

Subtype Distribution of Human Papillomavirus in HIV-Infected Women With Cervical Intraepithelial Neoplasia Stages 2 and 3 in Botswana

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Summary

Human papillomavirus (HPV) vaccines containing types 16 and 18 are likely to be effective in preventing cervical cancer associated with these HPV types. No information currently exists in Botswana concerning the HPV types causing precancerous or cancerous lesions. Our goal was to determine the prevalence of HPV types associated with precancerous cervical intraepithelial neoplasia (CIN) stages 2 and 3 in HIV-infected women in Gaborone, Botswana. HIV-infected women referred to our clinic with high-grade intraepithelial lesion on the Pap smear were enrolled in the study. HPV typing was only performed if the histopathology results showed CIN stage 2 or 3 disease using linear array genotyping (CE-IVD, Roche Diagnostics). One hundred HIV-infected women were identified with CIN stages 2 or 3 between August 11, 2009 and September 29, 2010. Eighty-two of 100 women enrolled had coinfection by multiple HPV subtypes (range, 2 to 12). Of the remaining 18 women, 14 were infected with a single high-risk subtype and 4 had no HPV detected. Overall, 92 (92%) women were infected with at least 1 high-risk HPV subtype, and 56 were coinfecting with more than 1 high-risk HPV type (range, 2 to 5). Fifty-one (51%) women had HPV subtypes 16, 18, or both. HPV 16 and 18 are the most common types in HIV-infected women with CIN 2 or 3 in Gaborone, Botswana, suggesting that the implementation of HPV vaccination programs could have a significant impact on the reduction of cervical cancer incidence. However, given the relative lack of knowledge on the natural history of cervical cancer in HIV-infected women and the significant prevalence of infection and coinfection with other high-risk HPV types in our sample, the true impact and cost-effectiveness of such vaccination programs need to be evaluated.

Keywords

HIV; HPV; CIN; Cervical cancer

Cervical cancer is a preventable cause of morbidity and mortality worldwide and the most common cancer among women in Sub-Saharan Africa (1–3). It is estimated that approximately 250,000 women die from cervical cancer each year, 80% of them living in resource-limited countries (1). The incidence of cervical cancer in Botswana is estimated at

22.2/100,000 in 2008, making it the most common cancer among women in Botswana and second in terms of cancer mortality in both sexes after Kaposi sarcoma (4,5).

Cervical cancer is associated with persistent human papillomavirus (HPV) infection, which is the most common sexually transmitted infection in the world (6). However, the risk of progression to cancer varies depending on the HPV subtype. Subtypes considered to have the highest oncogenic risk are HPV 16, 18, 31, 33, 45, 52, and 58, with subtypes 16 and 18 being implicated in approximately 70% of cervical cancers worldwide (6–8). HPV types 35, 39, 51, 58, 59, 68, 73, and 82 have also been associated with the development of cervical neoplasia (6–8). Low-risk subtypes, usually leading to the development of nonmalignant lesions, are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89. Limited data exist for the HPV types 26, 53, and 66 (6,8,9).

Immunocompetent individuals have low and slow rates of progression to cervical cancer, partly secondary to an appropriate suppression of HPV replication (10–12). In contrast, premalignant lesions in HIV-infected individuals seem to progress more rapidly and are associated with higher morbidity and mortality (13). Rates of infection and abnormal cytology increase as CD4⁺ T-cell counts decrease, as do the number of oncogenic HPV subtypes involved in infection (14–17). In addition, the longer life span associated with increased access to lifesaving antiretroviral therapy (ART) permits progression to cancer in more patients (13,18).

Determining the frequency of HPV subtypes is important to evaluate the potential impact of an HPV vaccine, particularly as the life expectancy of patients with HIV infection from the region increases. There is limited information of the distribution of HPV subtypes causing precancerous or cancerous lesions in HIV-infected women in Sub-Saharan Africa and no information currently exists in Botswana, one of the countries most severely affected by the HIV pandemic (9,19). The goal of this study was to determine the HPV subtypes detected in HIV-infected women with precancerous cervical intraepithelial neoplasia (CIN) stages 2 and 3 in Gaborone, Botswana.

Materials and Methods

Setting

This study was implemented at the Botswana-UPenn Partnership Women's Health Clinic located within the Princess Marina Hospital, the largest referral hospital in the country.

Study Design and Participant Enrollment

We conducted a descriptive study to determine the prevalence of HPV subtypes in HIV-infected women in Botswana. HIV-infected women referred to our clinic with high-grade intraepithelial lesion on the Pap smear were enrolled in the study. All HIV-infected adult women (aged 21 yr or older) with CIN2 or CIN3 were eligible for enrollment. Women were enrolled consecutively as they arrived at the clinic. All women were referred by the treating physician (no self-referrals). Enrollment continued until the predetermined sample size of 100 women with histopathologic CIN2 or CIN3 was accrued.

Specimen Collection and Processing

Two specimens were obtained from all participants. Dry cervical swabs and tissue specimens preserved in formalin were shipped daily to the Bioanalytical Research Center in Johannesburg, South Africa, at room temperature. All processing and testing of samples occurred at this site. HPV testing was performed exclusively on cervical swab specimens from women who had histopathology results showing CIN2 or CIN3 disease. Swabs were

lysed in 500 μL of the kit lysis buffer for 30 minutes at room temperature. The MagNa Pure external lysis protocol was used to extract DNA from the lysis buffer into a 100- μL eluate. Fifty microliters of the eluate was used for screening (Roche Amplicor HPV test; Roche Diagnostics, Branchburg, NJ) and 50- μL eluate was used for genotyping (Roche Linear Array Genotyping test; Roche Diagnostics) (1,20,21). Two internal control probes for high- and low-level expression of the human beta-globin gene were run on each strip of DNA extraction and amplification. Results were considered acceptable only if both internal controls were clearly visible. None of the 100 samples had a negative internal betaglobin polymerase chain reaction control; therefore, all positive results were genotyped. This standardized polymerase chain reaction-based method can detect 13 high-risk HPV genotypes (i.e. genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 24 low-risk HPV genotypes (i.e. genotypes 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108).

Variables, Data Sources, and Measurement

All demographic, clinical and behavioral data were collected prospectively using standardized questionnaires administered by the study personnel. Clinical data regarding HIV history were also abstracted from the electronic medical records.

Sample Size

The sample size of 100 participants was selected based on the fact that it is possible that types 31, 33, 35, 45, 52, 56, 58, or other HPV types occasionally detected in cervical cancers may predominate in Botswana. On the basis of a precision of point estimate of a prevalence of 50% for HPV type 16 or 18 infection, 100 samples would enable us to detect 50 cases caused by type 16 or 18, with a 95% confidence interval to detect between 40 and 60 cases.

Statistical Analysis

Data analysis was carried out using STATA 11.0 (StataCorp, College Station, TX). Comparisons were performed using the Mann-Whitney rank sum test or the Student t-test according to the distribution of the variables. Association between variables was determined by χ^2 tests.

Results

A total of 100 HIV-infected women were identified with CIN stage 2 or 3 between August 11, 2009 and September 29, 2010. All patients invited to participate in the study consented for enrollment. The median age was 36 years (interquartile range, 33–39 yr) and the mean and median CD4^+ T-cell count at the time of CIN2 or CIN3 diagnosis were 458.5 (95% confidence interval, 414.9–502.2) and 420 cells/ mm^3 (interquartile range, 295–579 cells/ mm^3), respectively. The mean and median CD4^+ T-cell count of women at the time of HIV diagnosis increased significantly by the time they were diagnosed with CIN2 or CIN3 ($P < 0.001$). Table 1 lists the main demographic characteristics of the women enrolled in this study.

Forty-nine of 100 (49%) women with premalignant lesions had the diagnosis of CIN 2, whereas 51 (51%) had CIN3. One or more HPV subtypes were isolated from 96 (96%) women. Ninety-two (92%) women had coinfection with multiple HPV subtypes (range, 2–12), at least one of them being a high-risk subtype. Fifty-six (56%) women were coinfecting with more than 1 high-risk HPV subtype (range, 2–5) (Fig. 2).

All women infected with a single subtype had a high-risk HPV subtype (Figs. 1, 2). Among these women, 4 were infected with HPV 16, 2 with HPV 18, 1 with HPV 33, 3 with HPV

35, 1 with HPV 45, and 3 with HPV 52 (Fig. 1). Fifty-one (51%) women had HPV types 16 or 18 and 4 (4%) had both subtypes. Among the other high-risk subtypes, HPV subtypes 33, 35, and 58 were the most prevalent, detected in 18, 40, and 35 participants, respectively (Fig. 3). The probe for HPV subtype 52 can lead to false-positive reports of HPV types 33, 35, and 58 by cross-reactivity. The number of coinfections with HPV subtype 52 was evaluated to assess potential misclassification. Five of the 8 women infected with HPV subtype 52 were also infected with HPV subtypes 33, 35, or 58, suggesting possible false-positive reports for these 3 subtypes.

Discussion

High-risk HPV subtypes were found in 92% of the patient samples. Together, HPV 16 and 18 were the most common types in HIV-infected women with CIN2 or CIN3 in Gaborone, Botswana, found in 51% of the women, with 8% having coinfection with both subtypes. This prevalence is similar to other reports in resource-limited settings (6,9,19,22–26,28). In Sub-Saharan Africa, this prevalence varies from 43% to 90% in HIV-negative women and from 53% to 68% in HIV-infected women (19,27,28). Other high-risk HPV subtypes account for approximately half of the CIN2 or CIN3 lesions in Botswana. Consistent with reports from the region, HPV subtypes 35 and 58 were highly prevalent (19,27,28). Multiple reports have documented an increase in the worldwide prevalence of HPV subtypes 52 and 58 over the last decade (29). Although the high prevalence of HPV subtype 58 in our sample is consistent with this world trend, it may be an overestimation secondary to cross-reactivity with HPV type 52. However, due to the high percentage of women coinfecting with HPV subtype 52 and HPV subtypes 33, 35, or 58, we believe the degree of misclassification secondary to cross-reactivity was not high in our study (30).

Coinfection with multiple HPV subtypes was common. However, the significance of these findings on the risk for progression to cervical cancer is unknown as our study was not designed to distinguish persistent versus transient HPV infections. Other investigators have reported an increase in coinfection with different HPV subtypes in HIV-infected women compared with HIV-negative women (17,23,31). It remains to be determined whether this finding is secondary to higher risk sexual practices, increased predisposition to infection, decreased clearance of HPV, or other undefined factors in HIV-infected women (32).

The mean and median CD4⁺ T-cell count at the time of HIV diagnosis, in this sample, was low (238 CD4⁺ cells/mm³) and it significantly increased by the time when CIN2 or CIN3 was diagnosed (458 CD4⁺ cells/mm³). This increase in CD4⁺ T-cell count is likely a reflection of the large number of women who were either taking ART at the time of CIN2 or CIN3 diagnosis, or had taken ART in the past. In addition, the mean number of years that these women were on ART by the time of CIN2 or CIN3 diagnosis (3.4 yr) would allow enough time for immune reconstitution. The effect of ART on the natural history of cervical cancer remains unknown. As opposed to other HIV-associated conditions, most studies suggest that the administration of ART has no effect on cervical cancer prevention (18,33). Although our study was not designed to look at the role of ART on the development of CIN2 or CIN3, the fact that the CD4⁺ T-cell counts were high (458) and 73% of women were on ART is consistent with the hypothesis that ART does not prevent progression to cervical cancer.

This cross-section of women treated in one clinic is likely representative of HIV-infected women diagnosed with CIN2 and CIN3 in Botswana (4,5). The demographics and general characteristics of our samples were comparable with those reported in prior series evaluating the distribution of HPV subtypes in Sub-Saharan Africa and with those of women diagnosed with premalignant cervical lesions through routine screening in Botswana and in the region

(1,4,5). The mean and median age of women in our sample were similar to those reported in other Sub-Saharan studies (1,3,19,34).

Progression to cervical cancer has been universally associated with persistent HPV infection, and in particular, infection with high-risk HPV subtypes. Therefore, the failure to identify any HPV subtype on 4 (4%) samples and a high-risk HPV subtype on 4 (4%) additional samples likely represents false-negative results, even though a highly sensitive HPV DNA polymerase chain reaction-based technology was used for detection and HPV typing. However, secondary to the overall high rate of identification of HPV subtypes, and in particular, the high frequency of identification of multiple subtypes in single samples, we believe the impact of false-negative results on our estimates is likely minimal. The relatively small number of samples tested also decreased the accuracy of our point prevalence estimates and the generalizability of our results and suggests the need for larger studies to confirm our findings.

This study is the first report of the prevalence and distribution of HPV subtypes among HIV-infected women diagnosed with CIN2 or CIN3 in Botswana. Our results are consistent with previous reports from the region and suggest that vaccination for HPV subtypes 16 and 18 could potentially prevent at least half of the cervical cancers in the country. However, the current limited knowledge regarding the natural history of cervical cancer in patients infected with HIV prevents us from drawing stronger conclusions. Given the relatively unknown role of infection, coinfection, and reinfection with HPV subtypes other than 16 and 18 on cervical carcinogenesis in HIV-infected individuals, the true impact of HPV vaccination on cervical cancer in settings similar to ours is difficult to predict and needs to be evaluated. The high prevalence of primary infection, coinfection, and reinfection with other HPV subtypes should be taken into consideration and be monitored if vaccination programs are to be implemented, as other subtypes may replace HPV 16 and 18 as major causes of cervical cancer and decrease the impact and cost-effectiveness of such interventions.

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References

1. Wong FK, Ching JC, Chow JK. Comparison of the DiagCor GenoFlow human papillomavirus array test and roche linear array HPV genotyping test. *Open Virol J.* 2010; 4:169–74. [PubMed: 21270940]
2. Denny L. Cervical cancer: the South African perspective. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet.* 2006; 95:S211–4. [PubMed: 17161160]
3. Parkin DM. The role of cancer registries in cancer control. *Int J Clin Oncol.* 2008; 13:102–11. [PubMed: 18463952]
4. Botswana National Cancer Registry. 2007
5. Central Statistics Office. Botswana: 2007.
6. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer.* 2007; 121:621–32. [PubMed: 17405118]

7. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens-Part B: biological agents. *Lancet Oncol.* 2010; 10:321–2. [PubMed: 19350698]
8. Clifford G, Smith J, Aguado T, et al. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Br J Cancer.* 2003; 89:101–5. [PubMed: 12838308]
9. Bruni L, Diaz M, Castellsague X, et al. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis.* 202:1789–99. [PubMed: 21067372]
10. Ho GY, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med.* 1998; 338:423–8. [PubMed: 9459645]
11. Rodriguez AC, Schiffman M, Herrero R, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst.* 102:315–24. [PubMed: 20157096]
12. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA.* 2001; 286:3106–14. [PubMed: 11754676]
13. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. *J Natl Cancer Inst.* 2005; 97:577–86. [PubMed: 15840880]
14. McKenzie ND, Kobetz EN, Hnatyszyn J, et al. Women with HIV are more commonly infected with non-16 and -18 high-risk HPV types. *Gynecol Oncol.* 116:572–7. [PubMed: 19906410]
15. Luque AE, Demeter LM, Reichman RC. Association of human papillomavirus infection and disease with magnitude of human immunodeficiency virus type 1 (HIV-1) RNA plasma level among women with HIV-1 infection. *J Infect Dis.* 1999; 179:1405–9. [PubMed: 10228061]
16. Piper MA, Severin ST, Wiktor SZ, et al. Association of human papillomavirus with HIV and CD4 cell count in women with high or low numbers of sex partners. *Sex Transm Infect.* 1999; 75:253–7. [PubMed: 10615312]
17. Levi JE, Kleter B, Quint WG, et al. High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J Clin Microbiol.* 2002; 40:3341–5. [PubMed: 12202576]
18. De Vuyst H, Lillo F, Broutet N, et al. HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy. *Eur J Cancer Prev.* 2008; 17:545–54. [PubMed: 18941376]
19. Louie KS, de Sanjose S, Mayaud P. Epidemiology and prevention of human papillomavirus and cervical cancer in sub-Saharan Africa: a comprehensive review. *Trop Med Int Health.* 2009; 14:1287–1302. [PubMed: 19772550]
20. Bulkmands NW, Berkhof J, Rozendaal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet.* 2007; 370:1764–72. [PubMed: 17919718]
21. Black CC, Bentley HA, Davis TH, et al. Use of a linear array for the detection of human papillomavirus genotypes in head and neck cancer. *Arch Pathol Lab Med.* 2010; 134:1813–7. [PubMed: 21128780]
22. Arbyn M, Sankaranarayanan R, Muwonge R, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. *Int J Cancer.* 2008; 123:153–60. [PubMed: 18404671]
23. Clifford GM, Goncalves MA, Franceschi S. Human papillomavirus types among women infected with HIV: a meta-analysis. *Aids.* 2006; 20:2337–44. [PubMed: 17117020]
24. Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer.* 2003; 88:63–73. [PubMed: 12556961]
25. De Vuyst H, Franceschi S. Human papillomavirus vaccines in HIV-positive men and women. *Curr Opin Oncol.* 2007; 19:470–5. [PubMed: 17762573]
26. Reiter PL, Pendergraft WF, Brewer NT. Meta-analysis of human papillomavirus infection concordance. *Cancer Epidemiol Biomarkers Prev.* 2010; 19:2916–31. [PubMed: 20833971]
27. De Vuyst H, Gichangi P, Estambale B, et al. Human papillomavirus types in women with invasive cervical carcinoma by HIV status in Kenya. *Int J Cancer.* 2008; 122:244–6. [PubMed: 17764116]

28. Sahasrabudde VV, Mwanahamuntu MH, Vermund SH, et al. Prevalence and distribution of HPV genotypes among HIV-infected women in Zambia. *Br J Cancer*. 2007; 96:1480–3. [PubMed: 17437020]
29. Li N, Franceschi S, Howell-Jones R, et al. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer*.
30. Averbach SH, Gravitt PE, Nowak RG, et al. The association between cervical human papillomavirus infection and HIV acquisition among women in Zimbabwe. *AIDS*. 2010; 24:1035–42. [PubMed: 20397287]
31. Banura C, Franceschi S, Doorn LJ, et al. Infection with human papillomavirus and HIV among young women in Kampala, Uganda. *J Infect Dis*. 2008; 197:555–62. [PubMed: 18237268]
32. Nicol AF, Nuovo GJ, Salomao-Estevez A, et al. Immune factors involved in the cervical immune response in the HIV/HPV co-infection. *J Clin Pathol*. 2008; 61:84–8. [PubMed: 17483251]
33. Minkoff H, Ahdieh L, Massad LS, et al. The effect of highly active antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women. *AIDS*. 2001; 15:2157–64. [PubMed: 11684935]
34. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005; 55:74–108. [PubMed: 15761078]

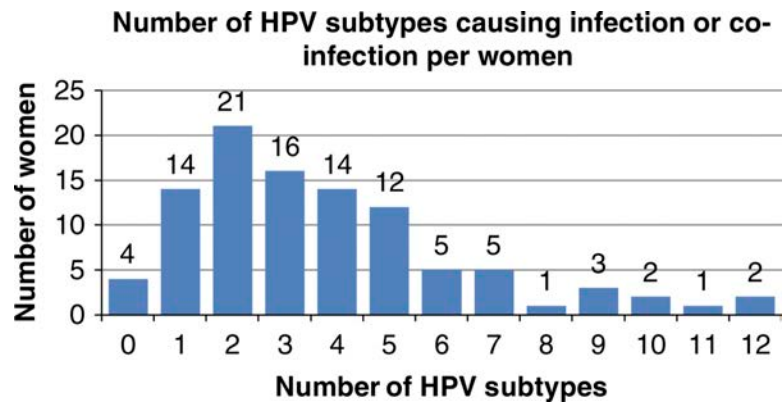


Fig. 1. Number of human papillomavirus subtypes causing infection or coinfection per women.

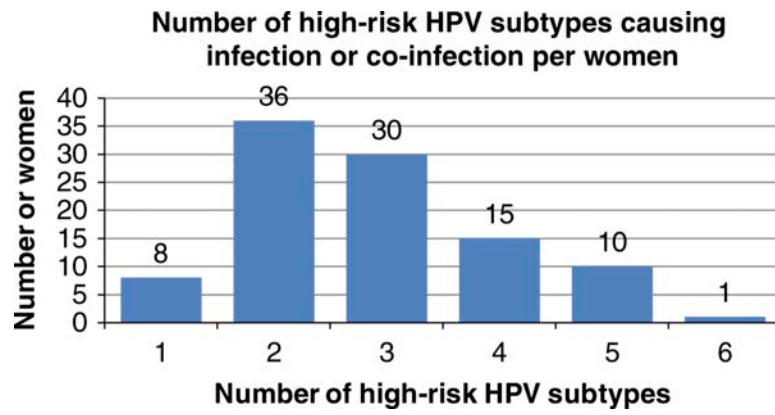


Fig. 2. Number of high-risk human papillomavirus subtypes causing infection or coinfection per women.

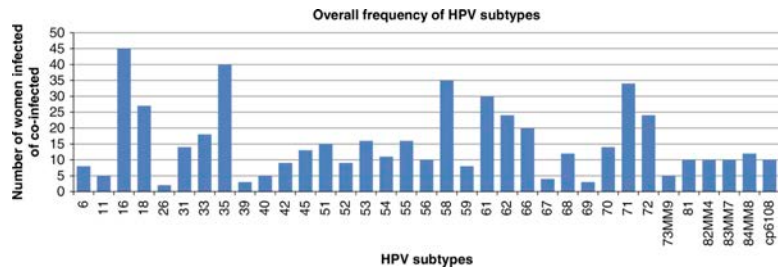


Fig. 3.
Overall frequency of human papillomavirus subtypes.

Table 1
Basic demographics and patient's characteristics

	Median/Number Interquartile Range/Percentage
Age	36 (33–39)
Latest CD4 cell count cells/mm ³	420 (295–579)
CD4 cell count at the time of HIV diagnosis	192 (84–346)
Time on HAART (yr)	3.4 * (2.84–3.97) [†]
HAART	
Never taken	9 (9%)
Taken in the past (not now)	18 (18%)
Currently on HAART	73 (73%)

* Mean.

[†] 95% confidence interval.

HAART indicates highly active antiretroviral therapy.