

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

By

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Abstract

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

8 **Material & Methods:** All women diagnosed with VIN and managed between January 2002
9 and December 2011 were included and followed up until December 2017. They were
10 identified by searching histopathology and diagnosis records in hospital colposcopy
11 databases. Symptoms at the time of presentation, subsequent treatment and outcomes
12 were collated, including the influences of multifocality and multicentricity on time to disease
13 recurrence.

14 **Results:** A total of 90 women with were VIN identified, from which 78 records were
15 recovered indicating focality and centricity. 15 patients were HIV+ and 75 were HIV-. HIV+
16 women presented with fewer symptoms than the HIV- women. Multicentricity caused a
17 more rapid recurrence of disease than unicentricity ($p=0.006$), whereas multifocality
18 increased the risk of recurrence more than unifocality ($p<0.0001$). Viral load in the HIV+
19 patients was not associated with time to disease recurrence but the number of CD4+
20 lymphocytes present in HIV+ patients was.

21 **Conclusion:** Both focality and centricity have an effect on interval to recurrence and final
22 patient outcome, with multifocal disease having a poorer prognosis. Centricity and focality
23 should be recorded at the time of diagnosis and act as a concern for disease recurrence.
24 HIV+ VIN patients with multifocal disease and/or known immunosuppression (low CD4+
25 lymphocyte counts) should be regarded as 'high-risk' patients and treated accordingly.

26 **Keywords:** Vulva, intraepithelial neoplasms, focality, centricity, HIV, disease recurrence, CD4

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

30 **Author's contributions:** T. Ayakannu was responsible for designing and drafting the
31 original manuscript and revision for important intellectual content. J. Chatterjee and
32 D. Lyons conceptualized the original study, were involved in manuscript revision,
33 supervised data collection and also for providing important intellectual input into the
34 work. They take responsibility for the conduct of the study. S. Murugesu, P. Sokhal
35 and R. Limandhee were responsible for collection of data and presenting some of the
36 work at national and international conferences. A.H. Taylor aided in the re-analysis
37 of some of the data and CSM Wilhelm-Benartzi was involved in the statistical
38 analysis for the paper. All authors contributed to the design of the paper and have
39 approved the final draft.

Introduction

40 Vulval intraepithelial neoplasia (VIN) is a condition in which changes occur in the skin
41 covering the vulva of female external genitalia. It can change from a condition that is
42 relatively benign (VIN1) into one that has the potential to become invasive (VIN3), affecting
43 all surface tissues of the pelvic floor (mons into perianal region). In 1986, the International
44 Society for the Study of Vulvovaginal Disease (ISSVD) devised a classification system for VIN,
45 which was updated in 2004 and remains the most commonly used system in literature [1].
46 Pre-invasive abnormalities in vulval tissue are categorised as VIN 1–3, depending on the
47 level of dysplasia present, which is similar to the current grading of cervical intraepithelial
48 neoplasia (CIN), a related and often coincident (multicentric) finding during clinical
49 examination and diagnosis. It is widely believed that VIN 1 has a low malignant potential and
50 is not a precursor of VIN 2 or 3, which have high malignant potential, often presenting with
51 or developing into invasive squamous cell carcinoma (SCC).

52 Since the 1980s, the incidence of VIN as a disease entity has been reported to have
53 increased in several countries and in particular within the younger female population [2].
54 Even so, VIN remains a relatively uncommon condition, with an unclear aetiology. Younger
55 women tend to have the 'usual-type' VIN that is characterized by previous or existing
56 exposure to human papillomavirus (HPV), whereas older women tend to have the 'unusual-
57 type' VIN (also called differentiated VIN), which is not related to HPV exposure, but is related
58 to chronic dermatological conditions, in particular vulval lichen sclerosis [3]. The symptoms
59 reported by patients with VIN are itching, burning, dyspareunia and the appearance of
60 leucoplakic patches in any part of the vulva. Often patients are asymptomatic as well and
61 suspected VIN is observed during colposcopy for cervical abnormality or during general
62 gynaecological examination. Emerging evidence suggest that the type of VIN and recurrence
63 of disease may be related to the presence of viruses other than HPV, such as human

64 immunodeficiency virus (HIV) and in immunocompromised patients, suggesting that
65 immunomodulation may have a prognostic effect in some, but not all, forms of VIN [4].

66 Due to the multi-factorial and heterogeneous nature of VIN, there is no single characteristic
67 or pathognomonic feature that can facilitate the diagnosis of VIN. If VIN is suspected, visual
68 inspection of the vulva and surrounding tissues (cervix, vagina, perineum, anus, rectum and
69 gluteal folds) with vulvoscopy guiding the collection of vulval biopsy and confirmation of the
70 disease is made by histological examination. VIN in more than one part of the vulval tissue is
71 defined as ***multifocal***, whilst the presence of lesions in more than one genital site is defined
72 as being ***multicentric disease***. The importance of vulvoscopy is based on the observed
73 prevalence of microscopic abnormalities adjacent to the gross lesion that becomes
74 pronounced with the uptake of acetic acid. In some series, additional areas of VIN have been
75 found in 80% of the areas adjacent to the primary lesion [5]. This high rate of concurrent
76 disease is most characteristic of younger women.

77 There are numerous standard treatments for VIN and for the prevention of VIN2/3
78 progressing to vulval cancer [6-9]. The gold standard treatment for high-grade vulval
79 intraepithelial lesions is surgery, either localized or radical excision or laser ablation [10].
80 Alternatively, immune modulators such as imiquimod [11-13] can be used as adjunctive
81 therapy, although the efficacy and side effects of this combined treatment remain
82 undetermined.

83 The primary aim of this study was to determine the impact of lesion focality and centrality at
84 VIN presentation in relation to patient outcome and disease recurrence. In particular, the
85 effectiveness of different treatment modalities on disease free duration, disease recurrence,
86 and failure rates, based on focality and centrality of the disease at presentation was

87 assessed. In addition, this study also assesses how VIN presentation and outcomes varied
88 with immune status, specifically HIV status was used to interrogate this.

89 **Methods**

90 This retrospective cohort study was conducted over 10 years in a tertiary University Hospital
91 setting (the West London Cancer Centre, Imperial College Hospitals NHS Trust) by examining
92 the records of women at Hammersmith and St Mary's Hospitals between January 2002 and
93 December 2011. The women were identified through a search of histopathology and
94 colposcopy databases. All women diagnosed with VIN and managed within this period were
95 included; women were suitable for inclusion irrespective of VIN type or grade of the disease.
96 A search by histological diagnosis of VIN was performed and hospital numbers obtained. St.
97 Mary's Hospital data was collected from the colposcopy database 'Excelicare' and pathology
98 database 'Telepath'. Hammersmith Hospital data was obtained from patients' paper medical
99 and histopathology records.

100 Symptoms at the time of presentation were collated, together with patient age at the time
101 of initial presentation, smoking status, HPV and HIV status, CD4+ lymphocyte count and viral
102 load (only in the HIV+ patients), and if the lesions present were unifocal/multifocal and
103 unicentric/multicentric. Viral load was determined using an immunoassay (IA) that
104 simultaneously detects both antibody to human immunodeficiency virus (HIV) and HIV p24
105 antigen (Architect HIV Ag/Ab Combo) and confirmation was made using LIAISON® XL MUREX
106 HIV Ab/Ag HT. CD4 positivity was determined using fluorescence activated cell sorting on a
107 BD FACS Canto analyser (BD Biosciences, San Jose, CA). The initial, subsequent and
108 adjunctive treatment regimen(s), whether the patient remained disease free or if disease
109 recurred (until December 2017), the time from treatment to recurrence and final patient
110 outcome(s) were all recorded.

111 Univariate analysis using permutation χ^2 tests (10 000 permutations; R version 2.10) were
112 used to evaluate statistical significance with respect to the effect of treatment on VIN
113 recurrence and patient outcomes, whilst Fisher's exact test and linear regression analysis
114 (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA,
115 www.graphpad.com) were used to determine the influences of multifocality and
116 multicentricity on time to disease recurrence after treatment. Demographic data were
117 analyzed with unpaired Student's t-test with Welch's correction for non-uniform variances
118 (Prism version 7.00).

119 **Results**

120 A total of 90 women with a diagnosis of VIN were identified during the 10 years study
121 period. The mean (\pm SD) age at presentation was 44.8 ± 15.1 years (range 20-86) for the
122 patient cohort. Of these, 15 patients (16.6%) were HIV+ and 75 (83.3%) were HIV-. The ages
123 of these two groups at presentation were not significantly different (40.4 ± 8.8 years (range
124 27-57) and 45.7 ± 15.9 (range 20-86) respectively, ($p=0.313$).

125 At the time of presentation, 61% of the HIV- patients were smokers whilst only 23% of the
126 HIV+ patients were smokers – probably should put total numbers in brackets, alongside
127 percentages. Although those who smoked in the HIV+ group smoked less than 20
128 cigarettes/day and some of the HIV- group - insert number in brackets(6%) smoked more
129 than 20 cigarettes/day, analysis showed that smoking was not a confounding factor in later
130 analyses for either group.

131 The HIV+ subgroup presented with fewer symptoms than the HIV- group, and more patients
132 were asymptomatic (Figure 1a). The presence of a lesion, pruritus, pain or a combination of

133 these symptoms were similar in both groups, although 'soreness' was only reported in the
134 HIV- group. The type of lesion present and initially diagnosed was similar in both groups,
135 with 76% of the HIV- group and 93% of the HIV+ group, respectively presenting with VIN3 or
136 invasive disease (Figure 1b). Furthermore, 60% of HIV+ patients had a coincidental diagnosis
137 of cervical intraepithelial neoplasia (CIN) and or vaginal intraepithelial neoplasia (VAIN), in
138 contrast to only 28% of HIV- patients (Figure 1c). The majority HIV+ patients (87%) had a
139 previous diagnosis of CIN/VAIN, compared to only 48% of the HIV- patients (Figure 1c). –
140 insert numbers as well as percentages This data was not statistically significantly different
141 ($p=0.56$; Fisher's exact test).

142 Histological diagnosis confirmed the presence of VIN in all patients, with 24 women (37%)
143 having unifocal disease and 54 (69%) having multifocal disease. Furthermore, 30 patients
144 (38%) had unicentric disease and 48 (61%) had multicentric disease (Table 1) and in 12 cases
145 (15%), the number and positions of lesions were not recorded. Since multiple combinations
146 are possible at diagnosis, these possible combinations are presented together in Table 1.

147 There were nine different management plans put in place at initial presentation (Table 2)
148 and none of the patients were treated with cidofovir or photodynamic therapy; 39 patients
149 were managed conservatively. Of this group, one went on to have examination under
150 anaesthesia (EUA) and one went on to develop invasive disease. Laser treatment as initial
151 treatment was used on 23 patients and of these, 7 had recurrent disease within a year and
152 15 within 2 years. Diathermy ablation was used to treat 12 patients and 3 patients had
153 diathermy excision. Only one patient in our cohort who was treated with imiquimod alone,
154 relapsed and had recurrence of disease – could define time in this as only one patient. In this
155 case, the patient did not require any further treatment (Table 2). One patient had
156 radiotherapy (following diagnosis of invasive cancer), one referred to a cancer centre and 1

157 had a vulvectomy. At the time of writing, only 3 (20%) HIV+ patients and 30 (38.5%) HIV-
158 patients are disease free, whilst 12 patients (13.3%) have been lost to follow-up. One
159 patient died of Hodgkin's lymphoma and 3 died of causes that were not recorded in their
160 notes and two developed invasive vulval carcinoma (Table 2). Of the 78 patients that had
161 detailed notes available, 12 out of the 15 HIV+ group (80%) and 30 out of the 63 remaining
162 HIV- patients (47.6%) went on to have recurrent disease (Figure 2).

163 An analysis of the effect of centricity and focality on the time to disease recurrence indicated
164 that both factors had a significant effect on the rate of recurrence; multicentricity was more
165 rapid than unicentricity ($p=0.006$; Fisher's exact test) and multifocality was more rapid than
166 unifocality ($p<0.0001$, Fisher's exact test) (Table 1) in relation to disease recurrence and
167 progression. A total of 31 patients presented with multifocal and multicentric disease and 23
168 presented with multifocal and unicentric disease. These multifocal-multicentric patients had
169 a significantly ($p=0.0005$) shorter time to disease recurrence (Table 1). The average time to
170 disease recurrence in HIV+ patients was 3.2 years, compared to 5.4 years in the HIV-
171 patients, with 73% of the HIV+ patients presenting with multifocal disease compared to only
172 61% of the HIV- patients.

173 In order to dissect the cause of the accelerated disease recurrence in HIV+ patients, CD4+
174 lymphocyte counts and viral load were examined. The data showed a significant positive
175 correlation between CD4+ lymphocyte count at diagnosis and time to recurrence (Figure 3a).
176 By contrast, no significant relationship between viral load (at time of diagnosis or at time of
177 disease?) and time to recurrence of VIN could be observed (Figure 3b). The slope of the line
178 for CD4+ lymphocyte count and time to recurrence was 0.0039 years per CD4+ lymphocyte
179 cell identified. This provides an estimate of time to disease recurrence in the HIV+ patient

180 population based on initial CD4+ lymphocyte counts, e.g. 1000 CD4+ cells predicts a 3.9 year
181 delay in disease recurrence.

182

183 **Discussion**

184 The data presented here shows that both focality and centrality of disease at initial diagnosis
185 have a statistically significant effect on both interval to recurrence and final outcome for the
186 patient diagnosed with VIN. Recurrence within 1 year was highest overall in those with
187 multifocal/multicentric disease and also 6 patients (7%) of this cohort developing invasive
188 forms of vulval cancer. This has been reported previously in only a small set of studies [14-
189 18].

190 The majority of women presented with VIN 2/3, and the main concern with VIN 2/3 is its
191 potential to progress to cancer of the vulva. A woman's risk of developing cancer of the
192 vulva by the age of 75 years varies between countries, and ranges from 0.01% to 0.28%
193 although the true rate of progression to invasive vulval cancer in women with untreated
194 high-grade VIN is debatable, with some studies suggesting a rate as high as 9% [19]. The
195 rates and the risk of progression in treated lesions has been reported as between 2% and 5%
196 [2], with an increase in vulval cancer in women under the age of 50 years being increasingly
197 documented [6, 20]. This has been linked to an increasing incidence of VIN in younger
198 women, which has been attributed to infection with HPV, smoking or poor immunological
199 status especially in HIV+ women [4, 14, 21]. Treatment modality did not seem to have any
200 significant effect on outcome. This is similar to previous studies where radical vulvectomy or
201 combination therapy had no significant effect on patient outcomes [7, 8, 22]. What is clear
202 from previous work is that immunocompromised patients are at a higher risk of recurrent
203 disease [23].

204 In this study, 17% of the patient group were HIV+, which is significantly higher when
205 compared to the general female population of West London aged 21-86, during the
206 diagnostic period, where 0.1% were known to be HIV+. This suggests that VIN may occur as a
207 consequence of HIV infection, possibly through the loss of CD4+ lymphocytes or increased

208 viral load. The corollary of this would be that patients that have increased viral loads or were
209 HIV+ at the time of initial presentation might have a greater susceptibility to disease
210 recurrence. These ideas were examined and viral load did not seem to have any effect on
211 the rate of disease recurrence, but CD4+ lymphocyte count did in our patient cohort. In fact,
212 the data (albeit from a small sample) suggests that CD4+ count could be a good predictor of
213 disease recurrence in HIV+ women with VIN, although these data need confirmation in a
214 larger sample for any useful prognostic value.

215 Symptoms at presentation were very similar in both HIV+ and HIV- patients, with 60%
216 presenting with a lesion alone or alongside other symptoms including pruritus and vulval
217 pain. We noted a greater number of HIV+ patients (93%) had the more advanced form of
218 VIN (VIN3) when compared to only 76% of the HIV- patients, suggesting that the presumably
219 higher CD4+ lymphocyte count in the HIV- patients provides suitable immune surveillance
220 and prevention of conversion to malignancy – I WOULD PROBABLY SAY THIS MAY HAVE
221 PREVENTED PROGRESSION TO HIGHER GRADE DISEASE, RATHER THAN CONVERSION TO
222 MALIGNANCY. This is supported by the observation that the majority (85%) of HIV+ patients
223 had a synchronous or previous diagnosis of CIN/VAIN, whilst synchronous or metachronous
224 CIN/VAIN were only diagnosed in <50% of HIV- patients. These data suggest that HIV+
225 patients have a greater propensity for the development of such neoplasms. Radical
226 vulvectomy did not seem to show any improvement over any other treatment modality
227 suggesting that a conservative approach in younger women is an acceptable treatment
228 option.

229 **Conclusions**

230 The centricity and focality of VIN lesions at the time of diagnosis should be determined and
231 the presence of both parameters act as a warning for the gynaecologist/ gynaecology

232 oncologist to initiate close monitoring for disease recurrence. We believe that the presence
233 of both parameters may eventually be used to predict those women at high risk of VIN
234 recurrence and progression, which may influence and guide treatment choices.

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

Table 1: Interval to VIN disease recurrence based on focality and centrality

Lesion type	Interval to Recurrence (Years)				Totals
	<1	2	3-5	>5	
Unifocal and unicentric	1	2	2	2	7
Unifocal and multicentric	14	2	0	1	17
Multifocal and unicentric	3	10	4	6	23
Multifocal and multicentric	11	10	4	6	31
Totals	29	24	10	15	78
Permutation χ^2 p-value	0.0005				

Fisher's exact test indicated that multicentric disease significantly shortened the interval to disease recurrence ($p=0.0063$) and multifocal disease significantly shortened the interval to disease recurrence ($p<0.0001$) when compared to their unicentric or unifocal counterparts. The permutation χ^2 p-value for the comparison of multifocal and multicentric disease *versus* multifocal and unicentric disease is also shown.

Table 2: The effect of treatment modality on the interval to recurrence and final patient outcome in December 2017

Treatment	Interval from treatment to recurrence (years)				Totals	Final Outcome*				Totals
	≤ 1	1-2	3-5	>5		Follow up	Re-treated/EUA	Invasive	Died	
None	1	2	3	0	6	6	0	0	0	6
Observation	17	8	1	4	30	31	1	1	0	33
Laser	7	8	3	5	23	13	9	1	3	26
Diathermy ablation	3	4	2	3	12	6	6	0	0	12
Diathermy excision	0	1	1	1	3	0	2	0	1	3
Imiquimod	0	1	0	0	1	1	0	0	0	1
Radiotherapy	0	0	0	1	1	0	1	0	0	1
Referral to cancer centre	1	0	0	0	1	0	1	0	0	1
Vulvectomy	0	0	0	1	1	0	1	0	0	1
Totals	29	24	10	15	78	57	21	2	4	84
Permutation χ^2 p-value	0.14				0.12					

*Follow up means a patient with chronic VIN, but no progression of disease and so on long-term observation only; Re-treatment/EUA means a different treatment modality was applied either after evaluation under anaesthesia (EUA) or independent of re-diagnosis, invasive means VIN had progressed to vulval cancer.

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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 methods and histological confirmation 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.

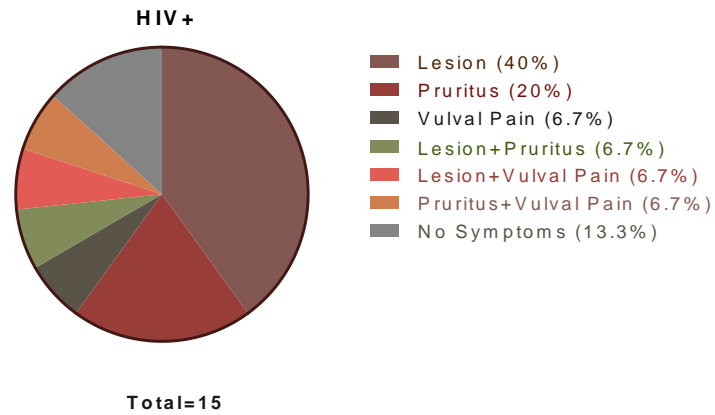
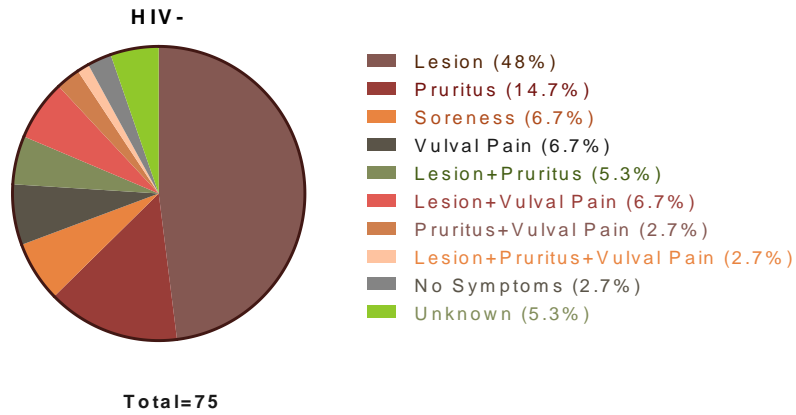
Figure 3. The effect of viral load and CD4+ lymphocyte count on the time to VIN recurrence in HIV+ patients.

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

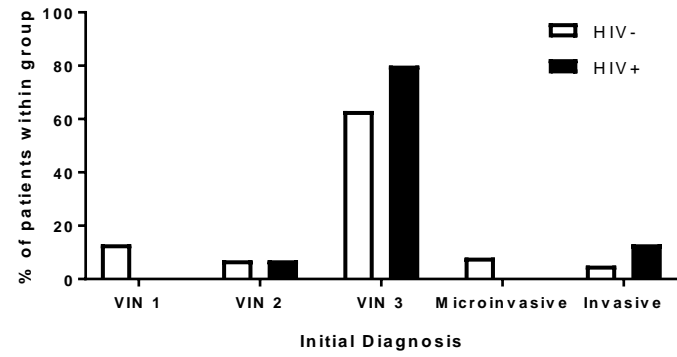
fluorescence activated cell sorting and is presented as number of CD4+ lymphocytes per 10^9 cells. Linear regression was used to calculate potential relationships between viral load (n=7) and CD4+ lymphocyte count (n=7) and time to recurrence. Data are not shown when encompassed by another symbol. Pearson correlation co-efficient and p-values were calculated using Prism version 7.00 software.

Ayakannu et al. Figure 1.

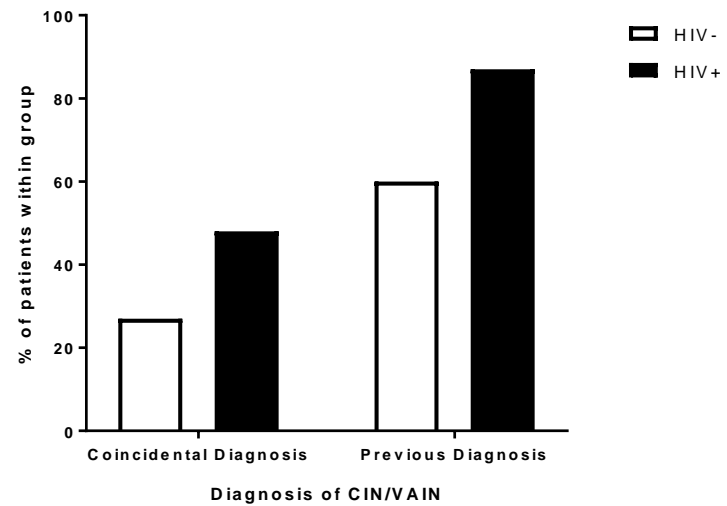
a



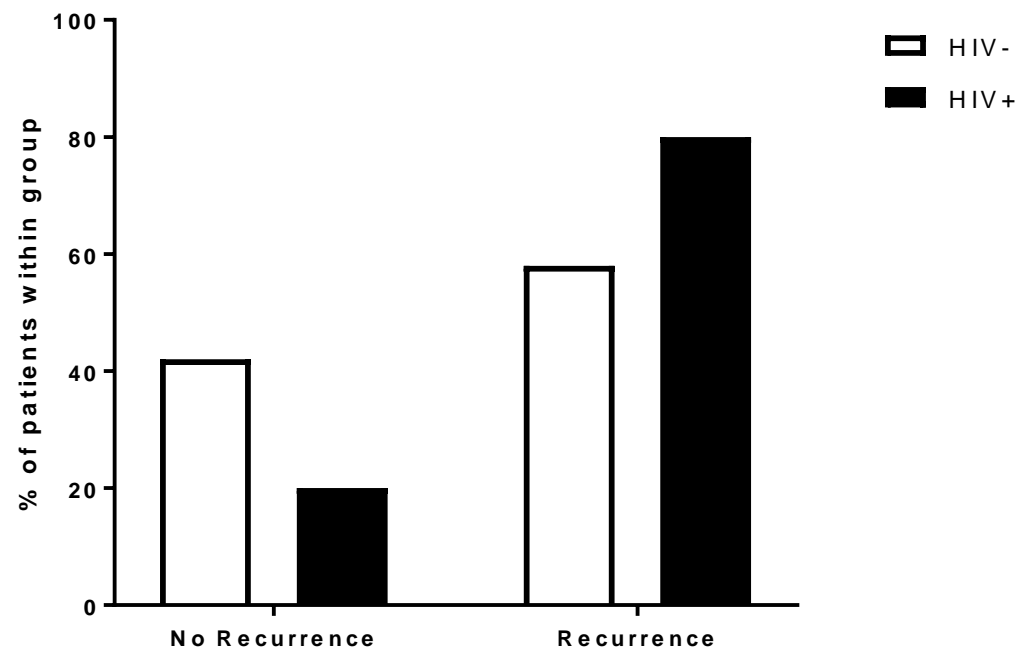
b



c

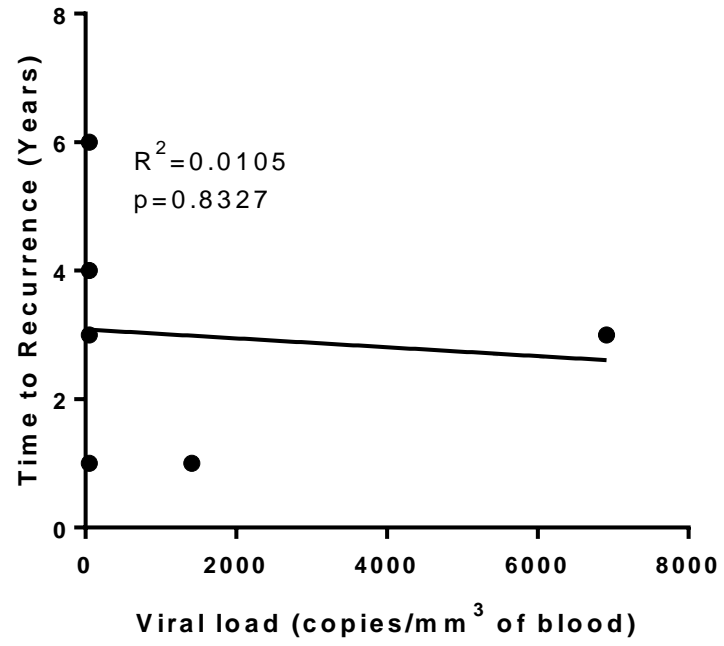


Ayakannu et al. Figure 2.



Ayakannu et al. Figure 3.

a



b

