

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

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1 **Title page**

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

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29 **Running Title:** Local disease recurrence in vulval squamous cell carcinoma

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32

33 **Abstract**

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

41

42 **Main body of text**

43 **Background**

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

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

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

68 **The dual pathobiology of VSCC**

69 Like squamous cell carcinoma of the head and neck (HNSCC), VSCC is known to arise through HPV-
70 dependent and independent routes (see Figure 1). The current disease paradigm holds that
71 following persistent infection with high-risk (HR)-HPV strains, women are at risk of developing usual
72 or classical type vulvar intraepithelial neoplasia (uVIN), which subsequently progress into basaloid or
73 warty type squamous cell carcinoma (SCC)^{12 13}. It is estimated that 40% of all VSCC cases arise
74 through the viral-dependent route; interestingly, the prevalence of HR-HPV positive tumours is 20%
75 higher in the United States compared to the UK¹⁴⁻¹⁹. Most cases of the tumour test positive for
76 HPV16 and, to a lesser extent, HPV18 and HPV33²⁰. HPV-associated tumours typically affect younger
77 women, aged <65 years, and the incidence in this age group is reportedly increasing in the UK and
78 elsewhere¹. This increase is a reflection of the rising incidence of the precursor lesion, uVIN, in
79 young women, due in part, to the rise in the prevalence of infection with HR-HPV strains²⁰. Although
80 women with uVIN often suffer debilitating physical and psychosexual symptoms, the risk of
81 progression to VSCC is substantially lower than that of cervical intraepithelial neoplasia; current
82 estimates of disease progression are less than 10%²¹.

83 The virus independent route is associated with the development of keratinising tumours in a
84 background of differentiated intraepithelial neoplasia (dVIN) or Lichen Sclerosus (LS)^{12 13}. It is
85 thought that the primary trigger of carcinogenesis in this setting is chronic inflammation, which
86 results in repeated injury, scarring and ultimately, sclerosis of the affected epithelium. The sustained
87 episodes of cell renewal and repair, which accompanies chronically inflammation, are associated
88 with DNA damage and a high probability of mutation or silencing of tumour suppressor genes (TSGs),
89 which, over time can result in oncogenic transformation²². Nevertheless, it remains unclear if LS
90 gives rise to dVIN as there is no clear-cut connection between the two conditions. Similarly, it is also
91 unclear whether dVIN, like uVIN, is a precursor lesion in HPV-negative VSCC. Women within this age
92 group are usually older (> 65 years) and critically they are also more likely to have other medical co-
93 morbidities, which may pose particular challenges in managing their cancer.

94 Although the current theory suggests that VSCC may arise through these two distinct pathways, our
95 recent study has shown that resected tumour specimens from almost a third of patients were found
96 to have LS, uVIN and dVIN co-existing with each other⁷. This finding raises the question as to

97 whether the two routes to VSCC development are mutually exclusive. Understanding the underlying
98 pathobiology which leads to the development of VSCC is crucial as many studies have found that the
99 presence of NNEDs found adjacent to the primary tumour appears to influence the rate and pattern
100 of local recurrence^{7-10 23 24}. Furthermore, in other HPV-associated cancers, such as HNSCC and anal
101 cancer, there is compelling evidence to suggest that HPV-positivity confers a survival advantage.
102 However, despite this clear-cut correlation in these two diseases, studies on VSCC have failed to
103 demonstrate that HR-HPV positivity is an independent predictor of disease-free survival^{19 25-27}. The
104 difficulty in revealing the expected association with HPV status in women with VSCC may flow in part
105 from the frequency with which uVIN co-exists alongside LS and dVIN, both of which impose an
106 increased risk of LVR development⁷. It is also worth noting that the detection HR-HPV DNA in
107 tumour specimens does not necessarily indicate the presence of transcriptionally active virus given
108 that the virus might have undergone integration, become methylated and transcriptionally silent²⁸.
109 Alternatively, the presence of HR-HPV DNA might constitute a transient reactivation or new infection
110 that is not necessarily related to viral-driven oncogenesis²⁹. Due to the complexity of the HPV life
111 cycle, the significance of HR-HPV DNA positivity in VSCC remains unclear. Further studies are
112 required to measure the levels of expression of the HR-HPV oncogenes and its surrogate markers (E7,
113 p16^{INK4a} and MCM7); these biomarkers would confirm if oncogenesis is driven through the HR-HPV
114 route.

115 **Topography of VSCC recurrence**

116 Like HNSCC, our recently published study, along with two others, has identified two different
117 patterns of local recurrence in VSCC (Figure 2). A local vulval recurrence can occur on a site
118 previously occupied by or distant to the primary tumour^{7 8 23}. This pattern of local recurrence was
119 first described in SCC of the oral cavity and upper respiratory tract³⁰, and, like VSCC, the former can
120 be derived from both HPV-dependent and HPV-independent routes. Molecular profiling of HNSCC
121 has identified three distinctive patterns of local recurrence. Tumours that arise on a site previously
122 occupied by the primary tumour are termed a local relapse (LR), and are thought to be a true local
123 recurrence, while tumours that occur at least 2cm or more away from the primary tumour are
124 termed second field tumours (SFT) or second primary tumours (SPT) and are thought to constitute
125 new tumours that could be genetically related (SFT) or unrelated (SPT) to the primary tumour³¹.
126 Although still speculative, it is thought that both SFT and SPT arise within an area of genetically
127 altered pre-neoplastic epithelium contiguous with the primary tumour that has a propensity to
128 undergo malignant transformation³².

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

139 **Clinico-pathological determinants of LVR**

140 Tumour-free pathological margins of 8mm or more, measured after formalin fixation, is considered
141 to be the gold standard practice to minimise local disease recurrence. The current surgical practice
142 advocates the removal of at least 15mm of disease-free tissue, lateral and deep margins, so that
143 after fixation a ≥ 8 mm histological cancer-free margins can be achieved to avoid LVR³⁶. This
144 recommendation is based on a study conducted by Heap *et al.* on a small retrospective cohort³⁷.
145 The study found that none of the patients with pathological margins of ≥ 8 mm had recurrent disease,
146 and local recurrence was only found in those with pathological margins of < 8 mm. While a number
147 of independent studies support these findings^{23 38}, other more recent studies, which interrogated
148 pathological margins in addition to other clinical-pathological determinants, dispute the notion that
149 inadequate excision margin is the sole reason that contributes to LVR⁵⁻⁷. After an extensive review
150 of the literature, we have identified 27 independent retrospective cohort studies which have
151 assessed the clinicopathological factors that determine LVR (see Table 1). Collectively, these studies
152 found, that in addition to inadequate excision margins, there were other clinical determinants that
153 influenced the risk of LVR. These included: groin node metastasis; the presence of Lichen Sclerosus
154 (LS) and vulvar intraepithelial neoplasia (usual and differentiated type VIN) adjacent to the primary
155 tumour; older age group; tumour size; tumour multifocality; histology grade; lymphovascular
156 invasion (LVSI); perineural invasion; site of tumour; the type of surgery performed; and others^{4-7 9 11}
157 ^{23 33 34 37-52}. However, it remains unclear which of the risk factors best predict LVR, as each study
158 identified different predictors, and none were in total agreement with each other.

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

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

182 **Field cancerization and LVR**

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

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

199 As more than two-thirds of VSCC arise on a background of atypical skin in the form of uVIN, dVIN, or
200 LS¹¹, it is plausible that these non-neoplastic epithelial disorders arise from molecularly altered
201 epithelium that is generated through virus-dependent and independent routes. As such, NNEDs may
202 constitute pathological biomarkers which indicate the presence of a molecularly altered field of
203 epithelium. In the case of uVIN, these lesions are derived from HR-HPV infected epithelium that has
204 acquired additional molecular changes that have progressed to high-grade VIN. Several studies
205 performed on HIV-infected women revealed the presence of multifocal HPV-associated warts and
206 uVIN lesions/condylomata in the genital tract of HIV-positive women pointing to the existence of a
207 cancer field in these patients^{53 54}. Using molecular analyses involving X chromosome inactivation,
208 Rosenthal and colleagues revealed that high-grade VIN lesions contiguous with VSCC were of clonal
209 origin, raising the possibility that these VSCCs were derived from molecularly altered clones within
210 the VIN lesions⁵⁵. However, the question of whether HR-HPV infection *per se* generates a cancer
211 field is currently unclear. Although data for VSCC is unavailable, a recent study performed in HNSCC
212 has revealed that normal epithelium obtained from resection margins were uniformly HPV negative,
213 suggesting that at least in this disease, HR-HPV may not generate a field of molecularly altered
214 epithelium. This finding supports the notion that unlike HPV negative HNSCC, HR-HPV-positive
215 HNSCC exhibits lower rates of local recurrence⁵⁶.

216 While uVIN is a putative precursor lesion for HPV-positive VSCC, it is still debatable whether LS is a
217 precursor lesion for the HPV-negative counterpart. Although recent evidence shows that residual LS
218 that remains after excision of the primary tumour increases the risk of local recurrence⁷⁻⁹, the
219 absolute risk of recurrence in these women is not well defined. The notion that LS generates a field
220 of cancerization, much like that observed in HPV-negative HNSCC, is a strong but as yet unproven
221 concept. However, such an idea is not without foundation. It is now well established that chronic
222 inflammation, coupled with sustained episodes of wound-healing, can predispose epithelial tissue to
223 oncogenic transformation⁵⁷. It is still unclear whether inflammation plays a permissive or promoting
224 function in the generation or expanding “initiated” (i.e. mutated) cells. Chronic inflammation is
225 associated with abnormal cytokine and growth factor production which can fuel the expansion of
226 molecularly altered or premalignant cells. A number of studies have shown that LS lesions
227 overexpress p53 protein and, in a significant proportion of cases, harbour mutated TP53 genes^{22 58}

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

243 **The challenges in managing local VSCC recurrence**

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

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

261 naïve or in those who are physically unfit for surgery⁶⁷. Neoadjuvant chemoradiotherapy followed
262 by surgery is still superior to chemoradiotherapy alone in treating local recurrences, as overall
263 survival is significantly better in those who can have surgery⁶⁷. Concurrent chemoradiotherapy may
264 also be used to reduce the tumour volume before surgery, sparing those patients who required an
265 exenterative surgery from having a simple radical excision; but defunctioning colostomy may be
266 necessary in cases where the tumour recurs in close proximity to the anorectal canal².
267 Nevertheless, as VSCC mostly affects the elderly population, only a small number of patients are
268 physically fit enough to endure such forms of aggressive triple therapies that involve chemo-
269 radiation and surgery. As currently chemotherapeutic agents are used as an adjunct to radiotherapy
270 or surgery and in palliative setting, there is a need to look for new chemotherapeutic drugs that can
271 be used as a lone therapy for VSCC so that we are less reliant on surgery.

272 **Conclusion**

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

291

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294 **Disclosure of Interests**

295 The authors declare no conflict of interest

296

297 **Contribution to Authorship**

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

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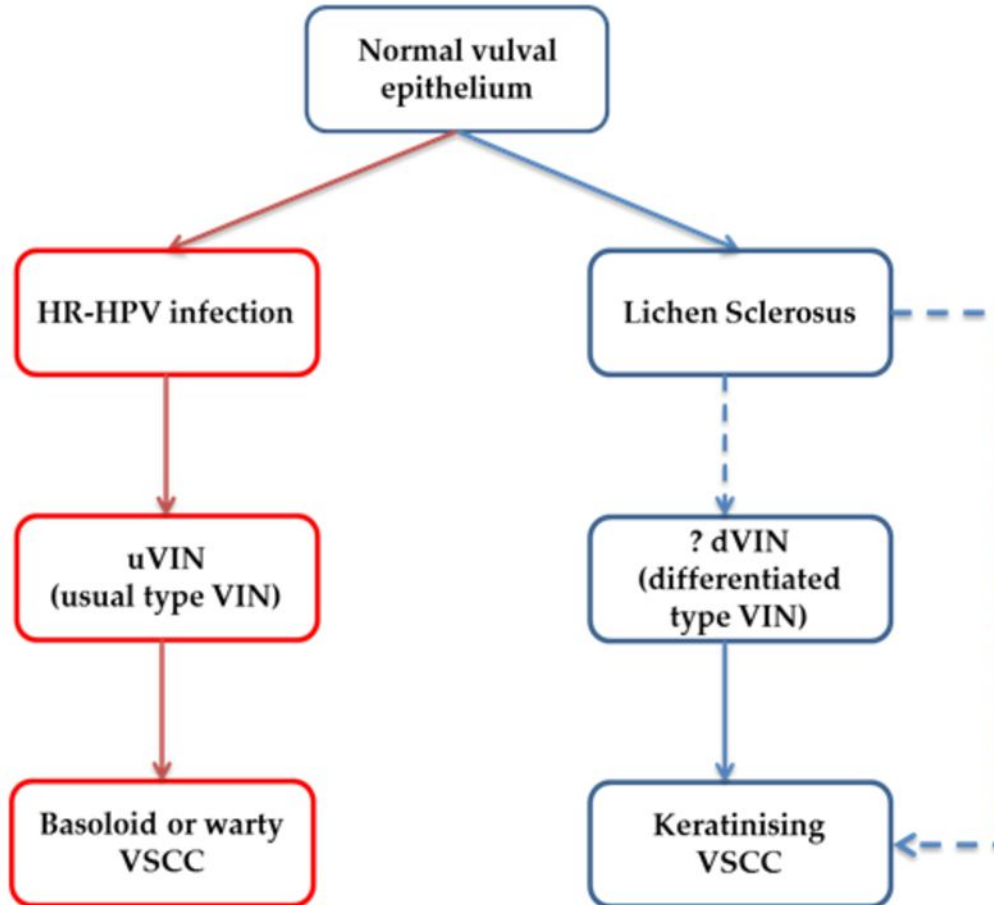
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510

Natural history of VSCC



Sites of Local recurrences

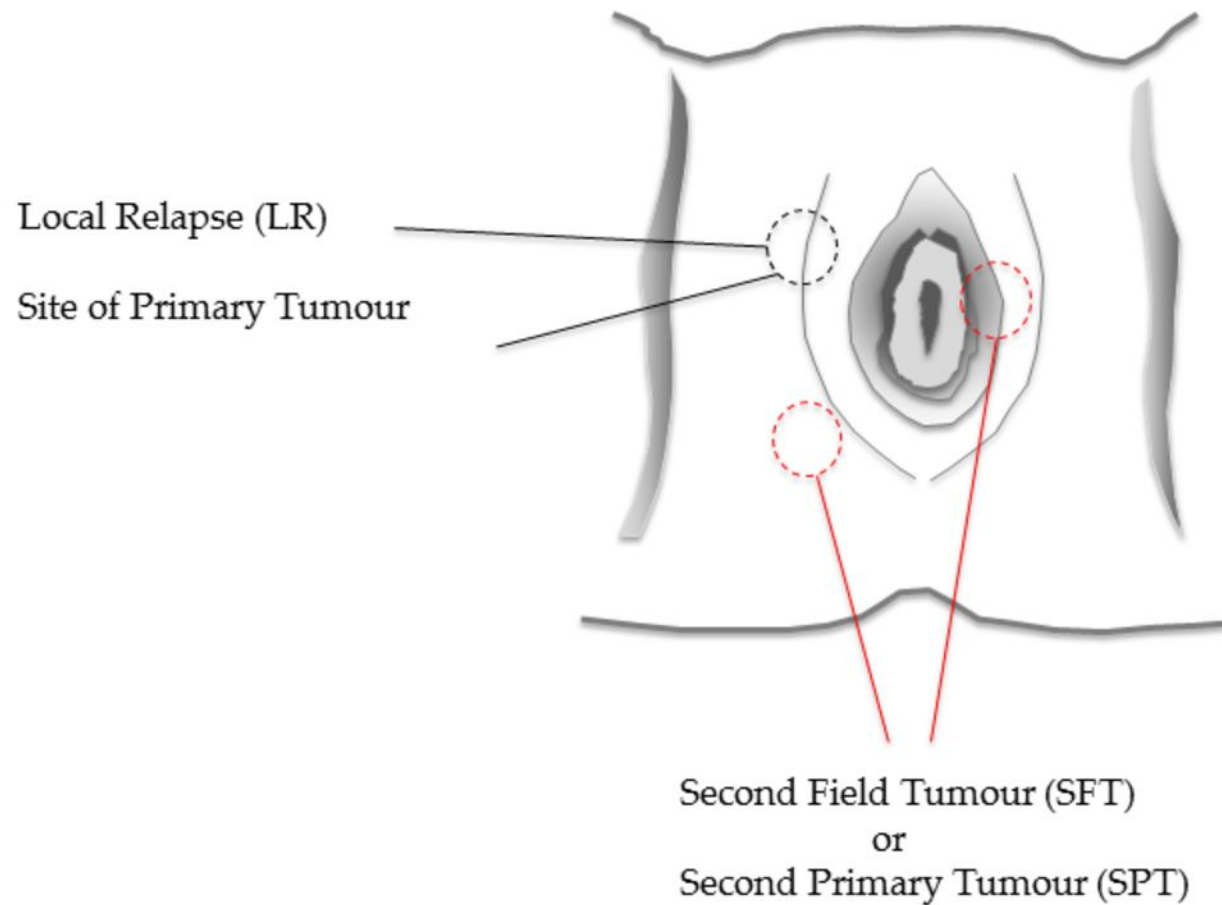


Table 1: Clinico-pathological determinants associated with local VSCC recurrence (LVR)

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

^asite of LR not defined; ^bthe appearance of tumour in a new location after treatment, or in the same location after a minimum disease-free period of 6 months; ^crecurrence defined as 'vulva'; ^dde novo: >3 months after definitive surgery; ^eLR defined as tumour recur at a site remote from initial tumour; ^fstatistical analysis of recurrence included distant metastasis; ^grecurrence: development of SCC in a previously treated vulva/groin within 5 years, reoccurrence: development of SCC in vulva/groin after 5 years; ^hLR: at or near the site of vulvectomy scar; ⁱprimary tumor site recurrence (up to and including 2 cm from the vulvectomy scar); ^jLR: >2cm from vulvectomy scar; ^k'recurrence' was defined as new appearance of tumour after therapy with radical intent, unsure if also encompassed distant recurrence

