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Recurrence of anal high-grade squamous intraepithelial neoplasia post-treatment in HIV-positive women

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RECURRENCE OF ANAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL NEOPLASIA POST-TREATMENT IN HIV-POSITIVE WOMEN

by

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ABSTRACT

BACKGROUND: The incidence of anal cancer in HIV-positive (HIV+) women has increased in the past 3 decades. Anal cancer prevention strategies include the detection and treatment of high-grade squamous intraepithelial lesions (HSIL) of the anus (anal precancer) in order to prevent progression to anal cancer. Studies have evaluated the effectiveness of anal HSIL treatment in HIV+ Men who have Sex with Men (MSM); little is known about the effectiveness of treatment in HIV+ women.

OBJECTIVE: To determine the recurrence rate of anal HSIL in HIV+ women after ablative or topical treatment.

METHODS: A retrospective chart review was performed on all HIV+ women seen at Boston Medical Center (BMC) between 2004 and 2017 who received treatment for anal HSIL and had at least one follow up visit with high-resolution anoscopy (HRA). The time to recurrence was measured as the time between the first treatment procedure to the date of biopsy-proven HSIL.

RESULTS: 84 HIV+ women were diagnosed with anal HSIL. 48 (57%) had treatment of anal HSIL and at least one follow up anal evaluation and were included in the study. The median age of the study cohort was 53 years. The median follow-up time was 3.79 years, with a range of 0.44 to 13.39 years. 8 participants (16.67%)

received hyfrecation in clinic, 26 (54.17%) received ablation in the OR, 9 (18.75% received IRC, 2 (4.16%) received imiquimod, and 2 (4.16%) received 5-FU. HSIL recurrence occurred among 29 (60.4%) women. Mean time to recurrence of 25.4 months (95% CI: 15.4-35.4). The probability of anal HSIL recurrence was 26% after 12 months, 46% after 24 months, and 60% after 60 months (5 years). One (2%) study participant developed anal SCC.

CONCLUSIONS: Recurrence rates of anal HSIL post-treatment are high in HIV+ women. This group would benefit from close surveillance and long-term follow up.

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LIST OF ABBREVIATIONS

- ..... Negative

+ ..... Positive

5-FU 5-Flurouracil

AIDS ..... Acquired Immunodeficiency Syndrome

AIN ..... Anal Intraepithelial Neoplasia

ALT ..... Alternative Lengthening of Telomere

AMC ..... AIDS Malignancy Consortium

ANCHOR ..... Anal Cancer HSIL Outcomes Research

ASC-US ..... Atypical Squamous Cells of Undetermined Significance

ASCCP ..... American Society of Colposcopy and Cervical Pathology

ASIL ..... Atypical Squamous Intraepithelial Lesions

ATZ ..... Anal Transitional Zone

BMC ..... Boston Medical Center

CAP ..... College of American Pathologists

CDK ..... Cyclin-Dependent Kinase

CDW ..... Clinical Data Warehouse

CIN ..... Cervical Intraepithelial Neoplasia

CRZ ..... Colorectal Zone

DARE ..... Digital Anal Rectal Exam

DNA ..... Deoxyribonucleic Acid

E6AP..... E6 Associated Protein

EMR.....Electronic Medical Record

HAART..... Highly Active Antiretroviral Therapy

HDAC.....Histone Deacetylases

HIPAA..... Health Insurance Portability and Accountability Act

HIV..... Human Immunodeficiency Virus

HPV..... Human Papillomavirus

HR..... High Risk

HRA..... High Resolution Anoscopy

HSIL..... High-grade Squamous Intraepithelial Lesions

hTERT..... Human Telomerase Reverse Transcriptase

LAST.....Lower Anogenital HPV-associated Squamous Terminology

LR..... Low Risk

AIDS..... Acquired Immunodeficiency Syndrome

MRN.....Medical Record Number

MSM..... Men who have Sex with Men

NCI..... National Cancer Institute

NET.....Neuroendocrine Tumors

ORF..... Open Reading Frame

pRB..... Phosphorylated Retinoblastoma Protein

PV..... Papillomavirus

qHPV.....Quadrivalent Human Papillomavirus Vaccine

SAS ..... Statistical Analysis System

SCC ..... Squamous Cell Carcinoma

SCJ ..... Squamocolumnar Junction

SEER ..... Surveillance, Epidemiology and End Results

SQZ ..... Squamous Zone

URR ..... Upstream Regulatory Region

VAIN ..... Vaginal Intraepithelial Neoplasia

VIN ..... Vulvar Intraepithelial Neoplasia

INTRODUCTION

Anal Cancer is a disease in which there is unregulated proliferation of cells in the anal canal. Most anal cancers are linked to infection with the Human Papillomavirus (HPV), the most common sexually transmitted infection globally. Anal cancer has many similarities to cervical cancer. Like cervical cancer, anal cancer is associated with HPV, frequently starts in the histological metaplastic zone between squamous and columnar epithelium, and frequently co-exists with anal HSIL. Because of these similarities, it is believed that the progression from anal HPV infection to anal cancer is analogous to that of the cervix. In the cervix, most HPV infections are transient; however, some HPV infections will persist and may cause changes in the epithelium termed high grade squamous intraepithelial lesions (HSIL), which may ultimately develop into cervical cancer. Prevention of cervical cancer includes the detection and treatment of cervical HSIL, preventing progression to cancer. Therefore, the management of HPV-associated lesions in the anal canal are modeled on the successful clinical management of cervical lesions in preventing progression to cancer. The vast majority of research conducted on anal cancer prevention has focused on the high-risk population of Human Immunodeficiency Virus-positive (HIV+) Men who have Sex with Men (MSM). Because HIV+ women are also at increased risk of developing anal cancer, it is important to develop anal cancer prevention strategies for this patient population.

Epidemiology

The American Cancer Society predicts that in 2018, in the United States, there will be 8,580 new anal cancer cases (5,620 in women and 2,960 in men) and 1,160 anal cancer-related deaths (680 in women and 480 in men).[1] Although a rare cancer, only currently accounting for 0.5% of new cancer cases in the United States, anal cancer rates have been on the rise in the past 4 decades. From 1975 to 2013, the incidence increased from 0.8 to 1.9 cases per 100,000 persons per year.[2] Older individuals are at greater risk for developing anal cancer. The peak age range is 55-64 years, with a median age of 60. Only 1.1% of anal cancers are diagnosed before age 35.[3] Racial differences are also present, with the incidence greatest in White and lowest in Asian/Pacific Islander women.[4] Survival from anal cancer is highly dependent on the stage at diagnosis. Based on data from the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute (NCI), from 2007 to 2013, the 5-year survival rate for anal cancer at all stages was 66.9%. For cancers confined to the primary site (48% of total), survival was 81.3%. For cancers that spread to regional lymph nodes (32%), survival was 62.1%. For metastatic cancer (13%) survival was 29.6%.[2]

Widespread implementation of national screening programs have proven successful in decreasing both the incidence and mortality rates of cervical SCC.[5] Because of the rising incidence of anal cancer and the pathophysiological and epidemiological similarities between anal and cervical cancers, a body of research

has been done in the past decade focusing on developing screening programs for anal cancer prevention.

There are certain known risk factors for anal cancer. Immunocompromised individuals, including those that are HIV+ or recipients of organ transplants are at greater risk. A study examining the incidence of anal cancer in HIV+ men and women between 1996 and 2012 found that individuals with HIV, including those with Acquired Immunodeficiency Syndrome (AIDS), are 19.1 times more at risk of developing anal cancer when compared to the general population.[6] This relationship could be explained by the fact that as HIV infection progresses, the number of T-cells decline and HPV infection is cleared through a cell-mediated pathway involving T-cells.[4] A compromised immune system results in greater HR-HPV persistence in the anal canal, increasing the risk of developing anal precancer that might ultimately transition to cancer.

Unlike several other opportunistic infections and cancers, anal cancer incidence did not decrease with the advent of highly active antiretroviral therapy (HAART), a treatment for HIV that uses a combination of drug therapies to effectively suppress the viral load, improving immune function. In fact, longer life expectancies for HIV+ individuals in the HAART era contributed to increased acquisition and persistence of anal HPV infection, contributing to increased anal cancer risk.[7] Other risk factors include a history of cervical vaginal or vulvar cancer, genital warts, or high-grade cervical, vaginal or vulvar intraepithelial neoplasia (CIN, VAIN or VIN). Based on data from the SEER database, from 1973 to 2007, of the

189,206 cases of either in situ or invasive cervical vulvar or vaginal carcinoma, 255 women developed anal cancer, indicating a 13-fold increase in anal cancer incidence when compared to the general population.[8] The risk of developing anal cancer is also increased with cigarette smoking. A study determining relative risks for anal cancer by comparing various risk factors found that smoking more than 15 cigarettes per day was associated with double the risk of anal SCC incidence.[9]

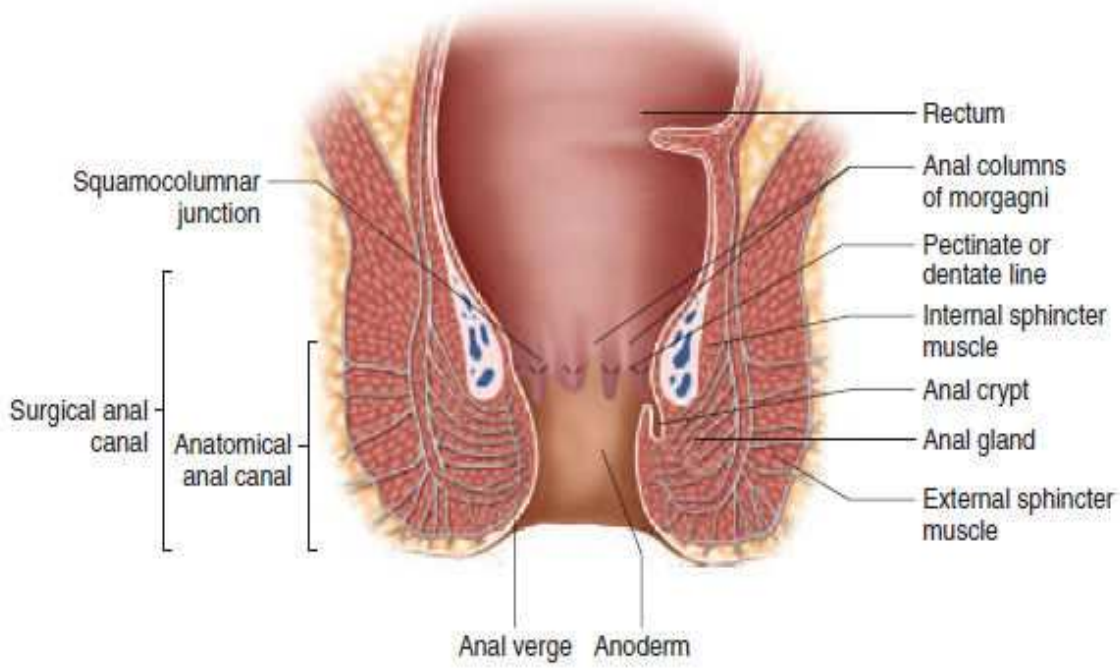

Figure 1: Anatomy of the Anal Canal. The anal canal is nonkeratinizing squamous epithelium in between the columnar epithelium of the rectum and the keratinized squamous epithelium of the anal verge. Reprinted from *The American Society of Colon and Rectal Surgeons (ASCRS) Textbook of Colon and Rectal Surgery* (p. 184), by M. Luchtefeld and R. E. Hoedema, 2016, Arlington Heights, IL: Springer International Publishing. Copyright 2016 by Springer International Publishing. Reprinted with permission.

The Anal Canal

The anal canal refers to the terminal end of the gastrointestinal tract. Surgically, it starts at the anorectal ring, a fusion of the puborectalis muscle, deep external anal sphincter and internal anal sphincter, located at the pelvic floor. The anal canal extends 3 to 4 centimeters distally to the anal verge, identified as the junction where the mucosal epithelium of the anal canal transitions to the cutaneous epithelium of the anal margin. The anal margin, or perianal skin, is the 5 centimeter region of squamous keratinized epithelial tissue around the anal verge, containing hair follicles and sweat glands. In the middle of the anal canal, is the dentate or pectinate line, a structure formed by transverse folds of the mucosa. This line divides the anal canal into an upper region (two thirds of the canal) and lower region (one third of the canal). Anatomically, the anal canal is the region between the dentate line and anal verge.

The anal canal can be divided into three histologically distinct regions. The colorectal zone (CRZ) is the region immediately distal to the rectum, measuring 1 to 2 centimeters long. It consists of non-squamous epithelium, including simple columnar epithelium continuous with the rectal mucosa and transitional epithelium. The anal transformation zone (ATZ), also known as the intermediate or cloacogenic zone, is the region of squamous metaplasia immediately distal to the CRZ, consisting of four to nine layers of cells, including squamous, colorectal, and endocrine cells, melanocytes, and anal glands. The ATZ is the transitional region between the uninterrupted columnar mucosa, located proximally, to the uninterrupted

squamous mucosa located distally and is the region of the anal canal most susceptible to HPV infection. It starts at the squamocolumnar junction (SCJ) and

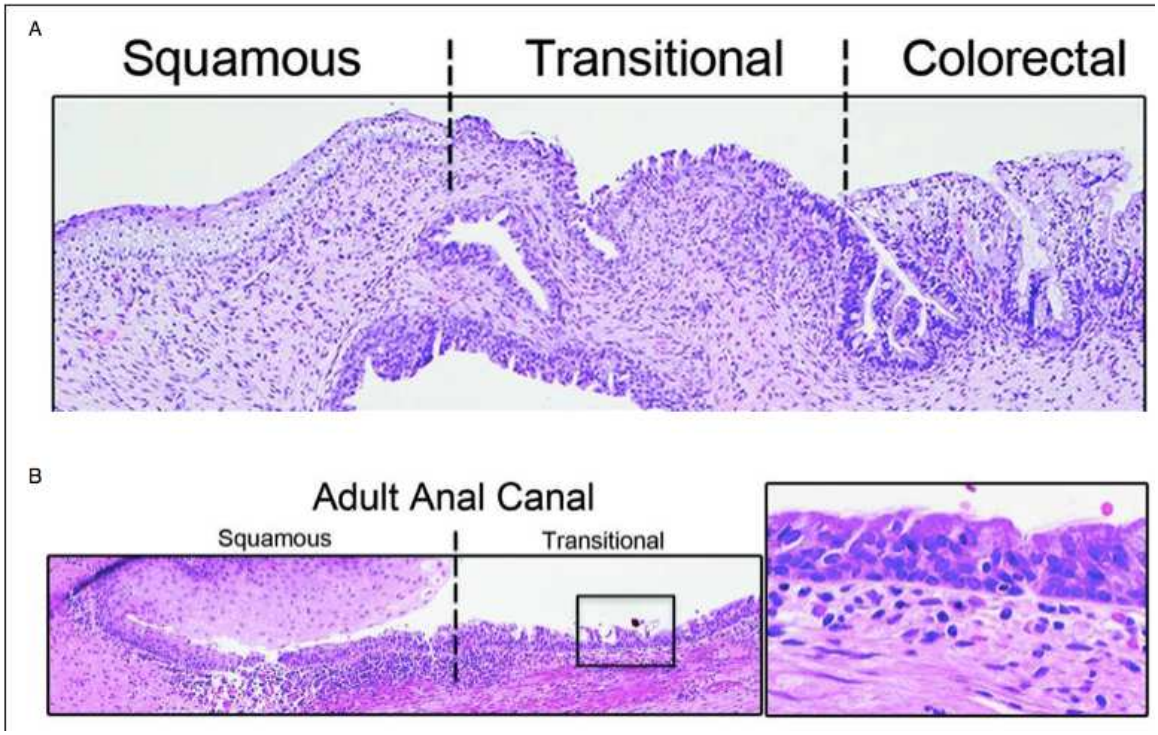

Figure 2: Micrographs of the Anal Canal: A: A longitudinal section of a fetal anal canal showing the three zones of the anal canal: Colorectal Zone (CRZ), Anal Transitional Zone (ATZ), and Squamous Zone (SQZ). B. A longitudinal section of the adult anal canal, showing the SQZ and TRZ. Adapted from "Microanatomy of the cervical and anorectal squamocolumnar junctions: a proposed model for anatomical differences in HPV-related cancer risk." by E. J. Yang et al., 2015 *Modern Pathology, 28(7),* p. 994-1000. Copyright 2015 by Springer Nature. Adapted with permission.

ends at the dentate line. The basal layer of the ATZ consists of small cells with nuclei arranged perpendicularly to the basement membrane, while the surface layer consists of a variety of epithelial cells. The Squamous Zone (SQZ) lines the lower third of the anal canal, immediately inferior to the dentate line, extending to the anal verge. It consists of both non-keratinizing squamous epithelium and the squamous

keratinized epithelium of the distal zone, free of hair, sebaceous glands and sweat glands.[10]

Because of the complexity of this region, several different tumors can arise in the anal canal, with SCC being the most common. Tumors that arise from the CRZ are usually non-keratinizing SCC from transitional epithelium or adenocarcinomas from non-squamous epithelium.[11] Tumors that originate from the ATZ include adenocarcinomas of the colorectal and anal glandular type, non-keratinizing SCC, neuroendocrine tumors (NET), and melanomas.[10] Tumors from the squamous zone include SCC and the rare melanoma.

When combined, keratinizing (also known as basaloid) and non-keratinizing SCC account for around 80% of tumors of the anal canal.[10] Both types of SCCs behave similarly and are treated similarly.[11] Adenocarcinomas are estimated to account for 10% of anal cancer incidences.[10] However, it is possible that this is an overestimate due to misclassification of rectal adenocarcinomas as anal adenocarcinomas. Anal adenocarcinomas mostly arise from the region above the dentate line or in the anal margin, with the latter arising from anal glands or fistula tracts. They are treated similarly to rectal cancers. With the exception of SCC and adenocarcinomas, other tumors of the anal canal are relatively rare.

Human Papillomavirus

HPV is a member of the *Papillomaviridae* family. Of the 189 known papillomavirus (PV) types, over 100 are human-PVs.[12] However, not all HPV types

cause anal cancer. Of the five genera: alpha-, beta-, gamma-, mu- and nu-PVs; those that are responsible for anogenital and upper aero-digestive tract infections are mostly classified under the alpha-PV genus. HPV strains can be further divided into "low-risk" (LR) and "high-risk" (HR) types. HPVs 6, 11, 40, 42, 43, 44, 53, 54, 61, 70, 72, 81, and 89 have been identified as LR-HPVs. Infection with LR-HPV types results in benign tumors, such as condylomas, or no detectable lesions, and are not expected to progress to malignant tumors even without treatment. HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 have been identified as HR-HPVs, and have the potential to cause malignant transformation. HPV types 16 and 18 account for 79% of anal SCC.[13]

Due to the worldwide detection of HR-HPV strains in cervical carcinomas, it has been determined that HPV is a necessary cause of invasive cervical cancer worldwide.[14] A review of the global health burden of infection-associated cancers found that HPV was responsible for 4.8% of all cancers diagnosed in 2008. Of the 530,000 reported cases of cervical cancer in 2008, 100% were attributable to HR-HPV infection. This strong correlation was independent of other risk factors and consistent in several different countries. The presence of HR-HPV in the tumor tissue of penile (50%), vulvar (43%), vaginal (70%) and anal carcinoma (88%) suggested that HR-HPV played a causal role in these cancers as well.[15] The 23,760 HPV-associated anal cancer cases diagnosed in 2008, were evenly distributed among developing and developed countries. The distribution of HPV-related anal

cancer cases in males and females were 11,000 (45.8%) and 13,000 (54.2%) respectively.[15]

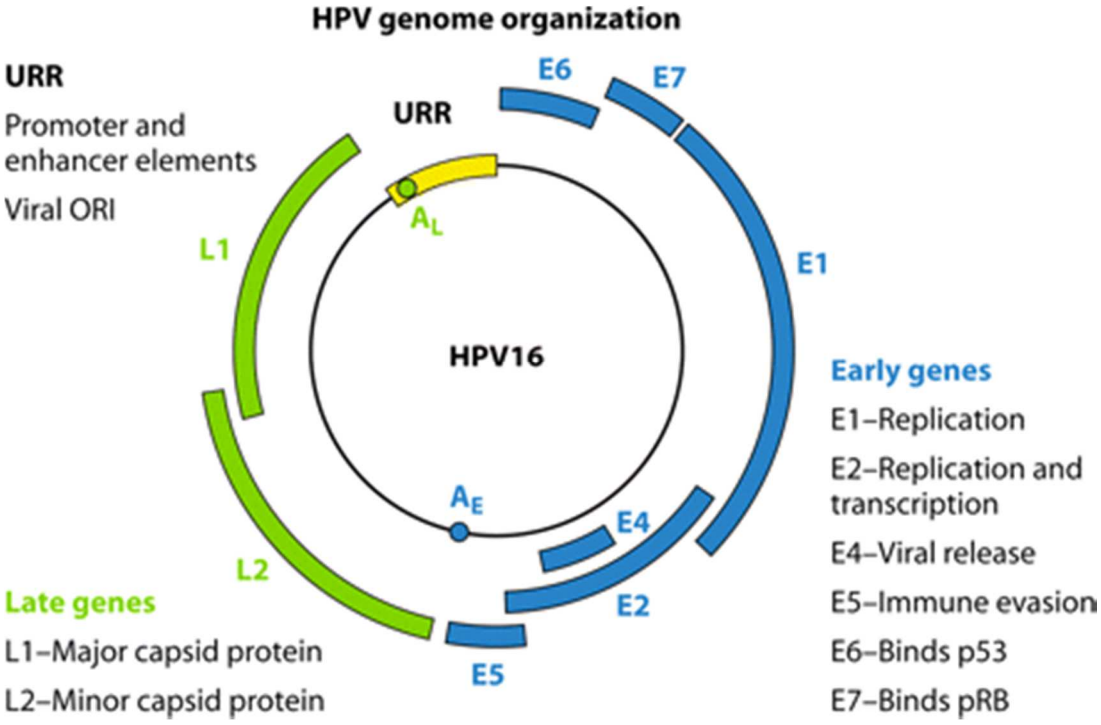

Figure 3 Genomic organization of HPV16: Illustration of the genome of a high-risk HPV strain showing the upstream regulatory region (URR), early region and late region. Reprinted from "Epithelial Cell Responses to Infection with Human Papillomavirus" by M. A. Stanley, 2012, *Clinical Microbiology Reviews, 25(2),* p. 216. Copyright 2012 by the American Society for Microbiology. Reprinted with permission.

HPV consists of a relatively small, circular, double stranded deoxyribonucleic acid (DNA) genome, 8 kb in size, surrounded by non-enveloped caspids. The genome of all HPV strains contain 3 regions: an upstream regulatory region (URR), a late region and an early region. The URR is a non-coding region that includes sequences that regulate DNA replication by transcription of protein-coding sequences in the early region. The late region contains sequences that code for L1, the major capsid

protein, and L2, the minor capsid protein. Together, these proteins form the structure of the virion that houses the DNA. The early region contains a series of open reading frames (ORFs) such as E1, E2, E4, E6 and E7. It determines the pathogenicity of the virus.[13]

In mucosal tissue, HPV targets the cells that compose the basal layer of the stratified squamous epithelium. When the basal cells divide, they form new basal cells as well as transit amplifying cells that detach from the basal layer and make their way up to the surface layer, differentiating as they progress. In the basal cell, the viral DNA is maintained in a low-copy number. As the transit amplifying cells differentiate, the virus uses the host cell's machinery for DNA amplification. As the host cell terminally differentiates and exits the cell cycle, proteins E6 and E7 facilitate viral DNA replication. The genetic product of E6 and E7 genes are viral oncoproteins that extend the lifespan of supra-basal human keratinocytes and facilitate immortalization. E6 and E7 proteins from HR-HPV strains are constitutively expressed in tumors. Differentiation of HPV-infected cells result in dysplasia, which can progress to cancer.

E7 is a multifunctional protein that binds to cell cycle regulatory proteins to alter their actions and allow for unscheduled cell cycle entry. One target of E7 is the tumor suppressing phosphorylated reintoblastoma protein (pRB), a key regulator of the E2F family of transcription factors. HR-HPV E7 interacts more efficiently with pRB and related "pocket proteins" p107 and p130 than LR-HPV E7. The E2F family plays a major role in the G1/S transition in the mammalian cell cycle. Binding of pRb

to E2F transcription factors prevents them from interacting with the cell's transcription machinery and inhibits E2F-regulated gene expression. E7 targets pRB and other "pocket proteins" for ubiquitination and subsequent proteosomal degradation. The removal of the inhibiting influence on the E2F-regulated gene results in hyperproliferation.[16] Other targets of E7 are histone deacetylases (HDAC). HDACs deacetylate lysine residues on histones, resulting in repression of transcription via chromatin remodeling. Interaction between E7 and HDAC specifically prevents HDAC from binding to the E2F2 promoter, resulting in active expression.[17] E7 also binds to cyclin-dependent kinase (CDK) inhibitors p21 and p27, which are greater expressed when keratinocytes differentiate and lose contact with the basal layer. CDK2 when combined with Cyclin E or Cyclin A promotes the G1 to S progression. Binding of E7 to p21 and p27 allows the overriding of p21- and p27- mediated growth arrest.[16] E7 is able to interfere with the mechanisms of telomere maintenance by the alternative lengthening of telomere (ALT) pathway to allow for tumor progression by extending the host cell's lifespan.[17]

The E7-mediated changes to the suprabasal epithelial layers results in p53-mediated signals for growth arrest and/or apoptosis. The HR-HPV E6 protein mediates p53 ubiquitination and degradation via E6 associated protein (E6AP), an E3 ubiquitine ligase which blocks E7 apoptosis. E6-mediated degradation of PDZ proteins also occurs via E6AP, resulting in a loss of cell polarity and an increase in cell proliferation. HR-HPV E6 also induces expression of human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, contributing to the

immortalization of the host cell by maintaining telomere length.[16] Through the cooperation of both the E6 and E7 proteins, the virus is able to disrupt the G1-S cell cycle checkpoint control and cause the cell to evade senescence.

Natural history of anal HPV infections:

Much of what we know about the development of anal cancer stems from research done on cervical cancer due to the common causative agent (HPV) and the cytological and histological similarities of HPV-associated damage in the anus and cervix. In the cervix, there is a SCJ, similar to, but not exactly like, the ATZ of the anal canal. This area is between two histologically distinct regions: the simple columnar epithelium of the endocervix and the nonkeratinized squamous epithelium of the exocervix. In both the anus and cervix, the transitional zones are highly susceptible to HPV infection and can develop HPV-related abnormalities in the epithelial tissue. In most cases, these abnormalities are transient. Some, however, progress to cancer.

The Bethesda System, established in 1988, standardized classification of HPV-related abnormalities in the cervix to decrease variability of reported results and allow for a uniform standard for cytological interpretation.[18] HPV-related abnormalities in the cervix can be referred to as dysplasia, cervical intraepithelial neoplasia (CIN), or atypical squamous intraepithelial lesions (ASIL). CIN is graded by increasing severity as CIN1, CIN2 or CIN3. Lesions are classified as LSIL when there is mild dysplasia, or CIN1, whereas moderate to severe dysplasia or carcinoma in situ, graded as CIN2 or CIN3, is classified as HSIL. In both the anus and cervix,

LSIL reflect active HPV replication and may resolve on its own or progress to HSIL, which reflects HPV-induced transformation with persistence of certain HR-HPV infection.[19] HSIL are considered to be premalignant.

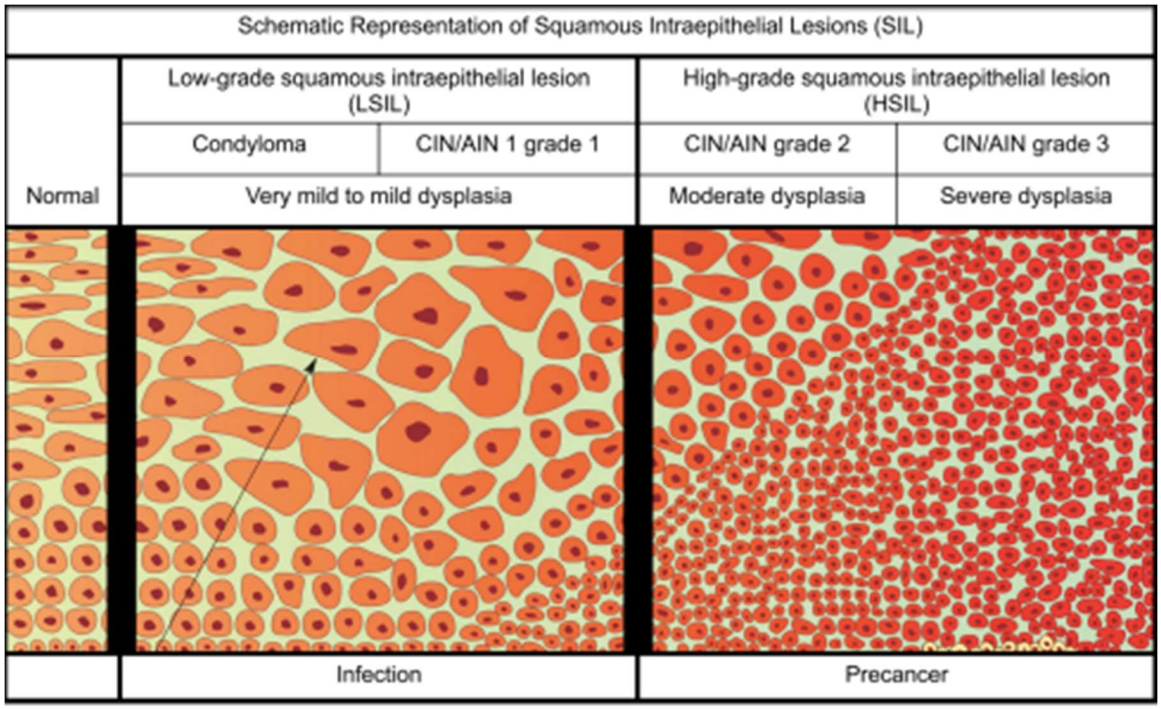

Figure 4: Anal Epithelial Cells with Increasing Severity of Atypia. Illustration of epithelium of the anal canal showing the cellular changes associated with increasing grades of dysplasia. Reprinted from "Human Papillomavirus in the HIV-Infected Host: Epidemiology and Pathogenesis in the Antiretroviral Era," by C. Brickman and J. M. Palefsky, 2015, *Current HIV/AIDS Reports* 12(1) p. 9. Copyright (2016) by Springer Science + Business Media. Reprinted with permission.

The Lower Anogenital HPV-associated Squamous Terminology (LAST) project, conducted by the American Society of Colposcopy and Cervical Pathology (ASCCP) and the College of American Pathologists (CAP), provided evidence-based recommendations to standardize the classification of dysplasia along the anogenital tract.[20] Because of the commonality between the HPV-associated lesions in

squamous sites of the lower genital tract in both men and women, it is recommended that the terminology used to diagnose the histopathology of the cervix be used to classify anal intraepithelial neoplasia (AIN) as well as other lesions of the anogenital tract.

Anal Cancer Screening

Anal cancer screening is based on the success of cervical cancer screening programs. Implementation of these programs, which involve the detection and removal of precancerous lesions, significantly reduced the incidence and mortality of cervical cancer. A review of SEER data showed a 54% decrease in the incidence of invasive cervical cancer in the United States between the years 1973 and 2007.[21] A similar study, based in Taiwan, measured the national disease burden of cervical cancer after the implementation of a national screening program in 1996 and found a 54.7% reduction in incidence between 1996 and 2007.[22] This is mainly attributed to the introduction of the Papanicolaou Test in the 1940s. The Papanicolaou test, or "pap smear" is performed by collecting cells at the transformation zone of the cervix and examining them under a microscope to detect intraepithelial abnormalities associated with HPV. An abnormal cytology result is followed up with by a colposcopy, a diagnostic procedure in which a speculum is inserted into the cervix and 5% acetic acid is applied to the mucosal surface of the cervix, which is carefully viewed under magnification via colposcope for changes that suggest the presence of HSIL. Biopsies are taken of abnormal appearing lesions for further pathological

review. If the biopsies show cervical HSIL, treatment is performed with ablation or excision of the SCJ of the cervix. In 2012, the highest incidence rates of cervical cancer globally were found in medically underserved regions including parts of sub-Saharan Africa, Latin America and the Caribbean, mainly due to lack medical infrastructure to support cervical cancer screening programs.[23]

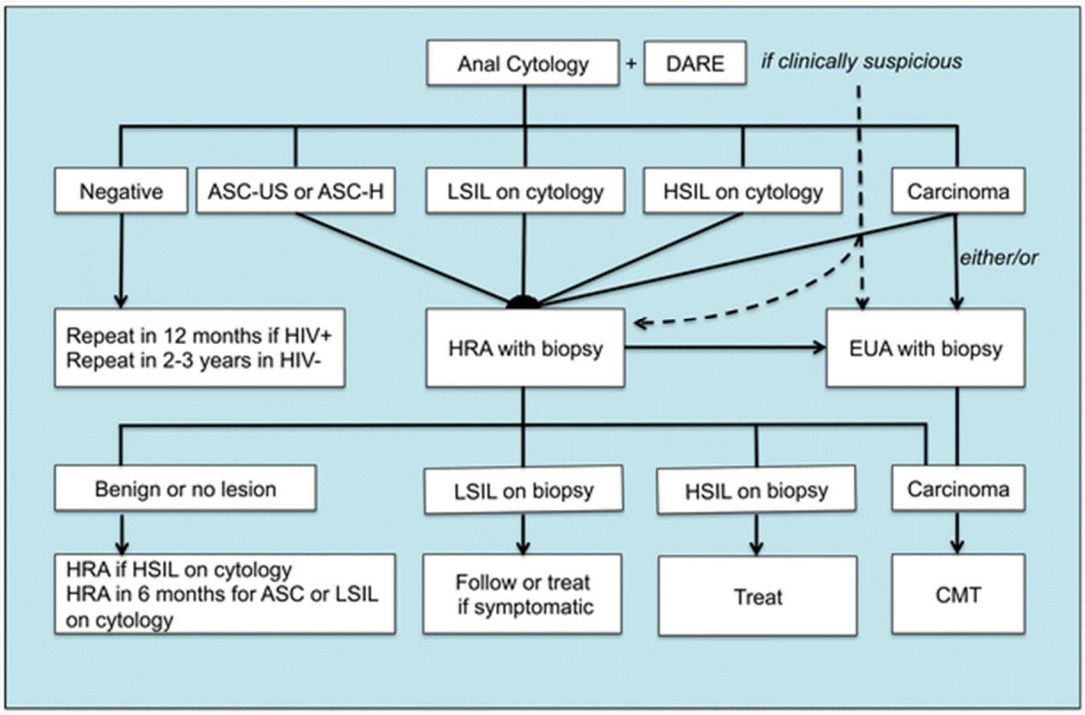

Fig. 3
Anal cancer/HSIL screening algorithm. *ASC-US* atypical squamous cells of undetermined significance, *ASC-H* atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, *CMT* combined modality therapy, *DARE* digital anorectal examination, *EUA* examination under anesthesia, *HRA* high-resolution anoscopy, *HSIL* high-grade intraepithelial lesions, *LSIL* low-grade squamous intraepithelial lesions. Adapted from Current Infectious Disease Reports, 2010

Figure 5: Proposed Screening Guidelines for High-Risk Populations Note, no formal guidelines currently exist for anal cancer screening. Screening consists of anal cytology, DARE, HRA with Biopsy and possible treatment. Reprinted from "Human Papillomavirus in the HIV-Infected Host: Epidemiology and Pathogenesis in the Antiretroviral Era," by C. Brickman and J. M. Palefsky, 2015, *Current HIV/AIDS Reports* 12(1) p. 9. Copyright (2016) by Springer Science + Business Media. Reprinted with permission.

Anal Pap Test:

One method of screening for anal cancer, adopted from cervical cancer screenings, is the use of cytology. Similar to the cervical pap test, the anal pap test is used to detect the presence of abnormal cells on the surface of mucosal tissue. The cells are collected via a moistened Dacron swab that is inserted into the anal canal and rotated along the length of the anal canal. The swab is then submerged in liquid cytology medium and agitated to disperse the cells in the vial. Debris such as mucus is removed from the sample and a thin layer of anal squamous cells are deposited on a microscope slide and stained using the Papanicolau stain for examination by a pathologist. Concurrent HPV testing may be performed to detect the presence of HR-HPV DNA in anal epithelial cells. The anal pap test has been shown to be a reliable way to detect the presence of atypical-SIL but is limited in its diagnostic ability. In a study comparing the cytology results of 200 anal smears to the corresponding pathology reports of subsequent anal biopsies, it was found that anal smears had a 98% success rate in detecting ASIL but were only able to predict the grade of abnormality of the biopsy 50% of the time. It was found that patients with cytology results that reported atypical squamous cells of undetermined significance (ASC-US) or LSIL had anal biopsies that showed HSIL 46-56% of the time.[24]

Digital Anal Rectal Exam (DARE) and the High Resolution Anoscopy (HRA)

Further examination of the anus includes the DARE and HRA. The DARE involves a provider inserting a lubricated and gloved finger into the anus to feel for

abnormalities such as thickened, raised, tender, hardened or ulcerated masses. The HRA is similar to the colposcopy used to screen for cervical cancers. During an HRA, a small plastic tube termed an anoscope is inserted into the anal canal to widen the area and the application of 5% acetic acid and Lugol's iodine solution is applied to the mucosal surface to allow for the detection of A-SIL under magnification via a colposcope. Positioning of the anoscope allows for visualization of different regions of the anal canal, including the ATZ.

The normal appearance of anal epithelium is shiny, translucent and pink. The application of acetic acid removes the superficial mucus layer and the acid penetrates into the cells of the surface layer of the epithelium. Physical changes that occur in these cells include dehydration of the intracellular compartment as well as coagulation of intracellular proteins. This alters the appearance of cells when exposed to light.[25] In the normal stratified squamous epithelium, the surface layer is composed of squamous keratinocytes that are flat and contain small nuclei. The application of acetic acid does not significantly change the appearance of these cells and under magnification the epithelium maintains its pink color. The SCJ contains a variety of cell types, including cells that are transitioning from the simple columnar tissue of rectal epithelium to the stratified squamous epithelium of the anal canal. These cells typically have large nuclei and more protein in their intracellular compartments. With the application of acetic acid, the cells of the SCJ become denser, reflecting more light, and appear slightly grey under magnification. HPV-infected cells have large nuclei as well and the intracellular compartment is filled

with viral proteins. Depending on the HPV type, the infection can involve the entire thickness of the epithelium. With the application of acetic acid, this region appears dense and absorbs light poorly.[25] The term "acetowhite epithelium" describes such regions that are perceived as white due to the poor light reflection. Staining with Lugol's iodine solution further differentiates HSIL and LSIL and helps determine the margin of disease. Lugol's iodine solution is a dark brown liquid and is taken up by glycogenated cells such as normal anal epithelium. ASIL, especially HSIL do not contain glycogen and do not stain.[25] HRA-directed biopsies are able to take advantage of the physical characteristics of the tissue after application of acetic acid and Lugol's solution to identify regions of suspected HSIL. Histopathology then reliably confirms the diagnosis.

Treatment Modalities

It is hypothesized that therapeutic management of anal HSIL is effective in reducing the risk of progression to anal cancer, as is the case with cervical HSIL. The Anal Cancer HSIL Outcomes Research (ANCHOR) study conducted by the AIDS Malignancy Consortium (AMC) is an ongoing prospective trial that aims to discover if this is indeed the case by randomizing 5,085 HIV+ men and women diagnosed with anal HSIL into either an active monitoring or treatment arm to compare the incidence of anal cancer in both groups.

Unlike the treatment of cervical HSIL, which involves either ablation or excision of the entire SCJ, treatment of anal HSIL is only directed at the HSIL lesions.

Treating the entire anal SCJ is not an option due to complications such as scarring and stenosis. The course of treatment largely depends on size, number, location and grade of the ASIL. Other contributing factors include the availability of resources, the patient's likely adherence to treatment and anticipated treatment-related side effects.[26]

Although no standard of care currently exists, several medical and surgical interventions have been developed in the past decade to treat anal HSIL. HRA-guided procedures, such as infrared coagulation and electrocautery can be performed on an outpatient basis or in the operating room, and allow for ablation or fulguration of high grade lesions while preserving the deeper layers of tissue. In infrared coagulation, pulses of infrared light are applied to the lesions via a probe resulting in thermal coagulation and tissue necrosis. Hemostasis is achieved spontaneously. In electrocautery or hyfrecation, an electric probe is applied to the lesions to cauterize the tissue. Both methods allow for targeting lesions specifically. Another alternative are patient-applied topical creams, 5-Flurouracil (5-FU) and Imiquimod for treating extensive HSIL. 5-FU is an antimetabolite that interferes with the synthesis of DNA. Imiquimod is an immunomodulating agent with antiviral and antitumor properties.[26] Surgical excision is another option to treat HSIL but this procedure is not ideal for patients with extensive or multifocal HSIL and may have adverse outcomes related to anal function.

Due to the rising incidence of anal cancer in high-risk populations, it is increasingly important that screening, monitoring and treatment of anal HSIL be

standardized. Although several treatment modalities exist to treat anal HSIL, these lesions often persist or recur and follow up care is necessary. Studies determining the recurrence rate of anal HSIL in HIV+ populations largely focus on MSM. Data on recurrence rates for HIV+ women is limited and determining cost and medically effective management of anal HSIL, including follow up care, from data extrapolated from studies focusing on MSM is not ideal. This study will contribute necessary information for the development of guidelines for screening and treating anal HSIL in HIV+ women.

METHODS

Data Source and Patient Selection

With approval from the institutional review board after expedited review at Boston Medical Center (BMC) and in accordance with the Health Insurance Portability and Accountability Act (HIPAA), a retrospective chart review was performed of all HIV+ women diagnosed with anal HSIL (AIN 2 or 3) at BMC from the start of anal screening with HRA in 2004 to the year of our request, 2017.

We identified 90 patients through BMC's Clinical Data Warehouse (CDW). After receiving our preliminary data set, we sorted through the list of patients using the matrix shown in Figure 6. The electronic medical charts of the study population identified went through a preliminary review to confirm eligibility based on our inclusion/exclusion criteria. Eligible subjects had to be female, have a diagnosis of HIV, and a pathology report confirming diagnosis of anal HSIL (AIN2 or AIN3). This review excluded 6 subjects: 4 were found to have anal cancer along with HSIL for their baseline pathology report, 1 had a pathology report of AIN1 that could not rule out HSIL and 1 was a male to female transgender. Additional inclusion criteria included having treatment for anal HSIL and at least one follow-up visit following anal HSIL treatment that included HRA. Participants that were never treated (n=24) or had no follow-up after treatment (n=12) were also excluded. A total of 48 patients were included in this study.

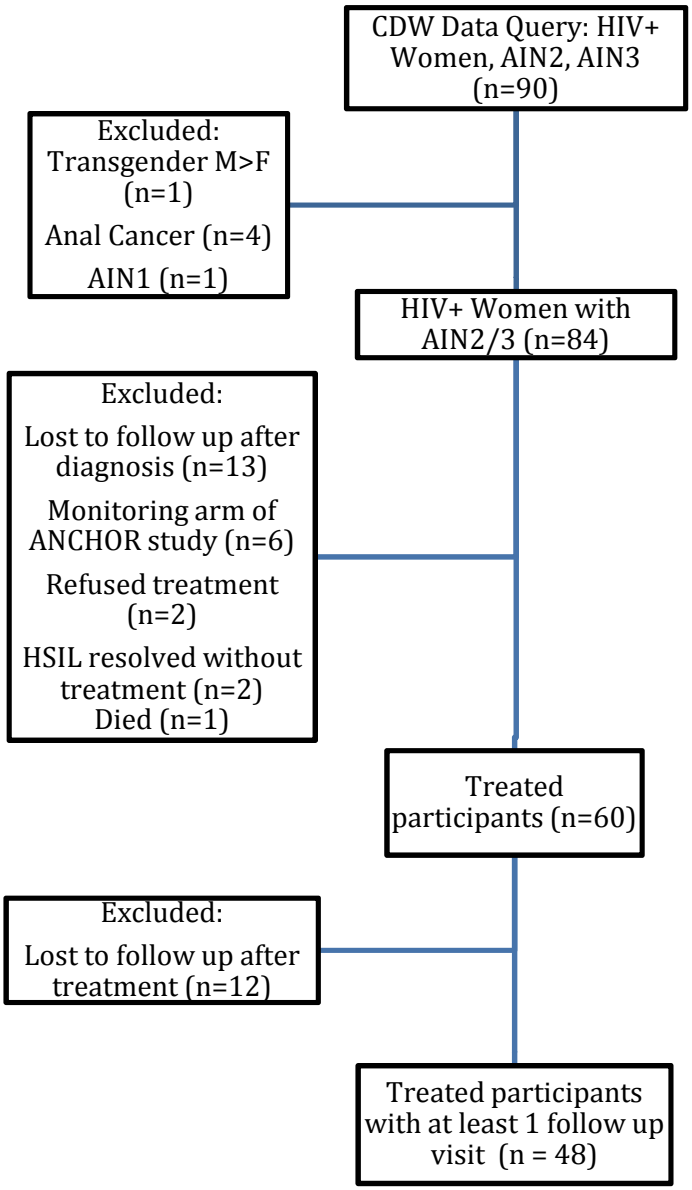

Figure 6: Flowchart of patient inclusion and exclusion

Demographic and Clinical Data

A master list of medical record numbers (MRN) linked to subject identification numbers for participants that met inclusion and exclusion criteria was maintained during data collection. Informed consent was waived by the IRB. Demographic and clinical information of eligible participants was obtained from the BMC electronic medical record (EMR) system. A manual chart review of eligible patients was sequentially performed and data was collected in REDcap, a secure web-based data management and survey tool.

The initial dataset included relevant demographic information (age, ethnicity, race, marital status, and country of origin) and baseline clinical information (smoking status, year of HIV diagnosis, history of AIDS defining illnesses, nadir CD4 count and HPV medical history (history of HPV vaccine, anogenital dysplasia diagnosis and treatment). Subjects were then followed from their initial visit, defined as the date of diagnosis of HSIL, to either their last anal evaluation or to the diagnosis of anal cancer, whichever came first. Data was obtained from progress notes and corresponding lab reports. Data obtained included anal, cervical and vaginal cytology, HR-HPV testing, anal pathology, status of anti-retroviral therapy (ART) compliance, absolute CD4 count, and records of any treatments for anal HSIL or non-anal HPV-associated dysplasia.

Recurrence

For the purposes of this study, anal HSIL persistence and recurrence were considered identical. Recurrence was defined as biopsy-proven HSIL following treatment for anal HSIL. A histological diagnosis of HSIL was defined as all AIN2 or AIN3 lesions. Time to recurrence was measured in months from day of first treatment to recurrence or until censoring occurred.

Statistical Analysis

Statistical analyses were performed using the Statistical Analysis System (SAS) v 9.4 (SAS Corp., Cary, NC, USA). Categorical variables were described as number and percentage. Continuous variables were described using mean (standard deviation), median and range. 95% CIs were calculated for the mean time to recurrence. The probability of recurrence was determined by the Kaplan-Meier estimate for cumulative incidence. Patients lost to follow up or patients who did not recur by their last visit were censored.

RESULTS

From January 2003 through June 2017, 84 HIV+ women were diagnosed with anal HSIL at BMC. Of these 84 women, 36 were excluded due to loss of follow up or no history of treatment. Figure 6 details the inclusion and exclusion of study participants. A total of 48 patients met inclusion criteria and were included in this study. Table 1 describes the demographic data of the study cohorts. All patients were female, 30 (62.5%) were African American or Black and 32 (68.1%) were single. The median age of participants was 53 years with a range of 35-66 years.

Table 2 describes the baseline clinical information of the study cohort. At the age of anal HSIL diagnosis, participants had been living with HIV for an average of 12.64 years. Patients with any history of smoking accounted for 68.7% of the population. Nadir CD4 counts averaged at 211 cells/mm3 (SD 205.15) with 58% of participants with a nadir CD4 count of less than 200. Only 14.6% of the population had a history of AIDS defining illnesses and 95.8% were currently on HIV medication. At their baseline visit, study participants had an average absolute CD4 count of 506.22 (SD 330.67) with a range of 78 to 1853. Of the 48 participants, 47 (98%) did not receive the HPV vaccine in the past. Of the reported cases of HPV-related dysplasia (23.91% with VIN2/3, 2.1% with VAIN2/3, 50% with CIN2/3 and 15.2% with genital warts), treatment was received for 100% of VIN2/3 and VAIN2/3, 95.65% of CIN2/3 and 57.13% of genital wart cases.

Table 1: Participant Demographics (N=48)

Measure	*n*	%
Ethnicity		
Hispanic/Latino	13	27
Not Hispanic/Latino	35	73
Race		
Black or African American	30	62.5
White	10	20.8
Unknown	8	16.7
Place of Origin		
North America	26	54.2
South America	2	4.2
Africa	10	20.8
Europe	1	2.1
Unknown	9	18.8
Age in years		
30-39	2	4.2
40-49	15	31.3
50-59	21	43.8
60-69	10	20.8
Marital Status		
Single	32	68.1
Married	10	21.3
Divorced	4	8.5
Widowed	1	2.1

Table 2: Baseline Clinical Information

Measure	*n*	%
Age at HSIL Diagnosis (years)		
Mean	51.75 (7.86)	
Median	53	
Range	35-66	
Year of HIV diagnosis		
1980-1989	8	18
1990-1999	19	42
2000-2009	16	35.6
2010-2018	2	4.4
Years of HIV at HSIL Diagnosis		
Mean	12.64 (8.64)	
Median	12	
Range	0 - 30	
Nadir CD4		
Mean	211 (205.15)	
Median	181	
Range	2-931	
> 200	20	41.7
< 200	28	58.3
CD4 at HSIL Diagnosis		
Mean	506.2 (330.7)	
Median	447.5	
Range	78-1853	
> 200	41	85.4
< 200	7	14.6

Table 2: Baseline Clinical Information *Continued*

Measure	*n*	*%*
History of Genital Dysplasia (reported)		
VIN 2/3	11	23.91
VAIN 2/3	1	2.17
CIN 2/3	23	50.0
Genital Warts	7	15.22
History of Treatment for Dysplasia (reported)		
VIN 2/3	11	100
VAIN 2/3	1	100
CIN 2/3	22	95.65
Genital Warts	4	57.13
Smoking History		
Never Smoker	*13*	28.3
Current Smoker	*22*	45.8
0-20 pack years	*10*	45.5
20-40 pack years	*6*	27.3
41+ pack years	*3*	13.6
Unknown	*3*	13.6
Past Smoker	*11*	22.9
0-20 pack years	*6*	54.5
20-40 pack years	*2*	18.2
41+ pack years	*0*	0
Unknown	*3*	27.3
Unknown	*2*	4.2

Table 2: Baseline Clinical Information *Continued*

Measure	*n*	*%*
History of HPV Vaccine		
Yes	1	2
No	47	98
History of AIDS Defining Illness		
Yes	7	14.6
No	41	85.4
Currently on HIV medication		
Yes	46	95.8
No	2	4.2

Study participants were followed for an average of 3.79 years (SD 2.97) from the date of anal HSIL diagnosis to the date of last follow up, and had an average of 7.85 anal evaluations (SD 4.92) during this time with a range of 2 to 22 visits. Treatment occurred on the day of HSIL diagnosis for 10 participants (20.8 %). The other 38 participants (79.2%) received their first treatment at a follow up visit. Days between diagnosis and treatment of anal HSIL averaged 204.7 days (SD 354.54) and ranged between 0 and 1890 days. For the first treatment procedure, 8 participants (16.67%) received hyfrecation in the clinic, 26 (54.16%) received ablation in the OR, 9 (18.75%) received IRC, 2 (4.16 %) received imiquimod, and 2 (4.16 %) received 5-FU. Participants were treated an average of 1.98 (SD 1.31) times after diagnosis of HSIL, with a range of 1-5 times during the period they were being followed. One

participant developed anal SCC during follow up. Visit details are summarized in table 3.

Table 3: Visit Information (N=48)

Measure	*n*	%
Number of Visits		
Mean	7.85 (4.92)	
Median	6 (4.5 – 10.5)	
Range	2-22	
Years in Follow up		
Mean	3.79 (2.97)	
Median	2.62 (1.56 -5.51)	
Range	0.44-13.39	
Days Between Diagnosis and Treatment		
Mean	204.67 (354.54)	
Median	87.5 (41.5-189)	
Range	0-1890	
Number of treatments after diagnosis		
Mean	1.98 (1.31)	
Median	1	
Range	1-5	
Time to first recurrence (days) (n=29, 60.4 %)		
Mean	772.9	
Median	441 (194-1061)	
Range	91-3325	
Type of treatment		
Hyfrecation in clinic	8	16.67
Ablation in OR	26	54.16

Table 3: Visit Information (N=48) *(Continued)*

Measure	*n*	%
Type of treatment *(Continued)*		
IRC	9	18.75
Imiquimod	2	4.16
5-FU	2	4.16
Other	1	2.08
Baseline Anal Cytology (n=25)		
Normal	8	32
ASC-US	8	32
LSIL	5	20
HSIL	4	16
Baseline anal HR-HPV(n=15)		
Yes	12	80
No	3	20

Of the 48 women included in the study, recurrence occurred in 29 (60.4%) participants, with a mean time to recurrence of 25.4 months (95% CI 15.4 – 35.4). Figure 7 demonstrates the probability of recurrence over time. The Kaplan Meier curve predicted that the probability of anal HSIL recurrence was 26% at 12 months, 46% at 24 months, 53% at 36 months, and 60% after 48 and 60 months.

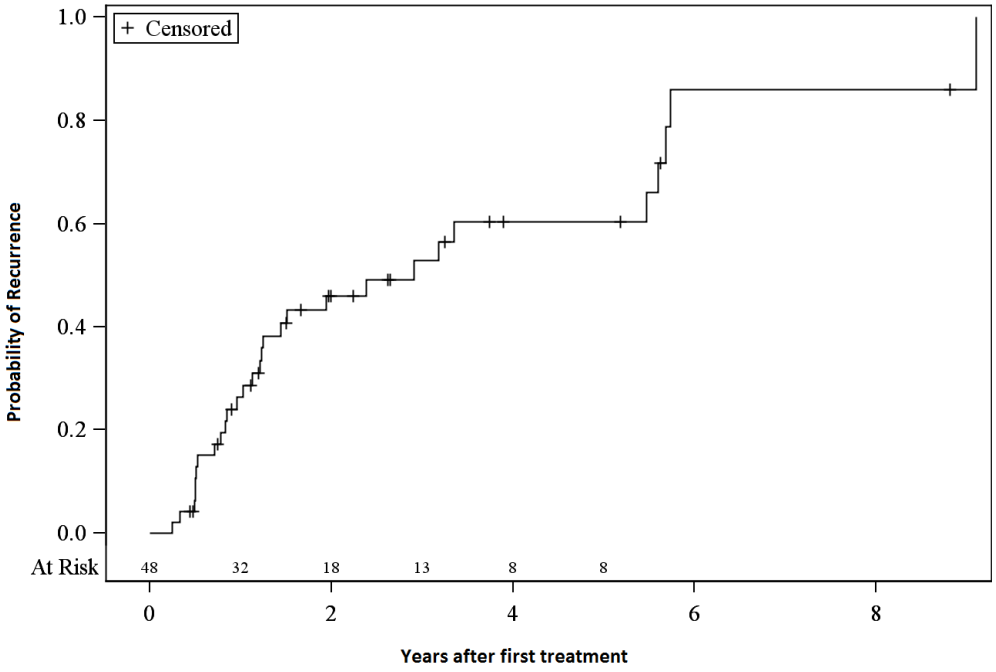

Figure 7: HSIL Recurrence in HIV-positive women after anal HSIL treatment

DISCUSSION

With the increase of anal cancer incidence in the past few decades, there is a strong need to develop a standardized approach for management of anal HSIL. Most research on the effectiveness of anal HSIL treatment in the HIV+ population has been done on HIV+ MSM and little is known about anal HSIL management in HIV+ women. In this retrospective study, we followed 48 HIV+ women that received either topical or ablative treatment for anal HSIL and we were able to demonstrate that the post-treatment recurrence rate of anal HSIL in HIV+ women is high.

Increasing evidence supports that anal cancer is preceded by HSIL. Retrospective studies show an 8.5 to 13% progression to anal SCC when HSIL is monitored but not treated.[27] It is hypothesized that the cervical cancer model of treatment or removal of HSIL to prevent the progression to cancer in the cervix can successfully be applied to the anal canal to prevent anal cancer. In our study, one person out of the 48 followed (2.08%) unfortunately developed anal SCC. We expect that this number would have been higher had we observed HIV+ women that were being monitored but not treated. This individual was a 40-year-old female with a history of AIDS defining illness, anal fistulas and abscesses, VIN3, and CIN3, who received treatment of anal HSIL via ablation in the OR. She was later found to have a cancerous mass near the internal opening of an anal fistula tract.

In our study, patients who received treatment for anal HSIL were at high risk for recurrence. The median time to recurrence was 14.5 months after treatment. A

total of 60.4% of our 48 participants recurred during follow up. The probability of recurrence was 24.5% at 12 months and 59% at 60 months. Because of the limited data available for the effectiveness of anal HSIL treatment in HIV+ women, we compared our data with data from studies looking at the effectiveness of anal HSIL treatment in HIV+ MSM as well as the general HIV+ population. These studies varied in study design, treatment modalities, definitions of recurrence and outcomes measured (HSIL vs ASIL), duration and method of follow up. This was taken into consideration when comparing the information to our results.

The first prospective study of the surgical management of anal HSIL, conducted by Chang et al. in 2002, followed 29 HIV+ MSM that received ablation in an OR setting and found that 79% had persistent/recurrent HSIL with a mean time to recurrence of 12 months, with risk of recurrence approaching 100% by 50 months.[27] The time to recurrence was similar to our median time of 14.5 months. This study only included participants with a large volume disease and this may have contributed to the high recurrence rate. In 2016, Burgos et al studied the effectiveness of in-office electrocautery ablation of anal HSIL in HIV+ MSM and found 25.5% recurrence and 33.7% persistence. It was estimated that the probability of recurrence was 27.6% at 12 months and 48.3% at 24 months.[28] The study involved multiple ablative treatments as opposed to our study which followed patients after one course of treatment.

Studies have shown that treatment outcomes and overall recurrence rates for IRC and electrocautery ablation are similar. Goldstone et al. compared anal HSIL recurrence rates after IRC ablation in 44 HIV+ and 52 HIV- MSM and found that HIV+ MSM were 1.9 times more likely than HIV- MSM to recur after their first IRC treatment. They also determined that HIV+ MSM had recurrences twice as quickly as HIV- MSM, with a median time to recurrence in HIV+ MSM of 12 months and HIV- MSM of 24 months.[26] Recommendations from this study suggested that HIV status be a factor when determining the length of surveillance intervals after IRC. Marks and Goldstone followed 132 HIV+ MSM that received in-office electrocautery and found 63% of HIV+ MSM recurred over a median of 20 months, similar to earlier data regarding treatment outcomes after IRC.[29] Although we did not look at the different treatment modalities separately due to the small sample size, the overall recurrence rate is consistent with these findings.

In a retrospective study looking at the effectiveness of IRC in both HIV+ men and women, Sirera et al. found only 7 patients out of 56 (12.5%, 2 women and 5 men) had recurrence of anal HSIL after a mean follow up of 25 months post- IRC treatment. It should be noted that the evaluation of recurrence was by cytology and DARE; given the known poor correlation of anal cytology for the detection of anal HSIL, it is likely that the results represent a lower bound estimate. In addition, the study explained the low recurrence rate by the characteristics of the study population: most patients had only one high-grade lesion to treat and had good

immunological status.[30] The immunological statuses of our study participants were more assorted. 58.3% had a nadir CD4 of less than 200, 14.6 % had a history of an AIDS defining illness, and years of HIV diagnosis at the time of HSIL diagnosis ranged from 0 to 30.

Unlike the similarity between electrocautery and IRC treatment outcomes, past research shows that the recurrence rate of topical treatments tends to be consistently higher than ablative treatments. Richel et al. compared treatment response between imiquimod (n=24), 5-FU (n=16) and electrocautery (n=10) in HIV+ MSM in a randomized controlled trial where participants were assigned to receive either intra-anal or perianal treatments for 16 weeks via imiquimod, 5-FU or electrocautery. A complete response occurred in 13 out of the 54 patients (24%) in the imiquimod group, 8 of the 48 (17%) in the 5-FU group and 18 of the 46 (39%) patients in the electrocautery group. No response occurred in 26 (48%) patients in the imiquimod group, 29 (60%) in the 5-FU group and 15 (33%) in the electrocautery group. Recurrence occurred in 22 out of 48 (46%) participants with responses. This included 47% of patients assigned to the imiquimod arm, 50% assigned to 5-FU and 43% assigned to electrocautery. It was shown that imiquimod and 5-FU had lower complete responses and higher recurrence rates when compared to electrocautery. Perianal AIN responded better to the topical treatments than intra-anal AIN.[31] Unlike our study, this study included, and grouped together, participants with low-grade and high-grade lesions, making it difficult to

compare our recurrence rates to theirs. Also unlike our study, participants in the electrocautery group received multiple treatments as opposed to one in-office or OR procedure.

Limitations

Our small sample size and the relatively short follow up for a number of patients reduced the power of our study and increased the margin of error. Our sample size was limited due few number of people diagnosed with HSIL as well as the number of patients that were lost to follow up. Between 2004 and 2017, only 84 HIV+ women were diagnosed with anal HSIL at BMC. Patients were excluded from analysis due to being lost to follow up either after diagnosis or immediately after treatment. Inclusion criteria only required that participants had at least one follow up visit after treatment. Some patients were followed for only a short amount of time, reducing the sample size available as the time from treatment increased. A larger cohort being followed for a greater amount of time would have led to more significant findings. Moreover, our study only includes patients that were seen at BMC and it is not known if these findings are generalizable other populations of HIV+ women.

Because recurrence was determined by biopsy-proven HSIL, the rate of recurrence may have been underestimated due to HSIL present that was not detected during the HRA, patients deferring HRA and biopsies during their anal evaluation, or patients missing visits. Since the size of the lesions or presence of

multifocal lesions were not recorded, we were unable to assess the impact this information had on recurrence rates.

Future Directions

Because of the small sample size, we were unable to look at the impact potential factors such as length of HIV at anal HSIL diagnosis and nadir CD4 counts on treatment outcomes. Future studies could analyze the impact of such factors to determine if a patient's baseline clinical data has a significant impact on treatment outcomes.

Studies have shown that despite high recurrence rates, there is a decrease in incidence of anal HSIL with multiple ablative treatments.[32] Our study only looked at outcomes following the first treatment of anal HSIL but it would be informative to see recurrence rates following the second and third treatments. Additionally, this study looked at several different treatment modalities but did not differentiate between them during data analysis. Given a larger sample size, future studies can look at the treatment-specific recurrence rates and determine if one mode of treatment results in a longer recurrence-free survival than the others.

Possible future steps could include determining the effect of the qHPV on anal HSIL recurrence in HIV+ women. Studies suggest that the Quadrivalent Human Papillomavirus (qHPV) vaccine reduces the rate of recurrence of anal HSIL in MSM and cervical, vulvar and vaginal HSIL in women.[33] However, a recent prospective trial found that qHPV vaccination of adults with HIV did not prevent persistent anal

HPV infections nor improve anal HSIL outcomes, although it was possible that there might have been improved outcomes with longer follow up.[34]

Conclusion

Because of the high recurrence rates in HIV+ women, this high-risk population would benefit from close surveillance, similar to HIV+ MSM. Furthermore, as can be seen in our survival analysis, probability of recurrence approaches 100% over time, suggesting that long-term recurrence-free survival is unlikely in HIV+ women and this population needs long-term follow up.

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CURRICULUM VITAE