infectious Diseases Laboratory Services, Murdoch Children's Research Institute, Parkville; and [4]Vulvar Disorders and Dermatology Clinic, Royal Women's Hospital, Melbourne; [5]Riverina Dermatology, Wagga Wagga; and [6]Anatomical Pathology, Pathology North, Hunter New England, Newcastle, New South Wales; and [7]Faculty of Medicine, Dentistry, and Health, University of Melbourne, Parkville, Victoria, Australia

Reprint requests to: Tania Day, MD, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Rd, New Lambton Heights NSW 2305 Australia. E-mail: tania.day@hnehealth.nsw.gov.au

The authors have declared they have no conflicts of interest.

Approved by Hunter New England Research Ethics and Governance unit (14/09/10/5.04).

MagNA Pure elution buffer (Roche, Basel, Switzerland). Each sample was tested for human β-globulin to confirm extraction efficiency and assess for PCR inhibitors. The total nucleic acids were universally positive for 18s DNA, so the amplicons were sent for Sanger sequencing, which was reported as consistent with multiple organisms of fungal and/or yeast origin.[10] We used a previously described candidal assay with a broad range of detection for *Candida dubliniensis*, *guillermondii*, *parapsilosis*, and *tropicalis*, and specific detection of *C. albicans, krusei*, and *glabrata*.[11] A previously described multiplex dermatophyte assay was modified to omit the specific probes for each species and replaced with the Roche Syber green master mix, which allows the investigator to see all amplified products from one set of primers.[12] Although this removes the ability to identify species within the sample, it permits a melt profile that can distinguish between genera that have major differences in their amplicons. This primer set reported detection of 12 species of *Trichophyton*, 5 species of *Microsporum*, and *Epidermophyton floccosum*. Melt profiles of control cultures of *Trichophyton* and *Microsporum* were assessed, but a pure culture of *Epidermophyton* was unobtainable.

RESULTS

Twenty-seven biopsies met inclusion criteria, with 3 excluded because of unobtainable clinical data. The clinical, histopathological, microbiological, and PCR results are summarized in Table 1. The mean age was 60 years, and the median interval from symptoms to treatment was 24 months (range = 5–120). Four women initially attended a general gynecologist; the remainder saw dermatologists (5) or gynecologists (15) specialized in vulvovaginal disorders. Fifteen women (62.5%) had a lichenoid dermatosis, and 1 had contact dermatitis related to incontinence. All cases except 2 had a risk factor for mycosis, including 20 (83%) using topical corticosteroids, 5 with diabetes mellitus, 5 postmenopausal women using estrogen, 2 with urinary incontinence, and 3 with overhanging abdominal pannus; 11 (46%) had more than 1 risk factor. Microbiological testing occurred in 16 (67%) and yielded 9 positive results for *Candida* species and 1 for *Trichophyton rubrum*; 14 had a vulvovaginal swab, 1 had both swab and skin scrapings, and 1 had scrapings alone. There were no differences in case characteristics when stratified by microbiological results or presence of lichenoid dermatosis. Histopathology was the sole source of the diagnosis of mycosis in 7 (29%) of 24 of women.

Of the 12 unsuspected cases of mycosis, 6 had clinical findings of erythema and excoriation or erosion, with provisional diagnoses of psoriasis, dermatitis, lichen sclerosus, high-grade intraepithelial neoplasia, or Paget disease (see Figure 1A). The other 6 had areas of pallor, thought to be lichen sclerosus or vitiligo. In the cases of suspected mycosis, 6 had diffuse erythema with edema or fissures, one had lichenification, and 5 had erythematous scaly plaques over the groin or labia majora (see Figure 2).

TABLE 1. Comparison of the Clinical, Histopathological, Microbiological, and PCR Results of Women with Vulvar Biopsy Showing Cutaneous Mycosis

	Age	Final presumed diagnosis	Skin site	Skin comorbidity	Risk factors	Organisms on H&E/PAS	Culture	PCR
1	31	Candidosis	Hairless	LS[a]	TCS, DM	Sparse/sparse	*C. albicans*	Negative
2	45	Candidosis	Hairless	LS LP[a]	TCS	Sparse/sparse	Not done	Negative
3	62	Candidosis	Hairless	LS LP[a]	TCS	Nil/sparse	Negative	Negative
4	66	Candidosis	Hairless	LP[a]	TCS	Nil/sparse	Not done	Negative
5[b]	74	Candidosis	Hairless	LS LP[a]	TCS, estrogen	Nil/sparse	Negative	Negative
6	77	Candidosis	Hairless	None	TCS, DM, estrogen	Sparse/sparse	*C. albicans*	Negative
7	80	Candidosis	Hairless	LS[a]	TCS	Sparse/sparse	Negative	Unassessable
8	84	Candidosis	Hairless	LS LP[a]	TCS, DM, incontinence	Moderate/profuse	Not done	*C. albicans*
9[b]	25	Candidosis	Hair-bearing	None	TCS	Nil/moderate	*C. albicans*	Negative
10[b]	40	Candidosis	Hair-bearing	LS	TCS	Sparse/sparse	*C. albicans*	Negative
11	48	Candidosis	Hair-bearing	LP	TCS	Moderate/profuse	*C. albicans*	Negative
12[b]	60	Candidosis	Hair-bearing	None	TCS	Sparse/sparse	*C. albicans*	*C. glabrata*
13[b]	63	Candidosis	Hair-bearing	None	DM, obesity	Nil/sparse	*C. albicans*	Unassessable
14[b]	64	Candidosis	Hair-bearing	LS	TCS	Sparse/sparse	Not done	Dermatophyte
15	75	Candidosis	Hair-bearing	LS[a]	TCS, estrogen	Nil/sparse	*C. glabrata*	Negative
16[b]	81	Candidosis	Hair-bearing	LP	TCS	Nil/moderate	Not done	Unassessable
17	87	Candidosis	Hair-bearing	Dermatitis	TCS, incontinence	Nil/moderate	*Candida* species	*C. albicans*
18	24	Pityriasis versicolor	Hair-bearing	None	None	Moderate/profuse	Not done	Negative
19[b]	48	Tinea cruris	Hair-bearing	LS	TCS, obesity	Nil/sparse	*T. rubrum*	Negative
20[b]	49	Tinea cruris	Hair-bearing	LS	TCS, estrogen	Moderate/profuse	Negative	*C. albicans*
21[b]	56	Tinea cruris	Hair-bearing	LS	TCS, obesity	Sparse/sparse	Not done	Dermatophyte
22[b]	67	Tinea incognito	Hair-bearing	None	TCS, DM, estrogen	Moderate/profuse	Negative	Negative
23[b]	67	Tinea cruris	Hair-bearing	None	Tinea pedis	Moderate/profuse	Negative	*C. krusei*
24	68	Tinea cruris	Hair-bearing	None	None	Moderate/profuse	Not done	Dermatophyte

[a]Histopathological confirmation of the dermatosis concurrent to mycosis.

[b]Cases with a clinical suspicion noted before biopsy.

PCR indicates polymerase chain reaction; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; LS, lichen sclerosus; TCS, topical corticosteroids; DM, diabetes mellitus; LP, lichen planus.

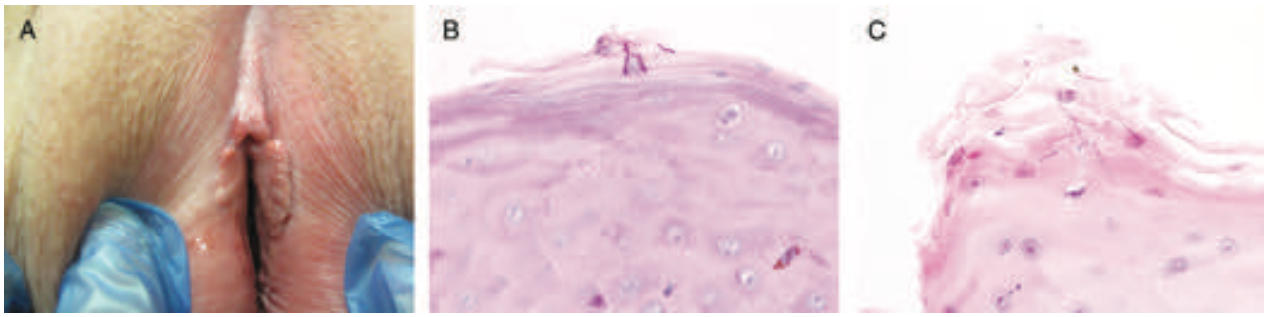

FIGURE 1. A, Architectural change, pallor, erythema, and erosion, consistent with lichen sclerosus and candidosis. B, Organisms clearly seen in vertical to oblique orientation. PAS, original magnification ×40. C, Sparse organisms in parakeratosis without inflammatory cells. H&E, original magnification ×40. H&E indicates hematoxylin and eosin; PAS, periodic acid-Schiff.

Organisms on PAS appeared as magenta outlines of filaments and round forms in the stratum corneum (see Figures 1B and 3A). No aleurioconidia were seen. A tendency for vertical or oblique orientation helped distinguish organisms from inspissated serum, which appeared as PAS-positive linear deposits aligned horizontally between keratin layers without morphological details such as capsular staining. The quantity of organisms on PAS was sparse in 14 (58%), moderate in 3 (12.5%), and profuse in 7 (29%) cases. On H&E, organisms appeared in 15 cases (62.5%) as pale basophilic outlines with the same morphology as on PAS (see Figures 1C and 3B). Spores could not be distinguished from cross-sections of hyphae. Pseudohyphae could not be differentiated from true hyphae because the diameters were too similar and it was not possible to discern constrictions and septae. No morphological or histopathological features distinguished *Candida* species from dermatophytes.

Two cases had otherwise normal epithelium; one had pityriasis versicolor, and the other had tinea incognito. An acanthotic tissue reaction with hyperkeratosis, parakeratosis, and elongated rete ridges was seen in 14 (58%). There was histopathological confirmation of the lichenoid dermatosis in 12 (80%) of 15–4 diagnoses were from a previous biopsy, and 8 were concurrent to the mycosis. The latter showed basal layer vacuolar change, squamatization, and band-like upper dermal lymphocytic infiltrate; 6 also had superimposed lichen simplex chronicus. The seven nonconfirmatory samples were all from hair-bearing skin; in contrast, 5 (62.5%) of the lichenoid specimens were from hairless skin. Inflammation was moderate to dense in 16 cases (67%).

The *Candida* assay showed low detectable levels of DNA in 5 cases, 3 of *C. albicans* and 1 each of *C. krusei* and *C. glabrata*; of 9 cultures showing *Candida* species, 2 (22%) had a genus-concordant positive PCR. The dermatophyte assay showed low detectable levels of DNA in 3 cases, but these did not follow the melt profile of the *Trichophyton* and *Microsporum* controls, suggesting either presence of *Epidermophyton* or nonspecific binding of primers to human or bacterial DNA. The one case of positive *Trichophyton* culture had a negative PCR. Three samples were nondetectable for human β-globin gene, because of either insufficient tissue or PCR inhibition. Two cases had discrepant clinicopathological impression and PCR results; 1 woman with clinical tinea and a negative vulvovaginal swab had *C. albicans* on PCR, and 1 woman with tinea pedis, scaly erythematous plaques on the groin, and negative swab and scraping had *C. krusei* on PCR.

Despite a histopathology report of mycosis, 3 women received no antimycotic therapy until seen by a vulvovaginal specialist. Treatment duration recommendations ranged from 2 weeks to 6 months. Seven women were managed with topical therapies including ketoconazole, miconazole, nystatin, terbinafine, and boric acid. Five women had short courses of fluconazole in combination with topical miconazole or ketoconazole. The remainder was prescribed oral nystatin, griseofulvin, itraconazole, or fluconazole. Regimens of the latter included 50 mg daily for 6 months, 100 mg twice a week for 3 months, and 150 mg weekly for 2 months. Nystatin was provided only to women with culture-positive *Candida*. Griseofulvin and terbinafine were prescribed in 3 cases of presumed tinea; 1 did not improve until given clotrimazole. Three women did not have documented resolution: 1 used intermittent clotrimazole and never saw a vulvovaginal specialist, 1 cancelled her follow-up appointment, and 1 died of vulvar squamous cell carcinoma. No other woman had intraepithelial neoplasia or cancer during follow-up.

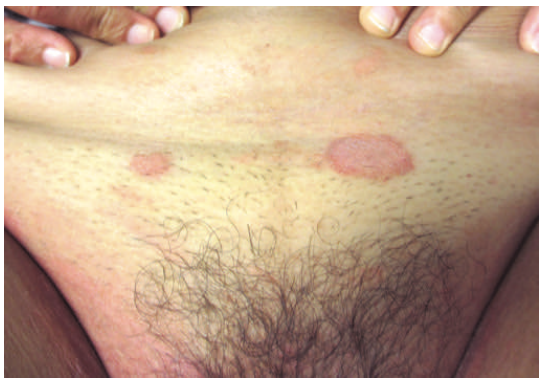

FIGURE 2. Well-demarcated erythematous scaly plaques with central clearing located over groin, mons, and labia majora, consistent with tinea cruris.

DISCUSSION

There is scant previous work specific to definitive diagnoses of cutaneous mycosis of the vulva. At least 3 distinct categories of vulvovaginal candidal infection exist: sporadic acute VVC, chronic recurring VVC (CRVVC), and intracrural candidosis occurring primarily in obese postmenopausal women.[1] Few studies attempt to classify women according to severity of skin involvement or describe treatment strategies specific to the vulva.[13] The clinical presentation of CRVVC is variable; findings may be limited to subtle erythema, edema, or fissures, and vaginal discharge may seem normal.[1,3,14,15] The prevalence, management, and prognosis of both vulvar tinea and intracrural candidosis are poorly documented, perhaps because of use of nonspecific terminology (intertrigo) and a tendency to treat without performing diagnostic tests.[1,16]

The prolonged interval between symptoms and definitive diagnosis has been noted in other vulvovaginal disorders.[17–19] In

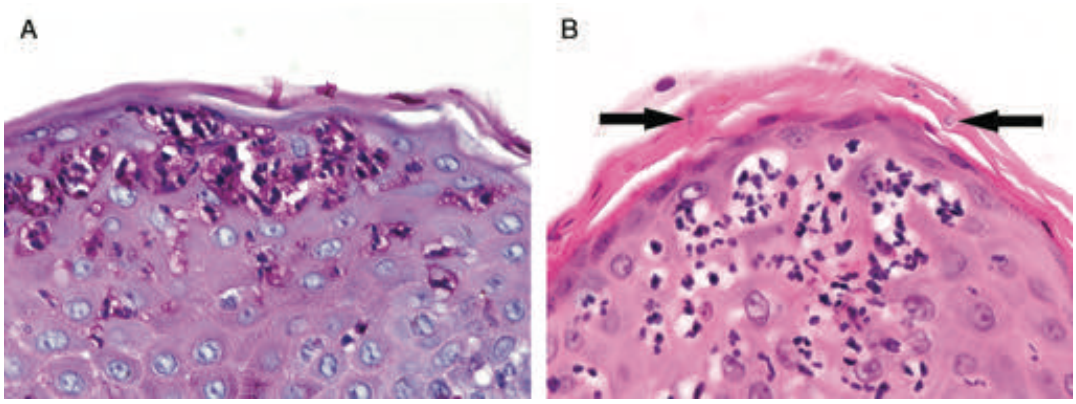

FIGURE 3. A, Subcorneal abscess with organisms in overlying parakeratosis. PAS, original magnification ×40. B, Same location as A showing scarcely visible organisms (arrows). H&E, original magnification ×40. H&E indicates hematoxylin and eosin; PAS, periodic acid-Schiff.

this group of women with histological evidence of mycosis, the diagnosis was suspected clinically in only 50% of cases. This is consistent with previous studies noting an accurate provisional diagnosis in 14% to 83%.[6,7,9] Although risk factors such as obesity, diabetes, and incontinence may increase clinical suspicion, these conditions are common to many dermatological problems.[16,20,21] Women with vulvar dermatoses often have multiple risk factors for mycosis: compromised barrier function, exogenous estrogen, and topical corticosteroids, which may result in an atypical appearance of the mycosis.[20] Treatment resistance or exacerbation of a previously stable dermatosis may signal candidal superinfection.[17–19,22]

In these 24 cases, 2 had a skin scraping and 16 had a vulvovaginal swab. None had point-of-care potassium hydroxide microscopy; this is not routinely performed in local gynecology and vulvar dermatology practices. The asymptomatic candidal colonization rate in reproductive-age women is 70% over 1 year, which may result in erroneous disease attribution.[3,23] Limiting assessment to a vulvovaginal swab will miss dermatophytes, and false-negative findings may result from easy access to over-the-counter topical antimycotics.[3] In a study of dermatophytosis cases with positive PCR-reverse line blots for pathogenic fungi, microscopy and culture of skin scrapings were positive in 33% and 48%, respectively, with better performance for *T. rubrum* than other species.[12] Although an important component of the evaluation, culture is insufficiently sensitive and specific to be considered a criterion standard.

Routine histopathology with H&E and PAS provided the diagnosis in 29% of these cases, yet organisms may be scant in number, subtle in appearance, and easily missed if oriented horizontally. Studies of extragenital skin biopsy have recommended the universal use of PAS to improve the diagnostic rate of tinea.[6,7,9] In 1 study, detection of organisms on H&E improved from 45% to 68% when the pathologist was informed of the positive PAS.[7] Pathologists should scrutinize PAS stains of vulvar biopsies with an acanthotic tissue reaction, particularly if superimposed on a lichenoid process.

Unfortunately, PCR was unhelpful in distinguishing between candidosis and dermatophytosis when applied to FFPE vulvar biopsies. The dermatophyte and *Candida* assays modified for this study were initially tested on 145 samples of nails, skin scales, and hair and on blood from 9 patients with candidemia, respectively.[11,12] The sensitivity for PCR detection of fungi is as high as 97% in nonembedded tissue but falls to 60% to 70% for FFPE specimens.[8] The discrepancy is primarily caused by suboptimal DNA extraction, which relates to the small volume of pathogen compared with human DNA, the challenge of digesting the fungal cell wall without disrupting contained nucleic acid, and the presence of PCR inhibitors. Samples with multiple organisms may generate false-negative results, as polymerases preferentially amplify a more abundant target.[24] False-positive findings may occur because of phylogenetic diversity within the *Candida* genus, which may generate cross-reactivity with environmental contaminants such as *Aspergillus*, *Saccharomyces*, and *Fusarium*.[24]

The ability to distinguish between dermatophytosis and candidosis has important clinical implications. Tinea-associated fungi are keratinophilic parasitic organisms transmittable between hosts through touch or exposure to fomites. Tinea cruris frequently coexists with tinea pedis, tinea corporis, and onychomycosis, and a diagnosis at 1 site should provoke a generalized examination. A strategy of treating all sites may reduce recurrence.[2,25] The choice of topical versus oral antimycotics is influenced by multiple factors including the sites involved and infection severity.[2,25,26] Controversy exists with regard to extragenital sites, such as the gastrointestinal tract, serving as reservoirs for candidal relapse.[3,14,15] Reported concordance rates of rectal and vaginal *Candida* species vary, and attempts to eradicate gastrointestinal reservoirs with oral nystatin have been unsuccessful.[20] Some authors suggest that the lower genital tract is the likely reservoir in CRVVC, perhaps because of residual organisms with intermittent oral "-azole" therapy or inadequate vulvar treatment with intravaginal preparations; they thus recommend daily or alternate daily oral fluconazole or prolonged combined therapy.[1,3,14] In women with dermatoses, tablets may be preferred over topicals to reduce the risk of contact dermatitis.[27] Although a wide range of prescribing practice was identified in this study, the majority were prescribed oral agents, which reflects clinicians' concern for infections that are multifocal, severe, or likely to recur.

Access to biopsies from clinics specialized in vulvovaginal disorders permits the study of vulvar cutaneous mycosis but yields a predominance of complex cases and concurrent dermatoses. Most women with a rash attributed to *Candida* or tinea either self-medicate or receive a diagnosis and treatment in the community setting without collection of a biopsy. Moreover, histopathology is insensitive for VVC; 1 study abandoned its use after 3 in 10 biopsies had organisms visible in the stratum corneum.[14] The retrospective design permits a view into real-life management of vulvar mycosis, highlighting the variability of both assessment and treatment. Other authors note that the lack of a reliable diagnostic test for mycosis hinders both patient care and research in this field.[3,20]

This study demonstrates that vulvar cutaneous candidosis and dermatophytosis cannot be reliably distinguished on histopathological assessment or PCR analysis of FFPE biopsies. A

high index of suspicion combined with comprehensive clinical and microbiological assessment is essential to identify vulvar mycosis and distinguish between candidosis and dermatophytosis, which should inform treatment decisions and improve outcomes.

REFERENCES

1. Mendling W, Brasch J, Cornely OA, et al. Guideline: vulvovaginal candidosis (AWMF 015/072), S2k (excluding chronic mucocutaneous candidosis). *Mycoses* 2015;58(suppl 1):1–15.
2. Drummond C. Common vulval dermatoses. *Aust Fam Physician* 2011;40:490–6.
3. Fischer G. Chronic vulvovaginal candidiasis: what we know and what we have yet to learn. *Australas J Dermatol* 2012;53:247–54.
4. Weissenbacher T, Witkin SS, Ledger WJ, et al. Relationship between clinical diagnosis of recurrent vulvovaginal candidiasis and detection of *Candida* species by culture and polymerase chain reaction. *Arch Gynecol Obstet* 2009;279:125–9.
5. Levitt JO, Levitt BH, Akhavan A, et al. The sensitivity and specificity of potassium hydroxide smear and fungal culture relative to clinical assessment in the evaluation of tinea pedis: a pooled analysis. *Dermatol Res Pract* 2010;2010:764843.
6. Mohan H, Bal A, Aulakh R. Evaluation of skin biopsies for fungal infections: role of routine fungal staining. *J Cutan Pathol* 2008;35:1097–9.
7. Al-Amiri A, Chatrath V, Bhawan J, et al. The periodic acid-Schiff stain in diagnosing tinea: should it be used routinely in inflammatory skin diseases? *J Cutan Pathol* 2003;30:611–5.
8. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;24:247–80.
9. Park YW, Kim DY, Yoon SY, et al. "Clues" for the histological diagnosis of tinea: how reliable are they? *Ann Dermatol* 2014;26:286–8.
10. Fujita SI, Senda Y, Nakaguchi S, et al. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *J Clin Microbiol* 2001;39:3617–22.
11. Innings A, Ullberg M, Johansson A, et al. Multiplex real-time PCR targeting the RNase P RNA gene for detection and identification of *Candida* species in blood. *J Clin Microbiol* 2007;45:874–80.
12. Bergmans AM, van der Ent M, Klaassen A, et al. Evaluation of a single-tube real-time PCR for detection and identification of 11 dermatophyte species in clinical material. *Clin Microbiol Infect* 2010;16:704–10.
13. Mendling W, Schlegelmilch R. Three-day combination treatment for vulvovaginal candidosis with 200mg clotrimazole vaginal suppositories and clotrimazole cream for the vulva is significantly better than treatment with vaginal suppositories alone – an earlier, multi-centre, placebo-controlled double blind study. *Gerburtshilfe Frauenheilkd* 2014;74:355–60.
14. Beikert FC, LE MT, Koeninger A, et al. Recurrent vulvovaginal candidosis: focus on the vulva. *Mycoses* 2011;54:e807–10.
15. Hong E, Dixit S, Fidel PL, et al. Vulvovaginal candidiasis as a chronic disease: diagnostic criteria and definition. *J Lower Gen Tract Dis* 2014;18:31–8.
16. Kalra MG, Higgins KE, Kinney BS. Intertrigo and secondary skin infections. *Am Fam Physician* 2014;89:569–73.
17. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Lower Gen Tract Dis* 2013;17:28–32.
18. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust N Z J Obstet Gynaecol* 2010;50:148–52.
19. Kapila S, Bradford J, Fischer G. Vulvar psoriasis in adults and children: a clinical audit of 194 cases and review of the literature. *J Lower Gen Tract Dis* 2012;16:364–71.
20. Sobel JD. Vulvovaginal candidosis. *Lancet* 2007;369:1961–71.
21. Scheinfeld NS. Obesity and dermatology. *Clin Dermatol* 2004;22:303–9.
22. McPherson T, Cooper S. Vulval lichen sclerosus and lichen planus. *Dermatol Ther* 2010;23:523–32.
23. Beigi RH, Meyn LA, Moore DM, et al. Vaginal yeast colonization in nonpregnant women: a longitudinal study. *Obstet Gynecol* 2004;104: 926–30.
24. Khot PD, Fredricks DN. PCR-based diagnosis of human fungal infections. *Expert Rev Anti Infect Ther* 2009;7:1201–21.
25. El-Gohary M, van Zuuren EJ, Fedorowicz Z, et al. Topical antifungal treatments for tinea cruris and tinea corporis. *Cochrane Database Syst Rev* 2014;8:CD009992.
26. Gupta AK, Cooper EA, Ryder JE, et al. Optimal management of fungal infections of the skin, hair, and nails. *Am J Clin Dermatol* 2004;5:225–37.
27. Pullen SK, Warshaw EM. Vulvar allergic contact dermatitis from clotrimazole. *Dermatitis* 2010;21:59–60.

4.3 The interpretation of non-diagnostic vulvar biopsies

Several studies have shown that more than half of women who present with vulvar pain have dermatologic disease demonstrable on vulval biopsy. However, there are no previous studies that address the problem of suspected dermatologic disease and a non-diagnostic biopsy. This conundrum is especially pressing in LS and erosive LP, both of which are chronic diseases often managed with lifelong topical steroids and accompanied by ongoing surveillance. Mismatch between clinical impression and biopsy result is relatively common in vulval disease, and this study arose out of the resulting frustration felt by clinicians and pathologists. If the biopsy report is falsely negative and the clinician reverses the diagnosis, the result is inadequate treatment and discharge to primary care. Conversely, if the biopsy is a true negative but the clinician persists in the diagnosis of dermatologic disease, there may be unnecessary exposure to topical and systemic immunosuppressives and failure to provide a more effective treatment. The study's aim was to explore the clinical course and histopathologic findings of cases with discrepancy between the provisional diagnosis and the biopsy result. The results demonstrated that, despite careful review and follow-up by specialised clinicians and pathologists, diagnostic discordance persisted in the majority of cases. This finding underscored the need for additional research in multiple areas, particularly the histology of the vestibule and MCJ, the significance of focal basal layer change, and the clinical course of women with presumed LP unsupported by biopsy. Also highlighted was the association between non-diagnostic biopsy of the MCJ and microbiologic positivity for *Candida albicans*, implicating mycosis as a potential aetiology of vulval pain and erythema with a non-specific histopathologic appearance. Future prospective studies of women with clinical LP should aim for universal histopathology, obtain data on those with non-diagnostic results, and compare that to women with biopsy-proven disease.

Interpretation of Nondiagnostic Vulvar Biopsies

Tania Day, MD,[1,2] Veronica Knight, MBBS,[1] Delwyn Dyall-Smith, FACD,[3] Graeme Dennerstein, FRCOGFRANZCOG,[4] Ross Pagano, FRANZCOG,[5,6] Hong Tran, FRANZCOG,[5,6] Yasmin Tan, FRANZCOG,[7] Desiree Yap, FRANZCOG,[8] Julie Weigner,[9] and James Scurry, FRCPA[2,9]

Abstract: Objective: The aim of the study was to assess clinical and histopathologic characteristics of symptomatic women who underwent a nondiagnostic biopsy of the inner vulva.

Materials and Methods: Consecutive nondiagnostic biopsies from medial labia minora, posterior fourchette, and vestibule obtained from symptomatic women between 2011 and 2015 were reviewed for this retrospective histopathologic case series. Histopathologic assessment included site, basal layer appearance, lymphocytic infiltrate, and presence of fibrosis or sclerosis. Examination findings, treatment, initial impression, and final clinical diagnosis were recorded. Descriptive statistics were performed; clinical and histopathologic characteristics were compared with Fisher exact test.

Results: There were 85 cases; mean age was 53 years. Most women presented with painful erythema and underwent biopsy to confirm (30, 35%) or exclude (43, 51%) lichen planus. After clinical follow-up and histopathologic review, most cases had persistent diagnostic discordance. Final clinical diagnoses were available in 70 women: lichen planus in 27 (38%), vulvodynia in 15 (21%), and the other 28 (40%) had LS (8), plasma cell vulvitis (5), psoriasis (4), dermatitis (4), candidosis (3), estrogen deficiency (3), and aphthosis (1). Histopathologic review highlighted the difficulty in distinguishing mucosa-associated lymphoid tissue from an inflammatory infiltrate in 23 (27%) of cases. Compared with other sites, biopsies from the mucocutaneous junction were more likely to be associated with a positive culture for *Candida albicans*.

Conclusions: Nondiagnostic biopsies from the inner vulva should prompt thoughtful multidisciplinary review, but more research is required to resolve the problem of clinicopathologic discordance through better understanding of vulvar histology and pathophysiology.

Key Words: vulva, nondiagnostic biopsy, lichen planus, vulvodynia, candidosis

(*J Low Genit Tract Dis* 2018;22: 74–81)

Standard indications for vulvar biopsy include diagnostic uncertainty, failed empiric treatment, and exclusion of neoplasia.[1–3] Some authors advise a low threshold for biopsy to mitigate the problem of unsuspected dermatologic disease, which has been reported at 50% for mycosis and 60% for chronic vulvar pain.[4–6] There is scant research on the reverse situation—nonconfirmatory histopathology in women suspected to have dermatologic disease.

In particular, the problem of a 30% nondiagnostic biopsy rate in clinically diagnosed lichen planus (LP) raises the possibilities of overidentification by clinicians and underreporting by pathologists.[7–9] Vulvodynia, mycosis, and dermatitis present similarly to LP with painful erythema but have nonspecific histopathology. Thus, LP may not be the correct diagnosis in some women with a clinical suspicion unsupported by biopsy, a problem potentially compounded by the high placebo-response rate for vulvodynia.[10,11] In contrast, some suspected LP cases with negative histopathology may represent true disease; biopsy may be nondiagnostic if performed at the wrong site or in the setting of waning or treated disease. A lack of consensus histopathologic criteria for vulvar LP may contribute to false-negative results.[7,12–14] Clinicopathologic discordance presents a conundrum to the involved specialists and women who undergo an uncomfortable genital procedure without the benefit of a tissue diagnosis.

Several recent publications have highlighted site-specific challenges of histopathologic assessment of the inner vulva, including the presence of mucosa-associated lymphoid tissue (MALT), and epithelial regeneration as a manifestation of erosive LP.[13–15] Using this information, we aim to review the histopathologic findings and clinical trajectory of women with a suspicion of dermatologic disease and a nondiagnostic vulvar biopsy.

MATERIALS AND METHODS

The local pathology database was searched for consecutive nondiagnostic biopsies of the inner vulva obtained between 2011 and 2015. Inner vulva was defined as medial labia minora, posterior fourchette, fossa navicularis, and vestibule. Reports considered that nondiagnostic included normal, nonspecific lymphocytic infiltrate, vestibular gland, maceration, old hemorrhage, and fibrosis. Clinical notes were reviewed to assess that both symptoms and an examination abnormality were present. Exclusion criteria were age less than 18 years, vestibulectomy and labiaplasty specimens, and biopsies containing hair-bearing skin.

Data were collected on age, clinician specialty, initial impression, examination findings, other vulvar dermatoses and biopsy results, duration of follow-up, treatment, and response. For women with at least one follow-up visit, clinicians provided a final diagnosis and these responses were categorized into LP, vulvodynia, or others. The vulvodynia category incorporated cases attributed to "associated factors" including neuropathic or referred pain, pelvic floor dysfunction or hypertonicity, and psychosexual problems.[16] The Hunter New England Research Ethics and Governance unit approved this retrospective histopathologic case series (HREC 15/11/18/5.02), and signed written consent was obtained for use of the clinical photograph.

Histopathologic review was performed of slides stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). The pathologist (J.S.) was blinded to clinical impression and outcome. Biopsy site was recorded as hairless skin, mucocutaneous junction (MCJ), or squamous mucosa.[15] The epithelium was evaluated for spongiosis, noted as present or absent. Epithelial thickness was measured at the thinnest site. Both the stromal lymphocytic infiltrate and exocytosis (presence of leukocytes in the epithelium) were assessed semiquantitatively as absent, sparse, moderate, or

[1]Maternity and Gynecology, John Hunter Hospital, Newcastle, Australia; [2]Faculty of Health and Medicine, University of Newcastle, Australia; [3]Riverina Dermatology, Wagga Wagga, New South Wales, Australia; [4]Former director, Dermogynaecology Clinic, Mercy Hospital for Women, Heidelberg, Victoria, Australia; [5]Vulvar Disorders and Dermatology Clinic, Royal Women's Hospital, Parkville, Victoria, Australia; [6]Faculty of Medicine, Dentistry, and Health, University of Melbourne, Parkville, Victoria, Australia; [7]Royal Hospital for Women, Sydney, New South Wales, Australia; [8]Private practice, East Melbourne, Victoria, Australia; and [9]Pathology North, Hunter New England, Newcastle, Australia

Correspondence to: Tania Day, MD, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Road, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au

T.D. is supported by the Australian Government Research Training Program Scholarship.

This study was approved by Hunter New England Research Ethics and Governance unit (HREC 15/11/18/5.02).

DOI: 10.1097/LGT.0000000000000366

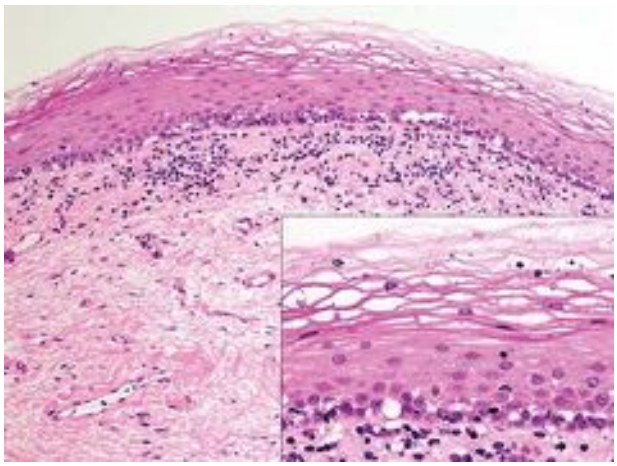

FIGURE 1. Nonspecific lichenoid tissue reaction: squamous mucosa with basal layer vacuolar change and a moderate closely applied mixed infiltrate (H&E ×100, inset H&E ×400).

dense; other cell types were also recoded. Stromal sclerosis or fibrosis was reported as present or absent. The presence of spores or hyphae was sought on PAS-stained slides.

The epithelial basal layer was scrutinized for vacuolar change, apoptotic bodies, and squamatization. Perilymphocytic clearing sometimes seen with exocytosis was attempted to be distinguished from hydropic change within basal cells. Squamatization was defined as a change in morphology of normal basilar keratinocytes to horizontally disposed cells with a mature squamous appearance.[17] Erosive LP required a diffuse change of absent rete ridges, epithelial thinning, a closely applied lymphocytic infiltrate, and either degenerative or regenerative basal layer change.[13,14,18] A diagnosis of lichen sclerosus (LS) required basal layer changes, sclerosis in the upper dermis, and an underlying band-like lymphocytic infiltrate.[18] Cases with a closely applied stromal lymphocytic infiltrate and basal layer damage that lacked the other diagnostic features of LP or LS were labeled nonspecific lichenoid reaction (see Figure 1).

In the setting of a normal basal layer, other dermatologic diagnoses were sought. Criteria for acute dermatitis included spongiosis and acanthosis, sometimes accompanied by epithelial and stromal eosinophils. Lichen simplex chronicus was diagnosed by orthokeratosis, hypergranulosis, acanthosis with broadening of the rete ridges, fibrosis of the papillary dermis, and absence of dermatophytes or other dermatosis.[19] Plasma cell vulvitis required epithelial thinning, spongiosis, exocytosis, a moderate or dense infiltrate rich in plasma cells, and stromal hemosiderin.[20–22] Stromal sclerosis with absent or sparse lymphocytes and a normal or squamatized epithelium yielded a diagnosis of vestibulovaginal sclerosis.[17,23]

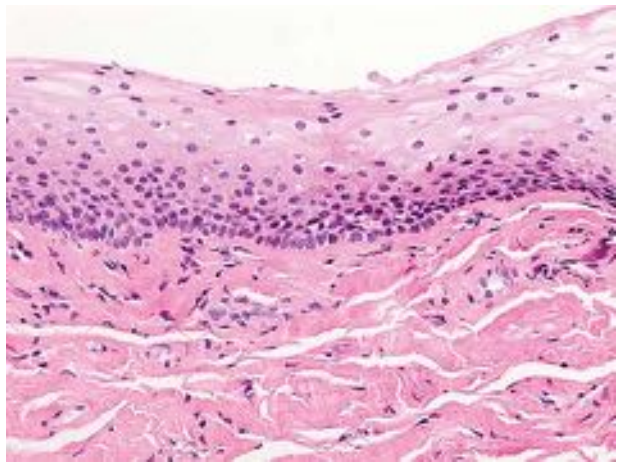

FIGURE 3. Scar: squamous mucosa with stromal fibrosis and absent infiltrate (H&E ×200).

Cases that did not meet dermatopathologic criteria for disease were categorized as vestibular gland, scar, and nondiagnostic with or without lymphocytic infiltrate. A minor vestibular gland appeared as a crypt of mucinous epithelium arising from squamous mucosa with periglandular mixed infiltrate (see Figure 2).[24] Absent leukocytes, normal or squamatized epithelium, and stromal fibrosis were categorized as scar (see Figure 3). Cases categorized as "nondiagnostic with lymphocytic infiltrate" had moderate to dense stromal and/or epithelial lymphocytes (see Figure 4).

Cases were then stratified by biopsy site and by final clinical diagnoses of LP, vulvodynia, and others. Descriptive statistics were performed; clinical and histopathologic characteristics were compared with Fisher exact test.

RESULTS

There were 85 cases of nondiagnostic biopsy obtained from the inner vulva of symptomatic women; during this time,

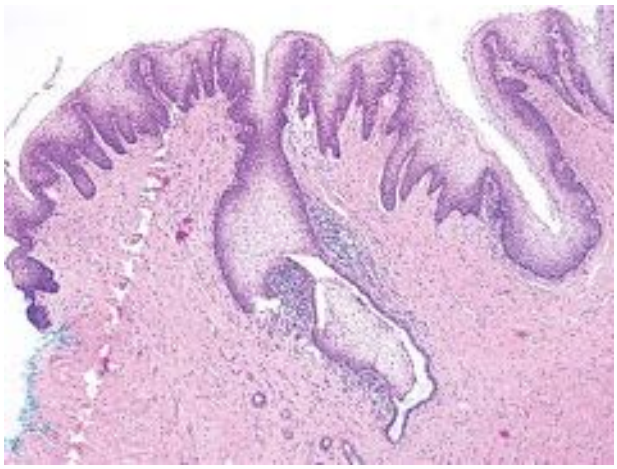

FIGURE 2. Vestibular gland: squamous mucosa with invagination leading to mucinous epithelium crypt and dense periglandular infiltrate (H&E ×40).

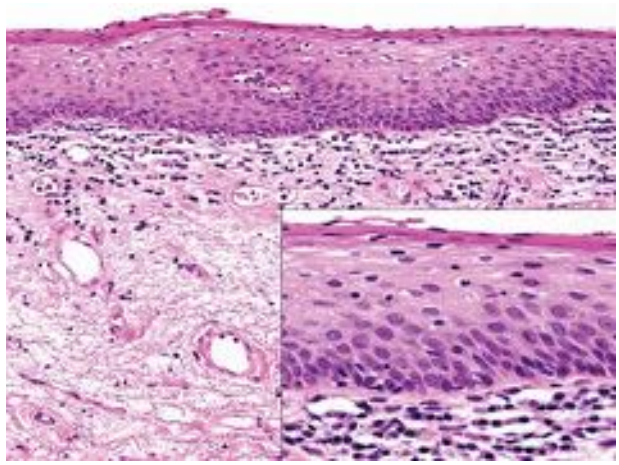

FIGURE 4. Nondiagnostic with lymphocytic infiltrate: MCJ with exocytosis and moderate mixed stromal infiltrate (H&E ×100, inset ×400).

approximately 830 biopsies were submitted from this anatomic zone. Mean age was 53 years, with 58% of women older than 50 years. The pathology request form contained a provisional diagnosis of LP, LS, or plasma cell vulvitis in 49%; in the remainder, the clinician wished to exclude dermatologic disease. Clinicians requested exclusion of LP in 31 (72%), of LS in 8 (19%), and in 4 (9%) noted a symptom (see Table 1). Seventy women (82%) had at least one follow-up visit and a final clinical diagnosis: LP in 27 (38%), some other vulvovaginal condition in 28 (40%), and vulvodynia in 15 (21%). The "other" category included diagnoses of LS (8), plasma cell vulvitis (5), psoriasis (4), dermatitis (4), candidosis (3), estrogen deficiency (3), and aphthosis (1).

Erythema was the examination abnormality in 76%; other descriptors included white, orange, mottled, macerated, shiny, lacy, induration, and atrophy. At time of presentation to the specialist, 64% used a medication that affects vulvar assessment, most commonly topical corticosteroids, hormonal preparations, and neuromodulators, but also three each on antibiotics and antifungals. Topical corticosteroids preparations used at time of biopsy were high potency in 57%, medium in 21%, low in 7%, and unspecified in 14%. Two women were taking oral methotrexate, one for systemic lupus erythematosus and the other for presumed vulvovaginal LP; none were on other topical or systemic immunosuppressive medication. Women with a final diagnosis of vulvodynia were more likely to be on a neuromodulator at the intake visit [4/15 (27%) vs 2/70 (3%), $p < .008$] (see Figure 5).

Of 27 women who returned after an initial impression of LP, specialists maintained the diagnosis in 23 (85%). Four women

TABLE 1. Clinical Characteristics of Symptomatic Women with Nondiagnostic Biopsies of Inner Vulva, Stratified by Final Clinical Diagnosis

	All cases ($N = 85$)	LP ($n = 27$, 32%)	Other ($n = 28$, 33%)	Vulvodynia ($n = 15$, 18%)	No follow-up ($n = 15$, 18%)
Age; mean (range)	53 (18–88)	59 (29–88)	51 (23–83)	51 (18–71)	47 (25–75)
Age >50 y, n (%)	49 (58)	21 (78)*	12 (43)	9 (60)	7 (47)
Clinician specialty, n (%)					
Gynecology	72 (85)	25 (93)	22 (79)	13 (87)	12 (80)
Dermatology	13 (15)	2 (7)	6 (21)	2 (13)	3 (20)
Primary symptom, n (%)					
Pain	60 (71)	20 (74)	17 (61)	14 (93)	9 (60)
Dyspareunia	17 (20)	5 (19)	8 (29)	1 (7)	3 (20)
Itch	8 (9)	2 (7)	3 (11)	0	3 (20)
Color, n (%)					
Red	65 (76)	24 (89)	14 (50)	14 (87)	13 (87)
White	12 (14)	3 (11)	7 (25)	1 (7)	1 (7)
Other	8 (9)	0	7 (25)	0	1 (7)
Architectural change, n (%)	23 (27)	18 (67)*	3 (11)	0	2 (13)
Medications at biopsy, n (%)†					
None	31 (36)	7 (26)	13 (46)	4 (27)	7 (47)
Topical corticosteroids	28 (33)	10 (37)	8 (29)	4 (27)	6 (40)
Exogenous hormones	22 (26)	10 (37)	6 (21)	3 (20)	3 (20)
Neuromodulators	5 (6)	1 (4)	1 (4)	4 (27)*	0
Other	8 (9)	2 (7)	1 (4)	2 (13)	2 (13)
Initial impression, n (%)					
Suspect LP	30 (35)	23 (85)*	2 (7)	2 (13)	3 (20)
Suspect lichen sclerosus	4 (5)	0	2 (7)	1 (5)	1 (5)
Suspect plasma cell vulvitis	8 (9)	0	5 (18)	1 (5)	2 (13)
Exclude dermatosis	43 (51)	4 (15)	19 (68)	12 (80)	9 (60)
Microbiologic results, n (%)					
Normal flora	33 (39)	7 (26)	10 (36)	8 (47)	8 (53)
Candida albicans	11 (13)	5 (19)	4 (14)	1 (7)	1 (7)
Nonalbicans candida	3 (4)	1 (4)	0	1 (7)	1 (7)
Gardnerella	1 (1)	1 (4)	0	0	0
No culture performed	37 (44)	13 (48)	14 (50)	5 (33)	5 (33)
Topical corticosteroids prescribed by specialist, n (%)					
Improved with topical	58 (68)	27 (100)	20 (71)	6 (40)	5 (33)
Steroid monotherapy	18 (21)	10 (37)*	8 (29)	0	—
Duration of follow-up, mean (range), mo‡	15 (1–48)	21 (3–48)	12 (1–48)	11 (2–28)	—
Not improved despite treatment, n (%)‡	8 (11)	4 (15)	2 (7)	2 (13)	—

LP indicates lichen planus.

*$p < .05$.

†Some women were on multiple medications.

‡Of 70 cases with follow-up.

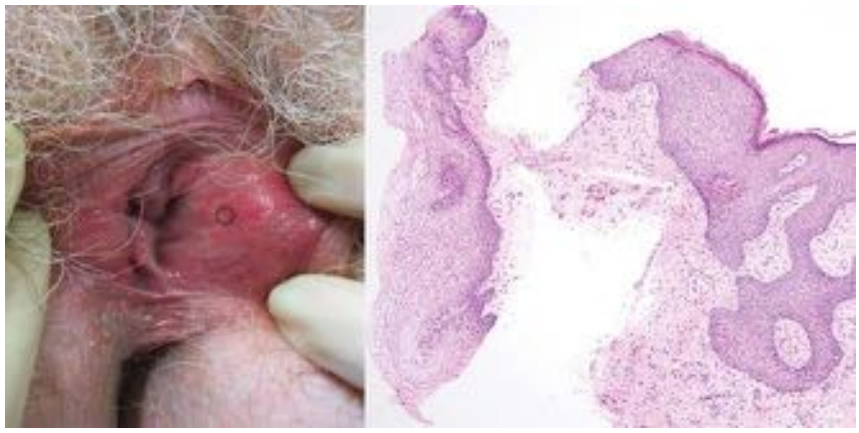

FIGURE 5. Bland vestibular erythema without erosion or demarcated border; clinical diagnosis was vulvodynia and biopsy (site indicated) was performed to exclude LP. Squamous mucosa and hairless skin were nondiagnostic without lymphocytic infiltrate (H&E ×40).

with a low suspicion of LP were later assigned that diagnosis based on their clinical course. Lichen planus cases were more likely to be older than 50 years ($p = .02$), demonstrated vulvar architectural change ($p = .001$), and improved with steroid monotherapy ($p = .003$). Previous or subsequent biopsies demonstrated vulvar LP in 3 (11%) of 27, a lichenoid reaction on hairless skin in 2 (7%), LS on hair-bearing skin in 2, and vestibulovaginal sclerosis in 1 (4%).

TABLE 2. Histopathologic Characteristics of Symptomatic Women With Nondiagnostic Biopsies of Inner Vulva, Stratified by Final Clinical Diagnosis

	All cases ($N = 85$)	LP ($n = 27$, 32%)	Other ($n = 28$, 33%)	Vulvodynia ($n = 15$, 21%)	No follow-up ($n = 15$, 21%)
Biopsy location, n (%)					
Vestibule	22 (26)	6 (22)	9 (32)	3 (20)	4 (27)
Posterior fourchette/fossa navicularis	27 (32)	10 (37)	6 (21)	6 (40)	5 (33)
Inner labium minus	36 (42)	11 (41)	13 (46)	6 (40)	6 (40)
Biopsy site, n (%)					
Squamous mucosa	32 (38)	8 (30)	10 (36)	7 (47)	7 (47)
MCJ	30 (35)	11 (41)	11 (39)	4 (27)	4 (27)
Hairless skin	23 (27)	8 (30)	7 (25)	4 (27)	4 (27)
Initial histopathology, n (%)					
Normal	54 (63)	18 (67)	14 (50)	12 (80)	10 (67)
Nonspecific infiltrate	22 (26)	7 (23)	9 (32)	3 (20)	3 (20)
Other	9 (11)	2 (7)	5 (18)	0	2 (13)
Revised histopathology, n (%)					
Nondiagnostic without lymphocytic infiltrate	40 (47)	12 (44)	12 (43)	9 (60)	7 (47)
Nondiagnostic with lymphocytic infiltrate	23 (27)	5 (19)	9 (32)	5 (33)	4 (20)
Lichenoid reaction†	11 (13)	7 (26)*	2 (7)	1 (7)	1 (7)
Other	11 (13)	3 (11)	5 (18)	0	3 (20)
Epithelial thickness, mean (SD), mm	0.08 (0.04)	0.08 (0.03)	0.07 (0.04)	0.1 (0.05)	0.09 (0.04)
Lymphocytic infiltrate, n (%)					
None to sparse	46 (54)	17 (63)	13 (46)	8 (53)	8 (53)
Moderate	30 (35)	9 (33)	11 (39)	5 (33)	5 (33)
Dense	9 (11)	1 (4)	4 (27)	2 (13)	2 (13)
Exocytosis, n (%)					
None to sparse	51 (60)	15 (56)	15 (56)	10 (67)	11 (73)
Moderate	29 (34)	12 (44)	10 (36)	4 (27)	3 (20)
Dense	5 (6)	0	3 (11)	1 (7)	1 (7)
Spongiosis, n (%)	16 (19)	2 (8)	7 (25)	2 (13)	5 (33)
Fibrosis or sclerosis, n (%)	10 (12)	6 (22)	0	2 (13)	2 (13)

LP indicates lichen planus; MCJ, mucocutaneous junction.

*$p < .05$.

†Includes cases interpreted as lichen sclerosus.

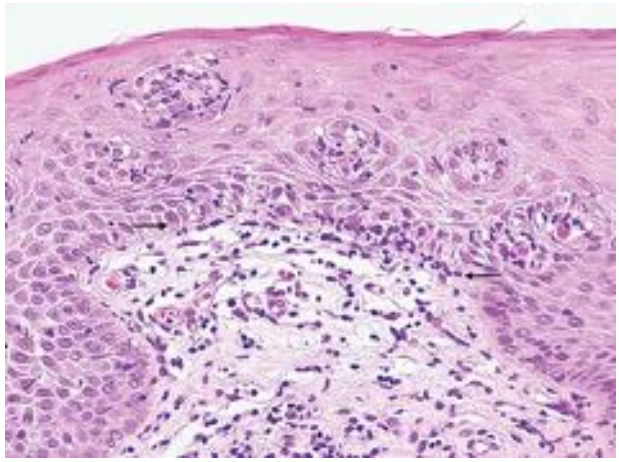

FIGURE 6. Focal vacuolar change in a woman with clinically diagnosed LP: MCJ with spongiosis, moderate exocytosis and stromal lymphocytic infiltrate, and basal layer degeneration limited to an area of less than 30 cells (between 2 arrows) (H&E ×200).

At the time of histopathologic review, 9 biopsies (11%) were interpreted as nonspecific lichenoid reaction and 2 (2%) as LS; 7 (64%) of these had basilar apoptotic bodies. Although none met criteria for LP, there was concordance with a clinical diagnosis of LP in 6 (22%) of 27 (see Table 2). An assessment of nonspecific lichenoid reaction or LS was more common in women with clinical LP than in the other diagnostic categories [7/27 (26%) vs 4/58 (7%), $p = .03$]. One case of clinically diagnosed plasma cell vulvitis showed typical stromal features of hemosiderin and plasma cells but lacked spongiosis. No case showed spores or hyphae on PAS.

Histopathologic review yielded several findings of unknown significance. Four cases showed focal basal layer vacuolar change or squamatization occurring for an area of 30 or fewer cells (see Figure 6). Eighteen cases showed marked perilymphocytic haloes; 3 of these had a neutrophil predominance (see Figure 7). In some of these, it was difficult to know whether the basilar clearing was entirely related to exocytosis and spongiosis or whether there also were hydropic keratinocytes. Suprabasilar apoptotic bodies were seen in two cases with a normal basal layer (see Figure 7).

Table 3 displays clinical and histopathologic characteristics grouped by type of epithelium. Biopsy of hairless skin was more likely from a circumferential abnormality [18/23 (78%) vs 16/62 (26%), $p < .001$], whereas the lesion was more likely to be focal when squamous mucosa was sampled [20/32 (62.5%) vs 16/53 (30%), $p = .025$]. Biopsies from MCJ were more likely to be associated with *Candida albicans* on culture [8/30 (27%) vs 3/55 (5%), $p = .014$] and to be nondiagnostic with lymphocytic infiltrate [14/30 (47%) vs 9/55 (16%), $p = .005$]. On histopathologic review, biopsies of hairless skin were more likely to be categorized as nondiagnostic without lymphocytic infiltrate [17/23 (74%) vs 23/62 (37%), $p = .003$], whereas squamous mucosa specimens were more likely to have some other diagnosis—vestibular gland in 3, vestibulovaginal sclerosis in 2, and 1 each with dermatitis, plasma cell vulvitis, and scar [8/32 (25%) vs 3/53 (6%), $p = .02$]. These three cases of vestibular gland were all described as multifocal or localized erythema with an initial impression of plasma cell vulvitis.

DISCUSSION

In this study of nondiagnostic biopsies of inner vulva from symptomatic women, initial clinicopathologic discordance was only partially corrected by careful clinical and histopathologic review. Although clinicians modified the diagnosis to vulvodynia in a few cases after negative biopsy and follow-up, usually they retained a diagnosis of chronic dermatosis, particularly LP, which was inconsistent with the pathology. It is possible that some women thought to have LP instead to have vulvodynia; clinical improvement due to placebo effect removes an early imperative for clinicians to modify their diagnoses. During the course of follow-up, additional treatments may be provided for presumed comorbid pain conditions, or symptoms may remit in keeping with the natural history of vulvodynia.[25]

However, 18% of clinical LP cases had previous or subsequent supportive histopathology, so clinicians should not reflexively dismiss a dermatologic diagnosis after a single negative biopsy. A potential explanation for nondiagnostic results is suboptimal site or timing of biopsy. There is scant literature regarding when and where to biopsy, beyond the traditional guidance to choose the lesion periphery or worst area. This study suggests several low-yield situations. Multifocal erythema near the hymenal base likely represents vestibular gland openings or vulvodynia.[11,26] Diffuse erythema around the MCJ is associated with candidosis; women with risk factors for mycosis may benefit from microbiologic assessment and treatment before biopsy.[4,27] The MCJ is also where lymphocytic infiltrates potentially representing MALT are commonly found; when the abnormality is diffuse, hairless skin may yield a specimen that is easier to interpret. When vulvar architecture is normal, biopsy of bland poorly demarcated erythema is unlikely to yield a diagnosis, particularly if the woman has comorbid musculoskeletal or neuropathic pain conditions.

From a pathological perspective, this study highlights a major problem in vulvar histopathology—the definition of normal. Fundamental knowledge gaps are exacerbated by the use of imprecise terminology. "Inflammation" is commonly used to describe the presence of stromal lymphocytes but actually refers to the pathologic process of leukocytes causing tissue damage. Stromal lymphocytes without evidence of epithelial injury may represent MALT or may be the infiltrate associated with dermatosis or mycosis.[26,28] Several studies have documented a moderate infiltrate in absence of dermatologic disease, at rates that vary by specimen type: 15% of perilesional specimens of hairless skin and squamous mucosa, 71% of vestibulectomies, and 73% of biopsies from the Bartholin gland opening of asymptomatic controls.[15,24,26] Features of local immune activation, such as germinal

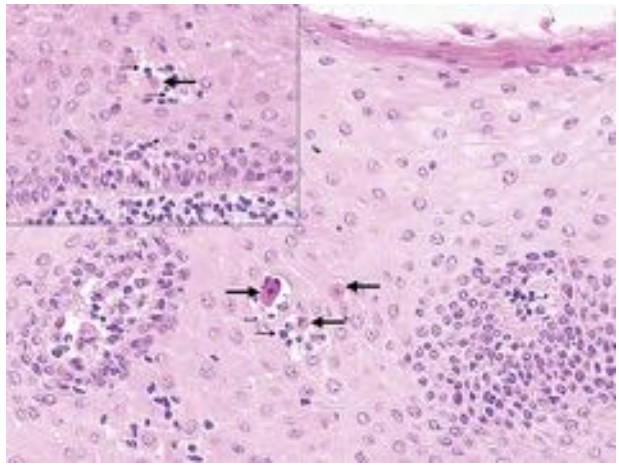

FIGURE 7. Suprabasilar apoptotic bodies in a woman with impression of plasma cell vulvitis and no follow-up visit: MCJ with normal basilar keratinocytes, exocytosis with perilymphocytic clearing (thin arrows), apoptotic bodies (thick arrows), and moderate mixed infiltrate (H&E ×400, inset H&E ×400).

TABLE 3. Clinical Characteristics and Histopathologic Diagnoses of Symptomatic Women With Nondiagnostic Biopsies of Inner Vulva, Stratified by Site

	Squamous mucosa (n = 32)	MCJ (n = 30)	Hairless skin (n = 23)
Biopsy location, n (%)			
Vestibule	18 (56)	4 (13)	0
Posterior fourchette/fossa navicularis	8 (25)	12 (40)	7 (30)
Inner labium minus	6 (19)	14 (47)	16 (70)
Lesion extent, n (%)			
Circumferential	6 (19)*	10 (33)	18 (78)
Multifocal	6 (19)	4 (13)	2 (9)
Localized	20 (63)	16 (53)	3 (13)*
Architectural change, n (%)	6 (19)	8 (27)	9 (39)
Microbiologic results, n (%)			
Normal	15 (47)	10 (33)	8 (35)
Candida albicans	2 (6)	8 (27)*	1 (4)
Nonalbicans candida	1 (3)	2 (7)	0
Gardnerella	0	1 (3)	0
No culture performed	14 (44)	9 (30)	14 (61)
Initial impression, n (%)			
Suspect LP	7 (22)	15 (40)	8 (35)
Suspect lichen sclerosus	1 (3)	0	3 (13)
Suspect plasma cell vulvitis	8 (25)	0	0
Exclude dermatosis	16 (50)	15 (50)	12 (52)
Final clinical diagnosis, n (%)			
LP	8 (25)	11 (37)	8 (35)
Other	10 (31)	11 (37)	7 (30)
Vulvodynia	7 (22)	4 (13)	4 (17)
No follow-up	7 (22)	4 (13)	4 (17)
Revised histopathology, n (%)			
Nondiagnostic without lymphocytic infiltrate	13 (41)	10 (33)	17 (74)*
Nondiagnostic with lymphocytic infiltrate	7 (22)	14 (47)*	2 (9)
Lichenoid reaction†	4 (12.5)	3 (10)	4 (17)
Other	8 (25)*	3 (10)	0

MCJ indicates mucocutaneous junction.

*$p < .05$

†Includes cases interpreted as lichen sclerosus.

centers, antigen-presenting dendritic cells, macrophages, and mast cells, have been documented both in controls and vulvodynia cases.[29,30] Currently, there is no reliable assay for lymphocytic activity that distinguishes normal immune function from a disease process. Thus, without histopathologic evidence of tissue damage, pathologists cannot reliably distinguish between inflammation and MALT.

Specimens with moderate lymphocytic infiltrate pose substantial challenges for pathologists. Exocytosis with perilymphocytic clearing can mimic or mask vacuolar change, especially when combined with spongiosis. A nearby vestibular gland may be the source of an infiltrate, but the crypt may not be contained within the specimen. Stromal capillaritis and hemosiderin accompanied by plentiful plasma cells likely represent plasma cell vulvitis, but pathologists may not assign this diagnosis in biopsies lacking epithelial changes. As a result of this complexity, histopathologic review sometimes yields a different interpretation of findings, especially when combined with the clinical photograph and context. We constructed an algorithm to assist with clinicopathologic assessment of these challenging cases (see Figure 8).

The limitations of this study are those inherent to the retrospective design, including incomplete clinical data and practice differences among clinicians. Some clinicians may be more likely than others to modify their initial impression after a nondiagnostic biopsy, but we were unable to ascertain any statistical differences because of the large number of referring providers. A comprehensive examination of the mouth and extragenital skin was not always documented. Forty-four percent of cases had no microbiological assessment, yet when performed, 31% had a pertinent positive result. Wet mounts are not routinely performed by many Australian dermatologists and gynecologists. There was substantial variation in the information written on pathology requisition forms. To facilitate clinicopathologic correlation, notes should provide a provisional diagnosis and competing differential diagnoses. Location should be described unambiguously, for example, "right inferior inner labium minus," rather than with a clock-face position or general terms such as introitus and vulva. Clinical photographs were not obtained or available in all cases and would have permitted a more nuanced description of anatomic location and clinical findings. Finally, our results and algorithm are most applicable to clinicians with access to a skilled vulvar pathologist.

There is broad scope for research in this area. Study of the variation in clinical appearance and histology of the inner vulva is urgently required to improve our understanding of the

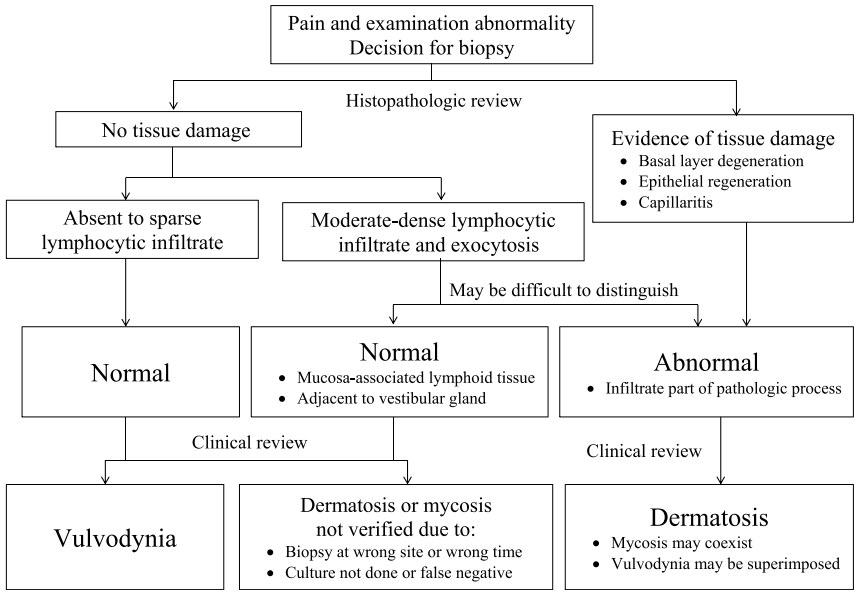

FIGURE 8. Algorithm for clinicopathologic review of nondiagnostic vulvar biopsies obtained from women with symptoms and perceived examination abnormality.

relationship between pain, erythema, and MALT. This may assist clinicians in distinguishing the erythema of chronic dermatoses from that due to neurogenic inflammation or from the physiologic erythema of some fair-skinned women and perhaps help them avoid unnecessary biopsy. It is possible that the histopathologic phenomenon of exocytosis with focal basal layer degeneration is a manifestation of MALT. There is scant literature to validate the description of plasma cell vulvitis in dermatopathology textbooks.[20–22] More information about the clinical course of women with presumed LP and nondiagnostic biopsy would be best obtained in prospective trials that require histopathology for inclusion and use this information to stratify response to interventions.[7,13]

In summary, nondiagnostic biopsies from the inner vulva should prompt thoughtful multidisciplinary review, but more research is required to resolve the problem of clinicopathologic discordance through better understanding of vulvar histology and pathophysiology.

ACKNOWLEDGMENTS

The authors thank Dr Jenny Bradford and Dr Judith Fleming for their assistance with access to clinical records.

REFERENCES

1. Edwards SK, Bates C, Lewis F, et al. 2014 UK national guideline on the management of vulval conditions. *Int J STD AIDS* 2015;26:611–24.
2. Van der Meijden WI, Boffa MJ, ter Harmsel B, et al. 2016 European Guideline for the Management of Vulval Conditions. *J Eur Acad Dermatol Venereol* 2017;31:925–41.
3. Simpson RC, Murphy R, Bratton DJ, et al. Systemic therapy for vulval erosive Lichen Planus (the 'hELP' trial): study protocol for a randomized controlled trial. *Trials* 2016;17:2.
4. Day T, Borbolla-Foster A, Phillips S, et al. Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis? *J Low Genit Tract Dis* 2016;20:267–71.
5. Bowen AR, Vester A, Marsden L, et al. The role of vulvar skin biopsy in the evaluation of chronic vulvar pain. *Am J Obstet Gynecol* 2008;199:e1–6.
6. Harris V, Fischer G, Bradford JA. The aetiology of chronic vulval pain and entry dyspareunia: a retrospective review of 525 cases. *Aust N Z J Obstet Gynaecol* 2017;57:446–51.
7. Neill SM, Lewis FM. Vulvovaginal lichen planus: a disease in need of a unified approach. *Arch Dermatol* 2008;144:1502–3.
8. Simpson RC, Littlewood SM, Cooper SM, et al. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. *Br J Dermatol* 2012;167:85–91.
9. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Low Genit Tract Dis* 2013;17:28–32.
10. Foster DC, Kotok MB, Huang LS, et al. Oral desipramine and topical lidocaine for vulvodynia: a randomized controlled trial. *Obstet Gynecol* 2010;116:583–93.
11. Donders GG, Bellen G. Cream with cutaneous fibroblast lysate for the treatment of provoked vestibulodynia: a double-blind randomized placebo-controlled crossover study. *J Low Genit Tract Dis* 2012;16:427–36.
12. Simpson RC, Thomas KS, Leighton P, et al. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br J Dermatol* 2013;169:337–43.
13. Day T, Moore S, Bohl TJ, et al. Cormorbid lichen planus and lichen sclerosus. *J Low Genit Tract Dis* 2017;21:204–8.
14. Day T, Bowden N, Jaaback K, et al. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *J Low Genit Tract Dis* 2016;20:174–9.
15. Day T, Holland S, Scurry J. Normal vulvar histology: variation by site. *J Low Genit Tract Dis* 2016;2:64–9.
16. Bornstein J, Goldstein AT, Stockdale CK, et al. 2015 ISSVD, ISSWSH and IPPS Consensus terminology and classification of persistent vulvar pain and vulvodynia. *Obstet Gynecol* 2016;127:745–51.
17. Day T, Burston K, Dennerstein G, et al. Vestibulovaginal sclerosis versus lichen sclerosus. *Int J Gynecol Path* 2017. Dec Epub ahead of print.
18. Wang W, Lazar A. Lichenoid and interface dermatitis, Chapter 7. In: Calonje E, Brenn T, Lazar A, et al, eds. *McKee's Pathology of the Skin With Clinical Correlations*. 4th ed. London: Elsevier; 2012:219–58.
19. Patterson JW, Hosler GA. Tissue reaction patterns, Section 2. In: Patterson JW, ed. *Weedon's Skin Pathology*. 4th ed. London: Churchill Livingstone Elsevier 2016; 37–135.

20. Calonje E, Neill S, Francis N, et al. Diseases of the anogenital skin, Chapter 12. In: Calonje E, Brenn T, Lazar A, et al, eds. *McKee's Pathology of the Skin With Clinical Correlations*. 4th ed. London: Elsevier 2012; 444–57.
21. Virgili A, Levratti A, Marzola A, et al. Retrospective histopathologic reevaluation of 18 cases of plasma cell vulvitis. *J Repro Med* 2005;50:3–7.
22. Scurry J, Dennerstein G, Brennan J, et al. Vulvitis circumscripta plasmacellularis, a clinicopathological entity? *J Reprod Med* 1993;38:14–8.
23. Fadare O. Vaginal stromal sclerosis: a distinctive stromal change associated with vaginal atrophy. *Int J Gynecol Path* 2011;30:295–300.
24. Pyka RE, Wilkinson EJ, Friedrich EG, et al. The histopathology of vulvar vestibulitis syndrome. *Int J Gynecol Path* 1988;7:249–57.
25. Reed BD, Legocki LJ, Plegue MA, et al. Factors associated with vulvodynia incidence. *Obstet Gynecol* 2014;123:225–31.
26. Lundqvist EN, Hofer PA, Oloffson JL, et al. Is vulvar vestibulitis an inflammatory condition? A comparison of histological findings in affected and healthy women. *Acta Derm Venereol* 1997;77:319–22.
27. Mendling W, Brasch J, Comely OA, et al. Guideline: vulvovaginal candidosis (AWMF 015/072), S2k (excluding chronic mucocutaneous candidosis). *Mycoses* 2015;58(Supl 1):1–15.
28. Tommola P, Butzow R, Unkila-Kallio L, et al. Activation of vestibule-associated lymphoid tissue in localized provoked vulvodynia. *Am J Obstet Gynecol* 2015;212:476:e1–8.
29. Falsetta ML, Foster DC, Bonham AD, et al. A review of the available clinical therapies for vulvodynia management and new data implicating proinflammatory mediators in pain elicitation. *BJOG* 2017; 124:210–8.
30. Micheletti L, Radici G, Lynch PJ. Provoked vestibulodynia: inflammatory, neuropathic or dysfunctional pain? A neurobiological perspective. *J Obstet Gynaecol* 2014;34:285–8.

4.4 Perianal lichen dermatoses: a review of 60 cases

The cross-disciplinary character of vulvovaginal disease is especially apparent when dermatosis occurs on the perianus. This study was inspired by a cluster of perianal biopsies showing hypertrophic LP, two of which elicited concern for SCC due to extensive PEH. Review of existing literature revealed that standard dermatopathologic terminology was not used to describe perianal skin conditions, and authors instead persisted with the outdated descriptive term 'pruritus ani'. Thus, the aim was to describe the relative frequency of LS, LP, and LSC on the perianus, determine if these diseases are focal or extend across the vulva, and comment on the likelihood of superimposed lichenification of perianal LS and LP. This is the first published study of the PhD, laying the groundwork for further investigation into comorbidity of lichenoid disorders, the importance of standard definitions and terminology in vulvar dermatoses, and the essential role of clinicopathological correlation prior to undertaking irreversible management decisions such as surgery.

Australasian Journal of Dermatology

Australasian Journal of Dermatology (2016) 57, 210–215 doi: 10.1111/ajd.12308

ORIGINAL RESEARCH

Perianal lichen dermatoses: A review of 60 cases

Tania Day,[1,2] Tanja Gizela Bohl[4] and James Scurry[2,3]

[1]*Maternity and Gynaecology, John Hunter Hospital,* [2]*University of Newcastle, and* [3]*Anatomical Pathology, Pathology North, Hunter New England, Newcastle, New South Wales, and* [4]*Solo Private Practice, Newborough, Victoria, Australia*

ABSTRACT

Objectives: To determine the diagnostic range of lichen dermatoses of the perianus, their extent, and response to treatment.

Methods: We reviewed perianal biopsies submitted to a tertiary referral pathology service between January 2010 and July 2014, interpreted as 'lichen' or 'lichenoid'. We collected data on patients' characteristics, referring specialty, extent of lesion and response to treatment.

Results: During the study period, 60 perianal biopsies met our inclusion criteria. The distribution of diagnoses was lichen sclerosus (LS) in 25/60 (42%), lichen simplex chronicus (LSC) in 23/60 (38%), lichen planus (LP) in 10/60 (17%), and a non-specific lichenoid reaction in 2/60 (3%). Eleven of 25 cases of LS (44%) showed superimposed LSC. Of 10 LP cases, nine (90%) were hypertrophic and three of these showed pseudoepitheliomatous hyperplasia; none were erosive LP. Compared with patients in the LS and LSC groups, those with LP were more likely to have a localised lesion. Topical steroids were prescribed in 91% cases with treatment data available, and 98% of treated patients who returned for follow up had improved or their disease was resolved.

Conclusions: We encountered a spectrum of perianal lichen dermatoses, with LS, LP and LSC all represented. LS biopsied at the perianus is often lichenified. Hypertrophic LP is a common form of LP at the perianus.

Key words: lichen planus, lichen sclerosus, lichen simplex chronicus, perianal, pseudoepitheliomatous hyperplasia.

INTRODUCTION

The perianus is the circumferential region extending 5 cm from the anal verge, although on inspection the lateral extent is not anatomically clear.[1,2] Patients with perianal symptoms are seen by a variety of clinicians, including colorectal surgeons, gynaecologists, dermatologists, and sexual health physicians, as well as general medical and nurse practitioners. With so many specialties involved, standard terminology is essential. While considerable progress has been made in the classification of genitoanal neoplasia related to human papillomavirus (HPV), terminology surrounding non-neoplastic disorders remains imprecise.[2,3] Few studies exist on perianal dermatoses, and descriptive phrases such as 'primary and secondary pruritus ani' continue to be advocated.[4,5]

There is a broad differential diagnosis for chronic perianal pruritus that includes the lichen dermatoses: lichen sclerosus (LS), lichen planus (LP), and lichen simplex chronicus (LSC). LS and LP are both T-cell mediated inflammatory dermatoses, while LSC is the clinicopathological manifestation of the itch-scratch cycle.[3,6] There is considerable overlap of the three diagnoses, as LS and LP may be comorbid and superimposed LSC may occur with any pruritic dermatosis.[3,7–9] To determine the diagnostic range, extent and response with treatment of the

Abbreviations:

HPV	human papillomavirus
LP	lichen planus
LS	lichen sclerosus
LSC	lichen simplex chronicus
PEH	pseudoepitheliomatous hyperplasia
SCC	squamous cell carcinoma

Correspondence: Dr Tania Day, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Road, New Lambton Heights NSW 2305, Australia. Email: tania.day@hnehealth.nsw.gov.au

Tania Day, FRANZCOG. Tanja Gizela Bohl, FACD. James Scurry, FRCPA.

Conflict of interest: none

Submitted 3 December 2014; accepted 23 December 2014.

perianal lichen dermatoses, we performed a clinicopathological review of 60 consecutive perianal biopsies reported as lichen.

MATERIALS AND METHODS

We identified perianal skin biopsies submitted to a tertiary referral pathology service between January 2010 and July 2014, with a histopathological result of lichen or lichenoid. The Hunter New England Research Ethics and Governance unit granted approval for the project (14/2/19/5.08). Cases were obtained by searching the Pathology North, Hunter New England database, which also provided the age, sex, referring specialist and requisition notes. Specimen processing included both H–E and Periodic acid-Schiff stains of all cases. The evaluation of the requisition notes resulted in the exclusion of six cases of vulvar intraepithelial neoplasia, one case of squamous cell carcinoma (SCC), one case of dermatophytosis, and 13 cases due to vulval biopsy sites. The remaining biopsies were then reviewed and classified histologically into LSC, LP and LS; we did not encounter additional cases of dermatophytosis or HPV. We then excluded five more cases: four had a revised result of normal or dermal scar, and one showed psoriasis without lichenification. In the cohort of 60 cases there were seven biopsies for which the pathologist requested additional tests, including immunoperoxidase stains for p53 and p16 in four, and one case each of the Ziehl–Neelsen stain for acid-fast bacilli, the Warthin-Starry stain for microorganisms, and immunofluorescence to exclude immunobullous disease. We did not require any special stains or studies as a part of this review. Clinical information on lesion distribution, treatment and response was obtained from electronic medical records or through communication with clinicians who submitted specimens. We performed descriptive statistics and compared categorical variables by the Fisher's exact test.

LSC was diagnosed by orthokeratosis, hypergranulosis, acanthosis with broadening of the rete ridges, fibrosis of the papillary dermis, and absence of dermatophytosis or any identifiable dermatosis.[10] LP was diagnosed by a closely applied dermal lymphocytic infiltrate accompanied by basal layer damage in the form of vacuolisation and multiple scattered keratin deposits (Civatte bodies), supported by wedge-shaped hypergranulosis and jagged, saw-tooth acanthosis. Dermal homogenisation was absent.[10,11] Erosive LP required erosion, defined as loss of the stratum corneum in skin or loss of the surface layers of squamous cells in squamous mucosa, in addition to basal layer damage and a band-like lymphocytic infiltrate, with the absence of dermal homogenisation.[10,12] Hypertrophic LP required the changes of LSC with the additional features of basal layer vacuolar change at the tips of elongated rete ridges and an adjacent lymphocytic infiltrate in the dermis.[11]

The diagnosis of LS required dermal homogenization appearing as a band of oedematous, hyaline or fibrotic collagen in the superficial dermis beginning at the dermoepidermal junction.[10,11] Hyperkeratosis, atrophy of the prickle cell layer, basal layer vacuolar change and a band-like dermal lymphocytic infiltrate beneath the homogenisation were supportive features of LS but not sufficient for a diagnosis in absence of dermal homogenisation.[10,13] When the epidermis showed acanthosis the diagnosis was LS with superimposed LSC.

We also looked for pseudoepitheliomatous hyperplasia (PEH) which may complicate LP, LS or LSC.[14] The diagnosis of PEH was made when an architecture resembling invasive SCC with separated nests and tentacles of squamous cells involving the dermis was present, but the nuclear atypia and inflamed desmoplastic reaction characteristic of SCC were absent.

RESULTS

In all, 60 cases met the inclusion criteria. Of the 60 specimens, gynaecologists submitted 32 (53%), dermatology provided 20 (33%), colorectal surgery and gynaecologic oncology three (5%) each, and two were from general surgery (3%). Although 27 different clinicians performed the biopsies in this cohort, 62% (37/60) of the specimens were submitted by six clinicians who provide subspecialty care in vulvovaginal disease. Indications for biopsies included suspicion or further investigation of a lichen diagnosis (50%), examination findings of erythema, erosion, or fissure (20%), concern for neoplasia (13%), pruritus (8%), suspicion of dermatitis or psoriasis (5%), and one case each of anal bleeding and polyp excision. Women comprised 93% of the sample and the median age was 61 years. The diagnoses, distribution and response to treatment are summarised in Table 1.

Of 25 (44%) LS cases, 11 showed superimposed LSC and none showed PEH. One case was notable for LS with

Table 1 Diagnosis, age, site and response to treatment of 60 cases of perianal lichen dermatoses, *n*, %

	Total, *n*, %	Median age, range	Site: perianus only,† *n*, %	Site: perianus and genital skin,† *n*, %	Improved after treatment, † *n*, %
Total	60	61, 22–90	29 (53)	26 (47)	42/43 (98)
Lichen sclerosus	25 (42)	63, 39–90	8 (38)	13 (62)	20/20 (100)
Lichen simplex chronicus	23 (38)	59, 22–80	12 (55)	10 (46)	15/16 (93)
Lichen planus	10 (17)	68, 41–80	9 (90)	1 (10)	6/6 (100)
Non-specific lichenoid reaction	2 (3)	64, 61–67	0	2 (100)	2/2 (100)

†Cases with unavailable clinical data excluded.

superimposed LSC at one end of the specimen while the other side did not demonstrate features of LS (Fig. 1). A histological diagnosis of LS may not have been made if the biopsy site had been shifted slightly. This 74-year-old woman reported intractable vulvar pruritus and clinical examination was consistent with confluent excoriated LS in a figure-of-eight distribution around the vulva and anus. The symptoms and lichenification subsided with topical steroids and vulvar care modification.

Nine of the 10 (90%) LP cases showed hypertrophic LP, with three of these demonstrating PEH (Table 2). One case of PEH was so severe that it could not be distinguished from SCC. The slides were sent for a second opinion, which likewise could not exclude cancer. The clinical appearance favoured a non-neoplastic condition, so the clinician initiated a trial of topical corticosteroids that resolved the lesion. In another case of hypertrophic LP and PEH, the presence of oral and vulvovaginal LP and known response to topical steroids helped to alleviate potential confusion with SCC (Fig. 2). Hypertrophic LP was comorbid with LS in the case of a 40-year-old woman with perianal pruritus; the examination showed a circumferential perianal erythematous plaque, controlled LS anteriorly and a perineal abnormality. A perianal biopsy showed hypertrophic LP (Fig. 3). Topical steroids were recommended, but she was lost to follow up.

Among the 23 biopsies of LSC we found no PEH. Three cases (13%) were consistent with lichenified psoriasis; histological findings in addition to LSC included subcorneal abscesses, focal parakeratosis and regular elongated rete ridges. In one case of a 53-year-old woman with genitoanal pruritus, the clinical assessment suggested an underlying dermatosis likely to be LS or psoriasis, but the histology showed only LSC (Fig. 4). She improved with topical steroids.

Non-specific lichenoid reaction was diagnosed in two patients (3%), one of whom had a clinical impression of LS. The other was a 68-year-old woman with Type 2 diabetes mellitus and chronic genitoanal pruritus and pain; the clinical impression was LS on keratinised skin and erosive LP at the vestibule. Topical steroids yielded improvement, limited by ongoing exposure to irritants (Fig. 5).

Extent. Information about the distribution of the lesion was available in 55 patients, with 29 (53%) localized to the perianus and the remainder generalised over other genital skin. Compared to the LS and LSC groups, those with LP were more likely to have a localised lesion: (9/10 [90%] vs 20/43 [47%]; P = 0.015).

Response to treatment. Of the 53 cases with information on treatment advice, topical steroids were recommended in 48 (91%) and five (9%) had other management, with one case each of observation, oestrogen cream, antifungals, excision and laser. Data on response to therapy were available in 43 cases, of which 42 (98%) were better or resolved and one (2%) case of psoriasis with superimposed LSC had persistent symptoms despite the use of topical steroids (Table 1). Eight patients did not follow up, of whom two had biopsies within 12 months and one died shortly after the biopsy due to non-dermatological causes. Eight had missing data due to our inability to acquire information from the clinician.

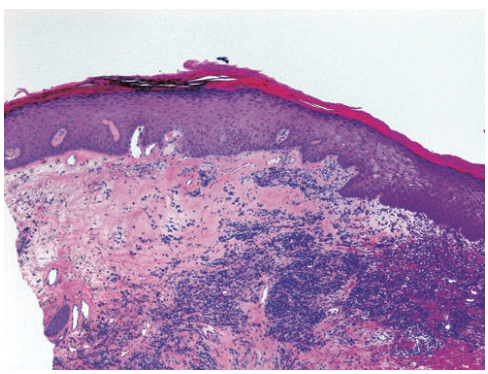

Figure 1 Lichen sclerosus with lichen simplex chronicus: epidermal hyperplasia and marked compact orthokeratosis with dermal inflammation and a thick layer of dermal hyaline. H–E x40.

DISCUSSION

We present a review of perianal lichen dermatoses in terms of standard dermatopathological terminology. Previous clinical studies of perianal pruritus offer insight into the problems of nomenclature and multiple contributory diagnoses. A classic evaluation of 200 patients referred for perianal pruritus reported 257 separate clinical diagnoses with intertrigo and haemorrhoids being most common, followed by contact dermatitis, erythrasma, candidiasis, LSC and psoriasis; no case of LS or LP was reported.[15] In another study, Kranke and colleagues described their approach to

Table 2 Details of nine hypertrophic lichen planus (LP) cases

Case	Age	M/F	Perianal only	Clinical comments	PEH	Treatment	Outcome
1	75	M	Yes	Anal bleed	Yes	None	Resolved
2	59	F	No	Oral and erosive LP	Yes	Topical steroids	Improved
3	62	F	Yes	Purple hyperkeratotic plaque	Yes	Topical steroids	Resolved
4	69	F	Yes	Purple streak	No	Topical steroids	Improved
5	78	F	Yes	Prior LP	No	Unknown	Unknown
6	67	F	Yes	Erythematous plaque	No	Topical steroids	Unknown
7	41	F	Yes	Violaceous plaque	No	Topical steroids	Unknown
8	80	M	Yes	Lichenified	No	Unknown	Deceased
9	67	M	Yes	Hyperkeratotic	No	Topical steroids	Resolved

M/F, male or female; PEH, pseudoepitheliomatous hyperplasia.

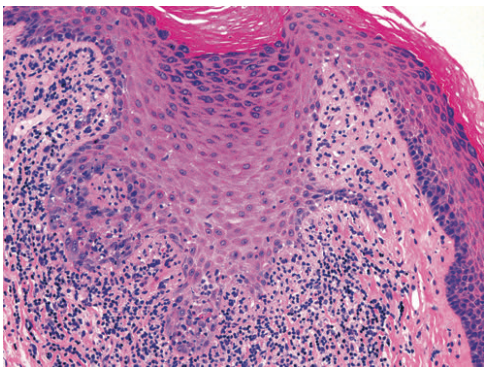

Figure 2 Pseudoepitheliomatous hyperplasia occurring in hypertrophic lichen planus: acanthosis with lobules of squamous cells protruding into the dermis. H–E ×100.

126 patients with perianal dermatitis, which included history, physical, microbiology, blood chemistry, proctoscopy, patch testing and a biopsy, as appropriate.[16] They most commonly diagnosed contact dermatitis (46%), followed by intertrigo (29%), atopic dermatitis (4%), 'pruritus ani' (4%), and psoriasis (2%), with only two cases of LS. They defined pruritus ani as 'a cutaneous sensation that induced a self-propagated itch-scratch cycle in the absence of any dermatologic disorder', and considered a positive *Candida* culture as supportive of intertrigo. It is unclear if the rarity of lichen dermatoses in these series reflects the male-predominant sex distribution, referral patterns or under-recognition of the diagnoses.

We sought to describe the spectrum of lichen dermatoses at the perianus and found that LS, LP and LSC were all represented. LS is frequently lichenified and LP is usually hypertrophic. Three previous studies of vulvar LS report the rates of superimposed lichenification: an analysis of epidermal thickness in LS found superimposed LSC in 32% (29/90), an audit of 285 cases of vulvar dystrophy reported that 46% (61/133) of LS was lichenified, and 35% of a cohort of 129 women with LS had severe hyperkeratosis on examination.[17–19] Our result of 44% suggests that perianal LS is not substantially more prone to superimposed LSC than vulvar LS. A subset of the LSC cases were likely to have been lichenified psoriasis but this diagnosis requires clinical correlation because the two processes are not easily distinguished on histopathology.[3,9,11] The provision of an accurate description of clinical findings, differential diagnosis and the exact location of the biopsy may facilitate clinicopathological correlation.

Hypertrophic LP represents a significant minority of perianal lichen disease, in contrast to the vulva, where erosive LP is reportedly the most common subtype.[20] Two cohort studies describe the spectrum of vulval LP. Bradford and Fischer encountered perianal LP in 8% of a cohort of 131 women, of whom 37% had LP located outside the vestibule and vagina.[7] Belfiore and colleagues systematically examined 42 women with oral LP and found 24 cases of vulval LP, of whom 30% had hypertrophic LP; three women had perianal or perineal disease.[8] Our study cannot

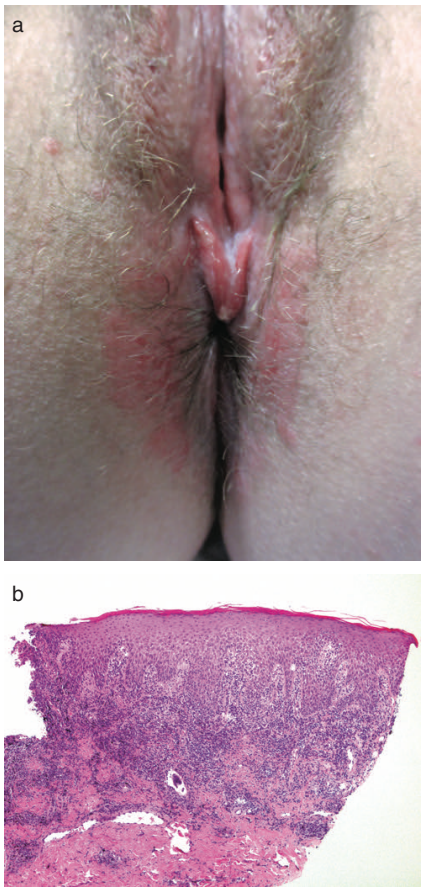

Figure 3 (a) Hypertrophic lichen planus appearing as circumferential perianal pink plaque with excoriations. (b) Hypertrophic lichen planus: marked acanthosis with lichenoid tissue reaction maximal at tips of elongated rete ridges. H–E ×40.

comment on the prevalence of genitoanal hypertrophic LP, but our rate of 15% suggests, along with these cohorts, that it may be an under-recognised entity. Several authors describe the potential for misdiagnosis, with erroneous initial impressions ranging from condyloma to psoriasis to SCC.[21–24] In our cohort, three of nine cases of hypertrophic LP showed PEH, a finding that may provoke concern for neoplasia. Clinicopathological correlation is essential prior to excision in these cases; obtaining an opinion from a subspecialised dermatopathologist may help to avoid misdiagnosis and overtreatment.

We found in 3% of patients there were insufficient findings of dermal homogenisation to diagnose LS and, simultaneously, inadequate alterations to rete ridges to diagnose LP. Studies of clinically diagnosed vulvar LS and LP report a substantial rate of non-diagnostic biopsy ranging from 12 to 29%.[7,19,25,26] The variety of morphological patterns seen in vulvar LS and LP complicates assessment, as does the controversy over which histopathological features must be present in order to make these diagnoses.[9,10,12,17,27,28] As a result, there may be significant inter-observer and intra-observer variability in histological assessment, as has been documented in oral LP.[29]

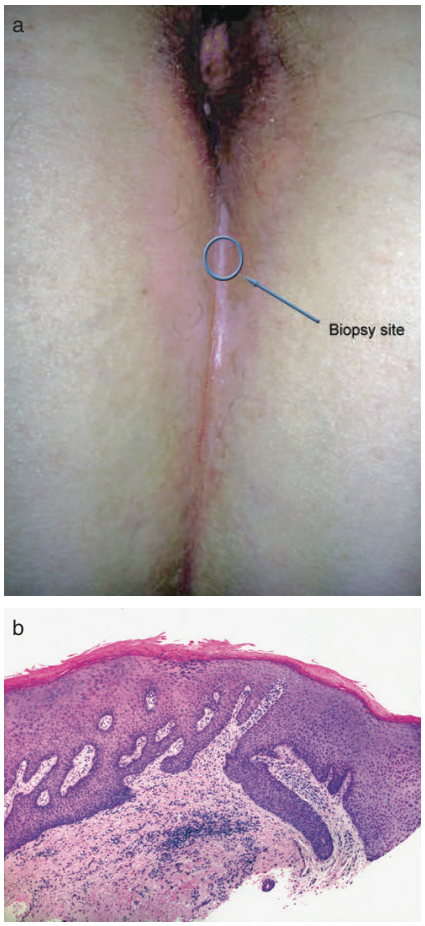

Figure 4 (a) Well-demarcated erythema, pallor and fissure extending up the natal cleft suggest an underlying dermatosis, rather than the pathological diagnosis of lichen simplex chronicus. (b) Lichen simplex chronicus: irregular acanthosis with absence of basal layer damage. H–E ×100.

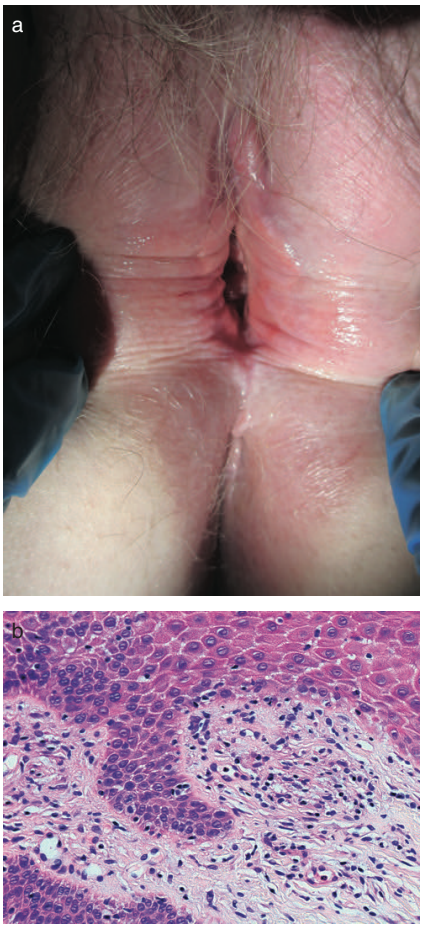

Figure 5 (a) Erythema and pallor contiguous with erythematous erosions at the vestibule suggest comorbid lichen sclerosus and erosive lichen planus. The vulval and perineal skin appearance is representative of the findings seen circumferentially at the perianus, consistent with a figure-of-8 pattern. (b) Non-specific lichenoid tissue reaction: basal layer damage, lymphocyte exocytosis of the suprapapillary plate but largely sparing the rete ridge, absence of dermal homogenisation. H–E ×200.

Access to biopsies from clinics across Australia specialised in vulvovaginal disorders permits the study of dermatoses in this discrete anatomical distribution, but yields a predominantly female cohort. Any retrospective histopathology review is limited to cases in which clinical features merit a tissue sample, but biopsy is often avoided at the perianus due to concerns about hygiene, comfort and healing. The issue of biopsy location is especially important in LS, which often has a figure-of-8 distribution. We had limited access to some variables of interest; in particular, microbiology results may have provided an aetiology for some LSC cases.

In summary, LS, LSC, and LP all occur at the perianus. Perianal dermatoses merit further study across the specialties involved, with a focus on clinicopathological correlation, the use of standard dermatopathological terminology and an appreciation of the multifactorial nature of genitoanal dermatological disease in order to gain meaningful insight into this challenging clinical problem.

REFERENCES

1. Palefsky JM. Practising high-resolution anoscopy. *Sex. Health* 2012; **9**: 580–6.
2. Darragh TM, Colgan TJ, Cox JT *et al*. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int. J. Gynecol. Pathol.* 2013; **32**: 76–115.
3. Lynch PJ. Lichen simplex chronicus (atopic/neurodermatitis) of the anogenital region. *Dermatol. Ther.* 2004; **17**: 8–19.
4. Markell KW, Billingham RP. Pruritus ani: etiology and management. *Surg. Clin. North Am.* 2010; **90**: 125–35.
5. Nasseri YY, Osborne MC. Pruritus ani: diagnosis and treatment. *Gastroenterol. Clin. North Am.* 2013; **42**: 801–13.
6. Bohl TG. Overview of vulvar pruritus through the life cycle. *Clin. Obstet. Gynecol.* 2005; **48**: 786–807.
7. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J. Low. Genit. Tract Dis.* 2013; **17**: 28–32.

8. Belfiore P, Di Fede O, Cabibi D *et al*. Prevalence of vulval lichen planus in a cohort of women with oral lichen planus: an interdisciplinary study. *Br. J. Dermatol.* 2006; **155**: 994–8.
9. O'Keefe RJ, Scurry JP, Dennerstein G *et al*. Audit of 114 non-neoplastic vulvar biopsies. *Br. J. Obstet. Gynaecol.* 1995; **102**: 780–6.
10. Weedon D, Strutton G, Rubin A. Tissue reaction patterns and Disorders of collagen. In: Weedon D (ed.). *Weedon's Skin Pathology 3rd En*. London: Churchill Livingstone Elsevier, 2010; 38–41, 86–7, 313–16.
11. Calonje E, McKee P, Brenn T *et al*. Spongiotic, psoriaform, and pustular dermatoses and lichenoid and interface dermatitis. In: Calonje JE, Brenn T, Lazar A, McKee P (eds). *McKee's Pathology of the Skin with Clinical Correlations. Vol. 1*. London: Elsevier, 2011; 207–11, 222–7.
12. Simpson RC, Thomas KS, Leighton P *et al*. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br. J. Dermatol.* 2013; **169**: 337–43.
13. Powell JJ, Wojnarowska F. Lichen sclerosus. *Lancet* 1999; **353**: 1777–83.
14. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. *Int. J. Gynecol. Pathol.* 2003; **22**: 57–62.
15. Bowyer A, McColl I. A study of 200 patients with pruritus ani. *Proc. R. Soc. Med.* 1970; **63** (Suppl.): 96–8.
16. Kranke B, Trummer M, Brabek E *et al*. Etiologic and causative factors in perianal dermatitis: results of a prospective study in 126 patients. *Wien. Klin. Wochenschr.* 2006; **118**: 90–4.
17. Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. *Am. J. Dermatopathol.* 2001; **23**: 413–18.
18. Ayhan A, Tuncer ZS, Kaya H. Vulvar dystrophy: an evaluation of 285 cases. *Eur. J. Gynaecol. Oncol.* 1997; **18**: 139–40.
19. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust. N. Z. J. Obstet. Gynaecol.* 2010; **50**: 148–52.
20. McPherson T, Cooper S. Vulval lichen sclerosus and lichen planus. *Dermatol. Ther.* 2010; **23**: 523–32.
21. Zindanci I, Kavala M, Zenginkinet T *et al*. Condyloma accuminata-like hypertrophic lichen planus. *Eur. J. Dermatol.* 2010; **20**: 416–17.
22. Fisher V, Fernandez MP, Krejci-Manwaring J. Lichen planus mimicking recalcitrant ulcerative psoriasis: when is it time to biopsy? *Cutis* 2013; **92**: 136–9.
23. Tan E, Malik R, Quirk CJ. Hypertrophic lichen planus mimicking squamous cell carcinoma. *Australas. J. Dermatol.* 1998; **39**: 45–7.
24. Pusiol T, Zorzi MG, Morichetti D *et al*. Pseudoepitheliomatous hyperplasia arising from hypertrophic lichen planus mimicking squamous cell carcinoma: limited value of immunohistochemistry. *Acta Dermatovenerol. Croat.* 2012; **20**: 112–14.
25. Santegoets LA, Helmerhorst TJ, van der Meijden WIA. retrospective study of 95 women with a clinical diagnosis of genital lichen planus. *J. Low. Genit. Tract Dis.* 2010; **14**: 323–28.
26. Simpson RC, Littlewood SM, Cooper SM *et al*. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. *Br. J. Dermatol.* 2012; **167**: 85–91.
27. Marren P, Millard P, Chia Y *et al*. Mucosal lichen sclerosus/lichen planus overlap syndrome. *Br. J. Dermatol.* 1994; **131**: 118–23.
28. Weyers W. Hypertrophic lichen sclerosus with dyskeratosis and parakeratosis – a common presentation of vulvar lichen sclerosus not associated with a significant risk of malignancy. *Am. J. Dermatopathol.* 2013; **35**: 713–21.
29. van der Meij EH, Reibel J, Slootweg PJ *et al*. Interobserver and intraobserver variability in the histologic assessment of oral lichen planus. *J. Oral Pathol. Med.* 1999; **28**: 274–77.

4.5 Vestibulovaginal sclerosis versus lichen sclerosus

The dogma is that LS is a disease of keratinised skin, and this is one of the ways it is differentiated from vulvovaginal erosive LP. Despite this, clinicians often fail to provide a specific site when labeling biopsies and writing request forms. In absence of appropriate clinical notes, the pathologist is left to identify site based on the microscopic appearance of the specimen. Abnormal epithelium of the vestibule and vagina may become keratinised, removing one of the most important tools used to determine biopsy location. Thus, identifying cases that challenge long-standing assumptions relies on a knowledgeable clinician recognizing the situation, performing a well-placed biopsy, and then describing the anatomic site for an expert vulval pathologist. Taking advantage of this combination of factors, cases of LS occurring on vestibule or vagina were collected. Guided by Fadare's description of 'vaginal stromal sclerosis,' vestibular and vaginal specimens containing isolated subepithelial sclerosis were sought. The plan was to evaluate the clinicohistopathologic characteristics these unusual cases. This study found that LS biopsied at the vestibule or vagina typically coexists with LS elsewhere on the vulva, and responds to topical steroids. In contrast, cases of isolated sclerosis are focal lesions, half of which were incidental findings, and treatment varies across clinicians. The name 'vestibulovaginal sclerosis' (VVS) was coined to describe sclerotic lesions that lack a lymphocytic infiltrate and basal layer degeneration. Although it was not possible to categorically reject the hypothesis that VVS is a subset of LS, the clinical trajectory of affected women was not consistent with a chronic inflammatory dermatosis.

International Journal of Gynecological Pathology
37:356–363, Lippincott Williams & Wilkins, Baltimore

Original Article

Vestibulovaginal Sclerosis Versus Lichen Sclerosus

Tania Day, M.D., F.R.A.N.Z.C.O.G., Kate Burston, M.B.B.S., Graeme Dennerstein, F.R.A.N.Z.C.O.G., Ross Pagano, F.R.A.N.Z.C.O.G., and James Scurry, F.R.C.P.A.

Summary: To determine if vestibulovaginal sclerosis and lichen sclerosus (LS) are 2 distinct entities. Biopsies obtained from the vagina or vulvar vestibule that contained abnormal subepithelial collagen were reviewed. Cases were categorized either as LS or vestibulovaginal sclerosis based on presence or absence of basal layer degeneration and lymphocytic infiltrate. Clinical data collected included examination findings, biopsy site and indication, previous vulvovaginal surgery, medications at time of biopsy, vulvar LS, treatment, and response. There were 15 cases with a mean age of 62 yr (range: 32–86 yr); 12 (80%) specimens were from vestibule and 3 from vagina. Nine cases were categorized as LS because of lymphocytic infiltrate in combination with basal layer degeneration, of these 8 had LS elsewhere on vulvar skin. Six cases were classified as vestibulovaginal sclerosis and had an absent or sparse lymphocytic infiltrate and essentially normal epithelium; none of these had vulvar LS. While vestibulovaginal sclerosis and lichen sclerosus are distinguishable clinically and histopathologically, further studies are needed to determine if vestibulovaginal sclerosis is a subset of LS or a different condition. **Key Words:** Vagina—Vulvar vestibule—Sclerosis—Lichen sclerosus.

Lichen sclerosus (LS) is a chronic dermatosis with a predilection for keratinized vulvar skin. It has 2 diagnostic histopathologic features: a lichenoid tissue reaction and dermal collagen homogenization. While LS is commonly found on skin, sometimes squamous mucosa may be affected. Vaginal LS has been described in 3 women with pelvic organ prolapse, all with vulvar LS and white plaques on exposed vagina (1,2). There is a single report of vaginal LS without prolapse in a 54-yr-old woman with prior hysterectomy, vulvar LS managed with topical corticosteroids, and a separate white plaque at the vaginal apex that did not require specific treatment (3). Although these reports describe histopathologic findings of LS, none of the 3 published images demonstrates all standard diagnostic features.

Fadare's (4) description of "vaginal stromal sclerosis" may provide an explanation for cases in which biopsy of a white lesion demonstrates abnormal subepidermal collagen without basal layer alterations. He reported 3 cases of postmenopausal women with dyspareunia, atrophic-appearing mucosa, and white plaques of < 1 cm diameter located in the distal vagina. None had lymphocytic infiltrate, while all had a thick paucicellular band of hyalinized collagen. Fadare hypothesized that focal injury of nonestrogenized vaginal mucosa results in scar

From the Department of Maternity and Gynecology, John Hunter Hospital (T.D., K.B.); Faculty of Health and Medicine, University of Newcastle (T.D., J.S.); Anatomical Pathology, Pathology North, Hunter New England (J.S.), Newcastle, NSW; Dermogynaecology Clinic, Mercy Hospital for Women, Heidelberg (G.D.); and Vulvar Disorders and Dermatology Clinic, Royal Women's Hospital, Melbourne (R.P.), Vic., Australia.

T.D. is supported by the Australian Government Research Training Program Scholarship. The remaining authors declare no conflict of interest.

Address correspondence and reprint requests to Tania Day, MD, FRANZCOG, Department of Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Road, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au.

DOI: 10.1097/PGP.0000000000000441

formation seen histopathologically as sclerosis, and that this is unrelated to LS.

This study aims to assess the clinical and histopathologic features of vaginal and vestibular biopsies with abnormal subepidermal collagen, to determine if lichen sclerosus and vestibulovaginal sclerosis are 2 separate entities.

MATERIALS AND METHODS

The Pathology North, Hunter New England database was searched between January 2010 and April 2017 for biopsies from the vagina or vulvar vestibule and the terms "sclerosis" and "sclerosus." Clinical notes from the biopsy request form and histologic slides were reviewed. All specimens were stained both with hematoxylin and eosin and periodic acid-Schiff. Biopsy locations described as "hymen," "introitus," or "fossa navicularis," were considered to be vestibule. Site was recorded as squamous mucosa or mucocutaneous junction (MCJ). Histopathologic features of MCJ included continuity with hairless skin or squamous mucosa, parakeratosis, absent granular cell layer, and reduced glycogen compared with squamous mucosa in estrogenized epithelium (5). Cases with keratinized epithelium and an inadequate clinical description of biopsy location were excluded, as it could not be determined if these represented hairless skin, or mucosa that had become keratinized. Vagina referred only to locations cephalad to the hymen, and by definition was squamous mucosa. Immunostaining with antibodies to D2-40, a lymphatic-specific marker, was performed if lymphangiectasia was suspected on histopathology. Hunter New England Research Ethics and Governance Unit approved this retrospective histopathologic case series (HREC 15/11/18/5.02).

Histopathologic assessment included epithelial thickness, papillary morphology, and lamina propria lymphocytic infiltrate, which was semiquantitatively assessed as absent, sparse, moderate, or dense. Basal layer alterations were recorded, to include vacuolar change, apoptotic bodies, squamatization, and lymphocytosis. Squamatization was defined as a change in morphology of normal basal keratinocytes to more horizontally disposed cells with a mature squamous appearance. Subepithelial collagen homogenization was characterized as edematous or hyaline.

Cases were categorized either as LS or vestibulovaginal sclerosis. Minimum additional criteria for LS were basal layer vacuolar change, apoptotic bodies, or squamatization, with a lamina propria lymphocytic infiltrate; lymphocytosis was supportive. A diagnosis of vestibulovaginal sclerosis was applied when epithelium lacked vacuolar degeneration or apoptotic bodies, and both the lymphocytic infiltrate and lymphocytosis were absent or sparse. Clinical information was obtained on examination findings, biopsy site and indication, previous vulvovaginal surgery, medications at time of biopsy, vulvar LS, treatment, and response. Descriptive statistics were performed and categorical variables were compared with the Fisher exact test.

RESULTS

There were 15 cases with a mean age of 62 yr (range: 32–86 yr). All biopsies were submitted by gynecologists. Twelve (80%) specimens were obtained from the vestibule, of which 9 (75%) were classified as squamous mucosa and 3 (25%) as MCJ. Confluent keratinization was noted on 1 vestibular specimen and the location of the biopsy was verified with the clinician. Parakeratosis occurred in 3 specimens classified as squamous mucosa—1 from vagina and 2 from vestibule. Nine cases were categorized as consistent with LS and 6 as vestibulovaginal sclerosis; Table 1 summarizes the histopathologic characteristics stratified by assigned diagnosis.

The clinical features of all cases are detailed in Table 2. Compared with vestibulovaginal sclerosis, LS cases were more likely to have LS elsewhere on vulvar skin [8/9 (89%) vs. 0/6; $P<0.002$]. The 1 case of LS restricted to the vestibule occurred in a 60-yr-old woman with 3 yr of pruritus who underwent 2 sessions of fractional carbon dioxide laser for presumed genitourinary syndrome of menopause. She had persistent symptoms and obtained a second opinion (case 4, Fig. 1). Histopathology of case 1 demonstrates LS across the transition from MCJ to estrogenized nonkeratinized epithelium with abundant glycogen well visualized on periodic acid-Schiff (Fig. 2). Case 2 had biopsies from labium minus and hymen, the former demonstrated LS of hairless skin and the latter showed LS across MCJ and squamous mucosa, as well as histiocytes and lymphangiectases confirmed by positive D2-40 in the endothelium of dilated vessels (Fig. 3). Case 6 was previously published as a case report (6). Steroid ointment was prescribed and yielded improvement in all cases categorized as LS and all women continued maintenance regimens.

Vestibulovaginal sclerosis cases were typified by an incidental finding of a white plaque. One case showed

TABLE 1. *Histopathologic Characteristics of Vestibular and Vaginal Biopsies With Abnormal Subepithelial Collagen*

	n (%)		
	Total	Lichen Sclerosus (N = 9)	Vestibulovaginal Sclerosis (N = 6)
Age [mean (SD)] (y)	62 (13)	63 (16)	61 (8)
Epithelial thickness [mean (SD)] (mm)	0.1 (0.05)	0.11 (0.07)	0.1 (0.03)
Keratinization status			
Nonkeratinized	8 (53)	4 (44)	4 (67)
Parakeratosis	6 (40)	4 (44)	2 (33)
Keratinized	1 (7)	1 (11)	0
Lymphocytic infiltrate			
Absent	6 (40)	0	6 (100)
Sparse	6 (40)	6 (67)	0
Moderate-dense	3 (20)	3 (33)	0
Abnormal collagen			
Edematous	5 (33)	4 (44)	1 (17)
Hyalinized	8 (53)	3 (33)	5 (83)
Both	2 (13)	2 (22)	0
Lymphocytosis	6 (40)	5 (56)	1 (17)
Basal layer			
Vacuolar change	5 (33)	5 (56)	0
Squamatization only	5 (27)	4 (44)	1 (17)
Normal	5 (40)	0	5 (83)

TABLE 2. *Clinical Characteristics of Vestibular and Vaginal Biopsies With Abnormal Subepithelial Collagen*

	Age (y)	Vulval LS	Prior Vulvovaginal Surgery	Medications at Biopsy	Indication for Biopsy	Biopsy Location	Treatment
Lichen sclerosus							
1	32	Yes	No	None	Dyspareunia, suspect LS	Vestibule	Topical corticosteroids
2	47	Yes	No	None	Dyspareunia, suspect LS	Vestibule	Topical corticosteroids
3	58	Yes	No	Topical corticosteroids	Suspect LS	Vestibule, keratinized	Topical corticosteroids
4	60	No	Vaginal fractional laser	None	Suspect LS	Vestibule	Topical corticosteroids
5	62	Yes	Vaginal hysterectomy, prolapse repair	Topical estrogen	Suspect LS	Vagina	Topical corticosteroids
6	70	Yes	Excisions of basal cell carcinomas	None	Suspect LS	Vestibule	Topical corticosteroids
7	73	Yes	Vaginal hysterectomy	Topical corticosteroids	LS exacerbation	Vestibule	Topical corticosteroids
8	79	Yes	No	Topical corticosteroids	LS exacerbation	Vestibule	Topical corticosteroids
9	86	Yes	Excision of differentiated vulvar intraepithelial neoplasia	None	Exclude neoplasia	Vagina	Topical corticosteroids
Vestibulovaginal sclerosis							
10	47	No	No	Topical estrogen	Dyspareunia, introital stenosis	Vestibule	Excision with flap repair
11	56	No	No	None	Incidental finding of white plaque on hymen	Vestibule	None
12	59	No	No	None	Dyspareunia, pallor at posterior fourchette	Vestibule	Oral tricyclic antidepressant
13	66	No	No	None	Incontinence-associated dermatitis, incidental finding of white plaque	Vagina	Topical corticosteroids
14	69	No	Vaginal hysterectomy, prolapse repair	None	Nodular scar seen at hysterectomy	Vestibule	Scar excised
15	70	No	Transobturator tape, pessary placement	Systemic hormone replacement	Incidental finding of suburethral white plaque	Vestibule	None

LS indicates lichen sclerosus.

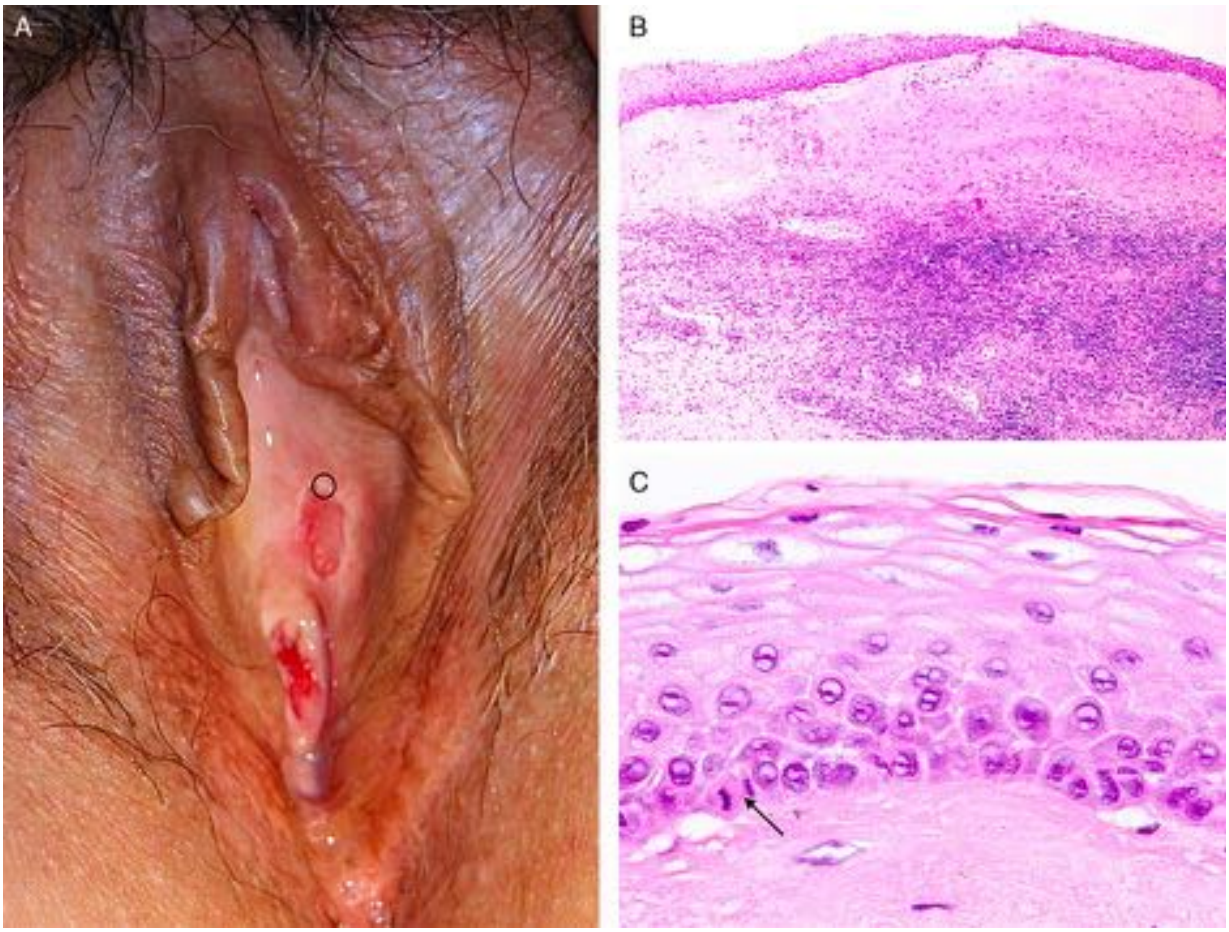

FIG. 1. (A) Pallor between clitoris and urethra, biopsy sited at the edge of an ulcer. (B) Histopathology consistent with lichen sclerosus: squamous mucosa with erosion, a band of hyalinized collagen, and dense lymphocytic infiltrate, hematoxylin and eosin 40 × . (C) Basal layer squamatization and mitosis (arrow), hematoxylin and eosin 400 × .

focal squamatization of thinned and parakeratotic epithelium overlying a sclerotic protruberance (case 14, Fig. 4). Focal lymphocytosis and spongiosis were seen in a biopsy of posterior fourchette, with absent lymphocytic infiltrate (case 12). The remaining sclerosis cases were characterized by normal epithelium (case 11, Fig. 5). Two (29%) women improved after excision of the lesion, 2 required no treatment, 1 (14%) had resolution of sexual pain with neuromodulators, and 1 used intermittent topical steroids for dermatitis.

DISCUSSION

Vestibular and vaginal biopsies with abnormal subepithelial collagen may be classified into 2 groups: those diagnostic of LS, and those without evidence of inflammation. The former demonstrates a lichenoid tissue reaction, which is the manifestation of basal layer damage mediated by the closely applied band of T cell-predominant lymphocytes (7). The latter shows abnormal collagen without evidence of interaction between the lymphocytes and the epithelium. The pathophysiology of the collagen change is not well understood. It appears that dermal homogenization begins as an edematous protein-rich exudate from blood vessels, which becomes hyalinized through dehydration, deposition of type 5 collagen, loss of elastic fibers, and accumulation of decomposed fibrin (8–11). In some cases, this may progress to fibrosis with loss of the inflammatory infiltrate. It is not known if vestibulovaginal sclerosis represents inactive LS that has lost the lymphocytic infiltrate either from treatment or spontaneous remission. Arguing against this, none of the 6 sclerosis cases in this study had LS on vulvar skin, and none were treated at time of biopsy. In contrast, 89% of women with LS on a vestibular or vaginal biopsy had LS

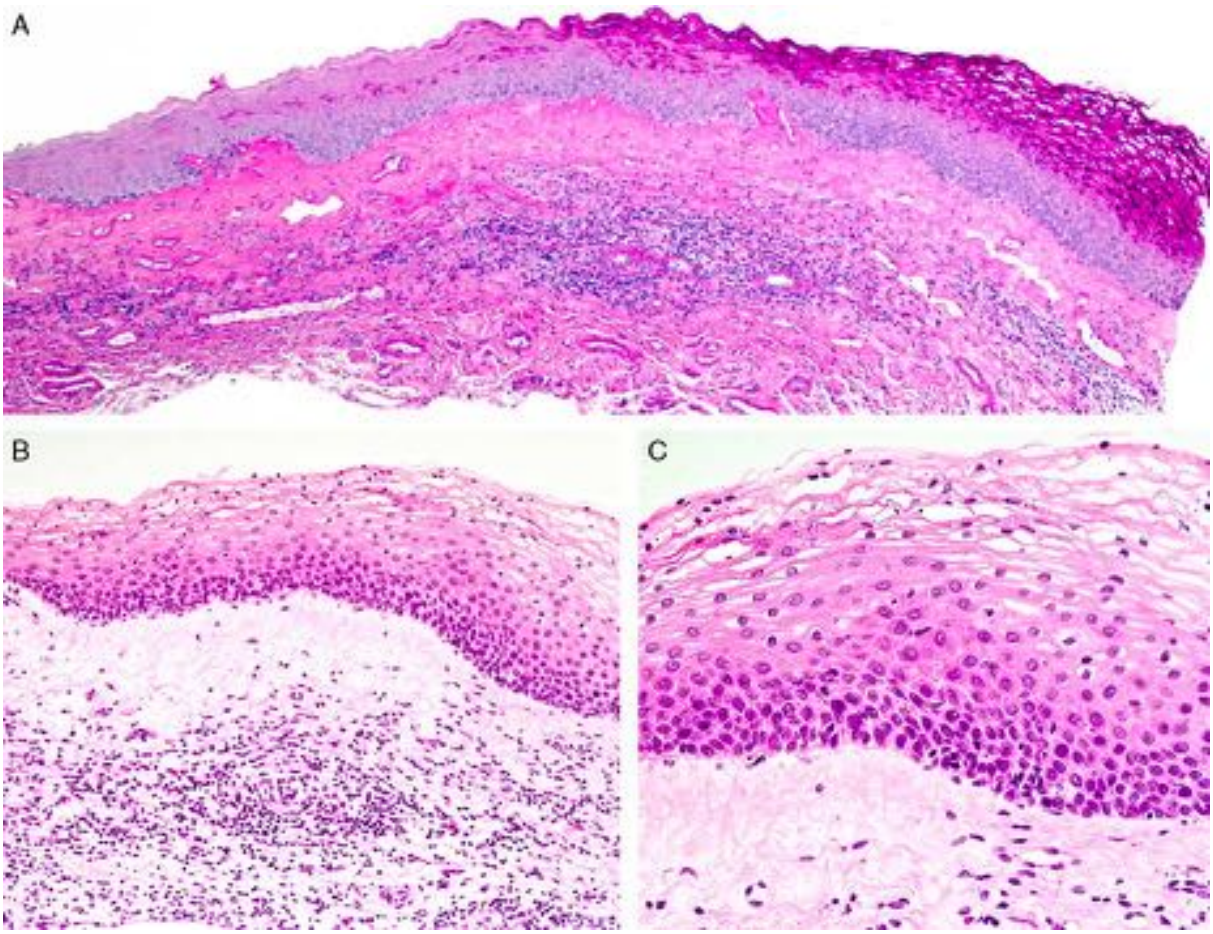

FIG. 2. Biopsy of pallor at vestibule consistent with lichen sclerosus: (A) mucocutaneous junction (left) and squamous mucosa with abundant periodic acid-Schiff positive glycogen (right) both show subepithelial sclerotic collagen overlying a band of lymphocytes, periodic acid-Schiff 40 ×, (B) squamous mucosa with basal layer degeneration overlying a band of edematous collagen, hematoxylin and eosin 100 ×, (C) basal layer with lymphocytosis and squamatization, hematoxylin and eosin 400 ×.

elsewhere on the vulva, and the 3 treated at time of biopsy still showed a lichenoid reaction.

Previous reports suggest that keratinization is required to establish susceptibility to LS. Of the 3 published images of vaginal LS, 1 shows parakeratosis and 2 show keratinized epithelium (2,3). A study of 99 men with LS of penile skin reported 14 biopsies showing LS at the navicular or penile urethra, all of which were keratinized (12). The authors hypothesized that urinary obstruction because of LS-related distal stenosis provided the irritant stimulus. In contrast, this study demonstrates that nonkeratinized epithelium may be affected by LS. Likewise, keratinization is variable in vestibulovaginal sclerosis. The histopathologic images in Fadare's (4) report suggest a nonkeratinized epithelium in 1 and keratinization in 2, while the 6 cases in this study were all nonkeratinized. It is possible that the Koebner phenomenon is one pathway toward abnormal subepithelial collagen, given that vulvovaginal surgery occurred in half of cases (13). The long-term impact of intracavitary fractional laser on vulvovaginal skin and mucosa is unclear, and there are no studies regarding outcomes in women with chronic inflammatory dermatoses.

Basal layer squamatization is infrequently mentioned in dermatopathology publications as a feature of the basal layer degeneration required for diagnosis of LS and lichen planus (14–16). In isolation, basal layer squamatization is not diagnostic of a lichenoid tissue reaction; thus we classified the case of nodular scar with a squamatized basal layer and absent lymphocytic infiltrate as vestibulovaginal sclerosis. One of the histopathologic images from Fadare's

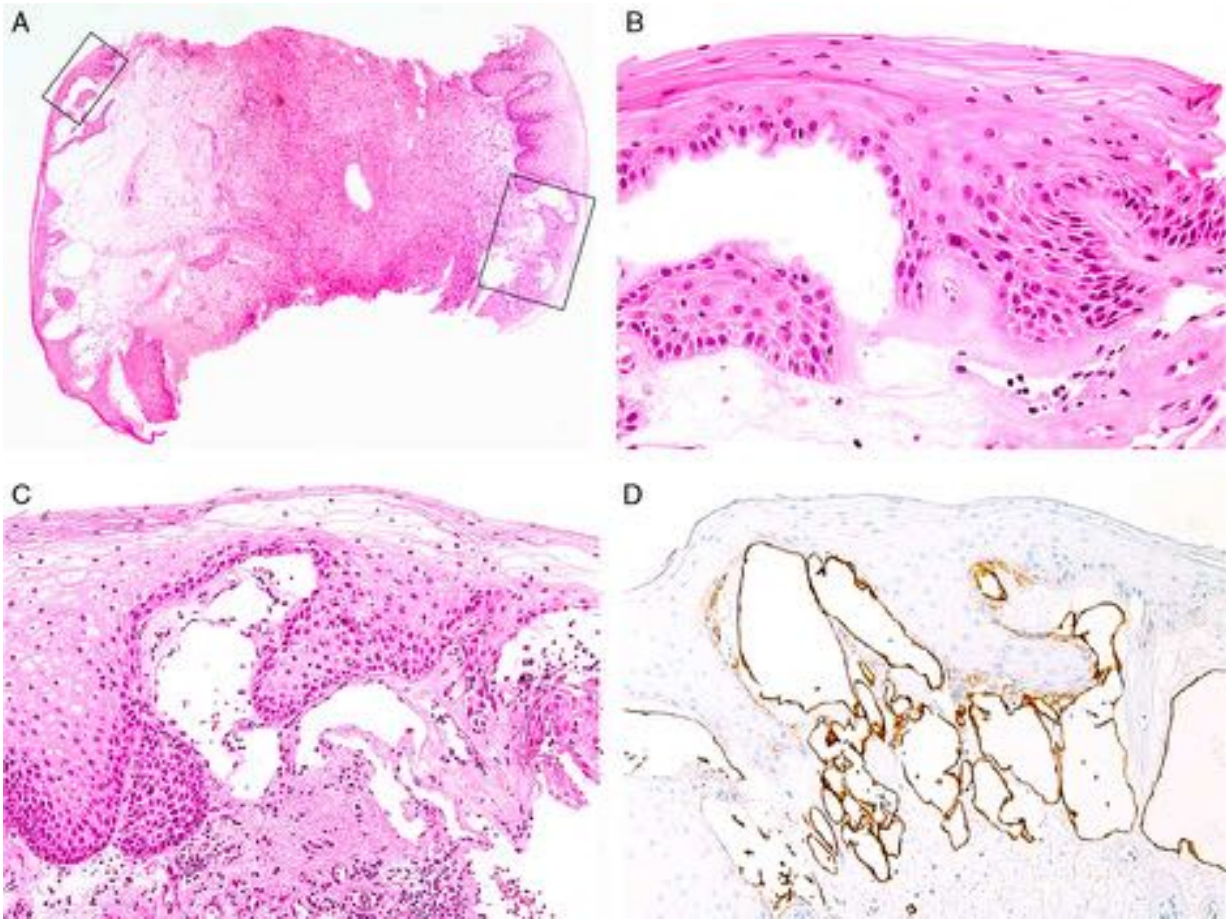

FIG. 3. Biopsy of pallor at hymen consistent with lichen sclerosus: (A) mucocutaneous junction (left) and squamous mucosa (right) with edematous and hyalinized subepidermal collagen and a moderate lymphocytic infiltrate, hematoxylin and eosin (H&E) 40×, (B) mucocutaneous junction with basal layer squamatization and dilated lymphatics, H&E 200× (C) squamous mucosa with basal layer squamatization and dilated lymphatics, H&E 100×, (D) positive D2-40 immunohistochemistry of the lymphatic vessel endothelium.

report also shows a squamatized basal layer. The combination of squamatization and abnormal dermal collagen yields a differential diagnosis that also includes dermal scar related to trauma or radiation, morphea, mycosis fungoides, and malignant atrophic papulosis (17–20). However, these conditions have multiple other clinicopathologic features that help distinguish them from LS and vestibulovaginal sclerosis.

Among the 9 cases of vestibular and vaginal LS, 1 had lymphangiectasia with histiocyte infiltration. Carlson et al. (21) raised the possibility of an association between lymphedema and LS in a study of the quantity and size of lymphatics in 18 LS cases compared with 9 controls, hypothesizing that dermal sclerosis disrupts lymphatic drainage leading to lymphostasis. However, neither the association nor the mechanism has been investigated by other authors.

There were no common exposures apparent in the 6 cases of vestibulovaginal sclerosis encountered in this study. This contrasts with the 3 cases described previously, all of whom were postmenopausal, not on hormone replacement, complained of dyspareunia, and had no other skin disease or vulvovaginal surgery (4). There may be exposures common to both studies that are difficult to obtain retrospectively, such as obstetric lacerations or sexual trauma. Estrogen deficiency may not be a prerequisite for vestibulovaginal sclerosis, as one third of cases in this study were on topical or systemic hormone replacement.

It is unclear if vestibular and vaginal LS is as rare as suggested by the sparse examples encountered in

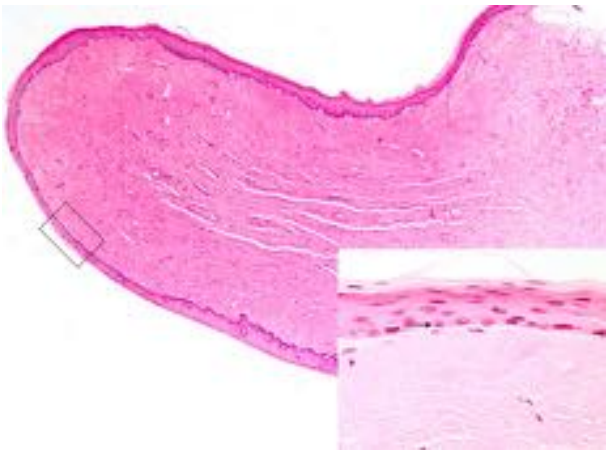

FIG. 4. Excision of nodular scar at hymen consistent with vestibulovaginal sclerosis: protuberance with marked hyalinized and fibrotic subepidermal collagen and absent lymphocytic infiltrate, hematoxylin and eosin 40 ×, inset shows overlying parakeratotic epithelium with basal layer squamatization (arrow), hematoxylin and eosin 200 ×.

the literature. White plaques at the vestibule may be interpreted as lichen planus, as its clinical appearance is described as glazed erythema with white striations or plaques at the periphery (22,23). When a skin disease is generalized, clinicians may avoid biopsy of the vestibule and vagina due to challenges with exposure and concerns about patient discomfort and healing. Speculum examination is unlikely to be performed unless the woman has symptoms attributable to the vagina, and nongynecologists may be hesitant to obtain a vaginal biopsy (2,22).

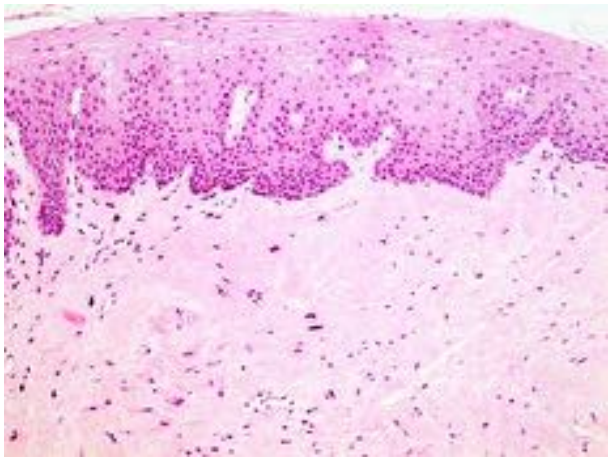

FIG. 5. Biopsy of pallor at vestibule consistent with vestibulovaginal sclerosis: squamous mucosa with normal basal layer, band of hyalinized collagen, and absent lymphocytic infiltrate, hematoxylin and eosin 100 ×.

Access to biopsies from specialist vulvovaginal clinics across Australia permits the study of uncommon diagnoses and unusual sites, but the total number of cases meeting inclusion criteria was small nevertheless. The limitations of this study are those inherent to the retrospective design including incomplete clinical data, differences in practice between clinicians, and access only to the cases in which a clinician detected an abnormality and decided to obtain a tissue sample. Most women with clinically diagnosed vulvar LS did not have biopsy verification of this diagnosis. Universal clinical photography would have permitted a more nuanced description of the difference in appearance of the 2 diagnostic categories.

In summary, the differential diagnosis for abnormal subepithelial collagen on vaginal or vestibular biopsies includes vestibulovaginal sclerosis and LS. Although the 2 may be distinguished clinically and histopathologically, further studies are needed to determine whether vestibulovaginal sclerosis is a subset of LS or is a different condition.

REFERENCES

1. Bhargava K, Lewis FM. Lichen sclerosus occurring on vaginal mucosa secondary to uterine prolapse. *J Obstet Gynaecol* 2013;33:319–20.
2. Zendell K, Edwards L. Lichen sclerosus with vaginal involvement: report of 2 cases and review of the literature. *JAMA Dermatol* 2013;149:1199–202.
3. Longinotti M, Schieffer YM, Kaufman RH. Lichen sclerosus involving the vagina. *Obstet Gynecol* 2005;106:1217–9.
4. Fadare O. Vaginal stromal sclerosis: a distinctive stromal change associated with vaginal atrophy. *Int J Gynecol Pathol* 2011;30:295–300.
5. Day T, Holland S, Scurry J. Normal vulvar histology: variation by site. *J Low Genit Tract Dis* 2016;2:64–9.
6. Kirkpatrick E, Dobriansky D, Scurry J. Gorlin syndrome, vulvar basal cell carcinomas, vismodegib, and lichen sclerosus: from the ISSVD Case Consultation Committee. *J Low Gen Tract Dis* 2016;20:e40–41.
7. Terlou A, Santegoets LA, van der Meijden WI, et al. An autoimmune phenotype in vulvar lichen sclerosus and lichen planus: a Th1 response and high levels of microRNA-155. *J Invest Dermatol* 2012;132:658–66.
8. Bushkell LL, Friedrich EG, Jordon RE. An appraisal of routine direct immunofluorescence in vulvar disorders. *Acta Derm Venerol* 1981;61:157–61.
9. Godoy CA, Teodora WR, Velosa AP, et al. Unusual remodeling of the hyalinization band in vulvar lichen sclerosus by type V collagen and ECM 2 protein. *Clinics (Sao Paolo)* 2015;70:356–62.
10. Machesney M, Tidman N, Waseem A, et al. Activated keratinocytes in the epidermis of hypertrophic scars. *Am J Pathol* 1998;152:1133–41.
11. Baird PJ. Lichen sclerosus et atrophicus (dissertation), Sydney: University of Sydney, Department of Pathology, 1981.
12. Barbagli G, Mirri F, Gallucci M, et al. Histologic evidence of urethral involvement in male patients with genital lichen sclerosus: a preliminary report. *J Urol* 2011;185:2171–6.

13. Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. *Am J Dermatopathol* 2001;23:413–8.
14. Fung MA, LeBoit PE. Light microscopic criteria for diagnosis of early vulvar lichen sclerosus: a comparison with lichen planus. *Am J Surg Pathol* 1998;22:473–8.
15. Regauer S, Liegl B, Reich O. Early vulvar lichen sclerosus: a histopathological challenge. *Histopathology* 2005;47:340–7.
16. Day T, Moore S, Bohl TG, et al. Comorbid vulvar lichen planus and lichen sclerosus. *J Low Genit Tract Dis* 2017;21: 204–8.
17. Yang S, Sun Y, Geng Z, et al. Abnormalities in the basement membrane structure promote basal keratinocytes in the epidermis of hypertrophic scars to adopt a proliferative phenotype. *Int J Mol Med* 2016;37:1263–73.
18. Guitart J, Peduto M, Caro W, et al. Lichenoid changes in mycosis fungoides. *J Am Acad Dermatol* 1997;36:417–22.
19. Fujimoto M, Basko-Plluska JL, Petronic-Rosic V, et al. Early morphea simulating patch-stage mycosis fungoides. *Am J Dermatopathol* 2015;37:490–12.
20. Regine Mydlarski PR, Barber DF, Robertson LH. Painless pink papules with central porcelain-white scars. *CMAJ* 2008;179:1171–3.
21. Carlson JA, Carlson GD, Murphy M, et al. Lichen sclerosus exhibiting histologic signs of lymphedema: an essential factor in the pathogenesis of verruciform xanthoma. *Arch Dermatol* 2012;148:148–260.
22. McPherson T, Cooper S. Vulval lichen sclerosus and lichen planus. *Dermatol Ther* 2010;23:523–32.
23. Moyal-Barracco M, Edwards L. Diagnosis and therapy of anogenital lichen planus. *Dermatol Ther* 2004;17:38–46.

4.6 Cormorbid lichen planus and lichen sclerosus

The occurrence of LP and LS together on the vulva has been mentioned in previous studies, usually as an item in a list of exclusion criteria, but not described. The lack of literature on this topic was surprising because several cases were encountered during the first two years of operation of the local vulval referral clinic. The topic was of particular interest because a potential explanation for erroneous attribution of cancer to LP is that these women in fact had unrecognized LS adjacent to the LP. This study demonstrated that comorbid LP and LS usually appears as central shiny erythema abutting peripheral pallor and textural change, with the colour transition occurring at inner labia minora and posterior fourchette. The LS had a standard histopathologic appearance. Despite having the same clinical appearance, LP demonstrated two different basal layer patterns - degenerative and regenerative. The regenerative pattern may be interpreted as atypical and erroneously reported as dVIN or HSIL. This is another potential cause for misattribution of neoplasia to LP. Again, the results suggested that well-placed vulval biopsies will aid in accurate diagnosis and clinical management, and should be part of inclusion criteria or protocols for prospective studies.

Comorbid Vulvar Lichen Planus and Lichen Sclerosus

Tania Day, MD,[1,2] Sarah Moore, MBBS,[1] Tanja Gizela Bohl, FACD,[3] and James Scurry, FRCPA[2,4]

Objectives: The aims of the study are to assess the histopathologic characteristics of vulvar biopsies consistent with lichen planus (LP) in women with a previous or concurrent histopathologic diagnosis of vulvar lichen sclerosus (LS) and to describe the clinical features of comorbid LP and LS.

Materials and Methods: Patients were included if a diagnosis of LP was confirmed after review of the hematoxylin and eosin slides and the histopathology reporting LS noted a band of abnormal collagen. Data were collected on anatomic site, clinical appearance, histopathology, microbiology, treatment, and follow-up.

Results: There were 31 cases with a mean age of 69.5 years. Thirty specimens showed erosive LP, of which 22 were from inner labium minus and 8 from vestibule. There were no significant differences between biopsy site in epithelial thickness, erosion, lymphocytic infiltrate, or basal layer pattern. One third of cases showed a regenerative pattern of LP. Of the 26 patients with clinical records available, erythema at the biopsy site was noted in all cases; in 23 the notes specified central erythema and peripheral pallor. Forty-six percent were prescribed topical corticosteroids before biopsy. All 26 were treated with topical corticosteroids, 23% were prescribed antimycotics, and 38% required other supplemental therapies.

Conclusions: Comorbid vulvar LP and LS are not rare; clinicians suspecting one should evaluate for the other and consider separate biopsies of morphologically distinct areas. Clinicopathological correlation is an invaluable tool in assessing biopsies when both diagnoses are suspected, because the regenerative pattern of LP may otherwise be overlooked or misdiagnosed.

Key Words: vulva, comorbid, lichen planus, lichen sclerosus, overlap

(*J Low Genit Tract Dis* 2017;21: 00–00)

Lichen sclerosus (LS) and lichen planus (LP) are T-cell–mediated inflammatory dermatoses directed against unknown epitopes on basal cells of squamous epithelium.[1] Typically, LS affects anogenital skin of women and girls but may also occur on extragenital sites and in males.[2] Vulvar LS usually presents with pruritus, pallor, and architectural change and may be lichenified because of provocation of an itch-scratch cycle. Lichen planus may affect any type of squamous epithelium from any site, although the vulva is a site of predilection. Three types of vulvar LP are recognized. Erosive LP is the most common and presents with pain and erythematous erosions on inner labia minora and vestibule; apposition of eroded surfaces may result in adhesions. The other 2 forms are hypertrophic, which appears as well-demarcated violaceous plaques, and typical, which resembles LP as found on extragenital skin.[3,4]

[1]Maternity and Gynaecology, John Hunter Hospital, Newcastle, New South Wales, Australia; [2]Faculty of Health and Medicine, University of Newcastle, New South Wales, Australia; [3]Solo Private Practice, Victoria, Australia; and [4]Anatomical Pathology, Pathology North, Hunter New England, Newcastle, New South Wales, Australia

Reprint requests to: Tania Day, MD, FRANZCOG, Maternity and Gynaecology John Hunter Hospital, 2 Lookout Road, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au

The authors have declared they have no conflicts of interest.

T.D. is supported by the Australian Government Research Training Program Scholarship.

This study was approved by Hunter New England Research Ethics and Governance unit (HREC 15/11/18/5.02).

DOI: 10.1097/LGT.0000000000000307

Vulvar LS is described with extragenital LP; the latter is typically oral disease and/or plaques on the extremities and torso.[5–8] Several studies on genital dermatoses have mentioned LS and LP as comorbid on the vulva.[4,9–12] The presence of LS is listed among exclusion criteria in a planned randomized trial on systemic therapy for erosive LP.[13] None of these publications describe in detail the clinical and histopathologic features of comorbid vulvar LP and LS.

This study aims to assess the histopathologic characteristics of vulvar biopsies consistent with LP in women with a previous or concurrent histopathologic diagnosis of vulvar LS and to describe the clinical features of these cases.

MATERIALS AND METHODS

The Pathology North, Hunter New England database was searched between January 2010 and January 2016 for vulvar biopsies diagnosed with LP from women who had a previous or concurrent vulvar biopsy showing LS. Cases were included if the diagnosis of LP was confirmed after review of the hematoxylin and eosin (H&E) slides and the histopathologic description of LS noted a band of abnormal collagen. In cases of a nonconcurrent histopathologic diagnosis of LS, those slides were not retrieved and reviewed. A diagnosis of superimposed lichen simplex chronicus was recorded if hyperkeratosis and acanthosis were described in addition to LS. The Hunter New England Research Ethics and Governance Unit approved this retrospective histopathologic case series (HREC 15/11/18/5.02); signed written consent was obtained for use of clinical photographs.

Biopsy site was recorded as hair-bearing skin, hairless skin, mucocutaneous junction (MCJ), or squamous mucosa. Histopathologic features of MCJ included continuity with hairless skin or squamous mucosa, parakeratosis, absent granular cell layer, and reduced glycogen compared with squamous mucosa in estrogenized epithelium.[14] This transition from hairless skin to squamous mucosa is also called Hart line, which circumferentially traverses the fossa navicularis, base of inner labia minora, and the inferior aspect of the clitoral frenulum.[15] Site was described as unsure if erosion-limited assessment and site-specific skin appendages were absent. Erosion was defined as loss of the stratum corneum in skin or loss of the surface layers of squamous cells with intraepidermal neutrophils in nonkeratinized epithelium. In skin biopsies with assessable stratum corneum, its morphology was assessed as basket weave, intermediate, or compact.[14] Drawings or clinical photographs were used when possible to identify biopsy location; this was assigned as vestibule if the clinician wrote "introitus," "hymen," or "fossa" and as labia minora if "fourchette" or "labia" were recorded. The dermal lymphocytic infiltrate was assessed as sparse, moderate, or dense. Epithelial thickness was measured at the thinnest site.

Cases of vulvar LP were grouped into 3 categories. Erosive LP could have a degenerative or regenerative pattern. Degenerative erosive LP was defined as erosion, a closely applied band-like lymphocytic infiltrate, absence of dermal homogenization, and evidence of basal layer damage such as squamatization or vacuolar change.[16] The regenerative pattern was characterized by reduced maturation, increased nucleus to cytoplasm ratio, and the presence of mitoses, in addition to erosion, the band-like lymphocytic infiltrate, and absence of dermal homogenization. Basal layer vacuolar change and scattered keratin deposits (civatte bodies) were

TABLE 1. Histopathologic Characteristics of LP Biopsies in Cases Comorbid With LS, Stratified by Biopsy Site[a]

	Labium minus (*n* = 22)	Vestibule (*n* = 8)
Epithelium type, *n* (%)		
Hairless skin	9 (41)	0
MCJ	5 (23)	1 (12.5)
Squamous mucosa	2 (9)	4 (50)
Unsure	6 (27)	3 (37.5)
Stratum corneum, *n* (%)	*n* = 14	*n* = 1
Parakeratosis	5 (36)	1 (100)
Compact	7 (50)	NA
Intermediate	1 (7)	NA
Basket weave	1 (7)	NA
Epithelial thickness, mean (range), mm	0.07 (0.02–0.2)	0.03 (0.01–0.07)
Erosion present, *n* (%)	21 (95)	6 (75)
Lymphocytic infiltrate		
Sparse	1 (4.5)	1 (12.5)
Moderate	4 (18)	2 (25)
Dense	17 (77)	5 (62.5)
Basal layer pattern, *n* (%)		
Degenerative	15 (68)	5 (62.5)
Regenerative	5 (23)	2 (25)
Both	2 (9)	1 (12.5)

[a]One case of LP on hair-bearing skin not included.
MCJ indicates mucocutaneous junction.

absent.[9] Hypertrophic LP was defined as hyperkeratosis, hypergranulosis, acanthosis with elongated rete ridges, basal vacuolar change predominantly at the tips of rete ridges, papillary dermal fibrosis, and a lymphocytic infiltrate.[4] Typical LP was defined similarly to that seen on the following extra-anogenital sites: hyperkeratosis, wedge-shaped hypergranulosis, jagged "saw-tooth" acanthosis, basal layer damage in the form of vacuolization and civatte bodies, and a closely applied dermal lymphocytic infiltrate.[16]

Clinical data collected included age, autoimmune disease, diabetes mellitus, examination findings, medication at biopsy, microbiological results, treatment and response, clinician-reported adherence to treatment recommendations, vulvar neoplasia, and duration of follow-up. χ^2 and Student *t* test were used to compare proportions and means, respectively (Stata v11.2; College Station, Tex).

RESULTS

Thirty-one cases of comorbid LP and LS met inclusion criteria with a mean age of 69.5 years (range = 43–90 years). Lichen sclerosus showed superimposed lichen simplex chronicus in 3 cases (10%). There were 30 cases of LP in biopsies of inner labium minus or vestibule; the histopathologic characteristics of these are summarized in Table 1. The 1 excepted case had typical LP at hair-bearing skin of the inferior labium majus and noncontiguous LS at hairless skin of labium minus. Biopsies labeled by clinicians as labium minus could be hairless skin, MCJ, or squamous mucosa; specimens labeled as vestibule were either MCJ or mucosa. The junction between LP and LS was displayed in 1 labial biopsy (see Figure 1). There were no significant differences between biopsy site in epithelial thickness, erosion, lymphocytic infiltrate, or basal layer pattern. One third (10/30) of the cases showed a regenerative pattern of LP.

In 26 cases with detailed clinical notes, erythema was noted at all biopsy sites. In 23 of the 26 cases, clinicians reported peripheral pallor in addition to central erythema. The pattern of erythema was described as circumferential in 20 (77%), localized in 4 (15%), and multifocal in 2 (8%). Clinical photographs were available in 9 cases; these suggested erythematous erosions of variable location and size that extended to or beyond the expected position of the MCJ. In some women, architectural distortion limited the assessment of the likely location of Hart line. In cases without a photograph, clinical descriptions were inadequate to draw conclusions about the boundaries of each disease.

The clinical appearance of LP did not differ between those with a degenerative versus regenerative pattern; this is illustrated by three cases. A 63-year-old woman with diabetes mellitus and a clinical diagnosis of vulvar LS presented with increased pain; examination suggested focal LP on a background of LS (see Figure 2A). The left inner labial biopsy demonstrated regenerative erosive LP at the MCJ and the perineal specimen showed LS (see Figures 2B, C). A 62-year-old woman presented with pruritus and

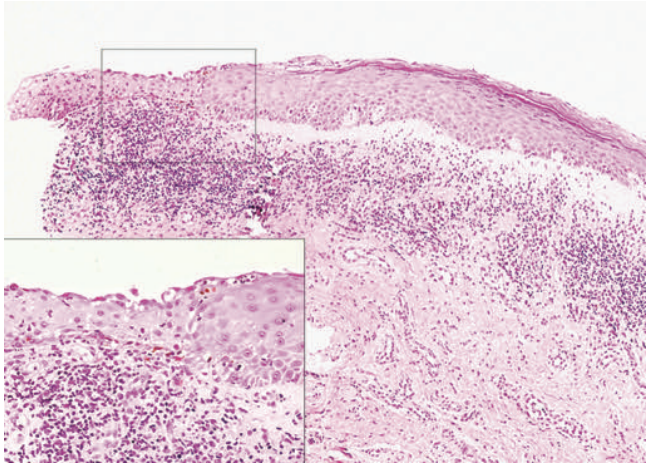

FIGURE 1. Inner labium minus—junction of LP and LS showing eroded epithelium on the left, hairless skin with subepidermal cell-poor hyalinized collagen on the right, and basal layer degeneration with a closely applied lymphocytic infiltrate throughout. H&E, ×40; inset H&E, ×200.

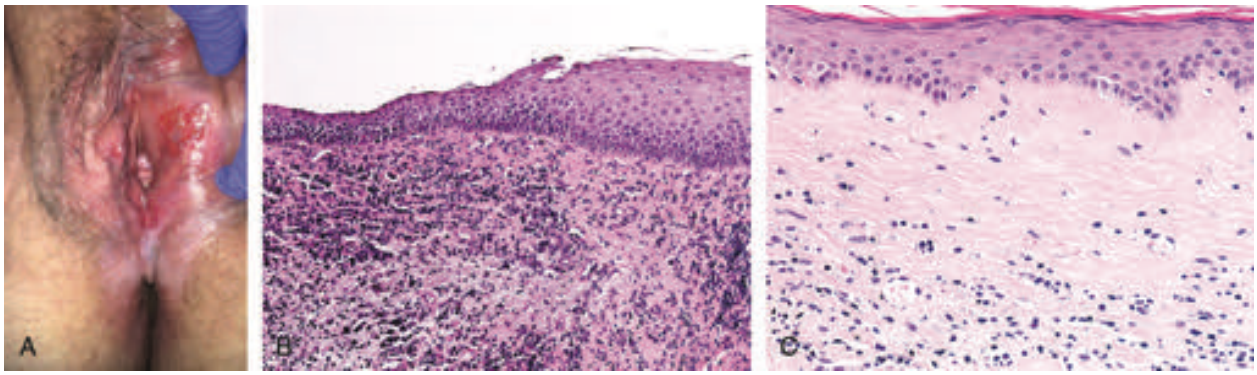

FIGURE 2. A, Pallor in a figure-of-8 distribution consistent with LS, erythematous erosion on inner left labium minus, consistent with erosive LP. B, Inner left labium minus—regenerative pattern of erosive LP on the left and MCJ on the right. H&E, ×100. C, Perineum—features of LS including squamatized basal layer, subepithelial hyalinized collagen, and moderate lymphocytic infiltrate. H&E, ×200.

pain, and examination was consistent with comorbid disease (see Figure 3A). The clinical photograph demonstrated multifocal erythematous erosions, including one that extended beyond the expected location of Hart line to the edge of the remnant right labium minus. The result of the biopsies taken from a vestibular erosion on the right and the left labium minus showed regenerative erosive LP and LS, respectively (see Figures 3B, C). A 79-year-old woman with longstanding extragenital LS presented with vulvar pain and pruritus and examination showed marked central erythema abutting pallor, in keeping with comorbid LP and LS (see Figure 4A). The photograph showed erythematous erosions extending over the entire residual inner labia minora. Histopathology confirmed LS at outer labium majus and a degenerative pattern of erosive LP at inner labium minus (see Figures 4B, C).

All but 1 woman (30/31, 97%) were postmenopausal and 19% (5/26) had diabetes mellitus (see Table 2). The following 3 women had notation of extragenital dermatoses: oral LP in 1, LS in 1, and psoriasis in 1. Of the 26 women with clinical notes, all were treated with topical steroid ointment, usually betamethasone dipropionate 0.05%. In Australia, clobetasol proprionate is only available through compounding pharmacies; this was requested in 3 cases. In addition to fluconazole and topical estrogen, other supplemental therapies included antihistamines (3 cases), clindamycin vaginal cream (1 case), systemic antibiotics for streptococcal superinfection (1 case), and physiotherapy (1 case). Clinicians obtained a vulvovaginal microbiological swab in 12 cases, of which 3 showed *Candida albicans*. The 3 surgeries performed for dermatosis were excision of a lichenified plaque to exclude neoplasia, division of anterior adhesions, and Fenton procedure (longitudinal division of posterior adhesions sutured transversely). There was no differentiated vulvar intraepithelial neoplasia or squamous cell carcinoma recorded in these cases for a mean follow-up of 41.5 months (range = 3–180 months), but 1 woman had excisions for both low- and high-grade squamous intraepithelial lesions of the vulva.

DISCUSSION

Using strict criteria at a single pathology service in 6 years, 31 cases of comorbid LP and LS were encountered, suggesting that this phenomenon is not rare. In view of the scant literature about this topic to date, it is likely that comorbid disease is underrecognized by clinicians. In a third of these cases, the epithelium shows basal layer regeneration, a pattern that may be confused with differentiated vulvar intraepithelial neoplasia or other neoplasia by pathologists unfamiliar with this entity.[9]

Lichen planus and sclerosus occurring on the same person have been called "overlap syndrome."[5] However, previous reports describe each diagnosis in a separate physical location; the 2 dermatoses do not overlap. This study documents that when both diagnoses occur on the vulva, almost invariably, the erosive LP occurs on inner labia minora and vestibule with LS peripherally

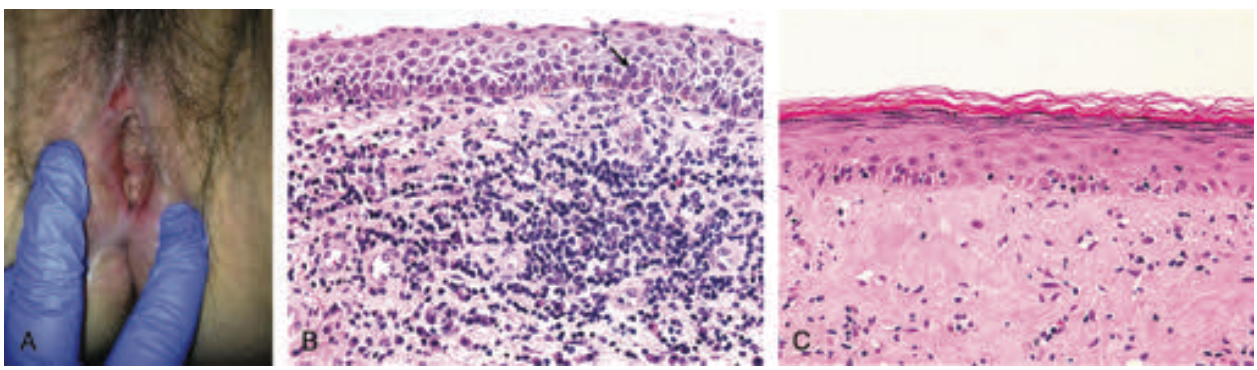

FIGURE 3. A, Abnormal vulvar architecture and pallor abutting multifocal erythematous erosions, consistent with comorbid LP and LS. B, Vestibule, site unsure due to erosion—features of the regenerative pattern of erosive LP: maturational change, increased nucleus to cytoplasm ratio, erosion, and a closely applied lymphocytic infiltrate. Arrow indicates mitosis. H&E, ×200. C, Labium minus—intermediate stratus corneum morphology, features of LS including basal layer degeneration, subepithelial hyalinized collagen, and scattered lymphocytes in the upper dermis. H&E, ×200.

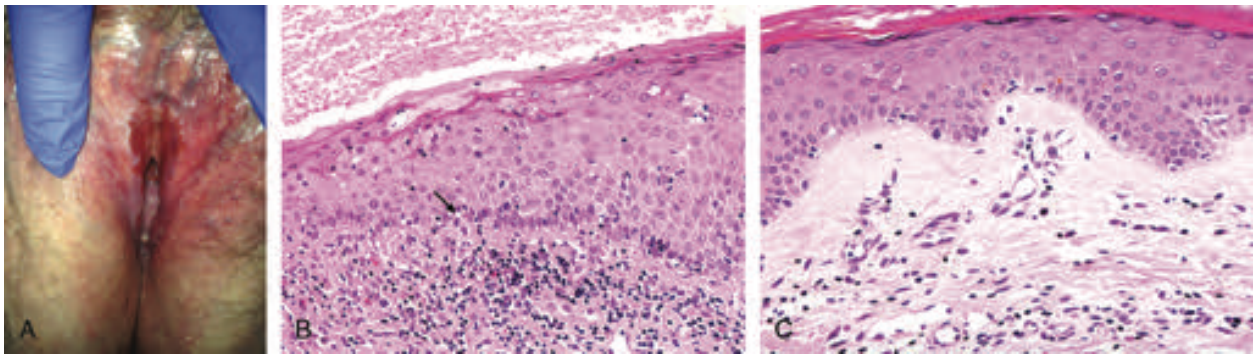

FIGURE 4. A, Abnormal vulvar architecture with pallor over labia majora abutting circumferential erythematous erosions over vestibule and inner labia minora, consistent with comorbid LP and LS. B, Inner labium minus—degenerative pattern of erosive LP on the left and eroded hairless skin on the right. Arrow indicates vacuolar change. H&E, ×200. C, Labium majus—basal layer degeneration, subepithelial hyalinized collagen, and deep to that a sparse lymphocytic infiltrate, consistent with LS. H&E, ×200.

contiguous to the LP. A fortuitous specimen showed the junction of LS and LP seen as an abrupt end to the dermal homogenization of LS where the epithelium becomes eroded. The data and clinical photographs presented in this study suggest that the transition point sometimes occurs on hairless skin, lateral to Hart line. The premise that vulvar erosive LP involves hairless skin deserves further investigation.

There are several potential explanations for inattention to comorbid LP and LS. If clinicians identify and treat 1 dermatosis, there is less impetus to look for another diagnosis. Clinicians may be unfamiliar with subtle differences in color and texture that help distinguish LS and LP on vulvar skin.[3,17] The pale skin peripheral to LP may be labeled as Wickham striae, a clinical term without well-documented histopathological correlation on vulvar skin. Conversely, in women with a diagnosis of LS, clinicians may interpret the erythema of LP as candidal superinfection or "atrophy with labial fusion."[8,18] The histopathologic diagnosis of comorbid LP and LS usually requires 2 genital biopsies, and both women and clinicians may be reticent to do this.

The lack of consensus-based histopathologic diagnostic criteria for genital LP is another challenge to recognition of comorbid LP and LS. The rate of nonconfirmatory biopsy in clinically diagnosed LP is at least 30%, and the regenerative pattern of erosive LP has only recently been described.[9,19–21] In this series, erosion could not be definitively diagnosed in 3 cases, but the other histopathologic criteria for vulvar LP were met. The interpretation of genital skin biopsies showing that a lichenoid tissue reaction without subepidermal abnormal collagen remains the subject of investigation and controversy.[22–24] Pathologists should inspect for subtle features such as focal hyalinization and favor LS if present.

It may be suggested that this study has little clinicopathological significance because LP and LS are both lichenoid dermatoses managed similarly with topical steroid ointment. However, nonrecognition of comorbid pathology has implications in the realms of clinical management, health care policy, and research. Failure to identify both diagnoses may be associated with inadequate or misplaced topical therapies, resulting in iatrogenic treatment failure. Anterior fusion or introital scarring ascribed to LS may instead relate to LP, and the potential for vaginal agglutination in LP may be overlooked. The extragenital sites associated with each diagnosis may not be examined if the vulvar disease goes unrecognized. The UK national guideline on vulvar conditions advises long-term specialist management for LP, while women with treatment-responsive LS are directed to ongoing care with a general practitioner.[25] Previous studies of vulvar LS and LP that have not considered the possibility of comorbid disease should be critically appraised. Interventional studies of steroid alternatives should take into account that these agents may have different efficacy and risks in each diagnosis.[21] In studies of the association between chronic dermatoses and vulvar cancer, failure to recognize both dermatoses may lead to misattribution of neoplastic risk.[9,17,26]

There are manifold implications for future research. It is unknown what proportion of vulvar LP cases is accompanied by LS and vice versa; this study did not aim to address that question. Because vulvar LP is less common than LS, it is possible that a significant fraction of LP cases have comorbid LS, whereas proportionally fewer LS cases are complicated by LP. In designing severity scores for assessment of LS and LP, comorbid disease might be considered within those tools rather than automatically excluded. This study augments the argument that clinical trials enrolling women with LS and LP should incorporate histopathology and use this information to stratify response to interventions.[21] It is unknown whether different treatment approaches may be useful for regenerative versus degenerative patterns of LP or whether all cases cycle between these 2 phases.

TABLE 2. Clinical Characteristics of Comorbid LP and LS

	***n* = 26**
Age, median (range)	69.5 (43–90)
Autoimmune disease, *n* (%)	3 (11.5)
Diabetes mellitus, *n* (%)	5 (19)
Medications at biopsy, *n* (%)	
Topical corticosteroids	12 (46)
Estrogen	4 (15)
Candidosis, *n* (%)	6 (23)
Candida albicans culture	3 (11.5)
Antimycotic for clinical suspicion	3 (11.5)
Cases requiring additional therapies, *n* (%)[a]	10 (38)
Compounded clobetasol, *n*	3
Estrogen, *n*	4
Other, *n*	6
Comorbid HSIL vulva, *n* (%)	1 (4)
Surgery for dermatosis, *n* (%)	3 (11.5)
Suboptimal compliance, *n* (%)	1 (4)

[a]Some cases required more than one of the therapies listed.
HSIL indicates high-grade squamous intraepithelial lesion.

Access to biopsies from clinics specialized in vulvovaginal disorders permits the study of comorbid LP and LS but introduces a selection bias of more difficult cases with recalcitrant symptoms or dramatic clinical findings. The limitations of this study are those inherent to the retrospective design including incomplete clinical data and differences in practice between clinicians. A comprehensive examination of the mouth and extragenital skin was not always documented. Less than half of cases had microbiological assessment; of those who did, one quarter had a candidal superinfection. Universal clinical photography would have permitted a more nuanced description of the anatomic location and architectural change associated with the dual diagnoses. The nonconcurrent LS biopsies were not retrievable in many cases, either because they went to other pathology services or occurred many years previously. The LS diagnosis was considered to be reliable in the context of a pathology report describing hyalinized collagen, another biopsy of a lichenoid tissue reaction, and a clinical diagnosis of LS usually made by a specialist.

In summary, comorbid vulvar LP and LS are not rare; clinicians suspecting one should evaluate for signs of the other and consider separate biopsies of morphologically distinct areas. Clinicopathological correlation is an invaluable tool in assessing biopsies when comorbid LP and LS are suspected, because the regenerative pattern of LP may otherwise be overlooked or misdiagnosed.

REFERENCES

1. Terlou A, Santegoets LA, van der Meijden WI, et al. An autoimmune phenotype in vulvar lichen sclerosus and lichen planus: a Th1 response and high levels of microRNA-155. *J Invest Dermatol* 2012; 132:658–66.
2. Funaro D. Lichen sclerosus: a review and practical approach. *Dermatol Ther* 2004;17:28–37.
3. Moyal-Barracco M, Edwards L. Diagnosis and therapy of anogenital lichen planus. *Dermatol Ther* 2004;17:38–46.
4. Day T, Bohl T, Scurry J. Perianal lichen dermatoses: a review of 60 cases. *Australas J Dermatol* 2016;57:210–5.
5. Marren P, Millard P, Chia Y, et al. Mucosal lichen sclerosus/lichen planus overlap syndromes. *Br J Dermatol* 1994;131:118–23.
6. Belfiore P, Di Fede O, Cabibi D, et al. Prevalence of vulval lichen planus in a cohort of women with oral lichen planus: an interdisciplinary study. *Br J Dermatol* 2006;155:994–8.
7. Holmes SC, Burden AD. Lichen sclerosus and lichen planus: a spectrum of disease? Report of two cases and review of the literature. *Clin Exp Dermatol* 1998;23:129–31.
8. Yahiro C, Oika M, Fukunaga A, et al. Mucosal lichen sclerosus/lichen planus overlap syndrome with cutaneous lesions of lichen sclerosus. *Eur J Dermatol* 2016;26:204–5.
9. Day T, Bowden N, Jaaback K, et al. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *J Low Genit Tract Dis* 2016;20:174–9.
10. Derrick EK, Ridley CM, Kobza-Black A, et al. A clinical study of 23 cases of female anogenital carcinoma. *Br J Dermatol* 2000;143:1217–23.
11. Kirtschig G, Wakelin SH, Wojnarowska F. Mucosal vulval lichen planus: outcome, clinical and laboratory features. *J Eur Acad Dermatol Venereol* 2005;19:301–7.
12. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust N Z J Obstet Gynaecol* 2010;50:148–52.
13. Simpson RC, Murphy R, Bratton DJ, et al. Systemic therapy for vulval Erosive Lichen Planus (the 'hELP' trial): study protocol for a randomised controlled trial. *Trials* 2016;17:2.
14. Day T, Holland SM, Scurry J. Normal vulvar histology: variation by site. *J Low Genit Tract Dis* 2016;20:64–9.
15. Lewis FM, Neill SM. Principles of examination, investigation and treatment. In Neill SM, Lewis FM, eds. Ridley's The Vulva, 3rd ed. London: Wiley-Blackwell 2009;34–43.
16. Calonje JE, McKee P, Brenn T, et al Lichenoid and interface dermatitis. In: Calonje JE, Brenn T, Lazar A, et al, eds. McKee's Pathology of the Skin With Clinical Correlations. London: Elsevier; 2011;222–7.
17. Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014;71:698–707.
18. Day T, Borbolla Foster A, Phillips S, et al. Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis? *J Low Genit Tract Dis* 2016;20:267–71.
19. Simpson RC, Littlewood SM, Cooper SM, et al. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. *Br J Dermatol* 2012;167:85–91.
20. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Low Genit Tract Dis* 2013;17:28–32.
21. Neill SM, Lewis FM. Vulvovaginal lichen planus: a disease in need of a unified approach. *Arch Dermatol* 2008;144:1502–3.
22. Weyers W. Hypertrophic lichen sclerosus sine sclerosis: clues to histopathologic diagnosis when presenting as psoriasiform lichenoid dermatitis. *J Cutan Pathol* 2015;42:118–29.
23. Fung MA, LeBoit PE. Light microscopic criteria for the diagnosis of early vulvar lichen sclerosus: a comparison with lichen planus. *Am J Surg Pathol* 1998;22:473–8.
24. Simpson RC, Thomas KS, Leighton P, et al. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br J Dermatol* 2013;169:337–43.
25. Edwards S, Bates CM, Lewis F, et al. 2014 UK National Guideline on the management of vulval conditions. *Int J STD AIDS* 2015;26:611–24.
26. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition? *Arch Dermatol* 2012;148:1314–6.

4.7 Classic and hypertrophic vulvar lichen planus

Most of the literature on vulvovaginal LP focuses on the erosive subtype, but classic and hypertrophic disease can also occur on the vulva. Knowledge about LP on keratinised skin is primarily extrapolated from non-genital disease. Classic LP appears with various morphologies, may spontaneously resolve, and is a diagnosis unfamiliar to non-dermatologists who may prescribe topical steroids without attempting to make a diagnosis. Thus, it is likely that vulval classic LP is often overlooked and undiagnosed; however, it is the least impactful of the three types and has not been implicated in neoplastic transformation. In contrast, non-genital hypertrophic LP has a dramatic appearance accompanied by severe symptoms and has an unclear neoplastic potential. On review of the vulval literature, the few reports or photographs of hypertrophic LP were inadequate to draw any conclusions regarding clinical appearance or behaviour. This study found that non-sclerotic lichenoid reactions may be grouped into 3 categories: classic LP, hypertrophic LP, and non-specific lichenoid reaction. Provisional diagnoses were correct in less than half of cases, again suggesting the importance of clinicopathologic correlation. A wide spectrum of appearances was documented for classic LP, while most cases of hypertrophic LP demonstrated a pattern of circumferential erythema extending over labia minora and transitioning to lichenification over the labia majora. Age, biopsy site, duration of symptoms, and previous treatment were similar across the three groups, so assumptions should not be made that non-specific cases represent ‘early LS’. Prospective research is required to better understand the clinical appearance and natural history of these lesions, as well as to assess the trajectory and neoplastic potential of hypertrophic LP.

OPEN

Classic and Hypertrophic Vulvar Lichen Planus

Tania Day, MD,[1,2] Julie Weigner, PhD,[3] and James Scurry, FRCPA[1,3]

Objectives: Three types of lichen planus (LP) occur on the vulva: erosive, classic, and hypertrophic. The latter 2 occur on keratinized skin and little is known about their clinicopathologic appearance.

Materials and Methods: Vulvar biopsies of keratinized skin reported as LP or "lichenoid" between 2011 and 2017 were reviewed. Inclusion required age of older than 18 years, a lichenoid tissue reaction, and insufficient abnormal dermal collagen to diagnose lichen sclerosus. Clinical and histopathologic data were collected and cases were categorized as hypertrophic, classic, or nonspecific lichenoid dermatosis. Descriptive statistics were performed and groups were compared with the Fisher exact test.

Results: Sixty-three cases met criteria for inclusion. Twenty-nine (46%) cases were categorized as hypertrophic LP, 21 (33%) as classic LP, and 13 (21%) as nonspecific lichenoid dermatosis. There were no significant differences in age, primary symptom, biopsy location, or duration of disease between the 3 groups. When compared with classic and nonspecific disease, hypertrophic LP was less likely to have comorbid dermatoses and more likely to be red, diffuse, have scale crust, and contain plasma cells in the infiltrate. Nonspecific disease had similar clinical features to classic LP but was less likely than the other 2 categories to have a dense lymphocytic infiltrate and exocytosis.

Conclusions: Vulvar LP on keratinized skin has a diversity of appearances and presents a clinicopathologic challenge. Further research is required to understand the natural history of hypertrophic LP and the underlying diagnosis of nonspecific lichenoid cases.

Key Words: vulva, hypertrophic lichen planus, classic lichen planus, nonspecific lichenoid, vulvar intraepithelial neoplasia

(*J Low Genit Tract Dis* 2018;22: 387–395)

Lichen planus (LP) is a T-cell mediated inflammatory dermatosis that affects both keratinized and nonkeratinized squamous epithelium.[1] Three types are described on the vulva: erosive, classic, and hypertrophic.[2,3] Previous research has focused on erosive LP, which usually occurs on nonkeratinized squamous epithelium of the vestibule and adjacent hairless skin of labia minora but may also extend into the vagina. It manifests as well-demarcated glazed erythema, often with a hyperkeratotic border. Histopathologic features of erosive LP include a thinned or eroded epithelium, evidence of basal layer degeneration or regeneration, and a closely applied band-like lymphocytic infiltrate.[4,5]

In contrast, LP on keratinized vulvar skin is infrequently discussed, and the histopathologic description is extrapolated from nongenital skin. Two cohort studies of vulvar LP that specified clinicopathologic subtype noted 6% to 29% cases were hypertrophic and 4% to 6% were classic.[2,6] The textbook description of classic LP is pruritic papules and plaques of variable color that occur anywhere and spontaneously resolve. Hypertrophic LP is usually characterized as thick violaceous plaques on extensor surfaces of lower extremities; perianal skin is reported as a site of predilection but this is not well documented.[3,7,8] Controversy continues regarding the association between LP and squamous cell carcinoma (SCC), with scant evidence for malignant transformation of both hypertrophic and erosive LP.[9–12]

This study aims to describe the clinical and histopathologic characteristics of LP on vulvar keratinized skin and categorize cases as classic or hypertrophic.

MATERIALS AND METHODS

The Pathology New South Wales, Hunter New England database was searched for "lichen planus," "lichenoid," and "vulva" between 2011 and 2017. Reports were reviewed to select biopsies from hairless and/or hair-bearing skin interpreted as LP or lichenoid tissue reaction. All cases were from women older than 18 years. Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) slides were reviewed. Immunohistochemistry for p16 and p53 was performed for standard indications—to assist in distinguishing between reactive change, dermatosis-associated neoplasia, and human papillomavirus (HPV)-dependent lesions. The Hunter New England Research Ethics and Governance Unit approved this retrospective histopathologic case series (HREC 15/11/18/5.02); signed written consent was obtained for use of clinical photographs.

Inclusion required histopathologic evidence of the lichenoid reaction: a closely applied band-like infiltrate along with basal layer degeneration seen as apoptotic bodies, vacuolar change, and/or squamatization.[13] Specimens with multifocal or diffuse homogenized collagen in the papillary dermis were considered to demonstrate lichen sclerosus (LS) and were excluded.[14] Findings of scant unifocal sclerosis or a thickened basement membrane were considered to be insufficient for diagnosis of LS. Cases were categorized as classic LP if a lichenoid reaction was accompanied by acanthosis seen as spiky, sawtooth, or irregular rete ridges.[15]

Hypertrophic LP was defined as a lichenoid reaction accompanied by parakeratosis or hypergranulosis and marked acanthosis; supportive findings included hyperkeratosis and papillary dermal fibrosis.[13,15] Specimens lacking these distinguishing features were classified as nonspecific lichenoid reaction.[7,16] Biopsies were inspected for pseudoepitheliomatous hyperplasia (PEH), which shows epithelial architecture resembling SCC with separated nests and tentacles protruding into the dermis, but lacks the nuclear atypia and inflamed desmoplastic reaction characteristic of neoplasia.[7,17] Specimens were also reviewed for areas

[1]Faculty of Health and Medicine, University of Newcastle, Callaghan, New South Wales, Australia; [2]John Hunter Hospital, Maternity and Gynecology, New Lambton Heights, New South Wales, Australia; and [3]Pathology New South Wales, Hunter New England, Newcastle, New South Wales, Australia

Reprint requests to: Tania Day, MD, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Rd, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au

T.D. is supported by the Australian Government Research Training Program Scholarship.

The authors have declared they have no conflicts of interest.

This study was approved by Hunter New England Research Ethics and Governance Unit (HREC 15/11/18/5.02)

DOI: 10.1097/LGT.0000000000000419

of verruciform morphology and premature maturation without sufficient basal layer atypia to meet criteria for differentiated vulvar intraepithelial neoplasia (dVIN).[18,19]

Site was recorded as hair-bearing skin or hairless skin. Anatomic location was grouped into the following 3 zones: (1) mons and labium majus, (2) labium minus and periclitoris, and (3) perineum and perianus. The location of basal layer changes was labeled as diffuse, at tips of rete ridges, or at tops of papillary processes. Involvement of hair follicles and skin appendages by the lichenoid reaction was noted. Exocytosis and the dermal lymphocytic infiltrate were semiquantitatively assessed as sparse, moderate, or dense, and cell types were recorded. The PAS was inspected for presence of yeast or dermatophytes. Scale crust, dermal pigment incontinence, and focal collagen abnormalities were recorded as present or absent.

TABLE 1. Clinical Features of Vulvar Lichen Planus on Keratinized Skin

	All cases ($N = 63$)	Hypertrophic ($n = 29$)	Classic ($n = 21$)	Nonspecific lichenoid ($n = 13$)
Age, mean (SD), range, y	63 (15), 21–88	60 (16), 21–88	63 (14), 31–78	66 (13), 43–84
Biopsy location, *n* (%)				
Labium minus	18 (29%)	9 (31%)	5 (24%)	4 (31%)
Labium majus or mons	28 (44%)	12 (41%)	13 (62%)	3 (34%)
Perineum or perianus	17 (27%)	8 (28%)	3 (14%)	6 (46%)
Specialty, *n* (%)				
Gynecology	39 (62%)	19 (66%)	12 (57%)	8 (62%)
Dermatology	18 (28.5%)	7 (24%)	8 (38%)	3 (34%)
Other	6 (9.5%)	3 (10%)	1 (5%)	2 (15%)
Primary symptom				
Itch	51 (81%)	23 (79%)	17 (81%)	11 (85%)
Pain	8 (13%)	3 (10%)	3 (14%)	2 (15%)
Nil or unknown	4 (6%)	3 (10%)	1 (5%)	0
Duration of symptoms				
≥5 y	18 (29%)	7 (24%)	6 (24%)	5 (38%)
1–5 y	16 (25%)	9 (31%)	5 (31%)	2 (15%)
< 1 y	25 (40%)	11 (38%)	9 (43%)	5 (38%)
Unknown	4 (6%)	2 (10%)	1 (5%)	1 (8%)
Provisional diagnosis, *n* (%)				
Lichen planus	26 (41%)	9 (31%)	12 (57%)	5 (38%)
Lichen sclerosus	19 (30%)	8 (28%)	5 (23%)	6 (46%)
Lichen simplex chronicus	5 (8%)	4 (14%)	1 (5%)	0
Psoriasis	5 (8%)	3 (10%)	1 (5%)	1 (8%)
VIN	5 (8%)	4 (14%)	0	1 (8%)
Other	3 (5%)	1 (3%)	2 (10%)	0
Color, *n* (%)				
Red	43 (38%)	24 (83%)[a]	11 (52%)	8 (61%)
White or pink	15 (24%)	5 (17%)	6 (29%)	4 (31%)
Purple or brown	4 (6%)	0	4 (19%)	0
Not available	1 (2%)	0	0	1 (8%)
Distribution, *n* (%)				
Localized	23 (37%)	6 (21%)	10 (48%)	7 (54%)
Multifocal or diffuse	39 (62%)	23 (79%)[a]	10 (48%)	6 (46%)
Not available	1 (2%)	0	1 (5%)	0
Comorbid dermatosis, *n* (%)				
None or unknown	46 (73%)	27 (93%)[a]	11 (52%)	8 (62%)
Lichen planus elsewhere	8 (13%)	0	5 (24%)	3 (34%)
Lichen sclerosus	5 (8%)	2 (7%)	2 (10%)	1 (8%)
Psoriasis	4 (6%)	0	3 (14%)	1 (8%)
Vaginal swab results, *n* (%)				
Not done or unknown	28 (44%)	14 (48%)	10 (48%)	4 (31%)
Normal flora	27 (43%)	11 (38%)	9 (43%)	7 (54%)
Candida albicans	6 (10%)	3 (10%)	2 (10%)	1 (8%)
Nonalbicans candida	2 (3%)	1 (3%)	0	1 (8%)

[a] $p < .05$.

VIN indicates vulvar intraepithelial neoplasia; SD, standard deviation.

Clinical data obtained included provisional diagnosis, lesion appearance, previous treatments, symptoms and their duration, dermatologic and autoimmune comorbidities, microbiologic results, treatment and outcome, and duration of follow-up. Descriptive statistics were performed and group comparisons were made with the Fisher exact test.

RESULTS

After histopathologic review, 63 cases were included in the study. Twenty-nine (46%) cases were categorized as hypertrophic LP, 21 (33%) as classic LP, and 13 (21%) as nonspecific lichenoid reaction. There was no significant difference in age, clinician

TABLE 2. Histopathologic Characteristics of Vulvar Lichen Planus on Keratinized Skin

	All cases (N = 63)	Hypertrophic (n = 29)	Classic (n = 21)	Nonspecific lichenoid (n = 13)
Site, *n* (%)				
Hairless skin	24 (38%)	13 (45%)	5 (24%)	6 (46%)
Hair bearing skin	39 (62%)	16 (55%)	16 (76%)	7 (54%)
Stratum corneum, *n* (%)				
Normal	10 (16%)	2 (3%)	2 (10%)	6 (46%)
Hyperkeratosis	40 (64%)	18 (62%)	15 (71%)	7 (54%)
Parakeratosis	4 (6%)	4 (14%)	0	0
Both HK and PK	9 (14%)	5 (17%)	4 (19%)	0
Scale crust, *n* (%)	8 (13%)	8 (28%)[a]	0	0
Granular cell layer, *n* (%)				
Absent/variable due to PK	8 (13%)	6 (21%)	2 (10%)	0
Normal (≤5)	11 (17%)	0	3 (14%)	8 (62%)
Diffuse HG	25 (40%)	15 (52%)	5 (24%)	5 (38%)
Wedge-shaped HG	19 (30%)	8 (27%)	11 (52%)[a]	0
Acanthosis, *n* (%)				
Not present or atrophic	3 (5%)	0	0	3 (23%)
Flat or regular	9 (14%)	2 (7%)	0	7 (54%)
Irregular or sawtooth	34 (54%)	24 (83%)	8 (38%)	2 (15%)
Spiky	17 (27%)	3 (10%)	13 (62%)[a]	1 (8%)
Exocytosis, *n* (%)				
Absent	3 (5%)	3 (10%)	0	0
Sparse	39 (62%)	17 (59%)	10 (48%)	12 (92%)
Moderate to dense	21 (33%)	9 (31%)	11 (52%)	1 (8%)[a]
Basal layer manifestations,[b] *n* (%)				
Apoptotic bodies	51 (81%)	26 (90%)	17 (81%)	8 (62%)
Vacuolar change	32 (51%)	11 (38%)	12 (57%)	9 (69%)
Squamatization	46 (73%)	22 (76%)	16 (76%)	8 (62%)
Site of basal layer changes, *n* (%)				
Diffuse	41 (65%)	16 (55%)	16 (76%)	9 (69%)
Rete tips only	14 (22%)	7 (24%)	4 (19%)	3 (23%)
Tops of papillary processes only	3 (5%)	2 (7%)	0	1 (8%)
Both tips and tops	5 (8%)	4 (14%)	1 (5%)	0
Infiltrate, *n* (%)				
Sparse	1 (2%)	0	0	1 (8%)
Moderate	13 (21%)	7 (24%)	1 (5%)	5 (38%)
Dense	49 (78%)	22 (76%)	20 (95%)	7 (54%)[a]
Cell types within infiltrate, *n* (%)				
Eosinophils	19 (30%)	10 (35%)	7 (33%)	2 (15%)
Plasma cells	15 (24%)	11 (38%)[a]	1 (5%)	3 (23%)
Neutrophils	5 (8%)	3 (10%)	2 (10%)	0
Pigment incontinence, *n* (%)	38 (60%)	11 (38%)[a]	17 (81%)	10 (77%)
Skin appendages involved, *n* (%)	5 (8%)	0	5 (24%)[a]	0
Scant focal sclerosis, *n* (%)	7 (11%)	4 (14%)	2 (10%)	1 (8%)
Fibrosis, *n* (%)	12 (19%)	7 (24%)	2 (10%)	3 (23%)

[a] $p < .05$.

[b] Each case may have more than 1 basal layer finding.

HK indicates hyperkeratosis; PK, parakeratosis; HG, hypergranulosis.

specialty, primary symptom, biopsy location, or duration of symptoms between the 3 categories (see Table 1). Nine cases had a systemic autoimmune disease identified: 4 (14%) in classic LP, 3 (10%) in hypertrophic LP, and 2 (15%) in nonspecific lichenoid. These included thyroid disease in 3, systemic lupus erythematosus in 2, and 1 each with Crohn disease, immune thrombocytopenic purpura, polymyalgia rheumatica, and an unclear autoimmune condition. Topical corticosteroids were prescribed before referral in 26 (41%) of cases with similar rates across the 3 disease types.

Clinicians identified a provisional diagnosis of LP in less than half of cases, instead suspecting LS, lichen simplex chronicus, psoriasis, VIN, Hailey-Hailey disease (1), granulomatous lesions (1), and estrogen deficiency (1). Body mass index was documented in 24 (38%) of 63, with a mean of 31 (range = 22–40). Of 35 (55%) cases with microbiology, 27 (77%) had normal flora, 6 (17%) grew *Candida albicans*, and 6% showed nonalbicans species; a swab was not obtained in 24 (38%) of 63 cases, and data were unavailable in 4 (6%). Women with a positive swab for *C. albicans* ranged in age from 62 to 77 years, of whom 3 used vaginal estrogen, 1 was on systemic estrogen, 1 had diabetes mellitus, and 1 had no reported risk factors. Treatment information was available in 59 (94%) cases; 58 (98%) were prescribed potent topical corticosteroid ointment and 1 declined further care after treatment for vulvar cancer. Adjunctive medications included antimycotics (7, 13%), topical or systemic estrogen (5, 9%), antibiotics (4, 7%), oral prednisone (1, 2%), and topical tacrolimus (1). Lesion resolution was documented in 6 (11%) cases, of which 5 were classic LP and 1 was nonspecific lichenoid. Five (9%) women were lost to follow-up, 4 (7%) had suboptimal response or adherence to treatment, and the remainder were improved on chronic therapy with a mean follow-up of 24 months.

Biopsy site of hairless skin versus hair-bearing skin was not significantly different across the 3 categories of disease (see Table 2). No case had evidence of mycosis on PAS. Most specimens showed hyperkeratosis (78%), hypergranulosis (70%), irregular or spiky acanthosis (81%), and a moderate to dense band-like lymphocytic infiltrate (98%). Exocytosis was lymphocytic in all but 3 cases—1 also had plasma cells and 2 had eosinophils, with neutrophils in 1 of these. All but 5 (8%) cases showed more than 1 manifestation of basal layer degeneration; the abnormality was confined to either rete tips or tops of papillary processes in 17 (27%). Lymphocytes and histiocytes were the primary cell types in the dermal infiltrate. Three cases had a granulomatous component to the infiltrate—2 were hypertrophic LP and 1 was nonspecific lichenoid. There were no differences across the 3 categories with regard to papillary dermal fibrosis and scant focal sclerosis.

The most common description of classic LP was a well-circumscribed, unilateral, homogenous, slightly raised plaque (see Figure 1). Classic LP lesions were red, purple, brown, or grey-white and sometimes noted to be "subtle" or "unusual." Comorbid dermatoses included psoriasis in 2, biopsy-proven LS in 2, orolabial LP in 2, classic LP of the eyelid in 1, scalp lichen planopilaris in 1, and vulvovaginal erosive LP in 1. Classic LP was more likely to demonstrate spiky acanthosis (13/21 [62%] vs 4/42 [10%], $p = .0001$) and wedge-shaped hypergranulosis (11/21 [52%] vs 8/42 [19%], $p = .01$) than the other 2 categories (see Figures 2, 3). Classic LP was the only type that involved the hair follicles and/or skin appendages (5/21 [24%] vs 0, $p = .003$) (see Figure 3). Two (10%) cases showed PEH, and there was no previous or concurrent VIN.

Clinical features of the nonspecific lichenoid category were similar to cases diagnosed as classic LP. No clinical photographs were available. Comorbid dermatoses included nongenital classic LP in 2, vulvar erosive LP in 1, and psoriasis in 1. One case was identified by perianal biopsy done concurrently with anterior vulvectomy for LS-associated SCC. A vulvar high-grade squamous intraepithelial lesion (HSIL) occurred in 1 woman, confirmed by block positive p16. One case was managed with imiquimod and LASER ablation after a pathology report of "VIN2," with subsequent clinicopathologic review demonstrating a lichenoid reaction and no evidence of HPV-dependent disease. The histopathologic features of nonspecific lichenoid reaction included hyperkeratosis (54%), a normal granular cell layer (62%), and flat or regular acanthosis (54%), although there was a spectrum of appearances (see Figure 4). Nonspecific lichenoid cases were less likely than classic and hypertrophic LP to have a dense lymphocytic infiltrate (7/13 [54%] vs 42/50 [84%], $p = .03$) and moderate to dense exocytosis (1/13 [8%] vs 20/50 [40%], $p = .04$). None had PEH.

Hypertrophic LP was more likely to be described as a red (24/29 [83%] vs 19/34 [56%], $p = .02$), diffuse abnormality

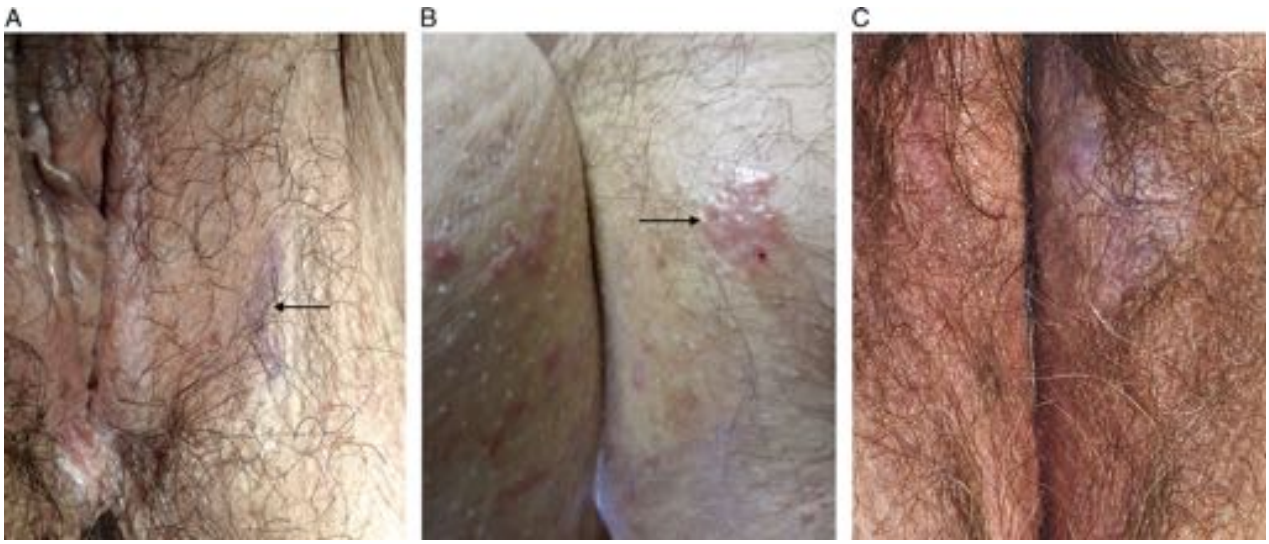

FIGURE 1. Classic lichen planus: subtle brown-purple plaque on left labium majus (A), red plaque on right mons pubis (B), and grey-pink plaque on left labium majus (C).

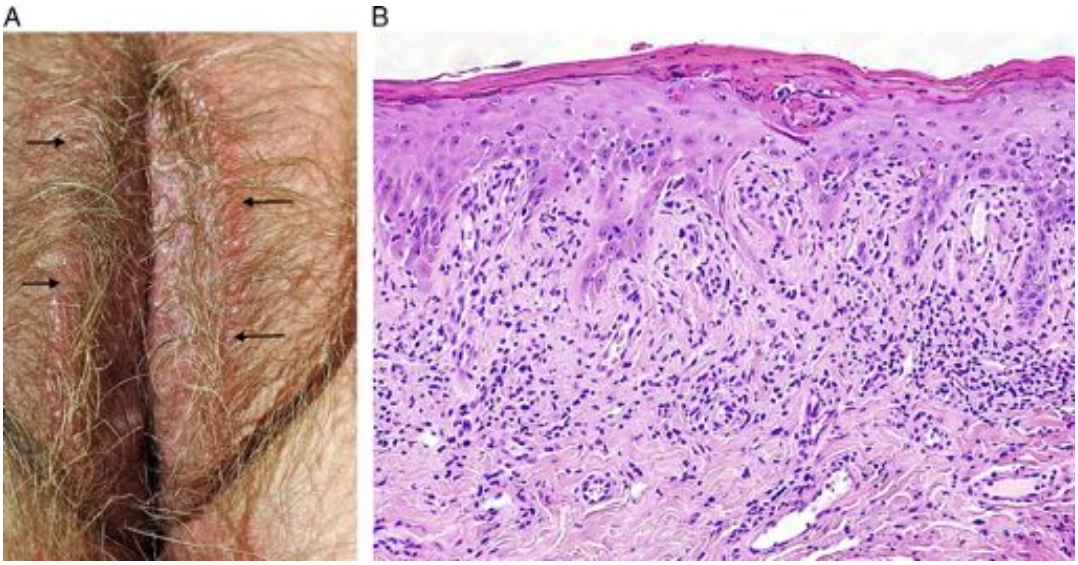

FIGURE 2. Classic lichen planus: circumferential erythematous plaque most prominent on hair bearing skin of labia majora (A), parakeratosis spiky acanthosis, and moderate lymphocytic infiltrate (B), H&E ×100.

(23/29 [79%] vs 16/34 [47%], $p = .01$) when compared with classic and nonspecific disease and was less likely to have other dermatoses identified (27/29 [93%] vs 19/34 [56%], $p = .001$) (see Figure 5). The 2 comorbid diagnoses were both biopsy-proven LS adjacent to perineal/perianal LP. Clinical photographs demonstrate a pattern of circumferential erythema extending over labia minora and partially across labia majora, transitioning to lichenification laterally (see Figures 6, 7). Compared with classic LP and nonspecific lichenoid, hypertrophic LP more often had scale crust (8/29 [28%] vs 0, $p = .001$) and plasma cells in the infiltrate (11/29 [38%] vs 4/34 [12%], $p = .02$) and was less likely to show pigment incontinence (11/29 [38%] vs 27/34 [79%], $p = .02$). Four (14%) specimens showed PEH. Two cases contained a differentiated verruciform lesion: 1 had previous treatment of microinvasive SCC and several subsequent excisions of dVIN in a field of nonspecific lichenoid dermatosis, whereas the other had lesion resolution after treatment with corticosteroids and antimycotics. p53 was obtained in 2 cases to distinguish reactive versus atypical nuclear changes, and both were wild-type.

DISCUSSION

Lichen planus on vulvar keratinized skin has a diversity of appearances and presents a diagnostic challenge to both clinicians and pathologists. Hypertrophic LP has the most dramatic clinical presentation and is the least described in the literature, perhaps accounting for low rates of accurate provisional diagnosis. Although its circumferential distribution is similar to LS, hypertrophic LP lacks porcelain-white pallor, and instead demonstrates beefy erythema and edema of inner vulva, often with a macerated or rind-like surface and transition to lichenification laterally.[3,20] Thick red plaques lead to confusion with psoriasis, extramammary Paget

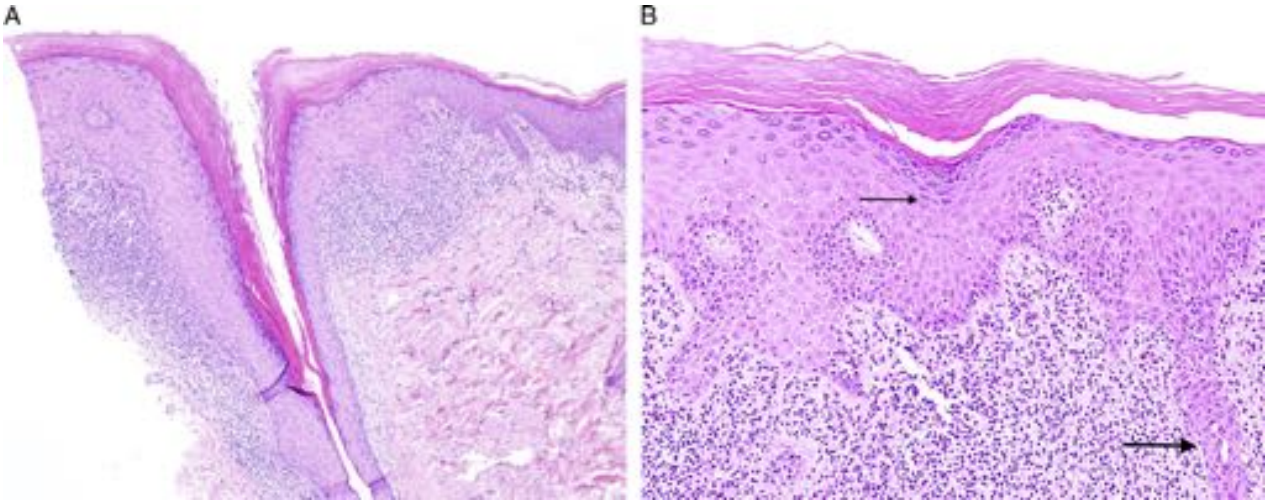

FIGURE 3. Classic lichen planus with appendageal involvement: hyperkeratosis, hypergranulosis, irregular acanthosis, and dense lymphocytic infiltrate, with involvement of the hair follicle (A), H&E ×40, and wedge shaped hypergranulosis (thin arrow), spiky acanthosis, and involvement of the eccrine gland (thick arrow) (B), H&E ×100.

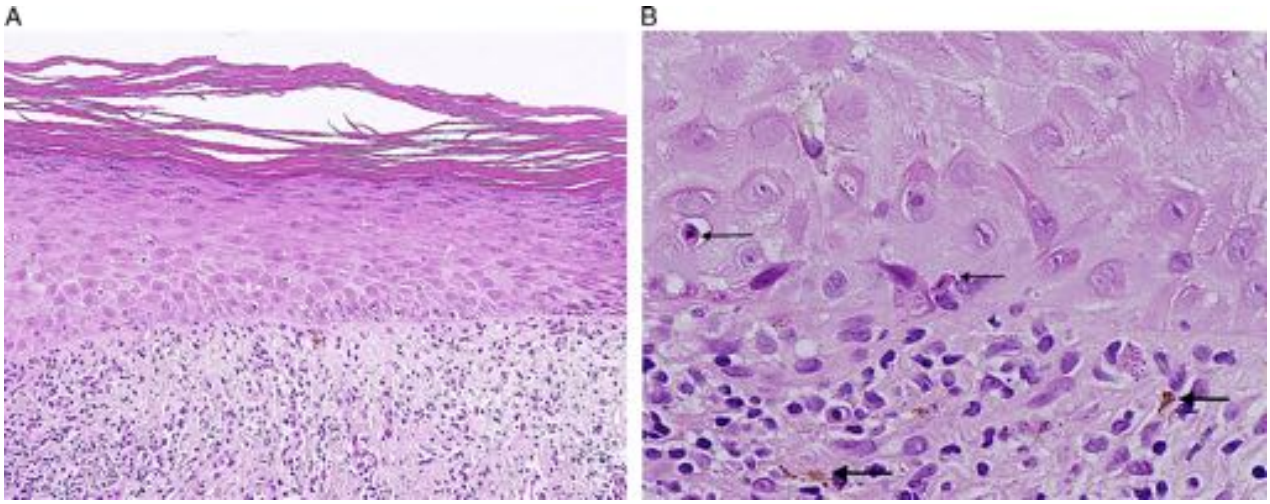

FIGURE 4. Nonspecific lichenoid reaction: hyperkeratosis, hypergranulosis, flat acanthosis, thickened basement membrane, moderate lymphocytic infiltrate, and normal dermal collagen (A), H&E ×100 and dermoepidermal interface with apoptotic bodies (thin arrows) and pigment incontinence (thick arrows) (B), H&E ×400.

disease, and HSIL, although the latter 2 usually display an asymmetric distribution and distinct histopathologic features. However, hypertrophic LP, nodular prurigo, and lichenified psoriasis represent a difficult differential diagnosis, because all demonstrate papillary dermal fibrosis and marked acanthosis. Among these 3, the sole factor that distinguishes hypertrophic LP is basal layer degeneration, which may be masked or mimicked by inflammation relating to superinfection. This study identifies that basal layer degeneration may be diffuse or confined to tops of papillary processes, in contrast to the textbook description of damage restricted to tips of rete ridges.[13] Thus, both "tips" and "tops" must be carefully inspected for vacuolar change, squamatization, and apoptotic bodies, with the latter being most useful when attempting to distinguish between marked exocytosis and true basal layer damage. In addition, PEH may be confused for microinvasive SCC and granulomatous infiltrates may be misinterpreted as systemic autoimmune or infectious diseases.[21]

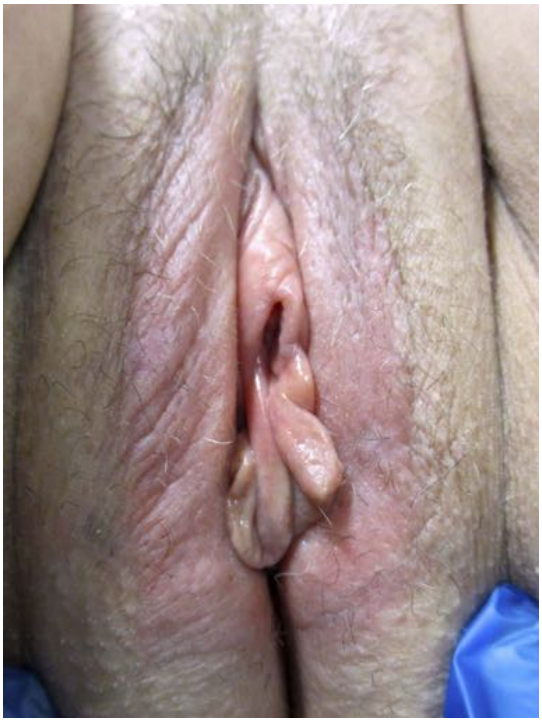

FIGURE 5. Hypertrophic lichen planus: circumferential erythema and edema extending midway across the labia majora.

Classic LP is a more straightforward clinicopathologic diagnosis, although the range of colors and patterns may be unfamiliar to nondermatologists.[20] A few cases have a normal stratum corneum and/or granular cell layer, so diagnosis relies on rete ridge abnormalities in combination with the lichenoid reaction. The 24% rate of resolution may be an underestimate related to duration of follow-up or misidentification of postinflammatory pigmentation as ongoing disease or could reflect a different natural history of vulvar versus nongenital disease.

A fifth of biopsies did not meet criteria for diagnosis of either hypertrophic or classic LP. It is unclear whether these nonspecific lichenoid cases are part of the spectrum of vulvar LP or whether they primarily represent LS in a nonsclerotic or minimally fibrotic phase.[14,22] Nonspecific lichenoid cases had fewer lymphocytes in both dermis and epidermis, perhaps indicating less severe inflammation. This cannot be explained by duration of disease or previous treatment, because these were similar across disease categories. Although not a statistically significant difference, the rate of perianal/perineal biopsies was highest in nonspecific lichenoid reaction; biopsies performed elsewhere might display more identifiable histopathologic features.[16]

Assessment of all 3 categories of nonerosive vulvar LP is complicated by high rates of comorbid dermatologic and infectious disease. All lichenoid dermatoses are associated with each other: however, each diagnosis and site have different management strategies and associated risks of neoplasia.[4,8,10,12,23,24] Thus, it is important to obtain the most accurate diagnoses across all locations, which often requires biopsies of morphologically distinct areas and thoughtful clinicopathologic correlation. Psoriasis was identified in 14% of classic LP cases, more than the 5% documented in a retrospective cohort of erosive LP.[25] The true prevalence of candidal superinfection of LP is unknown. Retrospective cohorts of LP have documented rates from 4% to 25%, but most studies make no comment on surveillance for

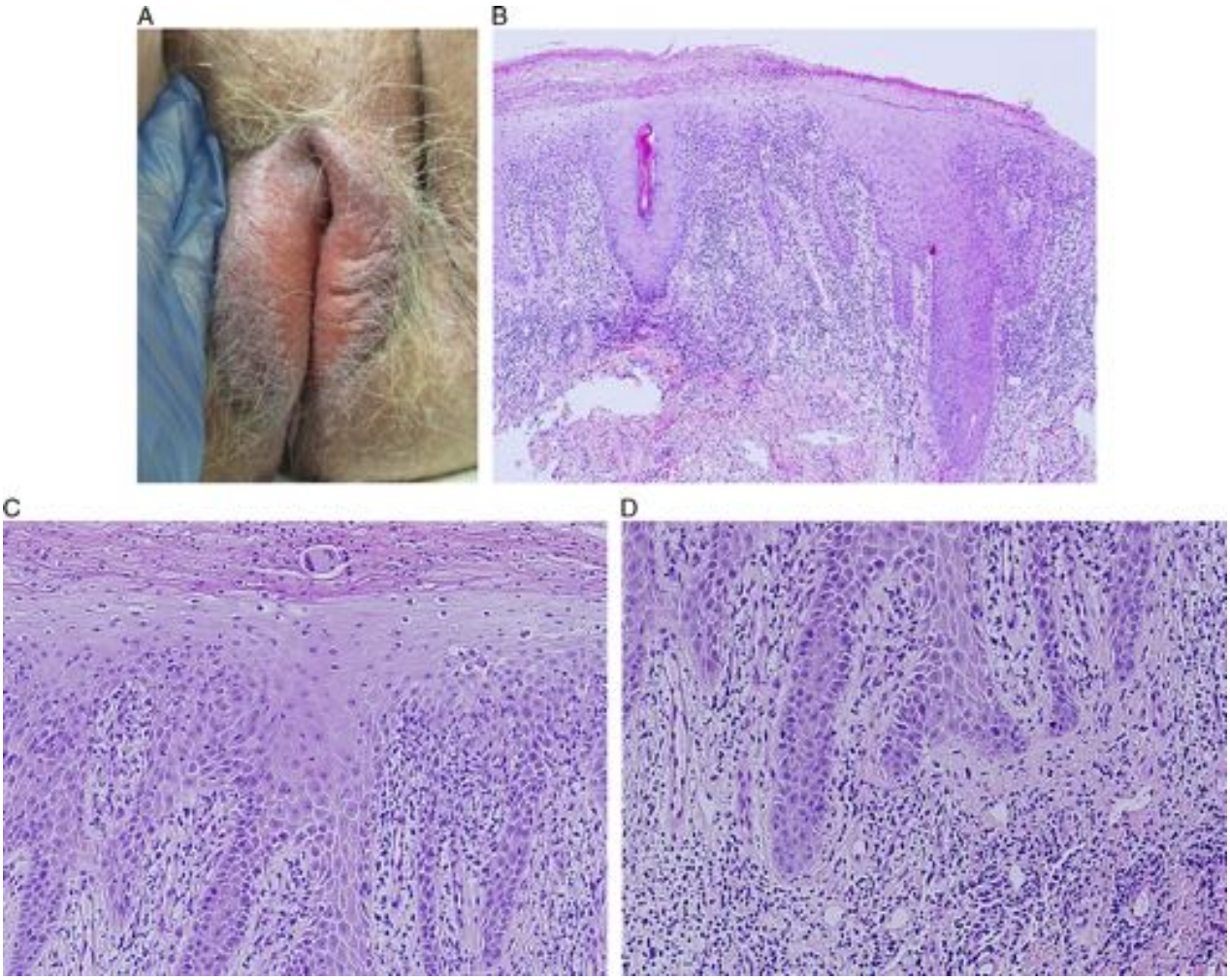

FIGURE 6. Hypertrophic lichen planus: erythema and edema of inner vulva transitioning to grey-pink lichenification of bilateral labia majora (A), scale crust accompanied by marked irregular acanthosis and dense infiltrate (B), H&E ×40, apoptotic bodies and squamatization predominantly involving the tops of papillary processes (C), H&E ×100, with minimal basilar abnormality at the tips of rete ridges (D), H&E ×100.

mycosis.[4,26,27] Likewise, it is unclear whether rates of candidosis relate to topical corticosteroids, exogenous estrogen, or medical conditions associated with lichenoid dermatoses.[27–29] These questions would best be addressed prospectively with a systematic approach to detection and detailed notation of medication exposures and risk factors.

There were 3 cases of vulvar neoplasia in this cohort: 1 case of HSIL (usual VIN), 1 of LS-associated SCC with a noncontiguous lichenoid reaction, and 1 with previous SCC and recurrent dVIN. Perilesional histopathology of the latter showed nonspecific lichenoid on 3 occasions and was diagnostic for hypertrophic LP once. Clinical photographs were consistent with hypertrophic LP, yet the long-standing clinical diagnosis was LS and biopsy was not obtained until concern for neoplasia arose. These 3 examples highlight the challenges in establishing the neoplastic risk of vulvar LP: LS and hypertrophic LP may be difficult to distinguish clinically, multiple diagnoses may coexist and be adjacent or noncontiguous, and some forms of HSIL and dVIN have a similar histopathologic appearance.[5,10,30] Differentiated verruciform lesions occurred in 2 cases of hypertrophic LP; these may be precursors to dVIN, may represent a distinct pathway to HPV-independent vulvar SCC, or may be an exaggerated but reversible response to inflammation and the itch-scratch cycle.[10,18]

Inherent to the retrospective design, limitations of this study include incomplete data, variations in individual practice patterns, and a bias toward unusual or difficult cases more likely selected for vulvar biopsy. Clinicians in obesity-endemic areas may be more likely to document body mass index than those in other settings. Nonperformance of microbiologic studies may be due either to well-informed low suspicion for mycosis or a lack of awareness of superinfection in chronic vulvar dermatoses. Universal clinical photography would allow for better representation of the patterns of each disease type and determination of best-fit diagnoses for nonspecific lichenoid reactions.

In summary, vulvar hypertrophic LP usually has a dramatic presentation of circumferential erythematous plaques seen on microscopy as a pronounced inflammatory band against markedly lichenified epithelium, whereas classic LP has a spectrum of lesion color and morphology seen as a lichenoid reaction with spiky or irregular acanthosis. Perhaps because of the unique vulvar milleu, both diseases may lack the pathognomonic findings of their nongenital counterparts. Research with a focus on clinicopathologic correlation is required to elucidate the underlying diagnosis of

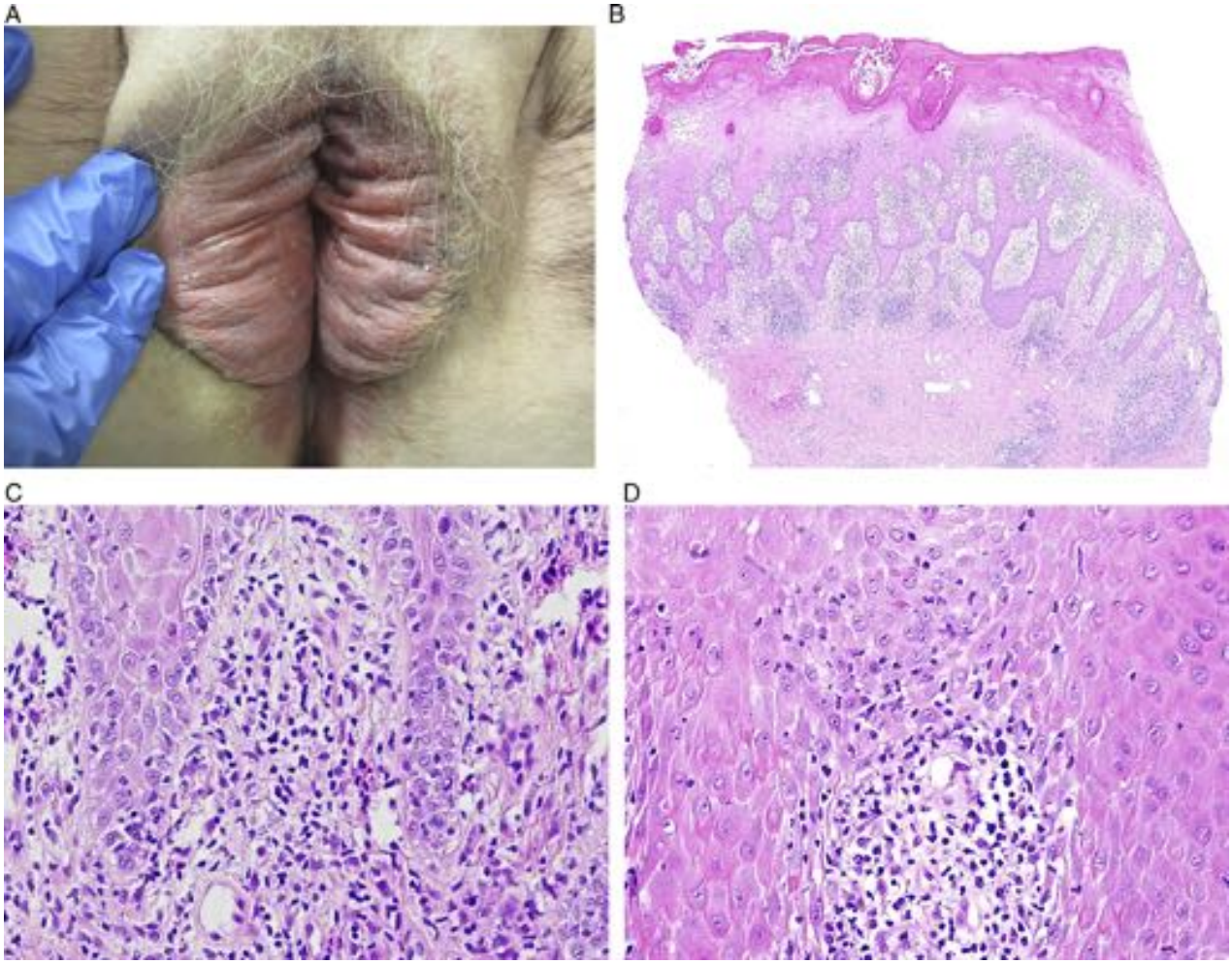

FIGURE 7. Hypertrophic lichen planus: dusky erythema, edema, and a macerated appearance of inner vulva, transitioning to grey-pink lichenification of bilateral labia majora (A), scale crust with parakeratosis, marked irregular acanthosis, and dense infiltrate (B), H&E ×40, with basal layer degeneration at the rete tips (C), H&E ×200, and tops of papillary processes (D), H&E ×200.

nonspecific lichenoid cases and to better describe the natural history and neoplastic potential of hypertrophic LP.

REFERENCES

1. Terlou A, Santegoets LA, van der Meijen WI, et al. An autoimmune phenotype in vulvar lichen sclerosus and lichen planus: a Th 1 response and high levels of microRNA-155. *J Invest Dermatol* 2012;132:58–66.
2. Chew A, Stefanato C, Savarese I, et al. Clinical patterns of lichen planopilaris in patients with vulval lichen planus. *Br J Dermatol* 2014;170: 218–20.
3. Goldstein A, Metz A. Vulvar lichen planus. *Clin Obstet Gynecol* 2005;48: 818–23.
4. Day T, Moore S, Bohl TG, et al. Cormorbid vulvar lichen planus and lichen sclerosus. *J Low Genit Tract Dis* 2017;21:204–8.
5. Day T, Bowden N, Jaaback K, et al. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *J Low Genit Tract Dis* 2016;20:174–9.
6. Belfiore P, Di Fede O, Cabibi D, et al. Prevalence of vulval lichen planus in a cohort of women with oral lichen planus: an interdisciplinary study. *Br J Dermatol* 2006;155:994–8.
7. Day T, Bohl TG, Scurry J. Perianal lichen dermatoses: a review of 60 cases. *Australas J Dermatol* 2016;57:210–5.
8. McPherson T, Cooper S. Vulval lichen sclerosus and planus. *Dermatol Ther* 2010;23:523–32.
9. Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014;71: 698–707.
10. Day T, Otton G, Jaaback K, et al. Is vulvovaginal lichen planus associated with squamous cell carcinoma? *J Low Genit Tract Dis* 2018;22:159–65.
11. Ramos-e-Silva M, Jacques C, Carneiro SC. Premalignant nature of oral and vulval lichen planus: facts and controversies. *Clin Dermtol* 2010; 28:563–7.
12. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition? *Arch Dermatol* 2012;148:1314–6.
13. Patterson JW, Hosler GA. Tissue reaction patterns, Section 2, and The dermis and subcutis, Section 4. In: Patterson JW, ed. *Weedon's Skin Pathology* 4th ed. London: Churchill Livingstone Elsevier. 2016; 37–80, 362–4.
14. Weyers W. Hypertrophic lichen sclerosus sine sclerosis: clues to histopathologic diagnosis when presenting with psoriasiform lichenoid dermatitis. *J Cutan Pathol* 2015;42:118–29.
15. Wang W, Lazar A. Lichenoid and interface dermatitis, Chapter 7. In: Calonje E, Brenn T, Lazar A, et al., eds. *McKee's Pathology of the Skin With Clinical Correlations*. 4th ed. London: Elsevier; 2012:219–58.

16. Day T, Knight V, Dyall-Smith D, et al. Interpretation of non-diagnostic vulvar viopsies. *J Low Genit Tract Dis* 2018;22:74–81.
17. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. *Int J Gynecol Pathol* 2003;22:57–62.
18. Watkins JC, Howitt BE, Horowitz NS, et al. Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA. *Mod Path* 2017;30: 448–58.
19. Nascimento AF, Granter SR, Cviko A, et al. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinioma? *Am J Surg Path* 2004; 28:638–43.
20. Moyal-Barracco M, Edwards L. Diagnosis and therapy of anogenital lichen planus. *Dermatol Ther* 2004;17:38–46.
21. Ashton KA, Scurry J, Rutherford J, et al. Nodular prurigo of the vulva. *Pathology* 2010;44:565–7.
22. Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. *Am J Dermatopath* 2001;23: 413–8.
23. Lewis FM, Tatnell FM, Velangi SS, et al. British Association of Dermatologists guideline for the management of lichen sclerosus, 2018. *Br J Dermatol* 2018;178:839–953.
24. Cooper SM, Wojnarowska F. Influence of treatment of erosive lichen planus of the vulva on its prognosis. *Arch Dermatol* 2006; 142:289–94.
25. Kirtschig G, Wakelin SH, Wojnarowska F. Mucosal vulval lichen planus: outcome, clinical and laboratory features. *J Eur Acad Dermatol Venereol* 2005;19:301–7.
26. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Low Genit Tract Dis* 2013;17:28–32.
27. Day T, Borbolla Foster A, Phillips S, et al. Can routine histopathology distinguish between vulvar cutaneous candidosis and dermatophytosis? *J Low Genit Tract Dis* 2016;20:174–9.
28. Virgili A, Borghi A, Cazzaniga S, et al. New insights into potential risk factors and associations in genital lichen sclerosus: data from a multicenter Italian study on 729 consecutive cases. *J Eur Acad Dermatol Venereol* 2017;31:699–704.
29. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust NZ. J Obstet Gynaecol* 2010;50:148–52.
30. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol* 2009;33: 1659–65.

4.8 Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia

The only previous mention of a regenerative pattern of vulval erosive LP occurred in an editorial on the need for a unified and multidisciplinary approach to the disease. The trigger for this study was a case involving a woman with diffuse vulval erythema and pain who had a biopsy interpreted as VIN. She underwent a partial skinning vulvectomy and the final specimen was negative for neoplasia; multidisciplinary review suggested a diagnosis of regenerative erosive LP. She subsequently was managed with topical steroids, had improvement in signs and symptoms, and has not had evidence of neoplasia on follow-up. The aim was to document the distinct clinical and histopathologic appearances of regenerative erosive LP compared to dVIN, in an effort to reduce the risk of similar cases occurring in future. Interestingly, LS was found in association with 4 of 5 cases of erosive LP and all cases of dVIN. IHC could not reliably distinguish between the two processes, although was helpful in two dVIN cases. Copy variant analysis was performed in one case in each category to add support to the clinicopathologic diagnosis. Although careful histopathologic assessment was important in identifying the correct diagnosis, even more critical was assessment of clinical features by an experienced vulvologist. Thus, in addition to duplicating Ordi's description of a 'basaloid' morphologic subtype of dVIN, this study underscores that prospective clinicopathologic correlation is essential prior to performance of an excisional procedure.

Distinguishing Erosive Lichen Planus From Differentiated Vulvar Intraepithelial Neoplasia

Tania Day, MD,[1,2] Nikola Bowden, PhD,[2,3] Ken Jaaback, CGO,[1] Geoff Otton, CGO,[1] and James Scurry, FRCPA[2,4]

Objective: Erosive lichen planus (LP) and differentiated vulvar intraepithelial neoplasia (dVIN) may display overlapping histopathologic features.

Materials and Methods: We searched the local pathology database for vulvar biopsies reported as dVIN or erosive vulvitis during 2011 to 2013 inclusive. After review of patient notes and slides, there were 5 cases with a clinical appearance and course consistent with erosive LP and histopathology showing epithelial regeneration. We then selected 5 cases of dVIN in which the clinical course and histopathology supported the diagnosis. We performed immunohistochemistry for p16 and p53 on all cases and did copy variant analysis on 1 case each of erosive LP and dVIN.

Results: Histopathology of the LP cases showed epithelial thinning, absent stratum corneum, lack of maturation, as well as nuclear changes of enlargement, pleomorphism, and hyperchromasia. Three LP cases (60%) showed a wild-type p53 pattern and 2 (40%) were confluent positive. Two dVIN cases (40%) showed full-thickness loss of differentiation. One case (20%) of dVIN was p53 negative, 2 (40%) were wild-type, 1 was confluent positive, and 1 showed dark suprabasilar staining. All cases were negative for p16. Compared with control, erosive LP epithelium showed a similar copy-number pattern, whereas the dVIN epithelium had many copy-number changes.

Conclusions: A small subset of clinically diagnosed vulvovaginal erosive LP will show on histopathology a regenerative erosive vulvitis with loss of epithelial maturation and nuclear changes, which requires clinicopathologic correlation to distinguish from dVIN.

Key Words: differentiated VIN, erosive lichen planus, atypia, regeneration, copy variant analysis

(*J Lower Gen Tract Dis* 2016;20: 174–179)

Vulvovaginal erosive lichen planus (LP) appears as painful, usually symmetric erythematous erosions on inner labia minora, vestibule, and/or vagina. Histopathologic diagnostic criteria are erosion, basal layer degeneration, which includes vacuolization, apoptotic bodies, and squamatization, and a band-like lymphocytic infiltrate in the lamina propria.[1,2] Studies of clinically diagnosed vulvovaginal LP report a nondiagnostic biopsy rate of 30% to 56%; 1 potential explanation is that the criteria for erosive LP are overly restrictive.[3,4]

Investigation into the histopathologic definition of oral LP (OLP) has yielded insights applicable to vulvovaginal erosive LP. T-cell–mediated basal layer damage is usually seen as degeneration, but regeneration may also occur and manifest as maturational disarray, nuclear enlargement, nuclear-cytoplasmic ratio reversal, and increased mitoses.[1,5,6] These changes also raise suspicion for neoplasia. Distinguishing between regenerative OLP, evolving neoplasia arising from OLP, and neoplasia related to other carcinogens is a challenge that inspires controversy among oral pathologists.[5]

The 2 squamous premalignant conditions of the vulva are high-grade squamous intraepithelial lesion (vulvar intraepithelial neoplasia [VIN], usual type) and differentiated VIN (dVIN). These are distinguishable on histopathology aided by immunohistochemistry (IHC) for p16, which is positive in human papillomavirus (HPV)–associated neoplasia and negative in dVIN. However, distinguishing dVIN from lichen sclerosus (LS) and LP may be difficult. One study reported that 42% of biopsies preceding non-HPV–related squamous cell carcinoma (SCC) read initially as LS were found to be dVIN on expert review.[7] Overexpression of p53 may occur in both dVIN and lichenoid dermatoses.[8]

We have observed that a subset of women with clinical vulvovaginal erosive LP show maturational disarray and nuclear changes on histopathology, which are difficult to distinguish from

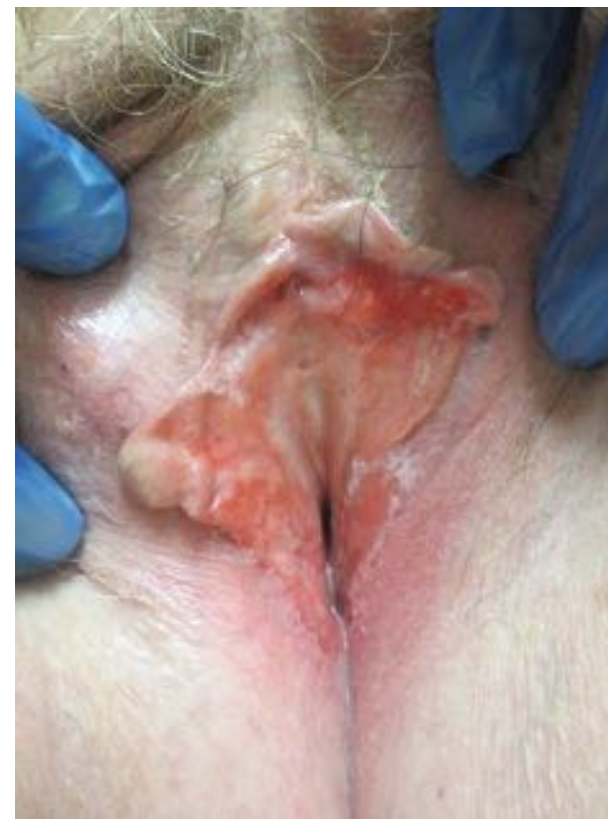

FIGURE 1. Case 1: symmetric erythematous erosions characteristic of vulvovaginal erosive LP.

[1]Gynaecology and Gynaecologic Oncology, John Hunter Hospital; [2]Faculty of Health and Medicine, University of Newcastle; [3]Hunter Medical Research Institute; and [4]Anatomical Pathology, Pathology North, Hunter New England, Newcastle, New South Wales, Australia

Reprint requests to: Tania Day, MD, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Rd, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au

The authors have declared they have no conflicts of interest.

The study was approved by Hunter New England Research Ethics and Governance unit (14/03/19/5.05).

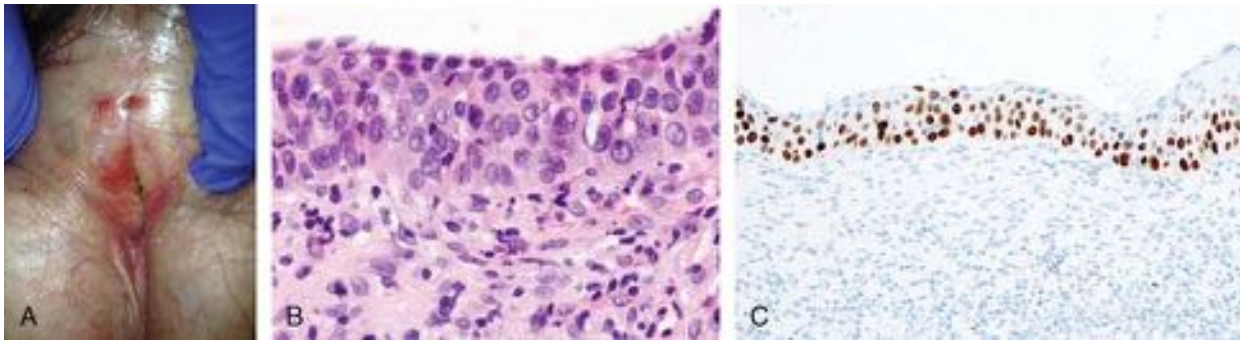

FIGURE 2. A, Case 2: symmetric erythematous erosions characteristic of vulvovaginal erosive LP. B, Case 2: histopathology showing erosion, atrophy, dermal lymphocytic infiltrate, and cellular atypia (H&E ×40). C, Case 2: IHC positive for p53 with confluent dark staining of basal and suprabasilar nuclei (×20).

dVIN. We present 5 of these cases, compare their clinicopathological features with 5 cases of dVIN, and use of copy variant analysis to discriminate between regeneration and neoplasia.[9]

METHODS

We searched the local pathology database for vulvar biopsies reported as erosive vulvitis or dVIN during 2011 to 2013 inclusive. The Hunter New England Research Ethics and Governance unit approved this retrospective histopathologic case series (14/03/19/5.05) and we obtained signed written consent for use of clinical photographs. Clinical information obtained included age, examination findings, specimen site, other vulvar dermatoses and biopsy results, duration of follow-up, treatment, and response. After review of patient notes and slides, there were 5 cases with a clinical appearance and course consistent with erosive LP and histopathology that showed a band-like lymphocytic infiltrate, erosion, and changes potentially consistent with regeneration: maturational disarray, increased mitoses, enlarged nuclei, and nuclear-cytoplasmic ratio reversal.[5]

We then selected 5 cases of dVIN in which the clinical course and histopathology supported the diagnosis. Features of dVIN include a hyperkeratotic or parakeratotic stratum corneum, elongated branching rete ridges, premature maturation, suprabasilar enlarged squamous cells with large vesicular nuclei, and basilar atypia characterized by pleomorphic nuclei, hyperchromasia, and increased and/or multipolar mitoses.[10] We also sought a dVIN variant, which displays full-thickness loss of differentiation with the epidermis composed entirely of homogenously abnormal keratinocytes.[11]

Unstained slides were cut from archived tissue blocks and all specimens had hematoxylin and eosin (H&E) and periodic acid–Schiff stains and IHC for p16 and p53. A positive p16 was defined as "block positive" according to the Lower Anogenital Squamous Terminology project.[12] A positive p53 was defined as confluent, strong nuclear staining of basal cells, sometimes with suprabasilar extension.[10] A wild-type p53 pattern was defined as scattered nuclei staining of variable intensity.[13,14]

All specimens were submitted for copy-number analysis; 1 case each of erosive LP and dVIN had sufficient DNA extracted to perform the test. Epithelium was isolated from 5 consecutive sections by needle macrodissection; this was the test specimen.[15] Likewise, a band of lymphocyte-rich stroma was isolated; this was the control. The QIAamp DNA micro kit (Qiagen, Hilden, Germany) was used to extract DNA, which was analyzed for whole genome copy number using Oncoscan formalin-fixed, paraffin-embedded assay kits (Affymetrix, Santa Clara, Calif). The results were analyzed with Nexus Express software (Affymetrix).

RESULTS

The mean (range) age was 74 (61–92) years in the erosive LP group and 78 (72–92) years in the dVIN group, with a mean follow-up of 18 and 20 months, respectively. Nine of

TABLE 1. Erosive Vulvitis Cases

Case no.	Age	Site	Appearance	Comorbid diagnosis	Other vulvar biopsy results	p53	p16	Treatment	Disease status (months of follow-up)
1	69	Inner labium minus	Erythema, erosion	Oral LP	Nonspecific inflammation	Wild-type	Negative	TCS	Improved (28)
2	77	Inner labium minus	Erythema, erosion	LS	Dermal fibrosis, band-like lymphocytic infiltrate	Positive	Negative	TCS	Improved, died of other causes (18)
3	69	Inner labium minus	Erythema, erosion	LS	LS, spongiotic dermatitis	Wild-type	Negative	TCS	Improved (16)
4	61	Fossa navicularis	Erythema, erosion	LS	Lichenoid tissue reaction, LSC	Wild-type	Negative	TCS	Improved (14)
5[a]	92	Inner labium minus	Erythema, erosion	LS	Acanthosis with altered differentiation	Positive	Negative	TCS	Improved (10)

[a]Case that underwent copy variant analysis.

LP indicates lichen planus; TCS, topical corticosteroid; LS, lichen sclerosus; LSC, lichen simplex chronicus.

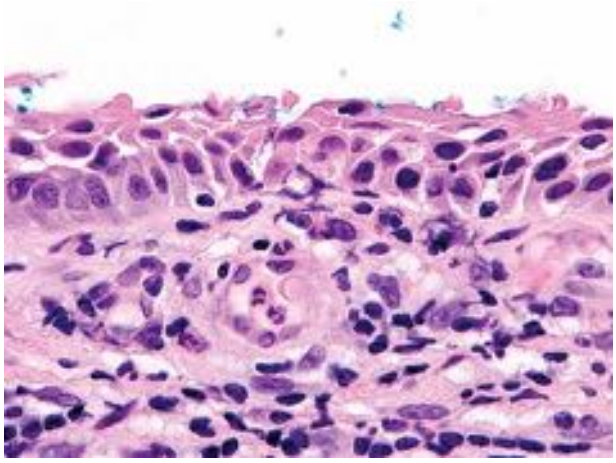

FIGURE 3. Case 3: histopathology showing erosion, atrophy, dermal lymphocytic infiltrate, and cellular atypia (H&E ×40).

10 cases were reviewed at a multidisciplinary gynecologic oncology meeting; a dermatologist specialized in vulvovaginal disorders referred in 1 LP case. All were negative for fungus or yeast on periodic acid–Schiff.

All patients with erosive LP had vulvar pain, symmetric erythematous erosion at the vestibule, and improvement with topical corticosteroids (TCS, see Figures 1, 2A) Four of 5 patients also had a clinical diagnosis of LS; of these, previous or concurrent biopsies of keratinized skin either diagnosed or favored LS (see Table 1). No case was associated with neoplasia during follow-up.

Histopathology of the erosive LP cases showed epithelial thinning, absent stratum corneum, lack of maturation, as well as nuclear changes of enlargement, pleomorphism, and hyperchromasia.(see Figures 2B, 3) Although mitotic activity was seen in all cases, no multipolar mitoses were seen. No apoptotic bodies or vacuolar changes were seen in the basal layer. The lamina propria showed a band-like predominantly lymphocytic infiltrate. No dermal collagen homogenization was seen. Because of erosion, it was not possible to determine whether specimens were from mucosa, mucocutaneous junction, or hairless skin. All were negative for p16. Two cases (40%) were positive for p53, whereas 3 cases (60%) were wild-type (see Figures 2C, 4).

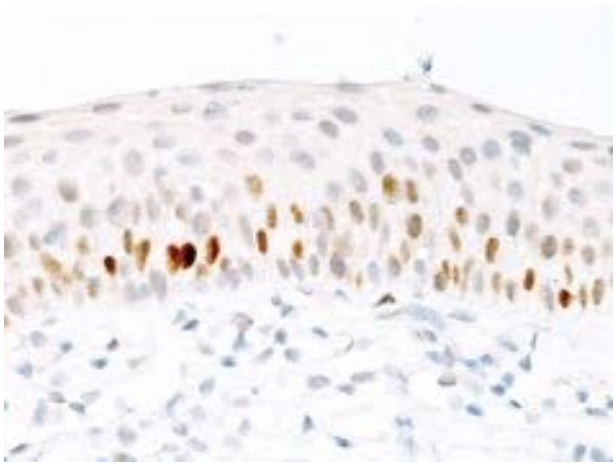

FIGURE 4. Case 4: IHC with p53 wild-type pattern with variable staining of scattered basal layer nuclei (×20).

TABLE 2. Differentiated Vulvar Intraepithelial Neoplasia Cases

Case no.	Age	Site	Appearance	Comorbid diagnosis	Pattern	p53	p16	Treatment	Disease status (months of follow-up)
6	72	Labium majus	Pallor, lichenification	LS, oral, and vulvar LP	Premature maturation	Wild-type	Negative	Excision, TCS	No recurrence, dermatoses controlled (15)
7	75	Outer labium minus	Pallor, lichenification	LS, previous SCC	Basaloid and premature maturation	Suprabasilar staining[a]	Negative	Excision, refused TCS	Recurrent SCC, groin nodes (27)
8[b]	74	Outer labium minus	Pallor, lichenification	LS, previous SCC	Basaloid and premature maturation	Negative	Negative	Excision, TCS	Recurrent SCC, palliation (22)
9	92	Labium majus	Pallor, erosion	LS, previous SCC	Premature maturation and acantholytic	Positive	Negative	Excision, TCS	Healed well (1)
10	75	Outer labium minus	Pallor, lichenification	LS, previous verrucous carcinoma	Premature maturation	Wild-type	Negative	Excision, TCS	Died of other causes (36)

[a]Pattern of uncertain significance.
[b]Case that underwent copy variant analysis.
LS indicates lichen sclerosus; LP, lichen planus; TCS, topical corticosteroid; SCC, squamous cell carcinoma.

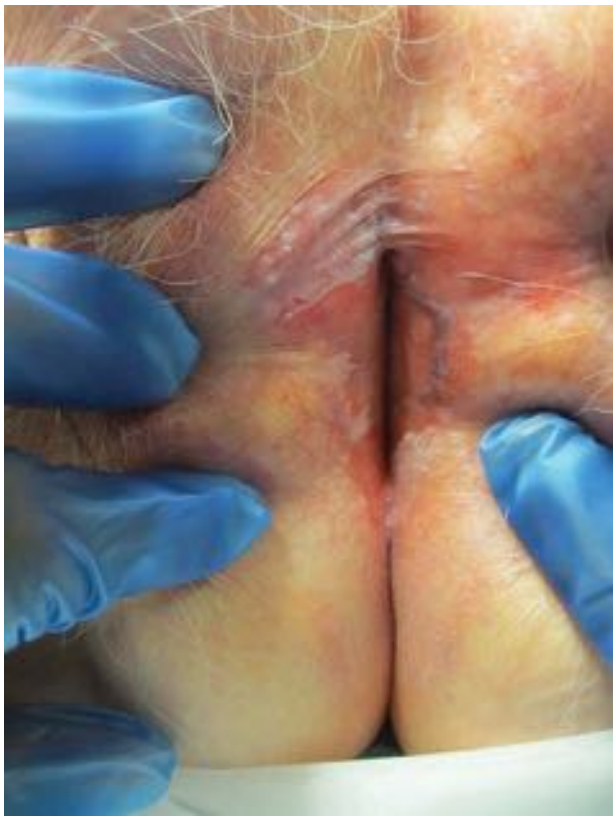

FIGURE 5. Case 6: lichenified plaque on a background of LS with comorbid LP, consistent with dVIN.

Of the dVIN cases, 4 had previous vulvar carcinoma, and all had both a clinical impression of LS and histopathologic evidence of LS within the dVIN specimen (see Table 2 and Figure 5). Two dVIN cases showed full-thickness loss of differentiation, but both also had areas of premature maturation (see Figures 6, 7A). All were negative for p16. One case (20%) was positive for p53, 2 (40%) were wild-type, and 1 (20%) was negative (see Figures 7B, 8). One case showed confluent dark suprabasilar p53 staining; a pattern is of uncertain significance.

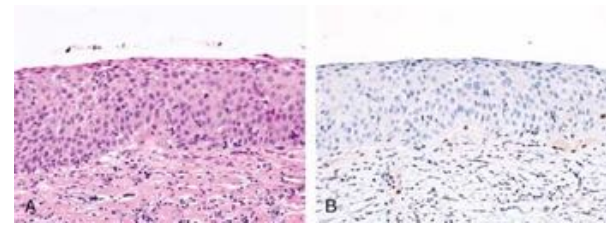

FIGURE 7. A, Case 8: histopathology showing full-thickness atypical keratinocytes, consistent with basaloid dVIN (H&E ×40). B, Case 8: IHC negative for p53 (×20).

The erosive LP epithelium that underwent copy variant analysis showed a similar pattern to the adjacent dermal control, with 0.9% and 0.2% of the genome showing copy-number alterations, respectively. In contrast, the epithelium affected by dVIN showed multiple copy-number alterations occurring in 23.8% of the genome, whereas the control had 0.3% affected. Chromosomes 3, 4, 9, 11, 12, 20, and X were most involved (see Figure 9).

DISCUSSION

We describe 5 cases of clinical vulvovaginal erosive LP in which the histopathology shows maturational and nuclear changes. The examination findings, improvement with TCS, and normal copy number in a representative case argue against a neoplastic etiology of the histopathologic appearance. We submit that these cases epitomize a small but important subset of erosive LP. This hypothesis raises several questions deserving of further investigation.

Mirroring the controversies in OLP, the implication of regenerative changes and a band-like lymphocytic infiltrate remains unclear. It is possible that the lack of consideration of regeneration in the diagnostic criteria of vulvovaginal erosive LP contributes to the high rate of nondiagnostic biopsy. Alternatively, this pattern may represent an entity with different pathophysiology or clinical behavior. In the mouth, the term "atypical lichenoid stomatitis" has been used when there are cellular and maturational abnormalities in combination with an inflammatory infiltrate, because this could "represent unusual reactive change or indicate early, evolving dysplasia."[5] However, the mouth is a carcinogen-rich environment with multiple pathways resulting in a common endpoint

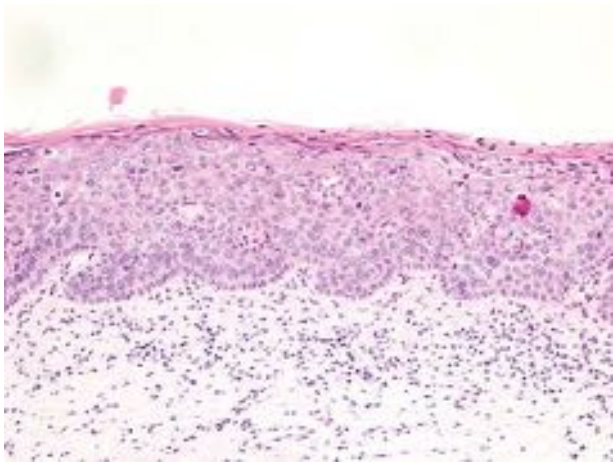

FIGURE 6. Case 7: histopathology showing full-thickness atypical keratinocytes, consistent with dVIN (H&E ×40).

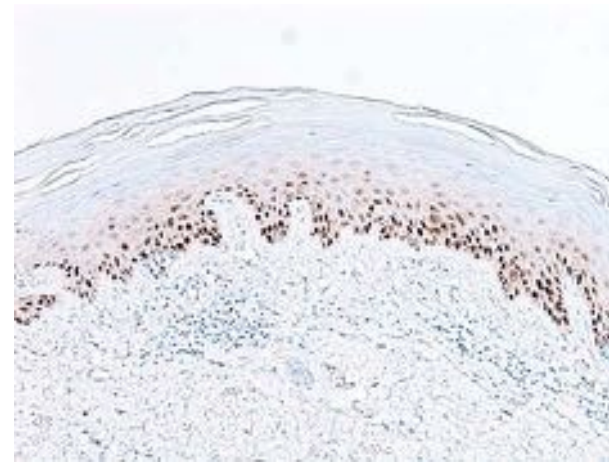

FIGURE 8. Case 9: IHC positive for p53 with confluent dark staining of basal and suprabasilar nuclei (×20).

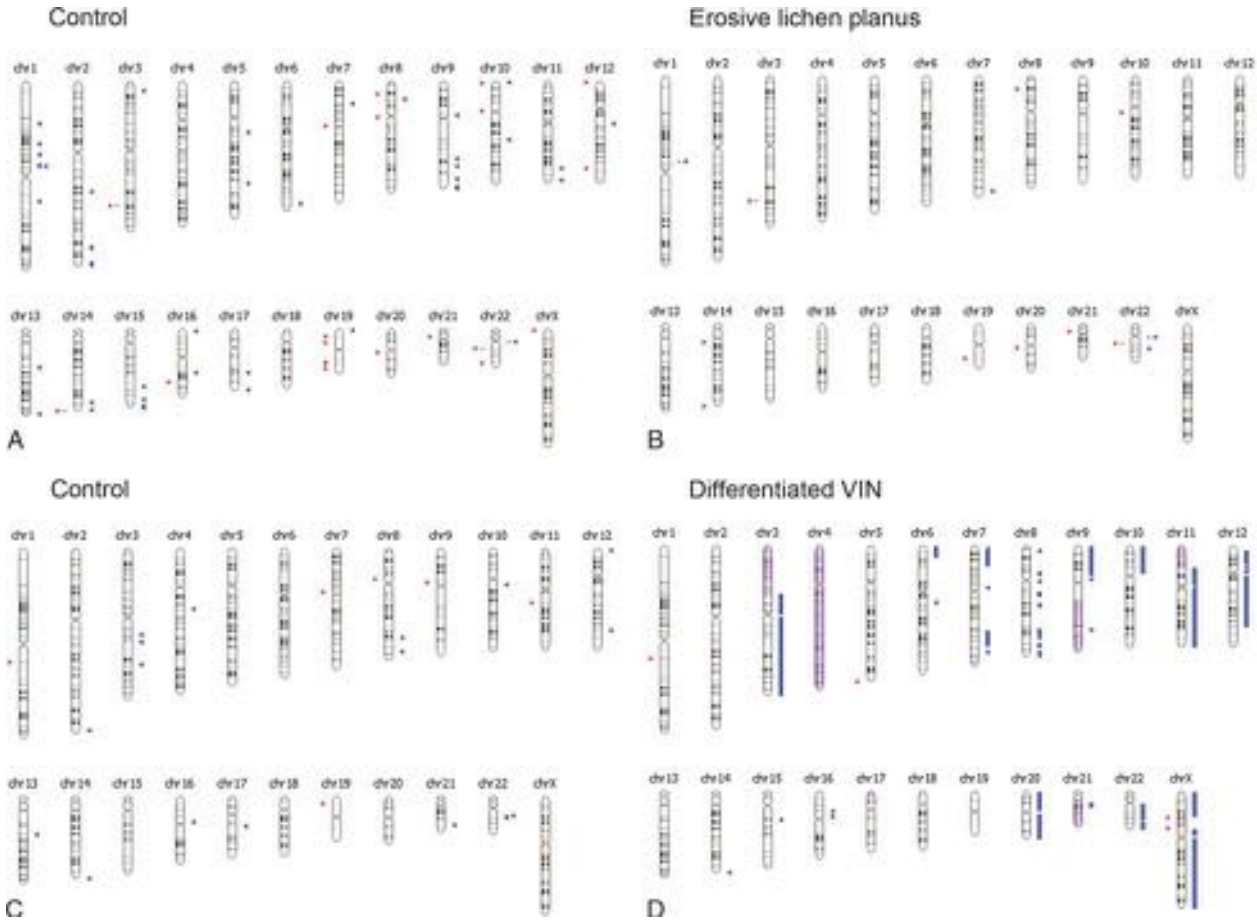

FIGURE 9. Copy variant analysis results.

of atypical epithelium. In contrast, vulvar squamous neoplasia is attributable to 1 of 2 etiologies—HPV or chronic inflammatory dermatoses. Thus, we do not endorse an analogous term such as atypical lichenoid vulvitis. Rather, we recommend clinicopathological correlation and IHC to separate lesions into reactive change or intraepithelial neoplasia.

The finding that 80% of erosive LP cases also carry a diagnosis of LS is noteworthy. Several authors have alluded to the coexistence of vulvar LS and LP, but the scant literature on this topic describes genital LS with oral and cutaneous LP.[16–19] Lichenified LS is well described as the major precursor for dVIN and multiple case reports describe cancers associated with LP on keratinized vulvar skin, but sparse documentation exists of cancer arising from mucosal LP.[20–23] In the largest series of 38 cancers associated with vulvovaginal LP, 13 reportedly arose from erosive disease, but the locations described may contain keratinized skin and 4 of 9 photographs suggest LS.[24] The lack of a conclusive association of LP and SCC in the mouth, the rarity of non-HPV–associated vaginal SCC, and the inability to apply the scar-cancer pathogenic mechanism of LS to erosive LP are reasons to question the association of mucosal vulvovaginal LP with SCC.[5,25]

This series underscores that distinguishing regenerative erosive vulvitis from dVIN is a challenging histopathologic problem and highlights the crucial role of a skilled vulvar pathologist. A negative p53 is helpful in diagnosing dVIN because this represents a p53 mutation but occurs in only 15% of cases.[10,26] Although p53 overexpression and reactive changes are described in oral lichenoid disorders, there is scant previous work specific to vulvovaginal erosive LP with regard to basal layer appearance, p53 staining patterns, and confusion with neoplasia.[6,27] Our study suggests that both erosive LP and dVIN may show p53 in a wild-type or confluent-positive pattern. Although marked copy-number alterations indicate a neoplastic process, currently, this strategy is hampered by prohibitive cost, diagnostic delay, and technical problems. Scant DNA is obtainable from a punch biopsy of thinned epithelium, and needle macrodissection may yield a contaminated sample because of intraepithelial inflammatory cells or stromal papillae. As methods of tissue sampling improve, the required amount of input DNA decreases, and costs decline, it may in future be achievable to apply this technique to clinical decision-making.

Meanwhile, we recommend that these challenging cases be evaluated collaboratively by clinicians and pathologists experienced in vulvar disorders. Erosive LP and dVIN are uncommon pathologies and the consequences of an erroneous diagnosis are grave. Circumferential or symmetric flat erosions on mucosa are consistent with erosive LP, whereas a unilateral erythematous plaque is worrisome for dVIN.[11] An erosion within a clinically resistant LS plaque is another suspicious circumstance for dVIN. Cases with a characteristic appearance of erosive LP and histopathology showing regenerative erosive vulvitis may be medically managed under close supervision.

In summary, a small subset of clinically diagnosed vulvovaginal erosive LP will show a regenerative erosive vulvitis on histopathology, which requires clinicopathologic correlation to distinguish from dVIN.

REFERENCES

1. Wang W, Lazar A. Lichenoid and interface dermatitis, Chapter 7. In: Calonje E, Brenn T, Lazar A, McKee P, eds. *McKee's Pathology of the Skin With Clinical Correlations*. 4th ed. London: Elsevier; 2012:219–58.
2. Simpson RC, Thomas KS, Leighton P, et al. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br J Dermatol* 2013;169:337–43.
3. Simpson RC, Littlewood SM, Cooper SM, et al. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. *Br J Dermatol* 2012;167:85–91.
4. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Lower Gen Tract Dis* 2013;17:28–32.
5. Eisenberg E. Oral lichen planus: a benign lesion. *J Oral Maxillofac Surg* 2000;58:1278–85.
6. Fernández-González F, Vázquez-Álvarez R, Reboiras-López D, et al. Histopathological findings in oral lichen planus and their correlation with the clinical manifestations. *Med Oral Patol Oral Cir Bucal* 2011;16:e641–6.
7. Van de Nieuwenhof HP, Bulten J, Hollema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;24:297–305.
8. Del Pino M, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology* 2013;62:161–75.
9. Allen DG, White DJ, Hutchins AM, et al. Progressive genetic aberrations detected by comparative genomic hybridization in squamous cell cervical cancer. *Br J Cancer* 2000;83:1659–63.
10. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000;24:429–41.
11. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol* 2009;33:1659–65.
12. Darragh TM, Colgan TJ, Thomas Cox J, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol* 2013;32:76–115.
13. Vanin K, Scurry J, Thorne H, et al. Overexpression of wild-type p53 in lichen sclerosus adjacent to human papillomavirus-negative vulvar cancer. *J Invest Dermatol* 2002;119:1027–33.
14. Liegl B, Regauer S. p53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). *Histopathology* 2006;48:268–74.
15. Kommoss S, Anglesio MS, Mackenzie R, et al. FOXL2 molecular testing in ovarian neoplasms: diagnostic approach and procedural guidelines. *Mod Pathol* 2013;26:860–7.
16. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust N Z J Obstet Gynaecol* 2010;50:148–52.
17. Day T, Bohl TG, Scurry J. Perianal lichen dermatoses: a review of 60 cases. *Australas J Dermatol* 2015.
18. Holmes SC, Burden AD. Lichen sclerosus and lichen planus: a spectrum of disease? Report of two cases and review of the literature. *Clin Exp Dermatol* 1998;23:129–31.
19. Yashar S, Han KF, Haley JC. Lichen sclerosus-lichen planus overlap in a patient with hepatitis C virus infection. *Br J Dermatol* 2004;150:168–9.
20. Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. *Int J Gynecol Cancer* 1999;9:89–97.
21. Lee A, Bradford J, Fischer G. Long-term management of adult vulvar lichen sclerosus: a prospective cohort study of 507 women. *JAMA Dermatol* 2015;151:1061–7.
22. Chiu TL, Jones RW. Multifocal multicentric squamous cell carcinomas arising in vulvovaginal lichen planus. *J Lower Gen Tract Dis* 2011; 15:246–7.
23. Jones RW, Rowan DM, Kirker J, et al. Vulval lichen planus: progression of pseudoepitheliomatous hyperplasia to invasive vulval carcinomas. *BJOG* 2001;108:665–6.
24. Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014; 71:698–707.
25. Carlson JA, Ambros R, Malfetano J, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. *Hum Pathol* 1998;29:932–48.
26. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol* 2010;23:404–12.
27. Ebrahimi M, Boldrup L, Coates PJ, et al. Expression of novel p53 isoforms in oral lichen planus. *Oral Oncol* 2008;44:156–61.

4.9 Is vulvovaginal lichen planus associated with squamous cell carcinoma?

The small body of evidence linking vulvar LP and SCC is methodologically flawed. The studies lack an adequate description of clinicopathologic diagnostic criteria, combine cancers with intraepithelial lesions, and fail to exclude other aetiologies of neoplasia. Previous work demonstrated several factors that could contribute to misattribution of vulval SCC to LP. These include the possibility of comorbid LP and LS, the potential for confusion between regenerative erosive LP and basaloid dVIN, and the similar appearances of hypertrophic LP and hypertrophic dVIN. In addition, since LS may occur on vestibule and vagina, cancers in that anatomic zone cannot be assumed to arise from LP. These new findings permitted a more informed assessment of perilesional LS and LP from excision specimens of HPV-independent SCC. LS was found in 95% of cancer excision specimens, while 5% had a previous or subsequent biopsy showing LS. Meanwhile, there was no evidence of LP in association with HPV-independent SCC. Review of these specimens also demonstrates that dVIN may be broadly categorised into three morphologies: basaloid dVIN, hypertrophic dVIN, and the standard form described by Yang and Hart. The study duplicates Watkins and colleagues' finding of a morphologic spectrum of lesions with verrucous acanthosis and abnormal differentiation, and augments the hypothesis that these lesions may represent precursors to dVIN.

OPEN

Is Vulvovaginal Lichen Planus Associated With Squamous Cell Carcinoma?

Tania Day, MD,[1,2] Geoff Otton, CGO,[3] Ken Jaaback, CGO,[3] Julie Weigner,[4] and James Scurry, FRCPA[1,4]

Objective: The aim of the study was to assess for the presence of vulvar lichen planus (LP) in association with human papillomavirus (HPV)–independent squamous cell carcinoma (SCC).

Materials and Methods: We performed a clinicohistopathologic review of consecutive vulvectomies and wide local excisions for HPV-independent vulvar or vaginal SCC from 2007 to 2017. Data collected included site of SCC, adjacent precursor lesions and dermatoses, dermatologic treatment, and outcome.

Results: There were 43 cases of primary HPV-independent vulvar SCC treated by excision, but no vaginal cancers. Eighteen women (42%) had a preoperative diagnosis of lichen sclerosus (LS); none had a diagnosis of LP. Topical corticosteroids were prescribed in 19 (44%) of 43, with 4 women placed on maintenance therapy. Tumors arose from the labia minora, labia majora, and periclitoris, but not from vestibule or perianus. On histopathological review, LS was present in 41 (95%) of 43 specimens, 1 had a nonspecific lichenoid reaction, and 1 had lichen simplex; both of the latter had subsequent biopsies showing LS. Lichen planus was not seen in association with SCC. Differentiated vulvar intraepithelial neoplasia (dVIN) was present in 38 (88%) of 43 specimens, whereas 1 had acanthosis with altered differentiation and 4 (9%) had no precursor lesion. Differentiated vulvar intraepithelial neoplasia had standard, basaloid, and hypertrophic morphology, superficially resembling erosive LP in 9 (24%) of 38 and hypertrophic LP in 6 (16%) of 38.

Conclusions: Lichen planus was not seen in association with HPV-independent vulvar SCC, whereas LS was underrecognized and inadequately treated in this group. Pathologists should be aware that dVIN may superficially resemble erosive or hypertrophic LP.

Key Words: lichen planus, lichen sclerosus, differentiated vulvar intraepithelial neoplasia, HPV-independent, vulvar squamous cell carcinoma

(*J Low Genit Tract Dis* 2018;22: 159–165)

There are 2 types of vulvar squamous cell carcinoma (SCC): approximately 30% of cases are human papillomavirus (HPV)–dependent and the other 70% are usually associated with lichen sclerosus (LS).[1] Multiple studies suggest that vulvar SCC occurs in 5% of women with LS, and a recent prospective cohort study suggested that effective treatment mitigates this risk.[2] Vulvovaginal lichen planus (LP) has also been described in association with vulvar neoplasia in several case reports and series, and SCC is occasionally noted in women with erosive LP during long-term follow-up.[3–6] Limitations of this literature include lack of histopathologic confirmation of the LP diagnosis, inadequate follow-up, and failure to identify if neoplasia could be related to HPV or other carcinogens.[3,4]

The largest study to date reported 38 cases of LP-associated SCC and differentiated vulvar intraepithelial neoplasia (dVIN), occurring at a single European center.[6] However, this series did not detail the histopathologic diagnostic criteria applied for the diagnosis of LP nor how LP was distinguished from LS and did not account for the possibility of comorbid LP and LS.[7] A recent publication describes the difficulty in distinguishing the basaloid variant of dVIN from regenerative erosive LP, raising the question of neoplastic precursor lesions being mistaken for LP.[8]

The aims of this study are to assess consecutive excisions of primary HPV-independent vulvar and vaginal cancers for peritumoral LP, LS, and precursor lesions and to place these findings in the context of clinical diagnosis and management of SCC-associated vulvar dermatoses.

MATERIALS AND METHODS

The Pathology New South Wales Hunter New England database was searched for vulvectomies and excisions of vulvar or vaginal cancer submitted between 2007 and 2017 inclusive. Cases of recurrent cancer were excluded. Human papillomavirus–independent status was confirmed by routine histopathology and, when in doubt, through nonblock staining of p16 on adjacent VIN. Any case with previous or concurrent high-grade squamous intraepithelial lesion and/or positive p16 was excluded. The Hunter New England Research Ethics and Governance Unit approved this retrospective histopathologic case series (HREC 15/11/18/5.02), and signed written consent was obtained for use of clinical photographs.

Histopathologic review was performed of slides stained with hematoxylin and eosin (H&E). The tumor morphology was assessed as keratinizing or verrucous SCC. Verrucous SCC is a well-differentiated nonmetastasizing neoplasia with minimal suprabasilar nuclear atypia; spread occurs through an expansile blunt interface.[9] The location of the tumor was determined through assessment of deep anatomic structures, site-specific stromal appendages, and the following perilesional epithelial types: squamous mucosa, mucocutaneous junction, hairless skin, and hair bearing skin. The peritumoral epithelium was then inspected for precursor lesions and adjacent dermatologic diseases.

Lichen sclerosus (LS) required basal layer degeneration seen as vacuolar change, apoptotic bodies, and/or squamatization, in combination with sclerosis or thick fibrosis of the papillary dermis and a closely applied band-like lymphocytic infiltrate.[10] Squamatization is defined as a change in morphology of normal basilar keratinocytes to horizontally disposed cells with a mature squamous appearance.[11] Classic LP required hyperkeratosis, hypergranulosis, sawtooth or spiky acanthosis, evidence of basal layer damage, a band-like lymphocytic infiltrate, and absence of dermal homogenization.[12] Hypertrophic LP required the changes

[1]Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW, Australia; [2]Maternity and Gynecology, John Hunter Hospital, New Lambton Heights, NSW, Australia; [3]Division of Gynaecologic Oncology, John Hunter Hospital, New Lambton Heights, NSW, Australia; and [4]Pathology New South Wales, Hunter New England, Newcastle, NSW, Australia

Reprint requests to: Tania Day, MD, Maternity and Gynaecology, John Hunter Hospital, 2 Lookout Rd, New Lambton Heights, NSW 2305, Australia. E-mail: tania.day@hnehealth.nsw.gov.au

The authors have declared they have no conflicts of interest.

T.D. is supported by the Australian Government Research Training Program Scholarship.

This study was approved by Hunter New England Research Ethics and Governance unit (HREC 15/11/18/5.02).

DOI: 10.1097/LGT.0000000000000384

of classic LP with superimposed lichenification seen as irregular elongated rete ridges and papillary dermal fibrosis.[10,12] Erosive LP required epithelial thinning, often with erosion, a closely applied lymphocytic infiltrate, and either the degenerative or regenerative epithelial patterns.[7,8] The regenerative pattern showed altered maturation, increased nucleus-to-cytoplasm ratio, and mitoses. Cases of a lichenoid pattern that lacked diagnostic features of LS or LP were labeled nonspecific lichenoid reaction.

Features of dVIN included a thickened hyperkeratotic or parakeratotic stratum corneum, acanthosis with elongated and branching rete ridges, premature maturation, enlarged squamous cells with large vesicular nuclei above the basal layer, and basal layer atypia characterized by the following 4 findings: increased mitoses, nuclear enlargement, pleomorphism, and hyperchromasia.[13] We also looked for the basaloid variant of dVIN in which the epidermis is replaced by a homogenous population of abnormal keratinocytes.[8,14] Basaloid dVIN was distinguished from regenerative erosive LP by the presence of marked nuclear pleomorphism, supported by an aberrant positive or negative p53; high-grade squamous intraepithelial lesion was excluded by a negative p16.[8] Vulvar acanthosis with altered differentiation (VAAD) is a descriptive term for an unusual epithelial appearance with marked verruciform hyperplasia, plaque-like parakeratosis, hypogranulosis, a layer of pale-staining squamous cells, premature maturation, and absence of basal atypia.[9] Vulvar acanthosis with altered differentiation was first described as a possible precursor lesion for verrucous SCC; alternatively, it may be associated with keratinizing SCC or may be a reactive phenomenon that resolves with treatment of the underlying dermatologic condition.

Clinical data collected included age, rurality, body mass index, diabetes mellitus status, tumor location and number, surgical stage, and outcome. Referrals and provider notes were reviewed for documentation of a clinical diagnosis of LS or LP occurring before cancer surgery. Additional data collected included previous and subsequent vulvar biopsies, treatment prescribed, and presence of a dermatologic diagnosis written on the pathology request form and in the histopathology report. Descriptive statistics were performed; clinical and histopathologic characteristics were compared with Fisher exact test.

RESULTS

There were 43 excisions for primary HPV-independent vulvar SCC; no vaginal cases were encountered. The mean age was 76 years, with 4 women 50 years or younger (Table 1). The diagnosis of LS was recorded in clinical notes before cancer surgery in 18 (42%) of 43, and there were no preoperative diagnoses of LP. There were no significant differences between women with and without a diagnosis of LS with regard to age, body mass index, diabetes status, rurality, notation of suspected or confirmed LS on the histopathology request form, surgical stage, and cancer outcome. A preoperative diagnosis was associated with postoperative documentation of LS (13/18 [72%] vs 7/25 [28%], $p = .006$) but no significant difference in treatment. Topical corticosteroids prescribed were high potency in 11 (58%) of 19, medium in 6 (32%), and unspecified in 2 (11%). One woman was referred postoperatively to a vulvar specialist for LS management.

Tumor locations included labia minora, labia majora, and periclitoral; cancers did not originate at the vestibule or perianus. On review of excision specimens, LS was present in 41 (95%) of 43, 1 had a nonspecific lichenoid reaction, and 1 had lichen simplex chronicus; both of the latter had subsequent biopsies showing

TABLE 1. Clinical Characteristics of Women With HPV-Independent Vulvar Cancer, Stratified by Preoperative Diagnosis of LS

	Total ($n = 43$)	Preoperative diagnosis of LS ($n = 18$)	No diagnosis ($n = 25$)
Age, mean (SD, range)	76 (12, 44–93)	73 (11, 50–85)	78 (12, 44–93)
Remoteness area, *n* (%)			
Metropolitan	20 (47)	7 (39)	13 (52)
Inner regional	16 (37)	7 (39)	9 (36)
Outer regional	7 (16)	4 (22)	3 (12)
BMI, mean (SD, range)	30 (8, 18–50)	32 (7, 21–48)	29 (7.8, 18–42)
Diabetes mellitus, *n* (%)	15 (35)	9 (50)	6 (24)
Stage, *n* (%)			
0	2 (5)	1 (6)	1 (4)
1	28 (65)	13 (52)	15 (63)
2	2 (5)	0	2 (8)
3	9 (21)	3 (17)	6 (24)
4	2 (5)	1 (6)	1 (4)
LS suspicion or diagnosis on pathology request, *n* (%)	10 (23)	5 (28)	5 (20)
LS documented postoperatively, *n* (%)	20 (47)	13 (72)	7 (28)
Topical corticosteroids prescribed, *n* (%)			
Intermittent	15 (35)	7 (39)	8 (32)
Maintenance	4 (9)	3 (17)	1 (4)
Never	24 (56)	8 (44)	16 (64)
Outcome, *n* (%)			
No evidence of disease	15 (35)	7 (39)	8 (32)
Recurrence	14 (33)	5 (28)	9 (36)
Dead of disease	11 (26)	4 (22)	7 (28)
No follow-up	3 (7)	2 (12)	1 (4)
Duration of follow-up, mean (SD, range), y	3 (2, 1–8)	2.9 (1.8, 1–8)	3.1 (1.2, 1–8)

HPV indicates human papillomavirus; BMI, body mass index; LS, lichen sclerosus.

TABLE 2. Histopathologic Characteristics of HPV-Independent Vulvar Cancer, Stratified by Preoperative Diagnosis of LS

	Total (n = 43)	Preoperative diagnosis of LS (n = 18)	No diagnosis of LS (n = 25)
Tumor origin, *n* (%)			
Periclitoral	12 (28)	2 (12)	10 (48)
Labium minus	18 (42)	9 (50)	9 (36)
Labium majus	13 (30)	7 (39)	6 (24)
Tumor histopathology, *n* (%)			
Keratinizing	37 (86)	16 (89)	23 (92)
Verrucous	2 (5)	1 (6)	1 (4)
Both	4 (9)	3 (17)	1 (4)
Tumors, *n* (%)			
1	32 (74)	12 (67)	20 (80)
2	6 (14)	3 (17)	3 (12)
≥3	5 (12)	3 (17)	2 (8)
LS noted on pathology report	33 (77)	16 (89)	17 (68)
Perilesional dermatosis on review of cancer excision, *n* (%)			
LS	41 (95)	18 (100)	23 (92)
Lichenoid, nonspecific	1 (2)	0	1 (4)
Lichen simplex chronicus	1 (2)	0	1 (4)
Precursor lesion, *n* (%)			
None	4 (9)	1 (6)	3 (12)
VAAD	1 (2)	0	1 (4)
dVIN	34 (79)	16 (89)	18 (72)
dVIN and VAAD	4 (9)	1 (6)	3 (12)
LS epithelium, *n* (%)			
Normal	14 (33)	4 (22)	9 (36)
Atrophic	5 (12)	3 (17)	2 (8)
Lichenified	22 (51)	11 (61)	12 (48)
Not applicable	2 (5)	0	2 (8)
LS stroma, *n* (%)			
Hyalinized	20 (47)	10 (56)	10 (48)
Fibrotic	3 (7)	1 (6)	2 (8)
Hyalinized and fibrotic	15 (35)	6 (33)	9 (36)
Hyalinized and edematous	3 (7)	1 (6)	2 (8)
Not applicable	2 (5)	0	2 (8)
Features of dVIN	(n = 38)	(n = 17)	(n = 21)
Morphology, *n* (%)			
Standard	23 (53)	9 (53)	14 (67)
Hypertrophic	6 (14)	4 (24)	2 (9.5)
Basaloid	4 (9)	2 (12)	2 (9.5)
Basaloid and other	5 (12)	2 (12)	3 (14)
Stromal sclerosis, *n* (%)	31 (72)	16 (94)	15 (71)

HPV indicates human papillomavirus; LS, lichen sclerosus; dVIN, differentiated vulvar intraepithelial neoplasia; VAAD, vulvar acanthosis and altered differentiation.

LS (Table 2). There were no significant differences in tumor characteristics, precursor lesions, or histopathologic appearance of LS, when stratified by preoperative LS diagnosis. One woman who underwent excision of a right labial SCC with adjacent LS and dVIN had a concurrent punch biopsy of a noncontiguous perianal lesion that showed classic LP.

There were 3 morphologies of dVIN identified: standard, basaloid, and hypertrophic, and more than 1 type could be seen in the same specimen (see Figures 1–3). Of 38 cases with dVIN, 14 (37%) had a negative p16 to substantiate the HPV-independent diagnosis; this included all specimens with basaloid dVIN and 5 with standard morphology. Subepithelial sclerosis, the cardinal histopathologic feature of LS, accompanied dVIN in 72% of cases. Verrucous carcinoma and hypertrophic dVIN appeared as a spectrum of disease, rather than 2 distinct entities. Vulvar acanthosis with altered differentiation was more often seen in combination with dVIN than in isolation and was observed in cases both with and without verrucous SCC (see Figure 4). In addition to VAAD as described by Nascimento et al.,[9] 2 cases demonstrated other morphologies of "acanthosis with altered differentiation" seen as marked hyperkeratosis and hypergranulosis disproportionate to the acanthosis or as parakeratosis without the underlying pale band of squamous cells (see Figure 5). Those cases showed mild basal nuclear enlargement and occasional mitoses but lacked nuclear pleomorphism and hyperchromasia, so they did not fulfill criteria for basal atypia as seen in dVIN.

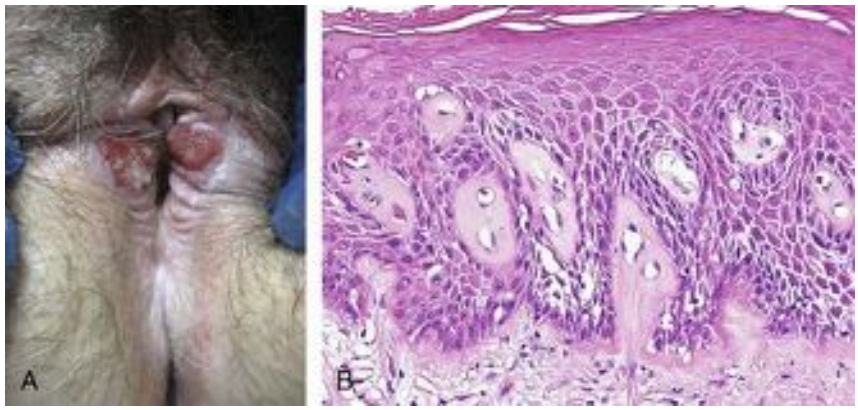

FIGURE 1. A, A 50-year-old with a 20-year history of LS and 2 opposing tumors of keratinizing SCC and dVIN in a field of LS. B, Standard dVIN morphology is accompanied by dermal sclerosis (H&E ×200).

The diagnosis of LS was written in 33 (77%) of pathology reports, but 14 (42%) of these cases never had a clinical diagnosis recorded. During postoperative follow-up, 8 additional women received diagnoses of LS by punch biopsy, 6 (75%) of whom were treated. Mean follow-up was 3 years (range = 1–8 years). Among 11 (26%) women who died of disease, the mean interval from diagnosis was 3.3 years. Eight (42%) of 19 women prescribed topical corticosteroids had no evidence of recurrent cancer during follow-up, compared with 7 (29%) of 24 of untreated women; this was not statistically significant.

DISCUSSION

For an 11-year period, 95% of excisions for HPV-independent vulvar SCC showed histopathologic evidence of LS. The other 5% had subsequent biopsies showing LS. There was no evidence of hypertrophic, classic, or erosive LP in the SCC specimens. These results suggest that vulvar SCC associated with LP is rare.

There are several reasons to doubt an association between vulvovaginal LP and SCC. Erosive LP is usually seen on nonkeratinized epithelium of vestibule and vagina, but primary HPV-independent vaginal cancer has not been reported and vestibular tumors are uncommon, although establishing an origin site medial to the mucocutaneous junction is difficult if there is dermatosis-associated architectural change or the cancer is locally advanced.[5] The proposed mechanism for LS-associated neoplasia is the scar-cancer hypothesis, in which the combination of altered epithelial-stromal interface and chronic epithelial damage and repair leads to accumulation of carcinogenic mutations.[15,16] Classically, scar cancers arise from burns and chronic ulcers; in the case of LS, the scar is the band of abnormal stromal collagen and the damaged epithelium results from T-cell–mediated attack on basilar keratinocytes.[15] Lichen planus lacks significant scarring, and chronic inflammation alone is a weak potential driver for carcinogenesis. Finally, controversy continues regarding the malignant potential of oral LP, with that literature likewise afflicted by problems with clinical and histopathologic diagnoses, documentation of comorbidities, and inadequate follow-up. The mouth is a carcinogen-rich environment in which multiple pathways result in a common end point of atypical epithelium; toxic exposures may cause antigenic alterations in mucosal basal cells, triggering a secondary lichenoid reaction.[4,17] In an effort to reduce the possibility of misattribution to LP, consensus criteria were constructed on diagnosis of oral lichenoid lesions.[17] No similar criteria exist for vulvovaginal disease.

Pathologic assessment of peritumoral dermatoses and precursor lesions is challenging and presents many opportunities for misdiagnosis. Previous studies have documented that dVIN may be misdiagnosed as LS and that p53 overexpression can occur both in benign and neoplastic conditions.[9,13,18,19] This study highlights several other pitfalls in assessment of these specimens. The basaloid variant of dVIN occurred in 21% of cancers and sometimes was the only precursor lesion present; clues to distinguish it from regenerative erosive LP include parakeratosis, minimal epithelial thickening, abnormal mitotic figures, and more nuclear hyperchromasia. The hypertrophic variant of dVIN was seen in 14% and is a mimic for hypertrophic LP; the presence of atypical nuclei away from the inflamed dermoepidermal junction is the major distinguishing characteristic. Another feature that may cause diagnostic confusion is pseudoepitheliomatous hyperplasia, a benign squamoproliferative condition that occurs within

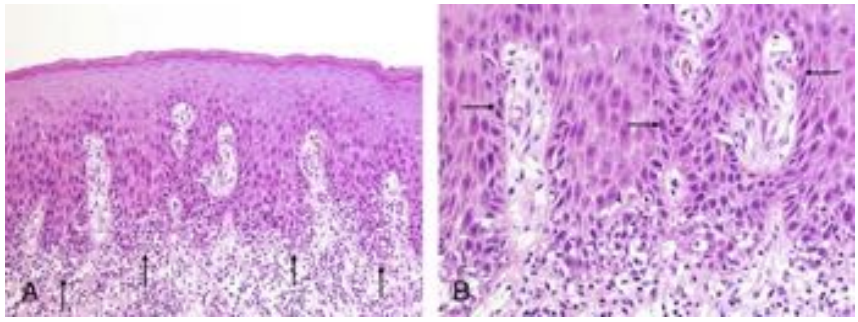

FIGURE 2. A, This example of hypertrophic dVIN displays basal layer degeneration at the tips of rete ridges (arrows) and dense lymphocytic infiltrate (H&E ×100). B, Basilar atypia away from the tips of rete ridges (arrows) differentiates dVIN from hypertrophic LP (H&E ×200).

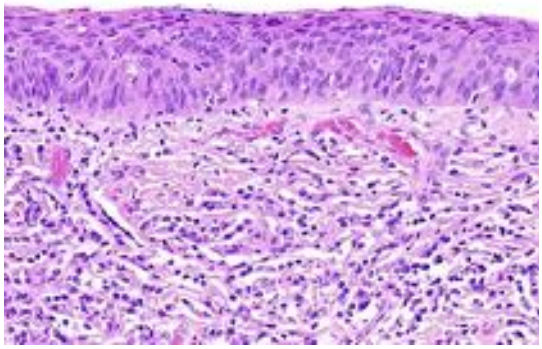

FIGURE 3. Basaloid dVIN seen as thinned epithelium, full-thickness atypia, stromal fibrosis, and a dense lymphoplasmacytic infiltrate; p16 was negative (H&E ×200).

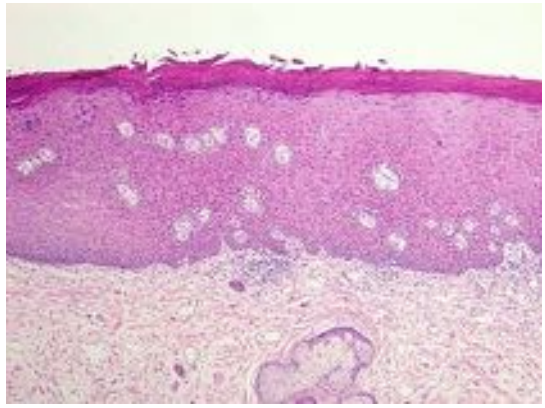

FIGURE 5. An area of acanthotic morphology without basal layer atypia showed a variable appearance of the stratum corneum and granular cell layer (H&E ×100).

LP, LS, and nodular prurigo, and histopathologically resembles SCC.[20] Mistaking pseudoepitheliomatous hyperplasia for SCC leads to unnecessary surgical intervention and attribution of the purported cancer to the adjacent dermatosis.[21] Finally, cases of LS with edematous, fibrotic, or localized collagen change may be misinterpreted as LP or a nonspecific lichenoid reaction.[7,22]

Distinguishing between LP and LS also presents difficulties to clinicians. The adage that LS is a disease of keratinized skin is incorrect as LS can extend into the vestibule and vagina and rarely may present as an isolated lesion in squamous mucosa.[11] Hypertrophic LP and lichenified LS both demonstrate a combination of erythema and pallor, often accompanied by architectural change, erosions, excoriations, and superinfection. The diagnosis of comorbid LP and LS requires a high index of suspicion and is confirmed with 2 well-placed biopsies; thus, this phenomena is likely underreported.[7] However, this study suggests that LS may be overlooked even when the clinical appearance is typical and peritumoral LS is noted on the pathology report. This is especially problematic because adjacent LS is associated with a three-fold risk of local recurrence of vulvar SCC and a five-fold risk of second field tumors. In contrast, margin status is not related to HPV-independent SCC recurrence risk.[23]

Despite recent evidence to suggest that a tailored long-term regimen of topical corticosteroids reduces the risk of primary vulvar cancer, only 4 women in this high-risk group were ever prescribed maintenance therapy.[2] Heterogeneity in international clinical guidelines likely contributes to a laissez-faire approach to long-term surveillance and treatment of LS. European guidelines state that "maintenance treatment with either topical steroids or calcineurin inhibitors is recommended as it seems to prevent severe relapses" and that frequency of steroid application is determined by symptoms.[24] British guidelines restrict maintenance treatment to women with persistent symptoms or appearance of active disease, with follow-up annually by a general practitioner for stable disease and specialist opinion for "atypical or poorly controlled" disease or previous neoplasia.[25] North American guidelines state that the evidence for maintenance therapy is conflicting and do not discuss referral to vulvar specialists.[26] Australian guidelines have recently been updated to advise chronic regular use of a topical corticosteroid potent enough to maintain remission and that management should be in consultation with an expert.[27] None of these documents specifies the role of gynecologic oncologists in management of vulvar dermatoses; moreover, there is no mention of LS treatment in national oncologic guidelines for management of vulvar cancer.[28,29] Most gynecologic oncologists have limited training and experience in long-term care of dermatoses, so involvement of a vulvar specialist in the follow-up of women with dVIN and HPV-independent SCC could offer advantages both in quality of life and reduced recurrence rates, with research urgently needed to assess this hypothesis.[30]

This study adds to the literature on the relationship between keratinizing SCC, verrucous SCC, and VAAD. The acronym "VAAD" was devised as a descriptive term for an unusual acanthotic lesion that lacks the basal atypia of dVIN and is remarkable for plaque-like parakeratosis with conspicuous underlying pallor; it was originally described as a precursor to verrucous SCC but has since been identified with keratinizing SCC.[9,31] We observed variants of "acanthosis with altered differentiation"

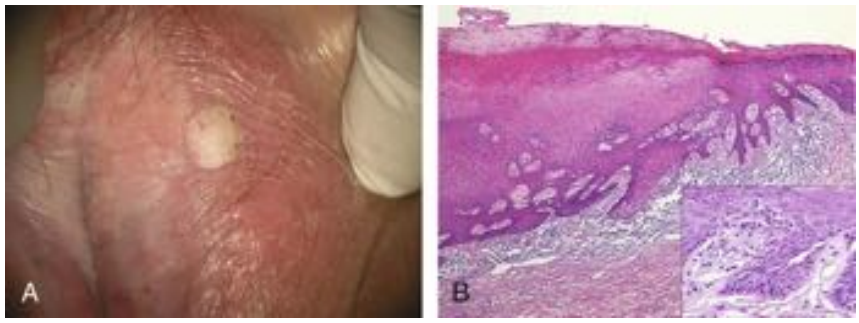

FIGURE 4. A, A 74-year-old with verrucous SCC, dVIN, and VAAD on background of pallor, lichenification, and architectural change characteristic of LS. B, VAAD adjacent to dVIN is demonstrated by thickened epithelium, parakeratosis, and an underlying pale band, with a sharp transition to the spiky rete ridges and basilar atypia (inset) of standard dVIN. Throughout there is a dense lymphocytic infiltrate and stromal sclerosis (H&E ×40, inset H&E ×400).

in cancer excision specimens that do not fulfill all criteria of the original VAAD description and agree that the nomenclature and definition should be revised to accommodate the various manifestations of this maturational abnormality.[31] The potential for reversibility of these lesions with aggressive treatment of the underlying dermatosis remains unclear. We found no clear histopathologic distinction between hypertrophic dVIN and verrucous SCC, and 4 (9%) of 43 cases showed coexistent verrucous and keratinizing SCC. Previous histopathologic studies of both vulvar and oral verrucous SCC specimens demonstrate foci of atypia or invasion consistent with conventional SCC in 20% to 35%; this is a high rate of comorbidity if these were synchronous-unrelated primaries.[32–34] From a clinical perspective, women with verrucous SCC have a substantial risk of concomitant or subsequent keratinizing SCC and should be managed similarly to women with dVIN.

Inherent to the retrospective design, limitations of this study include incomplete data, practice differences between clinicians, and the potential for selection bias. This study did not capture advanced vulvar or vaginal cancers confirmed by an external biopsy and referred directly for chemoradiation. A particular shortcoming of clinical notes was that details about LS symptoms and signs were lacking, as was documentation of the frequency of steroid use when prescribed "as needed." Women from outer regional areas often returned to their local doctor for ongoing care after several postoperative visits with gynecologic oncology; notes from these consultations were unavailable, which may have underestimated the number of women with diagnosis and treatment of LS during prolonged postsurgical follow-up. Immunohistochemistry for p16 was not universally obtained in cases of standard and hypertrophic dVIN; the potential for misdiagnosis was mitigated by the clinical context and expert pathology review. Universal clinical photography would have permitted a more nuanced description of tumor origin and location and severity of the adjacent dermatosis.

In summary, LP was not encountered in excision specimens for vulvar SCC occurring for more than a decade. Lichen sclerosus was histopathologically demonstrated in association with all HPV-independent SCC cases, either adjacent to the tumor or at a subsequent vulvar biopsy. Thus, several criteria should be satisfied before attributing vulvar cancer to LP: LS should be thoroughly considered and excluded, there should be clinicopathologic concordance in the diagnosis of LP, precursor lesions should be distinguished from LP, and the lesion should be a keratinizing or verrucous SCC arising in a field of LP.

REFERENCES

1. Chiesa-Vottero A, Dvoretsky PM, Hart WR. Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with an analysis of adjacent VIN types and lichen sclerosus. *Am J Surg Path* 2006;30: 310–8.
2. Lee A, Bradford J, Fischer G. Long-term management of adult vulvar lichen sclerosus: a prospective cohort study of 507 women. *JAMA Dermatol* 2015;151:1061–7.
3. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition? *Arch Dermatol* 2012;148:1314–6.
4. Ramos-e-Silva M, Jacques CD, Carneiro SC. Premalignant nature of oral and vulva lichen planus: facts and controversies. *Clin Dermatol* 2010;28: 563–7.
5. Derrick EK, Ridley CM, Kobza-Black A, et al. A clinical study of 23 cases of female anogenital carcinoma. *Br J Dermatol* 2000;143: 1217–23.
6. Regauer S, Reich O, Eberz B. Vulvar cancer in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014;71: 698–707.
7. Day T, Moore S, Bohl TJ, et al. Cormorbid lichen planus and lichen sclerosus. *J Low Genit Tract Dis* 2017;21:204–8.
8. Day T, Bowden N, Jaaback K, et al. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *J Low Genit Tract Dis* 2016;20:174–9.
9. Nascimento AF, Granter SR, Cviko A, et al. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinoma? *Am J Surg Pathol* 2004;28:638–43.
10. Patterson JW, Hosler GA. Tissue reaction patterns, Section 2, and The dermis, Section 4. In: Patterson JW, ed. *Weedon's Skin Pathology.* 4th ed. vol. 37–80. London: Churchill Livingstone Elsevier; 2016:36–54, 237–244.
11. Day T, Burston K, Dennerstein G, et al. Vestibulovaginal sclerosis versus lichen sclerosus. *Int J Gynecol Pathol*. 2017. Doi: 10.1097/ PGP.0000000000000441.
12. Wang W, Lazar A. Lichenoid and interface dermatitis, Chapter 7. In: Calonje E, Brenn T, Lazar A, et al, eds. *McKee's Pathology of the Skin With Clinical Correlations*. 4th ed. London: Elsevier; 2012:219–58.
13. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000;24:429–41.
14. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol* 2009;33: 1659–65.
15. Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. *Am J Dermatopathol* 2001; 23:413–8.
16. Carlson JA, Ambros R, Malfetano J, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. *Hum Pathol* 1998;29: 932–48.
17. Eisenberg E. Oral lichen planus: a benign lesion. *J Oral Maxillofac Surg* 2000;58:1278–85.
18. Van de Nieuwenhof HP, Bulten J, Hollema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;24:297–305.
19. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carincoma. *Mod Pathol* 2010;23:404–12.
20. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. *Int J Gynecol Pathol* 2003;22:57–62.
21. Ashton KA, Scurry J, Rutherford J, et al. Nodular prurigo of the vulva. *Pathology* 2010;44:565–7.
22. Weyers W. Hypertrophic lichen sclerosus sine sclerosis: clues to histopathologic disgnosis when presenting as psoriasiform lichenoid dermatitis. *J Cutan Pathol* 2015;42:118–29.
23. Yap JK, Fox R, Leonard S, et al. Adjacent lichen sclerosus predicts local recurrence and second field tumous in women with vulvar squamous cell carincoma. *Gynecol Oncol* 2016;142:420–6.
24. Kirtschig G, Becker K, Günthert A, et al. Evidence-based (S3) guideline on (anogenital) lichen sclerosus. *J Eur Acad Dermatol Venereol* 2015;29: e1–43.
25. Neill SM, Lewis FM, Tatnall FM, et al. British Association of Dermatologists' guideline for the management of lichen sclerosus. *Br J Dermatol* 2010;163:672–82.
26. Boardman L, Kennedy C. Diagnosis and management of vulvar skin conditions. *Obstet Gynecol* 2008;111:1243–53.

27. Anogenital Skin Conditions: lichen sclerosus. Victoria, Australia: Therapeutic Guidelines Ltd 2017. Available at: http://www.tg.com.au. Accessed December 1, 2017.
28. Koh WJ, Greer BE, Abu-Rustum NR, et al. Vulvar cancer, version 1.2017, clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2017;15: 92–120.
29. British Gynaecological Cancer Society and Royal College of Obstetricians and Gynaecologists. Guidelines for the diagnosis and management of vulvar carcinoma, May 2014. Available at: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/vulval-carcinoma-guidelines-for-the-diagnosis-and-management-of/. Accessed December 1, 2017.
30. Jones RW, Scurry J, Neill S, et al. Guidelines for the follow-up of women with vulvar lichen sclerosus in specialist clinics. *Am J Obstet Gynecol* 2008;198:e1–3.
31. Watkins JC, Howitt BE, Horowitz NS, et al. Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA. *Mod Pathol* 2017;30: 448–58.
32. Liu G, Li Q, Shang X, et al. Verrucous carcinoma of the vulva: a 20 year retrospective study and literature review. *J Low Genit Tract Dis* 2016;20: 114–8.
33. Haidopoulos D, Diakomanolis E, Rodolakis A, et al. Coexistence of verrucous and squamous carcinoma of the vulva. *Aust N Z J Obstet Gynaecol* 2005;45:60–3.
34. Shimizu A, Tamura A, Ishikawa O. Invasive squamous cell carcinoma arising from verrucous carcinoma: recognition of verrucous carcinoma of skin as an in-situ carcinoma. *Eur J Dermatol* 2006;16: 439–42.

5. Ethics

All of the included studies were either exempt or granted ethical approval from the Hunter New England Human Research Ethics Committee (HREC 14/2/19/5.08, 14/09/10/5.04, 14/03/19/5.05, and 15/11/18/5.02. The studies were also all registered with the University of Newcastle HREC.

Initial data collection required linkage of identifiable data between the pathology database, stored slides, and medical records held within John Hunter Hospital and clinicians' private rooms. After extracting and collating the data required for each study, it was de-identified and stored in Excel spreadsheet format on a password protected computer in a locked office. Data collection sheets are stored in a locked cabinet for five years from study completion date, in accordance with state Records Act 1998 (NSW) and the Joint NHMRC/ACC Statement and Guidelines on Research Practice.

6. Conclusions and recommendations

In 2008, an editorial by two eminent academic dermatologists described vulvovaginal LP as a "disease in need of a unified approach" [92]. They lamented the neglect of clinicopathologic correlation, the absence of diagnostic criteria, the deficiency of an evidence base for different treatments, and the "complete lack of consensus on a rational approach to therapy." In the interceding decade, much of the published work has not advanced the agenda they wisely proposed. However, several groups have made valuable contributions to the field. Simpson and colleagues' work on the electronic-Delphi model to establish consensus criteria for vulvovaginal erosive LP elucidated the clinical features thought by experienced clinicians to be diagnostic, while also revealing that more clinicopathologic work needed to be done to elaborate histopathologic criteria [132]. Sheinis' exploratory work on a standard outcome set for LS meanwhile demonstrated the impossibility of this task so long as there is no consensus on nomenclature and diagnostic criteria [90]. Bradford and Fischer's research into long-term outcomes of lichenoid dermatoses highlights the need to acknowledge the chronicity and comorbidities of these diseases, and brings the field closer to abandonment of a flawed 'treat and dismiss' approach to management [13,89,146]. Weyers has provided detailed insights into histopathologic features that distinguish LP and LS, and compellingly described the complex natural history of lichenoid dermatoses [111,121]. Studies published by Halonen, Scurry, Ordi, and Crum have substantially improved our understanding of the relative carcinogenicity of LS and LP and illustrated the spectrum of intraepithelial abnormalities that may precede invasive cancer [41,42,169,173,175].

The work contained in this thesis is offered as a remedy to the problematic neglect of clinicopathologic correlation. Each study addresses one of the many diagnostic unknowns in vulvovaginal lichenoid dermatoses. Similar methodology was employed in all nine studies, so they also share a set of limitations. The retrospective nature of the work results in several issues: some variables of interest were not available because they are not routinely documented, cases that underwent biopsy are more likely to be severe or complex, and differences in practice patterns complicate the interpretation of clinical results. In addition, the lack of a criterion standard for the diagnosis of vulval dermatoses

means that the validity of the work is founded upon the experience and intellectual humility of the investigators, as well as processes of international consensus-building and peer-review. The key conclusions from the collected studies are summarised.

a. Determination of anatomic site is fundamental to generation of a differential diagnosis and assessment of vulvovaginal specimens. The appearance of vulval epithelium varies by site: parakeratosis is normal at the MCJ, compact stratum corneum is common at hairless skin, and moderate lymphocytic infiltrate is often seen at the vestibule. Keratinisation at the vestibule or vagina is abnormal, with a differential diagnosis that includes VVS, LS, prolapse, or chronic trauma. Lichenoid dermatoses do not adhere to textbook definitions of disease location - LS may occur in the vestibule, erosive LP may extend onto hairless skin, and hypertrophic LP may affect both hairless and hair bearing skin of vulva and perianus. Clinicians and pathologists must identify anatomic site both in clinical documentation and in research activities.

b. Dermatologic and infectious comorbidities are common in lichenoid disorders. Comorbid LP and LS usually appear as glazed erythema over inner vulva, adjacent to circumferential pallor and textural change over hairless and hair bearing skin. Biopsies in morphologically distinct areas permit identification of both diagnoses. Neoplasia attributed to LP may instead represent unrecognized comorbid LS as the primary carcinogen. Mycotic superinfection is underappreciated by clinicians and the utility of microbiologic and histopathologic tests is limited by false negatives. Researchers should detail mycologic screening and treatment protocols in their methods, and document in their results the rates and treatments of candidosis and dermatophytosis.

c. Epithelial abnormalities consistent with erosive LP are categorised into two patterns: regenerative and degenerative. The clinical appearance is the same in both, and the patterns may occur together in the same lesion. Regenerative erosive LP may be interpreted by some pathologists as non-specific inflammation, contributing to the high non-diagnostic biopsy rate. Regenerative erosive LP is also easily mistaken for the

basaloid pattern of dVIN, and IHC is not helpful in distinguishing between the two. This issue may explain some cases of misattribution of vulval neoplasia to erosive LP.

d. The high non-diagnostic biopsy rate for presumed vulvovaginal LP is partially due to clinician factors. Suboptimal timing and site of biopsy contribute to falsely negative histopathology. Meanwhile, true negatives occur when vulvodynia and candidosis are mistaken for LP, as all three present with pain and vestibular erythema. Clinical studies that enroll women with presumed vulvovaginal LP should include a biopsy, and then stratify outcomes and treatment response based on histopathologic verification of disease. Alternatively, clinical studies should be restricted to subjects with a clinicopathologic diagnosis of disease, and criteria should be elaborated in the methods.

e. The clinicopathologic appearance of vulval classic and hypertrophic LP is more complex than disease on non-genital skin. Hypertrophic LP often demonstrates a pattern of central erythema, edema, and maceration transitioning laterally to pink-gray lichenification. Histopathology is difficult to distinguish from lichenified psoriasis because basal layer changes may be focal and masked by exocytosis; it is also easily mistaken for hypertrophic dVIN as cellular reactivity has a similar appearance to nuclear atypia. Studies of LP should specify site and clinicopathologic type of disease and report the natural history and treatment response of each category.

f. Clinicians and pathologists should exercise caution when evaluating cases of suspected LP-associated neoplasia. On careful histopathologic review, LS is found almost universally in excisions of HPV-independent vulvar cancer; its greater carcinogenic potential likely relates to the scar-cancer neoplastic pathway. Before attributing vulvar cancer to LP, a series of criteria must be met: there must be clinicopathologic concordance in the diagnosis of LP with certainty that LS is not present, the possibility of HPV-dependent disease must be excluded, precursor lesions should be distinguished from LP, and the lesion should be a keratinising or verrucous SCC arising in a field of LP.

Future work

Studies performed as part of this PhD inspired the creation of an ISSVD committee on 'difficult dermatologic diagnoses', tasked to write consensus criteria for the histopathologic diagnosis of LP, LS, and dVIN. This work has already begun, with a plan to present a draft document for comment at the 2019 Scientific Meeting. Meanwhile, ethics approval has been gained and data collection initiated for a comprehensive histopathologic and IHC assessment of over 100 vestibular biopsies from both healthy asymptomatic subjects and women afflicted by vulvovaginal pain. The goal of this project is to establish the definition of normal histology in this distinct anatomic zone, and determine if there are any features that distinguish between women with and without pain. Finally, analysis is underway of a study into the histopathologic appearance of lichenoid disorders comorbid with HSIL. Although this phenomenon has been documented by several authors, a description of the patterns of clinical presentation and each disease's impact on the appearance of the other has not been produced.

The recent convergence of multiple factors makes this an opportune time to progress in our understanding of vulvovaginal disease. Cultural changes have improved women's willingness to present to care for genital complaints, insist upon evaluation and treatment, advocate for research into their disease, and share their experiences and knowledge across an interconnected world. Scientists in collaboration with clinicians and pathologists are harnessing insights into the microbiome and genomics to investigate disease aetiology and treatment. Researchers are drawing upon lessons learned in other fields to define key problems and standardise definitions and outcome sets. Work performed over the next decade should provide a foundation for the long-desired 'unified approach' to vulvovaginal LP, and hopefully then be followed by breakthroughs in prevention and cure.

7. Bibliography

1. Terlou A, Santegoets LA, van der Meijden WI, et al. An autoimmune phenotype in vulvar lichen sclerosus and lichen planus: a Th1 response and high levels of microRNA-155. *J Invest Dermatol* 2012; 132:658–66.

2. Eisenberg E. Oral lichen planus: a benign lesion. *J Oral Maxillofac Surg* 2000;58:1278-85.

3. Wang W, Lazar A. Lichenoid and interface dermatitis, Chapter 7. In: Calonje E, Brenn T, Lazar A, McKee PH, eds. *McKee's Pathology of the Skin With Clinical Correlations* 4th ed. London: Elsevier; 2012:219-258.

4. Patterson JW, Hosler GA. Tissue reaction patterns, Section 2, and The dermis and subcutis, Section 4. In: Patterson JW, ed. *Weedon's Skin Pathology* 4th ed. London: Churchill Livingstone Elsevier 2016; 37-80, 362-364.

5. Bhargava K, Lewis FM. Lichen sclerosus occurring on vaginal mucosa secondary to uterine prolapse. *J Obstet Gynaecol* 2013;33:319-320.

6. Zendell K, Edwards L. Lichen sclerosus with vaginal involvement: a report of 2 cases and review of the literature. *JAMA Derm*;149:1199-1202.

7. Longinotti M, Schieffer YM, Kaufman RH. Lichen sclerosus involving the vagina. *Obstet Gynecol* 2005;106:1217-1219.

8. Moyal-Barracco M, Edwards L. Diagnosis and management of anogenital lichen planus. *Dermatol Ther* 2004;17:38-46.

9. Goldstein AT, Metz A. Vulvar lichen planus, *Clin Obstet Gynecol* 2005;48:818-823.

10. Atzmony L, Reiter O, Hodak E, Gdalevich M, Mimouni D. Treatments for cutaneous lichen planus: a systematic review and meta-analysis. *Am J Clin Dermatol* 2016;17:11-22.

11. Knacksteadt TJ, Collins LK, Li Z, Yan S, Samie FH. Squamous cell carcinoma arising in hypertrophic lichen planus: a review and analysis of 38 cases. *Dermatol Surg* 2015;41:1411-8.

12. McPherson R, Cooper S. Vulval lichen sclerosus and lichen planus. *Dermatol Ther* 2010;23:523-32.

13. Bradford J, Fischer G. Management of vulvovaginal lichen planus: a new approach. *J Low Genit Tract Dis* 2013;17:28-32.

14. Santegoets LA, Helmerhorst TJ, van der Meijden WIA. Retrospective study of 95 women with a clinical diagnosis of genital lichen planus. *J Low Genit Tract Dis* 2010;14:323-328.

15. Cooper SM, Wojnarowska F. Influence of treatment of erosive lichen planus of the vulva on its prognosis. *Arch Dermaol* 2006;142:289-294.

16. van de Nieuwenhof HP, Bulten J, Hollema H Dommerholt LM, van der Zee AGJ. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;24:297-305.

17. Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. *Int J Gynecol Cancer* 1999;9:89-97.

18. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000;24:429-441.

19. Carlson JA, Ambros R, Malfetano J, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. *Hum Pathol* 1998;29:932-948.

20. Van de Nieuwenhof HP, Bulten J, Hollema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;24:297-305.

21. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol* 2010;23:404-412.

22. Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014;71:698-707.

23. Simpson RC, Littlewood SM, Cooper SM et al. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. *Br J Dermatol* 2012;167:85-91.

24. Belfiore P, Di Fede O, Cabibi D et al. Prevalence of vulval lichen planus in a cohort of women with oral lichen planus: an interdisciplinary study. *Br J Dermatol* 2006;155:994-998.

25. Kirschig G, Wakelin SH, Wojnarowska F. Mucosal lichen planus: outcome, clinical and laboratory features. *J Eur Acad Dematol Venereol* 2005;19:301-7.

26. Edwards SK, Bates C, Lewis F, Sehti C. 2014 UK National Guideline on the Management of Vulval Conditions. *Int J STD AIDS* 2015;26:611-24.

27. Lewis FM, Tatnall FM, Velangi SS, Kumar A, Brackenbury MF et al. British Association of Dermatologists' guideline for the management of lichen sclerosus, 2018. *Br J Dermatol* 2018;178:839-53.

28. Boardman L, Kennedy C. Diagnosis and management of vulvar skin conditions. *Obstet Gynecol* 2008;111:1243-1253.

29. Anogenital Skin Conditions: lichen sclerosus. Victoria, Australia: Therapeutic Guidelines Ltd 2017 [cited 2017 Dec 1]. Available from: www.tg.com.au.

30. Kirtschig G, Becker K, Gunthert A et al. Evidence-based (S3) guideline in (anogenital) lichen sclerosus. *J Eur Acad Dematol Venereol* 2015;29:e1-43.

31. Jones RW, Scurry J, Neill S, MacLean AB. Guidelines for the follow-up of women with vulvar lichen sclerosus in specialist clinics. *Am J Obstet Gynecol* 2008;198:e1-3.

32. Koh WJ, Greer BE, Abu-Rustum NR et al. Vulvar cancer version 1.2017: clinical practice guidelines in oncology. *J Nat Comp Cancer Network* 2017;15:92-120.

33. British Gynaecological Cancer Society and Royal College of Obstetricians and Gynaecologists. Guidelines for the diagnosis and management of vulvar carcinoma, May 2014 [Cited 2017 Dec 1]. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/vulval-carcinoma-guidelines-for-the-diagnosis-and-management-of/.

34. Wallace HJ. Lichen sclerosus et atrophicus. *Trans St Johns Hosp Dermatol Soc* 1971;57:9-30.

35. Goldstein AT, Marinoff SC, Christopher K, Srodon M. Prevalence of vulvar lichen sclerosus in a general gynaecology practice. *J Repro Med* 2005;50:477-80.

36. Leibovitz A, Kaplun VV, Saposhnicov N, Habot B. Vulvovaginal examinations in elderly nursing home women residents. *Ach Gerontol Geriatr* 2000;1:1-4.

37. Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: a study of 723 patients. *J Am Acad Dermatol* 2002;46:207-14.

38. Eisen D. The evaluation of cutaneous, genital, scalp, nail, esophageal, and ocular involvement in patients with oral lichen planus. *Oral Surg Oral Med Oral Path Oral Radiol Endod* 1999;88:431-6.

39. Micheletti L, Preti M, Bogliatto F, Zanotto-Valentino MC, Ghiringhellp B, Massobrio M. Vulval lichen planus in the practice of a vulval clinic. *Br J Dermatol* 2000;143:1349-1350.

40. Nyirjesy P, Leigh RD, Mathew L, Lev-Sagie A, Culhane JF. Chronic vulvovaginitis in women older than 50 years: analysis of a prospective database. *J Low Genit Tract Dis* 2012;16:24-9.

41. Halonen P, Jakobsson M, Heikinheimo O, Riska A, Gissler M, et al. Lichen sclerosus and risk of cancer. *Int J Cancer* 2017;140:1998-2002.

42. Halonen P, Jakobsson M, Heikinheimo O, Riska A, Gissler M, et al. Cancer risk of lichen planua: a cohort study of 13,100 women in Finland. *Int J Cancer* 2018;142:18-22.

43. Lawton S, Littlewood S. Vulval skin conditions: disease activity and quality of life. *J Low Genit Tract Dis* 2013;17:117-124.

44. Nyirjesy P, Robinson J, Methew L, Lev-Sagie A, Reyes I et al. Alternative therapies in women with chronic vaginitis. *Obstet Gynecol* 2011;117:856-61.

45. Simpson RC, Thomas KS, Murphy R. Vulval erosive lichen planus: a qualitative investigation of UK clinician views and principles of management. *Br J Dermatol* 2013;169:226-227.

46. Simpson RC, Murphy R. Considerations for disease impact and outcomes measures in vulvar disease. *J Low Genit Tract Dis* 2012;16:460-3.

47. http://www.dermatology.org.uk/quality/dlqi/quality-dlqi.html. Accessed 4 March 2015

48. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI): A simple practical measure for routine clinical use. *Clin Exper Dermatol* 1994;19:210-216.

49. Basra MKA, Fenech R, Gatt RM, Salek MS, Finlay AY. The Dermatology Life Quality Index 1994-2007: a comprehensive review of validation data and clinical results. *Br J Dermatol* 2008;159:997-1035.

50. Melilli L, Shikiar R, Thompson C. Minimal clinically important difference in Dermatology Life Quality Index in moderate to severe plaque psoriasis patients treated with adalimumab. *J Am Acad Dermatol* 2006;54:AB221(P2894).

51. Shikiar R, Harding G, Leahy Michael, Lennow RD. Minimal Important Difference (MID) of the Dermatology Life Quality Index (DLQI): Results from patients with chronic idiopathic urticaria. *Health Qual Life Outcomes* 2005;3:36.

52. Van de Nieuwenhof HP, Meeuwis KAP, Nieboer TE, Vergeer MCM, et al. The effect of vulvar lichen sclerosus on quality of life and sexual functioning. *J Psychosom Obs Gynecol* 2010;31:279-84.

53. Lockhart J, Gray NM, Cruickshank ME. The development and evaluation of a questionnaire to assess the impact of vulval intraepithelial neoplasia: a questionnaire study. *BJOG* 2013;120:1133-1142.

54. Schwegler J, Schwarz J, Eulenburg C et al. Health-related quality of life and patient-defined benefit of clobetasol 0.05% in women with chronic lichen sclerosus of the vulva. *Dermatology* 2011;223:152-160.

55. Scurry J, Melville KR. Normal vulva and developmental anomalies. In: Heller DS, Wallach RC, eds. Vulvar Disease: A Clinicopathological Approach. Boca Raton, FL: Taylor and Francis Group LLC 2007;18-22.

56. Lewis FM, Neill SM. Principles of examination, investigation and treatment. In Neill SM, Lewis FM, eds. Ridley's The Vulva, 3rd ed. London: Wiley-Blackwell 2009;34-43.

57. Tommola P, Unkila-Kallio L, Paetau A, Meri S, Kalso E, Paavonen J. Immune activation enhanced epithelial nerve growth in provoked vestibulodynia. *Am J Obstet Gynecol* 2016;215:768.e1-8.

58. Harper WF, McNicol EM. A histologic study of normal vulval skin from infancy to old age. *BJOG* 1977;96:249-53.

59. Jones, IS. A histological assessment of normal vulval skin. *Clin Exp Dermatol* 1983; 8:513-521.

60. Barrett MM, Carlson JA. Vulvar lymphangiectasis, an indicator of lymph stasis, is an authentic sign of abnormal histopathology in labiaplasty specimens. *J Low Genit Tract Dis* 2014;18:13-20.

61. Scurry J, Marchitelli C, Moyal-Barracco M. Labiaplasty specimens: when should pathological findings be considered as abnormal? *J Low Genit Tract Dis* 2015;19:e49-50.

62. Brokenshire C, Pagano R, Scurry J. The value of histology in predicting the effectiveness of vulvar vestibulectomy in provoked vestibulodynia. *J Low Genit Tract Dis* 2014;18:109-114.

63. Halperin R, Zehavi S, Vaknin Z, et al. The major histopathologic considerations in the vulvar vestibulitis syndrome. *Gynecol Obstet Invest* 2005;59:75-79.

64. Lazar AJF, Wang W. Skin. In: Kumar V, Abbas A, Aster J, eds. Robbins Basic Pathology, 9th ed. Philadelphia, PA: Elsevier Saunders 2013;851-70.

65. Lundqvist EN, Hofer PA, Olofsson JL, Sjoberg I. Is vulvar vestibulitis an inflammatory condition? A comparison of histological findings in affected and healthy women. *Acta Derm Venereol* 1997;319-322.

66. Tommola P, Butzow R, Unkila-Kallio L, Paavonen J, Meri S. Activation of vestibule-associated lymphoid tissue in localized provoked vestibulodynia. *Am J Obstet Gynecol* 2015;212:476.e1-e8.

67. Simpson RC, Thomas KS, Murphy R. Outcome measures for vulval skin conditions: a systematic review of randomized controlled trials. *Br J Dermatol* 2013;169:494-501.

68. Rajar UD, Majeed R, Parveen N, Sheikh I, Sushel C. Efficacy of aloe vera gel in the treatment of vulval lichen planus. *J Coll Phys Surg Pak* 2008;18:612-4.

69. Thongprasom K, Luagjarmekorn L, Sererat T, Taweesap W. Relative efficacy of flucinolone acetonide compared with triamcinolone acetonide in treatment of oral lichen planus 1992. *J Oral Pathol Med*;21:456-8.

70. Burrows LJ, Creasey A, Goldstein AT. The treatment of vulvar lichen sclerosus and female sexual dysfunction. *J Sex Med* 2011;8:219-22.

71. Goldstein AT, Creasey A, Pfau R, Phillips D, Burrows LJ. A double-blind, randomized controlled trial of clobetasol versus pimecrolimus in patients with vulvar lichen sclerosus. *J Am Acad Dermatol* 2011;64:e99-104.

72. Orogoni M, Ferrari D, Rossi M et al. Topical oxatomide: an alternative approach for the treatment of vulvar lichen sclerosus. *Int J Gynaecol Obstet* 1996;55:259-64.

73. D'Antuono A, Bellavista S, Negosanti F et al. Dermasilk briefs in vulvar lichen sclerosus: an adjuvant tool. *J Low Genit Tract Dis* 2011;15:287-91.

74. Bracco GL, Carli P, Sonni L et al. Clinical and histologic effects of topical treatments of vulval lichen sclerosus: a critical evaluation. *J Reprod Med* 1993;38:37-40.

75. Paslin D. Treatment of lichen sclerosus with topical dihydrotestosterone. *Obstet Gynecol* 1991;78:1046-9.

76. Paslin D. Androgens in the topical treatment of lichen sclerosus. *Int J Dermatol* 1996;35:298-301.

77. Sideri M, Origoni M, spinaci L et al. Topical testosterone in the treatment of vulvar lichen sclerosus. *Int J Gynaecol Obstet* 1994;46:53-6.

78. Cattaneo A. Carli P, De Marco A et al. Testosterone maintenance therapy: effects on vulval lichen sclerosus treated with clobetasol propionate. *J Reprod Med* 1996;41:99-102.

79. Helgesen ALO, Warloe T, Pripp AH, et al. Vulvovaginal photodynamic therapy vs topical corticosteroids in genital erosive lichen planus: a randomized controlled trial. *Br J Dermatol* 2015;173:1156-62.

80. Wennberg AM. Vulvovaginal photodynamic therapy for genital erosive lichen planus. *Br J Dermatol* 2015;173:1119.

81. Simpson RC, Murphy R, Bratton DJ et al. Systemic therapy for vulval Erosive Lichen Planus (the 'hELP' trial): study protocol for a randomized controlled trial. *Trials* 2016;17:2. doi: 10.1186/s13063-015-1133-z.

82. Centre of Evidence Based Dermatology; hELP trial at https://www.nottingham.ac.uk/research/groups/cebd/projects/help-trial/index.aspx; accessed 6 March 2018.

83. Simpson RC, Murphy R, Bratton DJ et al. Help for future research: lessons learned in trial design, recruitment, and delivery from the "hELP" study. *J Low Genit Tract Dis* 2018; Jul 2. doi: 10.1097/LGT.0000000000000407.

84. Shi L, Miao F, Zheng LL et al. Comparison of 5-aminolevulinic aicd photodyanmic therapy and clobetasol propionate in treatment of vulvar lichen sclerosus. *Acta Derm Venereol* 2016;96:64-8.

85. Terras S, Gamichler T, Moritz RK, Stucker M, Kreuter A. UV-A1 phototherapy vs clobetasol propionate 0.05%, in the treatment of vulvar lichen sclerosus: a randomised clinical trial. *JAMA Dermatol* 2014;150:621-7.

86. Funaro D, Lovett A, Leroux N, Powell J. A double-blind randomized propsective study evaluating topical clobetasol propionate 0.05% versus topical tacrolimus 0.1% in patients with vulvar lichen sclerosus. *JAAD* 2014;71:84-91.

87. Virgili A, Borghi G, Toni S, Minghetti S, Corazza M. First randomized trial on clobetasol propionate and mometasone furoate in the treatment of lichen sclerosus: results of efficacy and tolerability. *Br J Derm* 2014;171:388-96.

88. Corazza M, Borghi A, Minghetti S, Toni G, Virgili A. Clobetasol propionate vs mometasone furoate in a 1-year proactive maintenance therapy of vulvar lichen sclerosus: results from a comparative trial. *J Eur Acad Dermatol Venereol* 2016;30:956-61.

89. Lee A, Bradford J, Fischer G. Long-term management of adult vulvar lichen sclerosus: a prospective cohort study of 507 women. *JAMA Derm* 2015;151:1061-1067.

90. Sheinis M, Selk A. Development of the adult vulvar lichen sclerosus severity scale – a Delphi consensus exercise for item generation. *J Low Genit Tract Dis* 2018;22:66-73.

91. Fahy CMR, Torgerson RR, Davis MDP. Lichen planus affecting the female genitalia: a retrospective review of patients at Mayo Clinic. *J Am Acad Dermatol* 2017;77:1052-1059.

92. Neill SM, Lewis FM. Vulvovaginal lichen planus: a disease in need of a unified approach. *Arch Dermatol* 2008;144:1502-1503.

93. Palefsky JM. Practising high-resolution anoscopy. *Sex Health* 2012;9:580-6.

94. Markell KW, Bilingham RP. Pruritus ani: etiology and management. *Surg Clin North Am 2010*;90:125-35.

95. Nasseri YY, Osborne MC. Pruritus ani: diagnosis and treatment. *Gastroenterol Clin North Am* 2013;42:801-813.

96. Bowyer A, McColl I. A study of 200 patients with pruritus ani. *Proc R Soc Med* 1970;63(Suppl.):96-8.

97. Kranke B, Trummer M, Brabek E *et al*. Etiologic and causative factors in perianal dermatitis: results of a prospective study in 126 patients. *Wien Klin Wochenschr* 2006;118:90-4.

98. Hadasik K, Hadasik G, Brzezinska-Wcislo L. Potential aetiologic factors in patients with persistent pruritus ani. *Postepy Nauk Med* 2015;3:173-6.

99. Chiesa-Vottero A, Dvoretsky PM, Hart WR. Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with an analysis of adjacent VIN types and lichen sclerosus. *Am J Surg Path* 2006;30:310-318.

100. Bornstein J, Bogliatto F, Haefner HK et al. The 2015 ISSVD Terminology of vulvar squamous intraepithelial lesions. *Obstet Gynecol* 2016;127:264-8.

101. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition? *Arch Dermatol* 2012;148:1314-16.

102. Ramos-e-Silva M, Jacques C, Carneiro SC. Premalignant nature of oral and vulva lichen planus: facts and controversies. *Clin Dermatol* 2010;28:563-567.

103. Derrick EK, Ridley CM, Kobza-Black A, McKee PH, Niell SM. A clinical study of 23 cases of female anogenital carcinoma. *Br J Dermatol* 2000;143:1217-1223.

104. Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: a clinicopathological study. *J Am Acad Dermatol* 2014;71:698-707.

105. Yap JK, Fox R, Leonard S et al. Adjacent lichen sclerosus predicts local recurrence and second field tumours in women with vulvar squamous cell carcinoma. *Gynecol Oncol* 2016;142:420-426.

106. Van Seters M, ten Kate FJ, van Beurden M et al. In he absence of (early) invasive carincoma, vulvar intraepithelial neoplasia associated with lichen sclerosus is mainly of undifferentiated type: new insights in histology and aetiology. *J Clin Path* 2007;60:504-9.

107. Rakislova N, Alemany L, Clavero O et al. Differentiated vulvar intraepithelial neoplasia-like and lichen sclerosus-like lesions in HPV-associated squamous cell carcinomas of the vulva. *Am J Surg Path* 2018;42:828-835.

108. High W, Tomasini C, Argenziano G, et al. Basic principles of dermatology. In: Jorizzo J, Schaffer J, Bolognia J, et al., eds. Dermatology. 3rd ed. Philadelphia, PA: Elsevier Saunders; 2012:1-42.

109. Dennerstein G, Scurry J, Brenan J, Allen D, Marin MG. The vulva and vagina manual 2005. BPA Print Group, Melbourne, Australia.

110. Fung MA, LeBoit PE. Light microscopic criteria for the diagnosis of early vulvar lichen sclerosus: a comparison with lichen planus. *Am J Surg Pathol* 1998;22:340-7.

111. Weyers W. Hypertrophic lichen sclerosus with dyskeratosis and parakeratosis – a common presentation of vulvar lichen sclerosus not associated with a significant risk of malignancy. *Am J Dermatopathol* 2013;35:713-21.

112. Bushkell LL, Friedrich EG, Jordon RE. An appraisal of routine direct immunofluorescence in vulvar disorders. *Acta Derm Venereol* 1981;61:157-61.

113. Godoy CA, Teodora WR, Velosa AP, et al. Unusual remodeling of the hyalinization band in vulvar lichen sclerosus by type V collagen and ECM 2 protein. *Clinics (Sao Paolo)* 2015;70:356-62.

114. Machesney M, Tidman N, Waseem A, et al. Activated keratinocytes in the epidermis of hypertrophic scars. *Am J Pathol* 1998;152:1133–41.

115. Baird PJ. Lichen sclerosus et atrophicus (dissertation), Sydney: University of Sydney, Department of Pathology, 1981.

116. Yang S, Sun Y, Geng Z, et al. Abnormalities in the basement membrane structure promote basal keratinocytes in the epidermis of hypertrophic scars to adopt a proliferative phenotype. *Int J Mol Med* 2016;37:1263-73.

117. Guitart J, Peduto M, Caro W, et al. Lichenoid changes in mycosis fungoides. *J Am Acad Dermatol* 1997;36:417-22.

118. Fujimoto M, Basko-Plluska JL, Petronic-Rosic V, et al. Early morphea simulating patch-stage mycosis fungoides. *Am J Dermatopathol* 2015;37:490-12.

119. Regine Mydlarski PR, Barber DF, Robertson LH. Painless pink papules with central porcelain-white scars. *CMAJ* 2008;179:1171-3.

120. Fadare O. Vaginal stromal sclerosis: a distinctive stromal change associated with vaginal atrophy. *Int J Gynecol Pathol* 2011;30:295-300.

121. Weyers W. Hypertrophic lichen sclerosus sine sclerosis: clues to histopathologic diagnosis when presenting as psoriasiform lichenoid dermatitis. *J Cutan Pathol* 2015;42:118-29.

122. Marren P, Millard PR, Wojnarowska F. Vulval lichen sclerosus: lack of correlation between duration of clinical symptoms and histological appearances. *J Eur Acad Dermatol Venereol* 1997;8:212-6.

123. Regauer S, Liegl B, Reich O. Early lichen sclerosus: a histopathological challenge. *Histopathology* 2005;47:340-347.

124. Ellis E, Fischer G. Pre-pubertal-onset vulvar lichen sclerosus: the importance of maintenance therapy in long-term outcomes. *Pediatr Dermatol* 2015;32:461-7.

125. Powell J, Wojanarowska F. Childhood vulvar lichen sclerosus: an increasingly common problem. *J Am Acad Dermaol* 2001;44:803-6.

126. Lagerstedt M, Karvinen K, Joki-Erkkila M, et al. Childhood lichen sclerosus - a challenge for clinicians. *Pediatr Dermatol* 2013;30:444-50.

127. Yousefi M, Pride H, Elston D. Case report: vulvar lichen sclerosus in a premenarchal girl with a complicated biopsy. *Pediatr Dermatol* 2009;26:357-8.

128. Topal IO, Sayilgan AT, Kalcin S. An uncommon cause of vulval pruritus in childhood: lichen sclerosus. *Turk Pediatri Ars* 2014;49:86-7.

129. Hassanein AM, Mrstik ME, Hardt NS, Morgan LA, Wilkinson EJ. Malignant melanoma associated with lichen sclerosus in the vulva of a 10-year-old. *Pediatric Dermatol* 2004;21:473-6.

130. Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. *Am J Dermatopath* 2001;23:413-418.

131. Niamh L, Naveen S, Hazel B. Diagnosis of vulval inflammatory dermatoses: a pathological study with clinical correlation. *Int J Gynecol Pathol* 2009;28:554-558.

132. Simpson RC, Thomas KS, Leighton P et al. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. *Br J Dermatol* 2013;169:337-343.

133. Cheng H, Oakley A, Rowan D, Lamont D. Diagnostic criteria in 72 women with erosive lichen planus. *Australas J Dermatol* 2015; doi 10.1111/ajd.12355.

134. ACOG Practice Bulletin No. 93: diagnosis and management of vulvar skin disorders (reaffirmed 2016). *Obstet Gynecol* 2008;111:1243-53.

135. O'Keefe RJ, Scurry JP, Dennerstein G et al. Audit of 114 non-neoplastic vulvar biopsies. *Br J Obstet Gynaecol* 1995;102:780-786.

136. Bowen AR, Vester A, Marsden L, et al. The role of vulvar skin biopsy in the evaluation of chronic vulvar pain. *Am J Obstet Gynecol* 2008;199:467e1-6.

137. Harris V, Fischer G, Bradford JA. The aetiology of chronic vulval pain and entry dyspaerunia: a retrospective review of 525 cases. *Aust NZ J Obstet Gynaecol* 2017;57:446-51.

138. Bornstein J, Goldstein AT, Stockdale CK et al. 2015 ISSVD, ISSWSH, and IPPS consensus terminology and classification of persistent vulvar pain and vulvodynia. *Obstet Gynecol* 2016;127:607-12.

139. Moyal-Barracco M, Lynch P. 2003 ISSVD terminology and classification of vulvodynia: a historical perspective. *J Reprod Med* 2004;49:772-7.

140. Micheletti L, Radici G, Lynch PJ. Is the 2003 ISSVD terminology and classification of vulvodynia up-to-date? A neurobiological perspective. *J Obstet Gynaecol* 2014;34:285-8.

141. Donders GG, Bellen G. Cream with cutaneous fibroblast lysate for the treatment of provoked vestibulodynia: a double-blind randomised placebo-controlled crossover study. *J Low Genit Tract Dis* 2012;16:427-36.

142. Foster DC, Kotok MB, Huang LS et al. Oral despramine and topical lidocaine for vulvodynia: a randomised controlled trial. *Obstet Gynecol* 2010;116:583-93.

143. Lynch PJ. Lichen simplex chronicus (atopic/neurodermatitis) of the anogenital region. *Dermatol Ther* 2004;17:8-19.

144. Kapila S, Bradford J, Fischer G. Vulvar psoriasis in adults and children: a clinical audit of 194 cases and review of the literature. *J Low Genit Tract Dis* 2012;16:364-71.

145. Gugliemetti A, Conlledo R, Bedoya J, Ianiszewski F, Correa J. Inverse psoriasis involving genital skin folds: successful treatment with dapsone. *Dermatol Ther* 2012;2:1-9.

146. Bradford J, Fischer G. Long-term management of vulval lichen sclerosus in adult women. *Aust NZ Obstet Gynaecol* 2010;50:148-52.

147. Fischer G. Chronic vulvovaginal candidiasis: what we know and what we have yet to learn. *Aust J Derm* 2012;53:247-254.

148. Sobel JD. Recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* 2016;214:15-21.

149. Hong E, Dixit S, Fidel PL, Bradford J, Fischer G. Vulvovaginal candidiasis as a chronic disease: diagnostic criteria and definition. *J Low Genit Tract Dis* 2014;18:31-38.

150. Nguyen Y, Lee A, Fischer G. Management of chronic vulvovaginal candidiasis: a long term retrospective study. *Australas J Dermatol* 2017;58:e188-92.

151. Mendling W, Brasch J, Comely OA et al. Guideline: vulvuvaginal candidosis (AWMF 015/072), S2k (excluding chronic mucocutaneous candidosis). *Mycoses* 2015;58 Suppl 1:1-15.

152. Beikert FC, LE MT, Koeninger A, et al. Recurrent vulvovaginal candidosis: focus on the vulva. *Mycoses* 2011;54:e807–10.

153. Toeima E, Sule M, Warren R, Igali L. Diagnosis and treatment of Zoon's vulvitis. *J Obstet Gynaecol* 2011;31:473-5.

154. Li Q, Leopold K, Carlson JA. Chronic vulvar purpura: persistent pigmented purpuric dermatitis (lichen aureus) of the vulva or plasma cell (Zoon's) vulvitis? *J Cutan Pathol* 2003;30:572-6.

155. Sobel JD. Prognosis and treatment of desquamative inflammatory vaginitis. *Obstet Gynecol* 2011;117:850-5.

156. Bradford J, Fischer G. Desquamative inflammatory vaginitis: differential diagnosis and alternate diagnostic criteria. *J Low Genit Tract Dis* 2010;14:306-310.

157. Murphy R, Edwards L. Desquamative inflammatory vaginitis: what is it? *J Repro Med* 2008;53:124-8.

158. Pereira N, Edlind TD, Schlievert PM, Nyirjesy P. Vaginal toxic shock syndrome triggering desquamative inflammatory vaginitis. *J Low Genit Tract Dis* 2013;17:88-91.

159. Veitch D, Kravvas G, Hughes S, Bunker C. A rare colocalization of lichen planus and vitiligo. *Case Rep Dermatol Med* 2015;doi:10.1155/2015/840193.

160. Attili VR, Attili SK. Acral vitiligo and lichen sclerosus – association or distinct pattern? A clinical and histopathologic review of 15 cases. *Indian J Dermatol* 2015;60:519.

161. Dennin MH, Stein SL, Rosenblatt AE. Vitligoid variant of lichen sclerosus in young girls with darker skin types. *Pediatr Dermatol* 2018;doi:10.1111/pde.13399.

162. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. *Int J Gynecol Pathol* 2003;22:57-62.

163. Ashton KA, Scurry J, Rutherford J, Otton G, Scott R, Bowden NA. Nodular prurigo of the vulva. *Pathology* 2010;44:565-567.

164. Levandoski KA, Nazarian RM, Asgari MM. Hypertrophic lichen planus mimicking squamous cell carcinoma: the importance of clinicopathologic correlation. *JAAD Case Rep* 2017;3:151-4.

165. Tan E, Malik R, Quirk CJ. Hypertrophic lichen planus mimicking squamous cell carcinoma. *Australas J Dermatol* 1998;39:45-7.

166. Kazlouskaya V, Junkins-Hopkins JM. Pseudoepitheliomatous hyperplasia in a red pigment tattoo: a separate entity or hypertrophic lichen planus-like reaction. *J Clin Aesthet Dermatol* 2015;8:48-52.

167. Jones RW, Rowan DM, Kirker J, Wilkinson EJ. Vulval lichen planus: progression of pseudoepitheliomatous hyperplasia to invasive vulval carcinomas. *BJOG* 2011;108:665-6.

168. Bornstein J, Bogliatto F, Haefner HK et al. The 2015 ISSVD terminology of vulvar squamous intraepithelial lesions. *Obstet Gynecol* 2016;127:264-8.

169. Nascimento AF, Granter SR, Cviko A, et al. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinoma? *Am J Surg Path* 2004;28:638-643.

170. Liu G, Li Q, Shang X et al. Verrucous carcinoma of the vulva: a 20 year retrospective study and literature review. *J Low Genit Tract Dis* 2016;20:114-8.

171. Haidopoulos D, Diakomanolis E, Rodolakis A et al. Coexistance of verrucous and squamous carcinoma of the vulva. *Aust N Z J Obstet Gynaecol* 2005;45:60-3.
172. Shimizu A, Tamura A, Ishikawa O. Invasive squamous cell carcinoma arising from verrucous carcinoma: recognition of verrucous carcinoma of skin as an in-situ carcinoma. *Eur J Dermatol* 2006;16:439-42.

173. Watkins JC, Howitt BE, Horowitz NS et al. Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA. *Mod Pathol* 2017;30:448-58.

174. Darraugh TM, Colgan TJ. Thomas Cox J, Heller DS, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Path* 2013;32:76-115.

175. Ordi J, Alejo M, Fuste V et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol* 2009;33:1659-1665.

176. Santos M, Montagut C, Mellado B et al. Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. *Int J Gynaecol Pathol* 2004;23:206-14.

8. Appendix

8.1 Coauthorship declaration forms

8.2 Copyright permissions

UON Graduate Research

Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

TITLE AND CITATION	Normal vulvar histology: variation by site. Day T, Holland S, Scurry J. Normal vulvar histology: variation by site. *Journal of Lower Genital Tract Disease* 2016;20(1):64-9.

1	Tarsi Day	Co-reviewed all slides Collected and analysed data Wrote manuscript	60%
2	Sean Holland	Generated list of eligible patients Collected and analysed data Wrote and presented abstract	25%
3	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	15%

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Wai Aih Chellen	Can routine histopathology distinguish between vulvar cutaneous candidiasis and dermatophytosis? Day T, Barbella-Foster A, Phillips S, Pagano R, Dyall-Smith D, Scurry J, Garland SM. Can routine histopathology distinguish between vulvar cutaneous candidiasis and dermatophytosis? *Journal of Lower Genital Tract Disease* 2015;20(3):267-71

Author position	Author	Nature of contribution to research and writing of the final paper	% contribution (if shared)	Signature
1	Tania Day	Co-reviewed all slides Collected and analysed data Provided clinical photographs Wrote manuscript	50	
2	Alba Barbella-Foster	Recruited [illegible] patients Co-reviewed all slides Collected and analysed data Wrote and presented abstract	15	
3	Sara Phillips	Performed molecular work Wrote segments of method and discussion related to molecular work	10	
4	Ross Pagano	Extracted clinical data Manuscript review	5	
5	Delwyn Dyall-Smith	Extracted clinical data Manuscript review	5	
6	Lyrian Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript review	10	
7	Suzanne Garland	Supervised molecular work Manuscript revision	5	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research
Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

TITLE AND CITATION	Day T, Knight V, Dyall-Smith D, Dennerstein G, Pagano R, Tran H, Tan Y, Yap D, Weigner J, Scurry J. The interpretation of non-diagnostic vulvar biopsies. *Journal of Lower Genital Tract Disease* 2018;22(1):74-81.

AUTHOR NUMBER	AUTHOR	SPECIFICS OF CONTRIBUTION TO RESEARCH REPORTED AND WRITING OF THE FINAL PAPER	% CONTRIBUTION TO PAPER	SIGNATURE
1	Tania Day	Co-reviewed all slides Collected and analysed data Wrote manuscript	64%	
2	Veronica Knight	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Wrote and presented abstract	15%	
3	Delwyn Dyall-Smith	Extracted clinical data Manuscript revision	2%	
4	Graeme Dennerstein	Extracted clinical data Manuscript revision	2%	
5	Ross Pagano	Extracted clinical data Manuscript revision	2%	
6	Hong Tran	Extracted clinical data Manuscript revision	2%	
7	Yasmin Tan	Extracted clinical data Manuscript revision	2%	
8	Desiree Yap	Extracted clinical data Manuscript revision	2%	
9	Julie Weigner	Extracted clinical data Manuscript revision	3%	
10	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	10%	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

[illegible]	Perianal lichen dermatoses: a review of 60 cases Day T, Bohl T, Scurry J. Perianal lichen dermatoses: a review of 60 cases. *Australasian Journal of Dermatology* 2016;57(3):210-15.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Tania Day	Co-reviewed all slides Collected and analysed data Provided clinical photographs Wrote manuscript	80%	
2	Tanja Bohl	Extracted clinical data and provided clinical photographs Manuscript revision	5%	
3	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	15%	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

TITLE AND CITATION	Vestibulovaginal sclerosis versus lichen sclerosus. Day T, Burston K, Dennerstein G, Pagano R, Scurry J. Vestibulovaginal sclerosis versus lichen sclerosus. *International Journal of Gynecological Pathology* 2018 Nov 30. doi: 10.1097/PGP.[illegible]

AUTHOR POSITION	AUTHOR	DETAILS OF CONTRIBUTION TO RESEARCH, REPORTS AND WRITING OF THE FINAL PAPER	% CONTRIBUTION TO PAPER	SIGNATURE
1	Tania Day	Co-reviewed all slides Collected and analysed data Wrote manuscript	60	
2	Kate Burston	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Wrote abstract	15	
3	Graeme Dennerstein	Extracted clinical data and provided clinical photographs Manuscript revision	5	
4	Rosa Pagano	Extracted clinical data Manuscript revision	5	
5	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	15	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

[illegible]	
[illegible]	Comorbid lichen planus and lichen sclerosus. Day T, Moore S, Bohl TJ, Scurry J. Comorbid lichen planus and lichen sclerosus. *Journal of Lower Genital Tract Disease* 2017;21(3):204-8.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Tania Day	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Wrote manuscript	70	
2	Sarah Moore	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Wrote abstract	15	
3	Tanja Bohl	Extracted clinical data and provided clinical photographs Manuscript revision	5	
4	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	10	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research
Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

[illegible]	Day T, Wolgner J, Scurry J. Classic and hypertrophic lichen planus.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Tania Day	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Provided clinical photographs Wrote manuscript	70%	
2	Julie Wolgner	Generated list of eligible patients Co-reviewed slides Manuscript revision	10%	
4	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	20	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

[illegible]	Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. Day T, Bowden N, Jaaback K, Otton G, Scurry J. Distinguishing erosive lichen planus from differentiated vulvar intraepithelial neoplasia. *Journal of Lower Genital Tract Disease* 2016;20(2):174-179.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Tania Day	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Provided clinical photographs Wrote manuscript	60%	
2	Nicola Bowden	Performed copy variant analysis Wrote segments of methods and discussion related to molecular work Manuscript revision	20%	
3	Ken Jaaback	Extracted clinical data Manuscript revision	5%	
4	Geoff Otton	Extracted clinical data and provided clinical photographs Manuscript revision	5%	
5	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	10%	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

UON Graduate Research

Co-authorship declaration

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

THE CANDIDATE	Is vulvovaginal lichen planus associated with squamous cell carcinoma? Day T, Otton G, Jasback K, Weigner J, Scurry J. Is vulvovaginal lichen planus associated with squamous cell carcinoma? *Journal of Lower Genital Tract Disease* 2018;22(2):159-65.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Tenia Day	Generated list of eligible patients Co-reviewed all slides Collected and analysed data Wrote manuscript	70	
2	Geoff Otton	Extracted clinical data Manuscript revision	5	
3	Ben Jasback	Extracted clinical data Manuscript revision	5	
4	Julie Weigner	Generated list of eligible patients Extracted clinical data Manuscript revision	10	
5	James Scurry	Co-reviewed all slides Provided histopathologic photographs Manuscript revision	10	

This co-authorship form is endorsed by the Assistant Dean of Research Training, Prof Bob Callister.

Signature

Date 8/7/18

RightsLink®

Home | Create Account | Help

Title:	Normal Vulvar Histology: Variation by Site
Author:	Tania Day, Seán Holland, and James Scurry
Publication:	Journal of Lower Genital Tract Disease
Publisher:	Wolters Kluwer Health, Inc.
Date:	Jan 1, 2016

LOGIN

If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials. Already a RightsLink user or want to learn more?

License Not Required

This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.

BACK | CLOSE WINDOW


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Can Routine Histopathology Distinguish Between Vulvar Cutaneous Candidosis and Dermatophytosis?
Author: Tania Day, Ailsa Borbolla Foster, Samuel Phillips, et al
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Jul 1, 2016
Copyright © 2016. Copyright © 2016 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials. Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Interpretation of Nondiagnostic Vulvar Biopsies
Author: Tania Day, Veronica Knight, Delwyn Dyall-Smith, et al
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Jan 1, 2018
Copyright © 2018, Copyright © 2018 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials.
Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

Title:	Perianal lichen dermatoses: A review of 60 cases
Author:	Tania Day,Tanja Gizela Bohl,James Scurry
Publication:	Australasian Journal of Dermatology
Publisher:	John Wiley and Sons
Date:	Mar 6, 2015

Logged in as:
Tania Day
LOGOUT

Review Order

Please review the order details and the associated terms and conditions.

No royalties will be charged for this reuse request although you are required to obtain a license and comply with the license terms and conditions. To obtain the license, click the Accept button below.

Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Australasian Journal of Dermatology
Licensed Content Title	Perianal lichen dermatoses: A review of 60 cases
Licensed Content Author	Tania Day,Tanja Gisela Bohl,James Scurry
Licensed Content Date	Mar 6, 2015
Licensed Content Pages	6
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	New insights into the clinical and histopathologic diagnosis of vulvar lichenoid dermatoses
Expected completion date	Aug 2018
Expected size (number of pages)	70
Requestor Location	

Publisher Tax ID	EU826007151
Total	0.00 AUD

Edit Order Details
Edit Requestor Location This location may be used to determine your tax liability.

☑ I agree to these terms and conditions.

☑ I understand this license is for reuse only and that no content is provided.


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Vestibulovaginal Sclerosis Versus Lichen Sclerosus
Author: Tania Day, Kate Burston, Graeme Dennerstein, et al
Publication: International Journal of Gynecological Pathology
Publisher: Wolters Kluwer Health, Inc.
Date: Jul 1, 2018
Copyright © 2018. ©2018International Society of Gynecological Pathologists
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials.
Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Comorbid Vulvar Lichen Planus and Lichen Sclerosus
Author: Tania Day, Sarah Moore, Tanja Bohl, et al
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Jul 1, 2017
Copyright © 2017. Copyright © 2017 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials.
Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Classic and Hypertrophic Vulvar Lichen Planus
Author: Tania Day, Julie Weigner, and James Scurry
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Jul 11, 2018
Copyright © 2018. Copyright © 2018 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials.
Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Distinguishing Erosive Lichen Planus From Differentiated Vulvar Intraepithelial Neoplasia
Author: Tania Day, Nikola Bowden, Ken Jaaback, et al
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Apr 1, 2016
Copyright © 2016, Copyright © 2016 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials. Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com


Copyright Clearance Center
RightsLink
Home
Create Account
Help
Wolters Kluwer
Title: Is Vulvovaginal Lichen Planus Associated With Squamous Cell Carcinoma?
Author: Tania Day, Geoff Otton, Ken Jaaback, et al
Publication: Journal of Lower Genital Tract Disease
Publisher: Wolters Kluwer Health, Inc.
Date: Apr 1, 2018
Copyright © 2018. Copyright © 2018 by the American Society for Colposcopy and Cervical Pathology
LOGIN
If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials.
Already a RightsLink user or want to learn more?
License Not Required
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.
BACK
CLOSE WINDOW
Copyright © 2018 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com