2019

Hpv And Hsv Associated Clinical Conditions Among People Living With Hiv-1 Infection

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HPV AND HSV ASSOCIATED CLINICAL CONDITIONS AMONG PEOPLE LIVING WITH HIV-1 INFECTION

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A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,

in partial fulfillment of the requirements for the degree of Doctor of Philosophy

BIRMINGHAM, ALABAMA

2019

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EPIDEMIOLOGY

ABSTRACT

This dissertation research focuses on investigating health burdens of HPV- and HSV- associated clinical conditions (CC) in people living with HIV-1 infection (PLWH) in the Southeastern United States. I designed a retrospective study using the UAB 1917 HIV Clinic Cohort. Electronic health records of PLWH attending the clinic for HIV-care between January 2006 and March 2019 were obtained and reviewed. I first examined incidence rates and trends of anogenital HPV- and HSV-associated CC. Then I adopted a new algorithm to group longitudinal CD4 cell counts based on their trajectories. I compared it with nadir and median CD4 cell counts in the associations of incident ulcers, warts, and anal high-grade squamous intraepithelial lesions (HSIL)/cancer. Last, I examined severities of high-grade anal intraepithelial neoplasia (HGAIN) and clinical practices of anal cancer screenings in PLWH in the region.

For the first aim, the diseases of interest included: HPV-associated anogenital warts, cervical, anal, penile low- and high- grade squamous intraepithelial lesions (LSIL and HSIL) and cancers, and HSV-associated anogenital herpetic ulcers. I observed 1,038 and 425 with HPV- and HSV-associated CC, respectively, and 163 patients with both

conditions. HPV-associated CC were more common in HIV-infected men than women, whereas HSV-associated CC were more prevalent in HIV-infected women. Rates of warts increased steadily over time. In the second aim, I used a group based trajectory modeling to group longitudinal CD4 counts. Trajectory CD4 groups more precisely predicted the risk of incident herpetic ulcers and anal HSIL/cancer. In the last aim, I observed substantially higher risks of HGAIN and anal cancer among HIV-infected men. However, over 44% of anal cancer patients did not receive a single anal/rectal Pap test during the study period. Also, the onset age of anal cancer was much younger for PLWH than the general US population.

PLWH bear higher risks of HPV- and HSV-associated CC in the region. However, except for cervical cancer, none of these conditions are AIDS-defining complications. Hence, screening programs for the conditions are lacking. The thesis expects to raise public and clinical awareness of the conditions in hopes of establishing better screening strategies.

Keywords: HIV, HPV, HSV, HSIL, cancer, rate

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DEDICATION

For my parents Ming and Min, and my grandparents Guanguang and Fengzhu.

ACKNOWLEDGEMENTS

The road towards my doctoral degree has been long and winding. Completion of the dissertation would not have been possible without the inspiration and support of a number of people.

I am deeply indebted to my advisor Dr. Sadeep Shrestha. Dr. Shrestha has been extremely inspiring, encouraging, and supportive. He motivated me to explore my thesis topic and branch out into new research areas. He gave me freedom to do whatever I would like to experiment, at the same time continuing his commitment to my work. Dr. Shrestha ensured that I would get sufficient resources to complete the thesis. Under his guidance, I have become more confident and open-minded in epidemiologic research. I simply cannot imagine a better advisor.

I gratefully acknowledge the contributions of my dissertation committee members, Drs.Inmaculada Aban, Stella Aslibekyan, Greer Burkholder, and Ashraf Khan. Without their expertise, support, and continuous optimism, this thesis would have been hardly achievable.

A very special gratitude goes out to Dr. Howard Wiener. Dr. Wiener introduced me to a new statistical program which has strengthened my analyses. He has been so patient and helpful with all my statistical problems.

My sincere thanks also goes to Drs. Marguerite Irivin and Nicole Wright. They went beyond their formal roles and helped me tremendously when I was recovering from a fall accident.

I would also like to say a heartfelt thank you to my family. I am especially grateful to my parents and grandparents, who supported me emotionally and financially. Their unconditional love has made me move forward fearlessly.

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LIST OF ABBREVIATIONS

AAPC	annual average percentage change
APC	average percentage change
ART	antiretroviral therapy
BIC	Bayesian information criterion
CC	clinical condition
CD4+	CD4+ T lymphocyte cells
GBTM	group-based trajectory modeling
HAART	highly active antiretroviral therapy
HGAIN	high-grade anal intraepithelial neoplasia
HIV	human immunodeficiency virus
HPV	human papillomavirus
HR-HPV	high risk- human papillomavirus
HSIL	high-grade intraepithelial lesion
HSV	herpes simplex virus

IR	incidence rate
LR-HPV	low-risk human papillomavirus
LSIL	low-grade intraepithelial lesion
M1	Model 1
M2	Model 2
MACS	Multicenter AIDS Cohort Study
MSM	men who have sex with men
NADM	non-AIDS-defining malignancy
OR	odds ratio
PAP	papanicolaou test
PLWH	people living with HIV
PY	person year
RISC	research and informatics service center
SCC	squamous cell carcinoma
SEER	Surveillance, Epidemiology, and End Results Program
STD	sexually transmitted disease
STI	sexually transmitted infection

SUN	Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy
VIF	variance inflation factors
VL	viral load

CHAPTER 1

INTRODUCTION

Human immunodeficiency virus infection (HIV) has caused global and domestic epidemics. According to the United States (US) Centers for Disease Control and Prevention (CDC), at the end of 2015, approximately 1.1 million people aged 13 and above were living with HIV infection (PLWH) in the US.[1] Nowadays, African Americans are the racial group most affected by HIV in the country.[1] There were over 17,670 African Americans diagnosed with HIV infection by the end of 2015.[1] More than half of them were homosexual and bisexual men.[1]

Human papillomavirus (HPV) and herpes simplex virus (HSV) cause two common sexually transmitted infections (STIs) in both PLWH and general populations.[2,3] The prevalence of anogenital HPV infection was about 42.5% among general U.S. adults aged 18–59 years between 2013 and 2014, with more than 14 million new diagnoses every year.[4] Speaking of similar epidemiologic commonality, the CDC estimated 1 in every 6 people aged 14-49 years have anogenital herpes.[5] In 2015, there were about 299,000 initial clinic visits due to anogenital herpes infections.[5]

HPV infection is extremely prevalent in the US, and most sexually active people will be infected with at least one type in lifetime.3 More than 40 types of HPV can infect anogenital tracts, although most infections can be cleared spontaneously within several

years after the initial infection.[4] Low-risk types of HPV (LR-HPV) cause warts and very mild cell changes in infected tracts, whereas high-risk types (HR-HPV) cause low- (LSIL) and high- grade squamous intraepithelial lesions (HSIL). Persistent HSIL may eventually lead to neoplasia. There are 12 types of LR-HPV (HPV-6, -11, -40, -42, -43, -44,-53, -54, -61, -72, -73 and -81) and at least 13 types of HR-HPV (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, 58, -59 and -68).[6] Persistent HR-HPV infection significantly promotes the initial intraepithelial dysplasia in the infected tract to become full-blown cancer cells. Anogenital cancers including 99.7% of cervical, 95% of anal, 65% of vaginal, 50% of vulvar, 35% of penile cancers are linked with HR-HPV infections.[7] HPV-associated cancers affect approximately 17,600 women and 9,300 men every year in the US.[8]

Anogenital herpetic ulcerations are caused by HSV -1 and -2. Although HSV-2 causes most of ulcerations in the anogenital tract, recent studies have demonstrated that HSV-1 can also infect anogenital tracts and cause ulcerations.[4] HSV infection may be persistent and induce a considerable amount of inflammatory responses. However, its mechanism of triggering persistent inflammation is still unclear. The national HSV-2 prevalence remains high at 16.2% in the general population.[4] The infection continues to burden African Americans with a prevalence of 39.2%. African American women account for 48.0% of the total infected.[5]

Both HPV and HSV- related illnesses are more common in PLWH than the general population. Invasive cervical cancer and persistent herpetic ulcers (>1 month's duration) are AIDS-defining conditions.[9] Genital HPV infection is 1.5-2.5 times higher in

women living with HIV- 1 infection than non-HIV women. Anal HPV infections among HIV infected women and men who have sex with men (MSM) are 3 times higher than their HIV negative counterparts. Similar findings were observed in HSV infection. Seroprevalence of HSV-2 among PLWH is varied between 50%-90% and it is overwhelmingly higher than immunocompetent populations.[10] The seroprevalence was reported 3 times greater among PLWH than the general US population. [11]

In the state of Alabama, African Americans are under the great impact of racial disparities regarding new HIV infections and STDs. According to the most recent report from the Department of Alabama Public Health, there were over 13,800 prevalent HIV cases by March 2017. [12] In the first quarter of 2017, over 70.5% of newly diagnosed and 64.3% of prevalent HIV cases were African Americans, while African Americans only represent about 26% of the total population of the state.[12] African American women and men are the most and second most rapid growing groups of incident HIV in Alabama among all races and genders[12] . Although we noted the high prevalence of HPV- and HSV-associated clinical conditions in the state, but most data were reported for the general population. There was a gap of knowledge in terms of the health burden caused by HPV and HSV infections among PLWH in the state of Alabama.

I obtained patients demographic, sociobehavioral, and clinical data through electronic health records (EHR) from the University of Alabama at Birmingham1917 HIV Clinic between January 2006 and March 2012. A total of 4,803 patients were retrospectively reviewed (Table1).The research used a retrospective cohort nested within the ongoing clinical cohort. The UAB HIV Clinic is the largest HIV clinic in the state of Alabama with extensive regional catchment and referral network. The prospective clinic

cohort has collected more than 7,000 patients' sociodemographic, psychosocial, comorbidities, medications, vital signs, laboratory results, and corresponding dates since the database establishment in 1992.[13,14] More than 3,500 patients currently receive their routine HIV care from the clinic, representing 30% of all PLWH in the state.[13]

Chapter 1 lays the foundation of the entire thesis by describing the health challenges of HPV- and HSV-associated clinical conditions among PLWH. My specific aim of Chapter 2 was to: *examine incidence rates of HPV-associated anogenital warts, cervical, anal, penile, LSIL, HSIL, and cancer , and HSV-associated anogenital herpetic, ulcers, stratified by race and gender (if applicable).* A new regression model, the Joinpoint regression was performed to study the changes of trends of incidence rates each year over the study follow-up.

Plasma CD4 T cell count is an important HIV prognostic indicator. Traditionally, nadir and median CD4 counts are widely used to assess the overall immune statue of PLWH. Clinically, they are also often used to evaluate the risks of diseases onset.[15–17] However, using one single-point CD4 counts for disease risk assessment might not be sufficiently informative and could be misleading due to the lack of trajectory information. The specific aim of Chapter 3 was to *compare the trajectory CD4 counts to median and nadir CD4 counts in the associations with incident anogenital herpetic ulcers, warts, and anal HSIL/cancer.* By using trajectory CD4 counts, I was able to demonstrate the benefits of using longitudinal CD4 to predict the disease onset.

Anal cancer is not an AIDS-defining malignancy. Thus, there are no mandatory guidelines for anal cancer screening and the treatments of the cancer are usually not

covered by the Ryan White program. [18] The specific aim of Chapter 4 was to *reveal the health burden of anal cancer among PLWH in a Southeastern US clinic and characterize the clinical practices of anal cancer screening in PLWH.* To our knowledge, this is the first study to explore the severity of anal cancer in PLWH in the region and how the current screening practices are differed by soceiobahavioral factors.

In summary, my thesis aims to explore the reveal the health burdens of HPV-and HSV- associated anogenital conditions among PLWH in the Southeastern US. I conducted comprehensive analyses by incorporating demographic, sociobehavioral, and clinical data to fill the clinical and epidemiologic gap. The findings represent the most realist risks of HPV- and HSV-associated conditions that PLWH in the region experienced as well as the applicable screening practices were offered to me. These findings contribute important clinical values to the field in hopes of raising public health awareness of the comorbidities and implementation better anogenital HPV- and HSV-related screening programs.

While the dissertation was written as Chapters 1-5, the major research part (Chapters 2-4) consists of 3 studies/manuscripts. For instance, if a table is labeled as (Table 2.1) in the dissertation, it refers to Table 1 in Manuscript 1.

Table 1. Baseline demographic, sociobehavioral and HIV-clinical indicator of the entire study population.

Characteristics	**Count (%)**
Total number obtained from Electronic health records	4803
Gender	
Men	1115 (76.3)
Women	3663 (23.2)
Transgender M-F	25 (0.5)
Race	
Black	2862 (59.5)
White	1744 (36.3)
Others	197 (4.1)
Age at HIV Diagnoses*	32.5 (25.8-40.8)
Nadir CD4*	174 (22-390)
Ever had HPV-related diagnoses	1277 (26.6)
Ever Had HSV-related diagnoses	827 (17.2)
Self-reported sexual risks	
MSM	2592 (56.8)
Heterosexual men	913 (20)
Heterosexual women	1059 (23.2)

*: median and interquartile range (25% -75%) reported

COMORBIDITIES ASSOCIATED WITH HPV AND HSV INFECTIONS AMONG PEOPLE LIVING WITH HIV-1 IN THE SOUTHEASTERN US: A RETROSPECTIVE CLINICAL COHORT STUDY

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Submitted to *BMC Infectious Diseases*

Format adapted for dissertation

Abstract

Background: The southeastern US is a domestic epicenter for incident HIV with high prevalence of human papillomavirus (HPV) and herpes simplex virus (HSV) co-infections. However, epidemics of HPV and HSV- associated clinical conditions (CC) among people living with HIV-1 infection (PLWH) are not fully known.

Methods: Electronic medical records (EMR) of PLWH attending one of the leading HIV clinics in the southeastern US between 2006 and 2018 were reviewed and analyzed. The retrospective study was nested within the University of Alabama at Birmingham HIV clinical cohort, which has electronically collected over 7000 PLWH's clinical and sociobehavioral data since 1999. Incidence rates of HPV-related CC including anogenital warts, penile, anal, cervical, and vaginal/vulvar low- and high-grade squamous intraepithelial lesions (LSIL and HSIL) and HSV-related CC including anogenital herpetic ulcers were estimated in per 10000 person years. Joinpoint regressions were performed to examine temporal changes in the trends of incident CC. All rates and trends were stratified by gender and race.

Results: Of the 4,484 PLWH eligible individuals (3,429 men, 1,031 women, and 24 transgender), we observed 1,038 and 425 patients with HPV-and HSV-related CC respectively, and 163 patients with both conditions. The mean log10 viral load (VL) was higher in all of the case groups than the non-cases with neither conditions (5.0) (whereas the median nadir CD4 counts (cells/uL) was higher in the non-cases than in any of the case groups ($P<0.05$). Anogenital warts, anal LSIL, HSIL, and cancer were more likely to be diagnosed among HIV-infected men than women. White men presented more frequently with anal LSIL and anal and penile cancers than black men ($P<0.03$). White women were also more likely to be diagnosed with cervical HSIL ($P=0.023$) and cancer ($P=0.037$) than black women By contrast, herpetic ulcers were more frequent in women than men.

Conclusions: There were significant differences between gender and race with incidence of HPV- and HSV-related CC among HIV patients. EMR-based studies provide insights on understudied epidemics; however, large-scale studies in other regions are needed to generalize current findings and draw public health attention to co-infection induced non-AIDS defining comorbidities among PLWH.

Background

Human papillomavirus (HPV) and herpes simplex virus (HSV) are two common sexually transmitted infections (STIs) in the general population and specifically among people were living with HIV infection (PLWH).[12] While both infections are treatable, they have chronic sequelae. The prevalence of anogenital HPV infection in 2013-2014 was estimated at 42.5% among US adults aged 18–59 years, with more than 14 million new diagnoses.[3] Genital HPV infection is 1.5-2.5 times higher in HIV+ women than HIV- women. Anal HPV infections among HIV+ women and MSM are 3 times higher than their HIV- counterparts. Likewise, CDC estimated that 1 in every 6 people aged 14-49 years have anogenital herpes.[3,4] In 2015, there were about 299,000 initial clinic visits due to anogenital herpes infections[4]. A multi-site prospective study, the Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy (SUN Study) reported HSV-seroprevalence was 3 times greater among PLWH than the general US population.[5]

HPV infection is common in the US, with over 80% of sexually active individuals being infected at least once during their lifetime[2]; however, most resolve on their own. There are 12 types of low-risk HPV (LR-HPV) and at least 13 types of high-risk HPV (HR-HPV).[6] LR-HPV cause warts and very mild cell changes in infected tracts, whereas persistent infection with HR-HPV cause low- (LSIL) and high- grade squamous intraepithelial lesions (HSIL) that can progress to cancer. Anogenital cancers, including 99.7% of cervical, 95% of anal, 65% of vaginal, 50% of vulvar, and 35% of penile cancers are linked to HR-HPV infections.[7] HPV-associated cancers are diagnosed in 17,600 women and 9,300 men every year in the US.[7,8]

Anogenital herpetic ulcerations are caused by HSV-1 and -2. Although HSV-2 causes most anogenital ulcerations, recent studies have demonstrated that HSV-1 can also be

transmitted to anogenital tracts and cause ulcerations.[3,4] HSV-2 prevalence is 16.2% in the general population,[3,4] and is much higher among PLWH, varying between 50-90% in different studies[9]. HSV infection may be persistent and induce considerable inflammatory responses. Similar to HIV, this infection continues to disproportionally burden blacks with a prevalence of 39.2% of the total HSV-2 diagnoses, with black women accounting for 48.0% of the total.[3,4]

In the state of Alabama, racial disparities in new HIV infections and STDs have been documented; black women and men are highly susceptible to incident HIV.[10] While the epidemiology of HIV infection in the state is well-studied and reported, HPV- and HSV-related clinical conditions (CC) among PLWH have not been comprehensively characterized. Neither the country nor the state of Alabama implements mandatory screening programs for anogenital HSV- and HPV-related (excluding cervical cancer) conditions. Therefore, there is a substantial lack of knowledge of the comorbidities among PLWH. In this study, we retrospectively studied PLWH at risk of HPV- and HSV-related CC for over 12 years from the patients in the University of Alabama at Birmingham (UAB) 1917 Clinic, an academic institute with the largest HIV patient catchment in Alabama, and estimated HPV- and HSV- CC incidence rates and comorbidity trends.

METHODS

Study Design and Population

Electronic health records between January 1st, 2006 and March 30th, 2018 from the UAB 1917 Clinic were reviewed. It is the largest HIV clinic in the state of Alabama[11] with

extensive referral network. The prospective clinic cohort has collected more than 7,000 patients' sociodemographic, psychosocial, and clinical information since its establishment in 1992.[12,13] More than 3,500 patients currently receive their routine HIV care from the clinic, representing 30% of all PLWH in the state[12].This retrospective study was nested within the UAB 1917 Clinic Cohort and approved by the UAB Institutional Review Board.

In this study, eligible patients were patients who: 1) attended the clinic at least twice for receiving primary HIV care during the 12-year study period; and 2) were at least 18 years old at HIV diagnosis.

Study Variables

HPV-related CC were categorized into nine groups: anal LSIL and HSIL; cervical LSIL and HSIL; anal, cervical, vaginal/vulvar, and penile cancers; and anogenital warts. HSV-related CC were defined as anogenital herpetic ulcers. Cases were verified by reviewing medical charts. Washout periods were given as two years for HPV-related CC and one year for HSV-related CC. Thus, we excluded patients with same HPV- and HSV- related CC (two years and one year, respectively) prior to the case presentation during the study period. A condition was recognized as an incident case only if the subject was free of the condition at baseline but developed the condition during follow-up.

Statistical Analysis

Univariate analyses were conducted to compare demographic and clinical characteristics between patients with HPV- and/or HSV-related CC to patients with neither

condition during the entire follow-up period. Chi-squared- and t-tests were used to compare categorical and continuous variables between the diseased and disease-free group, respectively.

Incidence rates (cases per 10,000 person-years) were computed for each HPV- and HSV-related CC separately and compared between different sexes and races. Annual incidence for each condition was estimated followed by trend analyses using the Joinpoint Trend Analysis Software program (JTAS)[14]. Briefly, the Joinpoint regression model started with the minimum number of joinpoints and kept adding more until the number of joins was sufficient to distinguish between two unique and consecutive linear trends[14]. Monte Carlo permutation and Bayesian Information Criterion (BIC) were used for the goodness-of-fit test to find the best fitted curves over time[15]. The permutation method identified a time point that revealed an apparent change in trend. The final selected model comprehended the minimum number of joinpoints and smallest value of BIC.

The annual percent rate change (APC), with an assumption of a constant percentage change of the rate of the previous year was computed by the joinpoint regression. Incidence was log-transformed to diminish the effects of potential outliers in the linear regression. All APCs were then summarized to estimate an average annual percent change (AAPC) over a fixed interval. The AAPC over any fixed interval was calculated using a weighted average of the slope coefficients of the underlying joinpoint regression line with the weights equal to the temporal length of each segment over the interval. The weighted average of slope coefficients was transformed to an annual percent change in the final step.[16] T-statistics were calculated for both APC and AAPC to assess the changes of slopes in the linear association.

Age-standardized incidence rates were initially estimated. The present study population was projected to the standard population of the 2016 US population from the Surveillance, Epidemiology, and End Results program (SEER). However, JTAS prohibits the calculation of age-adjusted rates with a dependent variable equal to zero cases under log transformation. Instead, crude incidence with a Poisson variance was used. Our main objective was to test whether there were any substantial changes on trends regarding incident HPV-and HSV-related CC during the study period.

RESULTS

A total of 4,803 PLWH attended the 1917 Clinic between January 1st 2006 and March 30th, 2017. However, 4,484 patients met inclusion/exclusion criteria with 3,333 (76.8%) men, 985 (22.7%) women, and 23 (0.5%) transgender individuals, 2,580 (59.4%) blacks, 1,588 (36.6%) whites, and 173 (4.0%) others. Among the patients, 1,038 (23.1%) presented with HPV-related CC and 425 (0.0022%) with HSV-related CC, 163 presented with both clinical conditions, and 3,098 PLWH had neither condition (Table 1). The mean ages at the time of HPV-and HSV-related CC presentation over the study follow-up were 41.8 (±10.6) years and 42.9 (±10.4) years, respectively. The median follow-up (years) since the enrollment was significantly shorter for non-cases (4.3 years) than HPV- (7.0 years) and/or HSV (8 years) and 9.4 years for both conditions ($P<0.05$). The mean log10 VL (copies/mL) were higher in all of the condition groups as compared to the non-case group (HPV CC:5.3±5.9 copies/mL; HSV CC:5.5±6.0 copies/mL; and both conditions: 5.6±6.2 vs. 5.0±0.4 copies/mL for neither) whereas the median nadir CD4 counts (cells/uL) were higher in the non-cases (318, IQR: 151-853) than

in any of the HPV CC (237 [IQR: 72-701]), HSV CC (268 [IQR:54-695]) or both conditions (130 [IQR:16-641]) (P< 0.05). Compared with women, men had much higher rates of HPV-related warts, anal LSIL, HSIL, and any HPV-related cancer (P<0.0001 for each comparison, Table 2). Overall, whites were more likely to be diagnosed with anal LSIL and cancer (P<0.05, Table 2). Among women, whites were more likely to present cervical HSIL and cervical, vaginal and vulvar LSIL (P<0.0001 for each comparison). In contrast with HPV-related CC, the rate of herpetic ulcers was much higher in women than men (P<0.0001).

HIV+ men had a higher rate than women to present anogenital warts (IR 190.4 vs 68.5 per 10,000 person-years), anal LSIL (188.2 vs 14.7 per 10,000 person-years), HSIL (43.2 vs 5.5 per 10,000 person-years), and cancer (25.8 vs 0 per 10,000 person-years) (Table 2). White men presented more frequently with anal LSIL and anal and penile cancers than black men (P<0.03 for each comparison) (Table 2). By contrast, IR of HSV-related anogenital ulcers was much higher in women than men (IR=216.8 vs 130.2 per 10,000 person years; P<0.0001) (Table 2). No racial disparity was observed in incident ulcers.

HPV-related anogenital warts showed significant upward trends in both genders (AAPC: 19.5, P<0.0001) and races (AAPC: 20.4, P<0.0001) (Figure 1). However, there were no distinct patterns between these two trends (P>0.05 for test for parallelism, Table 3). The AAPC of anal HSIL among black men also showed an increasing incidence trend (AAPC: 25.6, P<0.0001). Cervical HSIL and cancer did not show significant changes over time, but APC gave the joins that reflected periodic changes between 2006-2013 and 2016-2018 (P<0.05 for each period) (Table 3, Figure 2). Similarly, although anogenital herpetic ulcers failed to demonstrate statistically significant changes over the follow-up (P>0.05 for both gender and race

comparisons), there was a noticeable decrease between 2013 and 2016 for both races, followed by an increase after 2016 (APC=192, P<0.0001).

DISCUSSION

In the present study, among PLWH visiting a southeastern US HIV clinic between 2006 and 2018, HPV-related CC, particularly anal lesions and cancer were much more frequently diagnosed among men than women. By contrast, the rate of HSV-related ulcers was greatly elevated among HIV+ women compared to men. The incidence rate of anogenital warts constantly increased over the study period. Although cervical HSIL and herpetic ulcers did not have monotonic trends, significant periodic increases in trends were detected. Although crude rates were reported, we computed the age-adjusted rates for warts and herpetic ulcers, which did not generate 0 cases over the follow-up. The alternative rates were similar to our results (data not shown). These observations and trends have never been reported in a clinical setting and this study with sufficient follow-up provides the broader scenario of these conditions among PLWH in the area. Our findings attempt to help clinicians better understand the burden of these comorbidities and drive better care in clinical settings.

HPV-related conditions were observed predominantly in men as compared to women. Although the sub-population for HPV analyses only had 59.2% MSM, 64.4% of incident HPV cases were diagnosed among them. HIV+ men had almost a 3-fold greater risk of anogenital warts compared with women (Table 2), with no racial disparity observed. The trend of warts, however, increased approximately 20% each year (Table 3) regardless of gender and race.

HIV+ men were also 8 and 25 times more likely to be diagnosed with anal HSIL and cancers, respectively, than HIV+ women (Table 2). By contrast, HPV-related anal lesions and cancers are more common in women than men in the general population.[17] There is a huge gap in screening guidelines for non-AIDS defining comorbidities, such as HPV-related anal precancerous lesions and cancers.. The current HPV screening guidelines, the cervical cancer screening program, are only applicable to women.[18] The present study consisted of 76% men with limited anal cancer screenings. MSM were particularly susceptible for HPV-related CC. MSM are known to have an elevated risk of HIV acquisition. HIV+ MSM tend to be more likely infected with other STIs, such as HPV and HSV.[19]

One of the largest HIV cohort, Multicenter AIDS Cohort Study (MACS), reported an overall incidence rate of anal cancer of 7 per 10,000 person-years among HIV+ MSM between 1984 and 2006[20]. The finding from our study was over 3-fold greater than that rate (IR=25.8 per 10,000 person-years among men) between 2006 and early 2018. In spite of the better immune status of PLWH in our cohort compared to MACS, specifically before ART regimen in 1996[24], the median nadir CD4 counts were still significantly lower in patients with HPV-related CC than the non-cases (Table 1). Further, the rates of anal lesions and cancers increased exceptionally compared to the MACS. Geographically, the MACS, which predominantly includes white men, did not include a site in the Deep South of the US, and our findings provide evidence of the severity of HPV comorbidities in this high-risk population. Overall, there have not been many studies conducted among black MSM in the south regarding HIV and HPV comorbidities.

Although, we did not observe a monotonic trend of cervical HSIL or cancer, we were able to identify the periodic changes. For example, both conditions seemed to be growing

in numbers of new diagnoses between 2016 and 2018 (Table 3) in both races. However, we have to take the screening programs implemented into account. In March 2016, the US Health Resources and Services Administration issued new screening guidelines for cervical cancer among HIV+ women, which included both cytology pap smears and serologic testing.[18] As an academic clinic, the UAB 1917 Clinic actively advocates HPV-related screenings for HIV+ women. The implementation of the new screening program could temporarily boost the number of new diagnoses of HPV-related cervical lesions and cancers. However, it does not necessarily mean an increase in cervical HPV infections.

In contrast to HPV-related CC, our data suggest that more women with HIV experienced symptomatic anogenital herpetic ulcers than men. Unlike HPV-CC in the present study, the HSV-cohort consisted of 59.8% MSM and 51.8% of them presented incident herpetic ulcers during the follow-up. To our knowledge, only one study has reported that HIV+ men are more likely to develop HSV-related anogenital ulcers.[21] Most published studies implicate that anogenital herpetic ulcerations are more likely to be clinically manifested in HIV+ women.[22–24] Unlike HPV, HSV is not an oncogenic virus and does not generally lead to any fatal disease sequelae. Thus, preliminary studies have usually focused on its serologic impacts on PLWH. In our study, women showed a 1.8x higher incidence rate of anogenital ulcers than men. Similarly, a study in Uganda estimated the prevalence of anogenital ulcers was 1.4 times higher in women, and HIV viremia was found to be higher among people with symptomatic anogenital ulcers (mean log10 VL=4.4 copies/mL).[25] A similar association between higher viral load and symptomatic herpetic ulcers was observed in our cohort (mean log10 VL=5.5 copies/mL, $P<0.05$ compared with people with neither conditions). This could imply that PLWH with active HIV viral replication were more likely to have outbreaks of symptomatic anogenital ulcers. By

contrast, most previous studies only reported the risk of the patients who presented symptomatic herpetic ulcers before they became HIV infected.[22–24]

This southeastern US region bears a heavy public health burden of HIV and STDs.[26] The incidence rates of HPV- and HSV-related CC in our study were much higher among PLWH than the general population, based on national statistics. Both infections are incurable, but interventions alleviate symptoms and prevent the HPV-related neoplasia and chronic herpetic ulcers. We had a long clinical follow up in this clinical cohort, which allowed us to estimate incidence rates, while most other studies were only able to report incident HPV- and HSV-related CC as percentages of new cases among PLWH. We specifically used the Joinpoint regression analysis to examine trends, which enabled us to report the statistical significance of changes in trends as well as compare trends between different sexes and races.

It is important to note that clinical diagnoses were based on patient willingness to seek medical attention. Unlike cervical cancer screening, anogenital screenings and examinations are primarily recommended by providers and thus could reflect potential bias. While this can under-estimate the number of cases, with a pro-active screening approach in this academic clinic setting, our estimated incidence shows a substantially higher rate than the estimates from the previous HIV studies. HPV- and HSV- related CC are often initiated from self-reported pain and physical presentations of lesions, warts, and ulcers. It is common practice to make prompt diagnoses and immediate treatment for most HPV and HSV-related CC without testing for viral infections.

Conclusions

Impaired immune systems as shown by nadir CD4 count and high viral load exacerbate the HSV and HPV disease outcomes. It is known that HPV-related anogenital HSIL and HSV-related anogenital ulcers are more difficult to regress in PLWH than immunocompetent people[27]. In addition, PLWH face more complicated treatment options when the co-infections become symptomatic and develop chronic sequel. Since most of these infection related conditions except for cervical cancer and persistent herpetic ulcers are non-AIDS-defining, the Ryan White funding program provides limited coverage in the clinic. Additional comprehensive studies in other clinics in other areas of the country would be helpful to understand the hidden epidemic of clinical conditions caused by HPV and HSV infection among PLWH, which could help guide screening and prevention strategies.

List of abbreviations

HIV: human immunodeficiency virus; PLWH: people living with HIV infection; CC: clinical condition; HSV: herpes simplex virus; HPV: human papillomavirus

Declarations

Ethics approval and consent to participate

The study was approved by the UAB Institutional Review Board for Human Use (IRB-170329001), and performed in accordance with the ethical guidelines of the Declaration of Helsinki. All informed consent were signed by the patients. Animals were not used in the study.

Competing Interest

The authors declare no competing interests.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available upon request in https://www.uab.edu/medicine/1917cliniccohort/.

Funding

The ability to complete this work was supported by the UAB Center for AIDS Research (CFAR) [grant P30-AI27767 to MSS from the National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; the CFAR Network of Integrated Clinical Systems (CNICS) [grant 1R24 AI067039-1 to MSS from the National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; and the Mary Fisher CARE Fund (https://www.uab.edu/medicine/cfar/mary-fisher). This work was also supported by the Quetelet Endowed Professorship Research Fund (SS).

Author Contributions

YY, GB, and SS conceived the study. HW participated in statistical approach. YY processed and analyzed the data. YY and SS interpreted the data and wrote the manuscript. All authors have reviewed and approved the manuscript.

Acknowledgement

We thank all study patients from the UAB 1917 Clinic. We also thank the UAB Research and Informatics Service Center (RISC) for data access (https://www.uab.edu/medicine/1917cliniccohort/).

References

1. CDC. STD Fact- genital herpes. 2019. Available: https://www.cdc.gov/std/herpes/stdfact-herpes.htm.

2. CDC. STD Facts - Human papillomavirus (HPV). 2019. Available: https://www.cdc.gov/std/hpv/stdfact-hpv.htm.

3. CDC. Sexually Transmitted Diseases (STDs). 2018. Available: https://www.cdc.gov/std/stats16/other.htm#foot-5.

4. CDC. Prevalence of Herpes Simplex Virus Type 1 and Type 2 in Persons Aged 14-49: United States, 2015-2016. 2018. Available: https://www.cdc.gov/std/herpes/herpes-nhanes-2010.htm

5. Patel P E al. Prevalence and risk factors associated with herpes simplex virus-2 infection in a contemporary cohort of HIV-infected persons in the United States. Sex Transm Dis. 2012;39(2):154-60.

6. HPV information. High- and Low-Risk HPV Types. Available: http://www.hpvinformation.com/about-hpv/high-and-low-risk-hpv-types.

7. HPV and Cancer. National Cancer Institute. 2019. Available: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer.

8. CDC. Get HPV Vaccine for Your Child. 2019. Available: http://www.cdc.gov/features/preventhpv.

9. Strick LB, Wald A, Celum C. Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. Clin Infect Dis. 2006;43: 347–356.

10. Alabama Department of Health. Demogrpahics of HIV Infections among Individuals residing in Alabama at Diagnosis. 2018. Available: http://www.alabamapublichealth.gov/hiv/assets/hiv_aidsreport_1st_quarter_2017.pdf.

11. Koplon by S. The past and present of HIV: three decades of care at UAB. UAB News. Available: https://www.uab.edu/news/health/item/9074-the-past-and-present-of-hiv-three-decades-of-care-at-uab.

12. Guzman A. UAB. 1917 Clinic Cohort. UAB School of Medicine. Available: https://www.uab.edu/medicine/1917cliniccohort.

13. HIV Medicine Association. Ryan White Program. Available: https://www.hivma.org/uploadedFiles/HIVMA/Policy_and_Advocacy/Ryan_White_Medical_Providers_Coalition/Part_C_Funding_Profiles/Alabama_edit.pdf.

14. Joinpoint Regression Program. Joinpoint Trend Analysis Software. NIH Surveillance Research Program. Available: https://surveillance.cancer.gov/joinpoint.

15. Joinpoint Help System. Selecting the Final Model. Available: https://surveillance.cancer.gov/help/joinpoint/tech-help/frequently-asked-questions/selecting-the-final-model.

16. Joinpoint Help System. Average Annual Percent Change (AAPC) and Confidence Interval. Available: https://surveillance.cancer.gov/help/joinpoint/setting-parameters/method-and-parameters-tab/apc-aapc-tau-confidence-intervals/average-annual-percent-change-aapc.

17. Palefsky JM. Human Papillomavirus-Related Disease in Men: Not Just a Women's Issue. Journal of Adolescent Health. 2010. pp. S12–S19.

18. HRSA. HIV/AIDS Bureau Performance Measures. Available: https://hab.hrsa.gov/sites/default/files/hab/About/clinical-quality-management/adolescentadultmeasures.pdf.

19. Schim van der Loeff MF, Mooij SH, Richel O, de Vries HJC, Prins JM. HPV and anal cancer in HIV-infected individuals: a review. Curr HIV/AIDS Rep. 2014;11: 250–262.

20. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48: 491–499.

21. Phipps W, Nakku-Joloba E, Krantz EM, Selke S, Huang M-L, Kambugu F, et al. Genital Herpes Simplex Virus Type 2 Shedding Among Adults With and Without HIV Infection in Uganda. J Infect Dis. 2016;213: 439–447.

22. Celum C, Levine R, Weaver M, Wald A. Genital herpes and human immunodeficiency virus: double trouble. Bull World Health Organ. 2004;82: 447–453.

23. Harvard AIDS Initiatives. Herpes & HIV: Not What You Think. 2010. Available: https://aids.harvard.edu/herpes-hiv/.

24. HIV In site. Herpes Simplex Virus and HIV-1. 2006. Available: http://hivinsite.ucsf.edu/InSite?page=kb-05-03-02.

25. Gray R, Li X, Wawer M, et al. Determinants of HIV-1 load in subjects with early and later HIV infections, in a general-population cohort of Rakai, Uganda. 2004;189(7)1209-15.

26. CDC. HIV in the Southern United States.2019. Available: https://www.cdc.gov/hiv/pdf/policies/cdc-hiv-in-the-south-issue-brief.pdf.

27. Palefsky J, Holly E, Ralston M, Jay N, Berry J, Darragh T. High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. AIDS. 1998;12: 495–503.

Table 1. Demographic and clinical characteristics of study patients stratified by HPV- and HSV-related clinical condition (CC)[a] status

Characteristics	Ever with HPV CC (N=1038)	Ever with HSV CC (N=425)	Both (N=163)	Neither (Non-cases) (N=3098)
Age at baseline	38.3 (10.0)[c]	39.2 (9.8)[c]	37.9 (8.5)[c]	41.4 (11.5)
Age at HIV diagnosis	31.6 (8.8)[c]	32.0 (9.0)[c]	30.1 (7.6)[c]	35.5 (10.8)
Age at first HPV or HSV CC	41.8 (10.6)	42.9(10.4)	--	--
Race				
Black	596 (57.4)[c]	262 (61.7)[c]	98 (60.1)[c]	1861 (60.1)
White	430 (41.4)[c]	153 (36.0)[c]	64 (39.3)[c]	1084 (35.0)
Others	12 (1.2)[c]	10 (2.3)[c]	1 (0.6)[c]	153 (4.9)
Median years of follow-up	7.0 (4.3-12.1)[c]	8.0 (4.4-12.1)[c]	9.4 (5.5-12.1)[c]	4.3 (1.8-11.9)
Gender				
Male	779 (75.0)	273 (64.2)[c]	104 (63.8)[c]	2423 (78.2)
Female	249 (24.0)	151 (35.5)[c]	59 (36.2)[c]	662 (21.4)
Transgender	10 (1.0)	1 (0.3)[c]	0[c]	13 (0.4)
HIV risk factors				
MSM	646 (63.2)[c]	207 (49.0)[c]	91 (55.8)	1505 (50.2)
Heterosexual	299 (29.2)[c]	187 (44.2)[c]	59 (36.2)	1214 (40.5)
IVDU	77 (7.5)[c]	29 (0.68)[c]	13 (8.0)	275 (9.2)
Others	1 (0.1)[c]	0 (0.0)[c]	0 (0)	5 (0.1)
Mean Log VL(copies/mL)‡	5.3 (5.9)[c]	5.5 (6.0)[c]	5.6 (6.2)[c]	5.2 (5.8)
NadirCD4 (cells/µL)‡+	237 (72-701)[c]	268 (54-695)[c]	130 (16-641)[c]	318 (151-853)

[a]: Clinical conditions: clinically and/or symptomatic presented condition
***: All continuous variables in mean (SD); categorical variables in count (%).**
[+]: Median (25-75 percentiles)
[c]: P<0.05 when comparing the variable to the one in the "Neither" column
[‡]: Prior to HPV or HSV CC or last clinical visit for controls

Table 2. Incidence rates (IR) of HPV- and HSV-related conditions stratified by gender (if applicable) and race

	# Cases	# Total	Follow-up time (person-years)	IR (95%CI)	*P*-Value‡
HPV-related Clinical Conditions (by gender)					
Anogenital warts	478	4,484	29,646.2	161.2 (146.8-175.7)	--
Men	420	3,429	22,286.2	188.5 (170.4-206.5)	
Women	51	1,038	7,507.9	67.9 (49.3-86.6)	<0.0001
Anal LSIL	425	4,484	21,854.0	194.5 (176.0-213.0)	--
Men	411	3,429	21,842.3	188.2 (170.0-206.4)	
Women	8	1,031	5,436.6	14.7 (12.1-24.9)	<0.0001
Anal HSIL	75	4,484	22,195.0	33.8 (26.1-41.4)	--
Men	72	3,429	16,665.5	43.2 (33.2-53.2)	
Women	3	1,031	5,434.8	5.5 (0-11.8)	<0.0005
Anal cancer	43	4,484	22,188.2	19.4 (13.6-25.2)	--
Men	43	3,429	16,680.7	25.8 (18.1-33.5)	
Women	0	1,031	5,416.0	0	<0.0001
Bowen's disease	6	4,484	22,232.1	2.7 (0.55-4.9)	--
Men	5	3,429	16,701.2	3.0 (0.37-5.7)	
Women	1	1,031	5,436.3	1.8 (0-5.6)	0.66
HSV-related Ulceration (by gender)					
Anogenital ulcers	425	4,341	28,063.9	151,4 (137.0-165.8)	--
Men	273	3,333	20,975.7	130.2 (114.7-145.6)	
Women	151	985	6,965.9	216.8 (182.2-251.3)	<0.0001
HPV-related Clinical Conditions (by race)					
Anogenital warts	478	4,484	29,646.2	161.2 (146.8-175.7)	--
Black	312	2,676	18,360.7	169.9 (151.1-188.8)	
White	160	1,632	10,382.2	154.1 (130.2-178.0)	0.32
Anal LSIL	425	4,484	21,854.0	194.5 (176.0-213.0)	--
Black	182	2,676	12,892.1	141.2 (120.7-161.7)	
White	238	1,632	8,447.4	281.7 (207.8-317.5)	<0.0001
Anal HSIL	75	4,484	22,195.0	33.8 (26.1-41.4)	--
Black	40	2,676	13,053.3	30.6 (21.1-40.1)	
White	34	1,632	8,628.5	39.4 (26.5-53.3)	0.28
Anal cancer	43	4,484	22,188.2	19.4 (13.6-25.2)	--
Black	18	2,716	13,030.0	13.8 (7.4-20.2)	
White	24	1,632	8,643.7	27.8 (16.7-38.9)	0.025
Cervical LSIL	171	1,031	7352.3	232.6 (197.7-267.4)	--
Black	132	767	5679.6	232.4 (192.8-272.0)	
White	38	236	1610.2	250.3 (102.1-290.9)	0.69
Cervical HSIL	80	1,031	7404.7	108.0 (84.5-131.7)	--
Black	54	767	5721.3	94.4 (69.2-119.6)	
White	25	236	1529.5	163.5 (99.4-169.6)	0.023
Cervical cancer	12	1031	5304.8	22.62 (9.8-35.4)	--
Black	6	767	4018.1	14.9 (3.0-26.9)	
White	6	235	1203.4	49.9 (3.0-38.9)	0.037
Vaginal/Vulvar cancer	15	1,031	5,304.3	28.3 (14.0-42.6)	--
Black	11	767	4,018.1	27.4 (11.2-43.6)	
White	4	236	1,202.9	33.3 (0.67-65.8)	0.74
Penile cancer	4	3,429	16,493.2	2.3 (0.049-4.8)	
Black	3	886	8,769.5	3.4 (0-7.3)	
White	0	1,396	7,298.4	0	<0.0001
Bowen's disease	6	4,484	22,232.1	2.7 (0.55-4.9)	--
Black	3	2,676	13,070.1	2.3 (0-4,9)	

White	3	1,632	8,647.5	3.5 (0-7.4)	0.61
HSV-related Ulcerations					
Black	262	2,580	17,367.7	150.9 (132.6-169.1)	
White	153	1,588	9,813.1	155.9 (131.2-180.6)	0.75

‡P: comparison between races and genders

Table 3. Race and gender stratified trends of incident HPV-related anogenital warts, LSIL, HSIL, and cancer (men only), Cervical LSIL and HSIL, and cancer, and HSV-related anogenital ulcers between 2006-2018.

	Cases	Joins	Joinpoint Year	AAPC (95% CI)	*P*-Value
HPV-related CC					
Anogenital warts	--		--		
Men	420	0	--	19.5 (13.9-25.3)	<0.0001
Women	51	0	--	19.5 (13.9-25.3)	<0.0001
Black	312	0	--	20.4 (15.5-25.5)	<0.0001
White	160	0	--	20.4 (15.5-25.5)	<0.0001
Anal HSIL (men only)	71	0	--		
Black	38	0	--	25.6 (9.7-43.9)	0.0040
White	33	0	--	23.1 (-0.2-52.0)	0.052
Anal cancer (men only)	43	0	--	5.6 (-4.9, 17.2)	0.29
Black	18	0	--	--	--
White	24	0	--	---	--
Cervical HSIL		2	2013,2016	22.5 (-4.5-57.1)	0.11
Black	54	2	2013,2016		
White	25	2	2013,2016		
		--	2006-2013*	29.8 (11.6-50.9)	0.002
		--	2013-2016*	-38.3 (-73.9-45.7)	0.25
		--	2016-2018*	179.6 (20.9-546.7)	0.020
Cervical cancer	12	2	2012, 2016	15.9 (-9.7-48.7)	0.2
Black	6	2	2012, 2016	--	--
White	6	2	2012, 2016	--	--
		--	2006-2012*	17.1 (-2.4-40.6)	0.084
		--	2012-2016*	-26.4 (-58.7-31.3	0.27
		--	2016-2018*	177.5 (1.3-660.0)	<0.0001
HSV-related CC					
Anogenital ulcers					
Men	329	2	2013, 2018	8.5 (-9.5-30.2)	0.38
		--	2006-2013*	3.2 (-5.8-13.1)	0.42
		--	2013-2016*	-44.2 (-73.9-19.2)	0.11
		--	2016-2018*	250.1 (57.3-679.4)	0.010
Women	176	0	--	9.5 (-1.5-21.8)	0.089
Black	322	2	2013, 2016	9.6 (-10.8-34.7)	0.38
White	174	2	2013, 2016	9.6 (-10.8-34.7)	0.38
		--	2006-2013*	6.0 (-3.9-16.9)	0.23
		--	2013-2016*	-38.4 (-68.8-21.4)	0.15
		--	2016-2018*	192.9 (30.4-557.8)	0.013

*: when joinpoints were identified, APC were also reported for the periodic changes.

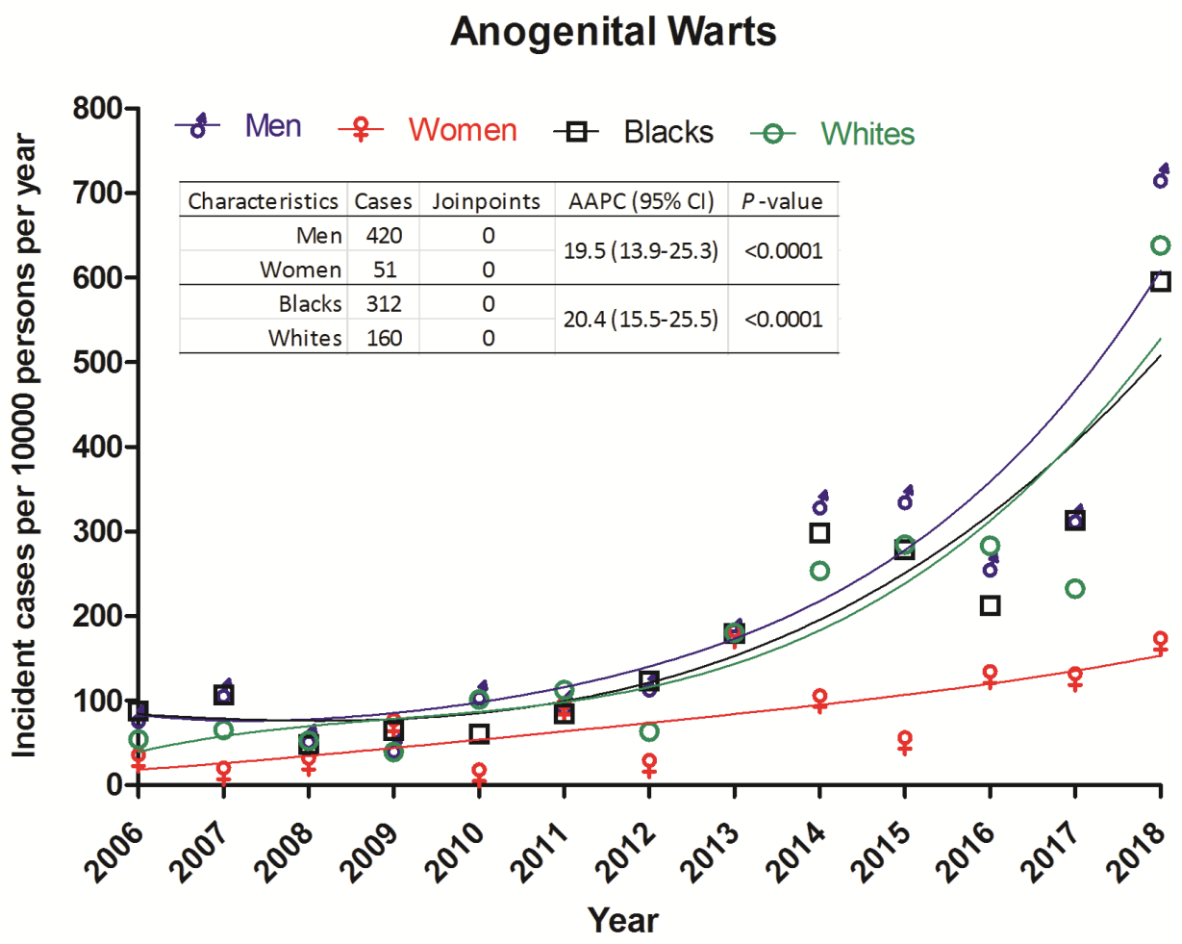
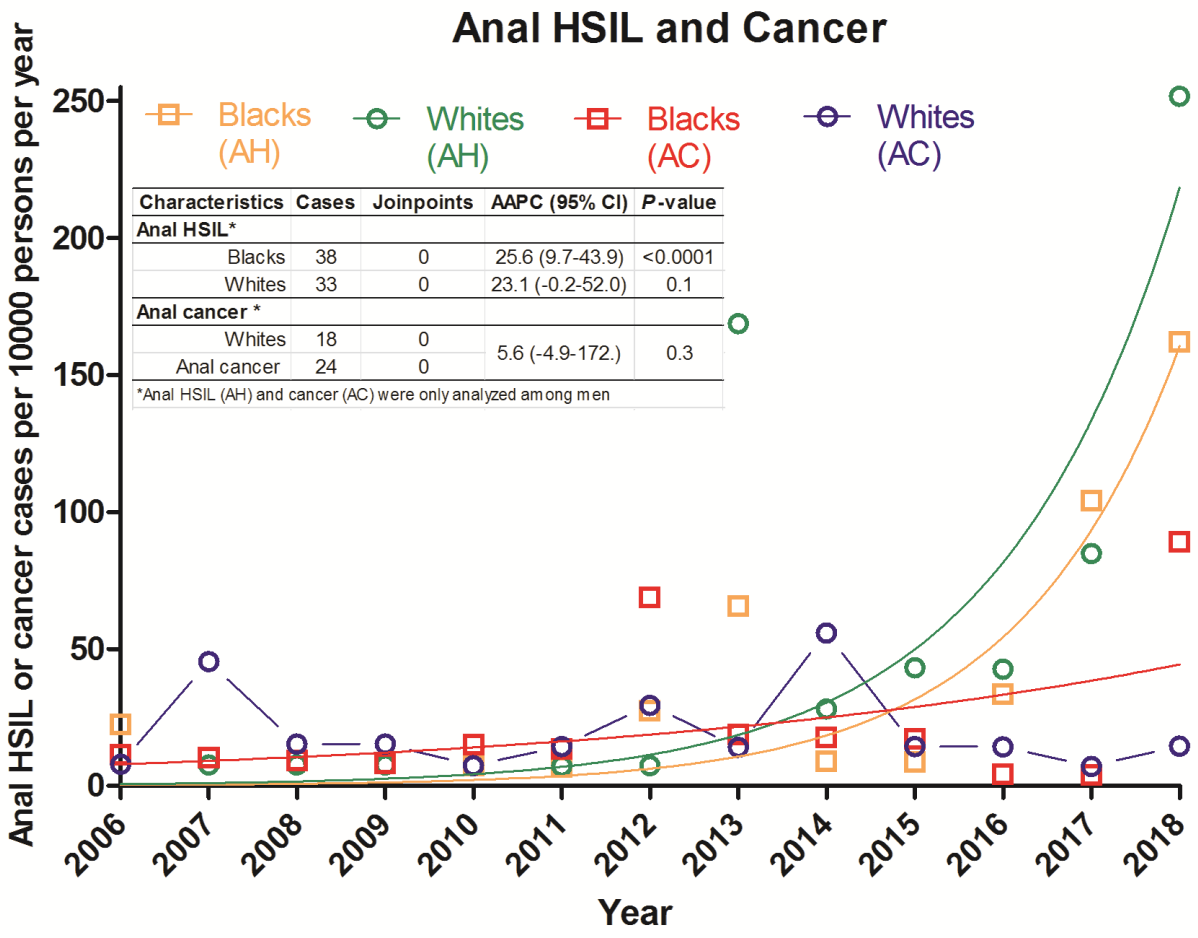


Figure 1. Trend of incident HPV-related anogenital warts stratified by genders and races between January 2006 and March 2018.



Characteristics	Cases	Joinpoints	AAPC (95% CI)	*P*-value
Anal HSIL*				
Blacks	38	0	25.6 (9.7-43.9)	<0.0001
Whites	33	0	23.1 (-0.2-52.0)	0.1
Anal cancer *				
Whites	18	0	5.6 (-4.9-172.)	0.3
Anal cancer	24	0		

*Anal HSIL (AH) and cancer (AC) were only analyzed among men

Figure 2. Trend of HPV-related incident anal HSIL and cancer stratified by genders and races between January 2006 and March 2018.

NADIR CD4, MEDIAN CD4, AND TRAJECTORY CD4 COUNTS AS ONSET INDICATORS OF INCIDENT HPV- AND HSV-RELATED ANOGENITAL CONDITIONS IN PEOPLE LIVING WITH HIV

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Submitted to *Open Forum Infectious Diseases*

Format adapted for dissertation

Abstract

Background

HPV- and HSV-associated clinical conditions are common in HIV individuals. Nadir and median CD4 counts are not fully informative for assessing the risks of these comorbidities. We compared the associations between median, nadir, and trajectory CD4 counts and anogenital ulcers, warts, and anal HSIL/cancer.

Methods

Retrospective sociobehavioral and clinical data from electronic health records of 4803 HIV patients from 2006-2018 were included. Six CD4 trajectory groups were constructed using the group-based trajectory modeling from all patients>18 years with ≥3 clinical visits. Univariate and multivariable logistic models were used to assess the associations with different conditions. Nadir and median CD4 counts were tested separately.

Results

A total of 331 ulcers, 408 warts, 102 anal HSIL/cancer were observed. Median CD4 (<200 cell/ul) was associated with ulcers (OR=2.1), warts (OR=2.2), and anal HSIL/cancer (OR=2.7) (each P<0.001). Low nadir CD4 (<200 cell/ul) was associated with warts (OR=1.8) and anal HSIL/cancer (OR=2.4) (each P≤0.001). Significant associations with different patterns (declining and sustained low CD4 counts) of CD4 trajectories were observed with all conditions.

Conclusion

Warts might be interpreted by one-time point immune dysfunction, however, different risks of ulcers and anal HSIL/cancer were dependent on CD4 trajectory over time although the median CD4 was similar.

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) - related morbidity and mortality have dramatically declined since the emergence of combination antiretroviral therapy (ART). People living with human immunodeficiency virus (HIV) infection-1 (PLWH) can expect nearly normal life expectancy, if early diagnoses and ART adherence are achieved.[1,2] With longer life expectancy, a growing number of non-AIDS defining conditions have been observed due to[3]. For example, incidence of anogenital clinical conditions resulting from human papillomavirus (HPV) and herpes simplex virus (HSV) infections are fairly common and regress less among PLWH[3].

HPV, a known etiologic factor in several cancers, is one of the most common sexually transmitted diseases (STDs) in the US.[4] More than 79 million Americans are currently infected with HPV, with an estimate of 14 million new diagnoses every year.[4] The prevalence of genital HPV infection is 1.5 to 2.5 times higher in women living with HIV, and anal HPV infection is 1.5 to 2 times more prevalent in all women and men who have sex with men (MSM) living with HIV compared to their HIV-negative counterparts.[5] HPV infection may lead to precancerous high- grade squamous intraepithelial lesions (HSIL) and squamous cell carcinomas (SCC) in the infected anogenital tract. Likewise, two strains of HSV can cause herpetic ulcers in anogenital tracts. PLWH co-infected with HSV experience more frequent episodes of mucosal shedding of HSV-2 and more severe ulceration than immunocompetent people.[6] Their herpetic ulcerations are also prone to be more persistent and thus result in chronic conditions. Consequently, anogenital ulcers have also been shown to contribute to faster HIV-1 disease progression and increased risk of HIV-1 transmission.[7]

Plasma CD4 T-cell count (CD4) is a key immune indicator in PLWH. It reflects the host's defensibility against pathogens, infections and illnesses and the extent of immune recovery.[8] Recovery and maintenance of an adequate CD4 count facilitate the necessary immune responses to control co-infections and reduce the risk of comorbidities in the course of HIV-1 infection.[8] Although CD4 is an important indicator to assess the prognosis of HIV progression among PLWH, its predictive ability of the onset of incident HPV- and HSV- related clinical conditions among PLWH is unclear. CD4 counts are often characterized as nadir and median CD4 counts (measured at a point during a defined period after HIV infection) in the existing literature.[9–11] By contrast, CD4 count trajectory requires longitudinal data, it has not been widely adopted, and no studies have compared the 3 forms of CD4 counts as predictors of incident HPV- and HSV-related clinical conditions over a long follow-up period. We, therefore, sought to model incident HPV- and HSV-related clinical conditions as a function of median and nadir CD4 counts and CD4 count trajectory over a 12-year follow-up among HIV patients from an academic clinic in Alabama.

METHODS

Study population and source of data

A retrospective study nested within an ongoing clinical cohort in the 1917 HIV Clinic at the University of Alabama at Birmingham was conducted using electronic health records (EHR) between January 1st 2006 and March 30th 2018. The UAB HIV Clinic is the largest HIV clinic in the state of Alabama with extensive regional catchment and referral network.[12] The prospective clinic cohort has collected more than 7,000 patients' sociodemographic,

psychosocial, comorbidities, medications, vital signs, laboratory results, and corresponding dates since the database establishment in 1992.[12,13] More than 3,500 patients currently receive their routine HIV care from the clinic, representing 30% of all PLWH in the state.[12] Data were obtained by query of the cohort's electronic database using MS SQL Server prior to analyses.

This present study was approved by the UAB Institutional Review Board.

Eligibility criteria

Patients who 1) attended the clinic at least 3 times during the study period, 2) were at least 18 years old at HIV diagnoses, 3) had 3 or more CD4 tests measured longitudinally were eligible for analyses. Patients who had the same HPV- or HSV-related clinical conditions at the 1 and 2 year time points, respectively before their study enrollments were excluded to avoid over-counting comorbidities. Thus, we were confident that eligible study participants were condition free at the baseline and that incident conditions were identified during the follow-up period.

Statistical Analysis

Outcome measures

The primary outcomes of interest included incident clinical conditions related to HPV- (anogenital warts, anal precancerous lesions (HSIL) and cancers) and HSV- (anogenital ulcers) infections, defined as the presence of the corresponding diagnoses on a patient's clinical chart dated within the defined period.

Univariate analyses

Univariate analyses were conducted to compare sociodemographic (gender, race, and sexual orientation) and clinical (mean age at HIV diagnosis, mean age at study entry, nadir and median CD4 T cell counts, and Log_{10} mean viral load between each defined HPV-related condition or herpetic ulcers to the condition-free groups (non-cases).Nadir and median CD4 counts were categorized as <200, 200-499, and >500 cells/uL. The active study follow-up was determined as the period between the study entry date until the onset of HPV-related and HSV-related clinical condition diagnoses for cases and last clinical visit for non-cases, respectively. Continuous variables were compared by t-tests, and categorical variables were compared by Chi-squared tests, with *P*-value<0.05 as the significance threshold.

Multivariable analyses

Multivariable regression models were used to compare the association of different forms of CD4 count and onset of HPV- and HSV- related conditions. All statistically significant factors from the univariate analyses were included in the multivariable models. Different CD4 measures were tested for multicollinearity. The variance inflation factors (VIF) demonstrated that at least 2 of the 3 CD4 measures were highly correlated in each group of the condition (VIF=2.4 and above). Both sets of models tested nadir CD4 and median CD4 counts and CD4 count trajectory separately and were adjusted for race, age at HIV diagnoses and study entry, and mean log10 VL. Furthermore, self-reported sexual orientation was highly correlated with gender (Pearson ρ=0.93). Therefore, we fit two separate tests of multivariable models: in addition to other covariates found significant in the univariate analyses, model 1 (M1) only included gender and model 2 (M2) only included sexual orientation. Survival analyses were first implemented using Cox proportional hazard regression. However, the Cox proportional hazards assumption could not be justified as the three CD4 counts metrics were heavily time dependent variables that did

not produce constant hazard ratios over time. Thus, logistic regression was used as an alternative method.

Group-based trajectory modeling (GBTM)

The group-based trajectory modeling (GBTM) approach identifies a finite number of groups of individuals exhibiting similar trajectories over a defined time of a single outcome.[14] GBTM has two key components: identifying the polynomial order (shape) of the trajectory and determining a potential trajectory group for each subject in the data.[14] Briefly, the selection process started with a model consisting of one group with the highest polynomial order allowed in the method (quartic degree) and added one group at a time until the best fitting model was found, as evidenced by the Bayesian information criterion (BIC). Subsequently, the polynomial order was reduced one at a time while a t-test was used to assess the fitness of each group to the polynomial order. Eventually, the lowest order with all significant groupings was determined and used as a new variable for multivariable regression.

The present study was derived from a clinical cohort, which had open enrollment instead of a unified study enrollment period. The number of CD4 tests among patients eligible for GBMT in our cohort varied between 3 and 50 in a maximum of 12-year follow-up (2006-early 2018). If all CD4 tests were used for trajectories, discrepancies among the counts of CD4 tests would bring in massive noise towards the later period of the follow-up, because not all participants had stayed in the study for a full 12-year period. Therefore, the median count of CD4 count was used for any model using CD4 count trajectories. To reduce the impact of random fluctuations, laboratory imprecision, and incomplete model accuracy,[15–17] CD4 series were square root-transformed in all subsequent analyses. The starting point of each individual's follow-up was

computed in subtraction of the subject's enrollment date. Thus, each subject would start from year 0.

In addition to the statistical reasoning employed in GBTM, clinical context was considered in grouping CD4 count trajectory in our analyses. An immunocompetent person without HIV-infection has a CD4 range between 500-1000 cells/μl.[18] Thus, PLWH with CD4 counts above 500 cells/μl are generally considered having a good immune recovery. Their overall morbidity and mortality rates may be comparable with uninfected individuals. Consistent with prior literature,[19,20] in the current analysis, all trajectory CD4 counts at and above 500 cells/μl were grouped into one trajectory.

RESULTS

A total of 4803 PLWH attended the 1917 Clinic between January 1, 2006 and March 30, 2018 (Table 1). During the follow-up, 331 incident cases of herpetic ulcers and 408 incident cases of anogenital warts were diagnosed. Moreover, 102 incident cases of anal HSIL and cancer were observed (Table 1).

Subjects who developed incident ulcers had lower median and nadir CD4 counts (<200 cells/μL) compared to those with CD4 counts of 200-499 cells/ μL and ≥500 cells/ μL (P<0.0001) (Table 1). Similar findings were also observed in anogenital warts (P<0.0001), anal HSIL and cancer (0.0001<P<0.05). Log_{10} viral loads were higher among individuals with any of the conditions than the comparable condition-free patients, with exception of anal HSIL and cancer (P=0.71). MSM were at the highest risk for being diagnosed with anal warts, HSIL and cancer. By contrast, more herpetic ulcers were observed in heterosexuals.

Models 1 (M1, adjusted for gender) and 2 (M2, adjusted for sexual orientation) for herpetic ulcers included 3947 and 3809 subjects, respectively. Nadir CD4 count was not associated with the onset of new herpetic ulcers in both models (M1: P=0.44, M2: 0.2) whereas median (M1: P=0.0002, M2: P<0.0001) and trajectory CD4 (M1: P=0.0033, M2: P=0.0008) counts were statistically associated with incident ulcers. Participants with median CD4 counts less than 200 (cells/μL) were more likely to present with anogenital ulcers than the subjects with ≥500 (cells/μL) (Table 2). Among the 6 trajectory CD4 count series (Figure 1a), individuals in group 1 with the lowest CD4 counts over the follow-up were most likely to have ulcers compared to group 6 (the highest CD4 trajectory) (Table 2). Individuals in group 4 with a substantial decline in CD4 counts were also at elevated odds of incident ulcers compared with group 6 (Table 2).

There were 4020 and 3877 patients included in M1 and M2 for warts-related analyses (Table 2). Unlike ulcers, all 3 CD4 measures were associated with incident warts in both models (Table 2). Patients with less than 200 (cells/μL) median CD4 count tended to be at higher risks of incident warts. Except for CD4 trajectory group 4, all the other trajectory groups appeared to have statistically higher odds of incident warts than group 6 with the optimal CD4 trajectory (Table 2).

Among 4035 and 3893 subjects in M1 and M2, respectively for anal HSIL/cancer analyses, all tested CD4 measures were associated with incident anal HSIL and cancers (Table 2). In both models, patients with nadir and median CD4 counts <200 (cells/μL) had much higher odds of incident anal HSIL/cancer than the ones with corresponding CD4 counts >500 (cells/μL). Similarly, the lowest trajectory CD4 groups were more likely to present with these conditions than the ones in group 6 with best CD4 recovery (Table 2).

DISCUSSION

The present study employed 3 CD4 count measures to evaluate prospective associations with incident anogenital herpetic ulcers, warts, anal HSIL, and cancer. Median CD4 count and CD4 count trajectory demonstrated strong associations with all defined HSV-and HPV-related conditions. However, nadir CD4, which is generally considered an important prognostic indicator in PLWH, was not associated with herpetic ulcers, and the effects were not consistent across CD4 groups.

Although most studies use nadir CD4 or median CD4 counts as key immune indicators for PLWH, the reliability of using one single CD4 test result to assess overall immunity is questionable under chronic HIV infection. Our study, instead, used both single time points of CD4 count as well as trajectory CD4 series over time as predictors of incident new HPV- and HSV-related conditions. Overall, nadir CD4 group was not in favor in comparison with the other 2 CD4 measures because of the lack of consistency (Table 2). By contrast, median CD4 and trajectory CD4 counts indicated statistically significant associations with incident HPV- and HSV-related conditions. Patients with < 200 median CD4 counts were at higher odds of HPV- and HSV- conditions due to the heavily suppressed immune systems. CD4 trajectory, in addition, gave more precise odds of these clinical conditions in each trajectory group (Table 2).

Nadir CD4 count is important for disease prognosis in PLWH. Low nadir CD4 is usually linked with high risks of developing AIDS-defining conditions.[21] In the present study, we did not observe the associations between nadir CD4 count and the onset of herpetic ulcers. Although several other studies found associations between low nadir CD4 counts and high risks of HSV-2

infections among HIV-infected MSM and men and women,[22,23] no clinically presented ulcers were found directly linked with low nadir CD4 counts in the current study.

Median CD4 count and CD4 count trajectory were both associated with all incident HPV- and HSV-related conditions. However, the interpretations of these two measures could be very different. For example, two subjects with opposing trajectories could possess the same median CD4 count. Therefore, using a single (e.g. median) CD4 count could result in misclassification and bias. For example, in the anogenital ulcers cohort, the median CD4 counts of subjects in trajectory groups 2 and 4 were 138 and 145 cells/μL, respectively. Therefore, both groups' median CD4 counts belonged to CD4 group <200 cells/μL. However, groups 2 and 4 had opposing trajectories: a steady increase in CD4 count in group 2, and a decline in group 4. This resulted in about a 76% increase in the odds of groups 4 than 2 in both M1 and M2 (Table 2; Figure 1a). Although the two group median CD4 counts were less than 200 (cells/μL), only trajectory CD4 counts were capable of differentiating the odds. Similar discrepancies were detected in anal HSIL and cancer as well. For instance, trajectory groups 3 and 4 had median CD4 counts in 200-500 cells/μL, however, their trajectories projected in opposite directions (Figure 1c). Group 4's odds elevated at least 158% compared with group 3's in both models sable (Table 2).

It did not seem that CD4 trajectory was more precise in linking immune recovery with incident anogenital warts compared with median CD4 (Table 2). For instance, trajectory group 4 (the declining group) failed to indicate higher odds of warts than the immune recovery group (group 6). Trajectory CD4 groups could not fully interpret the risk of warts with respect to immune status in the current study. In an early study of WHIS cohort, no evidence was observed in the association of genital warts and CD4 counts over time.[24] Thus, it suggested that using

longitudinal CD4 counts indicate that evaluating the risk of incident warts with this measure might not be ideal in clinical practice and could be potentially manifested from a single time-point immune event rather than over time.

CD4 counts may be acutely influenced by inactivity, lack of sleep, stress, smoking, or other medical treatments,[25–28] so using a single CD4 test to quantify the risk of the onset of HPV- and HSV-related conditions is insufficient. Rather than weigh significance to an individual test result, it makes good sense to monitor any trends in CD4 cell count over time.[8] Prognostic implications of trajectory CD4 count appeared to be more accurate than any one single CD4 count.

As with all clinical cohort studies, there are several limitations to this study. For example, using clinical data from the EHR could result in underestimating the number of HPV- and HSV-related cases, because seeking medical attention is solely based on patient willingness. In addition, entry of a diagnosis into the EHR by a provider is required and quality of medical record-keeping may vary between providers as well as for conditions (i.e. a provider may be more likely to enter a condition for which they have to take action such as a referral for anoscopy or prescription of a medication; in addition they may be more likely to enter cancer on a diagnosis list as opposed to HSIL. However, the current findings also reflect the actual HPV- and HSV-related health burden that PLWH in the southeastern US face. The southeastern US is the epicenter of the domestic HIV epidemic.[29] Thus, a better understanding of CD4 count measures in this population is valuable for the development of HSV- and HPV-related screening programs. Another limitation stems from the open nature of the clinical cohort: not all patients had the same length of follow-up and times of routine HIV care, such as CD4 and VL checks. In addition, some patients received more CD4 tests because they were participating in trials or were

suspected to have ART resistance. However, the GBTM method has been shown to be consistent with varying time-points with over 3 observations. Further, the numbers of cervical and vaginal HSIL/cancer were very small and not sufficient for statistical analyses in the current study.

Non-AIDS-related malignancies represent a large number of comorbidities and deaths among PLWH nowadays. While combination ART has decreased AIDS-related mortality among PLWH, the incidence of anal cancer is rising. HPV infection is common among PLWH and increases risk for anal cancer. In addition there is a high burden of HSV-2 infection among PLWH and they are at higher risk for outbreaks and have more prolonged symptoms.[6] CD4 count is a determinant for assessing the immune recovery. Overall, unsatisfactory immune recovery marks the high risks of HPV- and HSV-related anogenital conditions. Continued monitoring of CD4 count is critical. More studies are required to assess the CD4 trends over the entire disease course.

ACKNOWLEDGEMENTS

We thank all study participants from the UAB 1917 Clinic. We also thank the UAB Research and Informatics Service Center (RISC) for data access (https://www.uab.edu/medicine/1917cliniccohort/).

Funding

The study did not receive specific funding. However, the ability to complete this work was supported by the UAB Center for AIDS Research (CFAR) [grant P30-AI27767 to MSS from the National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; the CFAR Network of Integrated Clinical Systems (CNICS) [grant 1R24 AI067039-1 to MSS from the

National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; and the Mary Fisher CARE Fund (https://www.uab.edu/medicine/cfar/mary-fisher).

Ethics Statement

The study was approved by the UAB Institutional Review Board for Human Use, and performed in accordance with the ethical guidelines of the Declaration of Helsinki. Animals were not used in the study.

Author Contributions

YY, GB, and SS conceived the study. IA and HW participated in statistical approach. YY processed and analyzed the data. YY, SA, and SS interpreted the data and wrote the manuscript. All authors have reviewed and approved the manuscript.

Conflicts of Interest

Dr. Burkholder has received research support from Bristol-Myers Squibb, Definicare, LLC, and Amgen Inc, and has consulted for Medscape.

References

1. HIV Government. U.S. Statistics. 2018. Available: https://www.hiv.gov/hiv-basics/overview/data-and-trends/statistics.

2. CDC. HIV Care Saves Lives infographic. 2018. Available: https://www.cdc.gov/vitalsigns/hiv-aids-medical-care/infographic.html.

3. Stier EA, Baranoski AS. Human papillomavirus-related diseases in HIV-infected individuals. Current Opinion in Oncology. 2008;20(5):541–6.

4. CDC. HPV Vaccine for Human Papillomavirus Fact Sheet for Parents. 2018. Available: https://www.cdc.gov/vaccines/parents/diseases/teen/hpv.html.

5. Phanuphak N. HPV and HIV coinfection. Int J Infect Dis. 2012;16(S1):e62.

6. Strick LB, Wald A, Celum C. Management of Herpes Simplex Virus Type 2 Infection in HIV Type 1–Infected Persons. Clin Infect Dis. 2006;43: 347–356.

7. Gray RH, Li X, Wawer MJ, Serwadda D, Sewankambo NK, Wabwire-Mangen F, et al. Determinants of HIV-1 Load in Subjects with Early and Later HIV Infections, in a General-Population Cohort of Rakai, Uganda. J Infect Dis. 2004;189: 1209–1215.

8. AIDS Map. Factsheet CD4 cell counts. Available: http://www.aidsmap.com/CD4-cell-counts/page/1044596.

9. Lange CG, Lederman MM, Medvik K, Asaad R, Wild M, Kalayjian R, et al. Nadir CD4 T-cell count and numbers of CD28 CD4 T-cells predict functional responses to immunizations in chronic HIV-1 infection. AIDS. 2003;17(4). 2015–23.

10. Medscpe. When Interrupting Therapy, Mind the CD4 Nadir. 2005. Available: http://www.medscape.com/viewarticle/503955.

11. Mark M. CD4 Count at ART Start Rising Across Globe, But Median Still Below 350 / 25% start at below 200 CD4. 9th IAS Conference on HIV Science. 2017. Available: http://www.natap.org/2017/IAS/IAS_09.htm.

12. Koplon S. The past and present of HIV: three decades of care at UAB. UAB News. Available: https://uab.edu/news/health/item/9074-the-past-and-present-of-hiv-three-decades-of-care-at-uab

13. Guzman A. UAB 1917 Clinic Cohort. UAB School of Medicine. Available: https://www.uab.edu/medicine/1917cliniccohort/

14. Nagin DS E al. Group-based multi-trajectory modeling. Stat Methods Med Res. 2018;27(7):2015-23.

15. Bulkmans NWJ, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJP, Bulk S, 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.

16. Lange N, Carlin BP, Gelfand AE. Hierarchical Bayes Models for the Progression of HIV Infection Using Longitudinal CD4 T-Cell Numbers: Rejoinder. Journal of the American Statistical Association. 1992. p.631.

17. Mcneil AJ, Gore SM. Statistical analysis of zidovudine (AZT) effect on CD4 cell counts in HIV disease. Stat Med. 1996;15:75–92.

18. US Department of Veterans Affairs. CD4 count (or T-cell test). Available: https://www.hiv.va.gov/patient/diagnosis/labs-CD4-count.asp.

19. Lok JJ, Bosch RJ, Benson CA, Collier AC, Robbins GK, Shafer RW, et al. Long-term increase in CD4+ T-cell counts during combination antiretroviral therapy for HIV-1 infection. AIDS. 2010;24: 1867–1876.

20. Obirikorang C, Quaye L, Acheampong I. Total lymphocyte count as a surrogate marker for CD4 count in resource-limited settings. BMC Infect Dis. 2012;12: 128.

21. Miller E al V. Relations among CD4 lymphocyte count nadir, antiretroviral therapy, and HIV-1 disease progression: results from the EuroSIDA study. Ann Intern Med. 199;130(7):570-7.

22. Hidalgo-Tenorio C, Gil-Anguita C, Ramírez-Taboada J, Esquivias J, López-Ruz MA, Balgahata OM, et al. Risk factors for infection by oncogenic human papillomaviruses in HIV-positive MSM patients in the ART era (2010–2016). Medicine. 2017;96.

23. Patel P, Bush T, Mayer KH, Desai S, Henry K, Overton ET, et al. Prevalence and Risk Factors Associated With Herpes Simplex Virus-2 Infection in a Contemporary Cohort of HIV-Infected Persons in the United States. Sex Transm Dis. 2012;39: 154.

24. Luu HN, Amirian ES, Chan W, Beasley RP, Piller LB, Scheurer ME. CD4 Cell Count and HIV Load as Predictors of Size of Anal Warts Over Time in HIV-Infected Women. J Infect Dis. 2012;205(4):578-85.

25. Dang AK, Nguyen LH, Nguyen AQ, Tran BX, Tran TT, Latkin CA, et al. Physical activity among HIV-positive patients receiving antiretroviral therapy in Hanoi and Nam Dinh, Vietnam: a cross-sectional study. BMJ Open. 2018;8(5).

26. Dianatinasab M, Fararouei M, Padehban V, Dianatinasab A, Alimohamadi Y, Beheshti S, et al. The effect of a 12-week combinational exercise program on CD4 count and mental health among HIV infected women: A randomized control trial. J Exerc Sci Fit. 2018;16: 21.

27. Bopp M, Phillips D, Fulk J, Dudgeon D, Sowell R, Hand A. al. Physical activity and immunity in HIV-infected individuals. AIDS Care. 2005;16(3):387-93.

28. Madu AJ, Ocheni S, Ibegbulam OG, Aguwa EN, Madu KA. Pattern of CD4 T-lymphocyte Values in Cancer Patients on Cytotoxic Therapy. Ann Med Health Sci Res. 2013;3: 498.

29. CDC. HIV in the Southern United States. 2016. Available: https://www.cdc.gov/hiv/pdf/policies/cdc-hiv-in-the-south-issue-brief.pdf.

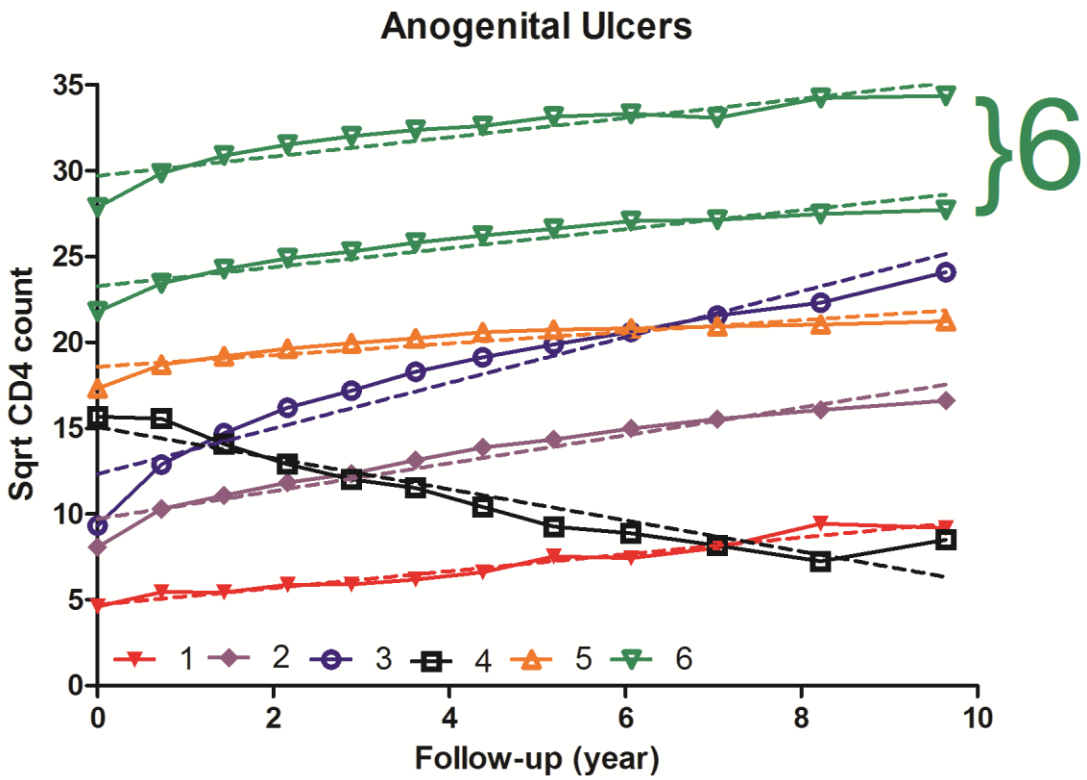
Table 1. Sociobehavioral and clinical data of the study population (2006-2018)						
Characteristics	No ulcers (N=3617)	HSV ulcers (N=331)	No warts (N=3612)	Warts (N=408)	No anal HSIL cancer (N=3934)	Anal HSIL/ cancer (N=102)
Gender	*	*	*	*	*	*
Men	2820 (93.1)	209 (6.9)	2669 (88.1)	359 (11.9)	2974 (96.8)	99 (3.2)
Women	776 (86.5)	121 (13.5)	897 (95.5)	42 (4.5)	937 (99.7)	3 (0.3)
Transgender individuals (M-F)	21 (95.5)	1 (4.5)	16 (69.6)	7 (30.4)	23 (100)	0 (0.0)
Race			‡	‡	‡	‡
Black	2167 (91.5)	200 (8.5)	2174 (97.2)	257 (2.8)	2397 (98.0)	50 (2.0)
White	1312 (91.3)	125 (8.7)	1294 (89.9)	146 (10.1)	1390 (96.5)	51 (3.5)
Others	138 (95.8)	6 (4.2)	144 (96.6)	5 (3.4)	147 (99.3)	1 (0.7)
HIV diagnosis age	34.8 (10.5)*	31.9 (8.7)*	35.1 (10.5)*	30.4 (8.0)*	34.7 (10.4)‡	32.4 (9.3)‡
Enrollment age	40.8 (11.3)‡	39.2 (9.6)‡	41.2 (11.2)*	36.8 (9.7)*	40.7 (11.2)	40.7 (10.8)
Median CD4(cells/μL)	*	*	*	*	‡	‡
<200	480 (86.3)	76 (16.7)	509 (84.7)	92 (15.3)	576 (96.2)	23 (3.8)
200-499	1339 (91.6)	123 (8.4)	1326 (88.9)	166 (11.1)	1458 (97.0)	45 (3.0)
≥500	1798 (93.2)	132 (6.8)	1777 (92.2)	150 (7.8)	1900 (98.2)	34 (1.8)
Nadir CD4 (cells/μL)	*	*	*	*	‡	‡
<200	430 (88.7)	55 (11.3)	1401 (87.4)	202 (12.6)	1554 (96.6)	54 (3.4)
200-499	1032 (92.0)	90 (8.0)	1528 (90.9)	153 (9.1)	1657 (97.9)	35 (2.1)
≥500	2155 (92.1)	186 (7.9)	683 (92.8)	53 (7.2)	723 (98.2)	13 (1.8)
Mean $\log_{10}$ viral load	3.3 (1.3)‡	3.6 (1.3)‡	3.3 (1.3)‡	3.6 (1.3)‡	3.3 (1.3)	3.3 (1.4)
Sexual orientation	N=3492	N=325	N=3489 *	N=396 *	N=3802*	N=99*
MSM	2032 (92.3)	169 (7.7)	1906 (86.6)	294 (13.4)	2123 (96.0)	89 (4.0)
Heterosexual	1452 (90.3)	156 (9.7)	1576 (94.0)	101 (6.0)	1671 (99.4)	10 (0.6)
Others	8 (100)	0 (0)	7 (87.5)	1 (12.5)	8 (100)	0 (0.0)

*: $P<0.0001$
‡: $0.0001<P<0.05$

Table 2. Associations between nadir, median and trajectory CD4 counts of the defined conditions between 2006-early 2018

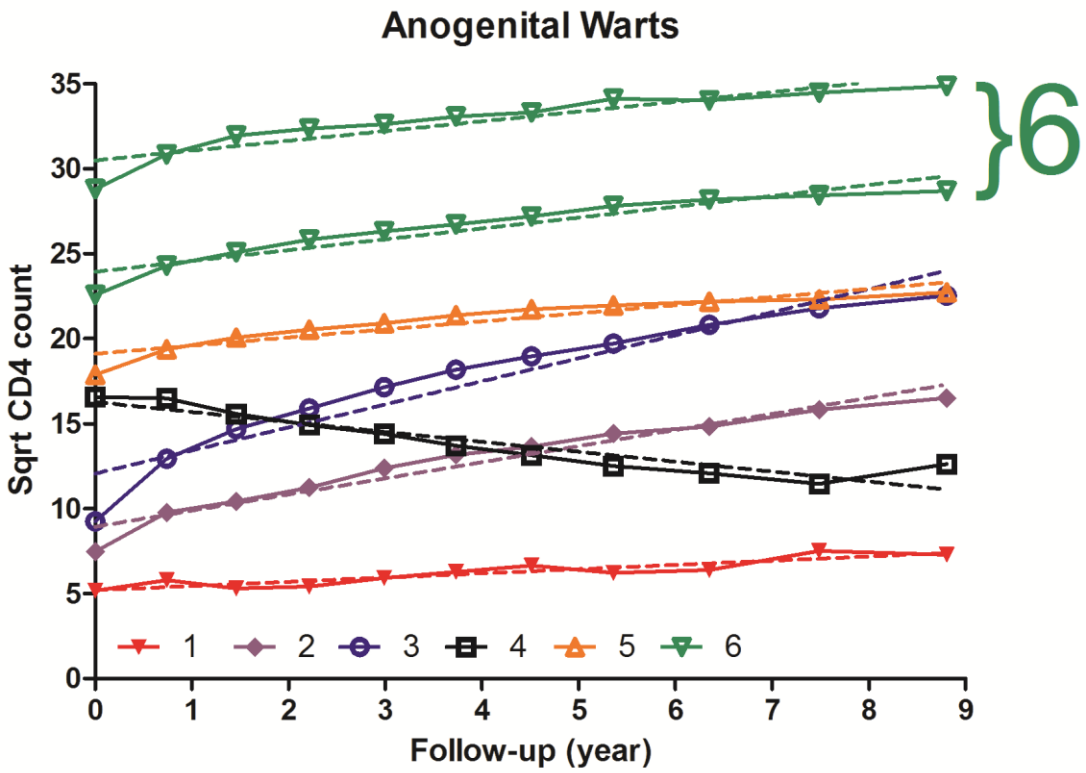
	Herpetic ulcers		Anogenital warts		Anal HSIL and cancers	
Model 1	(N=3947)		(N=4020)		(N=4035)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Nadir CD4 group	--	0.44	--	0.0002	--	0.0037
<200	1.2 (0.8-1.7)	0.34	1.8 (1.3-2.6)	0.0009	2.4 (1.2-4.7)	0.012
200-499	1.0 (0.8-1.3)	0.98	1.2 (0.9-1.7)	0.28	1.3 (0.7-2.5)	0.44
≥500	Reference					
Median CD4 group	--	0.0002	--	<0.0001	--	0.0035
<200	2.1 (1.5-3.0)	<0.0001	2.2 (1.6-3.0)	<0.0001	2.7 (1.5-5.0)	0.0014
200-499	1.3 (1.0-1.7)	0.052	1.5 (1.2-1.9)	0.0011	1.9 (1.2-3.0)	0.0098
≥500	Reference					
*CD4 trajectory group	--	0.0033	--	<0.0001	--	0.0023
Group 1	2.6 (1.5-4.4)	0.0004	2.4 (1.4-4.0)	0.0016	5.0 (2.0-12.9)	0.0008
Group 2	1.3 (0.8-2.0)	0.24	2.9 (2.0-4.3)	<0.0001	2.8 (1.3-5.8)	0.0061
Group 3	1.4 (0.9-2.0)	0.13	1.8 (1.2-2.5)	0.0016	1.9 (0.9-3.8)	0.081
Group 4	2.5 (1.4-4.3)	0.0012	1.3 (0.8-2.3)	0.33	4.9 (2.3-10.5)	<0.0001
Group 5	1.2 (0.9-1.6)	0.27	1.4 (1.0-1.8)	0.029	1.8 (1.0-3.0)	0.035
Group 6	Reference					
Model 2	(N=3809)		(N=3877)		(N=3893)	
Nadir CD4 group	--	0.20	--	0.0002	--	0.0038
<200	1.3 (0.9-1.9)	0.16	1.8 (1.3-2.7)	0.0011	2.4 (1.2-4.7)	0.013
200-499	1.0 (0.8-1.4)	0.74	1.2 (0.8-1.7)	0.36	1.3 (0.7-2.5)	0.47
≥500	Reference					
Median CD4 group	--	<0.0001	--	<0.0001	--	0.0013
<200	2.3 (1.6-3.2)	<0.0001	2.2 (1.6-3.1)	<0.0001	2.6 (1.4-5.0)	0.0025
200-499	1.3 (1.0-1.7)	0.041	1.5 (1.1-1.9)	0.0027	1.8 (1.1-2.9)	0.016
≥500	Reference					
*CD4 trajectory group	--	0.0008	--	<0.0001	--	0.0002
Group 1	2.9 (1.7-5.0)	<0.0001	2.7 (1.5-4.6)	0.0004	6.7 (2.6-17.6)	0.0001
Group 2	1.4 (0.9-2.2)	0.15	3.1 (2.1-4.5)	<0.0001	2.7 (1.3-5.7)	0.011
Group 3	1.5 (1.0-2.2)	0.066	1.8 (1.2-2.5)	0.002	1.7 (0.8-3.6)	0.14
Group 4	2.5 (1.4-4.5)	0.0018	1.4 (0.8-2.4)	0.3	5.1 (2.3-11.3)	0.033
Group 5	1.2 (0.9-1.6)	0.27	1.3 (1.0-1.7)	0.056	1.7 (1.0-2.9)	0.042
Group 6	Reference					

Ages at HIV diagnosis and study entry, mean Log_{10}VL, race and gender (M1) or sexual orientation (M2) were adjusted for each model.*: N for groups 1-6: herpetic ulcers=130,350,414,126,888,2049; warts=129,338,466,213,1061,1813; anal HSIL and cancer=133,338,468,213,1064,1819; Cervical, vaginal/vulvar HSIL and cancers: 24,74,86,207,340,207. All CD4 groups are in (cells/μL)



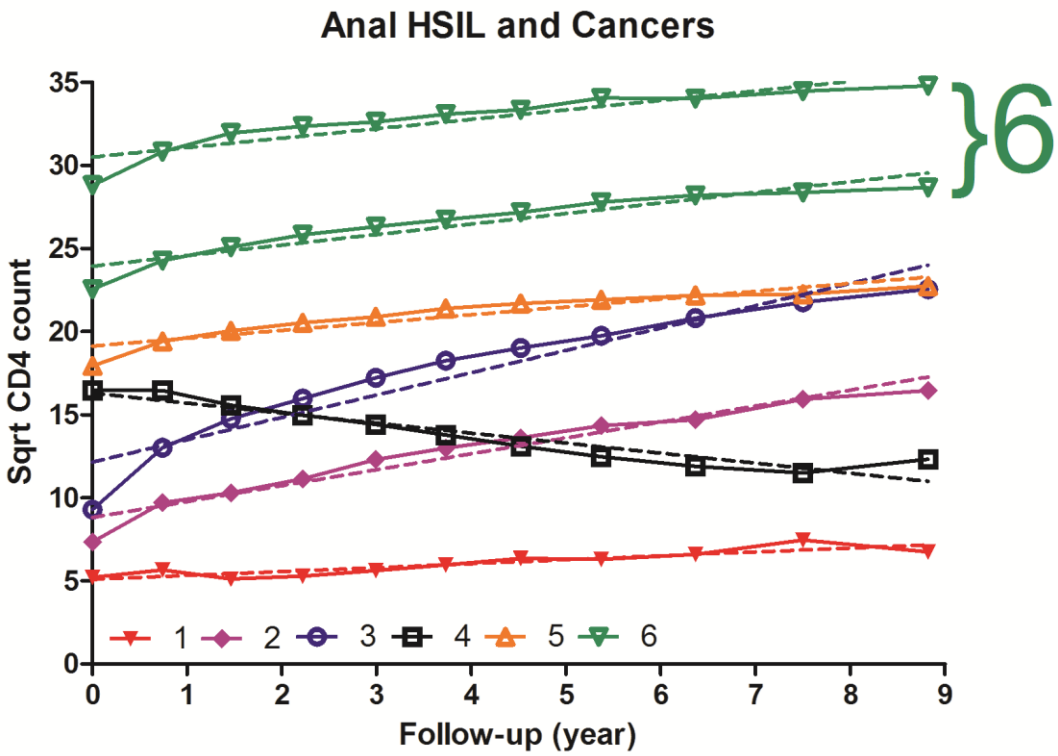
Group	Group membership	Cases in the group	Median CD4 counts
1	3.3	18.8	31
2	8.6	9	138
3	10.5	8.9	246
4	3.2	18.5	145
5	22.5	8.1	380
6	51.9	7.1	680

Figure 1a



Group	Group membership	Grouop morbidity	Median CD4 counts
1	3.2	17.0	30
2	8.4	18.4	121
3	11.6	11.9	237
4	5.3	10.0	225
5	26.4	9.9	410
6	45.1	7.8	712

Figure 1b



Group	Group membership	Cases in the group	Median CD4 counts
1	3.3	6.3	28
2	8.4	11.8	119
3	11.6	11.8	237
4	5.3	29.4	226
5	26.4	10.8	410
6	45.1	29.4	711

Figure 1c

Figure 1. Trajectory CD4 counts for anogenital ulcers (a), warts (b), and anal HSIL/ cancers (c). Percentages of trajectory group membership (%) and morbidity (%), and median CD4 count (cells/μL) of each trajectory group were given accordingly.

BURDEN OF ANAL CANCER AND PRECANCEROUS LESIONS IN PEOPLE LIVING WITH HIV-1 INFECTION: A SOUTHERN US SYNOPSIS

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In preparation for *Clinical Infectious Diseases*

Format adapted for dissertation

Abstract

Background

Infection with high-risk human papillomavirus plays an important role in development of anal cancer. Anal cancer is rare in general populations in both genders each year in US, but more evidence indicated an increase in anal cancer among people living with HIV-1 infection (PLWH). We studied high-grade anal intraepithelial neoplasia (HGAIN) and anal cancer regarding their incidence, sociodemographic and clinical characteristics,

Birmingham were reviewed retrospectively. Demographic, sociobehavioral, and HIV-clinical indicators were tested in univariate analyses between HGAIN and anal cancer to the condition-free group. Sociobehavioral data were also compared between the patients who ever received anal/rectal pap to those who never during the study follow-up. Ages at the onset of anal cancer were compared between the present cohort and the US general population reported by the National Surveillance, Epidemiology, and End Results Program.

Results

A total number of 79 HGAIN (96% men), 43 anal cancer (100% men) were observed along with 4367 non-HGAIN or AC patients (75.9% men). Gender ($P<0.0001$) and sexual orientation (HGAIN: $P<0.0001$; AC: $0.0001<P<0.01$) disparity were significant compared with the overall cohort. Low nadir ($0.0001<P<0.01$) and median ($P<0.0001$) CD4 counts were observed more often in AC than condition-free individuals. Only 44% anal cancer patients never received Paps compared to 29% in HGAIN patients ($P<0.0001$). People between 55-64 years (31.1%) contributed to the most anal cancer in the general population, while 45-54 years (58.1%) appeared at the highest risk in the study. Only 39.5% of total anal cancers were diagnosed under 55 years in the general population, but 79% of anal cancers were diagnosed under 55 years in our cohort. Incidence rates compared between the general population and the PLWH cohort were 1.9 and 258 per 100,000 person-years respectively.

Discussion

AC incidence among HIV-infected men was 161 times higher with an earlier onset than the general men. This study from an academic institute clinic in the Deep South US can be valuable for screening policies and program in the region.

BACKGROUND

Anal cancer is rare in the general US population, with an estimate of 8,300 new diagnoses (5,530 in women and 2,770 in men) in 2019.[1] The risk of being diagnosed with anal cancer is about 1 in 500 persons, with a higher risk in women than men in the general population.[1] However, new diagnoses have been increasing over the years in the high-risk populations, particularly among people living with HIV infection (PLWH). Certain risk factor, such as, high-risk HPV (HR-HPV) infection, co-infection with human immunodeficiency virus (HIV), being men who have sex with men (MSM) all increase the risk of anal cancer.

Most anal cancers are directly linked with persistent HR-HPV infection. HPV infection does not clear as spontaneously among immunocompromised individuals, resulting in a higher chance of becoming symptomatic and possibly leading to neoplasia in the infected tracts. A systematic review by Machalek et al in Lancet Oncology published in 2012 revealed the pooled incident anal cancer among HIV-infected MSM was 77.8 per 100,000 person-years (PY) in the post highly active antiretroviral therapy (HAART) era.[2] By contrast, the incidence of anal cancer was only 1.9 per 100,000 (PY) in the general US population.[3] The same meta-analysis also reported that the incident high grade anal intraepithelial neoplasia (HGAIN), an immediate precursor of squamous cell carcinoma (SCC) in the anal tract[4,5] varied between 8.5-15.4% per year among HIV-infected MSM.[1] However, incidence of anal cancer has been on the rise in recent reports warranting further research on demographic distribution, socio-behavioral risk factors and screening strategies and targets.

In 2017, over 52% of new HIV diagnoses were made in the South with a rate of 16.1 new cases per 100,000 person-years.[6] Since anal cancer is not AIDS-defining malignancy and its related medical costs are not fully covered by the Ryan White program, the health burden of it is not fully understood. It leads the incidence of HIV diagnoses in the US. In this study, we studied the epidemiology and clinical screening (rectal Pap test) of anal cancer and its precancerous lesions (HGAIN) over time in a HIV clinical cohort in southeastern US. Since it is not a standard clinical care routine, information from this study could identify clinical features that could help in formulating anal cancer screening guidelines in high-risk HIV patients.

METHODS

Study setting and population

A retrospective study nested within an ongoing clinical cohort in the 1917 HIV Clinic at the University of Alabama at Birmingham was conducted using electronic health records (EHR) between January 1st 2006 and March 30th 2018. The UAB HIV Clinic is the largest HIV clinic in the state of Alabama with extensive regional catchment and referral network[7]. The prospective clinic cohort has collected more than 7,000 patients' sociodemographic, psychosocial, comorbidities, medications, vital signs, laboratory results, and corresponding dates since the database establishment in 1992.[7,8] More than 3,500 patients currently receive their routine HIV care from the clinic, representing 30% of all PLWH in the state.[7] Data were obtained by query of the cohort's electronic database using MS SQL Server 2008 prior to analyses. The UAB 1917 Clinic

Cohort is a 100% quality controlled, institutional review board–approved prospective clinical cohort study that includes detailed sociodemographic, psychosocial, and clinical information from HIV-infected patients receiving primary HIV and subspecialty care at the clinic.[9] This present study was approved by the UAB Institutional Review Board.

Eligibility criteria

Patients included in the study who 1) attended the clinic between January 2006 and March 2018 at least twice for routine HIV-care during the study period, 2) were at least 18 years old at HIV diagnoses. Epidemiologically, only cases diagnosed during the study period is identified as the incident cases. Therefore, patients who had the same HPV- or HSV-related clinical condition 1 and 2 years respectively prior to the ones diagnosed during the study period were excluded to avoid over-counting incident comorbidities. Thus, we were confident that eligible participants were condition free at the baseline and that only incident conditions were identified during the follow-up period.

Patients included in the study were HIV patients who 1) attended the clinic between January 2006 and March 2018 at least twice for routine HIV-care, and 2) were at least 18 years at HIV diagnoses. All prevalent cases or those with history of anal cancer diagnoses were excluded from the study. Medical charts were reviewed for all cases to confirm the diagnoses.

Statistical analyses

Univariate analyses were performed to compare demographic, behavioral, and clinical characteristics between HGAIN and anal cancer cases to the non-anal dysplasia patients. Race, gender, self-reported sexual risks, ages at HIV diagnoses, study enrollment, receipt of standard anal/rectal pap test, CD4 T- cell count and viral load (VL) were assessed and summarized at the HGAIN or anal cancer presentations, In order to understand the clinical awareness of anal cancer screening, demographic and sexual risk factors were compared between the patients who received at least one standard anal/rectal pap test to those who never had during the 12-year follow up. Chi-squared tests were used to assess the differences of categorical variables and Fisher's exact test was used when the counts were less than 5. T-tests were used for the comparison of continuous variables.

Trends of HGAIN and anal cancer were analyzed every consecutive 4-year since patients' first ever clinical visit to the 1917 Clinic. Every 4 years was grouped as one period. The follow-up end at the last routine clinical visits for patient without diagnoses of HGAIN or anal cancer and the onset date of case diagnoses for patients confirmed with HGAIN or anal cancer. Cumulative risk was estimated as the incident HGAIN or anal cancer divided by the total population without the condition when they entered each period. The trend of cumulative risk was estimated using generalized log-linear regression model for each period. Log-transformed number of people at risk during the tested 4-year period was set as an offset for the model. Since it was not a full 20-year follow-up since the establishment of the 1917 Clinic Cohort (January 1999-March 2018),

we decided to exclude the period that contained the very few patients that have attended the clinic for more than 16 years (followed between 17-20 years).

In order to compare the age distribution of anal cancer in the present cohort to the general US population, the Surveillance, Epidemiology, and End Results Program by the National Cancer Institute (SEER) data was used[1]. Percentages of anal cancer in each standard age group were plotted side by side of the SEER's data.

RESULTS

There were 79 HGAIN and 43 anal cancer cases observed among 4,482 patients during the follow-up. Only 3 of HGAIN were women (4.0%), and none of the anal cancers were detected in women (Table 1). Gender distribution in HGAIN or anal cancer patients was different than the condition free patients ($P<0.0001$ for each). Self-reported MSM contributed most to HGAIN (93.5%) as well as anal cancer (82.9%) (Table 1). Median age at HGAIN diagnoses was 7.6 years younger than anal cancer diagnoses ($0.0001<P<0.05$). Nadir CD4 counts were only found significantly different between cancer and condition-free patients ($0.0001<P<0.05$). No significant differences were observed in receiving anal/rectal pap tests 1 year prior to the diagnoses between patients with HGAIN and cancer patients (Table 1). More HGAIN ($0.0001<P<0.01$) and anal cancer ($P<0.0001$) patients had low median CD4 counts than the condition free group.

Among the entire cohort of 4,482 patients, 1,648 (36.8%) ever received anal/rectal Pap tests and 2,834 (63.2%) did not during the study time. More men received the tests (47.0%) compared to very few women overall (1.5%) ($P<0.0001$). More than 15% Whites (46.8%) received the tests than Blacks (31.3%) ($P<0.0001$). Other races had

the lowest proportion of receiving the tests (27.3%). Based on self-reported sexual risks, only 8.4% heterosexual men received the tests while 62% MSM received ($P<0.0001$). Among HGAIN patients, 29.1% never received the tests, by contrast, 44.2% anal cancer patients never received one during the follow-up ($P<0.0001$) (Table 2). Although only 0.3% more Black MSM received tests compared to White MSM, about 1.7% more of black heterosexual men did not ($P<0.0001$) (Table 2).

According to the medical charts, there were only 2 patients who were diagnosed with HGAIN before they developed anal cancer during the follow-up. Among the 2 cases, 1 of them presented HGAIN within a year prior to the diagnosis of anal cancer.

The trends over 4 defined periods of HGAIN and anal cancer were in opposite directions. Cumulative incidence of HGAIN started to decline after year 12 since patient initial clinical visit dates, whereas, the incidence of anal cancer increased constantly overtime (Figure 1). The cumulative risk of AC increased constantly from 295 to 1267 per 100,000 persons, whereas, the cumulative HGAIN risk decreased from 697 to 507 per 100,000 persons (Figure 1).

Anal cancer was most frequently diagnosed in ages 55-64 (33.1%) in the general US population[1]. Instead, our cohort indicated most patients diagnosed with anal cancer occurred between 45-54 years (58.1%) (Figure 2). Only 26.5% of anal cancers among the general population are attributed to the ages 55-64 and under, however, 79% of anal cancers were found in the same age range among PLWH (Figure 2).

DISCUSSION

Our study revealed details of current social-behavioral and clinical facts of PLWH diagnosed with HGAIN and cancer. The premalignant and malignant lesions were mostly found in MSM, consistent with previous findings. Over 50% of the HGAIN patients had received anal/rectal pap tests a year within the onset of HGAIN, but only about 33% of cancer patients had been tested during the same time window. Key HIV-related prognostic indicators such as nadir CD4 count and median VL were important in terms of the onset of HGAIN and anal cancer diagnoses (Table 1).

Our early findings reported overall incidence rates of HGAIN and anal cancer were 338 and 194 per 100,000 person-years in both genders, respectively, with a rate of 258 anal cancer cases per 100,000 men-years (no women presented anal cancers in the study) (Chapter 2). By contrast, the incidence of anal cancer in the general US population is only 1.9 cases per 100,000 person-years, with 1.6 cases per 100,000 men-years.[1,3] These are alarming 161-fold increase among HIV-infected men in the study, compared with general men and 102 fold compared to both genders in the general population. The Adult and Adolescent Spectrum of HIV Disease Project and HIV Outpatient Study from 1992 to 2003 reported an increase of incidence of anal cancer from 19-78.2 per 100,000 person years.[10] The study suggested that the substantial elevation resulted from the increased chances of exposures to HPV infection because of the expanded life span.[10] Our study follow-up, instead, began in 2006 and ended in early 2018, during which ART has been widely prescribed and universally covered since 2012 in the US. PLWH were able to live a nearly normal life span during the study time because of ART. However, the risk of presenting anal cancer was substantially higher in

the current cohort. In 1996, the life expectancy for a 20-year-old person with HIV was only 39 years. By contrast, in 2011, the life expectancy has risen to about 70 years.[11] Anal cancer usually develops among people in their early 60s, and diagnoses is fairly rare in people younger than 35.[1] Thus, it would be extremely rare to see HIV diagnosed with anal cancer. The short life expectancy back then did not allow HPV-infection to be sufficiently persistent to undergo neoplasia. Although the wide coverage of ART greatly restores the impaired immune systems, it does not seem to reduce the risk of anal dysplasia and neoplasia.

The present findings also demonstrated that patients with nadir CD4 <200 (cells/μL) accounted for 45.6% and 62.8% of HGAIN and anal cancer, respectively (Table 1). Among cancer patients, only 23.3% of them had median CD4 counts between 200-499 (cells/μL) (Table 1) suggesting that a large proportion of anal cancer patients did not reach an optimal immune status, constantly having CD4 ≥500 before neoplasia.[12] The overall morbidity of non-AIDS-defining malignancies (NADM) was reported 10 times higher in patients with <50 cells/μL CD4 counts than those with >500 cells/μL and was closely associated with median CD4 between 200-500 (cells/μL).[13] Neither HGAIN or anal cancer are considered AIDS-related diseases, but we observe similar patterns

Anal cancer is more common in women than men in the general population[3]. By contrast, men accounted for 96.0% of HGAIN and 100% of anal cancer cases in the study, while the study cohort still consisted of 23% of women. Self-reported MSM presented most of HGAIN (93.5%) and anal cancer (32.9%) (Table 1). Our previous findings indicated that HIV-infected men had 7.9 times higher risk of incident HGAIN (432 per 100,000 person-years), compared with HIV-infected women (55 per 100,000

person-years) (Paper 1). More striking differences were noticed when comparing the rates of anal cancer between men and women. All 43 anal cancer cases occurred in men; it resulted in an incidence rate of 258 per 100,000 men-years (Chapter 2). Nevertheless, the general US women and men's rates of anal cancer are 2.2 and 1.6 cases per 100,000 person-years.[3] High-risk sexual behaviors among MSM have increased the risk of developing HGAIN and anal cancer. From a clinical standpoint, better cancer preventive methods should be evaluated and performed. For example, more rigorous anal/rectal pap tests should be recommended to HIV-infected men. Anal/rectal Pap test is considered the most common screening method for anal cancer.[3] The American Cancer Society suggested that annual anal/rectal pap could be recommended for the high-risk population, however, no specific screening schedule has been set.[3,14] The present findings demonstrated that at least half of HGAIN patients received at least 1 anal/rectal Pap test 1 year prior to the onset of HGAIN. However, over 67% of cancer patients did not receive a test within a year before the cancer diagnoses (Table 1). In other words, at least 33% of the cancer patients had missed the opportunities of screenings that could have captured the carcinoma in the precancerous stages. It is noticed that only 29.1% HGAIN patients never received anal/rectal pap tests during the study follow-up, but 44.2% of anal cancer patients missed the tests, resulting in a significant difference ($P<0.0001$) (Table 2). Although the degree of reduction of HGAIN to cancer by anal pap screening is not clear,[3,14] the present findings suggest that there was inadequate anal cancer screening among PLWH. Although there was only 0.3% more in Black MSM received anal/rectal pap tests than White MSM, 1.7% more in Black heterosexual man never received the tests compared with White heterosexual men ($P<0.0001$) (Table 2). Since sexual-

orientation is only based on self-report and we are aware MSM is at substantially higher risk of anal cancer, it is suggested that anal/rectal pap tests should also be proactively provided to heterosexual men, in case incorrect sexual risks are self-reported.

There were an absolutely higher number of HGAIN diagnoses at the 3 earlier four-year defined periods (Figure 1). It means more HGAIN were diagnosed when patients started receiving routine HIV care. However, anal cancer remained low in the first 8 years since they attended the HIV clinic and increased significantly after year 8 suggesting that there were missed opportunity of screening at pre-cancerous stages. Most anal cancers (91%) are directly linked with persistent HR-HPV infection,[3,14] clinically HGAIN is considered an immediate precursor of anal cancer. In the current study, the incidence of HGAIN diagnoses declined over time, while new diagnoses of anal cancer increased. It is likely that a great number of HGAIN cases were not detected. These undetected and untreated HGAIN failed to regress on their own and eventually progressed to cancer. Ideally, patients diagnosed with HGAIN should have been followed up to receive a wide surgical removal of the lesion area.[15] In the current study, we have noticed 44% of anal cancer patients never received anal/rectal pap tests during the study follow-up (Table 2). As the present study shows, the insufficient preventive procedures of anal cancer have afflicted the high risk population

Anal cancer is very similar to cervical cancer biologically.[16] Most of both cancers are preceded by persistent high grade squamous intraepithelial lesions caused by HR-HPV infection, and both are preventable through HPV vaccination and screening tests.[16] As cervical cancer is AIDS-defining condition, it has drawn much more attention which ultimately led to better screening guideline implementation. A great decline in new

cases of cervical cancer from 1999 (9.90 cases per 100,000 persons) to 2016 (7.91 cases per 100,000 persons) among general US women was reported by the Centers for Diseases Control and Prevention.[17] Women's cervical cancer screening programs are constantly up to date to reflect the current health burden caused by it. The routine check-up may potentially benefit women from all other HPV-induced cancer in anogenital tracts. All together these could potentially also be the reason why we observed less HGAIN and anal cancer among HIV-infected women, compared with men. By contrast, anal cancer is overlooked in the high risk population. Considering the benefits of cervical cancer screening program for HIV-infected women, we see the urgent needs of more rigorous and regular screenings for anal cancer, particularly among HIV-infected men.

It takes 15 to 20 years for cervical cancer to develop from untreated dysplasia in women with normal immune systems. However, it may take only 5 to 10 years for HIV-infected women.[18] As previously mentioned, PLWH's life expectancies have bumped about to 70 years from 39 years since the advent of ART.[11] Although we did not study the duration of progression to anal cancer since HGAIN in the current study due to the limited sample size, we observed a 7.6-year difference between the median ages at HGAIN and anal cancer diagnoses (Table 1). If we use cervical neoplasia as an analogy, the progression to anal cancer from dysplasia for PLWH might also be much shorter than the general population. However, anal cancer is rare in the general population, and hence, the average time needed for neoplasia is unclear. Further study is needed to retrospectively study the progression of anal neoplasia in PLWH.

The median age at anal cancer in the general US population is 62 with most frequent diagnoses made between 55-64 years (31.1%) (Figure 2). Instead, the median

age was 51.4 and people aged 45-54 (58.1%) were at the highest risk in the present study (Figure 2). An over 10-year younger median age of anal cancer was observed among PLWH. Approximately 79% of anal cancers were observed under 54 years in the current study; however, only 26.5% diagnoses were less than 54 years in the general population (Figure 1). We are aware that we have very few patients aged 74 years and older in the current HIV cohort, as they are slowly aging; thus, we focus more on the young and middle aged HIV population. High incidence of anal cancer in the younger HIV-infected men is alarming but until screening guidelines are implemented this target population will remain high risk

A few limitations of this retrospective study were noticed. For example, we used clinical diagnoses with no detailed laboratory information. Thus, we cannot give HPV infection information of HGAIN and anal cancer. However, there is no recommendation for HPV screening as part of the limited anal cancer screening program, and hence, PLWH are not mandated for its related examinations. Patients will only get treated if they seek medical attention. Although we might underestimate the numbers of HGAIN and anal cancer, we have already demonstrated substantially higher rates of both conditions compared with the national reported estimates in the general population. Without sufficient prevention, it is concerning that the incidence of anal cancer will stay high and even become higher.

Whether the observations from our study are specific to the southern region or HIV population in general needs more investigation. However, despite the number is small, the alarming rate needs to be carefully monitored before this becomes an epidemic problem in this targeted population. It may not be a public health priority to

implement anal cancer screening among the general population due to its rarity; however, the constant rise in anal cancer among PLWH makes it necessary to consider carrying out massive screenings in the target population. Our findings revealed the current public health burden in hopes of a quick approval and implementation of a mandatory anal cancer-related screening program among PLWH.

ACKNOWLEDGEMENTS

We thank all study participants from the UAB 1917 Clinic. We also thank the UAB Research and Informatics Service Center (RISC) for data access (https://www.uab.edu/medicine/1917cliniccohort/).

Funding

The study did not receive specific funding. However, the ability to complete this work was supported by the UAB Center for AIDS Research (CFAR) [grant P30-AI27767 to MSS from the National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; the CFAR Network of Integrated Clinical Systems (CNICS) [grant 1R24 AI067039-1 to MSS from the National Institute of Allergy and Infectious Diseases; https://www.niaid.nih.gov/]; and the Mary Fisher CARE Fund (https://www.uab.edu/medicine/cfar/mary-fisher).

Ethics Statement

The study was approved by the UAB Institutional Review Board for Human Use, and performed in accordance with the ethical guidelines of the Declaration of Helsinki. Animals were not used in the study.

Author Contributions

YY, GB, and SS conceived the study. IA and HW participated in statistical approach. YY processed and analyzed the data. YY, SA, and SS interpreted the data and wrote the manuscript. All authors have reviewed and approved the manuscript.

Conflicts of Interest

Dr. Burkholder has received research support from Bristol-Myers Squibb, Definicare, LLC, and Amgen Inc, and has consulted for Medscape.

References

1. American Cancer Society. Key Statistics for Anal Cancer. Available: Key Statistics for Anal Cancer. Available: https://www.cancer.org/cancer/anal-cancer/about/what-is-key-statistics.html.

2. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13: 487–500.

3. NIH Surveillance, Epidemiology, and End Results Program. Cancer of the Anus, Anal Canal, and Anorectum - Cancer Stat Facts. In: SEER. Available: https://seer.cancer.gov/statfacts/html/anus.html.

4. Pineda C, Welton M. Management of Anal Squamous Intraepithelial Lesions. Clinics in Colon and Rectal Surgery. 2009;22(2):94–101

5. Berry-Lawhorn M, Michael Berry-Lawhorn J, Palefsky M. Progression of anal high-grade squamous intraepithelial lesions to anal squamous cell carcinoma and clinical management of anal superficially invasive squamous cell carcinoma. Seminars in Colon and Rectal Surgery. 2017: 91–96.

6. CDC. HIV geographic distribution. Available: https://www.cdc.gov/hiv/statistics/overview/geographicdistribution.html.

7. Koplon S. The past and present of HIV: three decades of care at UAB. UAB News. Available: https://uab.edu/news/health/item/9074-the-past-and-present-of-hiv-three-decades-of-care-at-uab.

8. Guzman A. UAB 1917 Clinic Cohort. UAB School of Medicine. Available: https://www.uab.edu/medicine/1917cliniccohort/

9. Routman JS, Willig JH, Westfall AO, Abroms SR, Varshney M, Adusumilli S, et al. Comparative efficacy versus effectiveness of initial antiretroviral therapy in clinical trials versus routine care. Clin Infect Dis. 2010;50: 574–584.

10. Patel P, Hanson DL, Sullivan PS, Novak RM, Moorman AC, Tong TC, et al. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. Ann Intern Med. 2008;148: 728–736.

11. Scaccia A, Madell R. Facts About HIV: Life Expectancy and Long-Term Outlook. Healthline. 2018. Available: https://www.healthline.com/health/hiv-aids/life-expectancy.

12. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin Dev Immunol. 2012;2012: 670957.

13. Monforte AD, Abrams D, Pradier C, Weber R, Reiss P, Bonnet F, et al. HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malignancies. AIDS. 2008;22: 2143–2153.

14. American Cancer Soceity. Risk Factors for Anal Cancer. Available: https://www.cancer.org/cancer/anal-cancer/causes-risks-prevention/risk-factors.html.

15. ASCCP. Appopriate Strategies for Patientis with Anal Cancer. 2018. Available: www.asccp.org › Assets › 1125-j-michael-berry-lawhorn-pdf.

16. Palefsky J. Pathogenesis of anal cancer. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2016:38.

17. CDC. Cervical Cancer Statistics. 2019. Available: https://www.cdc.gov/cancer/cervical/statistics/index.htm.

18. WHO. Human papillomavirus (HPV) and cervical cancer. Available: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer

Table 1. Sociobehavioral and clinical characteristics of HAGIN and anal cancer cases compared with non-HGAIN or anal cancer subjects N=117 individuals

Race	HGAIN (N=79)	Anal cancer (N=43)	HGAIN and anal cancer free (N=4,367)
Blacks	42 (53.2)	18 (41.9)‡	2618 (60.0)
Whites	36 (45.6)	24 (55.8)‡	1575 (36.0)
Others	1 (1.2.)	1 (2.3) ‡	174 (4.0)
Gender			
Men	76 (96.0)*	43 (100)*	3315 (75.9)
Women	3 (4.0)*	0 (0)*	1028 (23.5)
Transgender individuals	0 (0)*	0 (0)	24 (0.50)
Self-reported sexual risks			
Heterosexual	5 (6.5)*	7 (17.1)‡	1826 (43.5)
MSM	72 (93.5)*	34 (82.9)‡	2360 (56.2)
Others	0 (0)*	0 (0) ‡	11 (0.30)
Age at HIV diagnoses	28.8 (24.6-37.7)‡	31.7 (25.6-38.8)	32.9 (26.4-54.0)
Age at study enrollment	38.4 (27.3-46.7)	44.9 (40.7-50.9)‡	40.9 (31.8-59.1)
Age at HGAIN or anal cancer‡‡	43.8 (31.8-54.2)	51.4 (46.9-54.6)	--
Nadir CD4 count (cells/µL)			
<200	36 (45.6)	27 (62.8)‡	1699 (38.9)
200-500	28 (35.4)	11 (25.6)‡	1819 (41.7)
≥500	15 (19.0)	5 (11.6)‡	849 (19.4)
Median CD4 count (cells/µL)			
<200	18 (22.8)‡	14 (32.6)*	612 (14.0)
200-500	28 (35.4)‡	19 (44.2)*	1606 (36.8)
≥500	33 (41.8)‡	10 (23.3)*	2149 (49.2)
Highest VL (copies/mL)			
<50 (undetectable)	13(16.5)	6 (14.0)	430 (9.9)
≥50 (detectable)	66 (83.5)	37 (86.0)	393 (90.1)
Median VL (copies/mL)			
<50 (undetectable)	44 (55.7)	21 (48.8)	2641 (60.5)
≥50 (detectable)	35 (44.3)	22 (51.2)	1726 (39.5)
Ever received anal/rectal pap 1 year before diagnosis			
Yes	40 (50.6)	14 (32.6)	--
No	39 (49.4)	29 (67.4)	

***: *P*<0.00010**
‡:0.0001<*P*<0.01
‡‡:0.0001<*P*<0.01 compared between HGAIN and anal cancer

Table 2. Practices of anal/rectal pap tests between January 2006-March 2018

	Ever received (N = 1,648)	Never received (N = 2,834)	*P*-value
Gender			
Men	1611 (47.0)	1816 (53.0)	<0.0001
Women	16 (1.5)	1015 (98.5)	
Transgender M-F	21 (87.5)	3 (12.5)	
Race			
Black	836 (31.3)	1839 (68.7)	<0.0001
White	764 (46.8)	867 (53.2)	
Others	48 (27.3)	128 (72.7)	
Self-reported sexual risk factors	N=1591	N=2683	<0.0001
MSM	1504 (61.7)	932 (38.3)	
Heterosexual men	72 (8.4)	781 (91.6)	
Heterosexual women	15 (1.5)	970 (98.5)	
HGAIN and cancer			
HGAIN	56 (70.9)	23 (29.1)	<0.0001
Cancer	19 (55.8)	24 (44.2)	
Race- sexual risks	N=1591	N=2683	
Black MSM	732 (62.3)	442 (37.7)	<0.0001
Black heterosexual men	51 (8.1)	578 (91.1)	
Black heterosexual women	10 (1.4)	731 (98.6)	
White MSM	729 (62.0)	446 (38.0)	
White heterosexual men	19 (10.6)	161 (89.4)	
White heterosexual women	5 (2.3)	213 (97.7)	
Other race MSM	43 (49.4)	44 (50.6)	
Other race heterosexual men	2 (4.6)	42 (95.4)	
Other race heterosexual women	0 (0)	26 (100)	

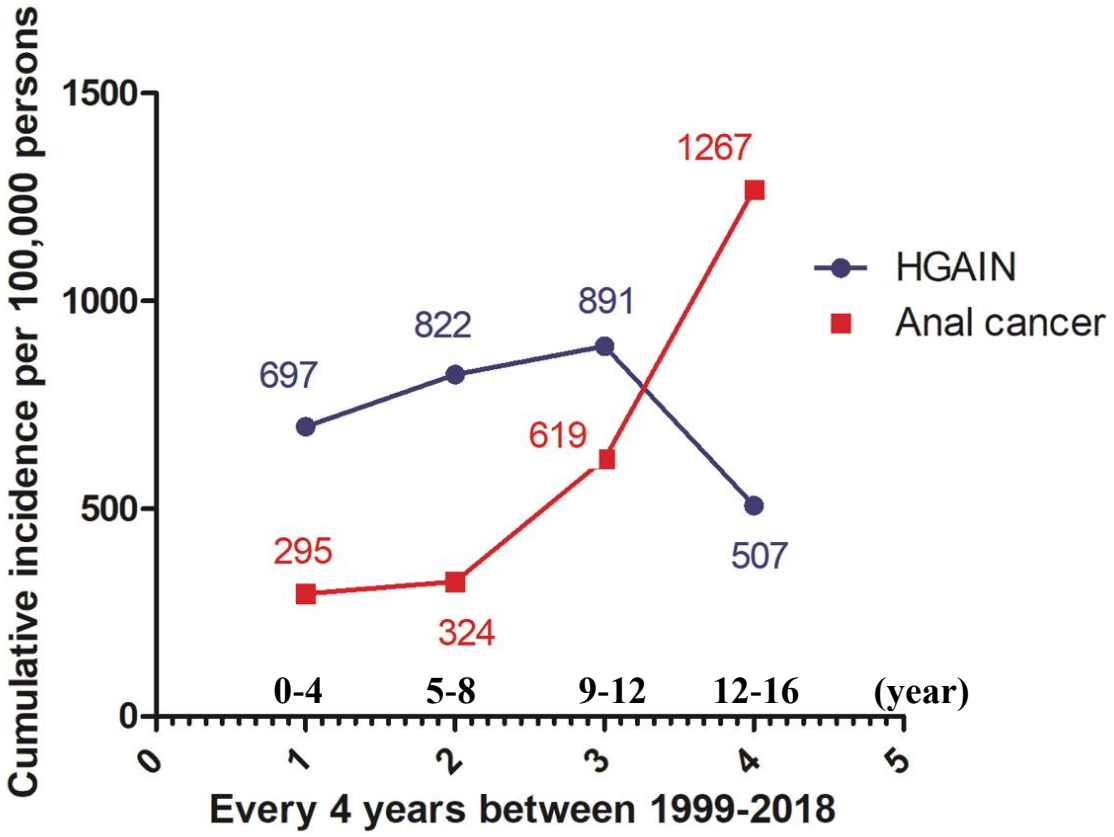

Figure 1. Cumulative risk of HGAIN or anal cancer every 4 years (0-4, 5-8, 9-12, 13-16 years) since patient initial clinical visit dates. The current cohort began in 1999 and ended in early 2018. The last period (17-20 years) were excluded due to an incomplete 4-year follow-up. Diagnoses of HGAIN declined while cancer cases increased during the follow-up.

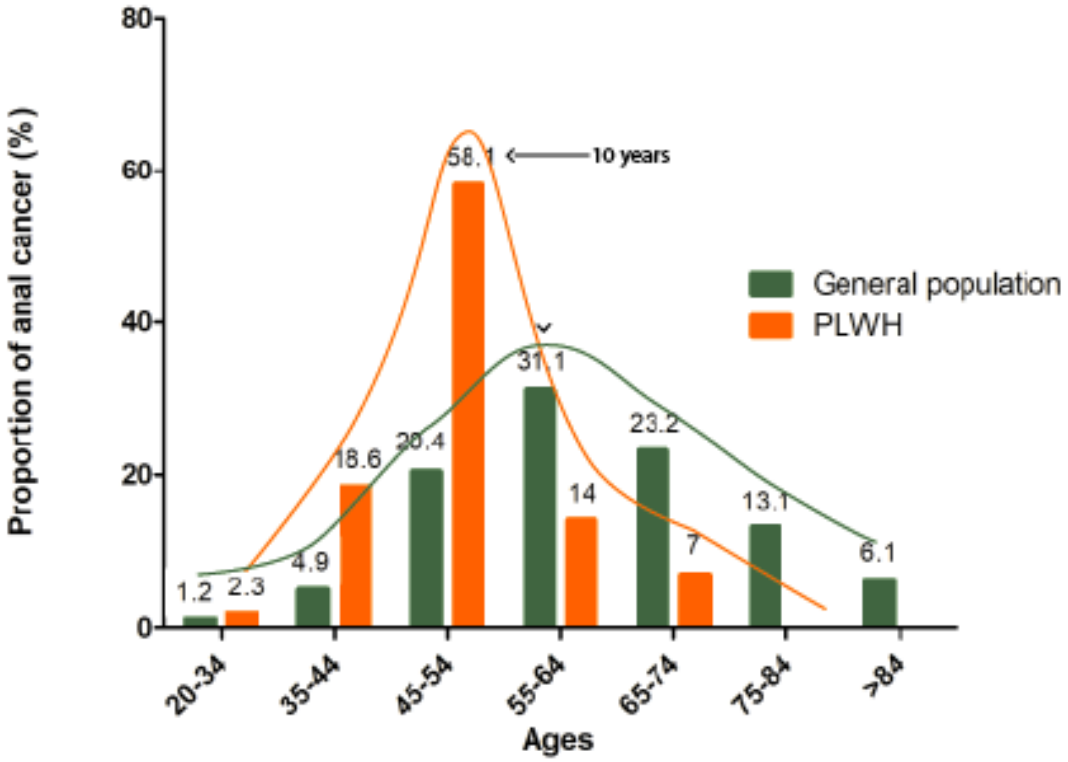

Figure 2. Ages at anal cancer diagnoses among PLWH compared to the general US population from the SEER. Percentage of each age group was given.

CHAPTER 6

SUMMARY AND DISCUSSION

People living in the Southeastern US bears high risks of HPV and HSV infection, along with increased incidence of HIV, disproportionately19,20. Reports in Chapter 2 and 3 revealed the current health burden of HPV- and HSV-associated clinical conditions that afflicts people living with HIV (PLWH) in the region. In hopes of discovering a more substantial biomarker that could represent the overall immune status of PLWH and to assess if they are associated with the onset of HPV- and HSV- associated conditions, I applied a new algorithm, trajectory CD4 counts to group longitudinal CD4 counts based on their trends, as described in Chapter 3. Then the trajectory CD4 groups were used to assess the risks of incident HPV- and HSV- associated conditions. Except for cervical cancer, all the other conditions investigated in the study are not AIDS-defining comorbidities, which are generally overlooked due to the limited research resources. However, more data have shown rises in a number of non-AIDS defining comorbidities. Anal cancer is one of the conditions that indicates a much higher risk in PLWH. Chapter 4 of the research focused on exploring sociobehavioral characteristics of HGAIN and anal cancer as well as well as the issues existing in the current screening practices.

HPV-associated anogenital conditions including warts, anal LSIL, HSIL and cancer were predominantly observed in HIV-infected men, compared with women. This

contradicts the findings in the general population.[21,22] Both anogenital warts and dysplasia are more common in general women than men in the general population. However, incidence rates of warts, anal LSIL, HSIL, and cancer (188.5, 188.2, 43.2, 25.8 per 100,000 person-years, respectively) were much higher in men than women (67.9, 14.7, 5.5, 0 per 100,000 person-years, respectively) in the HIV clinical cohort ($P<0.001$ for each) in Birmingham Alabama. Unfortunately, currently, there are no HPV-related anogenital screening guidelines recommended for HIV-infected men. By contrast, as part of the Women's Preventive Services Initiative established by the US Health Resources and Services Administration, screening for cervical cancer is mandatory for HIV-infected women.[23] The cervical screening protocol mandates the screenings for HIV-infected women as early as their sexual activity onsets regardless of age.[23] The screening program offers both cervical Pap (cytology) and HPV-serologic tests for HIV-infected women aged 30 years and above.[23] The major difference of the screening program between general and HIV-infected women is the screening will continue to be performed for HIV-infected women after the age of 65 years, but as for the general women, it ends at 65.[23] Under such rigorous screening guidelines, I did not observe a significant increase of cervical cancer in the study cohort. Although, the trend of cervical cancer in HIV-infected women has not been reported nationwide, the CDC reported a substantial decline in its new diagnoses from 1999 (9.90 cases per 100,000 persons) to 2016 (7.91 cases per 100,000 persons) in the general women.[24] Looking back at the much higher incidence rates of warts, anal LSIL, HSIL, and cancer among HIV-infected men, a comprehensive and mandatory anogenital related HPV-screening program should be warranted for HIV-infected men.

On the other hand, women (216.8 per 100,000 person-years) were at higher incidence risk of anogenital herpetic ulcers than men (130.2 per 100,000 person-years) in the present cohort (P<0.0001). Several other studies have also reported 1.2-2.0 times higher incidence rates of anogenital herpes or herpetic ulcers in HIV-infected women, compared with men.[25–27] Unlike HPV-associated conditions, findings relative to the ulcers are consistent with the general population reported by the CDC. According to the CDC's most recent STD fact report, the main etiology of anogenital herpetic ulcers, HSV-2 infection was more commonly detected in general women (15.9%) than men (8.2%).[28] It is known that HSV-2 causes majority of anogenital ulcers. Our findings were able to reflect the health issues caused by the infection.

There are discrepancies observed of HPV-associated conditions between the present HIV-cohort and general population, but the findings of HSV-associated ulcers are quite consistent between the two populations. The main difference in terms of prevention noticed is the implementation of the mandatory cervical cancer screening program (HPV screening) for HIV-infected women. It is not clear if the screening also benefits HIV-infected women from developing other HPV-associated conditions (i.e. warts and anal cancer) than cervical cancer.

CD4 T cell counts are a key HIV-related indicator that reflects the overall immune functions in PLWH. Tests of CD4 counts along with HIV VL are a routine part of standard HIV-care. According to the CDC, PLWH should have their CD4 counts and HIV VL tested every 3-6 months.[28,29] In general, CD4 counts <200 indicates the occurrence of severe immune damages.[30] PLWH with such low CD4 counts are considered AIDS patients.[30] On the contrary, CD4 counts ≥500 (cell/μL) usually

represents a complete immune recovery from the initial HIV infection.[26,31] An immunocompetent person generally has CD4 counts between 500-1400 (cell/μL).[26,31] Therefore, the overall morbidity and mortality rates of PLWH with CD4 counts ≥500 (cell/μL) are comparable to uninfected people.[26,31] Most of PLWH can reach CD4 counts ≥500 (cell/μL) eventually under treatments, specifically in the ART era.[32]

Nadir and median CD4 represent the lowest and median CD4 counts over a defined period of time, respectively. It is well known that nadir CD4 has a strong predictive value in terms of AIDS-related morality.[33,34] Clinically, nadir CD4 reflects the degree of immunosuppression and represents the lowest level of immunity. Although ART has been demonstrated to restore immunologic responses effectively, there are still comorbidities tightly associated with low nadir CD4 regardless of treatments.[32] It is noted that some body impairments related to low CD4 are not reversible, for example, neurocognitive damages.[32] Median CD4 is typically used in addition to nadir CD4 to summarize PLWH's overall immune statues. It is often used to predict the onset of other comorbidities and treatment responses.[35] It is as important as nadir CD4 in terms of prognosis after HIV infection.[35] However, both nadir and median CD4 counts are only one-single measure summary that are taken from a series of longitudinal CD4 tests. Although they are widely used in assessing the host defensibilities against other comorbidities, it is unclear how informative and precise these two measures represent in the context of HPV- and HSV- related outcomes

Since PLWH are required to have routine CD4 tests, I was able to use and examine different dimension of longitudinal CD4 measures as a potential biomarker to assess the risks of HPV- and HSV-associated conditions (Chapter 3). In the study, both

median and trajectory CD4 counts presented statistically significant associations with incident anogenital herpetic ulcers, warts, and anal HSIL/cancer (Table 4.2). By contrast, nadir CD4 was only associated with warts and anal HSIL/cancer (Table 4.2). The GBTM estimated 6 CD4 trajectory groups initially. As mentioned previously, PLWH achieving CD4 ≥500 (cell/μL) usually represent good immune recoveries. Therefore, trajectory groups 6 and 7 were decided to combine to 1 group and the new trajectory group 6 was then used as a reference in the analyses (Figure 3.1a-c). It is not surprising to find that patients with longitudinal CD4 <200 (cell/μL) had the highest odds of herpetic ulcers, warts, and anal HSIL/cancer (Table 3.2). Similar results were observed when using median CD4 as the main predictor (Table 3.2). However, significant differences in risks were noted when different groups of trajectory CD4 counts had similar median CD4. For example, median CD4 <200 (cell/μL) had the highest odds of ulcers in both Model 1 (M1:the model included gender and other controlled variables) (OR=2.6, P<0.0001) and Model 2 (M2: the model included self-reported sexual risks and other controlled variables) (OR=2.9, P<0.0001). Trajectory groups 2 and 4 had similar median CD4 counts of 138 and 145 (cell/μL), respectively. However, the 2 groups indicated different odds of incident ulcers. The odds of trajectory group 2, in which the PLWH started from CD4 <100 (cell/μL) and improved over time only had slightly higher odds of ulcers, compared with the healthy group 6. Also, the higher odds was not statistically significant. By contrast, group 4 started from CD4 over 200 (cell/μL) and decreased over time. The odds of group 4 (OR=2.5 in both M1 and M2) was even higher than the ones of median CD4 group<200 (cell/μL) (OR=2.1 and 2.3 in M1 and M2, respectively) (Table 3.2). More discrepancies of odds derived from trajectory groups but with similar median CD4

were observed in anal HSIL/cancer. Trajectory groups 3 and 4 had similar median CD4 counts of 237 and 226, respectively (Table 3.2). However, the odds of those 2 trajectory groups were completely different because of the opposite directions of the CD4 trends (Figure 3.1c). Trajectory group 4 had much higher odds of incident anal HSIL/cancer (M1: OR=4.9, $P<0.0001$, M2: OR=5.1, $P=0.033$), whereas, the higher odds of trajectory group 3 were not statistically significant (Table 3.2). Consequently, using 1 single median CD4 measure to determine the risk of incident herpetic ulcers and anal HSIL/cancer was not as informative as CD4 trajectories. In conclusion, PLWH with similar median CD4 counts could have CD4 trajectories in opposite directions, which would lead to significantly different risks of the disease outcomes.

Trajectory CD4 measures did not appear to be more precise when assessing the onset of new anogenital warts (Table 3.2). One other study that used mixed models to study CD4 counts over time also failed to demonstrate an association between longitudinal CD4 and incident warts.[36] They suggested that the baseline CD4 counts at HIV diagnoses seemed to be related to new warts; however, it still was not statistically significant.[36]

ART has substantially improved PLWH's survival by reducing AIDS-defining mortality rate by at least 40%.[37–39] Although the effectiveness of ART on viral suppression is well recognized, we are also aware that the treatment does not clear the infection and not all immune impairments caused by the virus can be restored. The life span of PLWH could be expected nearly as immunocompetent people; however, it is still shorter and PLWH suffer more comorbidities shift left, meaning they manifest in younger age.[40,41] Non-AIDS-defining complications, particularly non-AIDS defining malignancies

have become a rising public health concern among PLWH.[42] Infection-induced cancers, such as HPV-induced oropharyngeal and anal cancers have increased significantly and are expected to affect more long-term HIV surviviors.[42]

HR-HPV infection is linked with most of anal squamous cell carcinoma and found in at least 91% of all anal cancers.[21,43,44] Unlike oropharyngeal cancer, anal cancer is very rare in the general population, with an incidence rate of 1.9 per 100,000 person-years, with respect to 2.2 and 1.6 cases per 100,000 women-years and men-years.[21] Thus, not that many researches focused on it previously. However, this cancer caught my attention at the very early stage of my thesis. Incidence rates of anal cancer were 194 and 258 per 100,000 person-years and 100,000 men-years, respectively in the study (Table 2.2). All anal cancer cases were detected in men in the cohort (Table 2.2). It resulted in an alarming 102-fold increase in HIV-infected men and women and a 161-fold increase in HIV-infected men, compared with the general population and men, respectively.

Although there are no treatments to clear HPV infection, anal cancer is preventable through vaccination and screenings.[45,46] Anal/rectal Pap tests are one of the screening methods that have been applied in clinical setting to detect anal dysplasia. If abnormal cell changes are detected, a biopsy will be performed as a follow-up step to confirm the diagnosis.[47] A follow-up surgical excision of the abnormal tissues will be performed.[47] This anal/rectal Pap procedure is not invasive and only takes less than 5 minutes without causing major discomfort for patients.[47] However, a certain number of providers are unfamiliar with the procedure. Also, lack of triage and referral for related care followed by the detection of abnormal anal tissues challenges the values of the screening program.[47] To my knowledge, chapter 4 of my thesis is the first study

comprehensively investigating the association between clinical screening, anal precancerous dysplasia-HGAIN and cancer. Among the 79 HGAIN and 43 anal cancer cases diagnosed between January 2006 and March 2018, nearly 50% of HGAIN patients and over 67% patients did not receive anal/rectal Pap tests within a year prior to the case onset (Table 4.1). Although the comparison of receiving the Pap tests between HGAIN and anal cancer patents was not statistically significant (*P*=0,055), approximately 17% more anal cancer patients missed the important screening tests during their precancerous stage. Self-reported gender and sexual risks were both significantly associated with HGAIN and anal cancer, compared with HGAIN and anal cancer free patients (Table 4.1). It is well known that anal dysplasia and malignancies are mostly prevalent in HIV-infected MSM because of the sexual activities.[48] Interestingly, 17% of anal cancer patients self-reported as "heterosexual men", compared with 6.5% of in HGAIN patients (Table 4.1). It suggested that incorrect self-reported sexual orientation were observed in the study. A number of reasons could lead to this issue, and they are mostly related to social expectations and stigmas in the southern US.[49–51]

Although my research did not touch on the medical sociology of stigma of HIV-infected MSM, the self-reported sexual risks was as an important variable through my thesis as my main research interest is STI and HIV co-infections. This variable became more prominent when the research topic evolved to the screening practices of anal cancer. According to the EHR between January 2006 and March 2018, about 37% of PLWH received at least 1 anal/rectal Pap test (Table 4.2). Among those who received tests, 97% of them self-reported sexual orientations; among the never received tests, 95% self-reported sexual risks (Table 4.2). When looking at the overall study cohort, 16%

more Blacks never received test, compared with Whites (P<0.0001). Among those who self-reported sexual risks, about 38% White and 38% Black MSM never received tests; however, 1.7% more White heterosexual men received tests, compared with Black heterosexual men (P<0.0001). Among the 157 PLWH with self-reported sexual risks in other races, much less MSM (51%) never received tests (Table 4.3), compared with Black and White MSM (P<0.0001). It is more concerning that over 44% of anal cancer patients did not receive tests during the entire study follow-up, whereas, only 29% of HGAIN patients did not (P<0.0001). It suggests that 44% of anal cancer cases might have been captured when the patients were still at their precancerous stages.

Univariate analyses indicated there was lack of anal cancer screenings among cancer patients compared to HGAIN patients. The cumulative incidence of HGAIN started declining after year 12, while anal cancer increased since year 8 (Figure 4.1). We would have expected to see lower incidence of anal cancer when patients stay longer in the routine HIV-care, as more opportunities of screening could have been offered to the high-risk patients. HPV-associated anal cancer is not an acute disease and would not occur suddenly. Although no research has studied the duration since anal dysplasia to the onset of anal cancer, we can use cervical cancer as an example since both cancers are mostly caused by HPV infection and are biologically similar.[52] It takes 5-10 years for cervical neoplasia in HIV-infected women to develop to cancer.[53] If anal dysplasia requires similar length of time for neoplasia, the screenings should have captured the precancerous lesions before they progressed to cancers. Unfortunately, the current screening program has not been sufficiently implemented.

Last but not least, the earlier onset of anal cancer also caught my attention. According to the SEER, the median age of anal cancer diagnoses is 62 years with most cases found in ages between 55-64 years (31.1%) in the general population (Figure 4.2). By contrast, the mean age of anal cancer diagnoses is 51 years in PLWH and most cases (58%) were found in ages 45-54 years in the current cohort. Less than 27% of anal cancers were observed under 55 years in the general population, whereas, it was noted that over 90% of the diagnoses were made under the same age in PLWH (Figure 4.2). It is about 10 years earlier in terms of the onset of new anal cancer in PLWH, compared with the general population, suggesting indications of premature aging among PLWH. Earlier onset age of anal cancer among PLWH has never been reported before this study.

Throughout the development of my thesis, some strengths are noted. For example, the use of EHR from a clinical cohort reflects the real-world health challenges related to HPV- and HSV-associated conditions. The actual clinical practices in terms of the rate of disease diagnoses and screenings were analyzed and reported. This gave me insights into how PLWH in the southeastern US performed based on the routine HIV-care. Incidence rates of anogenital ulcers, warts, dysplasia and cancers have not been reported in this region. Also, anal cancer screening program was never evaluated in the region. Since it is not an AIDS-defining cancer, there are very limited resources that facilitate anal cancer related research. The entire research should help providers recognize the health burden of these conditions with possibilities of delivering better care. Speaking of methodology, Joinpoint regression models and GBTM were conducted in Chapters 1 and 2, respectively. Joinpoint regression does not make an assumption of monotonic linear trends of diseases outcomes and is able to compare trends between the stratified groups,

such as gender and race. Another new method used in the thesis is GBTM. GBTM has been widely used in clinical research to map the trends of biomarkers over time by incorporating longitudinal measures. However, very few HIV-related studies have used it to model longitudinal CD4 counts and interpreted it as a potential biomarker. This is the first study using trajectory CD4 as a biomarker to assess the risk of the onset of new anogenital ulcers, warts, anal HSIL/cancer. When using generalized linear model to evaluate the differences of incidence rates between different gender and race groups, both binomial and Poisson distributions were performed. However, the model failed to converge when using the binary distribution for multiple diseases outcomes, such as cervical and anal LSIL, HSIL, and cancers, and etc. Although the risk of these conditions were high in my study cohort, the absolute counts of the cases were not big. As an alternative approach, a Poisson distribution was used. They key benefit of using this distribution is to avoid any possibilities of non-convergence issues.[54] The method proposed by Zou, that a Poisson approach focused on the log count than the log probabilities.[55] Therefore, the linear predictor is not limited to be negative with a Poisson regression and positive fitted values were be generated.[54,55] Particularly, when modeling a rare disease as the outcome, the estimator approaches the log-binomial model, so it is acceptable to choose Poisson distribution and it should give equivalent estimates. [54]

Using EHR of a clinical cohort also presents a few weaknesses. For example, diagnoses could only be made if patients sought medical attention for the condition or providers recommended screenings. Thus, it is inevitable to underestimate the case numbers. However, the research has demonstrated alarmingly higher rates of HPV-and HSV- associated CC among PLWH in the region. Actions must be taken to better manage

the diseases for PLWH in the region. In addition, no HPV- and HSV-related laboratory tests were reported in the research. Since the study was conducted using an actual clinical cohort, all diagnoses were made from routine clinical practices. Except for cervical cancer/HPV screening, HPV-and HSV-related laboratory tests are usually not required for their associated diagnoses. The main task of such HIV-specialized clinic is to deliver healthcare to PLWH in a timely manner.

The thesis indicates PLWH in the southeastern US bear higher risks of HPV- and HSV-association CC. Large-scale studies should be performed to test the generalizability of the current findings.

References

1. CDC. HIV SURVEILLANCE REPORT Volume 28. Available: https://www.cdc.gov/hiv/pdf/library/reports/surveillance/cdc-hiv-surveillance-report-2016-vol-28.pdf.

2. CDC. STD Facts - Genital Herpes. 2017. Available: https://www.cdc.gov/std/herpes/stdfact-herpes.htm.

3. CDC. STD Facts - Human papillomavirus (HPV). 2017. Available: https://www.cdc.gov/std/hpv/stdfact-hpv.htm.

4. CDC. Other STDs - 2016 STD Surveillance Report. 2017. Available: https://www.cdc.gov/std/stats16/other.htm#foot-5.

5. CDC. Herpes Prevalence NHANES 2010. Available: https://www.cdc.gov/std/herpes/herpes-nhanes-2010.htm.

6. HPVinformation. High- and Low-Risk HPV Types. Available: http://www.hpvinformation.com/about-hpv/high-and-low-risk-hpv-types/.

7. National Cancer Institute. HPV and Cancer. Available: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-fact-sheet.

8. CDC. HPV in Communities of Color. 2017. Available: http://www.cdc.gov/features/preventhpv/.

9. CDC. Appendix A AIDS-Defining Conditions. 2008. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5710a2.htm.

10. Strick LB, Wald A, Celum C. Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. Clin Infect Dis. 2006;43: 347–356.

11. Patel P E al. Prevalence and risk factors associated with herpes simplex virus-2 infection in a contemporary cohort of HIV-infected persons in the United States. Sex Transm Dis. 2012;39(2):154-60.

12. Health AP. DEMOGRAPHICS OF HIV INFECTIONS AMONG INDIVIDUALS RESIDING IN ALABAMA AT DIAGNOSIS. 2017. Available: http://www.alabamapublichealth.gov/hiv/assets/hiv_aidsreport_1st_quarter_2017.pdf

13. Koplon S. News - The past and present of HIV: three decades of care at UAB. UAB News. Available: https://uab.edu/news/health/item/9074-the-past-and-present-of-hiv-three-decades-of-care-at-uab.

14. Guzman A. UAB - School of Medicine - 1917 Clinic Cohort - Home. Available: https://www.uab.edu/medicine/1917cliniccohort/

15. AIDS Map. Available: http://www.aidsmap.com/CD4-cell-counts.

16. Lange C, Lederman M, Medvik K, Asaad R, Wild M, Kalayjian R, et al. Nadir CD4+ T-cell count and numbers of CD28+ CD4+ T-cells predict functional responses to immunizations in chronic HIV-1 infection. AIDS. 2003;17: 2015–2023.

17. Keith H. When Interrupting Therapy, Mind the CD4 Nadir. AIDS Clinc Care. 2005;3(2):1.

18. University of Wisconsin Hospitals, Clinics Authority. Ryan White Coverage and Eligibility. UW Health. Available: https://www.uwhealth.org/hiv-aids/ryan-white-coverage/51671

19. Farley TA. Sexually transmitted diseases in the Southeastern United States: location, race, and social context. Sex Transm Dis. 2006;33: S58–64.

20. CDC. Geographic Distribution of HIA/AIDS. 2019. Available: https://www.cdc.gov/hiv/statistics/overview/geographicdistribution.html

21. Cancer of the Anus, Anal Canal, and Anorectum. Cancer Stat Facts. SEER. Available: https://seer.cancer.gov/statfacts/html/anus.html

22. Genital warts - Symptoms and causes. Mayo Clinic. 2016. Available: https://www.mayoclinic.org/diseases-conditions/genital-warts/symptoms-causes/syc-20355234

23. HIV/AIDS Bureau. Performance Measure Portfolio. Available: https://hab.hrsa.gov/clinical-quality-management/performance-measure-portfolio

24. CDC. Cervical Cancer Statistics. 2019. Available: https://www.cdc.gov/cancer/cervical/statistics/index.htm

25. Celum C, Levine R, Weaver M, Wald A. Genital herpes and human immunodeficiency virus: double trouble. Bull World Health Organ. 2004;82: 447–453.

26. Martha H. Herpes & HIV: Not What You Think. Harvard AIDS Initiative. 2010. Available: https://aids.harvard.edu/herpes-hiv

27. Herpes Simplex Virus and HIV-1. 2006. HIV InSite. Available: http://hivinsite.ucsf.edu/InSite?page=kb-05-03-02

28. CDC. STD Facts - Genital Herpes (Detailed version). 2019. Available: https://www.cdc.gov/std/herpes/stdfact-herpes-detailed.htm

29. CDC. UnderstandingHIV Care. 2019. Available: https://www.cdc.gov/hiv/basics/livingwithhiv/understanding-care.html

30. Mediline Plus. CD4 Lymphocyte Count. US National Library of Medicine. Available: https://medlineplus.gov/lab-tests/cd4-lymphocyte-count/

31. US Department of Veterans Affairs. CD4 count (or T-cell test) - HIV. Available: https://www.hiv.va.gov/patient/diagnosis/labs-CD4-count.asp

32. Bishop JD, DeShields S, Cunningham T, Troy SB. CD4 Count Recovery After Initiation of Antiretroviral Therapy in Patients Infected With Human Immunodeficiency Virus. Am J Med Sci. 2016;352: 239–244.

33. NEJM Journal Watch: Summaries of and commentary on original medical and scientific articles from key medical journals. [cited 30 Oct 2019]. Available: https://www.jwatch.org/na31794/2013/07/31/cd4-cell-nadir-before-art-and-serious-non-aids

34. Bray S, Gedeon J, Hadi A, Kotb A, Rahman T, Sarwar E, et al. Predictive value of CD4 cell count nadir on long-term mortality in HIV-positive patients in Uganda. HIV AIDS . 2012;4: 135–140.

35. May MT, Vehreschild J-J, Trickey A, Obel N, Reiss P, Bonnet F, et al. Mortality According to CD4 Count at Start of Combination Antiretroviral Therapy Among HIV-infected Patients Followed for up to 15 Years After Start of Treatment: Collaborative Cohort Study. Clin Infect Dis. 2016;62: 1571–1577.

36. Luu HN, Amirian ES, Chan W, Beasley RP, Piller LB, Scheurer ME. CD4+ Cell Count and HIV Load as Predictors of Size of Anal Warts Over Time in HIV-Infected Women. J Infect Dis. 2012;205: 578–585.

37. Detels R, Muñoz A, McFarlane G, Kingsley LA, Margolick JB, Giorgi J, et al. Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. JAMA. 1998;280: 1497–1503.

38. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection. New England Journal of Medicine. 1998. pp. 853–860. doi:10.1056/nejm199803263381301

39. Silverberg MJ, Wegner SA, Milazzo MJ, McKaig RG, Williams CF, Agan BK, et al. Effectiveness of highly-active antiretroviral therapy by race/ethnicity. AIDS. 2006. pp. 1531–1538. doi:10.1097/01.aids.0000237369.41617.0f

40. Lohse N, Hansen A-BE, Pedersen G, Kronborg G, Gerstoft J, Sørensen HT, et al. Survival of Persons with and without HIV Infection in Denmark, 1995–2005. Annals of Internal Medicine. 2007. p. 87. doi:10.7326/0003-4819-146-2-200701160-00003

41. Losina E, Schackman BR, Sadownik SN, Gebo KA, Walensky RP, Chiosi JJ, et al. Racial and sex disparities in life expectancy losses among HIV-infected persons in the

united states: impact of risk behavior, late initiation, and early discontinuation of antiretroviral therapy. Clin Infect Dis. 2009;49: 1570–1578.

42. Wang C-CJ, Silverberg MJ, Abrams DI. Non-AIDS-Defining Malignancies in the HIV-Infected Population. Current Infectious Disease Reports. 2014. doi:10.1007/s11908-014-0406-0

43. Jhaveri J, Rayfield L, Liu Y, Chowdhary M, Cassidy RJ, Madden NA, et al. Prognostic relevance of human papillomavirus infection in anal squamous cell carcinoma: analysis of the national cancer data base. J Gastrointest Oncol. 2017;8: 998.

44. How Many Cancers Are Linked with HPV Each Year? | CDC. 21 Aug 2019 [cited 31 Oct 2019]. Available: https://www.cdc.gov/cancer/hpv/statistics/cases.htm

45. Moscicki A-B, Darragh TM, Michael Berry-Lawhorn J, Roberts JM, Khan MJ, Boardman LA, et al. Screening for Anal Cancer in Women. J Low Genit Tract Dis. 2015;19: S26.

46. Stier EA, Chigurupati NL, Fung L. Prophylactic HPV vaccination and anal cancer. Hum Vaccin Immunother. 2016;12: 1348–1351.

47. UW Health. HPV and Anal PAP Testing. Available: https://www.uwhealth.org/healthfacts/diagnostic-tests/7056.pdf

48. Sheon N. Anal Cancer Risks Compared. The Anchor Study. Available: https://anchorstudy.org/anal-cancer-risk-among-hiv-positive-men-and-women

49. Bojko MM, Kucejko RJ, Poggio JL. Racial Disparities and the Effect of County Level Income on the Incidence and Survival of Young Men with Anal Cancer. Health Equity. 2018;2: 193.

50. Koskan AM, Fernandez-Pineda M. Anal Cancer Prevention Perspectives Among Foreign-Born Latino HIV-Infected Gay and Bisexual Men. Cancer Control. 2018;25: 1073274818780368.

51. Koskan AM, Leblanc N, Rosa-Cunha I. Exploring the Perceptions of Anal Cancer Screening and Behaviors among Gay and Bisexual Men Infected with HIV. Cancer Control. 2016;52–58.

52. Palefsky J. A-109 Pathogenesis of anal cancer. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2016;38.

53. WHO. Human papillomavirus (HPV) and cervical cancer. Available: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer.

54. Lumley T, Ma S, Kronmal R. Relative Risk, Regression in Medical Research: Models, Contrasts, Estimators, and Algorithms. U Washington Working Paper: BE Press; 2006.

55. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;10(7):702-6.

APPENDIX A

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on November 8, 2021. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	Shrestha, Sadeep
Co-Investigator(s):	Burkholder, Greer Anne Wiener, Howard William Ye, Yuanfan
Protocol Number:	**X170329001**
Protocol Title:	*HSV-2 and HPV Co-Infection Outcomes and Comorbidity Among HIV Patients*

The IRB reviewed and approved the above named project on 4/17/17. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 4-17-17

Date IRB Approval Issued: 4/17/17

IRB Approval No Longer Valid On: 4/17/18

HIPAA Waiver Approved?: Yes

Marilyn Dees

Expedited Reviewer
Member - Institutional Review Board
for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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Fax 205.934.1301
irb@uab.edu

The University of
Alabama at Birmingham
Mailing Address:
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BIRMINGHAM AL 35294-0104

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

PI: Shrestha, Sadeep
Protocol # X170329001

UAB IRB Approval of
Waiver of Informed Consent and/or Waiver of Patient Authorization

☑ **Approval of Waiver of Informed Consent to Participate in Research.** The IRB reviewed the proposed research and granted the request for waiver of informed consent to participate in research, based on the following findings:

1. The research involves no more than minimal risk to the subjects.
2. The research cannot practicably be carried out without the waiver.
3. The waiver will not adversely affect the rights and welfare of the subjects.
4. When appropriate, the subjects will be provided with additional pertinent information after participation.

Check one:
- ☑ **and** Waiver of Authorization (below)
- ☐ **or** Waiver of Authorization (below)
- ☐ Waiver of Authorization not applicable

☑ **Approval of Waiver of Patient Authorization to Use PHI in Research.** The IRB reviewed the proposed research and granted the request for waiver of patient authorization to use PHI in research, based on the following findings:

1. The use/disclosure of PHI involves no more than minimal risk to the privacy of individuals
   i. There is an adequate plan to protect the identifiers from improper use and disclosure.
   ii. There is an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, unless there is a health or research justification for retaining the identifiers or such retention that is otherwise required by law.
   iii. There is an assurance that the PHI will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of PHI would be permitted.
2. The research cannot practicably be conducted without the waiver or alteration.
3. The research cannot practicably be conducted without access to and use of the PHI.

—OR—

☐ **Full Review**
The IRB reviewed the proposed research at a **convened meeting** at which a majority of the IRB was present, including one member who is not affiliated with any entity conducting or sponsoring the research, and not related to any person who is affiliated with any of such entities. The waiver of authorization was approved by the majority of the IRB members present at the meeting.

Date of Meeting

Signature of Chair, Vice-Chair or Designee

Date

☑ **Expedited Review**
The IRB used an **expedited review procedure** because the research involves no more than minimal risk to the privacy of the individuals who are the subject of the PHI for which use or disclosure is being sought. The review and approval of the waiver of authorization were carried out by the Chair of the IRB, or by one of the Vice-Chairs of the IRB as designated by the Chair of the IRB.

4-17-17
Date of Expedited Review

Marilyn Davis
Signature of Chair, Vice-Chair or Designee

Date 4-17-17

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APPROVAL LETTER

TO: Shrestha, Sadeep

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)

DATE: 04-Apr-2019

RE: IRB-170329001
HSV-2 and HPV Co-Infection Outcomes and Comorbidity Among HIV Patients

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The IRB reviewed and approved the Continuing Review submitted on 12-Mar-2019 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review:	Expedited
Expedited Categories:	5
Determination:	Approved
Approval Date:	28-Mar-2019
Approval Period:	One Year
Expiration Date:	27-Mar-2020

The following apply to this project related to informed consent and/or assent:

- Waiver of Informed Consent
- Waiver of HIPAA

Documents Included in Review:

- ipr.190312