CD4⁺ Cell Count and HIV Load as Predictors of Size of Anal Warts Over Time in HIV-Infected Women

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Background. Little is known about the associations between CD4⁺ cell counts, human immunodeficiency virus (HIV) load, and human papillomavirus “low-risk” types in noncancerous clinical outcomes. This study examined whether CD4⁺ count and HIV load predict the size of the largest anal warts in 976 HIV-infected women in an ongoing cohort.

Methods. A linear mixed model was used to determine the association between size of anal wart and CD4⁺ count and HIV load.

Results. The incidence of anal warts was 4.15 cases per 100 person-years (95% confidence interval [CI], 3.83–4.77) and 1.30 cases per 100 person-years (95% CI, 1.00–1.58) in HIV-infected and HIV-uninfected women, respectively. There appeared to be an inverse association between size of the largest anal warts and CD4⁺ count at baseline; however, this was not statistically significant. There was no association between size of the largest anal warts and CD4⁺ count or HIV load over time.

Conclusions. There was no evidence for an association between size of the largest anal warts and CD4⁺ count or HIV load over time. Further exploration on the role of immune response on the development of anal warts is warranted in a larger study.

Human papillomavirus (HPV) is a necessary cause of cervical cancer [1, 2] and has also been shown to be strongly associated with anal cancer [3]. Although the high-risk HPV types 16 and 18 account for the majority of cancers [1, 2], low-risk HPV genotypes (eg, 6 and 11) are responsible for the development of anogenital warts [4, 5]. Recent estimates predict that approximately 1% of sexually active adults in the United States have visible genital warts [6], and the prevalence may be much higher among individuals infected with human immunodeficiency virus (HIV). A previous study reported that HIV-infected women were 9.32 times (95% confidence interval [CI], 3.04–38.00) more likely to have genital warts than HIV-uninfected women [7]. Furthermore, immunocompromised patients were found to have more recurrence of anogenital warts than immunocompetent persons [8].

Among risk factors shown to be associated with HPV infections and their clinical outcomes, CD4⁺ cell count and HIV load have been studied extensively, particularly in HIV-infected populations. Accordingly, several studies have reported that a person with a high CD4⁺ cell count and a low HIV load is less likely to be infected with HPV than a person with a low CD4⁺ cell count and a high HIV load [9–14]. Although there are numerous studies on how HPV high-risk types, CD4⁺ cell counts, and HIV loads collectively impact certain clinical outcomes (eg, cervical cancer), little is known about the associations with HPV low-risk types and important noncancerous clinical outcomes (ie, anogenital warts). As a result, the factors that predict changes in size of anal warts have not been identified. In 2002, Conley et al [15] reported that compared with an HIV-infected woman with CD4⁺ cell count >500 cells/mm³, a person with a CD4⁺ cell count <200/mm³ is 1.66 times
MATERIALS AND METHODS

Study Population

Data used for this study were obtained from the public dataset (release P09) provided by WIHS. WIHS is an ongoing prospective cohort study and has been described in detail elsewhere [19, 20]. In brief, WIHS included clinical consortia in 6 locations across the United States: Bronx/Manhattan, New York; Brooklyn, New York; Washington, District of Columbia; Los Angeles/Southern California/Hawaii; San Francisco/Bay Area, California; and Chicago, Illinois. WIHS has 2 enrollment phases: The first enrollment phase was between October 1994 and November 1995, during which 2059 HIV-infected and 569 HIV-uninfected women were recruited; the second enrollment phase was between October 2001 and September 2002, during which 1143 HIV-infected and 406 HIV-uninfected women were recruited.

The WIHS protocols include a baseline visit and follow-up every 6 months. During the baseline visit, a standardized in-person questionnaire was administered by trained interviewers. Self-reported information during the interview included general medical history, highly active antiretroviral therapy (HAART) use, obstetric and gynecologic history, alcohol and cigarette use, sexual behaviors, and history of physical and sexual abuse. Medical examination included height/weight/vital signs and examination of skin, abdomen, and breasts. Gynecologic examination included external genitalia, examination of internal vagina and cervix, cervical-vaginal lavage, bimanual and rectal examination, and colposcopy, biopsy, and dysplasia treatment if necessary. Follow-up visits [21] assessed key clinical characteristics such as CD4+ and CD8+ cell counts, HIV serostatus (HIV-uninfected women only), HIV load (HIV-infected women only), and Pap smear. For the current analyses, only those who had at least 1 anal wart over the study course were included.

Variables of Interest and Measurement

Outcome Variable

The outcome variable was the size of the largest anal wart at each visit. Presence of anal warts was recorded on the gynecologic exam form. The length and width (in millimeters) of the largest anal wart was measured and recorded. The physicians and physician assistants were instructed to complete the form and code the lesions and diagnoses. In current analysis, anal warts were defined as warts in one of the following locations: anus upper left, anus lower left, anus upper right, anus lower right, perineum left, and perineum right. The size of the anal wart was calculated by multiplying the width and length of the reported anal wart. In case of multiple warts, we assumed that the largest wart is an anal wart.

Independent Variables

Blood was drawn at each visit for determination of HIV serostatus, CD4+ cell count, and HIV load. The level of CD4+ cell count was quantified using flow cytometry at laboratories certified by the AIDS Clinical Trial Groups [19]. HIV load was measured in serum using a nucleic acid sequence-based amplification assay from Organon Teknika. HIV load tests were done at the National Institute of Allergy and Infectious Diseases AIDS Program, Virology Assurance HIV RNA Proficiency Program, National Institutes of Health [19].

Covariates

Covariates included in the current analysis were race/ethnicity (African American, white, and others); number of sex partners in the past 6 months (0 and ≥1); education level (less than high school education, high school education or general educational development test, some college, and college graduate or graduate school); annual household income (≤$6,000, $6,001–$12,000, $12,001–$24,000, and ≥$24,001); enrollment (enrollment 1 and enrollment 2); HAART use (yes/no); and marital status (married or living with partners, widowed, separated or divorced, and never married). The HAART use definition in the current analysis was based on the guidelines from the US Department of Health and Human Services, version 2002 [22] and the International AIDS Society Panel Antiretroviral Guidelines [23] and was consistent with previous WIHS analyses [24, 25]. A person was considered on HAART if she met 1 of the following criteria: (1) ≥2 nucleoside reverse transcriptase inhibitors (NRTIs) in combination with at least 1 protease inhibitor (PI) or

HIV Predictors of Anal Warts in Women • JID 2012:205 (15 February) • 579
1 nonnucleoside reverse transcriptase inhibitor (NNRTI); 
(2) 1 NRTI in combination with at least 1 PI and at least 1 NNRTI; 
(3) regimen containing ritonavir and saquinavir in combination with 1 NRTI and no NNRTI; and (4) an abacavir- or 
tenofovir-containing regimen of ≥3 NRTIs in the absence of both 
PIs and NNRTIs, except for the 3 NRTI regimens consisting of 
abacavir + tenofovir + lamivudine or didanosine + 
tenofovir + lamivudine. Combination of zidovudine and sta-
vudine with either a PI or NRTI were not considered HAART. 
Monotherapy is considered as taking 1 NRTI, or only PI, or 
only NNRTI [22, 23].

Statistical Analysis
The distributions of sociodemographic characteristics were 
examined. For continuous variables, means and their standard 
deviations were calculated. Incident cases of anal warts were 
defined as persons who did not have an anal wart at the base-
line visit but developed ≥1 anal warts during the follow-up 
period. Prevalence of anal warts for the entire WIHS cohort 
and in HIV-infected and HIV-uninfected women at baseline 
was calculated by taking respective number of persons with 
warts present at baseline divided by respective total samples. 
Incidence rates were calculated as the total number of in-
cident cases divided by total follow-up time (ie, person-years) 
for HIV-infected, HIV-uninfected, and seroconverters sepa-
rately. The 95% CIs for prevalence and incidence rates were 
based on a normal distribution if the number of cases was 
>30 and on the exact Poisson distribution if the number of 
cases was <30 [26].

CD4⁺ cell count and HIV load at each visit were provided 
as continuous variables in the WIHS public data set. During 
follow-up, some participants had HIV loads suppressed to 
undetectable levels. We used 10 copies/mL for undetectable 
level, as it was validated by Notermans et al [27]. In this 
analysis, CD4⁺ cell counts were categorized into 3 groups 
(<200, 200–500, and >500 cells/mm³), and HIV load was 
categorized into 4 groups (<4000, 4000–20 000, 20 001– 
100 000, and >100 000 copies/mL). These categories were 
chosen for consistency with previous WIHS analyses [24, 28–31]. 
Both CD4⁺ cell count and HIV load were used in each visit in 
the longitudinal modeling process.

The linear mixed model was chosen for the current analysis 
because of its ability to deal with missing values that are com-
mon in a longitudinal studies, deal with the highly correlated 
nature of repeated measurements within individuals and be-	ween individuals in a longitudinal study, and account for un-
balance measurements (ie, number of visits) of subjects and 
the time interval between measurements [32]. Unadjusted 
models were first constructed to determine the total variation 
in growth velocity [26]. In the adjusted models, time-dependent 
covariates included number of sex partners in the past 6 months, 
education level, marital status, annual household income, and 
HAART use. Each of these variables was entered into the 
model both as a main effect and as a product with time. The 
time-independent covariates in the adjusted models were race/
ethnicity and enrollment. Those covariates had been identified 
and used in previous analyses of WIHS [13, 24, 31, 33–35] and 
were treated as potential confounders in the current analysis. 
All statistical analyses were performed using command PROC 
 Mixed of the SAS 9.2 statistical package [36]. All tests were 
2-sided, and  P = .05 was used as the significance level.

RESULTS
WIHS consisted of 3766 HIV-infected and HIV-uninfected 
women. Participants were excluded from the current analysis 
if (1) they were HIV-negative ( n = 958); (2) they were sero-
converters ( n = 16) or had unknown HIV status ( n = 1); (3) they 
did not have any anal warts during follow-up ( n = 1777); or 
(4) they had received treatment for anal warts during the 
study period ( n = 38) (Figure 1). HIV-infected women who 
received treatment for their anal warts were excluded because 
these treatments could have greatly influenced the size of the 
anal wart, many different types of treatment were re-
ceived, and there were too few participants in the treatment 
group to perform a meaningful subanalysis.

Of the 1141 women who had at least 1 anal wart during 
follow-up, 477 women (441 HIV-infected vs 34 HIV-uninfected 
women) were identified with anal warts at baseline. Therefore,

![Figure 1. Flowchart of inclusion and exclusion of participants in current analysis. Abbreviation: HIV, human immunodeficiency virus.](https://academic.oup.com/jid/article-abstract/205/4/578/887042/figure-1)
the prevalence of anal warts at baseline was 12.67% (95% CI, 11.61–13.73%). The respective prevalences of anal warts at baseline in HIV-infected and HIV-uninfected women were 15.80% (95% CI, 14.45–17.15%) and 3.55% (95% CI, 2.38–4.72%). These prevalences were significantly different ($P < .0001$). The incidence rates of anal warts by HIV serostatus for the entire WIHS study are presented in Table 1. Because 724 incident cases of anal warts were identified, the incidence rate was 3.35 cases per 100 person-years (95% CI, 3.11–3.60 cases per 100 person-years). A statistically significant difference ($P < .0001$) was observed in the incidence rate of anal warts in HIV-infected women (4.15 cases per 100 person-years; 95% CI, 3.83–4.77 cases per 100 person-years) compared with HIV-uninfected women (1.30 cases per 100 person-years; 95% CI, 1.00–1.58 cases per 100 person-years).

Table 2 presents the baseline sociodemographic characteristics of study participants included in our analysis. Approximately 20% of participants had a CD4$^+$ cell count of $<200$ cells/mm$^3$, and 40% had a CD4$^+$ cell count of $>500$ cells/mm$^3$. Although 33% of participants had an HIV load of $<4000$ copies/mL, about 50% had an HIV load of $>20 000$ copies/mL.

Table 3 presents results on the association between size of anal warts and CD4$^+$ cell count. There was no significant relationship between size of the largest anal warts and CD4$^+$ cell count over time in either the unadjusted or adjusted model. At baseline, women with moderate CD4$^+$ cell counts had, on average, an anal wart size of 1.61 mm$^2$ larger than that of women with the highest CD$^+$ cell count. Similarly, the size of the anal wart of women with the lowest CD4$^+$ cell count was 5.98 mm$^2$ larger than that of women with the highest CD4$^+$ cell count. However, those results were not statistically significant ($P = .93$ and $P = .76$, respectively). Interestingly, the growth rate of anal wart size after each visit in women with the lowest and moderate levels of CD4$^+$ cell count were 0.30 mm$^2$ and 0.64 mm$^2$, respectively, less than that of women with the highest CD4$^+$ cell level. However, these differences were not statistically significant.

Table 4 shows estimates on the association between size of anal wart and HIV RNA. Similar to CD4$^+$ cell count, there was no significant association between size of the largest anal wart and HIV load over follow-up time. There appeared to be a significantly larger growth rate of anal wart size among women with HIV load of 20 001–100 000 copies/mL than women in the reference group (HIV load of $<4000$ copies/mL) ($P < .003$). However, this difference was diminished in the adjusted model. In the adjusted model, women with an HIV load of $>100$ 000 copies/mL had a larger wart size at baseline (20 mm$^2$) but a slower growth rate of change than those of women with an HIV load of $<4000$ copies/mL. Again, these differences were not statistically significant in the adjusted model.

**DISCUSSION**

The current analysis examines the possible association between size of anal warts and CD4$^+$ cell count as well as HIV load over time among HIV-infected women using the public dataset from WIHS, an ongoing longitudinal study in the United States. Our study did not provide evidence of an association between the size of the largest anal warts and CD4$^+$ cell count or HIV load over time in HIV-infected women.

However, we did find a higher prevalence of anal warts at baseline among HIV-infected women in the WIHS study than...
reported elsewhere [6, 32, 37]. For example, the prevalence of anal warts at baseline found in our analysis is 3 times higher than that in the 1996 survey of the Australian Longitudinal Study on Women’s Health, in which 3.1% of 14,762 women aged 18–23 years reported ever having been diagnosed with anal warts. Caution is warranted when comparing our finding with the prevalence of anal warts in Australia because warts were self-reported in that study and their population is younger than that of the WIHS. The incidence rate of anal warts among HIV-infected and HIV-uninfected women found in our analysis is similar to those in other studies [38–41]. For example, in a study also from the WIHS cohort in 2004, Massad et al [41] reported that the incidence of genital warts among HIV-uninfected and HIV-infected women in the WIHS study were 1.30 per 100 person-years, which is comparable to ours, and 5.41 per 100 person-years, which is somewhat higher than ours, respectively. One possible explanation for the higher incidence in their study is that the follow-up in our analysis is longer than the study by Massad et al [41], and as of October 2010, approximately 80% of WIHS participants had received HAART [21]. HAART was proven to be effective in reducing the incidence of anal warts [41], thus possibly explaining the reduced anal wart incidence in HIV-infected women reported herein.

To our knowledge, this is the first study to use the linear mixed model to address whether there is an association between the size of the largest anal warts over time and either CD4\(^+\) cell count or HIV load. Therefore, we cannot compare our findings directly with any other study. However, there are a few studies reporting the relationship between presence and incidence of genital warts with CD4\(^+\) cell count and genital warts. Accordingly, HIV-infected women with a CD4\(^+\) cell count of <200 cells/mm\(^3\) were 6.78 times more likely to have genital warts than HIV-uninfected women. The difference between our study and the study by Greenblatt et al [42] is that we examined the size of anal warts, whereas they looked at the presence...
of genital warts. Another difference is that in our study, we investigated the development (ie, progression or regression) of anal warts based on the size of the wart longitudinally, whereas the study by Greenblatt et al [42] was a cross-sectional analysis of baseline visit only.

We also found an inverse association between the size of anal warts and CD4$^+$ cell count at baseline visit with decreasing CD4$^+$ cell count category. One possible explanation is that because a person with a CD4$^+$ cell count of 500 cells/mm$^3$ probably has a larger anal wart than a person with a higher CD4$^+$ cell count, the growth rate would be slower when comparing the measurement of the size of anal warts between 2 visits. Even though we did not find an association between size of anal wart and CD4$^+$ cell count over time, the role of

### Table 3. Linear Mixed Model of Size of Anal Warts and CD4$^+$ Cell Count of the Women's Interagency HIV Study HIV-Infected Participants in Current Study

<table>
<thead>
<tr>
<th>Unadjusted Model</th>
<th>Adjusted Model$^a$</th>
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<tbody>
<tr>
<td></td>
<td>Estimates ± SD</td>
</tr>
<tr>
<td>Intercept</td>
<td>59.37 ± 66.69</td>
</tr>
<tr>
<td>Visit</td>
<td>−1.35 ± 5.31</td>
</tr>
<tr>
<td>CD4$^+$ &lt;200 cells/mm$^3$</td>
<td>−57.87 ± 78.06</td>
</tr>
<tr>
<td>CD4$^+$ 200–500 cells/mm$^3$</td>
<td>−42.45 ± 77.75</td>
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<tr>
<td>CD4$^+$ &gt;500 cells/mm$^3$</td>
<td>0$^b$</td>
</tr>
<tr>
<td>Visit (CD4$^+$ &lt;200 cells/mm$^3$)</td>
<td>6.41 ± 6.44</td>
</tr>
<tr>
<td>Visit (CD4$^+$ 200–500 cells/mm$^3$)</td>
<td>9.56 ± 6.35</td>
</tr>
<tr>
<td>Visit (CD4$^+$ &gt;500 cells/mm$^3$)</td>
<td>0$^b$</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; SD, standard deviation.

$^a$ Type 3, $P = .76$.

$^b$ Type 3, $P = .32$.

$^c$ Type 3, $P = .91$.

$^d$ Type 3, $P = .98$.

$^e$ Model adjusted for no. of sex partners in the last 6 months, race, highly active antiretroviral therapy use, enrollment, marital status, annual household income, and education level.

### Table 4. Linear Mixed Model of Size of Anal Warts and HIV Load of the Women's Interagency HIV Study HIV-Infected Participants in Current Study

<table>
<thead>
<tr>
<th>Unadjusted Model</th>
<th>Adjusted Model$^a$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Estimates ± SD</td>
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<tr>
<td>Intercept</td>
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<tr>
<td>Visit</td>
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<tr>
<td>Viral load &lt;4000 copies/mL</td>
<td>0$^a$</td>
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<tr>
<td>Viral load 4000–20 000 copies/mL</td>
<td>−17.35 ± 33.23</td>
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<tr>
<td>Viral load 20 001–100 000 copies/mL</td>
<td>−58.97 ± 30.15</td>
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<tr>
<td>Viral load &gt;100 000 copies/mL</td>
<td>−41.40 ± 29.05</td>
</tr>
<tr>
<td>Visit (Viral load &lt;4000 copies/mL)</td>
<td>0$^b$</td>
</tr>
<tr>
<td>Visit (Viral load 4000–20 000 copies/mL)</td>
<td>1.40 ± 3.06</td>
</tr>
<tr>
<td>Visit (Viral load 20 001–100 000 copies/mL)</td>
<td>8.97 ± 2.99</td>
</tr>
<tr>
<td>Visit (Viral load &gt;100 000 copies/mL)</td>
<td>3.67 ± 3.00</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

$^a$ Type 3, $P = .21$.

$^b$ Type 3, $P = .02$.

$^c$ Type 3, $P = .52$.

$^d$ Type 3, $P = .76$.

$^e$ Model adjusted for no. of sex partners in the last 6 months, race, highly active antiretroviral therapy use, enrollment, marital status, annual household income, and education level.

$^f$ Statistically significant at $P$ value $< .05$.  

HIV Predictors of Anal Warts in Women • JID 2012:205 (15 February) • 583
immunity cannot be ruled out because CD4\(^+\) cells play an important role in cell-mediated immunity against HPV infection. This is reflected by the increased incidence and progression of HPV infection among immunosuppressed persons. In a cohort of adolescent girls, Moscicki et al. [43] observed that the risk for incident Cervical intraepithelial neoplasia (CIN) among HIV-infected adolescents was due to the persistence of Low grade squamous intraepithelial lesions (LSILs). HIV-infected persons are more likely to have multiple recurrences of cervical CIN, chronic condylomatous changes [44], and increased incidence of both cutaneous and genital warts [45]. Additionally, de Jong et al. [46] observed a strong proliferative response against 1 or more peptide epitopes derived from HPV 16 E-2 T-cell antigen in peripheral blood mononuclear cell cultures of approximately half of healthy donors. They also found that most of these responses represented reactivity by memory CD4\(^+\) T-helper 1-type cells, which are able to secrete interferon-\(\gamma\) on antigenic stimulation.

We were unable to find other studies directly comparing size of anal wart by HIV load, but Dolev et al. [47] reported that HIV load was independently associated with the incidence of anogenital warts in the WIHS cohort. It is, however, noted again that the outcome used in our study is different from theirs because we used the size of anal warts and they used the presence of anogenital warts. Although HIV load has been identified as a risk factor for HPV infection [13, 14, 48, 49] and precancerous lesions of the cervix [14] caused by high-risk HPV, it has not been proven to play an important role in the development of anal warts caused by low-risk HPV types.

There are 2 strengths of our study. First, we used the linear mixed model to deal with the aforementioned difficulties inherent to longitudinal data. Second, the use of the linear mixed model to deal with the aforementioned difficulties in-...


