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Routine Anal Cytology Screening for Anal Squamous Intraepithelial Lesions in an
Ethnically Diverse Urban HIV Clinic

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
Hyman M. Scott
2006

ROUTINE ANAL CYTOLOGY SCREENING FOR ANAL DYSPLASIA IN AN ETHNICALLY DIVERSE URBAN HIV CLINIC

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Anal cancer, like cervical cancer, is associated with Human Papillomavirus (HPV) infection. HIV+ patients have 38-60 fold increased risk of anal cancer compared to HIV- patients prompting many to suggest routine screening given the success of cervical Pap screening. Our goal is to describe our experience with routine anal Pap screening, determine which patients are most likely to have abnormal results, if anal disease on physical exam is predictive of cytology, and correlate cytology with histology findings. Charts of all patients with an anal Pap followed at the Hospital of Saint Raphael HIV Clinic were reviewed. Demographics, immune status, sexually transmitted disease history, cytology and histology data was extracted from medical charts. Patients with an anal Pap between November 1, 2002-November 30, 2004 were included. Those with an insufficient sample were excluded. Analysis was done using χ^2 for comparison of proportions and student t-test for continuous variables. Overall, 265/560 HIV+ patients had at least one anal Pap. Seventy-four of these 265 patients had an abnormal anal Pap. Mean age was 44 yrs, and 68% were men. Fifty-nine percent were African American, 34% White, and 17% Hispanic. Those with an abnormal Pap were more likely to be White ($p=.03$), and be gay or bisexual men ($p=.02$). They were also more likely to have lower CD4⁺ nadir (142 vs 223, $p=.005$) and CD4⁺ at time of anal Pap (353 vs 497, $p<.001$). Those with an abnormal anal Pap also had more anal disease (30% vs 9%, $p<.001$), history of warts (23% vs 12%, $p=.02$) and herpes (35% vs 22%, $p=.02$). Anal disease on physical exam had a sensitivity of 56% and specificity of 77% for abnormal cytology findings. On histology two patients had Anal Intraepithelial Neoplasia (AIN) I, 2 AIN II, 3 AIN III, and 2 Squamous Cell Carcinoma In Situ. There was no correlation between cytology and histology. Routine anal cytology screening is a feasible tool to incorporate into an ethnically diverse HIV clinic for identifying precancerous anal lesions, a group which has been largely overlooked. Anal disease on physical exam is a poor predictor of abnormal cytology and there was no correlation between severity of disease on cytology and histology. However, further follow-up study is required to determine the impact on morbidity and mortality.

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Introduction

Since the advent of Highly Active Antiretroviral Therapy (HAART) there has been a significant decline in mortality and morbidity in western countries due to Human Immunodeficiency Virus (HIV) (1). Despite this improvement in the health of those infected with HIV, there are approximately forty thousand new infections each year in the United States (2). As many of these patients receive appropriate therapy and opportunistic infection prophylaxis, they are living longer with HIV and an incompletely reconstituted immune system. As a result, they are at increased risk for development of malignancies that have not historically been associated with HIV infection or Acquired Immunodeficiency Syndrome (AIDS) (3).

Since the early stages of the AIDS epidemic, an increased risk of malignancy has been associated with HIV infection. Two malignancies, Kaposi's sarcoma and primary CNS lymphoma, are currently part of the CDC's surveillance definition of AIDS (4). HIV positive women are at increased risk for invasive cervical cancer. Thus invasive cervical cancer was added to the case definition of AIDS in 1993 (4). Two of these diseases are strongly associated with viral infections, Human Herpes Simplex Virus (HHSV) 8 and Human Papillomavirus (HPV) for Kaposi's sarcoma and invasive cervical carcinoma respectively (5, 6). CNS lymphoma has been associated with Epstein Barr Virus (7). The role of HPV in the development of cervical cancer has been extensively studied in developed nations.

Human Papillomavirus and Cervical Cancer

Human Papillomavirus is an epitheliotropic virus and while initially included in the broader group of polyomaviruses, they were found to be molecularly distinct with later

molecular study (8). Currently there are more than one hundred genotypes of HPV which have been identified according to divergence in three of the virus's known genes, E6, E7, and L1. The classification of the different genotypes is dependent on the differences between the structural (L1 and L2) and the non structural genes (E1, E2, E4-E7). Genotype classification is based on 10% or greater divergence from known genotypes. Several of the genotypes have been classified as low, intermediate, and high risk in accordance with the degree of demonstrated oncogenicity. HPV has tropism for the cervical transitional zone where the epithelium transitions from columnar to squamous. It is within the transitional zone that the cervical dysplasia occurs and the site of targeted local excisional therapy. The most common high risk HPV types found in high cervical lesions are 16 and 18; however, other HPV types including 31 and 33 have also been associated with cervical dysplasia.

The natural history of HPV infection of the female genital tract has been elucidated primarily by cross-sectional and longitudinal studies (9-12). HPV infection in the female genital tract is fairly uncommon until women become sexual active during late adolescence or early adulthood. In the US, population prevalence studies have demonstrated that up to 10-20% of women between the ages of 15-49 have molecular evidence of infection (13). It is estimated that greater than 90% of these infections are subclinical, latent, or have been cleared (14). Cohort studies among college women found that high rates of women acquired HPV at some point during college (15, 16). However, only 9% of these women had persistent infection after two years of follow-up (16). Among those who developed cytological lesions, lesions often regressed and there is often spontaneous clearance of the virus (17). This is reflected in the declining

prevalence of HPV infection in middle-aged women (18). Although the occurrence of spontaneous cervical HPV clearance has been documented, its exact mechanism is unknown.

Despite this high rate of clearance, it's estimated that 10-70% of women with confirmed dysplastic lesions will eventually progress to high grade lesions or invasive cervical cancer depending on the initial lesion severity (19). Although less than 25% of women with high grade cervical lesions will progress to invasive cervical cancer, it appears that the persistent presence of HPV is a common risk factor for this eventual progression (20, 21). These lesions are often associated with the known high risk HPV types 16 and 18 (22, 23). The association between HPV infection, especially of high risk genotypes, and cervical cancer has been recognized and cervical cancer is now widely viewed as a sexual transmitted disease (11, 24, 25). Indeed, high risk type HPV infection and cervical cancer share many of the same risk factors of early first sexual intercourse, multiple sexual partners, and smoking (26). Fairly early in the epidemic, HIV induced immunosuppression has been associated with an increased risk of cervical cancer. This association led to the inclusion of invasive cervical as an AIDS defining diagnosis in the CDC 1993 revised AIDS Surveillance case definition.

The increased risk of cervical dysplasia and squamous cell carcinoma among HIV positive women has been well documented in the literature (27, 28). In one prevalence study, HIV positive women were found to have significantly higher rates of cervical lesions than HIV negative women, 26.5% and 7.5% respectively (28). HIV positive women who were more immunosuppression ($CD4^+ < 200$ cells/ml) had the highest rates of dysplasia. Although they were unable to demonstrate a significant direct interaction

between HPV and HIV, persistent HPV infection likely partially explains these increased rates of cervical dysplasia. Sun and colleagues showed that among a cohort of women in New York City that 56% of HIV positive women were HPV positive at initial examination compared to 31% of HIV negative women (29). Among these women, 24% of the HIV positive women had persistent infection (same HPV type detected on two or more examinations) compared to only 4% of HIV negative women. The HIV positive women also had higher rates of high risk HPV types 16 and 18 compared to their HIV negative counterparts. In their multivariate analysis HIV positive women, including those with CD4 counts above 500 cells/ml, being unmarried and having a history of injection drug use were all associated with an increase risk of persistent HPV infection. In addition to persistent infection, there is evidence that higher levels of high risk HPV viral replication occur in HIV positive women with dysplasia (30). The role of HIV on the increased rates of cervical dysplasia has yet to be elucidated, although it appears that it is secondary to immunosuppression and not a direct interaction between the two viruses (31). In addition to the increased prevalence of cervical dysplasia, HIV positive patients also have decreased rates of regression. Together, these factors have likely contributed to the higher rates of advanced cervical dysplasia and carcinoma in HIV positive women.

Although the rates in the general population have been relatively low since the introduction of routine screening, HIV positive women have continued to carry an excess burden of disease. Serraino and colleagues analyzed data from two European longitudinal databases, both part of a cohort of HIV positive women followed-up as part of a HIV Seroconverter Study (32). They found ten cases of invasive cervical carcinoma (ICC) among 2141 HIV positive women with an expected incidence of less than one. No

cases were identified among the 811 HIV negative women. Eight of these ICC cases were among HIV positive intravenous drug users. Similar increased rates have been described in the United States (33). However, in another US prospective study of women with or at risk for HIV infection, which included biannual Pap smears, only one incident case of ICC was found during 8260 woman-years of follow-up (34). Despite this controversy over prevalence, HIV positive women with cervical neoplasia have poorer outcomes and more relapse after therapy (35, 36).

Unlike the other AIDS associated malignancies, there has been debate over the effect of HAART in the incidence and natural history of cervical dysplasia. In a cohort study of women in several US cities followed with cervical Pap smears every six months, Minkoff and colleagues examined the impact of being on HAART on the short-term (at 6 months follow-up) cytological progression or regression of cervical lesions (37). They found that women who had initiated HAART were more likely to have regression of their lesions and less likely to have progression after adjusting for CD4 count and cervical Pap smear status. The positive impact on regression of cytological diagnosed lesions was confirmed by Ahdieh-Grant and colleagues who also found that HAART was associated with an increased likelihood of regression of lesions (38). However, they also noted that the majority of lesions did not regress to normal, even among those on HAART.

Several other studies have not demonstrated the beneficial effect of HAART on cervical dysplasia and invasive cervical cancer (3, 39-41). In a cohort of HIV positive women treated for a mean of 15.4 months, Lillo and colleagues did not find a difference in progression of cervical lesion between those receiving HAART and those who were not (42). Patients receiving HAART in this study experienced a significant increase in

CD4 count although no significant decrease in HIV viral load was noted. This was likely explained by the fact that those who were treated were often HAART-experienced at time of enrollment. Furthermore, in a meta analysis of twenty-three cohort studies with 1000 or more patients each, there was no trend toward a decrease in the incidence of cervical cancer after the introduction of HAART (41). A small study by Durruci and colleagues examined the incidence of invasive cervical cancer in an Italian cohort of women with known HIV seroconversion dates (39). Although there were only six cases of ICC, they found a significant increase in the number of cases during the two year post-HAART era.

Anal Cancer

The role of HPV in the development of cervical cancer has pointed toward the possibility of its role in the development of other anogenital cancers, notable anal carcinoma. Anal carcinoma shares many similarities with cervical cancer in anatomy and histology as well as its association with HPV infection (43-45). Anal cancer arises at the dentate line where the columnar epithelium transitions to squamous within the anal canal. Despite early opinion that anal cancer was secondary to chronic inflammation, there is considerable evidence that like cervical cancer it is caused by HPV infection (46-49). Initial reports demonstrated those with anal cancer had an increased risk of having had anal warts or other STDs (44). In addition, women with a history of cervical cancer have an increased risk for developing anal carcinoma. This risk has also been associated with the same high risk HPV subtypes as cervical cancer.

Among the general population, anal cancer is relatively rare with women having higher incident rates than men. Annual incident rates range between 0.9-1.3/100000 for women and 0.3-0.7/100000 for men (50-52). In addition to the increased risk of anal

cancer among women with a history of cervical cancer there has also been an increased risk of anal cancer among men who have sex with men (MSM) prior to the AIDS epidemic. Indeed while 8% of anal cancer cases were among MSM during 1959-67, this rose to 72% during 1978-86 (53). The current estimated incidence is 37/100,000 among men with a history of receptive anal intercourse and is estimated to be approximately twice as high among the same group of men who have HIV (51, 52, 54).

Although, anal intercourse has been implicated as increasing the risk of anal cancer, its exact causative role has been questioned. Frisch and colleagues conducted a population-based case control study to determine the role of sexual practices in the development of anal cancer in Denmark and Sweden (44). Women with more sexual partners and a history of anal intercourse were at higher risk for anal cancer compared to control patients with adenocarcinoma as well as the general population. Among men, there was a positive correlation between increasing number of sexual partners and risk of anal cancer, regardless of sexual practice. Approximately 90% of the cases and controls were positive for HPV and 73% of these cases had high risk HPV type 16. Although they found a history of anal sex increased the risk for anal cancer, most men and women who had anal cancer in this study did not engage in anal sex.

In addition, the presence of anal HPV infection in heterosexual men and women with no history of anal intercourse appears to point to another possible mechanism for anal HPV acquisition. Among heterosexual men with HPV positive female partners, 8% had anal HPV detectable (55). Furthermore, as many as 33% of heterosexual men presenting to a clinic with current or history of anal warts had clinical evidence of anal HPV infection (56). This relatively high infection rate was not correlated with receptive

anal intercourse or anodigital insertion. Although, HPV infection in the absence of obvious risk factors among heterosexual men and women implicates a potential field effect for infection, other studies have receptive anal intercourse in men as a risk factor (57-59).

Increased rates of anogenital cancer among renal allograft recipients first implicated the role of immunosuppression on cancer development. These patients have an 100 fold increased rate compared to the general population (60). High prevalence of HPV infection and multiple infections likely play an early role in this carcinogenesis (61). A similar association has been noted among HIV positive patients.

As noted earlier, increased rates of anal cancer were initially seen prior the beginning of the AIDS epidemic (59). During this time, a population based open-cohort study of never-married men in San Francisco demonstrated an increase in incidences of Kaposi's sarcoma and non-Hodgkin's lymphoma (59). The incidence of anal cancer was 9.9 times higher than expected during the period 1973-1979 and 10.1 times higher during 1988-1990. The increased incidence among a population with a large number of MSM prior to the AIDS epidemic suggest that there was an increased rate of HPV transmission in a high risk sexual group. However, in a study by Melbye and colleagues comparing AIDS registries and cancer registries found an increased relative risk of 63.4 for those with AIDS compared to the general population (62). Among those with AIDS, MSM had a relative risk of 84.1 and non-MSM of 37.7. Despite these large relative risks, the study's primary limitation was the small number of cases (31 anal cancer, 8 anorectal cancer). Other studies have supported these findings and also demonstrated increased rates of anal cancer among HIV positive men (58, 63). Palefsky and colleagues

demonstrated that 36% of HIV positive MSM had anal dysplasia compared to only 7% of HIV negative men (63). Identified risk factors associated with anal dysplasia were high levels HPV infection with oncogenic subtypes and reduced CD4 levels. Interestingly, among HIV positive men, significant risk of anal dysplasia was seen in the absence of severe immune suppression. Similarly, Critchlow et al demonstrated among a group of homosexual and bisexual men with initial negative cytologic and anoscopy findings that HIV positive men had higher rates of anal dysplasia compared to HIV negative patients (15.2% vs 5.4%) (58). They also were able to demonstrate HIV infection as an independent risk factor for development of anal dysplasia after controlling for HPV infection.

Increased rates of high risk HPV subtypes appear to partially explain this increased risk among HIV positive MSM. These men are more likely to have infections with multiple HPV types, acquire new HPV infections, and have less spontaneous clearance of HPV infection (64). In a community based longitudinal study, Critchlow and colleagues found that HIV positive men had higher rates of high-risk HPV subtypes (16, 18, 45, or 56) as well as higher HPV viral loads compared to HIV negative men. HIV seropositivity, however, was not associated with increased rates of low or intermediate risk HPV subtypes. HIV positive men were also more than twice as likely to acquire a new HPV subtype during follow-up, although this acquisition did not appear to be related to increased rates of unprotected receptive anal intercourse. Furthermore, HIV positive men were less likely to clear HPV infection on subsequent follow-up visits compared to HIV negative men (13% vs. 45%). This discrepancy was not related to HPV subtype. Among HIV positive men HPV persistence was associated with HIV DNA

presence in the anal canal and not with CD4⁺ count. Other studies have also shown that HIV associated immunosuppression increases the risk of anal HPV detection (43, 65, 66).

Furthermore, HIV positive men are more likely to have progression from low grade anal dysplasia to high grade dysplasia than HIV negative men (67-70). In a study by Palefsky and colleagues, HIV positive men had more than two-fold increase in relative risk for progression to high grade dysplasia compared to HIV negative subjects and were also less likely to have regression of lesions (67). Although baseline diagnosis of anal disease was not predictive of progression, subjects infected with one or more HPV subtypes were at increased risk regardless of HIV status. One cohort study has shown that as many as 49% of HIV positive homosexual and bisexual men developed high grade anal dysplasia over the course of a four year period compared to 17% of HIV negative homosexual and bisexual men (68). These high grade lesions are thought to be precancerous lesions with potential to progress to invasive carcinoma (45, 67, 71).

Treatment for anal cancer is limited and outcomes are poor with many patients have recurrence of disease (72). Surgical intervention was initially the treatment modality of choice and included local resection for limited disease and abdominal peritoneal resection with colostomy for more advanced disease. Although abdominal peritoneal resection was associated with significant morbidity, local resection is limited by inability to achieve complete resection and subsequent recurrence of disease (73). More recently, chemotherapy and radiation individually and in combination are being used with varying degrees of success (74, 75). However, combination therapy outcomes appear to be poorer among HIV positive patients, especially in the setting of severe immunosuppression (76-78).

Similar to cervical cancer, there is controversy on the impact of HAART on anal carcinoma with many of the studies limited by small sample size (79, 80). While Stadler and colleagues found a trend toward better outcomes in patients on HAART, their results were not significant. Linking cancer and AIDS registries, Diamond and colleagues found that anal cancer rates increased from 49/100,000 in the pre-HAART era to 122/100,000 in the post-HAART era (81). Although patients in the post-HAART era were as immunocompromised as the patients in the pre-HAART era, they had a longer duration of HIV diagnosis. It is likely that this longer immunosuppression allows prolonged HPV infection resulting in greater risk of carcinogenesis. Furthermore, patients that had been receiving HAART for a median of 32 months had similar rates of anal dysplasia compared to those not on HAART, despite immune recovery (82).

Routine cervical Papacounia (Pap) smear screening has been associated with a significant decrease in the incidence of cervical cancer from 40-50/100,000 to approximately 9.7/100,000 (83). Based on the success of the cervical cancer screening program many experts have suggested that routine anal cytology (anal Pap) screening should be performed on high risk individuals to detect and remove precancerous anal lesions (similar to the cervical Pap model) (84-86). It has been shown to be a cost-effective procedure with benefit comparable to other preventative medical protocols used in HIV care (87, 88).

Although most available data on anal carcinoma is for homosexual and bisexual men there is also an increased incidence among patients without a history of receptive anal intercourse (69, 89). As much of the evidence for anal cancer screening programs is among HIV positive MSM, there is not substantial data on anal dysplasia and anal cancer

screening in a diverse population of HIV positive patients seen in an urban HIV clinic. Our goals are to describe our experience with a routine anal Pap screening program in an urban HIV positive population, to determine if anal disease on exam is predictive of abnormal anal Pap findings, and if abnormal cytology correlates with anoscopy and histology findings.

Materials and Methods

This study was conducted with the approval of the Institutional Review Board, Hospital of Saint Raphael and the Human Investigations Committee, Yale School of Medicine. In November 2002 we implemented an anal cancer screening program, using anal Paps, into our HIV clinical care. All HIV positive patients seen at the Haelen Center were offered anal Pap smears as part of routine clinical care (Figure 1). Briefly, an anal Pap is performed using a moistened Dacron swab. The swab is inserted 2-3 cm into the anal canal to reach the anal verge. The swab is rotated 360 degrees as it is pulled out. After removal the sample was smeared onto a glass slide and fixative immediately applied. All cytology samples were read by one pathologist trained in interpreting anal cytology at Hospital of Saint Raphael Pathology Department and reported as one of the following: negative for dysplasia, Atypical Cells of Undetermined Significance (ASCUS), Anal Intraepithelial Neoplasia (AIN) I, AIN II, or AIN III. The cytology reports interpreted as AIN were recoded as Low-grade Squamous Intraepithelial Lesion (LSIL) for AIN I and High-grade Squamous Intraepithelial Lesion (HSIL) for AIN II and AIN III. Patients with abnormal anal Pap findings, classified as ASCUS or greater, were referred for surgical follow-up or received another screening anal Pap. Patients with an

abnormal anal cytology received high resolution anoscopy with biopsy of any obvious lesions seen with addition of 3% acetic acid. All samples were imbedded in paraffin for routine histopathologic examination. Biopsy results were reported as AIN I, AIN II, AIN III, or Squamous Cell Carcinoma In Situ (SCCIS) using Bethesda Staging criteria. We then retrospectively reviewed charts on all HIV positive patients that received anal Pap smears in the Haelen Center at Hospital of Saint Raphael between November 2002 and November 2004.

Medical Record Review

Outpatient medical charts were reviewed for 276 patients with anal Pap smears during a two year period between November 2002 and November 2004. The following data was extracted: Demographics [Age, Sex, Race, HIV risk factor(s)], CD4 count, CD4 nadir, HIV viral load, current use and history of antiretroviral therapy (ART), history of herpes simplex virus infection, history of genital warts, anal disease on physical exam, history and stage of cervical dysplasia for women, history of any other genital-urinary (GU) disease, result of anal Pap smear, and results of high resolution anoscopy findings for those patients with abnormal cytology findings. For patients with more than one anal Pap the following additional data was extracted for all subsequent Pap smears: reason for additional Pap, decline in CD4 since last anal Pap, and any new GU disease. Data for all patients with an anal Pap smear were coded and entered into a Microsoft Excel spreadsheet. Ten percent of charts were randomly selected and verified by a non-primary data collector with <5% variation between independent evaluations. Surgical follow-up data on all patients with abnormal Pap smears were collected beyond November 2004 to capture those with delayed surgical evaluation data. All primary data collection was

carried out by Hyman Scott and Joe Khoury, MD; surgical follow-up data was collected by Hyman Scott.

Statistical Analysis

For those patients with more than one anal Pap the highest grade cytological abnormality was used in the analysis. Eleven patients with insufficient or missing cytology results were excluded. For patients with biopsy results, the highest grade histology was used. The patients with complete high resolution anoscopy results were analyzed separately for anal dysplasia risk factors. Bivariate analysis was done using χ^2 for comparison of proportions and student T-test for continuous variables using SPSS software (SPSS Inc, Chicago IL). Comparisons were considered significant at $p < 0.05$ (two tailed). All statistical analysis was carried out by Brent A. Moore, Ph. D.

Results

Study Population

During the two year study period, 560 HIV+ patients were seen for at least one routine clinical visit (Figure 2). Two hundred seventy six patients (49%) received at least one screening anal Pap during this time. Eleven patients were excluded from analysis: ten for insufficient anal Pap sample and one for missing record data. Ninety-two patients had more than one anal Pap done during the study period. Of these 92 patients, 72 had two anal Paps, 19 patients had three anal Paps, and one patient had four anal Paps. Patients had repeat anal Paps for many different reasons. Fifty-one patients had only normal repeat anal Paps as part of annual screening. Four patients had a repeat Pap after an initial Pap showed insufficient sample. Thirty-four patients had a repeat Pap to

follow-up on an original abnormal anal Pap. Three patients had a repeat anal Pap for an undocumented reason. Table 1 describes the study population of the 266 patients who received an anal Pap smear. The demographics of the patients receiving an anal Pap are similar to the demographics of the entire clinic population (data not shown). Notably 66% were African-American or Hispanic and only 28% reported MSM as their HIV risk factor. This is an advanced HIV population which is heavily treated with antiretrovirals.

Normal vs Abnormal Pap Comparison

Seventy-four of 265 (27.9%) of subjects had at least one abnormal anal Pap smear (Figure 2). Twenty-four (32%) had ASCUS, 45 (61%) had LSIL and 5 (7%) had HSIL. The subjects with an abnormal anal Pap smear were significantly more likely to be white, have MSM as the HIV risk factor, have a lower CD4 nadir and lower CD4 count, as well as be on ART at the time of the anal Pap smear (Table 2). Forty-nine percent of the subjects with an abnormal anal Pap had heterosexual contact listed as their HIV risk factor. Patients with abnormal anal Paps were also more likely have anal disease on physical exam at the time of the anal Pap smear although only 30% of the patients with an abnormal anal Pap had anal disease on physical exam. Patients with abnormal Paps were also more likely to have a history of HSV or genital warts. Eighty-four women were included in the analysis and there was no significant difference in history of cervical dysplasia between those with abnormal anal Paps and those with normal anal Paps. However, complete data on cervical Pap smear and colposcopy results were not always accessible as many patients received gynecological care at different facilities. There was no difference between those with normal and abnormal anal Paps with respect to age,

gender, proportion with undetectable HIV viral load, history of ever having been on ART, or history of other GU disease beyond HSV or genital warts.

Predictive Value of Anal Exam

Twenty-two of thirty-nine patients had documented anal disease on physical exam at time of anal Pap and subsequently had an abnormal anal Pap. One hundred seventy four of two hundred twenty seven patients had no anal disease and a subsequent normal anal Pap. There were 17 patients with anal disease on exam and a normal anal Pap and 52 patients with no anal disease on exam and an abnormal anal Pap smear. The sensitivity of anal disease on exam for abnormal anal cytology is 56% and specificity of 77%. Anal disease on exam has a positive predictive value (PPV) of 30% and a negative predictive value (NPV) of 91% for predicting abnormal cytology.

Abnormal Anal Cytology Follow-up

As part of the routine screening program, patients with an abnormal anal Pap smear were to receive a surgical referral for high resolution anoscopy with biopsy of any suspicious lesions. Fifty of 74 patients (66%) with an abnormal anal Pap smear were referred for surgical evaluation (Figure 3). Of the 24 patients without a surgical referral, three patients were diagnosed with another malignancy, two patients moved away, and one patient was admitted to an extended care facility. Referral and follow-up data was lacking for the other 18 patients. Seven patients never showed-up for their surgical evaluation. Sixteen of the patients with a surgical referral did not receive an anoscopy. Two of these patients were unable to tolerate the anoscopy and the remaining 14 had either an anal exam or a repeat anal Pap. Fifteen patients had no abnormalities seen on anoscopy and three patients had lesions but no biopsy was taken (one patient was

pregnant and two for an undocumented reason). Nine patients had lesions seen on anoscopy and histology proven anal dysplasia (Table 3). Two patients were identified with AIN I, two with AIN II, three with AIN III, and 2 patients with squamous cell carcinoma in situ (SCCIS). Of these 9 patients, 8 with HIV risk factor information available (100%) were MSM compared to 7 (54%) of those patients with no lesions on anoscopy (two patients with no lesion on anoscopy and one patient with dysplasia had no HIV risk factor information available). In addition, 7 (78%) of the patients with biopsy proven dysplasia had anal disease on exam compared to 3 (20%) of the patients with no dysplasia. There was no difference between the two groups in age, CD4+ count at time of anal Pap, CD4+ nadir, or history of anogenital warts.

Cytology and Histology Association

In the follow-up data that was available, there was no association between cytology and histology findings (Table 3). Of the 2 patients with ASCUS on cytology who had an anoscopy, one had no lesions seen on anoscopy and the other had SCCIS. Similar discrepancies between the cytology and histological findings were seen in patients with LSIL and HSIL.

Discussion

We have demonstrated an ability to institute an anal cancer screening program as part of routine HIV care in an ethnically diverse inner city setting. During the study period we screened approximately half (49%) of the clinic population that was seen for HIV care which included a substantial number (38%) of women. Although, current recommendations are particularly targeted toward MSM for anal cancer screening, high

grade anal dysplasia has been demonstrated in 36% of intravenous drug users who have no history of receptive anal intercourse (70). In our study we found that 17% of patients with IVDU and 26% of patients with heterosexual sex as their only recorded HIV risk factor had abnormal anal Pap smears. In addition, 49% of those with an abnormal Pap smear had heterosexual contact listed as their HIV risk factor. This increased rate among heterosexual men and women is consistent with other published studies (69, 90).

However, the risk factors in our study were collected retrospectively and were based on coding by providers in clinic charts. Thus, we may underestimate the number of patients who have receptive anal intercourse. Nevertheless, our experience is more typical of a “real world setting” in which providers often do not routinely ask in-depth sexual history.

We found that patients with an abnormal anal Pap smear were more likely to have anal disease on physical exam compared to those with a normal anal Pap smear. However, despite a statistically significant difference, only 30% of those with an abnormal anal Pap smear had anal disease on physical exam. The vast majority (70%) of patients with abnormal Pap smear findings did not have anal disease at the time of their anal Pap smear. In addition, 17 (9%) of patients with a normal anal Pap had anal disease on physical exam. With a sensitivity of 56%, it would be a poor screening tool for determining which patients require routine anal cytology. Moreover, anal disease on physical exam is a poor predictor of abnormal cytology findings with a PPV of 30%. However, the absence of anal disease on physical exam had a NPV of 91% indicating that a negative anal exam may be a reliable predictor of negative cytology. Although this suggests that a negative anal exam may help identify which patients should receive a screening anal Pap, 23% of the patients without anal disease on exam had an abnormal

anal Pap. One explanation for lack of predictive value of anal disease on physical exam is that only the perianal area is visible on exam and whereas the anal Pap smear samples from within the anal canal, where anal dysplasia occurs within the transition zone. Furthermore, given that that HPV is considered the causative agent, the absence of obvious warts or other obvious lesions does not preclude the presence of HPV infection and epithelial dysplasia.

The patients with an abnormal anal Pap were also more likely to have a lower CD4 nadir and lower CD4 count at the time of the anal Pap smear compared to those with a normal anal Pap smear. Interestingly, these patients were more likely to be on antiretroviral therapy which reflects the treatment of their more advanced disease. However, although there is controversy whether anal carcinoma is responsive to highly active antiretroviral therapy (HAART), it does not appear to have an impact on anal HPV infection (70, 79, 80). HIV appears to have a local immunosuppressive effect in the anal mucosa that is not responsive to HAART even with increases in systemic CD4 counts and decrease in HIV viral load (43, 91, 92). This continued local immune suppression may be the mechanism via which HPV leads to anal carcinoma, as the decreased immune surveillance by dendritic cells allows transformed cells to grow unchecked (71).

The percentage of women with an abnormal anal Pap (36%) was proportionally equal to the percentage of women screened (32%) in clinic. It has been shown previously that women with a history of cervical dysplasia are at increased risk for developing anal dysplasia. We were not able to confirm this in our study, but this may be because cervical Pap smear or colposcopy results were not always available. Many of the women received gynecological care at another institution and records were not available for review.

Of the seventy four patients who were identified with cytological evidence of anal dysplasia, only twenty-four received high resolution anoscopy with biopsy of visible lesions. This highlights many of the barriers to instituting a cancer screening program into routine clinical care. The anal Pap smear was easily accepted by the patients and patients did not complain of untoward effects from this screening test. Yet, it was much more difficult to insure adherence with surgical anoscopy and biopsy follow-up. Barriers to surgical evaluation included patient-centered difficulties such as the perceived intolerability of the anoscopy procedure, fear of a cancer diagnosis, and general difficulties with maintaining clinic appointments. Furthermore, initially, as patients were referred for a surgical evaluation, there was confusion about the need and type of evaluation these patients required. Some patients were receiving high resolution anoscopy and aceto-whitening with biopsy of dysplastic lesions, while others received an anal exam or repeat anal Pap smear. With ongoing education to our surgical residents and focusing the anal dysplasia follow-ups to one clinic day a week (two clinic attendings) we were able to overcome this barrier although this led to a significant delay in follow-up (85, 93, 94). Lastly, even though the anal Pap smear was well accepted by patients only half the patients seen were actually screened. This is at least partly do to the complexity and advanced HIV disease stage of our patients. If other more urgent medical or HIV related problems were apparent (such as newly diagnosed malignancy), these would take priority and management of their anal disease would become a secondary issue. Despite these barriers, during the course of the screening program two patients (0.7%) were identified with squamous cell carcinoma in situ. These two patients have

been successfully treated with local therapy. They are currently being followed and are disease-free almost two years later.

Our data did not show consistency between cytology and histology findings. The three patients with HSIL on cytology had no lesions seen on anoscopies and the two patients with SCCIS on biopsy had ASCUS and LSIL on cytology, respectively. This finding is consistent with other published reports (85, 95). Palefsky et al have shown that anal Pap smear screening in gay and bisexual men has a positive predictive value of 38% and negative predictive value of 84% when ASCUS was included as abnormal as was done in with our study. The positive predictive value of the exam was driven by the higher disease prevalence in this patient population. In our study, only 38% of patients self-identified as gay or bisexual as their HIV risk factor so the positive predictive value of the anal Pap smear screening program would likely be lower. Given that all the patients with high grade lesions on histology were patients with ASCUS or LSIL, this study supports the recommendation of surgical evaluation of any patient with abnormalities, including ASCUS. One limitation is that only approximately thirty-two percent of the patients with abnormal cytology did not received anoscopy with biopsy. The majority of patients that did not receive an anoscopy were those with low grade cytological lesions. Other limitations of this study include its retrospective nature and the temporal distance between the anal Pap smear and anoscopy.

Of the twenty-four patients with anoscopy and biopsy, all those with histological confirmed dysplasia were gay or bisexual men and had higher rates of anal disease on physical exam than those with normal anoscopy findings. Given that all those with histological proven dysplasia were gay or bisexual men is telling, although this represents

only thirty percent of those with abnormal cytology. Since men with a history of receptive anal sex are at the increased risk for development of anal dysplasia, it is expected that a higher percentage of patients with anal dysplasia would be gay or bisexual men. However, since there are data that show a significant percentage of HIV positive men without a history of receptive anal sex also have anal dysplasia, routine screening is still warranted among those who do not self-identify as gay or bisexual (69). More follow-up data would be required to determine the predictive value and utility of screening an entire HIV population if MSM are the only patients who have histology confirmed anal dysplasia.

In summary, we were able to institute anal Pap smear screening as part of routine HIV care in an urban HIV clinic setting with diverse risk factors. The anal Pap smear was easy to do and well tolerated by patients. Anal exam proved to be a poor predictor of those with cytological evidence of dysplasia. Anal cancer screening should be performed on all HIV patients regardless of HIV risk factors and gender. Although only MSM were found to have histological evidence of dysplasia in our study, full follow-up evaluation of all patients with abnormal anal Pap smears is needed. Despite the identification of those with anal dysplasia via the screening program, its impact on morbidity and mortality of patients remains unknown, and warrants further study.

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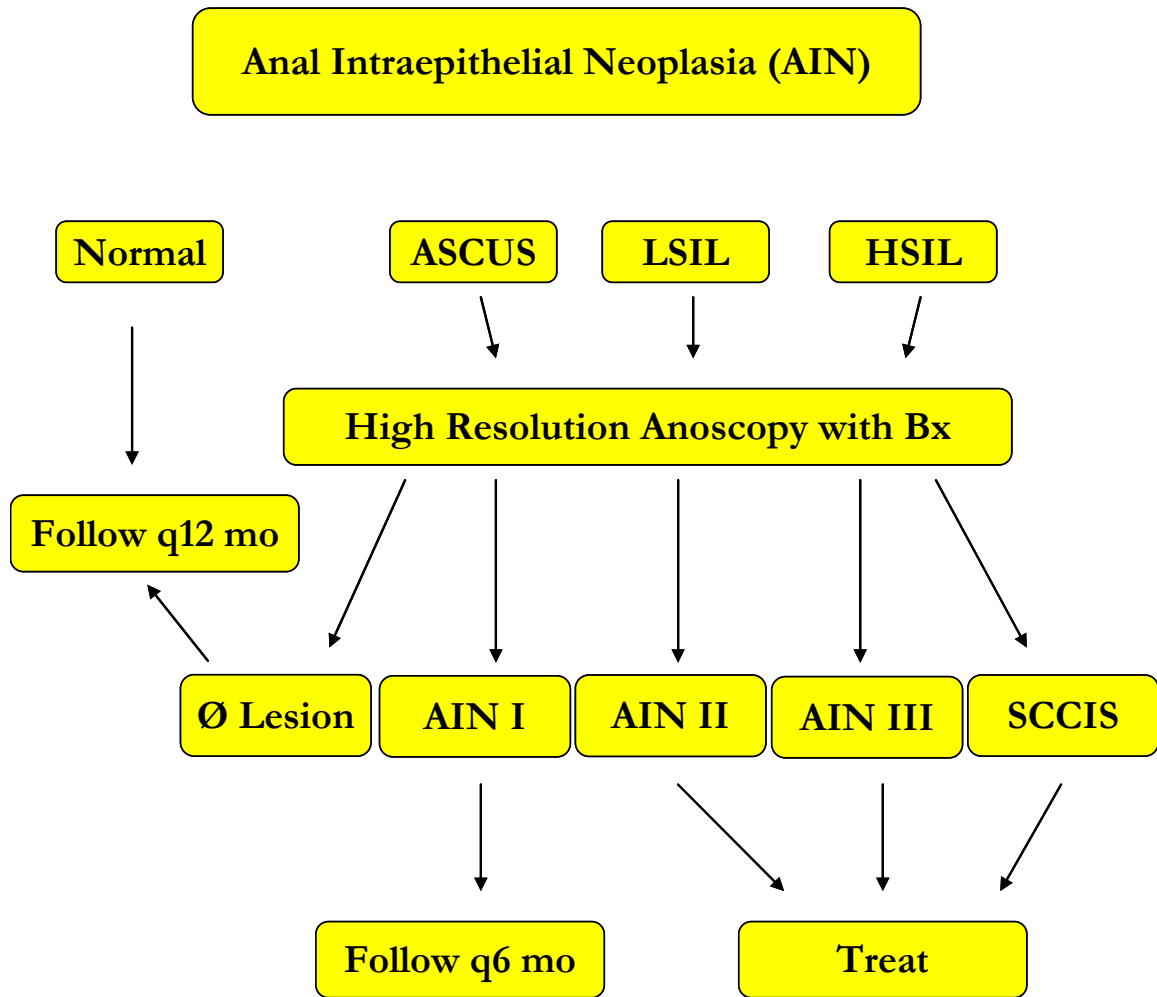


Figure 1: Anal Intraepithelial Neoplasia Screening Program*

Patients were offered routine anal Pap smear screening and the above protocol was used for follow-up or further evaluation.

ASCUS-Atypical Cells of Undetermined Significance; LSIL-Low-grade Squamous Intraepithelial Lesion; HSIL- High-grade Squamous Intraepithelial Lesion; Bx-Biopsy; AIN- Anal Intraepithelial Neoplasia; SCCIS-Squamous Cell Carcinoma In Situ.

* *Adapted from Chin-Hong and Palefsky CID 35:1127-1134

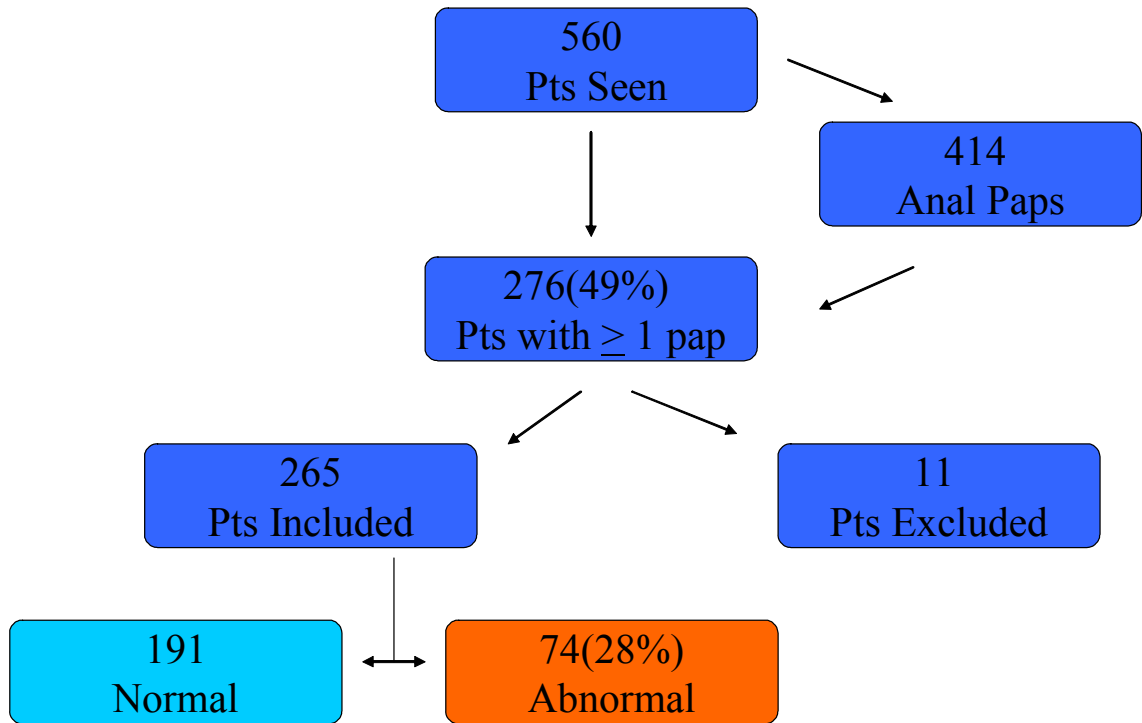


Figure 2: Total Number of Patients Screened During Two Year Study Period.

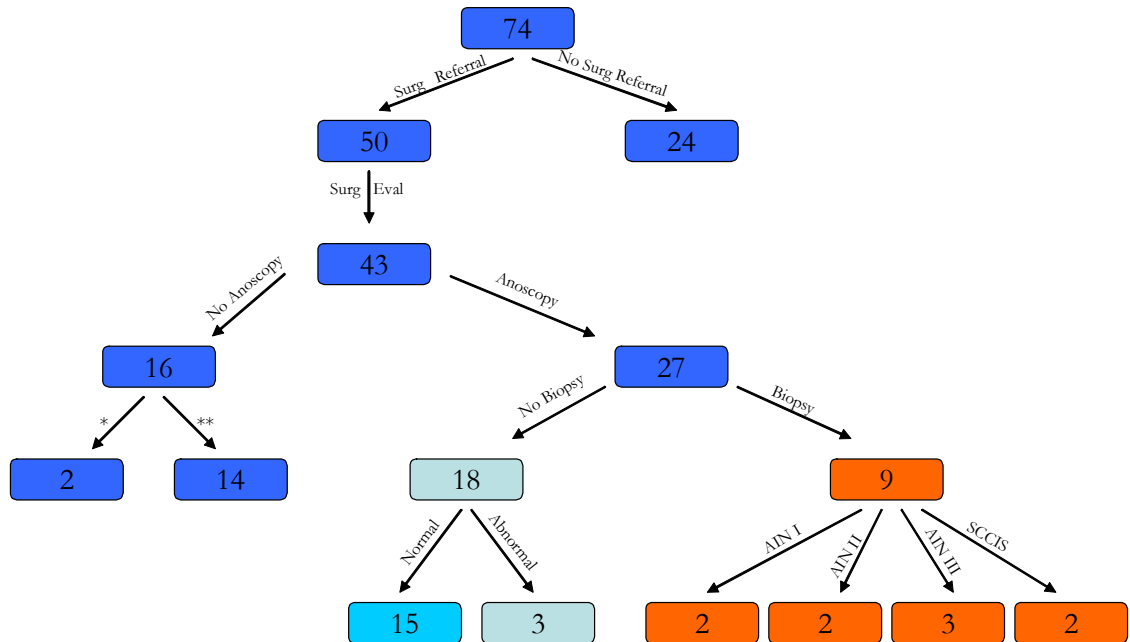


Figure 3: Surgical referral and evaluation of the 74 patients with at least one abnormal anal Pap.

* These two patients were unable to tolerate anoscopy.

** These patients had an anal exam and/or a repeat anal Pap.

Surg Referral-Surgical Referral, Surg Eval-Surgical Evaluation,

AIN- Anal Intraepithelial Neoplasia

Table 1: Baseline demographics of the patients screened during the study period.

Baseline Characteristics	
Mean Age (range)	44.1 yrs (26-77)
Gender	68% Male
Race	49% African American 34% White 17% Hispanic
HIV Risk Factors	49% Heterosexual 28% MSM 18% IVDU
% on ART	75%
% ever on ART	91%
Mean CD4 Nadir (sd)	201 (206)
% CD4 Nadir <200	59%
Mean CD4 at Pap (sd)	458 (323)
% CD4 <200	20%
% with undetectable VL (<400 copies/ml)	57%
Mean VL at Pap (sd)	46,844 (153,577)

MSM-Men who have sex with men; IVDU-Intravenous Drug Use; ART-HIV Antiretroviral therapy; Undetectable VL-Undetectable HIV Viral Load

Table 2: Comparison of patients with normal or abnormal anal Paps by age, race, sex, HIV risk factor, immune status, history of sexually transmitted disease, anal disease, and history of cervical dysplasia for women.

Characteristic	Normal (n=191)	Abnormal (n=74)	p-value
Mean Age (SD)	44.6 (9.0)	42.6 (8.3)	0.10
Race			0.03
% White	30% (57)	45% (33)	
% African American	54% (103)	35% (26)	
% Hispanic	16% (31)	19% (14)	
Male	69% (133)	64% (48)	0.49
HIV Risk Factor			0.02
MSM	25% (45)	40% (29)	
IVDU	22% (39)	11% (8)	
Heterosexual	53% (95)	49% (34)	
CD4 Nadir mean (SD)	223 (211)	142 (185)	0.005
CD4 Nadir <200	53% (100)	76% (56)	0.001
CD4 at Pap	497 (331)	353 (274)	<0.001
CD4 Pap <200	14% (27)	37% (27)	<0.001
VL <400	57 % (109)	63% (46)	0.33
Anal Disease	9%(17)	30%(22)	<0.001
ART at Pap	70%(135)	85%(63)	0.02
ART ever	89%(171)	96%(70)	0.10
History of HSV	22%(42)	35%(26)	0.02
History of Anogenital Warts	12%(23)	23%(17)	0.02
Other GU disease	35%(68)	30%(22)	0.42
History of cervical dysplasia (n =84)	50%(29)	46%(12)	0.74

P<0.05 considered significant.

MSM-Men who have sex with men; IVDU-Intravenous Drug Use; VL- HIV Viral Load; ART-HIV Antiretroviral therapy; HSV-Herpes Simplex Virus; GU-Genitourinary disease

Table 3: Comparison of cytological results to histological results for the 24 patients with complete anoscopy results.

<i>Cytology</i>	<i>Anoscopy</i>				
	No lesions seen on anoscopy	AIN I	AIN II	AIN III	SCCIS
ASCUS	1	0	0	0	1
LSIL	11	2	2	3	1
HSIL	3	0	0	0	0

ASCUS-Atypical Cells of Undetermined Significance; LSIL-Low Grade Squamous Intraepithelial Lesion; HSIL-High Grade Intraepithelial Lesion; AIN-Anal Intraepithelial Lesion; SCCIS-Squamous Cell Carcinoma in Situ.

APPENDIX

Anal Pap Data Collection Sheet #1

1. Reviewer/ 1a. Date of review:	1. 1a.
2. Study Number:	2.
3. Age at Pap (years):	3.
4. Race: 1= White 2=AA 3= Hispanic 4=Asian 5=ative American 6=Other	4.
5. Sex: 1=male 2=female	5.
6. HIV Risk Factor: 1=homo/bisexual 2=MSM/IDU 3=IDU 4=hetero 5=hetero/IDU 6=Transfusion 7=unknown	6.
7. CD4 nadir:	7.
8. Date CD4 nadir:	8.
9. CD4 at Anal Pap Test	9.
10. Date CD4 from item #9:	10.
11. Viral load at Anal Pap Test:	11.
12. Date viral load from item #11:	12.
13. On ART at Anal Pap Test:	13.
14. Ever on ART: 1=no 2=yes	14.
15. Anal Disease on physical examination: 1=no 2=yes	15.
16. History of HSV; 1=no 2=yes	16.
17. History of genital warts 1=no 2=yes	17.
18. History of abnormal cervical Pap: 1=no 2=yes	18.
19. Date abnormal cervical Pap:	19.
20. Abnormal cervical Pap result:	20.
21. Other anal/GU disease (type):	21.
22. Date Anal Pap:	22.
23. Results of Anal Pap:	23.
24. Date HRA:	24.
25. Results HRA:	25.

Repeat Anal Pap Collection Data Sheet

26. Repeat Anal Pap #:	26.
27. Date repeat Anal Pap:	27.
28. Age at repeat Pap (years):	28.
29. Reason repeat Anal Pap: 1=annual screening 2=f/u of abn pap 3=previous insufficient sample 4=other(explain)	29.
30. CD4 nadir:	30.
31. Date CD4 nadir:	31.
32. CD4 at repeat Anal Pap Test	32.
33. Date CD4 from item #9:	33.
34. Decline in CD4 since last Pap: 1=no 2=yes	34.
35. Viral load at this Anal Pap Test:	35.
36. Date viral load from item #11:	36.
37. On ART at this Anal Pap Test:	37.
38. Newly on ART since last Pap: 1=no 2=yes	38.
39. Anal Disease on physical examination at Pap: 1=no 2=yes	39.
40. New dx of HSV since last Pap: 1=no 2=yes	40.
41. New dx of Genital warts since last Anal Pap: 1=no 2=yes	41.
42. New abnormal Pap since last Anal Pap: 1=no 2=yes	42.
43. Date new abnormal cervical Pap:	43.
44. Abnormal cervical Pap result:	44.
45. Other new anal/GU disease (type):	45.
46. Results of Anal Pap:	46.
47. Date HRA:	47.
48. Results HRA:	48.