The Impact of Focality and Centricity on VIN Disease Progression in HIV+ patients: A 10-Year Retrospective Study

By

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Abstract

**Background:** The impact of lesion focality and centricity in relation to patient outcome and disease recurrence of vulvar intraepithelial neoplasia (VIN) is an understudied area of research, especially in immunocompromised women. The prevalence and incidence of VIN have increased steadily since the 1980s, because of the co-existence of human papilloma virus (HPV) and human immunodeficiency virus (HIV). In this study, we have retrospectively examined the records of VIN patients to determine the effect of lesion focality and centricity with respect to the interval to disease recurrence.

**Material & Methods:** All women diagnosed with VIN and managed between January 2002 and December 2011 were included (n=90) and followed up until December 2017. Symptoms at the time of presentation, including HIV positivity (n=75) were collated, including the influences of multifocality and multicentricity on time to disease recurrence.

**Results:** Multicentricity caused a more rapid recurrence of disease than unicentricity (p=0.006), whereas multifocality increased the risk of recurrence more than unifocality (p<0.0001). Viral load in the HIV+ patients was not associated with time to disease recurrence, but the reduced number of CD4+ lymphocytes present in HIV+ patients was. Treatment modalities had no effect on disease recurrence.

**Conclusion:** Both focality and centricity have effects on interval to recurrence and final patient outcome, with multifocal disease having a poorer prognosis. Centricity and focality should be recorded at the time of diagnosis and act as a warning for disease recurrence. HIV+ VIN patients with multifocal disease and/or known immunosuppression (low CD4+ lymphocyte counts) should be regarded as ‘high-risk’ patients and treated accordingly.
Keywords: Vulva, intraepithelial neoplasms, focality, centricity, HIV, disease recurrence, CD4

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Running title: Lesion focality/centricity effect on risk of VIN recurrence
Introduction

Vulval intraepithelial neoplasia (VIN) is a condition in which changes occur in the skin covering the vulva of female external genitalia from one that is benign into one that has the potential to become invasive, affecting all tissues of the pudendal region of the pelvic floor.

In 1986, the International Society for the Study of Vulvovaginal Disease (ISSVD) devised a classification system for VIN, which was updated in 2004 and remains the most commonly used system in literature (1). Pre-invasive abnormalities in the vulval tissue are categorised as VIN 1–3, depending on the level of dysplasia present, which is similar to the current grading of cervical intraepithelial neoplasia (CIN), a related and often coincident finding during clinical examination and diagnosis. It is widely believed that VIN 1 has a low malignant potential and is not a precursor of VIN 2 or 3, which have high malignant potential, often presenting as or developing into invasive squamous cell carcinoma (SCC).

Since the 1980s, the incidence of VIN has increased in several countries and in particular within the younger female population (2). Despite this, VIN remains a relatively uncommon condition, with an unclear aetiology. Younger women tend to have the ‘usual-type’ VIN that is characterized by previous or existing exposure to human papillomavirus (HPV), whereas older women tend to have the ‘unusual-type’ VIN, which is not related to HPV exposure, but is related to chronic dermatological conditions, in particular vulval lichen sclerosis (3). The symptoms reported by patients with VIN are itching, burning, dyspareunia and the appearance of leucoplakic patches in any part of the vulva, but often patients are asymptomatic and suspected VIN is often observed during annual sexual health examination. Emerging evidence suggest that the type of VIN and recurrence of disease may be related to the presence of viruses other than HPV, such as human immunodeficiency virus (HIV) or in immunocompromised patients, suggesting that immunomodulation may have a prognostic effect in some, but not all, forms of VIN (4).
Due to the multi-factorial and heterogeneous nature of VIN, there is no single characteristic or pathognomonic feature that can facilitate the diagnosis of VIN. If VIN is suspected, visual inspection of the vulva and surrounding tissues (cervix, vagina, perineum, anus, rectum and gluteal folds) with vulvoscopy or dermatome aids the collection of the vulval biopsy and confirmation of disease is made by histological examination. VIN in more than one part of the vulval tissue is defined as *multifocal*, whilst lesions in more than one genital site is defined as being *multicentric* disease. In some series, additional areas of VIN have been found in 80% of the areas adjacent to the primary lesion (5). This high rate of concurrent disease is mostly characteristic of younger women.

There are numerous standard treatments for VIN and for the prevention of VIN progressing to vulval cancer (6-9). The gold standard treatment for high-grade vulval intraepithelial lesions is surgery, either localized or radical excision or laser ablation (10). Alternatively, immune modulators such as imiquimod (11-13) can be used as adjunctive therapy, although the efficacy and side effects of this combined treatment remain undetermined.

The primary aim of this study was to determine the impact of lesion focality and centricity at VIN presentation in relation to patient outcome and disease recurrence. In particular, the effectiveness of different treatment modalities on disease free duration, disease recurrence, and failure rates, based on focality and centricity of the disease at presentation. In addition, how VIN presentation and outcomes varied with immune status, specifically HIV status was also determined.
Methods

This retrospective cohort study was conducted in a tertiary University Hospital setting (the West London Cancer Centre, Imperial College NHS Trust) over 10 years by examining the records of all women at Hammersmith and St Mary’s Hospitals diagnosed with VIN between January 2002 and December 2011. All women diagnosed with VIN (n=90) and managed within this period were included; women were suitable for inclusion irrespective of VIN type or grade of the disease. A search by histological diagnosis of VIN was performed and hospital numbers obtained. St. Mary’s Hospital data was collected from the colposcopy database ‘Excelicare’ and pathology database ‘Telepath’. Hammersmith Hospital data was obtained from patients’ paper medical records. Symptoms at the time of presentation (Figure 1) were collated, together with patient age at the time of initial presentation (mean ± SD; 44.8 ± 15.1 years; range 20-86), smoking status, HPV and HIV status (Figure 1), CD4+ lymphocyte count and viral load (only in the HIV+ patients), initial diagnosis (Figure 1), and if the lesions present were unifocal/multifocal and unicentric/multicentric. Viral load was determined using an immunoassay (IA) that simultaneously detects both antibody to human immunodeficiency virus (HIV) and HIV p24 antigen (Architect HIV Ag/Ab Combo) and confirmation using LIAISON® XL MUREX HIV Ab/Ag HT, whilst CD4 positivity was determined using fluorescence activated cell sorting on a BD FACSCanto analyser (BD Biosciences, San Jose, CA).

Next, the initial treatment regimen, whether the patient remained disease free or if disease recurred (until December 2017), the time from treatment to recurrence and final patient outcome were all recorded.
Univariate analysis using permutation $\chi^2$ tests (10 000 permutations; SPSS version 24; IBM Corp. Released 2016, Armonk, NY; ibm.com/products/spss-statistics) were used to evaluate statistical significance with respect to the effect of treatment on VIN recurrence and patient outcomes, whilst Fisher’s exact test and linear regression analysis (Prism version 7:00 for windows, GraphPad Software, La Jolla, California, USA, www.Graphpad.com) were used to determine the influences of multifocality and multicentricity on time to disease recurrence after treatment. Demographic data were analyzed with unpaired Student’s t-test with Welch’s correction for non-uniform variances (Prism version 7) and interactions between prediction variables for disease recurrence, including treatment options, was assessed using binomial logistic regression (Minitab version 18 (2019), Minitab, LLC. State College, Pennsylvania, USA, minitab.com/en-us).

**Results**

Histological diagnosis confirmed the presence of VIN in all patients, with 24 women having unifocal disease and 54 having multifocal disease. Furthermore, 30 patients had unicentric disease and 48 had multicentric disease (Table 1) and in 12 cases, the number and positions of lesions were not recorded. Since multiple combinations are possible at diagnosis, these possible combinations are presented together in Table 1.

At the time of presentation, 61% of the HIV- patients (n=15) were smokers whilst only 23% of the HIV+ patients (n=75) were smokers. Although those who smoked in the HIV+ group smoked less than 20 cigarettes/day, some of the HIV- group (6%) smoked more than 20 cigarettes/day. Analysis showed that smoking was not a confounding factor for later analyses for disease recurrence in either group.

The HIV+ subgroup presented with fewer symptoms than the HIV- group, and more patients were asymptomatic (Figure 1a). The presence of a lesion, pruritus, pain or a combination of
these symptoms were similar in both groups, although ‘soreness’ was only reported in the HIV- group. The type of lesion present and initially diagnosed was similar in both groups, with 76% of the HIV- group and 93% of the HIV+ group, respectively presenting with VIN3 or invasive disease (Figure 1b). Furthermore, 60% of HIV+ patients had a coincidental diagnosis of cervical intraepithelial neoplasia (CIN) and or vaginal intraepithelial neoplasia (VAIN), in contrast to only 28% of the HIV- patients (Figure 1c). The majority of HIV+ patients (87%) had a previous diagnosis of CIN/VAIN, compared to only 48% of the HIV- patients (Figure 1c).

Nine different management plans were put in place on initial presentation (Table 2), with 39 patients being managed conservatively, and of this group one went on to have examination under anaesthesia (EUA) and one went on to develop invasive disease. Laser treatment as initial treatment was used on 23 patients and of these, 7 had recurrent disease within a year and 15 within 2 years. Diathermy ablation was used to treat 12 patients and 3 patients had diathermy excision. Only one patient in our cohort who had imiquimod therapy relapsed and had recurrence of disease, in this case the patient did not require any further treatment (Table 2). One patient had radiotherapy (following diagnosis of invasive cancer), one referred to a cancer centre and 1 had a vulvectomy. In all cases, a recurrence of disease occurred (Table 2). Only 3 (20%) of the HIV+ patients and 30 (38.5%) of the HIV- patients were disease free in December 2017, whilst 12 patients (13.3%) were lost to follow-up. One patient died of Hodgkin’s lymphoma and 3 died of causes that were not recorded and two developed invasive vulval carcinoma (Table 2). Of the 78 patients that had detailed notes available, 12 out of the 15 HIV+ group (80%) and 30 out of the 63 remaining HIV- patients (47.6%) had recurrent disease (Figure 2). Binomial logistic regression indicated that none of the clinical interventions were effective in preventing recurrence of disease, and that none of the symptoms and signs at diagnosis except multicentricity ($p=0.01$) and multifocality ($p=0.001$) were significantly linked to disease recurrence ($p<0.0001$). CD4+ lymphocyte
count (p=0.903) and HIV status (p=0.432) were not significantly associated with disease recurrence in the logistic analysis.

An analysis of the effect of centricity and focality on the time to disease recurrence indicated that both factors had a significant effect on the rate of recurrence; multicentricity was more rapid than unicentricity (p=0.006; Fisher’s exact test) and multifocality was more rapid than unifocality (p<0.0001, Fisher’s exact test) (Table 1) in relation to disease recurrence and progression. A total of 31 patients presented with multifocal and multicentric disease and 23 presented with multifocal and unicentric disease. It was these patient groups who had a significantly (p=0.0005) shorter time to disease recurrence (Table 1). The average time to disease recurrence in HIV+ patients was 3.2 years, compared to 5.4 years in the HIV- patients, with 73% HIV+ patients presenting with multifocal disease compared to only 61% of HIV- patients.

In HIV+ patients, viral load and time to disease recurrence was not related (Figure 3a), but a significant (p=0.02) positive correlation ($R^2=0.665$) between CD4+ lymphocyte count at diagnosis and time to recurrence was observed (Figure 3a).

**Discussion**

The data presented here shows that both VIN focality and centricity at initial diagnosis have a significant effect on both interval to recurrence and final outcome for the patient diagnosed with VIN, and despite the nine different treatment options used, only 12 women were disease free after 5 years. Recurrence within 2 years was highest overall in those women with multifocal/multicentric disease with 7% of this cohort developing invasive forms of vulval cancer. This degree of effect has been reported previously by others (14-18),
suggesting that both where and how many lesions there are at presentation should be uppermost in the clinician’s mind.

The majority of women presented with VIN 2/3, and the main concern with VIN 2/3 is its potential to progress to vulval cancer. Although a woman’s risk of developing cancer of the vulva by the age of 75 years varies between countries, and ranges from 0.01% to 0.28%, in some studies the progression to invasive vulval cancer with untreated high-grade VIN may be as high as 9% (19). For treated lesions, this rate is between 2% and 5% (2), with an increase in vulval cancer in women under the age of 50 years being increasingly documented (6, 20) and has been linked to an increasing incidence of VIN in younger women, attributed to infection with HPV, smoking or poor immunological status especially in HIV+ women (4, 14, 21). Observations made herein indicate that although there is not real difference in VIN recurrence between HIV+ and HIV- women, the rate to recurrence is higher in the HIV+ women. The suggestion here is that HIV+ women are particularly sensitive to the development and recurrence of VIN and ultimately vulval cancer. In this study, 17% of the patient group were HIV+, which was significantly higher than that of the general female population of London at the time, where only 0.1% were known to be HIV+. This suggests that VIN may occur as a consequence of HIV infection, possibly through the loss of CD4+ lymphocytes but not because of increased viral load (Figure 3). However, because of the small numbers of patients that had a viral load or HIV positivity test in this study, firm conclusions on this cannot be made at this time, even though immunocompromised patients are at a higher risk of recurrent disease (23).

Symptoms at presentation were very similar in both HIV+ and HIV- patients, with 60% presenting with a lesion alone or alongside other symptoms including pruritus and vulval pain. We noted a greater number of HIV+ patients (93%) had the more advanced VIN3
disease compared to only 76% of the HIV- patients, suggesting that the presumably higher
CD4+ lymphocyte count in the HIV- patients provides suitable immune surveillance and
prevention of conversion to malignancy. This is supported by the observation that the
majority (85%) of HIV+ patients had a coincidental or previous diagnosis of CIN/VAIN, whilst
CIN/VAIN were only diagnosed in <50% of HIV- patients. These data suggest that HIV+
patients have a greater propensity to the development of such neoplasms.

Treatment modality did not seem to have any significant effect of outcome. This is similar to
previous studies where radical vulvectomy or combination therapy had no significant effect
on patient outcomes (7, 8, 22). Radical vulvectomy in our cohort did not seem to show any
improvement over any other treatment modality suggesting that a conservative approach in
younger women is probably an acceptable treatment option.

Conclusions

Multicentricity and multifocality of VIN lesions at the time of diagnosis should be
determined and the presence of both parameters act as a warning for the gynaecologist/
gynaecology oncologist/dermatologist to initiate close monitoring for disease recurrence.
We believe that after further, larger studies, the presence of both parameters may
eventually be used to predict those women at high risk of VIN recurrence and progression to
vulval cancer, which may influence and guide treatment choices.

Immunosuppressed groups and HIV+ patients, are more likely to present with multifocal and
more advanced disease (VIN2/3), and as such, HIV+ VIN patients with multifocal and/or
known immunosuppression (demonstrated by a low CD4+ lymphocyte count) should be
regarded as ‘high-risk’ patients and treated accordingly. Such groups may be appropriately
managed in clinics with access to multi-disciplinary services, including dermatologists, whose experience with the use of imiquimod may change the treatment choice.
References


Figure Legends

Figure 1. The effect of HIV status on symptoms at the time of presentation, the type of lesion present and presence of co-morbidities.

Panel a shows the symptoms described by HIV- patients (upper pie chart) and those described by HIV+ patients at the time of initial presentation. The numbers under each pie chart indicate the numbers of HIV- and HIV+ patients. The percentages are values for each patient group. Panel b shows the effect of HIV status on lesion type diagnosed at initial presentation. Visual and histological methods were used to diagnose lesion type and related to previous diagnosed HIV status. Microinvasive/invasive indicate the presence of vulval cancer. Panel c shows whether diagnosis of CIN or VAIN or both were present prior to initial diagnosis of VIN or were coincidental findings on the day of initial diagnosis. Data are presented as the % of the entire patient cohort based on HIV status.

Figure 2. The effect of HIV status on recurrence of VIN at any time after treatment.

Differential diagnosis of VIN recurrence within the period January 2002 to December 2017 (as reported by the consultant histopathologist) was recorded. Data are presented as the % of the entire patient cohort.

Figure 3. The effect of viral load and CD4+ lymphocyte count on the time to VIN recurrence in HIV+ patients.

Panel a shows the effect of viral load measured by an immunoassay (IA) that simultaneously detects both antibody to human immunodeficiency virus (HIV) and HIV p24 antigen (Architect HIV Ag/Ab Combo) and confirmation using LIAISON® XL MUREX HIV Ab/Ag HT, at the time of VIN recurrence. The time to recurrence was measured as the calendar year from initial diagnosis to report of a new lesion. CD4+ lymphocyte counts were measured using fluorescence activated cell sorting and is presented as number of CD4+ lymphocytes per 10⁹
cells. Linear regression was used to calculate potential relationships between viral load (n=7) and CD4+ lymphocyte count (n=7) and time to recurrence. Data are not shown when encompassed by another symbol. Pearson correlation co-efficient and p-values were calculated using Prism version 7 software.