Current insights into the aetiology, pathobiology, and management of local disease recurrence in squamous cell carcinoma of the vulva

O'Neill, D; Nagenthiran, S; Dawson, Christopher; Luesley, David; Yap, Jason

DOI:
10.1111/1471-0528.14560

Title page

Current insights into the aetiology, pathobiology and management of local disease recurrence in squamous cell carcinoma of the vulva: a review paper

JKW Yap [θΔ†], D O'Neill[†], S Nagenthiran[†], CW Dawson[Δ], DM Luesley[Δ†]

[θ]Corresponding author:
Dr Jason KW Yap
Institutes of Cancer and Genomic Sciences
College of Dental and Medical School
University of Birmingham
Birmingham B15 2TT
United Kingdom
Email: j.yap@bham.ac.uk
Tel: 0044 121 507 5337

Affiliations:
[Δ]Institute of Cancer and Genomic Sciences
College of Dental and Medical School
University of Birmingham
Edgbaston B15 2TT
United Kingdom

[†]Department of Obstetrics and Gynaecology
Birmingham City Hospital
Sandwell and West Birmingham Hospitals NHS Trust
Dudley Road
Birmingham B18 7QH
United Kingdom

Running Title: Local disease recurrence in vulval squamous cell carcinoma

Abstract

Squamous cell carcinoma of the vulva is predominantly a disease of the elderly, where the mainstay of treatment is radical surgery. Local vulval recurrence (LVR) is a significant problem for these patients, and the rates of recurrence have not improved over the last three decades. Disappointingly, we still lack an understanding of how LVRs develop and the best approach to prevent and manage the condition. This review discusses recent insights into the key prognostic factors that influence the risk of recurrence, focusing on the role of tumour-adjacent non-neoplastic epithelial disorders, which are thought to play a causative role.

Main body of text

Background

Vulval cancer comprises only 6% of all gynaecological malignancies reported in the UK, with squamous cell carcinoma (VSCC) making up 90% of all cases. It is predominantly a disease of the elderly with three-quarters of cases affecting those aged over 60 years [1]. Radical vulvectomy is the mainstay of treatment for VSCC, with the extent of surgery depending on a number of factors that include: the size of the tumour; its location and proximity to vital organs; fitness to tolerate major surgery; FIGO stage; and wishes of the patient. Recurrent disease is common following primary treatment in VSCC with more than half of the cases recur locally involving the vulvoperineal area [2 3]. The rate of local vulval recurrence (LVR) has not changed over time and affects at least 1 in 4 patients following primary treatment [2 4]. Inadequate surgical excision has always been thought to be the main reason attributed to the development of LVR, but this belief is increasingly being challenged by new evidences [5 6]. Furthermore, a number of studies have showed that other clinicopathological factors are equally important in determining the timing, pattern and frequency of LVR following surgery; in particular, the presence of non-neoplastic but dysplastic epithelium found adjacent to the primary tumour [7-10]. The latter is of particular interest given that more than two-thirds of VSCC cases arise in a background of histologically abnormal or dysplastic epithelium such as vulval intraepithelial neoplasia (VIN) or Lichen Sclerosus (LS) [11].

Managing LVR can be challenging especially in the elderly population who often have other medical comorbidities and in those who have previously received extensive surgery or exposure to radiotherapy. Further surgery is often associated with physical and psychosexual comorbidities and, in some instances, can result in the loss of urinary and bowel functions. Disappointingly, we still lack an understanding of how LVRs develop and the best approach to prevent and manage the condition.

This review discusses recent insights into the key prognostic factors that influence the risk of LVR and focuses on the role of non-neoplastic epithelial disorders (NNEDs), which are thought to arise from a field of molecularly altered epithelium termed a "field of cancerization".

The dual pathobiology of VSCC

Like squamous cell carcinoma of the head and neck (HNSCC), VSCC is known to arise through HPV-dependent and independent routes (see Figure 1). The current disease paradigm holds that following persistent infection with high-risk (HR)-HPV strains, women are at risk of developing usual or classical type vulvar intraepithelial neoplasia (uVIN), which subsequently progress into basaloid or warty type squamous cell carcinoma (SCC) [12 13]. It is estimated that 40% of all VSCC cases arise through the viral-dependent route; interestingly, the prevalence of HR-HPV positive tumours is 20% higher in the United States compared to the UK [14-19]. Most cases of the tumour test positive for HPV16 and, to a lesser extent, HPV18 and HPV33 [20]. HPV-associated tumours typically affect younger women, aged <65 years, and the incidence in this age group is reportedly increasing in the UK and elsewhere [1]. This increase is a reflection of the rising incidence of the precursor lesion, uVIN, in young women, due in part, to the rise in the prevalence of infection with HR-HPV strains [20]. Although women with uVIN often suffer debilitating physical and psychosexual symptoms, the risk of progression to VSCC is substantially lower than that of cervical intraepithelial neoplasia; current estimates of disease progression are less than 10% [21].

The virus independent route is associated with the development of keratinising tumours in a background of differentiated intraepithelial neoplasia (dVIN) or Lichen Sclerosus (LS) [12 13]. It is thought that the primary trigger of carcinogenesis in this setting is chronic inflammation, which results in repeated injury, scarring and ultimately, sclerosis of the affected epithelium. The sustained episodes of cell renewal and repair, which accompanies chronically inflammation, are associated with DNA damage and a high probably of mutation or silencing of tumour suppressor genes (TSGs), which, over time can result in oncogenic transformation [22]. Nevertheless, it remains unclear if LS gives rise to dVIN as there is no clear-cut connection between the two conditions. Similarly, it is also unclear whether dVIN, like uVIN, is a precursor lesion in HPV-negative VSCC. Women within this age group are usually older (> 65 years) and critically they are also more likely to have other medical co-morbidities, which may pose particular challenges in managing their cancer.

Although the current theory suggests that VSCC may arise through these two distinct pathways, our recent study has shown that resected tumour specimens from almost a third of patients were found to have LS, uVIN and dVIN co-existing with each other [7]. This finding raises the question as to

whether the two routes to VSCC development are mutually exclusive. Understanding the underlying pathobiology which leads to the development of VSCC is crucial as many studies have found that the presence of NNEDs found adjacent to the primary tumour appears to influence the rate and pattern of local recurrence [7-10 23 24]. Furthermore, in other HPV-associated cancers, such as HNSCC and anal cancer, there is compelling evidence to suggest that HPV-positivity confers a survival advantage. However, despite this clear-cut correlation in these two diseases, studies on VSCC have failed to demonstrate that HR-HPV positivity is an independent predictor of disease-free survival [19 25-27]. The difficulty in revealing the expected association with HPV status in women with VSCC may flow in part from the frequency with which uVIN co-exists alongside LS and dVIN, both of which impose an increased risk of LVR development [7]. It is also worth noting that the detection HR-HPV DNA in tumour specimens does not necessarily indicate the presence of transcriptionally active virus given that the virus might have undergone integration, become methylated and transcriptionally silent [28]. Alternatively, the presence of HR-HPV DNA might constitute a transient reactivation or new infection that is not necessarily related to viral-driven oncogenesis [29]. Due to the complexity of the HPV life cycle, the significance of HR-HPV DNA positivity in VSCC remains unclear. Further studies are required to measure the levels of expression of the HR-HPV oncogenes and its surrogate markers (E7, $p16^{INK4a}$ and MCM7); these biomarkers would confirm if oncogenesis is driven through the HR-HPV route.

Topography of VSCC recurrence

Like HNSCC, our recently published study, along with two others, has identified two different patterns of local recurrence in VSCC (Figure 2). A local vulval recurrence can occur on a site previously occupied by or distant to the primary tumour [7 8 23]. This pattern of local recurrence was first described in SCC of the oral cavity and upper respiratory tract [30], and, like VSCC, the former can be derived from both HPV-dependent and HPV-independent routes. Molecular profiling of HNSCC has identified three distinctive patterns of local recurrence. Tumours that arise on a site previously occupied by the primary tumour are termed a local relapse (LR), and are thought to be a true local recurrence, while tumours that occur at least 2cm or more away from the primary tumour are termed second field tumours (SFT) or second primary tumours (SPT) and are thought to constitute new tumours that could be genetically related (SFT) or unrelated (SPT) to the primary tumour [31]. Although still speculative, it is thought that both SFT and SPT arise within an area of genetically altered pre-neoplastic epithelium contiguous with the primary tumour that has a propensity to undergo malignant transformation [32].

Unlike HNSCC, a detailed examination of the topography of local recurrences in vulval cancer has not been adequately described. As such, very few retrospective cohort studies have attempted to categorise LVR based on the site and time at which the disease recurs following primary surgery. Bosquet *et al*. defined "recurrence" as a disease which relapses within five years of treatment while those that relapse after five years were termed a "re-occurrence" [33]. Both Regauer *et al*. and Oonk *et al.* postulated that disease which recurs locally within 3 months of treatment is primarily due to treatment failure, while van der Velden *et al.* described a "true" local recurrence as a disease which recurs within 2cm of or "near" to the excision scar [9 34 35]. However, it is important to note that the definitions of local recurrence used by these authors are purely hypothetical and based on observational studies and, unlike the case for HNSCC, were not based on molecular profiling.

Clinico-pathological determinants of LVR

Tumour-free pathological margins of 8mm or more, measured after formalin fixation, is considered to be the gold standard practice to minimise local disease recurrence. The current surgical practice advocates the removal of at least 15mm of disease-free tissue, lateral and deep margins, so that after fixation a ≥8mm histological cancer-free margins can be achieved to avoid LVR [36]. This recommendation is based on a study conducted by Heap *et al.* on a small retrospective cohort [37]. The study found that none of the patients with pathological margins of ≥8mm had recurrent disease, and local recurrence was only found in those with pathological margins of <8mm. While a number of independent studies support these findings [23 38], other more recent studies, which interrogated pathological margins in addition to other clinical-pathological determinants, dispute the notion that inadequate excision margin is the sole reason that contributes to LVR [5-7]. After an extensive review of the literature, we have identified 27 independent retrospective cohort studies which have assessed the clinicopathological factors that determine LVR (see Table 1). Collectively, these studies found, that in addition to inadequate excision margins, there were other clinical determinants that influenced the risk of LVR. These included: groin node metastasis; the presence of Lichen Sclerosis (LS) and vulvar intraepithelial neoplasia (usual and differentiated type VIN) adjacent to the primary tumour; older age group; tumour size; tumour multifocality; histology grade; lymphovascular invasion (LVSI); perineural invasion; site of tumour; the type of surgery performed; and others [4-7 9 11 23 33 34 37-52]. However, it remains unclear which of the risk factors best predict LVR, as each study identified different predictors, and none were in total agreement with each other.

The inconsistencies in the findings from each retrospective study can be attributed to a number of possibilities. Firstly, different methodologies were used in each study to collect and analyse its results; secondly, the majority of these studies were conducted in a single institution where clinical

practice in managing VSCC can be substantially different; thirdly, there was a lack of consistency in the clinical determinants used in each study; fourthly, the definition for LVR varies between each study and, at times, used interchangeably with distant metastasis; and lastly, there was lack of consensus in defining what constituted a true LVR. As a result, these studies failed to identify the common prognostic variable(s) involved in LVR. Taking into the account the limitations of these studies, we conducted an analysis of our cohort to evaluate all potential clinicopathological determinants previously implicated in the development of LVR [7]. We also dichotomized local recurrences into LR or SFT/SPT, according to the definitions obtained from molecular studies on HNSCC. Interestingly, our results showed that more than half of the cases of local recurrence occurred at a site distant to the primary tumour; we also found that the presence of LS appeared to be the only clinical determinant that reliably predicts LVR. These patients were not at greater risk of developing distant metastasis when compared to other clinical determinants evaluated, suggesting that local disease recurrence probably occurs as a result of the ongoing chronic inflammatory dermatosis associated with the residual LS. Although we have yet to perform molecular profiling of the tumour specimens obtained in our study, we believe that LVR (both SFT and SPT) originate from a “field" of molecularly altered epithelium that has acquired the necessary genetic changes to undergo malignant change. Contrary to previous beliefs, they do not occur as a result of inadequate excision margins as described by Heap and colleagues. It is also worth highlighting that Heap et al. drew their inferences solely from unadjusted estimates, and their findings could be confounded by other clinicopathological variables that were not evaluated in their study.

Field cancerization and LVR

The concept of field cancerization was first proposed by Slaughter et al. in 1953, who studied the histology of dysplastic epithelial tissue at tumour-adjacent surgical margins in an attempt to explain the reason for the development of multiple primary tumours and local recurrence in the oral cavity and upper respiratory tract [30]. In the original study, histological examinations were performed on normal tissue at surgical margins adjacent to the tumour. This study revealed the presence of multiple independent primary lesions and evidence of hyperplastic or atypical epithelium in seemingly histologically normal tissue contiguous with the primary tumour. Since the development of molecular biology, the concept of field cancerization has now been redefined in molecular terms. Mutation or epigenetic silencing of growth promoting or tumour suppressor genes predisposes epithelium to undergo oncogenic transformation, allowing genetically altered cells to expand and colonise large areas of the epithelium. This phenomenon partly explains the multifocality of tumours, as secondary tumours or local recurrences, such as SFT and SPT, emerge some years later

after removal of the primary tumour. The multifocality and multicentricity of vulval neoplasia, its propensity to recur locally but at sites distant from the primary disease, point to this tumour arising within a field of cancerization in which at least some of the molecular abnormalities present in the primary tumour will be detected in adjacent histologically normal epithelium.

As more than two-thirds of VSCC arise on a background of atypical skin in the form of uVIN, dVIN, or LS [11], it is plausible that these non-neoplastic epithelial disorders arise from molecularly altered epithelium that is generated through virus-dependent and independent routes. As such, NNEDs may constitute pathological biomarkers which indicate the presence of a molecularly altered field of epithelium. In the case of uVIN, these lesions are derived from HR-HPV infected epithelium that has acquired additional molecular changes that have progressed to high-grade VIN. Several studies performed on HIV-infected women revealed the presence of multifocal HPV-associated warts and uVIN lesions/condylomata in the genital tract of HIV-positive women pointing to the existence of a cancer field in these patients [53 54]. Using molecular analyses involving X chromosome inactivation, Rosenthal and colleagues revealed that high-grade VIN lesions contiguous with VSCC were of clonal origin, raising the possibility that these VSCCs were derived from molecularly altered clones within the VIN lesions [55]. However, the question of whether HR-HPV infection *per se* generates a cancer field is currently unclear. Although data for VSCC is unavailable, a recent study performed in HNSCC has revealed that normal epithelium obtained from resection margins were uniformly HPV negative, suggesting that at least in this disease, HR-HPV may not generate a field of molecularly altered epithelium. This finding supports the notion that unlike HPV negative HNSCC, HR-HPV-positive HNSCC exhibits lower rates of local recurrence [56].

While uVIN is a putative precursor lesion for HPV-positive VSCC, it is still debatable whether LS is a precursor lesion for the HPV-negative counterpart. Although recent evidence shows that residual LS that remains after excision of the primary tumour increases the risk of local recurrence [7-9], the absolute risk of recurrence in these women is not well defined. The notion that LS generates a field of cancerization, much like that observed in HPV-negative HNSCC, is a strong but as yet unproven concept. However, such an idea is not without foundation. It is now well established that chronic inflammation, coupled with sustained episodes of wound-healing, can predispose epithelial tissue to oncogenic transformation [57]. It is still unclear whether inflammation plays a permissive or promoting function in the generation or expanding “initiated” (i.e. mutated) cells. Chronic inflammation is associated with abnormal cytokine and growth factor production which can fuel the expansion of molecularly altered or premalignant cells. A number of studies have shown that LS lesions overexpress p53 protein and, in a significant proportion of cases, harbour mutated TP53 genes [22 58]

[59]. The induction of p53 is most likely associated with a DNA damage response, induced through the production of reactive oxygen species (ROS) or by ischaemic stress, both of which are produced during chronic inflammation. Increased levels of ROS are associated with the recruitment of the epigenetic modulator, DNMT1, to CpG-rich islands upstream of promoters of both growth regulatory (i.e. $p16^{INK4A}$) and genes involved in the DNA damage response [60]. Chronic or sustained bouts of inflammation also cause alterations to the underlying stroma, converting normal fibroblasts into myofibroblasts which produce cytokines, chemokines and growth factors that can promote the growth of pre-malignant epithelial cells. An overwhelming body of evidence now supports a key role of the stromal microenvironment in field cancerization and the development of both primary tumours and local recurrences [60]. This is particularly relevant as previous clinical studies which evaluated the risk of LVR following an en bloc vulvectomy, and a triple incision, showed no difference in risk despite the removal of less "normal" tissue in the latter [61-64]. Therefore, removing excessive non-neoplastic skin during primary surgery may not have prevented the development of LVR as the adjacent skin bought together to close the wound may have already undergone a "field transformation" that may eventually give rise to an LVR.

The challenges in managing local VSCC recurrence

The treatments for LVR have not changed over the last three decades, and surgical excision continues to be the only treatment modality for cure [2 65]. Surgery, however, may not be suitable for all patients and the procedures can be challenging especially in those who have previously had wide radical excision or radiotherapy. Reconstructive surgery is often required following primary excision to restore anatomy and function, as extensive scarring from previous surgery often reduces tissue volume and renders its flexibility to achieve primary closure. As a result, a skin flap is often harvested to cover the defect left after radical surgery. For a tumour which recurs and encroaches the urethra, anus or vaginal, pelvic exenteration followed by reconstructive surgery may be required to remove the disease completely if the patient is physically fit enough to undergo the operation. For those patients who previously had radiotherapy to their vulval, wound breakdown following subsequent surgery is common because irradiated skin often has an inadequate blood supply and a slow healing rate, making skin grafting unsuitable for most of them.

Squamous cell carcinomas, in general, are radiosensitive, but several studies have revealed poor treatment responses for large tumours when used alone without surgery [2]. However, radiotherapy alone has been used successfully to treat low volume disease which recurs in the vulva [66]. The use of concurrent chemotherapeutic agents such as 5-flurouracil, Mitomycin C and platinum agents with irradiation have proved effective in managing large volume disease in patients who are radiotherapy

naïve or in those who are physically unfit for surgery [67]. Neoadjuvant chemoradiotherapy followed by surgery is still superior to chemoradiotherapy alone in treating local recurrences, as overall survival is significantly better in those who can have surgery [67]. Concurrent chemoradiotherapy may also be used to reduce the tumour volume before surgery, sparing those patients who required an exenterative surgery from having a simple radical excision; but defunctioning colostomy may be necessary in cases where the tumour recurs in close proximity to the anorectal canal [2]. Nevertheless, as VSCC mostly affects the elderly population, only a small number of patients are physically fit enough to endure such forms of aggressive triple therapies that involve chemo-radiation and surgery. As currently chemotherapeutic agents are used as an adjunct to radiotherapy or surgery and in palliative setting, there is a need to look for new chemotherapeutic drugs that can be used as a lone therapy for VSCC so that we are less reliant on surgery.

Conclusion

Currently, there is a paucity of knowledge regarding the timing, topography and aetiology of local VSCC recurrence. The notion that inadequate surgical excision margins are the driver for local recurrence is increasingly being challenged by studies utilising more sophisticated statistical analysis to evaluate the clinical determinants which predict LVR. Based on current evidence, we hypothesise that LVR arises within a field of molecularly altered epithelium that is generated as a result of chronic inflammation or infection with oncogenic HPV strains. We suggest that LVRs develop in a pre-existing field of molecularly altered epithelium from clones that have acquired the necessary mutations to undergo malignant transformation. Future studies should utilise molecular profiling techniques to identify the molecular changes present in these pre-cancerous fields so that potential biomarkers or gene signatures can be determined, and these used to stratify patients into those who are most likely at risk of developing local recurrences. Unlike HNSCC, the contiguous nature and ease of accessibility of the vulva made this organ an ideal model to study how the field of cancerization develops and the key molecular changes that predispose cells within the field to tumour formation. This analysis would allow us to develop field therapies that could be administered short- or long-term to delay or prevent local VSCC recurrence. In the case of LS-associated VSCC, where chronic inflammation appears to play a vital role in disease pathology and tumour recurrence, the use of topical steroids may prevent or delay local recurrences by reducing inflammation and re-establishing a more ”normal” stromal microenvironment.

Acknowledgements

Not applicable

Disclosure of Interests

The authors declare no conflict of interest

Contribution to Authorship

JKWY, CWD and DML conceived the idea for the review, participated in its design and coordination, and provided final approval of the version to be published. JKWY, DO and SN performed a systematic review of the literature. JKWY and DO wrote the paper. CWD and DML critically reviewed the manuscript and contributed intellectual opinion. All authors read and approved the final manuscript.

Details of ethics approval

Not applicable

Funding

Cancer Research UK (CRUK) Clinical Research Training Fellowship funding to JKWY

References

1. Cancer Research UK Cancer Statistics. Vulval cancer incidence statistics. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/vulval-cancer/incidence
2. Coulter J, Gleeson N. Local and regional recurrence of vulval cancer: management dilemmas. *Best practice & research. Clinical obstetrics & gynaecology* 2003;17(4):663-81.
3. Salom EM, Penalver M. Recurrent vulvar cancer. *Curr Treat Options Oncol* 2002;3(2):143-53.
4. Fonseca-Moutinho JA, Coelho MC, Silva DP. Vulvar squamous cell carcinoma. Prognostic factors for local recurrence after primary en bloc radical vulvectomy and bilateral groin dissection. *The Journal of reproductive medicine* 2000;45(8):672-8.
5. Woelber L, Choschzick M, Eulenburg C, Hager M, Jaenicke F, Gieseking F, et al. Prognostic value of pathological resection margin distance in squamous cell cancer of the vulva. *Ann Surg Oncol* 2011;18(13):3811-8.
6. Groenen SM, Timmers PJ, Burger CW. Recurrence rate in vulvar carcinoma in relation to pathological margin distance. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2010;20(5):869-73.
7. Yap JK, Fox R, Leonard S, Ganesan R, Kehoe ST, Dawson CW, et al. Adjacent Lichen Sclerosis predicts local recurrence and second field tumour in women with vulvar squamous cell carcinoma. *Gynecologic oncology* 2016;142(3):420-6.

8. Tantipalakorn C, Robertson G, Marsden DE, Gebski V, Hacker NF. Outcome and patterns of recurrence for International Federation of Gynecology and Obstetrics (FIGO) stages I and II squamous cell vulvar cancer. *Obstetrics and gynecology* 2009;113(4):895-901.
9. Regauer S. Residual anogenital lichen sclerosus after cancer surgery has a high risk for recurrence: a clinicopathological study of 75 women. *Gynecologic oncology* 2011;123(2):289-94.
10. Preti M, Ronco G, Ghiringhello B, Micheletti L. Recurrent squamous cell carcinoma of the vulva: clinicopathologic determinants identifying low risk patients. *Cancer* 2000;88(8):1869-76.
11. Eva LJ, Ganesan R, Chan KK, Honest H, Luesley DM. Differentiated-type vulval intraepithelial neoplasia has a high-risk association with vulval squamous cell carcinoma. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2009;19(4):741-4.
12. Kurman RJ, Toki T, Schiffman MH. Basaloid and warty carcinomas of the vulva. Distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses. *The American journal of surgical pathology* 1993;17(2):133-45.
13. Ueda Y, Enomoto T, Kimura T, Yoshino K, Fujita M. Two distinct pathways to development of squamous cell carcinoma of the vulva. *J Skin Cancer* 2011;2011:951250.
14. Knopp S, Nesland JM, Trope C, Holm R. p14ARF, a prognostic predictor in HPV-negative vulvar carcinoma. *American journal of clinical pathology* 2006;126(2):266-76.
15. Iwasawa A, Nieminen P, Lehtinen M, Paavonen J. Human papillomavirus in squamous cell carcinoma of the vulva by polymerase chain reaction. *Obstetrics and gynecology* 1997;89(1):81-4.
16. Pinto AP, Schlecht NF, Pintos J, Kaiano J, Franco EL, Crum CP, et al. Prognostic significance of lymph node variables and human papillomavirus DNA in invasive vulvar carcinoma. *Gynecologic oncology* 2004;92(3):856-65.
17. Skapa P, Zamecnik J, Hamsikova E, Salakova M, Smahelova J, Jandova K, et al. Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. *The American journal of surgical pathology* 2007;31(12):1834-43.
18. Lee YY, Wilczynski SP, Chumakov A, Chih D, Koeffler HP. Carcinoma of the vulva: HPV and p53 mutations. *Oncogene* 1994;9(6):1655-9.

19. Lindell G, Nasman A, Jonsson C, Ehrsson RJ, Jacobsson H, Danielsson KG, et al. Presence of human papillomavirus (HPV) in vulvar squamous cell carcinoma (VSCC) and sentinel node. *Gynecologic oncology* 2010;117(2):312-6.
20. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *International journal of cancer. Journal international du cancer* 2009;124(7):1626-36.
21. van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. *Gynecologic oncology* 2005;97(2):645-51.
22. Carlson JA, Ambros R, Malfetano J, Ross J, Grabowski R, Lamb P, 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. *Human pathology* 1998;29(9):932-48.
23. Rouzier R, Haddad B, Plantier F, Dubois P, Pelisse M, Paniel BJ. Local relapse in patients treated for squamous cell vulvar carcinoma: incidence and prognostic value. *Obstetrics and gynecology* 2002;100(6):1159-67.
24. Spiryda LB, Fuller AF, Goodman A. Aggressive locally recurrent vulvar cancer: review of cases presented to Massachusetts General Hospital 1990 to present. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2005;15(5):884-9.
25. Pinto AP, Signorello LB, Crum CP, Harlow BL, Abrao F, Villa LL. Squamous cell carcinoma of the vulva in Brazil: prognostic importance of host and viral variables. *Gynecologic oncology* 1999;74(1):61-7.
26. Monk BJ, Burger RA, Lin F, Parham G, Vasilev SA, Wilczynski SP. Prognostic significance of human papillomavirus DNA in vulvar carcinoma. *Obstetrics and gynecology* 1995;85(5 Pt 1):709-15.
27. Bloss JD, Liao SY, Wilczynski SP, Macri C, Walker J, Peake M, et al. Clinical and histologic features of vulvar carcinomas analyzed for human papillomavirus status: evidence that squamous cell carcinoma of the vulva has more than one etiology. *Human pathology* 1991;22(7):711-8.
28. Leonard SM, Wei W, Collins SI, Pereira M, Diyaf A, Constandinou-Williams C, et al. Oncogenic human papillomavirus imposes an instructive pattern of DNA methylation changes which

parallel the natural history of cervical HPV infection in young women. *Carcinogenesis* 2012;33(7):1286-93.

29. Leonard SM, Pereira M, Roberts S, Cuschieri K, Nuovo G, Athavale R, et al. Evidence of disrupted high-risk human papillomavirus DNA in morphologically normal cervices of older women. *Sci Rep* 2016;6:20847.

30. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer* 1953;6(5):963-8.

31. Braakhuis BJ, Brakenhoff RH, Leemans CR. Second field tumors: a new opportunity for cancer prevention? *Oncologist* 2005;10(7):493-500.

32. Dakubo GD, Jakupciak JP, Birch-Machin MA, Parr RL. Clinical implications and utility of field cancerization. *Cancer Cell Int* 2007;7:2.

33. Gonzalez Bosquet J, Magrina JF, Gaffey TA, Hernandez JL, Webb MJ, Cliby WA, et al. Long-term survival and disease recurrence in patients with primary squamous cell carcinoma of the vulva. *Gynecologic oncology* 2005;97(3):828-33.

34. van der Velden J, Schilthuis MS, Hyde SE, Ten Kate FJ, Burger MP. Squamous cell cancer of the vulva with occult lymph node metastases in the groin: the impact of surgical technique on recurrence pattern and survival. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2004;14(4):633-8.

35. Oonk MH, de Hullu JA, Hollema H, Mourits MJ, Pras E, Wymenga AN, et al. The value of routine follow-up in patients treated for carcinoma of the vulva. *Cancer* 2003;98(12):2624-9.

36. Royal College of Obstetrician and Gynaecologist: Guidelines for the diagnosis and management of vulval cancer; 2014.

37. Heaps JM, Fu YS, Montz FJ, Hacker NF, Berek JS. Surgical-pathologic variables predictive of local recurrence in squamous cell carcinoma of the vulva. *Gynecologic oncology* 1990;38(3):309-14.

38. Chan JK, Sugiyama V, Pham H, Gu M, Rutgers J, Osann K, et al. Margin distance and other clinico-pathologic prognostic factors in vulvar carcinoma: a multivariate analysis. *Gynecologic oncology* 2007;104(3):636-41.

39. Sznurkowski JJ, Emerich J. Characteristic features of recurrences of squamous cell carcinoma of the vulva. *Ginekol Pol* 2010;81(1):12-9.

40. Larsson GL, Helenius G, Andersson S, Elgh F, Sorbe B, Karlsson MG. Human papillomavirus (HPV) and HPV 16-variant distribution in vulvar squamous cell carcinoma in Sweden. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2012;22(8):1413-9.

41. Stankevica J, Macuks R, Baidekalna I, Donina S. Midline involvement as a risk factor for vulvar cancer recurrence. *Asian Pacific journal of cancer prevention : APJCP* 2012;13(10):5237-40.
42. Woolderink JM, de Bock GH, de Hullu JA, Davy MJ, van der Zee AG, Mourits MJ. Patterns and frequency of recurrences of squamous cell carcinoma of the vulva. *Gynecologic oncology* 2006;103(1):293-9.
43. Holthoff ER, Jeffus SK, Gehlot A, Stone R, Erickson SW, Kelly T, et al. Perineural Invasion Is an Independent Pathologic Indicator of Recurrence in Vulvar Squamous Cell Carcinoma. *The American journal of surgical pathology* 2015;39(8):1070-4.
44. Ayhan A, Velipasaoglu M, Salman MC, Guven S, Gultekin M, Bayraktar O. Prognostic factors for recurrence and survival in primary vulvar squamous cell cancer. *Acta Obstet Gynecol Scand* 2008;87(11):1143-9.
45. Cheng X, Zang R, Wu X, Li Z, Cai S, Zhang Z. Recurrence patterns and prognostic factors in Chinese patients with squamous cell carcinoma of the vulva treated with primary surgery. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2009;19(1):158-62.
46. De Hullu JA, Hollema H, Lolkema S, Boezen M, Boonstra H, Burger MP, et al. Vulvar carcinoma. The price of less radical surgery. *Cancer* 2002;95(11):2331-8.
47. Lingard D, Free K, Wright RG, Battistutta D. Invasive squamous cell carcinoma of the vulva: behaviour and results in the light of changing management regimens. A review of clinicohistological features predictive of regional lymph node involvement and local recurrence. *The Australian & New Zealand journal of obstetrics & gynaecology* 1992;32(2):137-45.
48. Look KY, Reisinger M, Stehman FB, Miser M, Ehrlich CE, Sutton GP. Blood transfusion and the risk of recurrence in squamous carcinoma of the vulva. *American journal of obstetrics and gynecology* 1993;168(6 Pt 1):1718-21; discussion 21-3.
49. Maggino T, Landoni F, Sartori E, Zola P, Gadducci A, Alessi C, et al. Patterns of recurrence in patients with squamous cell carcinoma of the vulva. A multicenter CTF Study. *Cancer* 2000;89(1):116-22.
50. Yoder BJ, Rufforny I, Massoll NA, Wilkinson EJ. Stage IA vulvar squamous cell carcinoma: an analysis of tumor invasive characteristics and risk. *The American journal of surgical pathology* 2008;32(5):765-72.

51. Iacoponi S, Zapardiel I, Diestro MD, Hernandez A, De Santiago J. Prognostic factors associated with local recurrence in squamous cell carcinoma of the vulva. *J Gynecol Oncol* 2013;24(3):242-8.

52. Woelber L, Mahner S, Voelker K, Eulenburg CZ, Gieseking F, Choschzick M, et al. Clinicopathological prognostic factors and patterns of recurrence in vulvar cancer. *Anticancer research* 2009;29(2):545-52.

53. Chiasson MA, Ellerbrock TV, Bush TJ, Sun XW, Wright TC, Jr. Increased prevalence of vulvovaginal condyloma and vulvar intraepithelial neoplasia in women infected with the human immunodeficiency virus. *Obstetrics and gynecology* 1997;89(5 Pt 1):690-4.

54. Moodley JR, Constant D, Hoffman M, Salimo A, Allan B, Rybicki E, et al. Human papillomavirus prevalence, viral load and pre-cancerous lesions of the cervix in women initiating highly active antiretroviral therapy in South Africa: a cross-sectional study. *BMC cancer* 2009;9:275.

55. Rosenthal AN, Ryan A, Hopster D, Jacobs IJ. Molecular evidence of a common clonal origin and subsequent divergent clonal evolution in vulval intraepithelial neoplasia, vulval squamous cell carcinoma and lymph node metastases. *International journal of cancer. Journal international du cancer* 2002;99(4):549-54.

56. Rietbergen MM, Braakhuis BJ, Moukhtari N, Bloemena E, Brink A, Sie D, et al. No evidence for active human papillomavirus (HPV) in fields surrounding HPV-positive oropharyngeal tumors. *J Oral Pathol Med* 2014;43(2):137-42.

57. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer* 2011;11(1):9-22.

58. Rolfe KJ, MacLean AB, Crow JC, Benjamin E, Reid WM, Perrett CW. TP53 mutations in vulval lichen sclerosus adjacent to squamous cell carcinoma of the vulva. *British journal of cancer* 2003;89(12):2249-53.

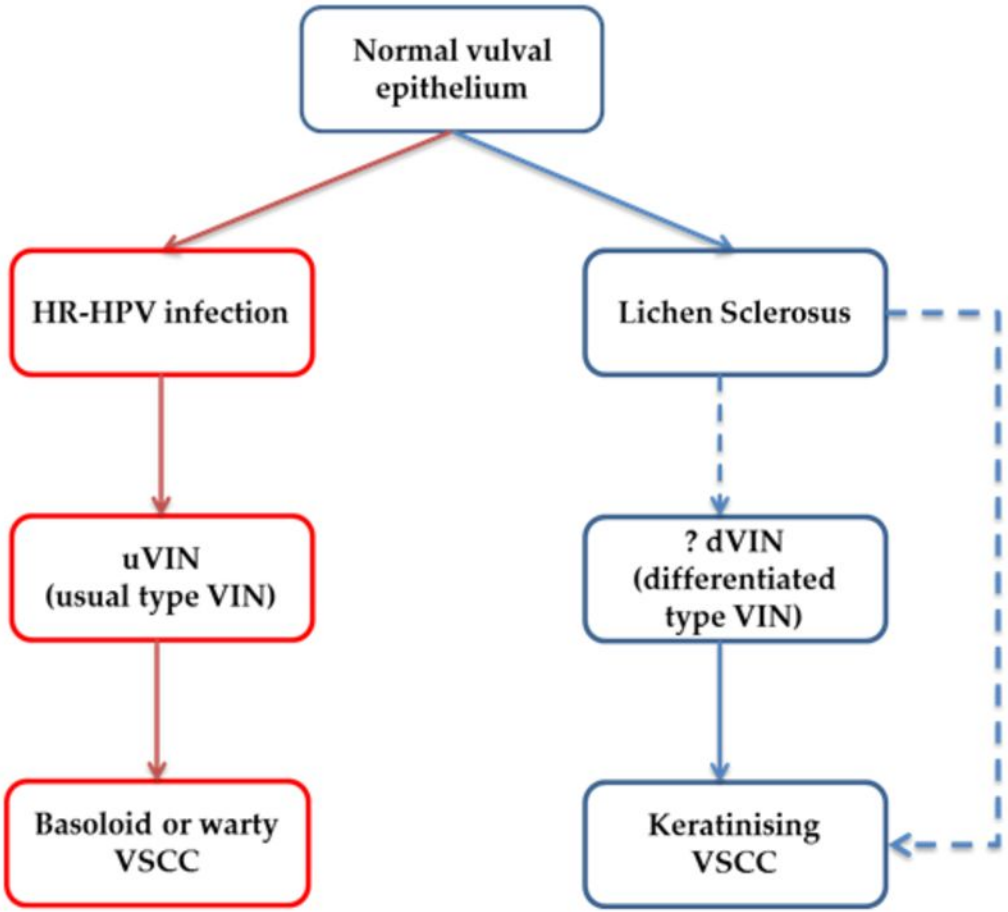
59. Vanin K, Scurry J, Thorne H, Yuen K, Ramsay RG. Overexpression of wild-type p53 in lichen sclerosus adjacent to human papillomavirus-negative vulvar cancer. *J Invest Dermatol* 2002;119(5):1027-33.

60. Dotto GP, Rustgi AK. Squamous Cell Cancers: A Unified Perspective on Biology and Genetics. *Cancer Cell* 2016;29(5):622-37.

61. Flannelly GM, Foley ME, Lenehan PM, Kelehan P, Murphy JF, Stronge J. En bloc radical vulvectomy and lymphadenectomy with modifications of separate groin incisions. *Obstetrics and gynecology* 1992;79(2):307-9.

62. Scheistroen M, Nesland JM, Trope C. Have patients with early squamous carcinoma of the vulva been overtreated in the past? The Norwegian experience 1977-1991. *Eur J Gynaecol Oncol* 2002;23(2):93-103.
63. Farias-Eisner R, Cirisano FD, Grouse D, Leuchter RS, Karlan BY, Lagasse LD, et al. Conservative and individualized surgery for early squamous carcinoma of the vulva: the treatment of choice for stage I and II (T1-2N0-1M0) disease. *Gynecologic oncology* 1994;53(1):55-8.
64. Siller BS, Alvarez RD, Conner WD, McCullough CH, Kilgore LC, Partridge EE, et al. T2/3 vulva cancer: a case-control study of triple incision versus en bloc radical vulvectomy and inguinal lymphadenectomy. *Gynecologic oncology* 1995;57(3):335-9.
65. Simonsen E. Treatment of recurrent squamous cell carcinoma of the vulva. *Acta Radiol Oncol* 1984;23(5):345-8.
66. Prempree T, Amornmarn R. Radiation management of squamous cell carcinoma of the bladder. *Acta Radiol Oncol* 1984;23(1):37-42.
67. Landoni F, Maneo A, Zanetta G, Colombo A, Nava S, Placa F, et al. Concurrent preoperative chemotherapy with 5-fluorouracil and mitomycin C and radiotherapy (FUMIR) followed by limited surgery in locally advanced and recurrent vulvar carcinoma. *Gynecologic oncology* 1996;61(3):321-7.

Natural history of VSCC



Sites of Local recurrences

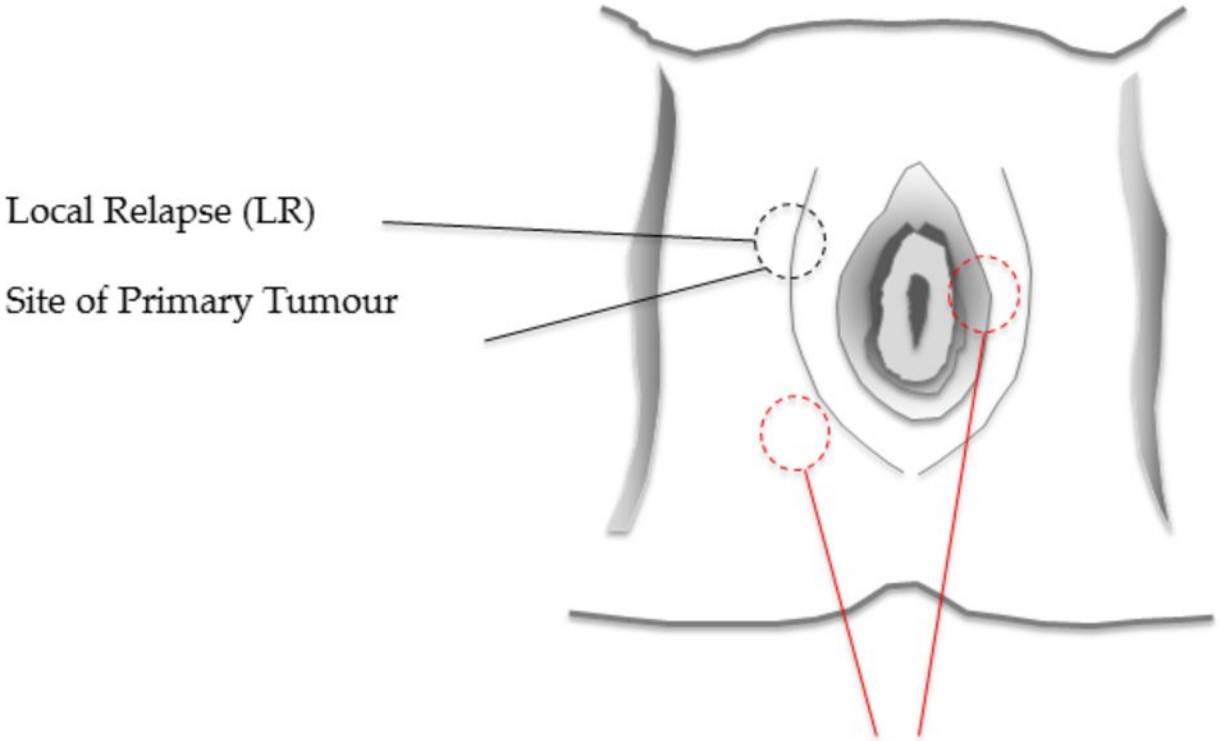


Table 1: Clinico-pathological determinants associated with local VSCC recurrence (LVR)

Author	Year	Cohort size (n)	Location of LVR (n)	Determinants associated with LVR
Yap *et al.* [7]	2016	201	LVR= 66 episodes, LR= 29 episodes; SFT= 26 episodes	LR and SFT: Lichen Sclerosis
Holthoff *et al.* [43]	2015	94	LR[a] recurrence in primary tumour = 31 'recurrent tumour' = 9	Perineural invasion
Iacoponi *et al.* [51]	2013	87	LR[b]= 23	Tumour size
Larsson *et al.* [40]	2012	133	LR[c]= 31	None identified
Stankevica *et al.* [41]	2012	107	LR[a]= 65	Site of primary cancer (midline disease)
Woelber* *et al.* [5]	2011	102	LR[a]= 10	Excision margins not significant
Regauer [9]	2011	75	LR[d]= 35	Presence of Lichen Sclerosus adjacent to main tumour
Sznurkowski *et al.* [39]	2010	59	LR[a]=10	Multifocal disease
Groenen *et al.* [6]	2010	93	LR[a]= 18	Excision margins not significant
Tantipalakorn *et al.*[8]	2009	121	LR=26 (primary[h]=13, remote[j]=13)	Primary recurrence= excision margins<8mm; remote recurrence= Presence of Lichen Sclerosus adjacent to main tumour
Woelber* *et al.* [52]	2009	103	LR[c=] 8	None identified
Cheng *et al.* [45]	2009	100	LR[a]= 20	Lymphovascular invasion, lymph node metastasis
Eva *et al.* [11]	2008	200	LR[a]= 34 (estimated)	Presence of dVIN adjacent to main tumour
Ayhan *et al.* [44]	2008	91	LR[a]= 8	Surgery type, lymph node metastasis, advanced stage disease, ulcerative lesion, tumour size
Yoder *et al.* [50]	2008	78	LR[e]= 11	Histological grade, incomplete resection, depth of invasion
Chan *et al.* [38]	2007	90	LR[i]= 13	Excision margins, groin node metastasis
Woolderink *et al.* [42]	2006	125	LR[a]= 29	Age >74 years
Bosquet *et al.* [33]	2005	330	LR[g=] 64 (30= reoccurrence; 34= recurrence)	Recurrence: Inguinal nodal metastasis; Re-occurrence: None identified
Van der Velden *et al.* [34]	2004	76	LR[h]= 15	Triple incision (vs en bloc)
Rouzier *et al.* [23]	2002	215	LR[i]= 13; distant recurrence= 13, Skin bridge recurrence= 7	Depth of invasion, incomplete resection margins
De Hullu *et al.* [46]	2002	253	LR[a]= 18 at 2 years; 32 at 4 years	Excision margins <8mm
Maggino *et al.* [49]	2000	502	'perineal' =94	FIGO stage, lymph node metastasis, Lymphovascular space invasion
Preti *et al.* [10]	2000	101	LR[a]= 18	VIN 2/3, FIGO stage, multifocal disease , lymphovascular space invasion, incomplete tumour resection
Fonseca-Moutinho *et al.* [4]	2000	56	LR[a]= 11 at 2 years LR[a] 15 at 5 years	FIGO stage IVa, groin node metastasis
Look *et al.* [48]	1993	154	'recurrence'[k]= 25	Lymph node metastasis
Lingard *et al.* [47]	1992	90	LR[a]= 16	Multifocal disease, tumour size (stage), inadequate excision margins
Heaps *et al.* [37]	1990	135	LR[a]= 21	Excision margins <8mm, depth of invasion, tumour thickness, lymphovascular space invasion, keratinizing tumour, mitotic activity

*Potential duplication of cohorts; LR – local recurrence, LVR – local vulval recurrence, SFT – second field tumour

[a]site of LR not defined; [b]the appearance of tumour in a new location after treatment, or in the same location after a minimum disease-free period of 6 months; [c]recurrence defined as 'vulva'; [d]de novo: >3 months after definitive surgery; [e]LR defined as tumour recur at a site remote from initial tumour; [f]statistical analysis of recurrence included distant metastasis; [g]recurrence: development of SCC in a previously treated vulva/groin within 5 years, reoccurrence: development of SCC in vulva/groin after 5 years; [h]LR: at or near the site of vulvectomy scar; [i]primary tumor site recurrence (up to and including 2 cm from the vulvectomy scar); [j]LR: >2cm from vulvectomy scar; [k]'recurrence' was defined as new appearance of tumour after therapy with radical intent, unsure if also encompassed distant recurrence